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Efficient Livestock and Low Emissions (ELLE) from southern grazing systems

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Executive summary

In this project we have addressed the reducing methane emissions priority to identify a wider range of farming practices by which landholders can reduce methane emissions and emissions intensity from grazing systems. Forty commercial, 'pipeline' and/or experimental species of annual and perennial grasses and legumes were used to: (i) identify species with antimethanogenic properties and; (ii) identify species/genotypes with potential to reduce methane emissions intensity via improved nutritional traits including the temporal pattern of digestibility and (iii) demonstrate the effects of the most promising species *in vivo.*We hypothesized that there would be differences in the potential of pasture species to improve livestock production and reduce methane, and that these differences can be associated with temporal patterns of forage yield and feed quality, or more directly through manipulation of methanogenic bacteria in the rumen.

A total of 450 plots (1.2 m x 8 m) were established of 150 accessions from 109 species, of which 40 were chosen for ELLE. A subset of these plants that werechosen to examine the effect of site and season and to grow material for a proof-of-concept *in vivo* experiment, were established at two sites (SA and WA) and sampled over years 2 and 3. In addition, individual spaced plants of six chicory cultivars, nine wild accessions and genotypes that have been selected for persistence and grazing tolerance were used to examine intraspecific variation. We measured the herbage production, nutritive value and *in vitro* methane production on all samples collected across season (years), site and phenological stage (over 4385 samples). We also used these samples to examine the potential for NIR spectrometry to provide rapid and inexpensive predictions of methanogenic properties of pasture species. To ensure these laboratory-based measurement and predictions of reduced methane and improved efficiency will translatedinto the field, we assessed *in vivo* the effects of the most promising plant species on ruminal methane production and animal productivity such as intake, diet selection, nitrogen balance and growth.

There are significant differences across annual and perennial species of grasses and legumes in days to flowering, maturity group, development and biomass. Of the nutritive traits, there was significant variation between accessions for dry matter digestibility(DMD) (hence predicted M/D -digestible energy content at maintenance, MJ/kg), crude protein (CP), acid detergent fibre (ADF), neutral detergent fibre (NDF), hemicellulose and ash. There were clear differences in the temporal pattern of biomass production and nutritive value that enable the identification of commercially-available and regionally-appropriate species with the potential to be used more widely by landholders to reduce methane emissions and emissions intensity. Biserrula was consistently the most bioactive species reducing methane emissions identified at all sampling times and was chosen for more detailed invetigations in the longer-term *in vitro*, Rusitec, system and *in vivo*. When Biserrula was tested *in vivo*, the methane yield expressed as g/MJ ME from sheep fed the legume hays decreased linearly as the proportion of Biserrula hay in the diet increased. Of the perennial species *D. hirsutum*, *D. glomerata* and *T. pratense* consistently produced less methane than the mean of other perennial species tested although in the case of *D. hirsutum* this was probably associated with its very low DMD. In chicory, some accessions had greater persistence than others and differed in seasonal dry matter (DM) distribution. Choice was the only cultivar to provide high yields throughout the year. The nutritional value of chicory was high with mean DMD of 75.1, 63.7 and 72.4 % in spring, summer and autumn. A large amount of variation between and within cultivars, accessions and selections was found for all nutritional parameters, however the differences in their methanogenic potential were limited. Correlations between methane and other *in vitro* fermentation parameters have been estimated and correlationsbetween NIR spectra with nutritive traits and *in vitro*methane production have been calculated. Powerful prediction equations have been developed for the majority of traits. When predicting samples from the collection that were not included in equation development, the statistics of prediction were; total N - r^2 0.96, RPD 5.3, *in vitro*DMD - r^2 0.93, RPD 3.7, ADF - r^2 0.93, RPD 3.9 and NDF - r^2 0.95, RPD 4.3.With only limited samples (n=170), we could predict total methane with reasonable accuracy; r^2 0.89, RPD 3.1.

In conclusion, we have established a comprehensive database of baseline measurements of biomass, nutritive value, *in vitro* fermentabilty and bioactivity across seasons, sites and phonological stage for the key commercially available and pipeline pasture species most suitable for southern Australian grazing systems. There is evidence that there are commercially-available and regionally-appropriate species with the potential to be used more widely by landholders to reduce methane emissions and emissions intensity. There are some species that should be considered further for

their potential to reduce methane emissions directly and others that should be considered because they have attractive temporal patterns for improving the quantity and quality of feed at critical times of the year, extending the growing season and offering opportunities to reduce emissions intensity. There are other species that should be considered as candidates for selective breeding because they show promise, and useful variation, for biomass, nutritive value and fermentative traits that could help reduce emissions and emissions intensity. We have also developed NIR calibration equations that will be a powerful tool to predict nutritional value of samples from the feedbase of southern Australia. The data generated during this project should be used to improve productivity and reduce methane emissions intensity from sheep in southern Australia.

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Background

The demand on agriculture to produce food more efficiently is unprecedented, with the world's population projected to reach 9.6 billion by 2050, and increasing anthropogenic greenhouse gas (GHG) emissions leading to climate change. Livestock GHG emissions form the largest proportion of emissions from agriculture, and comprise 10% of total GHG emissions in Australia. Depending on the diet it is estimated that cattle and sheep typically convert around 6-7% gross energy intake into methane and can produce 250-500 L methane/day (Johnson and Johnson 1995). It is therefore imperative that new methods to produce more meat and milk with lower emissions in a more variable climate are identified (Harrison *et al* 2014). In this project, we address the reducing methane emissions priority to identify a wider range of farming practices by which landholders can reduce methane emissions and emissions intensity from grazing systems.

In addition to being a major contributor towards anthropogenic greenhouse gas emissions, methane represents a significant loss of dietary energy and reducing enteric methaneproduction may also improve feed efficiency and lead to benefits for livestock production (Beauchemin *et al*, 2008). Previous assessments have revealed interventions to livestock management, pasture quality and animal genotype that hold promise for reducing emissions intensity (Martin *et al* 2010; Alcock and Hegarty 2011; Harrison *et al.,* 2014). It is also being recognised more widely that diet composition and intake are the main factors affecting methane production by ruminants (Archimede *et al* 2011) and as outlined by Beauchemin *et al* (2008), manipulating the feedbase is the most cost effective way to provide effective, persistent and significant abatement while maintaining productivity of livestock industries, particularly in the more intensive grazing systems.

In much of southern Australia, deterioration of pasture quality over summer and then the low availability of high quality feed in autumn and winter (McKiernan *et al.,* 2005) coincides with a period where livestock lose condition. The resulting lag in growth rate creates a substantial delay in the time it takes for animals to reach target weight and carcass parameters for slaughter. The slower grow-out of young livestock also increases the time it takes them to reach reproductive age, and this has been identified previously as a key constraint to reducing emissions intensity (Hegarty *et al.,* 2010; Alcock *et al.,* 2014). Pasture species with high total or seasonal dry matter production combined with high feeding value (digestibility plus intake) are required to increase livestock production efficiency. In the Mediterranean and temperate climates of southern Australia, pasture species may either extend the growing season with forage produced using subsoil moisture or respond quickly to out of season rainfall. An alternative strategy is *in situ* conservation of forage produced in spring, whereby plants retain green leaves or maintain high levels of digestibility in reproductive or senesced forage into the summer months. Identifying plants that employ a range of strategies to fill feed gaps and deliver constant feed supply will become increasingly important with predictions for increasing temperatures and changes in rainfall threatening the capacity of the feedbase to meet livestock demand (Moore 2013).

The potential of the Australian temperate feedbase to reduce methane production (either per unit of feed intake or per unit of animal production) has not been thoroughly investigated. In this project we aim to benchmark the variation between and within species of temperate, herbaceous forage species for antimethanogenic effects and identify variability in the provision of digestible nutrients at times of the year where they typically limit animal production. In this project we have investigated a comprehensive selection of commercial, 'pipeline' and/or experimental species using *in vitro*fermentation, wet chemistry and NIR to identify (i) species with antimethanogenic properties and; (ii) species/genotypes with potential to reduce methane emissions intensity via improved nutritional traits including the temporal pattern of digestibility.

Our aims were to: 1) benchmark the variation between and within species of temperate, herbaceous forage species for anti-methanogenic effects; 2) identify variability in the provision of digestible nutrients at times of the year where they typically limit animal production; and 3) quantify *in vivo* animal responses to pasture-based mitigation strategies. One of the most significant outputs from this work has been the ability to assess the potential for NIR spectrometry to provide rapid and inexpensive predictions of methanogenic and chemical properties of pasture species.To ensure these laboratory predictions of reduced methane and improved efficiency will translate to the field, we have assessed*in vivo* the effects of the most promising plant species on ruminal methane production and

animal productivity such as intake, diet selection, nitrogen balance and growth.We hypothesize that there are differences in the potential of pasture species to improve livestock production and reduce methane, and that these differences can be associated with temporal patterns of forage yield and feed quality, or more directly through manipulation of methanogenic bacteria in the rumen.

This report is divided into 6 sections that have been written in a format that can be modified quickly to submit as a series of papers. The first section in this series focuses on the growth and nutritional characteristics of annual legumes, herbs and grasses that are currently used commercially in Australia, or have the potential to be commercialised in the future. The second section focuses on the growth and forage quality of herbaceous perennial grasses, legumes and herbs. Section 3 is focused on the potential of annual and perennial grass and legume pasture species to directly inhibit *in vitro* methane production and section 4 is reports on the development of NIR calibration equations for the key nutritional value measurements and *in vitro* methane measurements that have been obtained. Section 5 deals with the intraspecific variability in growth and nutritional value of chicory and the results from an *in vivo* experiment designed to examine the feeding value and antimethanogenic potential of Biserrula and French serradella is reported in the final section (Section 6).Numbering of tables and figures are specific to each section and not continuous from one section to another.

SECTION 1 Temporal changes in biomass production and nutritional value of annual legumes, grasses and forbs in the feedbase of southern Australia

1.1 Introduction

Diet composition, intake and nutritional value are the main factors affecting the proportion of ingested energy used for animal maintenance and thus methane production per unit of production by ruminant enterprises (Archimede *et al* 2011). Enteric methane represents a significant loss of dietary energy from ruminants as well as a major component of anthropogenic greenhouse gas emissions. Strategies to reduce total emissions include selecting ruminants with higher inherent efficiency or manipulating the diet with plants and/or compounds that alter or inhibit methanogenesis pathways in the rumen. An alternative approach is to reduce methane production per unit of ruminant product by improving feed efficiency; examples include finishing young animals faster, reducing the time taken to reach the first reproduction/lactation event and increasing reproductive rate (Hegarty *et al.,* 2010; Alcock *et al.,* 2014). Feeding strategies that increase profitability to producers are likely to lead to the most significant long-term impact on emissions as they will be actively adopted by industry.

In the Mediterranean and temperate climates of southern Australia, poor pasture quality over summer and autumn and limited biomass availability in winter restrict ruminant growth unless supplements are provided. Pasture species with high total or seasonal dry matter production combined with high nutritional value are required to increase livestock production efficiency. Traits that would be useful include rapid growth during establishment, an ability to extend the growing season by using deep roots to access subsoil moisture and a slower rate of decline in nutritional value through reproduction and senescence.

The diversity of annual pasture species within the feedbase of southern Australia for nutritional and methane traits remains largely unexplored. This paper examines the nutritional value of 67 species of annual plants, comprising the major cultivars and promising experimental lines of legumes, grasses and forbs. We hypothesize that there will be significant differences in biomass production, nutritional value at various development stages and differences in the rate of nutritional decline after flowering. A companion paper (see section 3) explored the potential for methane abatement.

1.2 Materials and methods

To evaluate temporal changes in forage yield and quality, 90 accessions of annual legumes, grasses and forbs were grown in two adjacent, replicated experimental plots in South Australia (legumes separated from grasses and herbs). A subset of 16 accessions were planted in replicated plot experiments in Western Australia across two growing seasons.

Germplasm

The germplasm was selected to represent the complete range of annual pasture species that have been commercialized and promising experimental selections. The material comprised 60 annual legume accessions (from 50 species) 17 grasses (from 7 species) and 12 forbs (9 species) herbs. Accessions are listed in Table 1a and 1b.

Table 1a. Annual grasses and forbs included in experimental planting in South Australia.

Type	Group	Scientific name	Variety/entry	Common name
Forb	Brassica	*Brassica campestris var. rapa*	Hunter	Forage turnip
		Brassica napus	Hyola 50	Canola
			Taurus	
			43Y85	
		Brassica napus x oleracea	Titan	Rape
		Brassica oleracea	Kestrel	Kale
		Brassica rapa	*New York	Forage turnip
		Brassica rapa x oleracea	Winfred	Rape
		Brassica tournefortii	SA 42783	Wild turnip
	Chia	*Salvia hispanica*	Chia Black	Black chia
			Chia White	White chia
	Saltbush	*Atriplex semibaccata*	SA 45507	Creeping saltbush
Grass	Cereal	*Avena sativa*	*Winteroo	Forage oat
		Hordeum vulgare	Moby	Barley
		Secale cereale	Sthn Green	Ryecorn
		Triticosecale X	Crackerjack2	Tritacale
		Triticum aestivum	*Wedgetail	Wheat
	Ryegrass	*Lolium multiflorum* (D)	Eclipse	Italian ryegrass
			Mverick GII	
			*Dargo	
		Lolium multiflorum (T)	Feast ll	
			Tama	
		Lolium multiflorum X (D)	Fesper	
			Turbo	
		Lolium rigidum (T)	Sungrazer	Annual ryegrass
			Zoom	
		Lolium rigidum(D)	Progrow	
			Safeguard	
			Wimmera	

Varieties marked with an * were in the subset that were grown in Western Australia

Table 1b. Annual legumes included in experimental planting in South Australia.

Group	Scientific name	Variety/entry	Common name
Biserulla	*Biserulla pelecinus*	*Casbah	Biserulla
Clover	*Trifolium alexandrinum*	Memphis	Berseem clover
	Trifolium dasyurum	Sothis	Eastern star clover
	Trifolium diffusum	Tas 511/348	Diffuse clover
	Trifolium glanduliferum	Prima	Gland Clover
	Trifolium glomeratum	Tas 1630/1807	Cluster clover
	Trifolium hirtum	SARDI rose	Rose clover
	Trifolium incarnatum	Blaza	Crimson clover
	Trifolium isthmocarpum	SA 20009	Moroccan clover
	Trifolium lappaceum	Tas 2129	Lapp clover
	Trifolium michelianum	*Frontier	Balansa clover
	Trifolium nigrescens	SA 15896	Ball clover
	Trifolium purpureum	Paratta	Purple clover
	Trifolium resupinatum	Lightening; SARDI Persian	Persian clover
	Trifolium spumosum	*Bartolo	Bladder clover
	Trifolium squarrosum	SA 36400	Spike clover
	Trifolium striatum	Tas 1698	Striated clover
	Trifolium subterraneum subsp. brac.	*Antas; Clare	Subclover
	Trifolium subterraneum subsp. sub.	*Urana; Denmark	Subclover
	Trifolium subterraneum var. yan.	Gosse, Trikkala	Subclover
	Trifolium tomentosum	SA 35654	Woolly clover
	Trifolium vesiculosum	Cefalu	Arrowleaf clover
Fenugreek	*Trigonella balansae*	*SA 5045; SA 32999	
	Trigonella caerulea	SA 32200	Blue fenugreek
	Trigonella calliceras	SA 32202	
	Trigonella coelesyriaca	SA 19767	
	Trigonella foenum-graecum	*Wimmera Sungold; Might	Fenugreek
Hedysarum	*Hedysarum flexuosum*	SA 32504	Hedysarum
Lotus	*Lotus ornithopodioides*	ITA 8a	
Medic	*Medicago arabica*	*SA 8774; SA 36809	Spotted medic
	Medicago littoralis	Angel; Herald	Strand medic
	Medicago orbicularis	Bindaroo	Button medic
	Medicago phrygia	SA 32612	
	Medicago polymorpha	*Scimitar	Burr medic
	Medicago rotate	Highlander	Wheel medic
	Medicago rugosa	Paraponto	Gama medic
	Medicago scutellata	Essex	Snail medic
	Medicago sphaerocarpos	Orion	Sphere medic
	Medicago italica	Tornafield	Disc medic
	Medicago truncatula	Caliph	Barrel medic
Melilotus	*Melilotus albus*	Jota	White sweetclover
	Melilotus elegans	SA 37228	Elegent sweetclover
	Melilotus siculus	*SA 40002	Messina
Ononis	*Ononis alopecuroides*	SA 8577	Harrow
Pea	*Lathyrus cicera*	Ceora	Chickling Vetch
Serrdella	*Ornithopus compressus*	*Santorini	Yellow serradella
	Ornithopus pinnatus	Jebala	Slender seradella
	Ornithopus sativus	Cadiz	French seradella
Vetch	*Astragalus hamosus*	Ioman	European milkvetch
	Vicia benghalensis	Popany	Purple vetch
	Vicia sativa	*Languedoc	Subterranean vetch
	Vicia villosa	Namoi	Large Russian vetch

Varieties marked with an * were in the subset that were grown in Western Australia

Management of South Australian site

The field site was located in the Australian Pastures Genebank field nursery at the Waite Institute in South Australia. The fine sandy loam at this site is a red-brown earth (Stace *et al.,* 1968) of the non-sodic Urrbrae series (Litchfield 1951). The upper 0.10m contains 18% clay, increasing to 32% in the A2 horizon (Prescott 1931). Soil pH (in $CaCl_2$) was 6.2 and there was negligible calcium carbonate (Grace *et al.,*1995). The site had subsurface drip irrigation, with two lines running 0.5 m apart, 0.2 m beneath each plot, and with drip intervals of 0.5 m. In the first year (September 2012 to 31 January 2013), irrigation was with weekly applications equivalent to 25 mm of rainfall between November and April. No irrigation was used in the second year of the experiment (1 April 2013 to 31 January 2014). Rainfall data is presented in Fig 1.

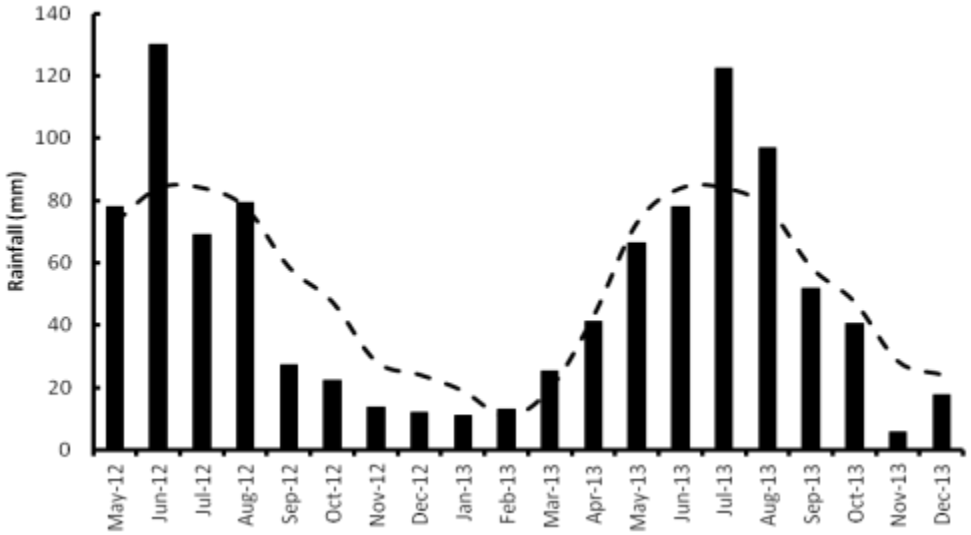

Fig 1. Monthly rainfall data (bars) at the South Australian site during the experimental period. The dashed line is the long-term average.

In South Australia, the annual legumes, grasses and herbs were evaluated in two separate experiments to allow broadleaf herbicide applications and nitrogen fertiliser to be applied separately to two different areas. For each experiment, the accessions were sown in a completely randomised and blocked design with 18 columns and 10 rows (3 replications with 6 columns and 10 rows per replication) for the annual legumes and 9 columns and 10 rows (3 replications with 3 columns and 10 rows per replication) for the herbs and grasses. Each species was sown into 1 x 8 m plots with a self propelled Wintersteiger small plot seeder at the highest recommended rate from the seed supplier, adjusted for percent germination. Fertilizer application is presented in Table 2. The legumes were inoculated with the recommended class of rhizobia for the species the day before planting.

Table 2. Fertiliser application at the South Australian research site.

Date	Fertiliser	Applied to
29/08/2012	100 kg/ha urea	All annual grasses/herbs (except chia's and quinoa)
		Annual legumes –Trigonella's; SA 32200, SA 32202, SA 19767; Medicago SA 32612; Melilotus SA 37228; Hedysarum SA 32504; Ononis SA 8577 and Astragalus Ioman
8/11/2012	100 kg/ha urea	Chia black and white, quinoa SA 45507
8/11/2012	100 kg/ha urea and 100 kg/ha ammonium sulphate	Brassica's; Winfred, Titan and Kestrel

In South Australia, the annual legumes, grass and herbs were sown on 11 June 2012. Forage yield was assessed every 3 weeks after an initial establishment phase of 77 days, when growth was sufficient to justify cutting. Plots were cut at 3 cm above ground using a 1m wide sickle (finger) mower with reciprocating blades to provide a 1 m^2 forage sample. This sample was weighed, sub-sampled, freeze dried and weighed a second time to calculate dry weight forage yield.

For the first season cuts, subsequent measurements of forage yield (cuts 2−7) were taken on the adjacent 1 m^2 section of plot, such that repeat measurements of yield were not taken from the same area. At each measurement of forage yield, plant development stage was assessed using the protocol developed by Metcalfe and Nelson (1985) (Table 3). In the second season (2013), forage yield of regenerating plots of annual legumes was assessed on 24 June and between 11 September and 16 October on the section of plot used in cut 1 (unless it was a species that did not recover from this cut, then cut 7 was used as it had the next greatest potential to produce seed).

Table 3. Morphological descriptors for growth stages of forage grasses and legumes (from Metcalfe and Nelson, 1985).

Grasses	
1	Vegetative (Leaves only; stems not elongated)
2	Stem elongation (Stems elongated)
3	Boot (Inflorescence enclosed in flag leaf sheath and not showing)
4	Heading (Inflorescence emerging or emerged from flag leaf sheath, but not shedding pollen)
5	Anthesis (Flowering stage; anthers shedding pollen)
6	Milk stage (Seed immature, endosperm milk)
7	Dough stage (Well-developed seed; endosperm doughy)
8	Ripe seed (Seed ripe; leaves green to yellow brown)
9	Postripe seed (Seed postripe; some dead leaves; some heads shattered)
10	Stem-cured (Leaves cured on stem; seed mostly cast)
Legumes & herbs	
1	Vegetative (No buds visible)
2	Bud (Buds visible, but no flowers)
3	First flower (First flowers appear on plants)
4	Flower (Plants flowering)
5	Pod (or green seed) Green seedpods developing
6	Ripe seed (Mostly mature brown seedpods with lower leaves dead and some leaf loss)
7	Senescence (Leaves cured or dropped, pod/seed mostly cast)

Management of Western Australian site

The field site in Western Australia was located on a commercial farm near Brookton. At this site, a range of 16 annual legumes, forbs and grasses were grown in two consecutive seasons. Plots were sampled every 3 weeks through the growing season and for 2 months after senescence. The first experiment was planted on 14 June 2013 and the second was planted on 28 May 2014 in an adjacent paddock. The soil had a pH level ($CaCl_2$) of 4.6, organic carbon of 1.09%, texture of 1.5 and the colour was lightbrown. The site was not irrigated and a basal application of fertiliser was applied to the entire paddock at sowing. The long-term mean annual rainfall in the area is 450 mm (Fig 2). In 2013 there was a total of 460 mm but an exceptionally dry June and wet spring. In 2014, the pattern largely followed the long-term average with the exception of a wetter May and drier June. Annual rainfall in 2014 was 370 mm, 80 mm below the long term average.

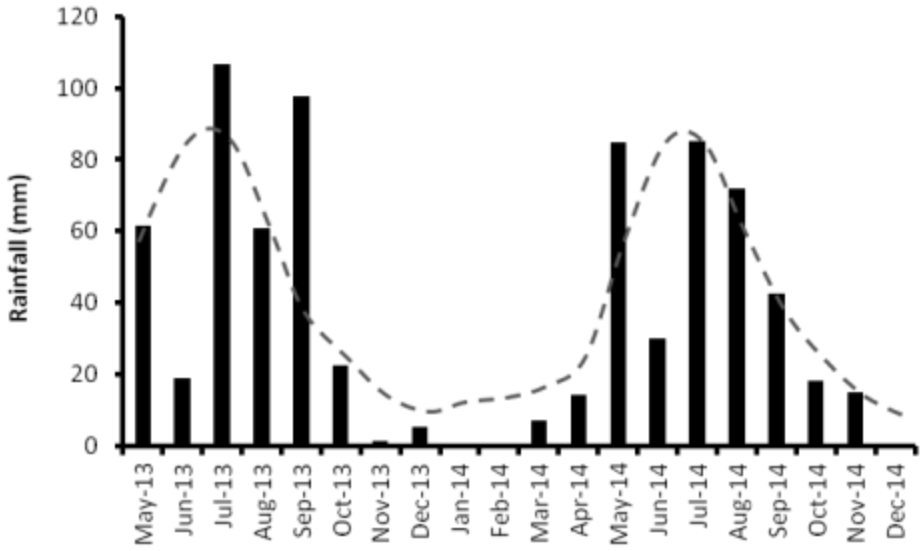

Fig 2. Monthly rainfall data (bars) at the West Australian site near Brookton during the experimental period. The dashed line is the long-term average.

The 16 accessions were planted in a randomized plot design with a replicate (1.2 x 8m) of each variety in each of the 3 blocks. Plots were sown with a cone seeder at the highest recommended rate from the seed supplier, and adjusted for percent germination (Table 4). The legumes were inoculated with the recommended class of rhizobia for the species the day before planting.

Samples were harvested from 30 x 30 cm quadrates using electric clippers as soon as there was sufficient biomass and every 3-4 weeks thereafter until late December. At each harvest a different section of the plot was sampled so individual plants were only harvested on one occasion.

Table 4. Germinability of seed, seeding rate, number of seeds sown/replicate and establishment density at the Western Australian site.

Common Name	Entry	Germination (%)	Seeding rate (kg/ha)	Seed sown/rep (g)	Establishment in 2013 (plants/m2)
Forage Oats	Winteroo	94	100	102.1	160
Italian ryegrass	Dargo	88	20	21.8	255
Forage turnip	New York	10	5	48.0	230
Biserulla	Casbah	66	10	14.5	100
Spotted medic	SA 8774	77	10	12.5	94
Burr medic	Scimitar	90	10	10.7	108
Messina	SA 40002	66	12	17.5	86
Yellow serradella	Santorini	90	10	10.7	110
French serradella	Cadiz	88	10	10.9	119
Sub clover	Antas	80	15	18.0	75
Sub clover	Urana	88	15	16.4	111
Balansa clover	Frontier	55	10	17.5	309
Bladder clover	Bartolo	52	10	18.5	107
Trigonella	SA 5045	70	10	13.7	180
Fenugreek	Wimmera Sungold	90	40	42.7	167
Common vetch	Languedoc	72	60	80.0	57

Sample processing

After collection and weighing, biomass samples were either immediately frozen for freeze drying or placed in a paper bag then oven dried at 60^{o}C. After drying, samples were ground to pass through a 1mm screen using either a Cyclotech or Retsch mill. A preliminary study revealed that there was little bias associated with the type of grinder.

NIRS

Nutritional value was estimated using chemistry and near infrared spectroscopy (NIRS; see review by Deaville and Flynn, 2000). Samples were scanned by NIRS (Unity Spectrastar 2500X- rotating top window system, Unity Scientific) and nutritional traits were predicted calibrations generated using partial least squares regression with the chemometric software package Ucal (Unity Scientific). Subsets of samples were set aside for chemical analysis. The broad, multispecies NIRS calibration for DMD, total N, ADF, NDF and OM (ash) was built on over 1000 samples with matching spectra and chemistry (see Section 3). Where a sample was analysed with chemistry, those data are used, otherwise data are predicted. Samples that did not fit within the calibration (as indicated by high global H and neighbourhood H values) were subject to chemistry. Global H and neighbourhood H tell if the model is suitable for the samples analysed ie very high distances mean that the samples are not yet represented in the calibration data set.

RPD tests the strength of the relationship between a constituents values and the error of the NIR predicted results and was calculated by;

$$\mathbf{RPD = 1 / (1 - r^2)^{0.5}}$$ (Williams 2014)

The larger the RPD value the greater its strength. We have adopted the forage RPD guide of Williams (2014) who suggested RPD values of 0.0–1.9 are very poor and not recommended for forage testing; RPD values of 2.0–2.4 are poor and only of use for rough screening; RPD values of 2.5–2.9 offer a fair screening potential; RPD values of 3.0–3.4 are good (quality control); RPD values of 3.5–4.0 are very good (suited to process control) and RPD values of 4.1+ are deemed excellent.

Samples within this paper were predicted with the following accuracy; total N - r^2> 0.98, RPD > 7.1; *in vitro* DMD - r^2> 0.96; RPD > 4.9, OM (ash) - r^2> 0.91; RPD > 3.3; ADF - r^2> 0.96, RPD > 4.8 and NDF - r^2> 0.95, RPD > 3.5. OM predictions are not as accurate as those for other traits and data interpretation should proceed with a degree of caution.

Chemistry

In vitro dry matter digestibility (DMD), adjusted to predict *in vivo* digestibility, was determined in duplicate using a modified pepsin-cellulase technique described by Clarke *et al.,* (1982). Modifications are outlined in Section 3. Duplicate samples of seven Australian Fodder Industry Association (AFIA) standards (AFIA 2007) with known *in vivo* DMD are included in each batch to allow raw laboratory values to be adjusted to predict *in vivo* digestibility using linear regression (see Fig. 1). The average standard error of the AFIA standards across the runs was 0.261%. The energy value of the sample (MJ/kg at maintenance level of feeding) was estimated by the equation: M/D = (0.172*DMD) - 1.707 (Standing Committee on Agriculture, 1990).

Concentrations of neutral detergent fibre (NDF) and acid detergent fibre (ADF) of the shrub material were measured sequentially, according to operating instructions, using an Ankom 200/220 Fibre Analyser (Ankom® Tech. Co., Fairport, NY, USA). Duplicate samples were analysed for each diet. The difference between NDF and ADF was deemed to be hemicellulose. An oaten hay samples was included in each of the 103 fibre analysis runs during the project and aross runs, this sample had NDF of 30.19 ± 0.1137 % DM and ADF of 19.71 ± 0.0665 %DM.

Total ash was measured on duplicate samples according to the methods of Faichney and White (1983). Total nitrogen and carbon was determined by combustion using a Leco CN628 N Analyser (Sweeney and Rexroad 1987). Where crude protein has been presented we have adopted the convention of CP = total N x 6.25.

Statistical analyses

Means of fixed accession effects for each response variate (forage yield, developmental growth stage) were calculated using spatial linear mixed models performed by Genstat 15 (Lawes Agricultural Trust 2012). Diagnostic plots of sample variograms and residuals were used in conjunction with REML log-likelihood ratios and Wald tests to fit new models that compartmentalised and removed random and fixed effects of variation (Smith *et al.,* 2005). The nutritional data was analysed using ANOVA with Genstat 15.No data sets required transformation. To model the pattern of digestibility change over time, logistic curves were fitted to the data for each entry within each year to give estimates of peak digestibility (at the vegetative phase), half-life digestibility loss, rate of digestibility loss at the point of inflection and digestibility after senescence. An example of a fitted model is presented in Fig 3.

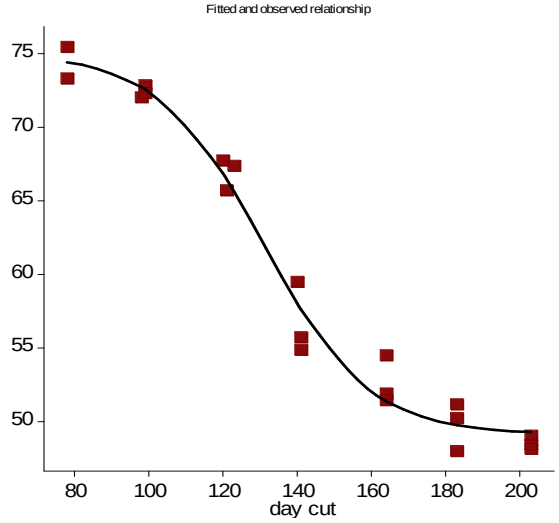

Fig 3. An example of a logistic curve fitted to data for Paratta purple clover showing changes in dry matter digestility (%) with days from seeding.

1.3 Results

Establishment and biomass production in South Australia

Mean plant density, days to flowering, maturity group and development stage for each cutting date for annuals at the South Australian site are presented in Tables 5a and 5b. There are statistically significant differences amongst the legumes for all of the measured traits. Sixvarieties failed to establish more than 45 plants per m^2 and were excluded from the analysis of biomass production. These were Orion medic, Ceora lathyrus, Sothis eastern star clover, *Trigonella calliceras* SA 32202, *Brassica tournefortii* and *Atriplex semibaccata.*

Biomass production was compared between plants within maturity groups. Annual plants that delay reproduction generally continue to produce vegetatve DM at the expense of seed production (thus persistence). We focus our comparison of biomass between accessionswithin maturity cohorts (ie plants that have a similar target growing season length). The maturity cohorts were as follows; 1 – Early with < 95 days to flowering (recommended for the 250-350 mm rainfall zone); 2 – Mid with 95-120 days to flowering (recommended for the 350-500 mm rainfall zone); Late with 121-240 days to flowering (recommended for the 500-700 mm rainfall zone) and Very Late with >240 days to flowering (recommended for the >700 mm rainfall zone).

Biomass growth for the majority of species are presented in Figs 4 to 7. Tables of biomass production with statistical comparisons are in Tables 6a and 6b. There were significant differences between accessions within maturity cohorts at all cutting times. For the early cohort of annual legumes, early winter herbage production was greatest for balansa clover cv Frontier and burr medic cv Scimitar. Both of these species, and Essex snail medic were the most productive at the site. The two fenugreek species, cv Might and cv Wimmera Sungold were unusual compared to other legumes in that they continued to produce more biomass despite early flowering (Fig 4, Table 6a). Of the mid maturity cohort of legumes, gland clover cv Prima had the greatest early growth. The most productive

accessions were the vetchs cv Languedoc and cv Namoi as well as purple clover cv Paratta. Crimson clover cv Cefalu, subterranean clover cv Antas and purple clover cv Paratta had the greatest biomass at the later cutting dates (Fig 5). The late and very late cohort of annual legumes clearly produced more biomass than the early cohort. The standout for early biomass production was another vetch cv Popany. *Melilotus elegens* (SA 37228), *Melilotus albus* cv Jota, Popanu vetch and the clover *Trifolium diffusum* were the most productive of the late cohort (Fig 6).

The grasses and forbs were generally more productive than the legumes. In the early to mid cohort, the forage oat cv Winteroo was clearly the most productive (over 9.5t DM on offer at its peak) and had the greatest early winter biomass production (Fig 7). Of the late maturing cohort triticale cv Crackerjack had over 14t DM at its peak. Other highly productive accessions included Ryecorn cv Southern Green and the tetraploid cv ryegrass Feast II (Fig 7).

Table 5a Mean plant density, days from planting to flowering, maturity group and development stage at each cutting date for annual legumes at the South Australian site in year 1

Species	Entry	Plant density[a]		Flowering	Maturity	Development stage by cutting date[b]						
		log trans.	Back trans.	(days)	Group[1]	d77	d 98	d120	d140	d163	d182	d203
Astragalus hamosus	Ioman	5.5	237	98	2	1.0	2.2	5.0	5.0	7.0		
Biserulla pelecinus	Casbah	4.4	81	106	2	1.0	1.1	5.0	5.0	6.6	6.0	6.5
Hedysarum flexuosum	SA 32504	4.3	74	140	4	1.0	1.0	1.1	2.7	5.0	6.1	7.0
Lathyrus cicera	Ceora	2.2	9	106	2	1.0	1.0	5.0	5.0	6.0	6.5	7.0
Lotus ornithopodioides	ITA 8a	5.6	278	98	2	1.0	3.9	5.0	5.0	7.0	7.0	
Medicago arabica	SA 36809	5.5	238	120	2	1.0	1.0	4.0	5.0	6.1	7.0	
Medicago arabica	SA 8774	5.7	301	98	2	1.0	3.9	5.0	5.0	7.0		
Medicago littoralis	Herald	4.9	134	91	1	1.0	4.8	5.0	5.0	7.0		
Medicago littoralis	Angel	5.3	209	85	1	1.0	5.0	5.0	6.0	7.0		
Medicago orbicularis	Bindaroo	5.1	158	85	1	1.5	5.0	5.0	6.7	7.0		
Medicago phrygia	SA 32612	5.7	306	120	2	1.0	1.0	2.4	5.0	5.6	6.6	
Medicago polymorpha	Scimitar	5.6	284	85	1	1.0	5.0	5.0	5.8	7.0		
Medicago rotata	Highlander	5.0	146	91	1	1.0	5.0	5.0	6.0	7.0		
Medicago rugosa	Paraponto	4.4	83	85	1	1.0	5.0	5.0	6.0	7.0		
Medicago scutellata	Essex	4.3	73	91	1	1.0	4.0	5.0	5.4	7.0		
Medicago sphaerocarpos	Orion	2.1	8	91	1	1.0	4.0	5.0	5.2	7.0		
Medicago tornata	Tornafield	5.4	221	98	2	1.0	3.7	5.0	6.0	7.0		
Medicago truncatula	Caliph	4.6	95	85	1	1.0	5.0	5.0	5.5	7.0		
Melilotus albus	Jota	5.7	303	147	4	1.0	1.0	1.0	1.8	4.5	5.4	6.1
Melilotus elegans	SA 37228	5.2	173	133	3	1.0	1.0	1.9	4.0	5.0	6.4	6.5
Melilotus siculus	SA 40002	4.8	126	98	2	1.0	2.9	5.0	5.0	7.0		
Ononis alopecuroides	SA 8577	5.9	358	120	2	1.0	1.0	3.0	5.0	6.1	7.0	
Ornithopus compressus	Santorini	4.5	91	98	2	1.0	2.2	5.0	5.0	6.5	6.5	7.0
Ornithopus pinnatus	Jebala	4.6	95	113	2	1.0	1.0	5.0	5.0	6.3	7.0	
Ornithopus sativus	Cadiz	5.3	194	106	2	1.0	1.1	5.0	5.0	6.0	6.1	7.0
Trifolium alexandrinum	Memphis	5.0	152	140	4	1.0	1.0	1.0	2.1	5.2	6.3	7.0
Trifolium dasyurum	Sothis	3.5	32	106	2	1.0	2.0	5.0	5.5	7.0		
Trifolium diffusum	Tas 511_348	6.3	567	147	4	1.0	1.0	1.0	1.9	5.0	6.0	6.5
Trifolium glanduliferum	Prima	6.7	781	98	2	1.0	2.5	5.0	5.3	7.0		
Trifolium glomeratum	Tas 1630/1807	6.1	445	142	4	1.0	1.0	1.0	4.9	6.0	6.4	7.0
Trifolium hirtum	SARDI rose	4.8	119	113	2	1.0	1.5	4.0	5.0	7.0		

Trifolium incarnatum	Blaza	5.0	145	120	2	1.0	1.0	3.2	5.0	7.0		
Trifolium isthmocarpum	SA 20009	6.2	493	120	2	1.0	1.0	2.4	5.0	6.5	6.7	
Trifolium lappaceum	Tas 2129	6.2	499	140	4	1.0	1.0	1.0	3.0	5.9	6.5	7.0
Trifolium michelianum	Frontier	6.6	746	91	1	1.0	2.3	5.0	6.0	7.0		
Trifolium nigrescens	SA 15896	5.7	300	106	2	1.0	1.8	5.0	6.0	6.5	7.0	
Trifolium purpureum	Paratta	6.3	534	120	2	1.0	1.0	2.2	4.4	5.5	6.5	6.8
Trifolium resupinatum	Lightening	5.5	246	133	3	1.0	1.0	1.3	4.5	6.0	6.5	7.0
Trifolium resupinatum	Persian	5.7	310	106	2	1.0	1.1	4.6	5.0	6.5	7.0	
Trifolium spumosum	Bartolo	5.2	185	113	2	1.0	1.6	5.0	5.6	7.0	7.0	
Trifolium squarrosum	SA 36400	5.7	299	147	4	1.0	1.0	1.0	2.2	5.5	7.0	
Trifolium striatum	Tas 1698	5.4	217	147	4	1.0	1.0	1.0	2.0	6.5	7.0	
Trifolium subterraneum	Clare	5.0	146	120	2	1.0	1.0	4.0	5.0	6.1	7.0	
Trifolium subterraneum	Antas	4.5	86	120	2	1.0	1.0	3.9	5.0	6.0	6.9	
Trifolium subterraneum	Denmark	5.0	142	126	3	1.0	1.0	3.0	5.0	6.0	6.5	7.0
Trifolium subterraneum	Urana	5.2	184	106	2	1.0	3.7	4.2	5.0	6.1	6.5	7.0
Trifolium subterraneum	Gosse	4.6	101	113	2	1.0	1.0	4.0	5.0	6.3	7.0	
Trifolium subterraneum	Trikkala	5.0	141	106	2	1.0	1.2	5.0	5.0	6.5	7.0	
Trifolium tomentosum	SA 35654	6.6	717	106	2	1.0	1.1	5.0	5.0	7.0		
Trifolium vesiculosum	Cefalu	5.5	251	120	2	1.0	1.0	2.1	4.0	5.2	6.1	6.5
Trigonella balansae	SA 32999	4.8	122	106	2	1.0	2.0	5.0	5.0	7.0		
Trigonella balansae	SA 5045	6.1	446	98	2	1.0	2.3	5.0	5.2	7.0		
Trigonella caerulea	SA 32200	5.3	202	133	3	1.0	1.0	1.2	4.0	5.5	7.0	
Trigonella calliceras	SA 32202	3.6	37	113	2	1.0	1.0	5.0	5.0	7.0		
Trigonella coelesyriaca	SA 19767	5.9	364	85	1	1.0	4.9	5.0	7.0	7.0		
Trigonella f-graecum	Might	4.1	63	91	1	1.0	3.0	5.0	5.0	6.5	7.0	
Trigonella f-graecum	Sungold	4.5	86	85	1	1.0	3.0	5.0	5.0	7.0		
Vicia benghalensis	Popany	4.1	62	126	3	1.0	1.1	2.2	5.0	6.3	7.0	
Vicia sativa	Languedoc	4.7	107	113	2	1.0	2.0	5.0	5.2	7.0		
Vicia villosa	Namoi	4.0	52	113	2	1.0	2.0	4.0	5.0	6.9	6.7	7.0
Grand Mean		5.1	228	111	2	1.01	2.1	3.8	4.8	6.4	6.6	7
F prob		<.001		<.001		<.001	<.001	<.001	<.001	<.001	<.001	<.001
LSD (5%)		0.42		0.81		0.00	0.21	0.10	0.21	0.20	0.13	0.06

[1]Maturity groups; 1 – Early with < 95 days to flowering (250-350 mm rainfall zone); 2 – Mid with 95-120 days to flowering (350-500 mm rainfall zone); Late with 121-240 days to flowering (500-700 mm rainfall zone) and Very Late with >240 days to flowering (>700 mm rainfall zone). [a]Established plants/m^2. [b]Days from planting.

Table 5b Mean plant density, days from planting to flowering, maturity group and development at each cutting date for annual grasses and forbs at the South Australian site in year 1

Species	Entry	Est[a] (p/m2)	Flowering (days)	Maturity Group[1]	Development stage by cutting date[b] d77	d98	d120	d140	d163	d182	d203
Atriplex semibaccata	SA 45507	37	120	2	1.0	1.0	1.0	1.0	1.0	2.0	2.0
Avena sativa	Winteroo	255	113	2	2.0	2.7	5.0	7.0	9.9	10.0	
Brassica campestris var. rapa	Hunter	117	113	2	1.0	1.0	2.9	5.0	5.6	7.0	
Brassica napus	Hyola 50	69	85	1	1.7	5.0	5.0	5.0	6.6	7.0	
Brassica napus	Taurus	56	120	2	1.0	1.0	2.2	5.0	5.1	6.0	6.5
Brassica napus x oleracea	Titan	100	151	4	1.0	1.0	1.0	1.5	4.4	5.1	6.2
Brassica napus	43Y85	116	85	1	2.0	5.0	5.0	5.0	6.5	7.0	
Brassica oleracea	Kestrel	89		4	1.0	1.0	1.0	1.0	1.0	1.1	1.0
Brassica rapa x oleracea	Winfred	85		4	1.0	1.0	1.0	1.3	1.6	2.6	2.7
Brassica rapa	New York	45	120	2	1.0	1.0	2.2	5.0	5.4	6.4	7.0
Brassica tournefortii	SA 42783	20	85	1	2.0	5.0	5.0	6.5	7.0	7.0	
Hordeum vulgare	Moby	215	98	2	2.0	3.1	5.0	7.0	9.4	10.0	
Lolium multiflorum (D)	Eclipse	612	151	4	1.2	2.0	2.1	3.3	6.2	9.5	
Lolium multiflorum (D)	Mverick GII	502	151	4	1.0	2.0	2.1	2.9	5.5	9.0	10.0
Lolium multiflorum (D)	Dargo	432	140	3	1.2	2.0	2.9	5.0	7.6	10.0	
Lolium multiflorum (T)	Feast ll	455	151	4	1.0	2.0	2.0	3.0	5.8	9.0	10.0
Lolium multiflorum (T)	Tama	390	151	4	1.4	2.0	2.0	3.1	6.0	10.1	
Lolium multiflorum X (D)	Fesper	409	151	4	1.1	2.0	2.0	3.2	6.3	9.0	10.0
Lolium multiflorum X (D)	Turbo	544	151	4	1.0	2.0	2.0	3.1	5.9	9.2	10.0
Lolium rigidum (T)	Sungrazer	383	142	4	1.5	2.0	2.0	4.8	7.3	9.7	10.0
Lolium rigidum (T)	Zoom	362	151	4	1.5	2.0	2.0	3.0	5.8	9.5	10.0
Lolium rigidum(D)	Progrow	545	140	3	1.0	2.0	2.0	3.4	6.3	9.3	10.0
Lolium rigidum(D)	Safeguard	349	98	2	1.0	3.1	5.0	7.0	9.2	10.0	
Lolium rigidum(D)	Wimmera	404	98	2	1.0	3.1	5.0	7.0	9.5	10.0	
Salvia hispanica	Chia Black	82		4	1.0	1.0	1.0	1.0	1.0	1.0	1.0
Salvia hispanica	Chia White	73		4	1.0	1.0	1.0	1.0	1.0	1.0	1.0
Secale cereale	Sthn Green	350	126	3	2.0	2.9	5.0	6.0	7.5	10.0	
Triticosecale X	Crackerjack2	197	140	3	2.0	2.0	3.1	5.0	7.5	10.0	
Triticum aestivum	Wedgetail	218	126	3	2.0	2.0	5.0	5.0	7.4	10.0	
Grand Mean		266	126		1.4	2.3	2.8	4.0	5.8	7.5	6.5
F prob		<.001	<.001		<.001	<.001	<.001	<.001	<.001	<.001	<.001
LSD (5%)		60	1.2		0.21	0.18	0.09	0.15	0.26	0.17	0.16

[1]Maturity groups; 1 – Early with < 95 days to flowering (250-350 mm rainfall zone); 2 – Mid with 95-120 days to flowering (350-500 mm rainfall zone); Late with 121-240 days to flowering (500-700 mm rainfall zone) and Very Late with >240 days to flowering (>700 mm rainfall zone).[a]Established plants/m^2. [b]Days from planting.

Table 5c Mean plant desity during establishment at Brookton, WA in 2013.

1.3	Genotype	Mean	sem
1.4	Antas subclover	74.9	10.5
1.5	Bartolo bladder clover	106.8	18.4
1.6	Cadiz French serradella	119.3	18.2
1.7	Casbah Biserulla	100.4	22.7
1.8	Dargo Italian reygrass	255.3	23.1
Frontier	Balansa clover	309.0	70.6
1.9	Languedoc vetch	56.9	15.3
1.10	Messina Melilotus	86.0	1.4
1.11	New York Forage Turnip	229.9	17.8
1.12	Spotted medic (SA8774)	94.4	39.1
1.13	Santorini yellow serradella	109.6	31.7
1.14	Scimitar burr medic	108.2	17.3
1.15	Trigonella balansae (SA5045)	180.4	29.7
1.16	Urana subclover	111.0	21.0
1.17	Wimmera sungold Fenugreek	166.5	26.0
1.18	Winteroo forage oats	159.6	7.3

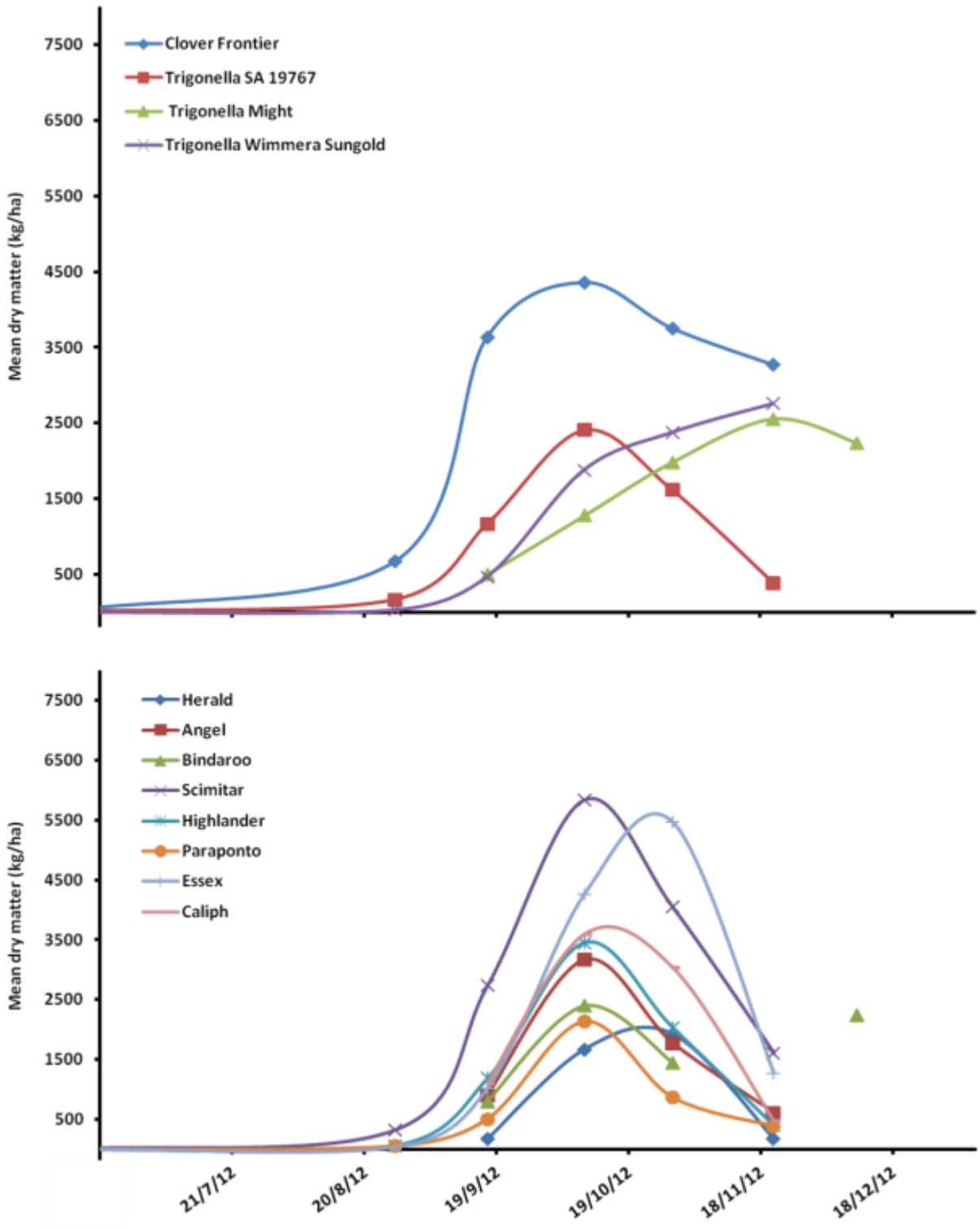

Fig 4 Pattern and quantity of biomass production in the early maturity cohort of annual legumes at the South Australian site in 2012.

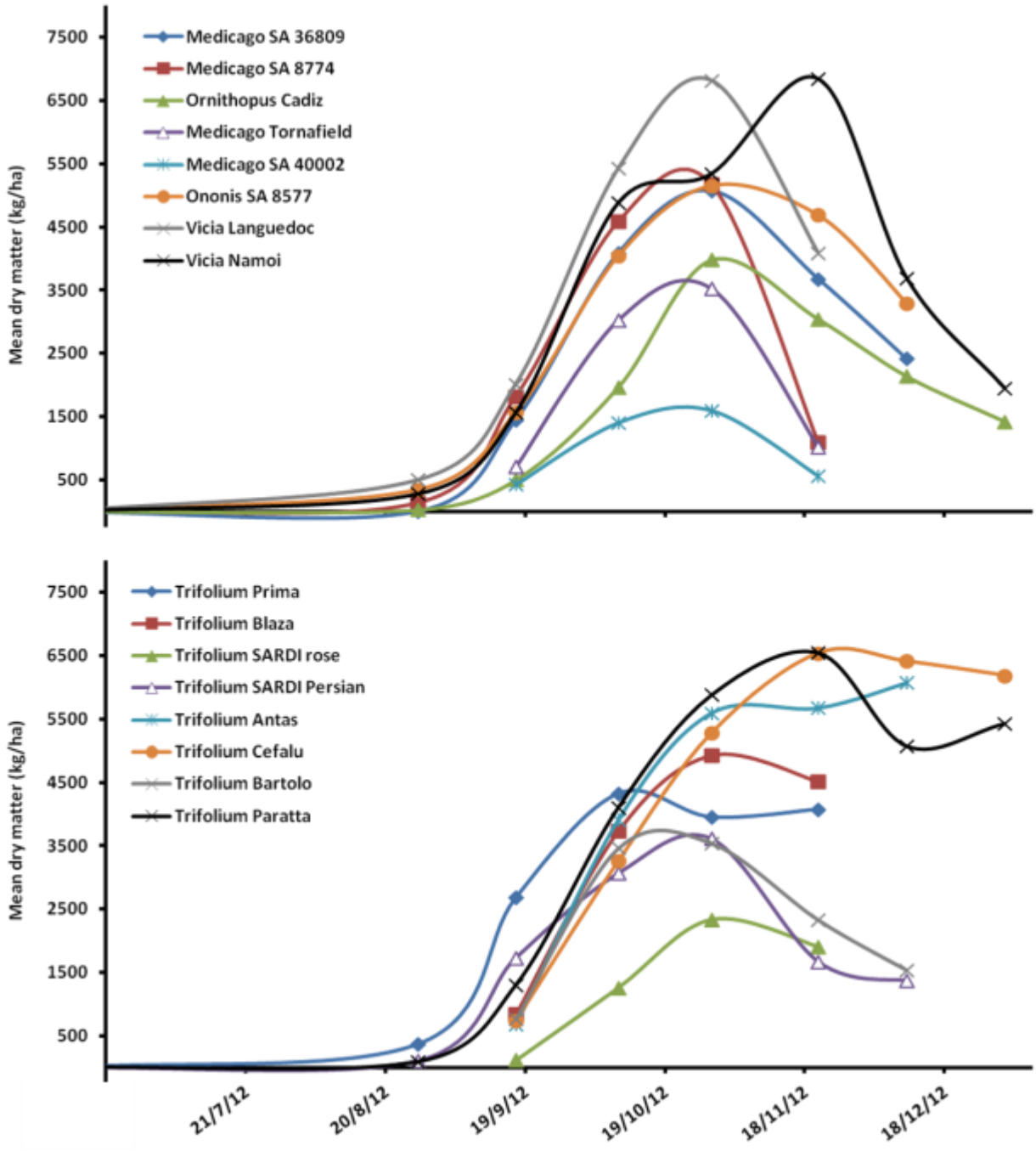

Fig 5 Pattern and quantity of biomass production in the mid maturity cohort of annual legumes at the South Australian site in 2012

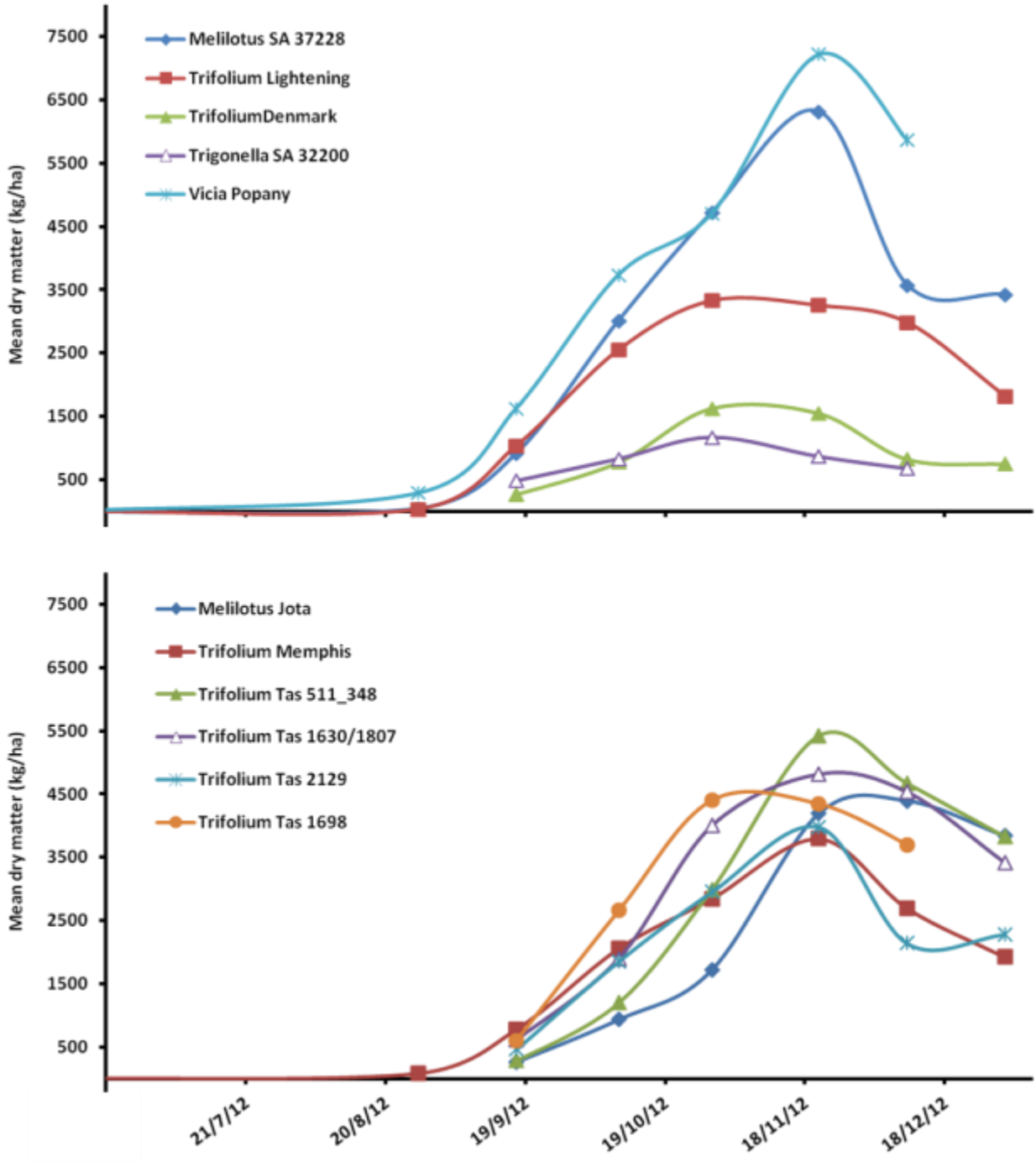

Fig 6 Pattern and quantity of biomass production in the late and very late maturity cohorts of annual legumes at the South Australian site in 2012

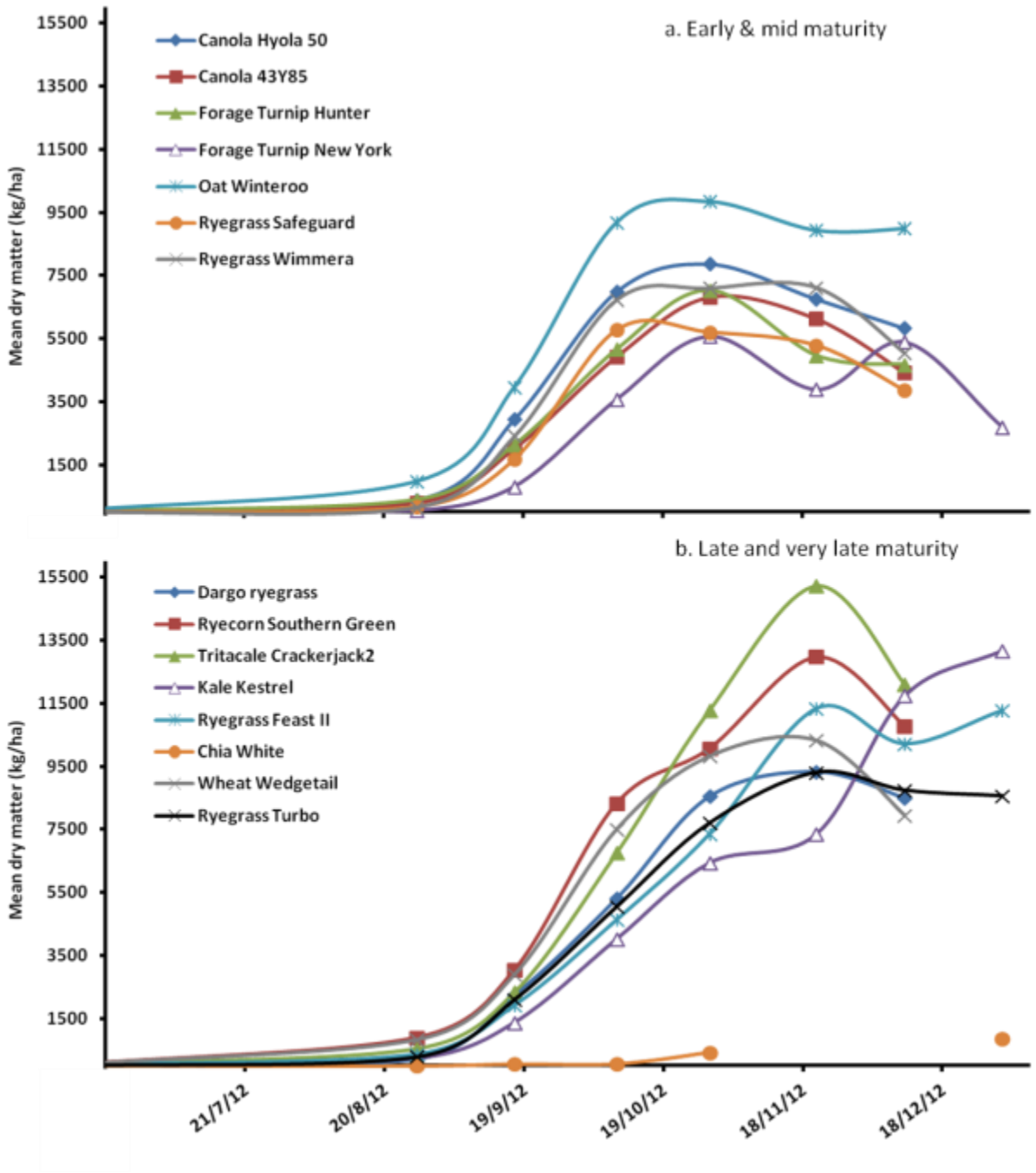

Fig 7 Pattern and quantity of biomass production (kg/ha) in the annual grasses and forbs at the South Australian site in 2012. Note doubling of the y scale compared to the previous legume graphs.

Table 6a. Biomass production of the annual legumes from the South Australian site in 2012

Species	Entry	Maturity Group	27/8/12 Day 77	17/9/12 Day 98	9/10/12 Day 120	29/10/12 Day 140	21/11/12 Log	21/11/12 Day 163	10/12/12 Day 182	31/12/12 Day 203	Total
Medicago littoralis	Herald	1		183	1676	1932	5.20	181			3972
Medicago littoralis	Angel	1		907	3170	1779	6.42	613			6469
Medicago orbicularis	Bindaroo	1		806	2404	1452	6.04	419			5081
Medicago polymorpha	Scimitar	1	326	2742	5844	4054	7.39	1616			14582
Medicago rotata	Highlander	1	69	1192	3459	2043	5.99	400			7163
Medicago rugosa	Paraponto	1	57	513	2139	871	5.97	393			3972
Medicago scutellata	Essex	1	30	1018	4263	5470	7.15	1269			12051
Medicago sphaerocarpos	Orion	1		149	679	1023					1851
Medicago truncatula	Caliph	1		1109	3604	3045	6.15	469			8227
Trifolium michelianum	Frontier	1	673	3643	4365	3759	8.09	3275			15714
Trigonella coelesyriaca	SA 19767	1	163	1168	2410	1620	5.96	386			5746
Trigonella foenum-graecum	Might	1		504	1279	1984	7.85	2558	2242		8567
Trigonella foenum-graecum	Wimmera Sungold	1	31	472	1882	2379	7.92	2760			7524
Astragalus hamosus	Ioman	2		282	866	1448	6.07	431			3027
Biserulla pelecinus	Casbah	2		160	302	1784	6.33	561	987	657	4451
Lathyrus cicera	Ceora	2		114	136	786	6.58	720	897	1226	3879
Lotus ornithopodioides	ITA 8a	2		500	1106	1646	6.92	1016	530		4798
Medicago arabica	SA 36809	2	9	1451	4088	5075	8.21	3681	2415		16719
Medicago arabica	SA 8774	2	139	1819	4585	5181	7.00	1091			12815
Medicago phrygia	SA 32612	2		107	150	343	6.39	593	447		1640
Medicago tornata	Tornafield	2		706	3026	3525	6.93	1021			8278
Melilotus siculus	SA 40002	2		426	1404	1598	6.33	563			3991
Ononis alopecuroides	SA 8577	2	350	1573	4045	5155	8.45	4689	3293		19105
Ornithopus compressus	Santorini	2		143	868	2013	6.38	588	763	1028	5403
Ornithopus pinnatus	Jebala	2		158	318	471	4.85	128	213		1288
Ornithopus sativus	Cadiz	2	27	499	1964	3980	8.02	3044	2140	1427	13081
Trifolium dasyurum	Sothis	2		299	1249	1512	5.75	315			3375
Trifolium glanduliferum	Prima	2	374	2681	4326	3958	8.31	4081			15420
Trifolium hirtum	SARDI rose	2		120	1262	2335	7.55	1908			5625
Trifolium incarnatum	Blaza	2		842	3735	4930	8.42	4519			14026
Trifolium isthmocarpum	SA 20009	2	23	1072	2236	3127	7.73	2280	1252		9990
Trifolium nigrescens	SA 15896	2		1336	3403	3352	7.86	2586	1229		11907
Trifolium purpureum	Paratta	2	89	1298	4103	5882	8.79	6549	5074	5432	28427
Trifolium resupinatum	SARDI Persian	2	113	1733	3067	3616	7.42	1674	1374		11578

Species	Entry	Maturity Group	27/8/12 Day 77	17/9/12 Day 98	9/10/12 Day 120	29/10/12 Day 140	21/11/12 Log	21/11/12 Day 163	10/12/12 Day 182	31/12/12 Day 203	Total
Trifolium spumosum	Bartolo	2		764	3463	3543	7.75	2329	1537		11636
Trifolium subterraneum	Trikkala	2		723	2211	2805	7.50	1808	1414		8962
Trifolium subterraneum	Antas	2		679	3914	5596	8.64	5676	6075		21940
Trifolium subterraneum	Urana	2	125	1576	3354	3366	7.66	2124	2276	1653	14474
Trifolium subterraneum	Gosse	2	11	875	2300	2365	7.57	1947	800		8298
Trifolium subterraneum	Clare	2		958	2727	3861	7.78	2399	2459		12404
Trifolium tomentosum	SA 35654	2	75	1323	3171	3233	7.82	2485			10286
Trifolium vesiculosum	Cefalu	2		743	3260	5279	8.79	6542	6411	6188	28423
Trigonella balansae	SA 32999	2		458	1292	1954	4.89	133			3837
Trigonella balansae	SA 5045	2	46	1348	3351	3049	7.33	1521			9315
Trigonella calliceras	SA 32202	2		52	150	685	5.39	219			1106
Vicia sativa	Languedoc	2	503	2016	5424	6822	8.32	4093			18858
Vicia villosa	Namoi	2	281	1565	4882	5344	8.83	6843	3688	1953	24556
Melilotus elegans	SA 37228	3	52	916	3013	4722	8.75	6317	3578	3431	22029
Trifolium resupinatum	Lightening	3	32	1032	2553	3333	8.09	3258	2988	1821	15017
Trifolium subterraneum	Denmark	3		270	780	1631	7.35	1552	825	749	5807
Trigonella caerulea	SA 32200	3		488	833	1171	6.77	868	677		4037
Vicia benghalensis	Popany	3	293	1626	3741	4705	8.89	7223	5868		23455
Hedysarum flexuosum	SA 32504	4		48	124	150	6.91	1001	555	718	2596
Melilotus albus	Jota	4		255	927	1711	8.34	4188	4388	3831	15300
Trifolium alexandrinum	Memphis	4	83	774	2052	2842	8.24	3790	2691	1918	14150
Trifolium diffusum	Tas 511_348	4		279	1198	2988	8.60	5421	4668	3825	18379
Trifolium glomeratum	Tas 1630/1807	4		608	1892	3996	8.48	4813	4529	3407	19244
Trifolium lappaceum	Tas 2129	4		459	1852	2949	8.29	3976	2138	2281	13655
Trifolium squarrosum	SA 36400	4		895	1858	2768	8.38	4368	2659		12548
Trifolium striatum	Tas 1698	4		601	2653	4396	8.38	4342	3697		15689
Grand Mean			**66**	**882**	**2435**	**2972**	**7.35**	**2468**	**2479**	**2444**	**10929**
F prob			<.001	<.001	<.001	<.001	<.001		<.001	<.001	
LSD (5%)			106	416	940	1301	0.00		1255.2	1389.7	

Table 6b. Biomass production of the annual grasses and forbs from the South Australian site in 2012

Species	Entry	Maturity Group	27/8/12 Day 77	17/9/12 Day 98	9/10/12 Day 120	29/10/12 Day 140	21/11/12 Day 163	10/12/12 Day 182	31/12/12 Day 203	Total
Brassica napus	Hyola 50	1	391	2939	6989	7866	6755	5827		30767
Brassica napus	43Y85	1	299	2020	4927	6814	6126	4418		24604
Brassica tournefortii	SA 42783	1	1	551	3816	2871	2440	2647		12326
Atriplex semibaccata	SA 45507	2	0	1	42	351		676	1620	6521
Brassica campestris var. rapa	Hunter	2	388	2130	5150	7021	4956	4672		24317
Brassica napus	Taurus	2	1	993	3085	5814	6335	3811	2960	22999
Brassica rapa	New York	2	49	807	3576	5579	3888	5397	2696	21992
Brassica napus x oleracea	Titan	4	314	1708	4000	6311	6867	7371	6971	33542
Brassica oleracea	Kestrel	4	232	1362	4013	6428	7343	11730	13167	44275
Brassica rapa x oleracea	Winfred	4	511	2670	4923	4989	5082	4314	6052	28541
Salvia hispanica	Chia Black	4	0	0	0	200			852	1052
Salvia hispanica	Chia White	4	0	56	60	417			861	1394
Avena sativa	Winteroo	2	969	3964	9187	9843	8930	8987		41880
Hordeum vulgare	Moby	2	924	2606	7040	7820	8153	7490		34033
Lolium rigidum(D)	Safeguard	2	183	1685	5775	5705	5287	3860		22495
Lolium rigidum(D)	Wimmera	2	158	2404	6730	7099	7121	5048		28560
Lolium multiflorum (D)	Dargo	3	211	2240	5305	8550	9334	8495		34135
Lolium rigidum(D)	Progrow	3	89	1667	5383	8414	8239	7022	6229	37043
Secale cereal	Sthn Green	3	887	3036	8330	10074	12976	10777		46080
Triticosecale X	Crackerjack2	3	527	2319	6742	11274	15227	12103		48192
Triticum aestivum	Wedgetail	3	815	2879	7496	9819	10322	7944		39275
Lolium multiflorum (D)	Eclipse	4	161	2259	5826	8396	10411	8282		35335
Lolium multiflorum (D)	Mverick GII	4	272	2099	4021	7190	7600	7896	5831	34909
Lolium multiflorum (T)	Feast ll	4	360	1910	4628	7355	11340	10221	11277	47091
Lolium multiflorum (T)	Tama	4	374	2199	4703	8338	7099	7048		29761
Lolium multiflorum X (D)	Fesper	4	78	1769	4504	8104	9092	7352	9097	39996
Lolium multiflorum X (D)	Turbo	4	284	2102	5047	7701	9301	8736	8558	41729
Lolium rigidum (T)	Sungrazer	4	522	2035	5188	8559	9094	7221	7719	40338
Lolium rigidum (T)	Zoom	4	358	2168	5157	6988	8547	9037	8412	40667
Grand Mean			**312**	**1821**	**4884**	**6755**	**7994.8**	**6977.1**	**6153.5**	
F prob			<.001	<.001	<.001	<.001	<.001	<.001	<.001	
LSD (5%)			291	857	1774	1783	1925	2257	2067	

Establishment and biomass production in Western Australia

The establishment data for Brookton in 2013 indicate that sufficient plants were established for the experiment (Table 5c). Biomass production was measured on 5 occasions during the 2013 growing season (23 September – 18 December) and on 6 occasions during the 2014 growing season (21 August – 16 December). There were significant differences in biomass production between accessions at each time of cutting at Brookton ($P<0.001$). All biomass data across the two years of measurement are presented in Fig 8. It is clear that 2014 was a better season than 2013 as the majority of species were more productive. The most productive species were the forage oat cv Winteroo (15 t/ha in 2013, 20 t/ha in 2014), vetch cv Langudoc (8/15 t/ha), Italian ryegrass cv Dargo (9/12 t/ha) and serradella cv Santorini (5/10 t/ha). The forage oat had standout early vigour and subclover cv Antas was the legume with greatest early vigour. At the end of the season, species with the most biomass included; vetch cv Langudoc, subclover cv Antas, Italian ryegrass cv Dargo, forage turnip cv New York and the bladder clover cv Bartolo. The medics were not well suited to the site with about 2 t DM/ha.

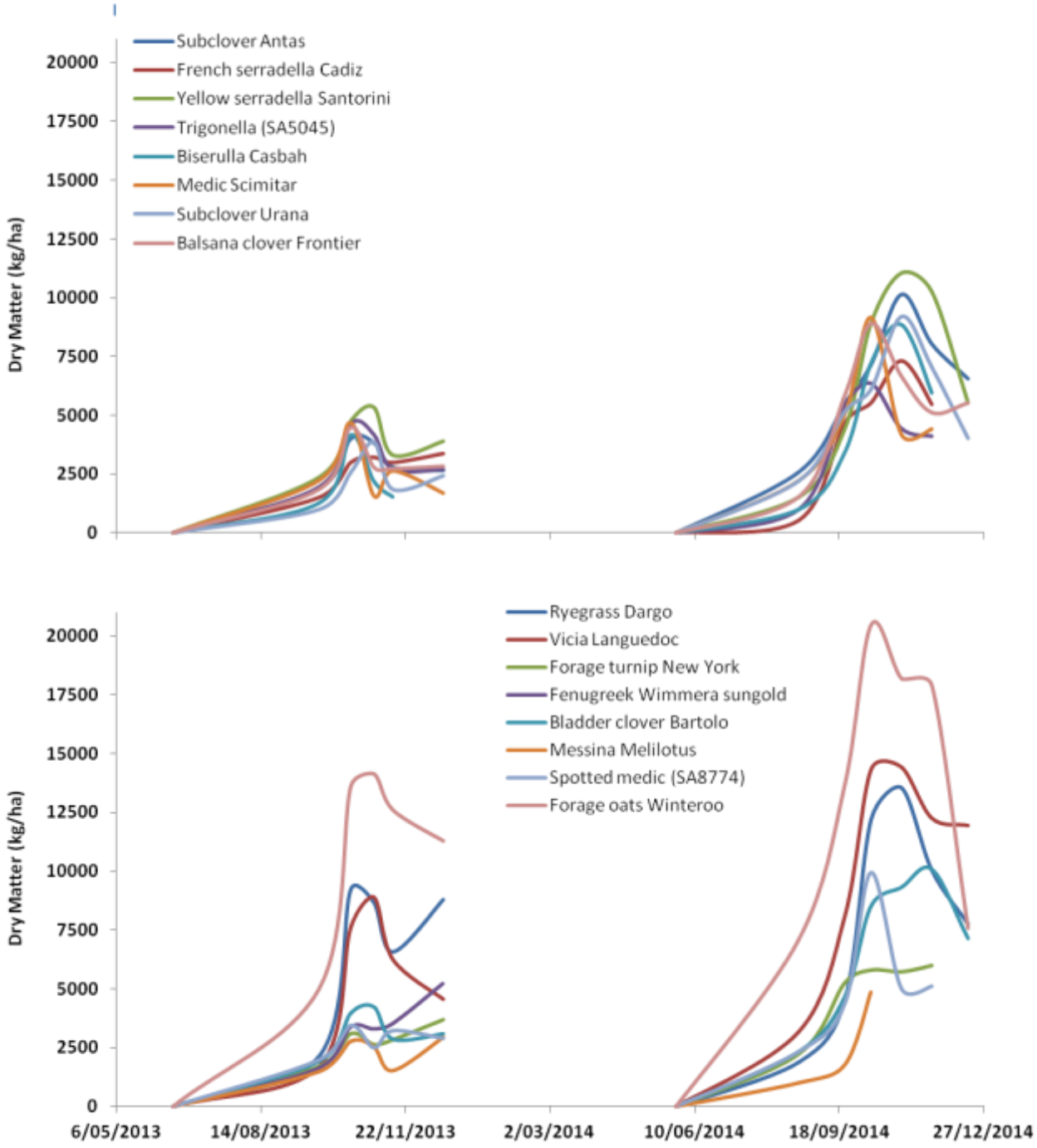

Fig 8. Pattern and quantity of biomass production in the accession grown at the Western Australian site in 2013 and 2014.

Nutritional value

Overall, there were significant differences in the nutritional parameters (DMD, N, ADF, NDF, hemicelluloses and ash) between accessions at each site within each season. The only exceptions are for DMD during the reproductive phase at the South Australian site in 2012, where differences between accessions were not significant.

Nutritional parameters for the South Australian site in year 1 are presented in Tables 7 a-f. All of the annual legumes at the vegetative development stages had high nutritional value (mean of 73.7% DMD and 3.8 % N) however there were significant differences (Table 7a). The species with the highest DMD were the clovers; Cefalu arrowleaf, SA 15896 ball and SARDI Persian (over 78% DMD). Two of the three *Trigonella* species also had high DMD. The species with the lowest DMD were the serradella species cv Cadiz and cv Santorini and the *Hedysarium* species. All had sufficient CP to support ruminant growth. Biserulla had unusually high ash content (25%) and it was similar across the replicates, suggesting it was not laboratory error. At the reproductive stages, all legume species had nutritional values that would support growth of sheep (Mean DMD 66.7%, N 2.7%, Table 7b). The subterranean clovers and several medics had the highest DMD (values over 72%) while the accessions with the poorest nutritional values were purple clover cv Paratta, serradella cv Jebala and Lappa clover cv Tas 2129. Of the senesced collection, purple clover cv Paratta and serradella cv Jebala still feature with poor nutritional value as do several medics (cv Caliph, cv Essex and cv Herald), the other serradellas (cv Cadiz and cv Santorini)and Melilotus (SA37228). All of these species have DMD and N values that would not support liveweight maintenance of mature sheep (<50 % DMD and >1.9% N), and ADF values that are excessive (Table 7c). The species with the highest nutritional values at the senesced phase include the clovers Bartolo (63.2 % DMD, 2.7% N), ball SA 15896 (61.4 % DMD, 2.1% N), and cluster clover (61.3% DMD, 2.5% N), Fenugreek cv Might (63% DMD, 2.7% N) and Hedysarium (61.7% DMD, 2.1% N).

The grasses and forbs had high nutritional value during the vegetative stages with a mean of over 75% DMD and 3.0% N (Table 7d). The greatest variation was amongst the forbs with 63% DMD for the Quinoa to 82% DMD for canola cv Hyola. The grasses all had DMD values above 73% and N values above 2.6%. During the reproductive phase there were no significant differences amongst the grasses and forbs in DMD and large differences between the ryegrasses in total N (1.1 to 3.0%; Table 7e). The senesced grasses all had nutritional profiles that would not support maintenance of dry sheep with DMD values below 55% and N less than 1.5%. The accessions with the highest nutritional value were barley cv Moby, Ryegrass cv Feast II and cv Turbo and triticale cv Crackerjack 2.

Mean DMD values of the accessions that were grown at both research sites over two seasons are presented in Fig 9. From the first vegetative cut (~ 90 days after sowing), forage Turnip cv New York had consistently higher DMD while the forage oat (cv Winteroo) had consistently lower DMD. These differences were also apparent at the next cuttings dates (130-140 days after sowing) and the serradella (cv Santorini) also had lower DMD than other species. By the next comparison at 150-160 days after sowing, Bartolo clover as well as forage turnip cv New York had relatively high DMD across sites. For senesced material (~180 days after sowing) Bartolo clover, forage turnip cv New York and the subterranean clovers had the highest DMD across sites. There appears to be less variation within and between sites of collection during the vegetative phase than the reproductive and senesced phases.

For the vast majority of annual species, the pattern of change in DMD and N followed a logistic breakdown pattern. The pattern of change and timing of inflection provides information about the ability of the accession to maintain nutritive value through their lifecycle under the conditions of the experiment. We successfully fitted logistic curves to 80 of the 90 annual species. For several species (the chias and Quinoa SA45507) we did not have enough diversity in sampling dates and for 5 others the data were too variable for a curve fit (Sulla SA 32504, Fenugreek cv Might, *Trigonella calliceras*, rape cv Winfred and slender serradella cv Jebala). Popany vetch and was better represented by an exponential decline curve as was Ncontent for 10 other species. We now have a database of 540 separate curves with associated statistics describing the fit and standard errors of parameters.

Digestibility curves are presented for the subset of 12 of the accessions that were grown across all of the sites (Fig 10). There are clear differences in initial digestibility, rate of digestibility loss over time and the final digestibility of senesced material. The DMD of the forage oat cv Winteroo declined at a faster rate than other species and reached a point of inflection (where rate of decline was highest) at 115 ± 5 days after sowing. At senescence the forage oat (cv Winteroo) and Italian ryegrass (cv Dago)

had the lowest digestibility. The annual legumes had similar vegetative digestibilities and varied in the time of the inflection, suggesting differences in the ability to maintain DMD through their lifecycle. Accessions with higher rates of decline included gland clover cv Prima (114 ± 2 days), burr medic cv Santiago (126 ± 4 days), purple clover cv Paratta (130 ± 3 days) and serradella cv Santorini (134 ± 5 days). Accessions that had a slower decline in DMD included subclover cv Antas (162 ± 2 days), subclover cv Urana (160 ± 3 days), forage turnip cv New York (144 ± 5 days) and bladder clover cv Bartolo (148 ± 6 days). Bartolo clearly had the highest DMD at the end of the season. For three accessions, curves are presented for DMD from 2012 (South Australia) and 2013 (Western Australia). Digestibility of the accessions over time appears to follow the same pattern despite different growing environments (Fig. 11). The curves from WA are always below that of SA, suggesting plants reached a greater digestibility potential at the SA site.

Table 7a. Mean nutritional traits of annual legumes in South Australia at the vegetative stages of development (development scores 1-2)

		DMD (%)		M/D (MJ/kg)		N (%)		Ash (%)		NDF (% DM)		Hemi (% DM)		ADF (%DM)	
Entry	n	Mean	sem	Mean	sem	Mean	sem	Mean	sem	Mean	sem	Mean	sem	Mean	sem
Biserulla Casbah	3	69.6	0.75	10.3	0.13	3.6	0.18	25	1.8	35	1.3	8	0.6	28	1.3
Clover Antas sub	7	70.6	0.94	10.4	0.16	4.0	0.12	12	1.7	28	1.1	9	0.5	19	0.6
Clover Bartolo bladder	10	73.0	1.23	10.8	0.21	4.2	0.14	12	1.1	30	1.4	9	0.8	21	1.0
Clover Blaza crimson	3	76.8	0.82	11.5	0.14	4.6	0.12	12	0.4	29	0.7	9	0.5	19	0.4
Clover Cefalu arrowleaf	6	78.3	1.59	11.8	0.27	3.7	0.39	10	0.8	27	1.3	7	0.8	20	0.5
Clover Clare sub	3	73.9	0.99	11.0	0.17	4.1	0.16	12	0.4	30	2.1	11	1.0	19	1.1
Clover Denmark sub	3	70.5	1.12	10.4	0.19	4.3	0.13	17	0.1	28	0.6	5	0.3	23	0.5
Clover Frontier balansa	12	76.0	0.41	11.4	0.07	4.0	0.17	11	1.1	28	0.8	9	0.5	19	0.7
Clover Gosse sub	4	76.9	0.83	11.5	0.14	4.3	0.08	11	0.7	27	0.8	9	0.7	18	0.5
Clover Lightening Persian	7	76.9	0.54	11.5	0.09	3.7	0.16	12	0.8	26	0.6	8	0.4	18	0.6
Clover Memphis berseem	11	70.5	0.96	10.4	0.17	3.1	0.26	10	0.6	30	0.5	10	0.5	19	0.5
Clover Paratta purple	8	70.9	1.22	10.5	0.21	3.0	0.22	12	0.7	30	1.0	8	0.4	22	0.7
Clover Prima gland	6	74.4	1.17	11.1	0.20	3.5	0.13	13	1.2	31	1.5	8	1.0	23	0.9

		DMD (%)		M/D (MJ/kg)		N (%)		Ash (%)		NDF (% DM)		Hemi (% DM)		ADF (%DM)	
Entry	n	Mean	sem	Mean	sem	Mean	sem	Mean	sem	Mean	sem	Mean	sem	Mean	sem
Clover SA 15896 Ball	3	79.4	0.66	11.9	0.11	4.4	0.07	12	0.5	28	0.4	9	0.3	18	0.4
Clover SA 20009 Moroccan	7	76.8	0.80	11.5	0.14	3.5	0.27	11	1.2	25	1.2	6	0.6	19	0.7
Clover SA 36400 spike	9	68.9	1.01	10.1	0.17	3.6	0.24	10	0.6	34	0.6	13	0.3	22	0.7
Clover SARDI Persian clover	4	78.1	0.75	11.7	0.13	3.8	0.11	13	1.0	26	0.9	7	0.6	19	0.6
SARDI rose	3	73.2	1.32	10.9	0.23	4.0	0.04	13	0.9	32	1.3	11	0.9	21	1.5
Clover Sothis eastern star	3	72.5	0.67	10.8	0.11	3.8	0.20	10	0.8	36	0.2	11	0.6	25	0.6
Clover Tas 1698 striated	9	74.1	1.60	11.1	0.28	3.7	0.23	11	0.8	30	1.1	9	0.5	21	0.8
Clover Tas 2129 Lappa	6	72.8	1.32	10.8	0.23	3.8	0.30	11	0.6	29	0.8	9	0.6	20	0.6
Clover Tas1630/1807 cluster	6	76.4	0.89	11.4	0.15	4.2	0.22	14	1.9	27	1.5	7	0.3	20	1.4
Clover Tas511_348 Difuse	8	73.2	1.29	10.9	0.22	4.2	0.26	11	0.7	34	1.0	13	0.4	20	0.7
Clover Trikkala sub	3	76.8	0.27	11.5	0.05	4.6	0.08	11	0.4	31	2.4	12	2.2	20	0.3
Clover Urana sub	8	69.2	2.98	10.2	0.51	3.8	0.19	12	1.4	27	1.4	8	0.9	18	0.5
Fenugreek	6	76.1	1.52	11.4	0.26	3.2	0.30	12	1.5	25	1.4	8	0.4	17	1.2

		DMD (%)		M/D (MJ/kg)		N (%)		Ash (%)		NDF (% DM)		Hemi (% DM)		ADF (%DM)	
Entry	n	Mean	sem	Mean	sem	Mean	sem	Mean	sem	Mean	sem	Mean	sem	Mean	sem
SA 32200 Fenugreek															
Wimmera	7	74.5	0.27	11.1	0.05	3.9	0.20	14	2.6	24	0.3	7	0.3	17	0.3
Hedysarium SA 32504	7	65.9	0.89	9.6	0.15	2.5	0.28	14	1.8	22	1.6	5	0.8	17	1.3
lathyrus Ceora	2	72.7	0.22	10.8	0.04	3.5	0.10	11	0.2	30	0.8	8	0.0	22	0.7
Lotus ornith ITA 8a	0														
Medic Angelstrand	3	69.8	0.59	10.3	0.10	4.8	0.07	14	0.2	31	0.3	11	0.5	20	0.2
Medic Caliphbarrel	3	71.6	0.47	10.6	0.08	4.8	0.13	13	0.5	30	0.6	10	0.2	20	0.4
Medic Paraponto gama	2	73.8	0.72	11.0	0.12	2.8	0.48	13	0.4	20	1.2	3	1.2	17	0.1
Medic SA 32612 phrygia	2	73.3	2.09	10.9	0.36	2.4	0.67	19	9.4	31	6.4	7	0.6	24	7.0
Medic SA 36809 spotted	4	76.8	0.93	11.5	0.16	4.4	0.21	13	0.4	26	0.4	7	0.3	18	0.4
Medic SA 8774 spotted	5	76.8	1.11	11.5	0.19	4.1	0.39	11	0.6	24	1.9	8	1.1	16	0.8
Medic Scimitarburr	6	75.9	0.80	11.3	0.14	4.6	0.16	13	1.8	26	0.5	9	0.7	17	0.4
Melilotus Jota	9	73.1	1.73	10.9	0.30	3.4	0.31	9	0.9	28	1.4	8	0.5	20	1.1
Melilotus SA 37228	7	72.0	2.17	10.7	0.37	3.2	0.35	9	0.9	27	2.5	7	0.6	21	1.9

		DMD (%)		M/D (MJ/kg)		N (%)		Ash (%)		NDF (% DM)		Hemi (% DM)		ADF (%DM)	
Entry	n	Mean	sem	Mean	sem	Mean	sem	Mean	sem	Mean	sem	Mean	sem	Mean	sem
Melilotus SA 40002	11	74.3	0.36	11.1	0.06	4.4	0.06	13	2.2	27	0.6	9	0.4	17	0.5
Ononis SA 8577	6	75.3	1.02	11.3	0.18	3.7	0.14	14	1.4	33	1.9	11	1.9	22	0.5
Serradella Cadiz	7	67.6	0.78	9.9	0.13	3.7	0.12	13	2.1	30	1.5	9	0.9	21	0.8
Serradella Santorini	6	68.2	0.72	10.0	0.12	3.7	0.12	13	2.9	33	2.5	10	0.7	23	2.2
Trigonella SA 19767	2	79.8	0.79	12.0	0.14	3.3	0.17	12	0.4	24	0.1	7	1.4	17	1.4
Trigonella SA 32999	8	77.8	0.64	11.7	0.11	4.2	0.15	13	1.0	28	1.1	9	0.5	19	1.2
Trigonella SA 5045	12	74.8	0.72	11.2	0.12	4.1	0.16	11	1.1	24	1.3	8	0.6	16	0.9
Vetch Ioman	3	75.2	0.17	11.2	0.03	3.7	0.21	11	0.1	23	1.3	7	1.5	16	0.5
Vetch Languedoc	12	71.1	0.54	10.5	0.09	4.3	0.13	11	1.0	31	0.8	8	0.4	22	0.5
Vetch Namoi	6	72.5	1.00	10.8	0.17	4.2	0.29	15	1.2	30	0.8	8	0.8	22	0.4
Vetch Popany	9	71.0	0.86	10.5	0.15	4.0	0.16	12	0.7	33	0.7	9	0.3	25	0.5
Grand Mean		**73.7**	**0.96**	**11.0**	**0.17**	**3.8**	**0.20**	**12**	**1.2**	**28**	**1.2**	**9**	**0.7**	**20**	**0.9**
LSD		6.4		1.1		1.2		7		7		4		5	
P		***		***		***		***		***		***		***	

*** P<0.001

Table 7b. Mean nutritional traits of annual legumes in South Australia at the reproductive stages of development (development scores 3-5)

		DMD (%)		M/D (MJ/kg)		N (%)		Ash (%)		NDF (% DM)		Hemi (% DM)		ADF (%DM)	
Entry	n	Mean	sem	Mean	sem	Mean	sem	Mean	sem	Mean	sem	Mean	sem	Mean	sem
Biserulla Casbah	14	67.9	1.38	10.0	0.24	3.1	0.20	11	1.8	22	1.4	11	1.0	33	2.4
Clover Antas sub	14	70.7	0.48	10.5	0.08	2.6	0.17	9	0.5	21	0.5	8	0.3	29	0.7
Clover Bartolo bladder	12	71.7	0.47	10.6	0.08	2.8	0.18	8	0.5	22	0.5	11	1.5	33	1.9
Clover Blaza crimson	6	65.4	3.18	9.5	0.55	2.7	0.16	8	0.5	26	2.2	10	0.7	36	2.8
Clover Cefalu arrowleaf	6	63.7	1.77	9.3	0.30	1.8	0.04	7	0.3	28	1.8	11	1.1	39	2.9
Clover Clare sub	6	71.3	1.16	10.6	0.20	2.6	0.22	8	0.6	22	0.6	11	0.3	32	0.8
Clover Denmark sub	6	71.1	1.14	10.5	0.20	3.5	0.22	11	0.2	20	0.7	10	0.5	30	1.0
Clover Frontier balansa	9	67.8	0.63	10.0	0.11	2.5	0.16	9	1.0	23	0.6	8	0.5	31	0.7
Clover Gosse sub	6	74.4	1.00	11.1	0.17	2.6	0.20	9	0.3	20	0.3	9	0.5	29	0.8
Clover Lightening Persian	3	70.4	0.49	10.4	0.08	2.6	0.12	9	0.3	22	0.5	9	0.3	31	0.3
Clover Memphis berseem	3	63.6	0.80	9.2	0.14	2.0	0.05	8	0.2	25	0.3	11	0.2	36	0.2
Clover Paratta purple	6	54.7	1.19	7.7	0.20	1.9	0.04	8	0.5	36	1.6	14	0.5	50	2.0
Clover Prima gland	5	60.6	1.50	8.7	0.26	2.1	0.11	8	0.3	32	1.3	12	1.6	44	2.8
Clover SA 15896 Ball	3	70.8	0.41	10.5	0.07	2.5	0.07	9	0.2	20	0.6	9	0.3	29	0.7
Clover SA 20009 Moroccan	3	66.0	0.41	9.7	0.07	2.5	0.12	8	0.4	25	0.5	9	0.3	35	0.8
Clover SA 36400 spike	3	60.4	1.01	8.7	0.17	2.3	0.09	8	0.5	30	0.6	14	0.5	43	1.1
Clover SARDI Persian	5	68.7	2.21	10.1	0.38	2.6	0.06	8	0.5	23	1.5	11	1.1	34	2.5
clover SARDI rose	5	66.2	1.41	9.7	0.24	2.7	0.13	8	0.3	26	1.4	12	0.5	38	1.8
Clover Sothis	6	62.8	0.71	9.1	0.12	2.6	0.07	6	0.5	28	0.9	15	1.4	43	2.2

		DMD (%)		M/D (MJ/kg)		N (%)		Ash (%)		NDF (% DM)		Hemi (% DM)		ADF (%DM)	
Entry	n	Mean	sem	Mean	sem	Mean	sem	Mean	sem	Mean	sem	Mean	sem	Mean	sem
eastern star Clover Tas 2129 Lappa	4	59.7	1.05	8.6	0.18	2.4	0.08	9	0.4	29	1.1	10	1.0	39	2.1
Clover Tas1630/1807 cluster	3	73.4	1.47	10.9	0.25	2.8	0.05	8	0.1	21	0.1	8	0.8	29	0.7
Clover Tas511_348 Difuse	3	61.5	0.74	8.9	0.13	2.1	0.05	7	0.2	27	0.3	13	0.1	40	0.2
Clover Trikkala sub	6	73.3	0.24	10.9	0.04	2.7	0.26	10	0.6	19	0.5	7	0.4	26	0.7
Clover Urana sub	18	70.5	0.41	10.4	0.07	2.9	0.18	10	0.5	21	0.4	9	0.4	30	0.6
Fenugreek Might	9	70.5	2.27	10.4	0.39	3.2	0.33	9	0.7	23	1.5	10	0.8	32	2.2
Fenugreek SA 32200	6	63.4	2.15	9.2	0.37	2.3	0.11	8	0.2	28	2.4	14	1.1	42	3.5
Fenugreek SA 32202	6	69.7	1.60	10.3	0.27	2.7	0.07	9	1.1	21	1.3	7	0.5	28	1.6
Fenugreek Wimmera	21	70.5	0.88	10.4	0.15	3.0	0.17	8	0.4	22	0.8	9	0.6	31	1.2
Hedysarium SA 32504	4	61.3	1.33	8.8	0.23	2.1	0.16	11	0.8	20	1.0	7	0.2	28	0.9
lathyrus Ceora	6	65.8	1.38	9.6	0.24	2.6	0.12	8	0.3	24	0.9	8	0.4	32	1.0
Lotus ornith ITA 8a	9	65.0	2.08	9.5	0.36	2.6	0.10	9	1.7	24	1.4	8	1.4	32	2.6
Medic Angel strand	6	67.0	1.71	9.8	0.29	3.6	0.41	10	1.5	24	1.4	9	0.5	33	1.7
Medic Bindaroo button	6	71.1	2.66	10.5	0.46	3.8	0.51	10	0.9	24	1.6	10	1.2	34	2.8
Medic Caliph barrel	8	62.3	2.94	9.0	0.51	3.2	0.39	10	1.4	29	2.5	12	1.5	41	4.0
Medic Essex snail	9	65.1	2.53	9.5	0.44	3.1	0.42	9	1.0	27	2.3	10	0.9	38	2.9
Medic Herald strand	9	65.7	2.65	9.6	0.46	3.3	0.35	10	1.3	27	2.2	11	1.3	39	3.5
Medic Highlander	6	68.7	2.19	10.1	0.38	3.1	0.36	9	0.8	25	1.1	10	0.8	35	1.9

		DMD (%)		M/D (MJ/kg)		N (%)		Ash (%)		NDF (% DM)		Hemi (% DM)		ADF (%DM)	
Entry	n	Mean	sem	Mean	sem	Mean	sem	Mean	sem	Mean	sem	Mean	sem	Mean	sem
rotata															
Medic Orion sphere	6	65.7	3.86	9.6	0.66	2.3	0.34	8	0.6	27	4.0	10	1.2	37	5.2
Medic Paraponto gama	6	71.7	1.46	10.6	0.25	3.4	0.32	11	0.9	23	0.4	8	1.0	31	1.3
Medic SA 32612 phrygia	5	65.5	1.41	9.6	0.24	2.5	0.15	9	0.5	26	1.8	13	1.6	39	3.4
Medic SA 36809 spotted	6	72.5	1.42	10.8	0.24	3.2	0.16	9	0.3	21	0.9	8	0.4	29	1.1
Medic SA 8774 spotted	16	67.4	1.62	9.9	0.28	3.6	0.26	10	0.8	25	1.3	10	0.5	35	1.6
Medic Scimitar burr	16	68.6	1.08	10.1	0.19	3.6	0.21	10	1.0	22	0.8	10	0.7	32	1.3
Medic Tornafield disk	6	72.7	1.77	10.8	0.30	4.1	0.43	11	1.0	22	0.9	9	0.6	31	1.5
Melilotus Jota	6	59.4	1.35	8.5	0.23	1.9	0.10	6	0.1	31	1.2	12	0.5	43	1.7
Melilotus SA 37228	6	61.9	0.70	8.9	0.12	2.0	0.07	6	0.2	32	0.9	12	0.9	44	1.6
Melilotus SA 40002	13	69.6	0.78	10.3	0.13	2.9	0.15	8	0.4	22	1.1	10	1.3	32	2.2
Ononis SA 8577	6	67.4	1.10	9.9	0.19	2.3	0.07	8	0.2	24	1.2	9	0.7	33	1.7
Serradella Cadiz	15	62.9	0.82	9.1	0.14	2.7	0.11	8	0.6	27	0.9	10	0.3	36	1.1
Serradella Jebala	5	59.7	1.23	8.6	0.21	2.5	0.15	10	0.6	24	1.3	11	0.5	36	1.7
Serradella Santorini	12	61.1	1.83	8.8	0.32	2.8	0.16	8	0.5	29	1.9	11	0.6	39	2.4
Trigonella SA 19767	6	72.4	1.05	10.7	0.18	3.2	0.32	9	0.6	21	0.5	9	1.0	31	1.1
Trigonella SA 32999	12	67.9	1.30	10.0	0.22	2.7	0.07	8	0.4	23	1.2	12	1.3	35	2.4
Trigonella SA 5045	13	67.1	0.72	9.8	0.12	2.9	0.15	8	0.4	23	0.7	11	0.8	34	1.4
Vetch Ioman	6	66.2	1.99	9.7	0.34	2.2	0.06	8	0.3	25	1.9	15	2.8	40	4.3
Vetch Languedoc	15	64.9	0.79	9.5	0.14	3.0	0.16	8	0.4	25	0.7	9	0.5	34	1.1
Vetch Namoi	6	65.6	1.33	9.6	0.23	3.1	0.12	9	0.5	24	0.8	10	0.7	34	0.9

		DMD (%)		M/D (MJ/kg)		N (%)		Ash (%)		NDF (% DM)		Hemi (% DM)		ADF (%DM)	
	n	Mea	sem	Mean	sem	Mean	sem	Mean	sem	Mean	sem	Mean	sem	Mean	sem
Entry		n													
Vetch Popany	3	68.0	1.76	10.0	0.30	3.0	0.11	9	0.5	27	1.8	10	0.3	37	1.9
Grand Mean		**66.7**	**1.40**	**9.8**	**0.24**	**2.7**	**0.18**	**9**	**0.6**	**25**	**1.2**	**10**	**0.8**	**35**	**1.8**
LSD		8.9		1.5		1.3		5		8		6		12	
P		***		***		***		***		***		***		***	

*** P<0.001

Table 7c. Mean nutritional traits of annual legumes in South Australia at the senesced stages of development (development scores 6-7)

		DMD (%)		M/D (MJ/kg)		N (%)		Ash (%)		NDF (% DM)		Hemi (% DM)		ADF (%DM)	
Entry	n	Mean	sem	Mean	sem	Mean	sem	Mean	sem	Mean	sem	Mean	sem	Mean	sem
Biserulla Casbah	16	55.9	0.78	7.9	0.13	2.2	0.07	7	0.4	56	1.7	19	0.8	37	1.0
Clover Antas sub	12	58.5	1.70	8.4	0.29	1.9	0.06	9	0.4	47	2.4	12	0.5	36	2.0
Clover Bartolo bladder	15	63.2	1.09	9.2	0.19	2.7	0.06	7	0.3	52	1.0	21	1.1	31	1.2
Clover Blaza crimson	3	54.0	2.02	7.6	0.35	2.1	0.28	8	0.9	56	1.8	18	0.3	37	2.1
Clover Cefalu arrowleaf	6	53.1	0.71	7.4	0.12	1.7	0.08	6	0.2	56	1.0	17	0.4	39	0.7
Clover Clare sub	6	55.7	2.10	7.9	0.36	1.4	0.05	10	0.3	52	2.7	16	0.3	37	2.4
Clover Denmark sub	9	57.0	1.56	8.1	0.27	2.2	0.04	11	0.3	45	2.6	13	0.9	31	1.8
Clover Frontier balansa	16	57.3	1.33	8.1	0.23	2.0	0.07	10	0.4	50	1.7	13	0.5	37	1.7
Clover Gosse sub	6	57.6	1.74	8.2	0.30	1.8	0.05	12	0.9	45	2.9	14	0.7	31	2.3
Clover Lightening Persian	9	59.2	1.04	8.5	0.18	1.9	0.11	9	0.4	51	1.7	16	0.4	35	1.4
Clover Memphis berseem	6	60.8	1.91	8.7	0.33	1.9	0.08	9	0.4	45	1.6	12	0.5	32	1.2
Clover Paratta purple	6	49.2	0.52	6.8	0.09	1.8	0.07	7	0.3	59	0.9	17	0.2	42	0.8
Clover Prima gland	4	53.9	1.90	7.6	0.33	1.8	0.14	7	0.3	56	2.3	16	1.2	40	2.1
Clover SA 15896 Ball	9	61.4	1.82	8.9	0.31	2.1	0.08	9	0.7	44	2.6	15	0.6	30	2.3
Clover SA 20009 Moroccan	6	59.8	1.36	8.6	0.23	2.2	0.12	9	0.8	47	1.5	14	0.3	33	1.6
Clover SA 36400 spike	3	54.8	0.53	7.7	0.09	1.7	0.09	10	1.1	52	1.0	14	0.8	39	0.5
Clover SARDI Persian	6	56.7	2.40	8.1	0.41	2.0	0.14	9	0.5	55	2.6	18	0.6	37	2.7
clover SARDI rose	4	55.5	1.03	7.8	0.18	2.0	0.13	8	0.6	58	1.2	21	0.3	38	1.1
Clover Sothis eastern star	3	55.3	0.67	7.8	0.11	2.1	0.11	7	0.5	56	0.5	20	0.6	36	1.0
Clover Tas 1698 striated	6	59.3	0.70	8.5	0.12	2.0	0.10	8	0.5	50	1.3	17	0.6	33	0.9
Clover Tas 2129 Lappa	8	49.9	1.54	6.9	0.26	1.8	0.09	8	0.3	54	2.0	15	0.4	40	1.7
Clover Tas1630/1807 cluster	9	61.3	1.01	8.8	0.17	2.5	0.09	9	0.4	48	1.8	15	0.6	32	1.2
Clover Tas511_348 Difuse	6	53.7	1.01	7.5	0.17	1.6	0.07	7	0.3	53	1.3	16	0.2	37	1.2
Clover Trikkala sub	6	59.6	2.87	8.5	0.49	1.8	0.10	10	0.9	46	3.9	15	1.4	31	2.5
Clover Urana sub	16	56.2	1.70	8.0	0.29	2.0	0.07	9	0.4	49	2.2	14	0.6	35	1.7
Fenugreek Might	6	63.0	0.45	9.1	0.08	2.7	0.08	6	0.6	50	0.8	21	0.4	29	0.6
Fenugreek SA 32200	3	55.7	0.61	7.9	0.11	1.9	0.08	8	0.1	56	0.5	18	0.2	38	0.5
Fenugreek SA 32202	3	57.6	0.83	8.2	0.14	2.4	0.08	7	0.2	54	0.5	19	0.5	35	0.6
Fenugreek Wimmera	9	58.0	2.08	8.3	0.36	2.1	0.11	6	0.4	54	1.8	19	1.0	35	2.1
Hedysarium SA 32504	6	61.7	2.72	8.9	0.47	2.1	0.18	12	0.6	39	3.6	8	1.3	31	2.4

		DMD (%)		M/D (MJ/kg)		N (%)		Ash (%)		NDF (% DM)		Hemi (% DM)		ADF (%DM)	
Entry	n	Mean	sem	Mean	sem	Mean	sem	Mean	sem	Mean	sem	Mean	sem	Mean	sem
lathyrus Ceora	9	59.1	0.47	8.5	0.08	2.5	0.03	6	0.2	41	0.8	14	0.3	26	0.7
Lotus ornith ITA 8a	6	52.7	0.49	7.4	0.08	2.0	0.05	5	0.3	57	0.6	20	0.3	38	0.7
Medic Angel strand	6	50.4	1.67	7.0	0.29	2.0	0.17	8	0.3	57	1.9	15	0.6	42	1.5
Medic Bindaroo button	6	53.7	1.38	7.5	0.24	2.2	0.19	9	0.7	57	1.9	18	0.4	39	1.7
Medic Caliph barrel	4	47.5	1.28	6.5	0.22	1.9	0.15	7	0.7	61	1.7	18	1.5	43	1.8
Medic Essex snail	3	47.6	0.42	6.5	0.07	1.8	0.04	7	0.3	59	0.3	15	0.5	44	0.3
Medic Herald strand	3	48.8	0.97	6.7	0.17	1.5	0.25	11	0.7	63	1.6	18	0.0	45	1.6
Medic Highlander rotata	6	52.6	1.58	7.3	0.27	2.0	0.18	7	0.5	58	2.3	18	0.4	40	2.0
Medic Paraponto gama	6	59.9	2.27	8.6	0.39	1.9	0.09	12	0.5	45	3.9	12	1.5	33	2.4
Medic SA 32612 phrygia	4	59.4	1.22	8.5	0.21	2.4	0.06	7	0.6	53	1.7	19	0.5	34	1.4
Medic SA 36809 spotted	6	58.1	1.32	8.3	0.23	2.5	0.19	8	0.3	51	2.6	16	0.5	36	2.1
Medic SA 8774 spotted	12	51.7	1.39	7.2	0.24	2.3	0.08	9	0.5	56	1.9	14	0.6	41	1.5
Medic Scimitar burr	14	53.6	1.43	7.5	0.25	2.4	0.10	8	0.4	51	2.0	15	0.7	36	1.5
Medic Tornafield disk	6	55.6	1.07	7.9	0.18	2.2	0.18	8	0.8	54	1.5	18	0.2	37	1.4
Melilotus Jota	3	59.9	1.85	8.6	0.32	2.5	0.18	5	0.5	45	1.5	14	0.2	31	1.4
Melilotus SA 37228	6	46.2	0.77	6.2	0.13	1.1	0.06	4	0.3	66	1.2	17	0.5	49	0.9
Melilotus SA 40002	12	56.9	1.48	8.1	0.25	2.2	0.16	5	0.3	54	1.9	17	0.5	37	2.0
Ononis SA 8577	6	52.4	0.72	7.3	0.12	1.6	0.03	8	0.3	56	1.1	14	0.4	41	0.9
Serradella Cadiz	15	49.6	1.96	6.8	0.34	1.6	0.11	6	0.4	59	2.5	15	0.5	44	2.1
Serradella Jebala	6	51.0	1.25	7.1	0.21	1.8	0.06	9	1.1	49	2.5	14	0.5	35	2.4
Serradella Santorini	16	44.4	0.75	5.9	0.13	1.7	0.04	4	0.5	65	1.5	16	0.4	49	1.2
Trigonella SA 19767	6	61.0	2.04	8.8	0.35	2.2	0.11	7	0.2	54	2.4	19	1.5	35	2.4
Trigonella SA 32999	6	57.3	2.58	8.2	0.44	2.2	0.26	8	0.3	55	2.6	20	0.3	35	2.7
Trigonella SA 5045	12	52.5	1.43	7.3	0.25	2.2	0.16	7	0.3	57	1.3	18	1.0	39	1.2
Vetch Ioman	3	57.0	0.57	8.1	0.10	1.8	0.04	8	0.2	54	1.1	20	1.3	34	0.3
Vetch Languedoc	9	53.5	2.43	7.5	0.42	2.2	0.17	5	0.5	46	2.7	14	0.3	33	2.6
Vetch Namoi	9	52.4	1.11	7.3	0.19	2.2	0.06	7	0.5	49	1.6	15	0.4	34	1.5
Vetch Popany	6	57.5	2.44	8.2	0.42	2.7	0.07	6	0.4	48	2.1	14	0.5	34	1.6
		55.5	**1.38**	**7.8**	**0.24**	**2.0**	**0.11**	**8**	**0.5**	**53**	**1.8**	**16**	**0.6**	**37**	**1.5**
		5.7		1.0		0.4		2		8		3		6	
		***		***		***		***		***		***		***	

*** P<0.001

Table 7d. Mean nutritional traits of annual grasses and forbs in South Australia at the vegetative stages of development (development scores 1-2)

		DMD (%)		M/D(MJ/kg)		N (%)		Ash (%)		NDF (%DM)		Hemi (%DM)		ADF (%DM)	
Entry	n	Mean	sem	Mean	sem	Mean	sem	Mean	sem	Mean	sem	Mean	sem	Mean	sem
Canola 43Y85	3	79.6	0.42	12.0	0.07	3.5	0.08	13	0.6	22	3.1	9	2.5	13	0.6
Canola Hyola 50	2	82.1	0.57	12.4	0.10	3.6	0.11	13	0.2	21	0.2	6	0.2	15	0.0
Canola Taurus	6	78.8	1.07	11.8	0.18	3.0	0.44	10	1.2	24	1.2	6	0.5	17	0.8
Chia Black	6	67.5	1.58	9.9	0.27	2.4	0.13	14	0.3	34	2.3	11	1.5	23	0.8
Chia White	6	65.4	1.21	9.5	0.21	2.3	0.12	14	0.2	35	2.0	12	1.1	23	0.9
Kale Kestrel	21	77.9	0.84	11.7	0.14	2.0	0.19	9	0.4	25	1.0	8	0.7	17	0.5
Quinoa SA 45507	6	63.5	3.07	9.2	0.53	3.4	0.41	7	0.4	32	3.4	7	0.8	25	2.7
Rape Titan	12	79.8	0.90	12.0	0.15	2.6	0.29	11	0.7	21	0.8	5	0.4	16	0.5
Rape Winfred	20	76.8	0.97	11.5	0.17	2.5	0.15	11	0.4	25	1.3	8	0.7	17	0.7
Turnip Hunter	7	79.2	0.55	11.9	0.09	3.4	0.17	16	1.2	24	0.7	6	0.5	18	0.6
Turnip New York	16	78.9	0.84	11.9	0.14	3.7	0.34	12	1.3	22	1.2	7	0.9	15	0.8
Barley Moby	3	77.2	0.55	11.6	0.10	2.7	0.35	11	0.2	37	1.1	15	1.1	23	0.2
Fesper	7	75.7	1.20	11.3	0.21	3.2	0.43	10	0.6	40	1.7	18	0.7	22	1.0
Oat Winteroo	9	72.1	1.27	10.7	0.22	2.4	0.14	10	0.5	40	1.7	17	0.8	23	1.0
Ryecorn Sthn Green	4	75.3	1.89	11.8	0.69	3.2	0.22	10	0.9	37	2.1	17	1.4	22	1.2
Ryegrass Concord	9	76.6	0.75	11.5	0.13	3.7	0.49	10	0.9	39	1.2	20	1.1	19	0.4
Ryegrass Dargo	17	74.3	0.80	11.1	0.14	2.8	0.25	11	0.8	39	1.3	17	0.7	21	0.8
Ryegrass Eclipse	8	75.2	0.92	11.2	0.16	3.1	0.31	11	1.0	40	1.8	18	0.9	23	1.0
Ryegrass Feast II	8	77.3	0.88	11.6	0.15	3.0	0.30	10	0.9	38	1.2	17	0.5	21	0.8
Ryegrass Maverick GII	10	74.3	1.13	11.1	0.19	2.7	0.31	10	0.9	41	1.0	19	0.8	22	0.5
Ryegrass Progrow	8	75.9	0.87	11.3	0.15	2.8	0.34	11	0.9	39	2.0	18	1.0	22	1.0
Ryegrass Safeguard	2	78.4	1.68	11.8	0.29	3.1	0.17	14	2.1	36	0.1	16	0.5	21	0.4
Ryegrass Sungrazer	9	77.2	1.08	11.6	0.19	2.6	0.21	11	0.5	36	1.5	16	0.8	20	0.9
Ryegrass Tama	9	77.7	0.79	11.7	0.14	2.7	0.19	10	0.9	36	0.9	16	0.5	20	0.4
Ryegrass Turbo	9	74.9	0.89	11.2	0.15	2.7	0.28	12	0.8	40	1.3	18	0.7	22	0.8
Ryegrass Wimmera	2	75.8	0.99	11.3	0.17	3.0	0.14	18	3.9	39	3.1	14	0.8	25	2.3
Ryegrass Zoom	9	77.6	0.80	11.6	0.14	2.8	0.30	11	0.5	36	1.7	16	1.1	20	0.7
Triticale Crackerjack2	6	75.9	1.71	11.4	0.29	3.7	0.18	11	0.4	38	1.8	17	0.5	21	1.4

		DMD (%)		M/D(MJ/kg)		N (%)		Ash (%)		NDF (%DM)		Hemi (%DM)		ADF (%DM)	
Entry	n	Mean	sem	Mean	sem	Mean	sem	Mean	sem	Mean	sem	Mean	sem	Mean	sem
Wheat Wedgetail	6	73.1	1.01	10.9	0.17	3.7	0.21	11	0.3	41	1.9	19	1.0	22	1.0
Grand Mean		75.7	1.08	11.3	0.20	3.0	0.25	11	0.8	33	1.5	13	0.8	20	0.8
Mean forbs		75.4		11.3		3.0		12		26		8		18	
Mean grasses		75.8		11.4		3.0		11		39		17		22	
LSD		4.9		0.9		1.3		4		7		4		4	
P		***		***		***		***		***		***		***	

*** P<0.001

Table 7e. Mean nutritional traits of annual grasses and forbs in South Australia at the reproductive stages (development scores 2-4 forbs and 3-5 grasses)

		DMD (%)		M/D(MJ/kg)		N (%)		Ash (%)		NDF (% DM)		Hemi (% DM)		ADF (%DM)	
Entry	n	Mean	sem	Mean	sem	Mean	sem	Mean	sem	Mean	sem	Mean	sem	Mean	sem
Canola 43Y85	9.0	59.8	3.13	8.6	0.54	2.6	0.35	8.9	0.94	41	1.8	11	0.5	30	1.4
Canola Hyola 50	9.0	63.0	3.12	9.1	0.54	2.3	0.35	8.8	1.07	40	1.9	10	0.5	30	1.5
Canola Taurus	6.0	61.5	2.68	8.9	0.46	1.9	0.12	7.5	0.75	44	2.1	14	0.9	30	1.4
Rape Titan	6.0	66.6	2.02	9.8	0.35	1.8	0.09	8.6	0.67	36	2.4	12	0.5	24	1.9
Turnip Hunter	8.0	63.3	3.11	9.2	0.54	1.9	0.11	9.8	0.77	42	3.7	13	1.3	29	2.5
Turnip New York	12.0	65.4	1.74	9.5	0.30	2.5	0.16	11.6	0.48	38	2.2	11	0.6	27	1.7
Wild turnip SA 42783	6.0	62.4	3.93	9.0	0.68	3.1	0.43	9.5	1.42	41	3.4	9	0.8	32	2.7
Barley Moby	6.0	67.4	3.09	9.9	0.53	2.3	0.41	7.7	0.81	46	1.9	20	1.0	26	1.0
Oat Winteroo	9.0	65.6	1.91	9.6	0.33	1.7	0.18	7.5	0.59	48	1.8	20	0.7	29	1.2
Ryecorn Sthn Green	5.0	63.7	2.97	9.2	0.51	2.1	0.42	9.0	0.56	51	2.7	20	0.9	31	1.8
Ryegrass Concord	3.0	65.6	1.62	9.6	0.28	1.8	0.10	9.0	0.33	48	1.6	25	0.5	23	1.1
Ryegrass Dargo	7.0	65.1	1.83	9.5	0.31	1.3	0.07	6.9	0.58	48	1.9	21	1.0	27	1.0
Ryegrass Eclipse	3.0	67.3	1.91	9.9	0.33	1.4	0.18	8.0	0.87	44	0.9	20	0.4	24	0.7
Ryegrass Feast II	5.0	65.5	1.49	9.6	0.26	1.3	0.06	8.8	0.28	48	1.9	23	1.5	25	0.6
Ryegrass Maverick GII	4.0	65.1	1.49	9.5	0.26	1.2	0.24	8.2	0.42	49	1.2	25	1.4	24	0.5
Ryegrass Progrow	3.0	67.4	1.70	9.9	0.29	1.5	0.16	6.7	0.77	46	1.8	21	1.0	25	1.1
Ryegrass Safeguard	6.0	71.5	1.85	10.6	0.32	2.9	0.55	10.6	0.54	42	0.8	19	0.2	24	0.7
Ryegrass Sungrazer	3.0	65.4	1.03	9.5	0.18	1.3	0.17	7.0	0.26	50	0.9	22	0.8	27	0.5
Ryegrass Tama	3.0	68.0	0.81	10.0	0.14	1.5	0.07	7.4	0.88	44	0.5	20	0.4	24	0.1
Ryegrass Turbo	4.0	65.4	2.32	9.5	0.40	1.1	0.10	7.7	0.13	47	2.9	22	1.9	25	1.4
Ryegrass Wimmera	6.0	68.6	3.19	10.1	0.55	3.0	0.52	10.5	0.75	48	2.0	21	0.7	27	1.6
Ryegrass Zoom	5.0	67.5	3.10	9.9	0.53	1.4	0.18	7.8	0.56	47	2.5	22	1.7	25	0.9
Triticale Crackerjack2	6.0	61.7	1.55	8.9	0.27	1.6	0.15	7.1	0.59	50	1.6	20	0.4	31	1.3
Wheat Wedgetail	6.0	63.2	1.38	9.2	0.24	1.6	0.19	7.1	0.49	48	1.2	21	0.9	27	0.8
Grand Mean		**65.3**	**2.24**	**9.5**	**0.39**	**1.9**	**0.23**	**8.5**	**0.65**	**45**	**1.9**	**18**	**0.9**	**27**	**1.2**
Mean forbs	4.8	63.1		9.2		2.3		9.3		40		11		29	
Mean grasses	4.8	66.1		9.7		1.7		8.1		47		21		26	
LSD		13.4		2.3		1.5		3.9		11		5		8	
P		ns		ns		***		***		***		***		***	

*** P<0.001; ns is not significant

Table 7f. Mean nutritional traits of annual grasses and forbs in South Australia at the mature stages (development scores 5-6 forbs and 7-9 grasses)

		DMD (%)		M/D(MJ/kg)		N (%)		Ash (%)		NDF (% DM)		Hemi (% DM)		ADF (%DM)	
Entry	n	Mean	sem	Mean	sem	Mean	sem	Mean	sem	Mean	sem	Mean	sem	Mean	sem
Barley Moby	3.0	53.3	1.23	7.5	0.21	1.2	0.06	5.2	0.41	52	2.4	24	0.7	28	1.8
Fesper	3.0	49.1	1.28	6.7	0.22	1.3	0.18	7.5	0.18	69	1.6	31	0.5	38	1.2
Ryecorn Sthn Green	3.0	46.6	1.78	6.3	0.31	1.0	0.08	5.6	0.18	59	3.2	26	0.6	33	2.7
Ryegrass Dargo	4.0	45.1	1.37	6.1	0.24	0.7	0.10	5.9	0.51	72	1.7	30	1.3	42	1.1
Ryegrass Feast ll	3.0	54.4	1.85	7.6	0.32	1.1	0.19	6.6	0.18	62	1.7	30	0.6	32	1.6
Ryegrass Maverick GII	3.0	58.1	0.80	8.3	0.14	1.2	0.15	6.9	0.39	58	1.7	29	0.5	29	1.2
Ryegrass Progrow	3.0	48.5	0.24	6.6	0.04	1.5	0.16	6.4	0.33	69	0.4	31	0.4	38	0.3
Ryegrass Safeguard	3.0	43.9	0.61	5.8	0.10	1.3	0.13	6.7	0.35	73	0.9	31	0.3	42	0.9
Ryegrass Sungrazer	4.0	49.9	0.44	6.9	0.08	1.4	0.17	7.3	0.26	66	0.8	30	0.7	36	0.9
Ryegrass Tama	3.0	49.2	0.89	6.8	0.15	1.0	0.15	7.4	0.27	66	1.2	31	0.3	35	1.1
Ryegrass Turbo	3.0	52.8	0.85	7.4	0.15	1.2	0.16	7.0	0.45	65	1.6	30	0.1	34	1.5
Ryegrass Wimmera	3.0	42.3	1.31	5.6	0.23	1.2	0.14	6.3	0.24	74	1.1	30	0.6	44	1.8
Ryegrass Zoom	3.0	50.6	0.39	7.0	0.07	1.2	0.12	6.8	0.54	66	0.7	31	0.2	36	0.5
Triticale Crackerjack2	3.0	53.9	0.30	7.6	0.05	1.0	0.05	5.4	0.29	48	1.8	24	0.8	24	1.1
Wheat Wedgetail	3.0	51.0	0.64	7.1	0.11	1.1	0.15	6.0	0.16	53	0.2	23	0.4	29	0.5
Grand Mean		49.8	0.95	6.9	0.16	1.2	0.13	6.5	0.33	64	1.5	29	0.5	35	1.3
LSD		2.6		0.5		0.3		0.8		4		2		3	
P		***		***		*		***		***		***		***	

*** P<0.001

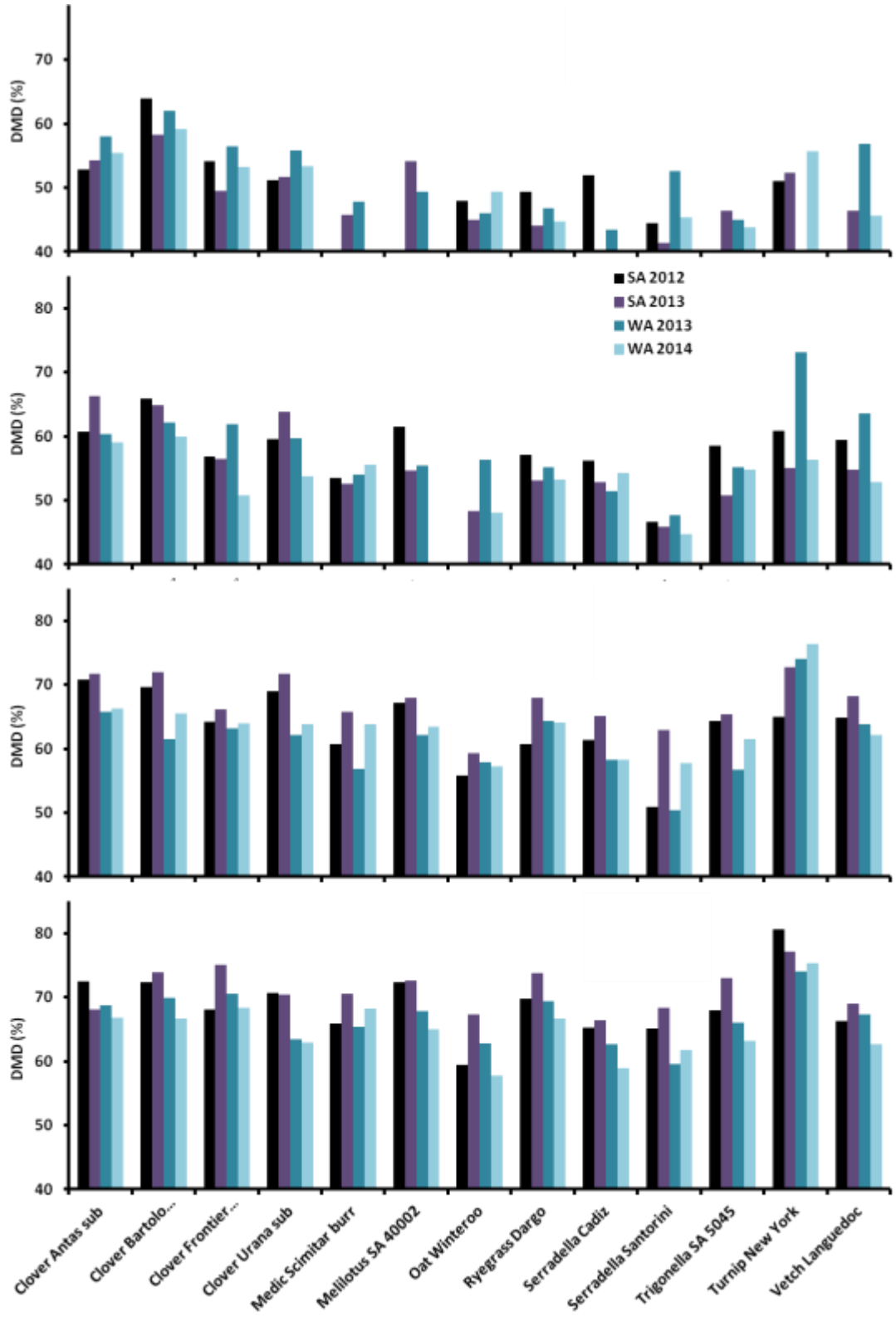

Fig 9. Mean DMD of the accessions that were grown at both research sites over two seasons. The bottom graph presents data from the first vegetative cut (~ 90 days after sowing), the next represents cuts at 130-140 days after sowing, the thirds presents cuts at 150-160 days after sowing and the top graph is for senesced material (~180 days after sowing).

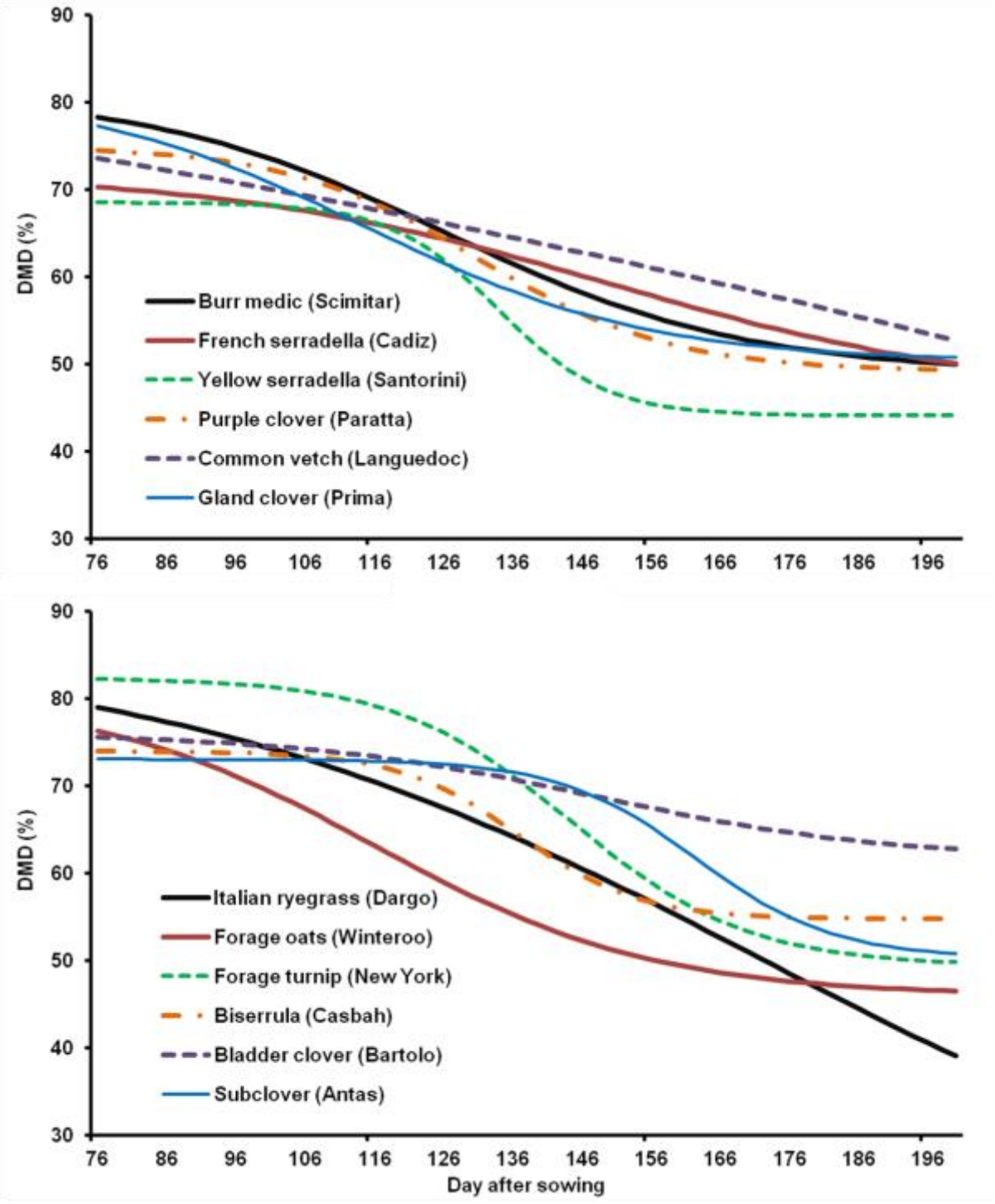

Fig 10. Changes in DMD of 12 of the accessions growing at the South Australian site, modeled with a logistic function (all curves were a statistically significant fit)

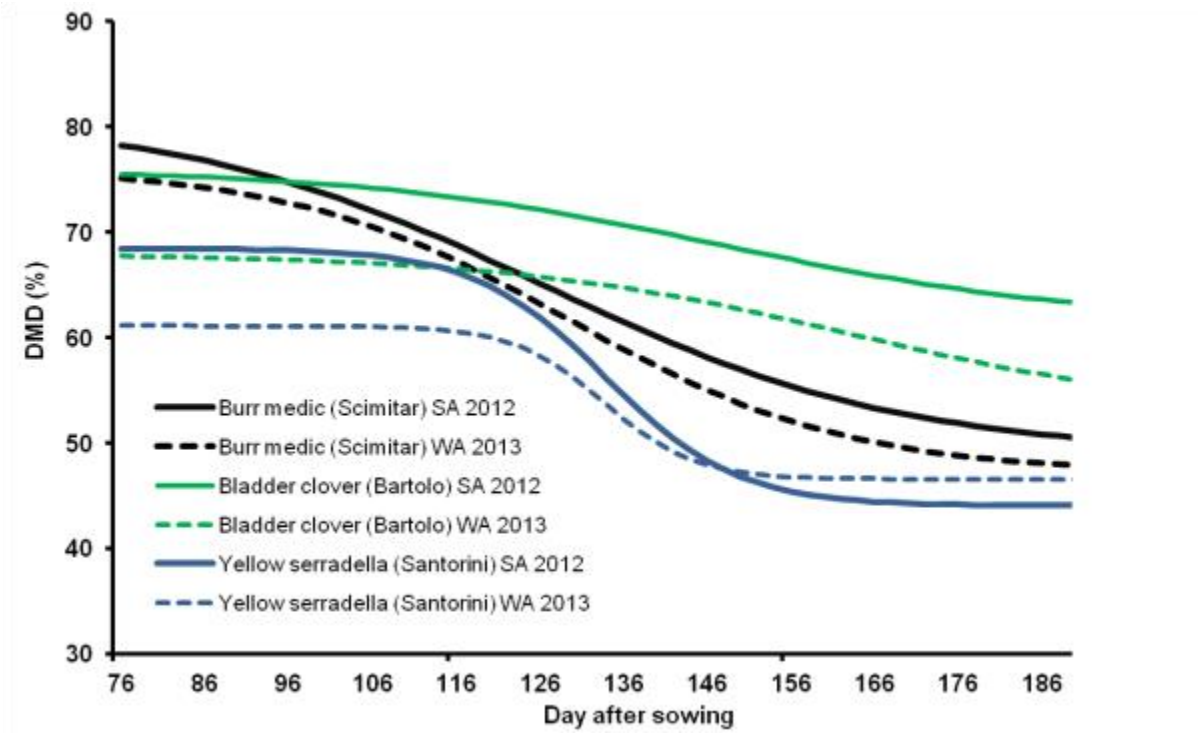

Fig 11. Changes in DMD of three of the accessions growing at the SA site in 2012 and the WA site in 2013, modeled with a logistic function (all curves were a statistically significant fit)

1.4 Discussion

The aim of this work was to identify a wider range of farming practices by which landholders can reduce methane emissions and emissions intensity from grazing systems. This is important as enteric methane is the most significant single source of greenhouse gas emissions from agriculture(Beauchemin *et al.,* 2008). As well as environmental implications, methane represents production inefficiency in the form of a loss of 6-7 % of gross energy intake of ruminants (Johnson and Johnson 1995). To date there has been few, if any, systematic comparisons of the feeding value of annual legumes, grasses and forbs in southern Australia. We compared 90 accessions from 66 species of legumes, grasses and forbs and found significant differences in biomass production and nutritional value at various development stages and in the rate of nutritional decline throughout reproduction and senescence. A subset of these plants was subject to batch culture fermentation to investigate potential to abate methane.

Within the context of the experimental design, there were clear differences in biomass production and nutritional value both within and between species, leading to differences in the likely feeding value to ruminants. The nutritive value of herbage mass was generally highest when the plants were vegetative and fell rapidly as plants senesced. This decline in quality was probably due to an increase in the stem:leaf ratio, changes in composition of the cell wall (Akin *et al.,* 1977) and loss of cell contents with maturity (Ballard et al 1990, Coleman and Henry, 2002).

The forage oat cultivar (cv Winteroo) was the most productive of the accessions tested across sites and years and had very good winter vigor. The nutritional value of the species however is not high and it declined very rapidly throughout the lifecycle of the plant. It is not suitable as a standing forage after seed set and should be grazed as young plant then cut for hay. In WA at the end of September 2013, this forage oat averaged 5000 kg/ha with a DMD of 72% thus yielding over 53 GJ ME/ha. The next most productive species, yellow serradella (cv Santorini) and burr medic (cv Scimitar), offered over 20 GJ ME/ha, but with a much higher crude protein content. It is clear the inclusion of a forage oat has significant potential to increase feeding value early in the season, but the legumes complement this by maintaining higher crude protein.

For the legumes, the vetch (cv Languedoc) demonstrated good biomass production and high nutritional value. When compared to some of the other annual legumes, for example the purple clover (cv Paratta) or yellow serradella (cv Santorini), the vetch had much greater potential to support ruminant growth late in the growing season. Another standout species was bladder clover (cv Bartolo) as it had a slow rate of DMD decline and high nutritional value in summer. This species, developed for the medium rainfall zone, has significant potential to extend the growing season for ruminants into summer. Bladder clover is widely distributed throughout the Mediterranean Basin and results of field experiments suggest the species offers significant agronomic advantages (in terms of biomass production and seed yield) to farmers with fine textured soils in the low to medium rainfall zone (300–450 mm annual rainfall) of southern Australia (Loi *et al.,* 2003). The seeds of this species are easily harvested with conventional cereal harvesting equipment (Loi *et al.,* 2003, 2005) and the pattern of hard seed breakdown within and between seasons favors long-term persistence (Norman *et al.,* 2002). Given the outstanding nutritional value and only one commercialized cultivar, this species may be worthy of further investigation.

Using the nutritional model Grazfeed (Freer *et al.,* 1997), we estimated intake and growth rates of mature, dry ewes offered unrestricted quantities of several accessions in December. Data from the South Australian site were used but they are similar to those collected in Western Australia. Predicted voluntary feed intakes and growth rates are in Table 8 for senesced forage species. Mature ewes could eat only half a kilo of the standing forage oats and would lose approximately 130 g of liveweight per day. If they were offered the purple clover they would average about 0.68 kg of intake but still lose 100 grams per day of liveweight. The bladder clover was the only species that would support growth with an estimated intake of 1 kg. In reality, the animals will select a diet that optimizes energy and protein intake, these numbers are only predictions, however ewes offered the bladder clover have greater potential to maintain bodyweight or grow. This is a crude and simpla way to compare the species, the information generated in this study needs to be used to test scenarios in whiole farm models to relaise the full differences in productivity and profitability (for example see Ghahramani and Moore, 2013).

Table 8. Predicted intakes (kg/day) and growth rates (g/day) of selected accessions of senesced forage from South Australia in 2012.

	Biomass (kg/ha)	DMD (%)	CP (%)	Intake (kg/day)	Growth (g/day)
Forage oat Winteroo	8987	46.1	1.2	0.50	-130*
Clover Paratta purple	5074	49.2	1.8	0.68	-100
Clover Antas subterranean	6075	53.5	2.2	0.78	-45
Vetch Langudoc	4093	58.5	1.9	0.90	-10
Clover Bartolo bladder	1537	63.2	2.7	1.00	15

*The animals must be given a protein supplement

Care must be taken when interpreting this information as the plants have not been managed to optimize either growth or nutritional value. The data simply reflect the potential of the plants as standing forage crops. Managing the plants to delay reproduction or conserving them as hay or silage (maintaining high nutritional value for use at times of nutrient deficit) will alter their feeding value within a system. Future work should investigate nutritional value within the context of the farming systems that utilize the cultivars.

Climate change scenarios have led to predictions of a decline in pasture production, decreases in forage quality, drought (Howden *et al.,* 2007) and greater risk of soil erosion and degradation due to a decline in ground cover (Cullen *et al.,* 2009; Ghahramani and Moore, 2013). Moore and Ghahramani (2013) used simulation modelling to investigate the impact of predicted climate change scenarios on livestock productivity in southern Australia. For the SRES A2 scenario of global change (IPCC 2000) and in the absence of adaptations other than adjusting stocking rates to avoid overgrazing, they estimated that total aboveground net primary productivity would decrease by an average of 9% in 2030, 7% in 2050 and 14% in 2070 from historical levels. Projected decreases in operating profit averaged 27% in 2030, 32% in 2050 and 48% in 2070. The proportional reductions in both pasture growth and profitability were greater at locations near the dry margin of the cereal–livestock zone. The information generated in this study could assist industry to mitigate these impacts. Ghahramani and Moore (2013) suggest lucerne is an option to maintain productivity in these areas however it is a difficult species to establish and maintain with little summer rainfall. Productive and high nutritional value annual species, such as bladder clover, vetches or Italian ryegrass, offer an opportunity to boost feeding value of the low to medium rainfall zone. A proportion of ley pastures may be replaced by directly seeded forage crops to meet the needs of livestock systems. There is much more work to be done to identify species and genotypes that are adapted to and productive within mixed farming systems in the mixed crop and livestock zone.

During the project we have generated a unique and extensive collection of data for biomass production and nutritive traits of annual forage species in southern Australia. In addition, the broad NIRS calibrations that were developed for this activity represent a valuable tool for further investigation of forage quality under different climatic and management systems.

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SECTION 2 Temporal changes in biomass and nutritional valueof temperate perennial legumes, grasses and herbs

2.1 Abstract

The temporal distribution of yield and nutritive value of 60 perennial grasses, herbs and legumes under irrigation and rainfed conditions was measured at the Waite Institute, South Australia. In the first nine months, under irrigation, the quality and yield of the pasture species was measured every three weeks on cumulative forage production, with the aim of measuring nutritive value through time and at different plant growth development stages. There were clear differences in digestibility between species and in genotypes within species at different times and in the pattern of digestibility change over time.

Forage yield under rainfed conditions was assessed in the second year to measure the distribution of production and identify plants that can extend the growing season or fill the autumn to winter feed gap. Lucerne had the highest feed value in the legume experiment, with SARDI 7 series 2 producing a total of 20922 kg/ha and 201 GJ ME/ha. The next highest forage legume was Hedysarum coronarium, with cv. Wilpena producing 14957 kg/ha, and a total of 144 MJ ME/ha. Chicory was the highest yielding grass or herb, with cv. Choice producing 15803 kg/ha and 180 MJ ME/ha. Lucerne and chicory were also the only entries to produce significant quantities of forage in late spring and summer, with 11745 kg/ha (56%) and 7661 kg/ha (44%) of their production in summer compared with < 1500 kg/ha in all other entries.The potential of perennial legumes, grasses and herbs to modify the diet of livestock to improve productivity and reduce emissions intensity is discussed.

2.2 Methods

Experimental design
Sixty perennial legumes, grasses and herbs were grown in two experiments (experiment 1 -legumes; experiment 2 - grasses and herbs) at the Waite Institute to evaluate their temporal changes in forage yield and quality. The first year of production (cuts 1-8) was irrigated, and used to assess the relationship between crop development stage and forage quality. The second year of production was used to identify the yield and feed value potential metabolisable energy produciton (ME/ha) under rainfed conditions.

Location and soil type
The field site was located in the Australian Pastures Genebank field nursery, at the Waite Institute, in South Australia. The fine sandy loam at this site is a red-brown earth (Stace *et al.,* 1968) of the non-sodic Urrbrae series (Litchfield 1951). The upper 0.10 m contains 18% clay, increasing to 32% in the A2 horizon (Prescott 1931). Soil pH (in $CaCl_2$) was 6.2 and there was negligible calcium carbonate (Grace *et al.,* 1995). The site had subsurface drip irrigation, with two lines running 0.5 m apart, 0.2 m beneath each plot, and with drip intervals of 0.5 m. In the first year (September 2012 to 31 March 2013), irrigation was with weekly applications equivalent to 25 mm of rainfall between November and April. No irrigation was used in the second year of the experiments (1 April 2013 to April 2014).

Germplasm
The germplasm in these experiments was selected to represent the complete range of perennial pasture species available commercially, plus new species that have been the focus of research in Australia over the last two decades. Commercial cultivars were sourced from a range of companies to provide an even representation and reduce potential bias. The 30 perennial legumes, and 30 grasses and herbs sown in Experiments 1 and 2 are listed in Table 3a and b.

Design of plots and planting
The perennial legumes, and grasses and herbs were evaluated in two separate experiments to allow broadleaf herbicide applications and nitrogen fertiliser to be applied separately to two different areas. For each experiment, 30 entries (hereafter cultivars) were sown in a completely randomised and blocked design with 18 columns and 5 rows and 3 replications (6 columns and 5 rows per replication). Each species was sown into 1 x 8 m plots on 11 August 2012 with a self propelled Wintersteiger small plot seeder at the highest recommended rate from the seed supplier, adjusted for percent germination (Table 3). Exceptions to this sowing regime were made for Bituminaria bituminosa cv. Tedera, which

was hand sown with tubestock at 32 plants/m^2 due to the unavailability of seed, and Austrodanthonia racemosa cv. Friend, which had the seed hand sown because it would not flow through the seeder.

Plant culture and evaluation protocols
Forage yield was assessed every 3 weeks after an initial establishment phase of 88 days. Plots were cut at 3 cm above ground using a 1 m wide sickle (finger) mower with reciprocating blades to provide a 1 m^2 forage sample. After collection and weighing, biomass samples were either immediately frozen for freeze drying (or placed in a paper bag then oven dried at 60^oC). After drying, samples were ground to pass through a 1mm screen using a Retsch Twister mill grinder mill.

For the first eight cuts, subsequent measurements of forage yield were taken on the adjacent 1 m^2 section of plot, such that growth was cumulative from the time of sowing and that repeat measurements of yield from the same area were not taken (Fig.1). At each measurement of forage yield, plant development stage was assessed using the protocol developed by Metcalfe and Nelson (1985, Table 1). After the 8th cut on 2 April 2013, the experiment was converted into a forage yield trial with two cutting frequencies (a split plot design with two 4 m^2 subplots). Subsequent forage yield harvests were repeated on the same area every 6−8 weeks in one split plot, and on the second, a hay fodder crop was simulated by locking it up between 20 June 2013 and 8 October 2013.

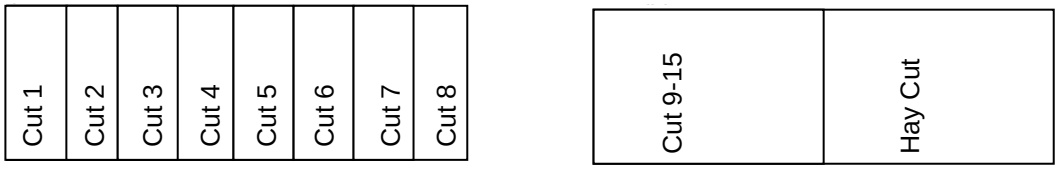

Fig.1. Representation of cutting schedule on each plot in perennial legume, and grass and herb experiments at the Waite Institute for (a) assessment of forage quality at each developmental growth stage with irrigation in year 1, first 8 cuts between 7 November 2012 and 6 March 2013, and (b) assessment of seasonal yield under frequent and infrequent (simulated hay cut replaces cuts 11 and 12) under rainfed conditions in year 2, cuts 9-15 between 20 March 2013 and 22 January 2014.

Near Infrared Spectroscopy (NIRS)

Nutritional value was estimated using chemistry and near infrared spectroscopy (NIRS; see review by Deaville and Flynn, 2000). Samples were scanned by NIRS (Unity Spectrastar 2500X- rotating top window system, Unity Scientific) and nutritional traits were predicted with calibrations generated using partial least squares regression with the chemometric software package Ucal (Unity Scientific). Subsets of samples were set aside for chemical analysis. The broad, multispecies NIRS calibration for DMD, total N, ADF, NDF and OM (ash) was built on over 1000 samples with matching spectra and chemistry (see Norman *et al.*, section 4). Where a sample was analysed with wet chemistry, that data is included, otherwise data are predicted. Samples that did not fit within the calibration (as indicated by high global H and neighbourhood H values – indicators of the samples multivariate distance from other samples in the calibration) were subject to wet chemistry analysis.

Table 1. Morphological descriptors for growth stages of forage grasses and legumes (Metcalfe and Nelson, 1985)

Grasses	
1	Vegetative (Leaves only; stems not elongated)
2	Stem elongation (Stems elongated)
3	Boot (Inflorescence enclosed in flag leaf sheath and not showing)
4	Heading (Inflorescence emerging or emerged from flag leaf sheath, but not shedding pollen)
5	Anthesis (Flowering stage; anthers shedding pollen)
6	Milk stage (Seed immature, endosperm milk)
7	Dough stage (Well-developed seed; endosperm doughy)
8	Ripe seed (Seed ripe; leaves green to yellow brown)
9	Postripe seed (Seed postripe; some dead leaves; some heads shattered)
10	Stem-cured (Leaves cured on stem; seed mostly cast)
Legumes & herbs	
1	Vegetative (No buds visible)
2	Bud (Buds visible, but no flowers)
3	First flower (First flowers appear on plants)
4	Flower (Plants flowering)
5	Pod (or green seed) Green seedpods developing
6	Ripe seed (Mostly mature brown seedpods with lower leaves dead and some leaf loss)
7	Senescence (Leave s cured or dropped, pod/seed mostly cast)

RPD tests the strength of the relationship between a constituents values and the error of the NIR predicted results and was calculated by;

RPD = 1 / (1 - r^2)$^{0.5}$ (Williams 2014)

The larger the RPD value the greater its strength. We have adopted the forage RPD guide of Williams (2014) who suggested RPD values of 0.0–1.9 are very poor and not recommended for forage testing; RPD values of 2.0–2.4 are poor and only of use for rough screening; RPD values of 2.5–2.9 offer a fair screening potential; RPD values of 3.0–3.4 are good (quality control); RPD values of 3.5–4.0 are very good (suited to process control) and RPD values of 4.1+ are deemed excellent.

The prediction statistics for the samples within this paper, separated into grasses, forbs and legumes are presented in Table 2. For the legumes and grasses, NDF (thus also hemicellulose) predictions are not as accurate as those for other traits and data interpretation should proceed with a degree of caution.

Table 2. Validation statistics for the NIRS predictions of nutritional value

Trait	Perennial grasses		Perennial legumes		Forbs	
	r2	RPD	r^2	RPD	r^2	RPD
NDF	0.818	2.3	0.767	2.1	0.980	7.1
ADF	0.927	3.7	0.880	2.9	0.983	7.7
DMD	0.927	3.7	0.887	3.0	0.981	7.3
OM	0.790	2.2	0.930	3.8	0.880	2.9
N	0.987	8.8	0.966	5.4	0.984	7.9

Chemistry

In vitro dry matter digestibility (DMD), adjusted to predict *in vivo* digestibility, was determined in duplicate using a modified pepsin-cellulase technique described by Clarke *et al.* (1982). Modifications are outlined in Norman *et al.* (section 4). Duplicate samples of seven AFIA standards (AFIA 2007) with known *in vivo* DMD are included in each batch to allow raw laboratory values to be adjusted to predict *in vivo* digestibility using linear regression (see Fig. 1). The average standard error of the AFIA standards across the runs was 0.261%. The energy value of the sample (MJ/kg at maintenance level of feeding) was estimated by the equation: M/D = (0.172*DMD) - 1.707 (Standing Committee on Agriculture, 1990).

Concentrations of neutral detergent fibre (NDF) and acid detergent fibre (ADF) of the shrub material were measured sequentially, according to operating instructions, using an Ankom 200/220 Fibre Analyser (Ankom® Tech. Co., Fairport, NY, USA). Duplicate samples were analysed for each diet. The difference between NDF and ADF was deemed to be hemicellulose. An oaten hay samples was included in each of the 103 fibre analysis runs during the project. The QC had NDF of 30.19 ± 0.1137 % DM and ADF of 19.71 ± 0.0665 %DM.

Total ash was measured on duplicate samples according to the methods of Faichney and White (1983). Total nitrogen and carbon was determined by combustion using a Leco CN628 N Analyser (Sweeney and Rexroad 1987). Where crude protein has been presented we have adopted the convention of CP = total N x 6.25.

Statistical analyses

Means of fixed entry effects for each response variate (forage yield, developmental growth stage, digestibility, protein, NDF and ADF) were calculated using spatial linear mixed models performed by GENSTAT 15 (Lawes Agricultural Trust 2012). Diagnostic plots of sample variograms and residuals were used in conjunction with REML log-likelihood ratios and Wald tests to fit new models that compartmentalised and removed random and fixed effects of variation (Smith *et al.,*2005).

2.3 Results

Rainfall

Rainfall in the first five months of the study was well below average (Fig. 2), but supplemented with approximately 25 mm of subsurface irrigation per week to ensure satisfactory establishment and development of all species. In 2013, above average rainfall was recorded in July and August, but this was followed by low spring rainfall including decile 1 November rainfall. There was a significant rainfall event near the end of the project, with 85 mm falling on 14−15 February 2014.

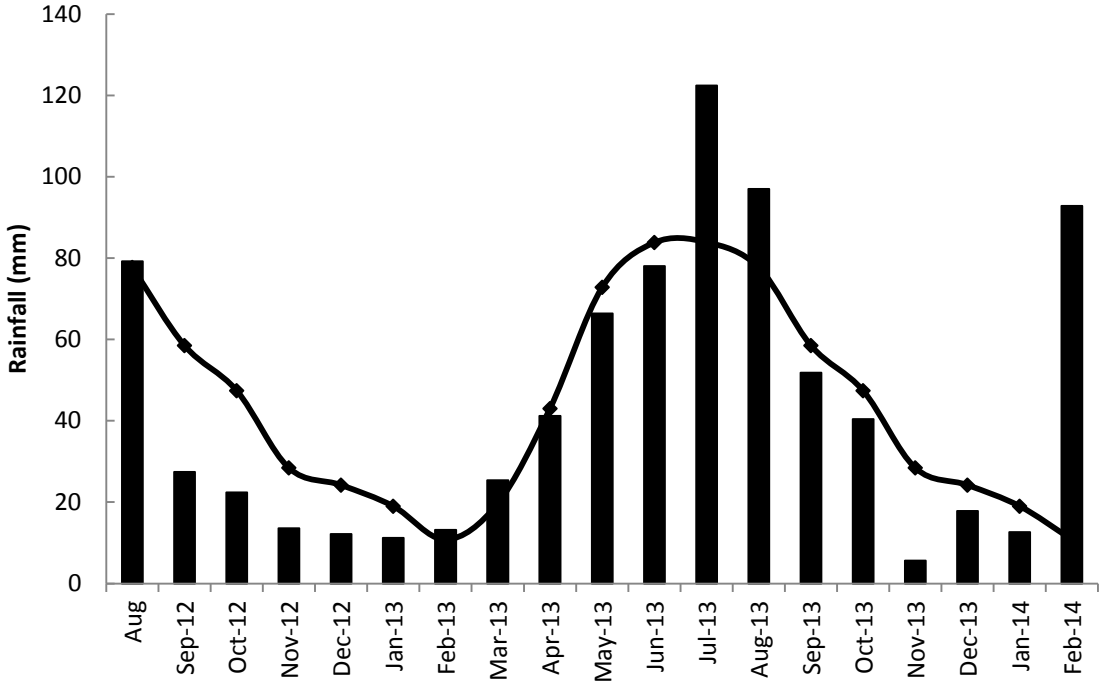

Fig. 2. Monthly (bar) and long term median rainfall (line) rainfall at Glen Osmond (Bureau of Meteorology station 23090) for the experimental period.

Air temperature

Air temperature for the experimental period at Kent Town (station 23090, 6 km away) is shown in Fig. 3. The Waite Institute is frost free, with lowest temperatures of 2˚C recorded. The highest air temperatures were 45˚C in January 2012 and in January and February 2013. The mean monthly maximum temperatures were approximately 30˚C in January and February each year and 18˚C in July 2013. The mean monthly minimum temperature ranged between 7˚C and 16˚C.

Fig 3. Monthly mean minimum (▲), mean maximum (∆), lowest (■) and highest (□) air temperatures (°C)at Kent Town (station 23090) at the Waite Institute for the experimental period

Establishment and persistence

All perennial grass and herb entries established at densities > 150 plants/m^2 (Table 3b) with the exception of Austrodanthonia racemosa cv. Friend (55 plants/m^2) and Ehrharta calycina cv. Mission (109 plants/m^2). Establishment density was highest in Lolium perenne cvv. AberMagic HSG (544 plants/m^2) and Victorian (555 plants/m^2). Most perennial grass and herb entries had final levels of ground cover (final plant frequency measured 19.5 months after establishment on 24 April 2014) above 70%, with the exception of Phleum pratense cv. 38843 (70%), Bromus coloratus cv. Exceltas (49%), Plantago lancolata cvv. Lancelot (52%) and Tonic (55%) and Puccinellia stricta cv. Menemen (64%).

The establishment density in the perennial legume experiment was 29−272 plants/m^2 (Table 3a). The species with lower plant density were generally those with the capacity to develop large individual plant biomass, such as Onobrychis, Cullen, Hedysarum and Astragalus, with the exception of Trifolium fragiferum cv. Palestine (69 plants/m^2), which is small seeded and established at high density. Ground cover at the end of the experiment was less than 30% in 18 of the 30 perennial legumes, and was lowest in Bituminaria bituminosa (0%), Lotus uliginosus cv. Maku (1%), L. australis cv. SA45714 (1%) and L. corniculatus cv. Lottas (6%), Trifolium pratense cv. Tuscan (5%) and Hedysarum coronarium cv. Aokau (6%). The perennial legumes with the highest final ground cover were Kennedia prostrate cv. SA41710 (which has prostrate foliage), and Medicago sativa cvv. SARDI 7 Series 2 (66%), Aurora (65%), WL925HQ (69%) and K202 (74%).

Table 3a. Sowing rate, establishment density and final density of perennial legumes sown at the Waite Institute

Species	Entry	Common name	Sowing rate kg/ha	Establishment Density 10	Final % ground cover, 30 April
Hedysarum coronarium	Aokau	Sulla	20	97	8
Medicago sativa subsp.	Aurora	Lucerne	12	209	76
*Dorycnium hirsutum**	Canaritas	hairy canary clover	10	98	18
*Lotus corniculatus**	Goldie	birdfoot trefoil	8	170	11
Trifolium hybridum	Hytas	Alsike clover	5	247	15
Medicago sativa subsp.	K202	Lucerne	12	224	81
Trifolium ambiguum	Kuratas	Caucasian clover	6	154	39
Lotus glaber	LosBanos	narrow leaf trefoil	8	317	20
Lotus corniculatus	Lottas	birdfoot trefoil	8	237	6
Lotus uliginosus	Maku	gr.birdfoot trefoil	6	95	1
Hedysarum coronarium	Moonbi	Sulla	20	117	15
Melilotus officinalis	Norgold	yellow sweet clover	12	174	52
Onobrychis viciifolia	Othello	Sanfoin	20	55	30
Trifolium fragiferum	Palestine	strawberry clover	3	69	44
Trifolium tumens	Permatas	talish clover	6	202	25
*Trifolium repens**	Quest	white clover	4	120	31
*Trifolium pratense**	Rubitas	red clover	8	272	23
Medicago sativa subsp.	S7S2	Lucerne	15	128	82
Dorycnium rectum	SA1231	erect *Dorycnium*	20	185	39
Astragalus cicer	SA38091	Milkvetch	8	38	18
Kennedia prostrata	SA41710	running postman	8	151	84
Lotus australis	SA45714	Australian trefoil	15	181	2
Lotus corniculatus	SA45718	birdfoot trefoil	12	213	59
Cullen australasicum	SA4966	native scurf pea	20	40	10
*Onobrychis viciifolia**	Shoshone	Sanfoin	4	46	18
Trifolium repens	Storm	white clover	12	170	42
Bituminaria bituminosa	Tedera	Tedera	*	29	0
Trifolium pratense	Tuscan	red clover	8	148	5
*Hedysarum coronarium**	Wilpena	Sulla	20	163	25
Medicago sativa subsp.	WL925HQ	Lucerne	12	209	74
F prob				<0.001	<0.001
LSD (5%)				58	18

Table 3b. Sowing rate, establishment density and final ground cover of perennial grasses and herbs sown at the Waite Institute.

Species	Cultivar	Common name	Sowing rate	Establishment Density 11	Final % ground
Lolium perenne	AberMagic	perennial ryegrass	20	544	74
Phalaris	Advanced	phalaris	3	475	90
Phalaris	Australian 2	phalaris	3	146	84
Lolium perenne	Banquet II	perennial ryegrass	20	482	89
Lolium perenne	Bealey	perennial ryegrass	20	415	88
Cichorium	Choice	chicory	5	193	78
Cichorium	Commander	chicory	5	156	63
Dactylis	Currie	cocksfoot	6	405	92
Lolium perenne	Drylander	perennial ryegrass	20	429	92
Agropyron	Dundas	tall wheat grass	12	205	94
Bromus	Exceltas	coloured brome	25	263	49
Festuca	Fraydo	tall fescue	15	459	91
Austrodanthonia	Friend	wallaby grass	2	55	60
Bromus	Gala	grazing brome	25	216	87
Phalaris	Holdfast GT	phalaris	3	168	92
Dactylis	Howlong	cocksfoot	6	353	77
Plantago	Lancelot	plantain	10	318	52
Bromus	Matua	prarie grass	25	240	87
Dactylis	Megatas	cocksfoot	6	208	76
Puccinellia	Menemen	puccinellia	10	660	64
Ehrharta	Mission	perennial veldt	2	109	80
Bromus	Nandu	grazing brome	25	261	84
Cichorium	Puna	chicory	5	187	78
Festuca	Quantum II	tall fescue	15	394	90
Festuca	Resolute	tall fescue	15	474	93
Phleum	SA 38843	timothy	8	171	41
Plantago	Tonic	plantain	10	240	55
Austrodanthonia	Trangie	ringed wallaby	2	158	83
Dactylis	Uplands	Mediterranean	6	164	93
Lolium perenne	Victorian	perennial ryegrass	20	555	86
Fprob				<0.001	<0.001
5% lsd				104	14

Temporal changes in feed value related to growth stage
Dry matter digestibility decreased over time (Figs 4a and 4b) and with progressive plant development stage (Figs 5a and 5b). There were clear differences in digestibility between species and in genotypes within species at different times, and in the pattern of digestibility change over time. The DMD, M/D, total N, ash, NDF, hemicellulose and ADF of perennial grasses, legumes and herbs for cuts 1 to 6 are tabled in the supplementary file (Tables 8a−9g).

Perennial grasses
The highest digestibility was recorded in perennial ryegrass cvv. Abermagic HSG (high sugar grass, 79%), Banquet II (76%) and Bealey (77%, Fig. 4a).Digestibility in these perennial ryegrass cvv.was 9−10% higher than drylander and Victorian at 4 Feb at the same development score. The ryegrass with the highest nutritive value at the mature plant vegetative stage was cv. Banquet II, with 10.97 MJ ME/kg DM, 25.7% CP, and the lowest NDF (41.4) and ADF (19.4) of all perennial grasses. Banquet II had significantly higher M/D than all other perennial ryegrass cvv., higher CP than Abermagic HSG and Bealey, and lower NDF, hemicelluloses and ADF compared to Abermagic HSG and Victorian.

Perennial ryegrass, tall fescue cvv. Resolute and Fraydo, coloured brome and tall wheat grass maintained vegetative (plant development score 1) for the first 208 days after sowing (Table 4a). The decline in digestibility for these species over time is related to leaf age, the impact of high temperature and remobilisation of soluble carbohydrates into new leaves.

The DMD of perennial veldt grass cv. Mission was relatively high through time and by growth development stage (Fig. 4a and 5a). Digestibility was 74% at cut 1, (88 DAS) and was still 61% on 4th February, 177 DAS at growth stage 9.0 (postripe seed with some dead leaves, refer Supplementary file, Table 8a). At the mature plant vegetative cut (7 May 2013, Table 5a), Mission veldt grass had high nutritive value, with 10.94 MJ ME/kg DM and 28.5 % crude protein (calculated as 6.25* total N). Mission veldt grass had the lowest hemicelluloses content ranking of all grasses (20.58%), low NDF (44.7) and average ADF (24).

Grazing brome grass cv. Nandu advanced quickly to growth development stage 4 (bud development) by the first cut, 88 days after sowing (Table 4a). The rapid advancement in flowering and fruiting was associated with digestibility that was low at each cutting time (Fig. 4a), but average when considered against other species at the same crop development stage (Fig. 5a). Similarly, prairie grass (Bromus willdenowii) cv. Matua was between boot and heading stage at the first cut, and its DMD declined fairly quickly with flowering and seed production (Fig. 5a). The vegetative DMD of both of these cultivars was relatively high when measured in the vegetative stage as a mature plant (Table 5a). In contrast, cvv. Gala had a much slower advancement in maturity (Table 4a) and consequently maintained higher DMD through time (Fig. 4a).

Table 4a. Plant development (Dev) and forage yield (Dry Weight, DW) of perennial grasses and herbs at the Waite Institute (year 1)

		Cut 1, 7/11/2012			Cut 2, 26/11/2012			Cut 3, 17/12/2012			Cut 4, 9/01/2013			Cut 5, 4/02/2013			Cut 6, 6/03/2013		
Cultivar	Common name	Dev	log DW	DW kg/ha	Dev	log DW	DW kg/ha	Dev	log DW	DW kg/ha	Dev	log DW	DW kg/ha	Dev	log DW	DW kg/ha	Dev	log DW	DW kg/ha
Choice	chicory	1	5.45	232	1	7.16	1289	2	8.22	3718	5	8.81	6688	6	9.30	10938	6	9.26	10541
Commander	chicory	1	4.84	127	1	6.29	539	1	7.50	1799	1	7.53	1856	1	8.20	3656	1	8.33	4134
Puna	chicory	1	4.67	106	1	6.28	536	1	7.91	2716	1	7.92	2738	1	8.61	5481	1	7.98	2922
Currie	cocksfoot	1	*	*	1	5.94	380	1	7.26	1422	1	7.34	1538	2	7.33	1525	2	6.70	815
Howlong	cocksfoot	1	4.62	101	2	5.52	248	3	7.08	1184	5	7.24	1390	6	7.77	2361	6	7.82	2482
Megatas	cocksfoot	1	*	*	1	5.58	265	1	6.45	633	1	6.22	503	2	6.30	545	2	6.75	857
Exceltas	coloured brome	1	*	*	1	5.33	205	1	6.26	522	1	6.81	903	1	7.13	1244	1	6.74	843
Gala	grazing brome	1	*	*	2	5.87	353	2	6.64	765	2	6.78	879	3	7.02	1118	3	7.01	1108
Nandu	grazing brome	4	6.25	515	5	7.43	1681	8	7.67	2150	9	8.03	3078	9	8.43	4573	*		*
Uplands	Med. cocksfoot	1	*	*	1	5.15	173	1	6.19	490	2	6.38	592	4	6.43	621	4	6.89	985
AberMagic	perennial ryegrass	1	4.90	134	1	6.14	463	1	7.06	1159	1	6.86	957	1	7.12	1236	1	7.08	1184
Banquet II	perennial ryegrass	1	4.81	123	1	5.73		307	1	6.43	621	1	6.31	551	1	6.62	749	1	6.51	674
Bealey	perennial ryegrass	1	5.75	313	1	6.58	720	1	7.12	1233	1	7.42	1674	1	7.58	1955	1	6.91	999
Drylander	perennial ryegrass	1	4.87	130	1	5.54	253	1	5.99	397	1	5.72	305	1	6.69	804	1	6.57	711
Victorian	perennial ryegrass	1	*	*	1	5.36	213	1	6.15	469	1	5.45	232	1	5.96	386	1	5.91	369
Mission	perennial veldt grass	2	4.32	75	4	4.98	145	7	5.77	319	9	6.41	609	9	7.10	1214	*		*

		Cut 1, 7/11/2012			Cut 2, 26/11/2012			Cut 3, 17/12/2012			Cut 4, 9/01/2013			Cut 5, 4/02/2013			Cut 6, 6/03/2013		
Cultivar	Common name	Dev	log DW	DW kg/ ha	Dev	log DW	DW kg/ha	Dev	log DW	DW kg/ha	Dev	log DW	DW kg/ ha	Dev	log DW	DW kg/ha	Dev	log DW	DW kg/ha
Advanced AT	phalaris	2	5.18	177	3	6.18	483	4	7.36	1566	5	7.79	2424	8	7.95	2833	8	8.11	3328
Australian 2	phalaris	1	*	*	1	*	*	2	5.88	358	2	6.37	585	7	7.07	1175	4	6.66	783
Holdfast GT	phalaris	2	4.72	112	3	5.01	149	4	6.39	597	5	7.16	1289	7	7.62	2047	9	8.26	3866
Lancelot	plantain	1	5.24	188	2	6.36	578	5	7.85	2573	6	8.01	3020	6	8.31	4064	*		*
Tonic	plantain	1	5.47	237	2	6.57	712	4	7.76	2354	6	8.01	3008	6	8.07	3194	*		*
Matua	prarie grass	4	5.51	248	5	6.70	816	8	7.35	1561	9	7.89	2670	9	8.18	3551	*		*
Menemen	puccinellia	3	5.11	166	4	5.32	204	4	5.38	217	8	5.71	302	9	6.04	421	*		*
Trangie	ringed wallaby grass	4	4.86	129	5	5.64	283	8	6.50	664	9	6.80	894	9	7.30	1485	*		*
Fraydo	tall fescue	1	*	*	1	5.20	181	1	6.00	402	1	6.30	543	1	6.94	1032	1	6.93	1026
Quantum II	tall fescue	1	5.00	149	1	6.10	445	1	7.16	1292	1	7.52	1843	2	7.74	2296	2	7.53	1869
Resolute	tall fescue	1	*	*	1	5.27	195	1	6.33	562	1	6.27	526	1	7.13	1251	1	6.97	1064
Dundas	tall wheat grass	1	*	*	1	4.99	148	1	6.53	684	1	7.26	1417	1	7.84	2535	1	7.68	2158
SA 38843	timothy	1	*	*	1	*	*	1	*	*	1	5.25	190	1	5.42	225	1	6.11	452
Friend	wallaby grass	1	*	*	3	*	*	7	5.59	267	8	6.19	485	9	5.99	400	*		*
Fprob		<0.001	<0.001		<0.001	<0.001		<0.001			<0.001			<0.001			<0.001		
sed		0.06	0.25		0.19	0.36		0.21	0.3		0.2	0.3		0.29	0.3		0.34	0.4	

Cultivar	Common name	Cut 1, 7/11/2012			Cut 2, 26/11/2012			Cut 3, 17/12/2012			Cut 4, 9/01/2013			Cut 5, 4/02/2013			Cut 6, 6/03/2013		
		Dev	log DW	DW kg/ha	Dev	log DW	DW kg/ha	Dev	log DW	DW kg/ha	Dev	log DW	DW kg/ha	Dev	log DW	DW kg/ha	Dev	log DW	DW kg/ha
5% lsd		0.11	0.47		0.35	0.68		0.40	0.67		0.4	0.72		0.55	0.6		0.64	0.8	

The digestibility of tall fescue cv. Fraydo declined quickly with time in comparison to cvv. Resolute and cv. Quantum II (Fig. 4a). Resolute and Fraydo are both Mediterranean tall fescues and had very similar patterns of growth in this experiment (Table 4a). Fraydo had higher vegetative digestilibility than Resolute (69% compared to 65.3%) at the mature plant vegetative stage (Table 5a), and this was associated with lower NDF, hemicelluloses and ADF fractions.

Timothy grass cv. SA38843 had poor early growth and consequently was not harvested until cut 4 (152 DAS, Table 4a). However, DMD for cuts 4−6, and 9 was very high (69−70%, Dev stage 1.0, Fig. 4a and Table 5a).

Ringed wallaby grass cv. Trangie had high DMD at the vegetative stage, but flowers very quickly in this environment (Dev 3.6 at first cut 88 DAS, Table 4a, perennial grasses) and quality deteriorates to below maintenance level for a dry sheep (cut 3, Dev 7.6 with 58% DMD, Fig. 4a). Mature plant vegetative DMD was also moderately high (67%), but NDF (53.3) and ADF (29.3) fractions were above average.

Australian 2 phalaris has lower digestibility at the initiation of stem elongation (Dev stage 2, 66%) and booting (Dev 3, 61%), in comparison to Holdfast GT (75% at Dev 2 and 67% at Dev 4) and Advanced AT (74% at Dev 2 and 67% at Dev 4), but then has a lower rate of decline during flowering and seed production (Dev 5 to 7) to maintain DMD above 61% in comparison to Holdfast GT and Advanced AT, which reduced to approximately 57% DMD (Fig. 5a). At the mature vegetative plant stage (cut 9, Table 5a), Advanced AT had lower DMD (68.67%) than Holdfast GT (71.5%) and higher Ash, NDF, hemicelluloses and ADF fractions.

The Mediterranean *hispanica* Cocksfoot cv. Uplands had lower DMD at growth development stages 1−2 compared with 'intermediate type' Howlong (developed from selections of Porto) during the first 6 cuts (seedling to reproductive, Fig. 5a). In the vegetative cut taken of the mature plant to measure the quality of autumn growth (7 May 2013, Table 5a), cv. Uplands had significantly greater DMD (70.28), compared with intermediate type cvv. Currie (67.83), and Howlong (Intermediate 67.57). Howlong had much higher NDF (58.6), hemicelluloses (31.76) and ADF (26.9) fractions compared to other intermediate types Currie and Megatas (Fig. 4a).

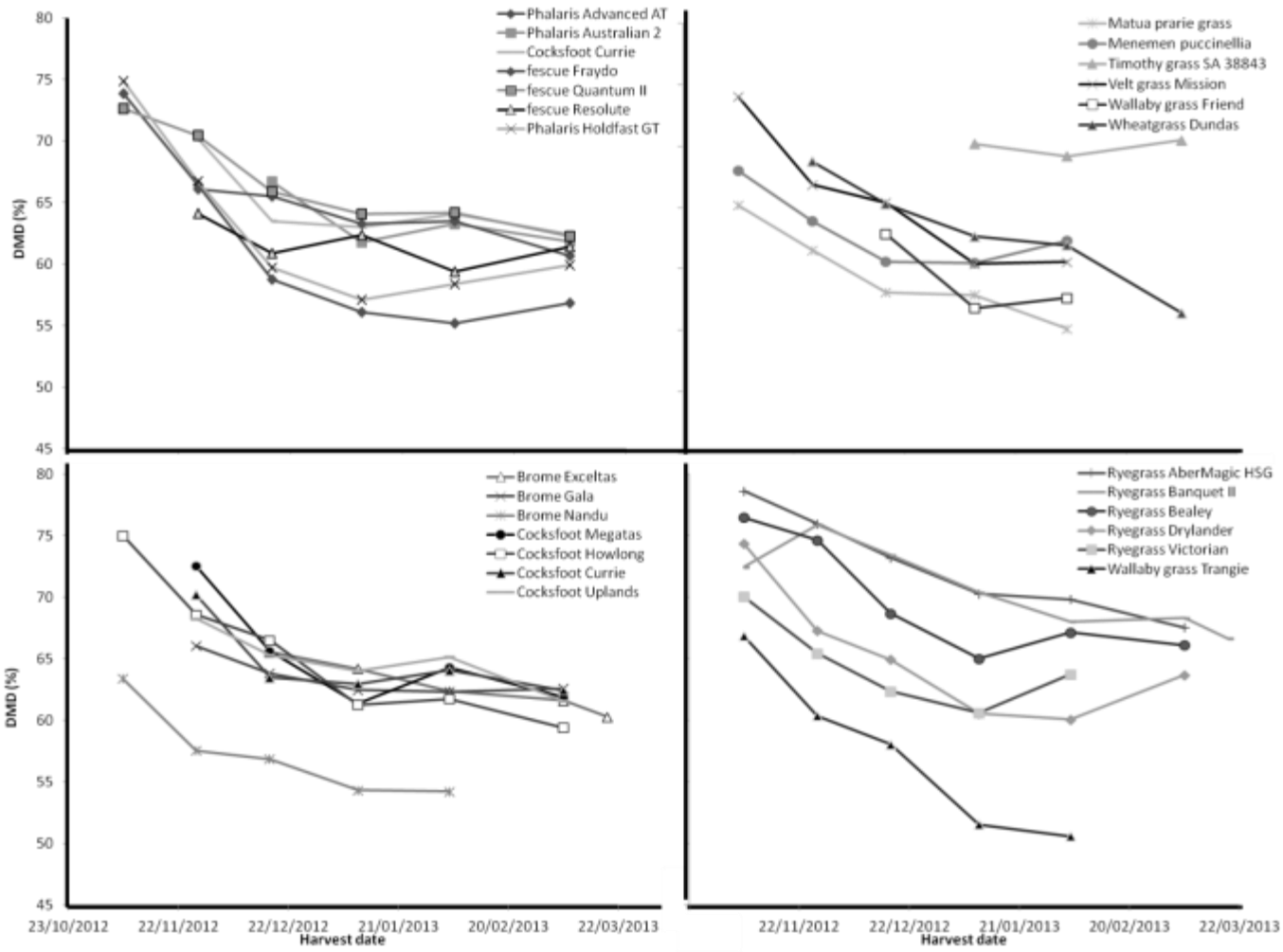

Fig 4a. Dry matter digestibility (DMD %) of the perennial grasses across sampling times.

Perennial herbs

Chicory has high DMD in the vegetative growth stage as immature plants (71−73% in cuts 1−3, supplementary file Table 10a), and as mature plants (75%, cut 9 in autumn 2013, Table 6c). The DMD of vegetative plantain was similar in cuts 1−3, and slightly lower as a mature plant at cut 9 (71-72.6%). Chicory also had lower NDF, hemicelluloses and ADF than plantain at cut 9 (Table 5c). Plantain had higher nutritive value during the reproductive phase (development scores 3−6, supplementary file, Table 10a), with 65% DMD and 26% ADF compared to 60% DMD and 29−31% ADF in chicory.

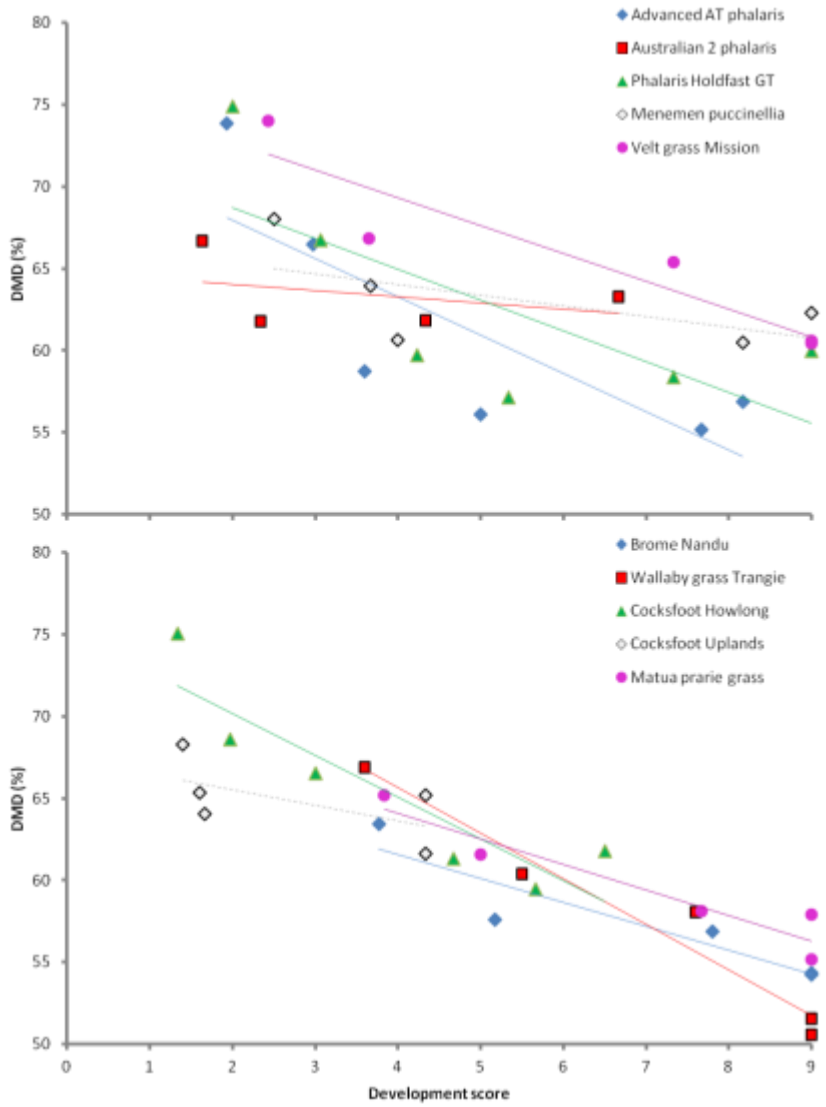

Fig 5a. Dry matter digestibility (DMD %) of the perennial grasses at different development scores.

Perennial legumes

The decline in digestibility with increasing time and growth development stage are shown in Fig. 4b and 5b. The digestibility of all clover species was still greater than 60% at ripe pod (development stage 6, Table 4b). The slope of DMD decline was less in current commercial clover, lucerne and sulla species than in legumes under recent development including Cullen, Dorynicum and Lotus.

Many of the perennial legumes were highly digestible at the mature plant vegetative stage (assessed using cut 9, May 2013) with alsike clover cv. Hytas, Caucasian clover cv. Kuratas, strawberry clover cv. Palestine, tallish clover cv. Permatas, white clover cvv. Quest, and Storm, Lotus cv. LosBanos, lucerne cvv. Aurora and K202, Melilotus cv. Norgold, sulla cv. Wilpena and Tedera 70−75% DMD.

There were also perennial legumes with poor mature plant vegetative DMD, including erect Dorycnium cv. SA1231 (58%), hairy canary clover cv. Canaritas (46%), running postman (57%), greater birdsfoot trefoil cv. Maku (58%), and Australian trefoil cv. SA45714 (55%).

Lucerne cv. Aurora had higher DMD than SARDI Seven series 2 (Fig. 4b). Aurora, K202 and SARDI Seven series 2 had lower NDF and ADF fractions than WL925HQ. The cv. WL925HQ is more winter active than the other cultivars, and thus would be expected to have lower forage quality (taller

cultivars have higher stem fractions resulting in higher fibre), particularly when cut on a fixed date and not a physiological or morphological basis.

Sulla cv. Wilpena had low vegetative DMD during the cumulative cuts 1-3 in the first year, (Fig. 4a, Perennial supplementary file Table 9a) but high DMD as a mature plant in the vegetative cut on 7 May 2013 (Table 5b). Sulla has a higher ash content (13−17%) compared to other perennial legumes (10−13% refer supplementary file, Table 9d).

Table 4b. Plant development (Dev) and forage yield (dry weight, DW) of perennial legumes at the Waite Institute (year 1).

Entry	Common name		Cut 1, 7/11/2012			Cut 2, 26/11/2012		Cut 3, 17/12/2012		Cut 4, 9/1/2013		Cut 5, 4/2/2013		Cut 6, 6/3/2013	
		DTF	Dev	log DW	DW (kg/ha)	Dev	DW (kg/ha)	Dev	DW (kg/h	Dev	DW (kg/h	Dev	DW (kg/h	Dev	DW (kg/h
Hytas	Alsike clover	126	1	9.0	161	1.1	545	3.2	1708	5.0	2816	6.0	2943	6.0	
SA4571	Australian	96	2	4.9	145	4.4	241	2.0	322	3.3	710	4.7	1833	4.8	1414
Goldie	birdfoot	126	1	3.9		1.9	345	0.9	1557	1.0	3487	1.0	3742	6.0	
Lottas	birdfoot	128	1	4.5	98	2.0	427	2.2	1654	2.0	2406	4.7	3739	6.0	
SA4571	birdfoot	112	1	6.8	905	2.6	1326	2.4	712	5.0	1658	5.7	2776	6.0	
Kuratas	Caucasian		1			1.0		5.0	78	5.0	255	6.0	121	1.0	41
SA1231	erect	151	1			1.0	174	5.0	450	5.0	603	6.0	1957	1.0	2128
Maku	gr.birdfoot	144	1			1.0	86	2.9	116	4.7	287	5.8	515	6.0	
Canarit	hairy canary	207	1			0.9	154	1.3	329	1.3	1296	1.0	2681	1.3	2814
Aurora	lucerne	112	1	6.3	583	2.4	1446	5.0	3299	5.0	4342	6.0	6113	6.0	
K202	lucerne	112	1	6.4	644	2.4	1601	3.3	3027	5.2	3517	6.0	5155	6.0	
S7S2	lucerne		1			1.0	178	4.4	3960	5.2	5749	5.8	7583	6.0	
WL925	lucerne	102	1	6.4	642	2.3	1270	5.0	3204	5.0	3974	6.0	5888	6.0	
SA3809	milkvetch		1			1.0		1.0	218	2.7	1211	3.2	2972	2.0	2777
LosBan	narrow leaf		1	4.4	88	1.3	581	1.0	2993	1.0	4750	1.0	5808	6.0	4888
SA4966	native scurf	134	1			1.3	251	2.0	424	2.6	1975	4.7		5.0	5020
Rubitas	red clover	131	1	4.9	135	1.0	490	3.0	1554	5.5	3467	6.0	4154	6.0	
Tuscan	red clover	134	1			1.0	312	2.0	1024	5.0	2884	5.8	4384	6.0	
SA4171	running	145	1			1.0		1.0	165	1.0	315	1.0	1015	1.0	1335
Othello	sanfoin	107	1	4.7	114	3.1	247	1.0	300	1.0	1203	1.0	1712	6.0	
Shosho	sanfoin	115	1			1.9	362	4.5	658	5.3	1322	6.0	2077	6.0	
Palestin	strawberry	126	1			1.0		4.6	223	5.4	382	6.0	244	6.0	
Aokau	sulla	134	1	4.8	123	1.4	855	2.4	2350	5.0	3487	6.0	4081	6.0	
Moonbi	sulla	130	1	5.1	177	2.3	808	1.9	2513	4.2	3456	5.0	4288	6.0	
Wilpena	sulla	123	1	5.2	189	1.3	565	2.6	1608	5.0	3548	6.0	3990	6.0	
Permat	talish clover		1			1.1	307	3.0	217	5.3	495	6.0	433	1.0	333
Tedera	tedera		1	5.4	230	1.1		1.2	179	1.0	648	1.1	1511	1.2	1677
Quest	white clover	126	1			1.4	286	1.0	727	1.0	907	1.0	815	6.0	
Storm	white clover	107	1	5.0	158	2.9	466	4.3	1154	5.5	1757	6.0	1491	6.0	
Norgold	yellow sweet		1	4.5	95	1.0	346	2.6	2350	5.2	4383	6.0	5581	1.0	2908
F prob		<0.0	<0.0	<0.		<.00	<0.001	<0.	<0.00	<0.0	<0.00	<0.0	<0.00	<0.0	<0.00
Averag		3.18	0.02	0.1		0.14	167	0.2	338	0.17	589	0.13	721	0.07	611
LSD		6.03	0.05	0.2		0.27	317	0.4	642	0.32	1119	0.24	1370	0.13	1161

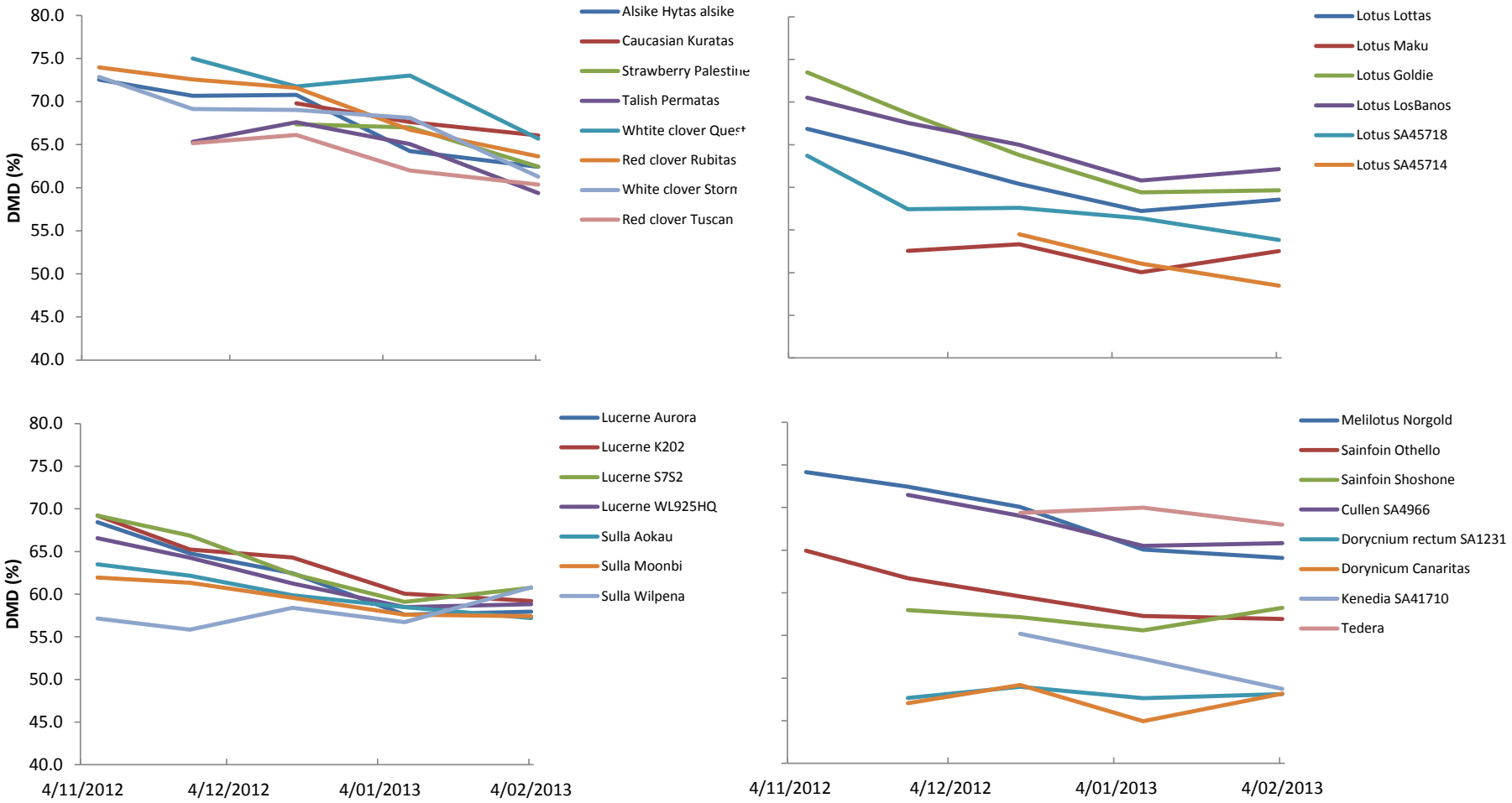

Fig. 4b. Dry matter digestibility (DMD, %) of the perennial legumes across sampling times

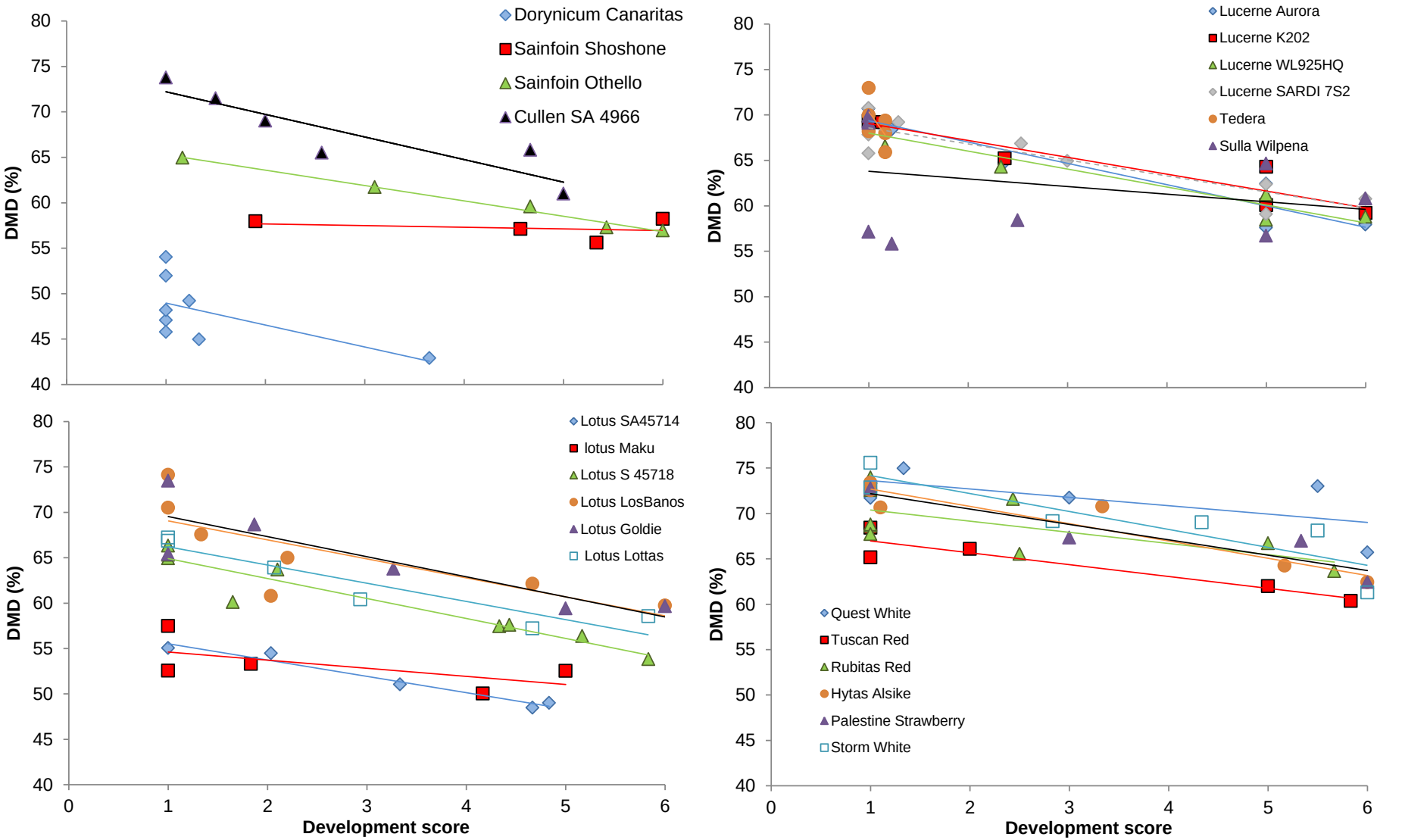

Fig 5b. Dry matter digestibility (%) of the perennial legumes at different development scores.

Table 5a. Nutritive traits of the perennial grasses, with 60 days regrowth, sampled on 7 May 2013 (269 days after sowing)

Type	Cultivar	Dev	DMD (%)		M/D (MJ/kg		Total N (%)		Ash (%)		NDF (% DM)		Hemicellulose (% DM)		ADF (% DM)	
		Mea	Mean	se	Mean	sem	Mean	sem	Mea	se	Mean	se	Mea	Se	Mean	se
Brome	Exceltas	1	66.04	1.0	9.65	0.17	4.22	0.24	12.9	0.5	47.76	1.6	24.1	0.9	23.7	0.8
	Gala	1	63.91	1.0	9.28	0.17	3.30	0.25	12.0	0.5	52.23	1.6	26.7	0.9	25.3	0.8
	Nandu	1	68.01	1.0	9.99	0.17	3.82	0.24	12.7	0.5	43.84	1.6	22.6	0.9	21.4	0.7
Cocksfoot	Currie	1	67.83	1.0	9.96	0.17	3.86	0.24	13.0	0.5	47.27	1.6	24.6	0.9	22.4	0.8
	Howlong	1	67.57	1.0	9.92	0.17	4.00	0.24	13.5	0.5	58.66	1.6	31.7	0.9	26.9	0.8
	Megatas	1	69.18	1.0	10.19	0.17	4.16	0.24	12.9	0.5	46.88	1.6	24.9	0.9	22.2	0.7
	Uplands	1	70.28	1.0	10.38	0.17	4.44	0.24	12.7	0.5	46.82	1.6	24.6	0.9	22.4	0.7
Fescue	Fraydo	1	69.06	1.0	10.17	0.17	3.66	0.24	11.6	0.5	47.94	1.6	24.0	0.9	23.4	0.8
	Quantum II	1	68.94	1.0	10.15	0.17	3.43	0.24	13.2	0.5	46.74	1.6	24.2	0.9	22.7	0.7
	Resolute	1	65.3	1.0	9.52	0.17	3.71	0.24	13.0	0.5	52.84	1.6	26.5	0.9	26.3	0.8
Phalaris	Advanced AT	1	68.67	1.0	10.1	0.17	4.24	0.24	15.4	0.5	51.87	1.6	26.2	0.9	25.6	0.8
	Australian 2	1	69.65	1.0	10.27	0.17	4.14	0.24	13.7	0.5	43.38	1.6	21.9	0.9	21.7	0.8
	Holdfast GT	1	71.5	1.0	10.59	0.17	3.96	0.24	13.5	0.5	42.78	1.6	22.0	0.9	20.9	0.7
Prarie	Matua	1	67.02	1.0	9.82	0.17	3.66	0.24	12.3	0.5	45.07	1.6	22.4	0.9	22.6	0.8
Puccinelli	Menemen	1	74.02	1.0	11.03	0.17	4.47	0.24	14.6	0.5	42.13	1.6	21.5	0.9	20.7	0.7
Ryegrass	AberMagic	1	71.07	1.0	10.52	0.17	2.98	0.24	11.7	0.5	49.98	1.6	26.4	0.9	23.9	0.7
	Banquet II	1	73.69	1.0	10.97	0.17	4.11	0.24	14.4	0.5	41.39	1.6	21.9	0.9	19.4	0.7
	Bealey	1	70.72	1.0	10.46	0.17	2.88	0.24	12.9	0.5	43.46	1.6	22.6	0.9	20.9	0.8
	Drylander	1	70.79	1.0	10.47	0.17	3.95	0.24	14.0	0.5	43.87	1.6	23.1	0.9	20.8	0.8
	Victorian	1	68.03	1.0	9.99	0.17	3.65	0.24	13.4	0.5	47.21	1.6	24.2	0.9	23	0.8
Timothy	SA 38843	1	71.11	1.0	10.52	0.17	3.75	0.24	15.2	0.5	48.22	1.6	24.2	0.9	24.1	0.8
Velt grass	Mission	1	73.56	1.0	10.94	0.17	4.57	0.24	14.2	0.5	44.7	1.6	20.5	0.9	24	0.7
Wallaby	Friend	1	64.56	1.0	9.4	0.17	3.55	0.24	16.4	0.5	56.56	1.6	25.8	0.9	31.1	0.7
	Trangie	1	66.97	1.0	9.81	0.17	3.82	0.24	14.4	0.5	53.3	1.6	24.1	0.9	29.3	0.7
Wheatgra	Dundas	1	65.76	1.0	9.6	0.17	3.69	0.24	13.7	0.5	53.81	1.6	24.2	0.9	29.4	0.8
Grand		1	68.93		10.15		3.84		13.5		47.95		24.2		23.76	
LSD			2.63		0.45		0.64		1.37		4.0147		2.35		1.921	
P value			***		***		***		***		***		***		***	

Table 5b. Nutritive traits of the perennial legumes, with 60 days regrowth, sampled on 7 May 2013 (269 days after sowing)

Genus	Cultivar	Dev	DMD (%) mean	sem	M/D (MJ/kg) mean	sem	Total N (%) mean	sem	Ash (%) mean	sem	NDF (% DM) mean	sem	Hemicellulose (% DM) mean	sem	ADF (% DM) mean	sem
Clover	Alsike Hytas alsike	1.0	73.4	0.8	10.9	0.1	4.3	0.03	13.3	0.4	34.1	0.5	12.9	0.1	21.2	0.4
	Caucasian Kuratas	1.0	74.6	1.0	11.1	0.1	4.2	0.18	13.1	0.5	32.4	0.6	12.3	0.3	20.1	0.3
	Strawberry Palestine	1.0	72.8	0.6	10.8	0.1	4.1	0.13	14.8	0.3	32.4	0.5	12.7	0.3	19.7	0.5
	Talish Permatas	1.0	72.7	1.1	10.8	0.2	4.8	0.13	15.4	1.1	35.5	1.1	14.3	0.9	21.1	0.2
	Whtite clover Quest	1.0	71.7	0.8	10.6	0.1	4.2	0.14	14.0	1.1	27.9	0.5	8.1	0.3	19.8	0.7
	Red clover Rubitas	1.0	68.6	0.3	10.1	0.0	4.4	0.05	12.6	0.1	33.3	2.5	13.7	2.2	19.6	0.5
	White clover Storm	1.0	75.6	2.0	11.3	0.3	4.2	0.26	14.8	2.1	30.4	1.2	11.4	0.3	18.9	1.1
	Red clover Tuscan	1.0	68.4	0.1	10.1	0.0	4.4	0.10	14.3	0.3	38.6	0.2	17.4	0.5	21.2	0.3
Lotus	Lotus Lottas	1.0	67.2	0.3	11.0	0.1	4.3	0.06	12.6	0.5	34.1	1.1	14.1	0.5	20.0	0.5
	Lotus Maku	1.0	57.5	1.0	8.2	0.1	4.3	0.29	13.4	0.5	26.5	1.6	7.8	1.2	18.7	0.5
	Lotus Goldie	1.0	65.4	0.2	6.2	0.0	3.3	0.01	10.5	0.0	39.5	1.6	11.3	1.3	28.2	0.4
	Lotus LosBanos	1.0	74.1	1.0	8.1	0.1	3.3	0.11	10.9	0.4	33.8	0.9	11.5	0.4	22.3	0.7
	Lotus SA45718	1.0	64.9	1.8	9.9	0.0	3.5	0.09	13.4	0.9	30.6	1.3	9.7	0.4	20.9	0.9
	Lotus SA45714	1.0	55.1	0.8	8.2	0.1	3.4	0.14	14.0	0.1	33.1	1.5	10.7	0.4	22.4	1.2
Lucerne	Lucerne Aurora	1.0	70.7	0.5	9.5	0.0	3.5	0.07	10.3	0.3	27.4	3.1	10.2	2.4	17.2	0.7
	Lucerne K202	1.0	69.4	1.0	11.0	0.1	3.9	0.08	11.9	1.5	29.2	1.0	11.1	0.2	18.0	0.8
	Lucerne SARDI 7S2	1.0	65.8	0.5	9.5	0.3	3.9	0.15	13.5	0.3	29.6	1.1	9.6	0.3	20.0	1.2
	Lucerne WL925HQ	1.0	69.9	1.0	7.8	0.1	3.7	0.02	13.2	0.7	36.4	0.7	11.7	0.3	24.7	0.4
Sulla	Sulla Aokau	1.0	66.7	0.9	10.5	0.1	4.3	0.11	12.2	0.3	35.3	1.1	12.3	0.6	22.9	0.5
	Sulla Moonbi	1.0	67.9	0.4	10.2	0.1	4.2	0.09	12.6	0.2	37.1	1.7	12.7	0.3	24.4	1.4
	Sulla Wilpena	1.0	69.9	0.3	9.6	0.0	4.6	0.12	12.0	0.0	35.2	0.8	9.8	0.4	25.4	0.7
Melilotus	Melilotus Norgold	1.0	72.8	2.3	10.3	0.1	3.9	0.06	12.6	0.3	36.3	1.5	12.6	0.4	23.7	1.1
Sainfoin	Sainfoin Othello	1.0	67.0	1.6	10.8	0.4	4.8	0.09	15.6	1.7	31.6	2.6	12.5	0.8	19.1	1.7
	Sainfoin Shoshone	1.0	62.5	1.0	9.8	0.2	3.7	0.03	12.4	1.4	29.7	1.4	10.1	0.7	19.5	0.8
Cullen	Cullen SA4966	1.0	73.8	0.6	9.0	0.1	3.9	0.23	11.4	0.7	31.2	1.0	9.2	0.2	22.0	1.2
Dorycniu	Dorycnium rectum	1.0	57.6	0.7	9.8	0.1	4.3	0.06	17.2	0.7	32.1	1.0	11.9	0.4	20.2	0.6
	Dorynicum Canaritas	1.0	45.8	0.1	10.0	0.0	4.2	0.14	16.3	0.2	31.2	0.7	11.4	0.5	19.7	0.4
Kenedia	Kenedia SA41710	1.0	57.2	0.9	10.3	0.0	3.9	0.04	16.1	0.8	23.9	1.1	6.2	0.2	17.6	0.9
Tedera	Tedera	1.0	73.0	0.7	10.8	0.1	3.8	0.18	11.6	0.4	32.2	0.1	11.6	0.5	20.6	0.3

Genus	Cultivar	Dev	**DMD (%)** mean	sem	M/D (MJ/kg) mean	sem	Total N (%) mean	sem	Ash (%) mean	sem	NDF (% DM) mean	sem	Hemicellulose (% DM) mean	sem	ADF (% DM) mean	sem
Vetch	SA 38091	1.0	75.7	0.4	11.3	0.0	4.5	0.07	14.3	0.2	33.5	0.7	13.2	0.5	20.3	0.3
	Grand mean	1.0	67.6		9.9		4.1		13.3		32.5		11.5		21.0	
	LSD		2.9		0.50		0.36		2.3		3.9		2.4		2.4	
	P value		***		***		***		***		***		***		***	

Table 5c. Nutritive traits of the perennial herbs with 60 days regrowth, sampled on 7 May 2013 (269 days after sowing)

Cultivar	Dev	DMD (%)		M/D (MJ/kg)		Total N (%)		Ash (%)		NDF (% DM)		Hemicellulose (% DM)		ADF (% DM)	
	Mean	Mean	sem	Mean	sem	Mean	sem	Mean	sem	Mean	sem	Mean	sem	Mean	sem
Choice	1	75.31	0.996	11.25	0.171	3.06	0.2466	19.54	0.507	27.84	1.6	9.86	0.9	17.8	0.7
Commander	1	75.43	1.005	11.27	0.173	2.95	0.2483	16.55	0.507	29.31	1.6	10.74	0.9	18.8	0.8
Puna	1	75.65	0.998	11.31	0.172	3.13	0.2478	16.35	0.5102	30.83	1.6	11.78	0.9	19	0.8
Lancelot Pla.	1	71.14	1.0	10.53	0.172	2.98	0.2469	15.08	0.507	38.12	1.6	16.49	0.9	21.6	0.7
Tonic Plantain	1	72.64	1.0	10.79	0.172	2.89	0.2453	20.05	0.5043	38.45	1.6	15.56	0.9	22.8	0.7
Grand Mean	1	74.03		11.03		3.00		17.51		32.91		12.89		20.00	
LSD		2.63		0.45		0.64		1.371		4.0147		2.3503		1.921	
P value		***		***		***		***		***		***		***	

White clover cv. Quest had low levels of hemicelluloses (5.7-9.5%) compared to mean of legumes (9-11%), and lower than cv. Storm at cut 3 (6.5% compared to 11.4%), cut 4 (8.1% compared to 11.1%) and cut 5 (8.1% compared to 11.4%). The highest levels of hemicellulose were found in tallish clover cv. permitas and red clover cv. Tuscan (supplementary file, Table 9f).

Biomass production and feed value in second year under rainfed conditions

Lucerne had the highest feed value in the second year of the study, producing 18628−20922 kg/ha and a total of 201 GJ ME/ha. The next highest forage legume was Hedysarum coronarium, with cv. Wilpena producing 14957 kg/ha, and a total of 144 MJ ME/ha. Lucerne was also the only forage legume to produce significant quantities of forage over summer, with SARDI 7 Series 2 producing 11745 kg/ha compared to < 1500 kg/ha in all other entries.

The hay cutting treatment (with a longer cutting interval, see Fig. 1) resulted in greater forage production in sulla, with cv. Wilpena producing 11968 kg/ha produced between 10/6/2013 and 23/10/2013, contributing to a total production of 14957 kg/ha compared with 8417 kg/ha with the management that included an additional cut on 27/08/2013. The forage quality of sulla cv. Wilpena was still quite high in the hay cut (64% DMD, 8 October 2013, supplementary file table 11b) despite the formation of green pod (development stage 6). Total production under the hay cutting management was also higher (approximately double) in Dorycnium rectum (11171 kg/ha) and alsike clover (6994 kg/ha). The nutritive value of Dorynicum rectum SA1231 was rarely above 55% DMD or 8 M/D required to maintain liveweight of a dry sheep (supplementary file, table 11b).

Chicory was the highest yielding entry in the second year of the grasses, legumes and herbs experiment under frequent cutting, with 13053 kg/ha in cv. Puna, 15803 kg/ha in cv. Choice and 17410 kg/ha in cv. Commander (Table 6a). Puna had lower development scores with fewer plants becoming reproductive in comparison with cvv. Choice and Commander (Table 6a). Chicory cv. Choice produced a total of 180 GJME/ha, in comparison to perennial ryegrass cv. Abermagic and Bealey, which produced 55 GJME/ha and plantain cv. Tonic, which produced 77 MJME/ha (calculated from Table 6a and supplementary file Table 11a).

Phalaris (7752 kg/ha), prairie grass (7372 kg/ha), tall fescue (8260 kg/ha), tall wheat grass (A. elongatum), 8897 kg/ha) were the highest yielding grasses under frequent cutting. The hay cutting treatment, which measured forage production with a longer winter cutting interval, resulted in greater forage production in every entry, with the exception of chicory cv. Puna and Phleum pretense cv. SA38843, which had similar yields. Phalaris and tall fescue were the highest yielding grasses when the less frequent hay cut was applied with total forage production of 14386 kg/ha in cv. Holdfast GT, 14074 kg/ha in cv. Resolute and 13387 kg/ha in cv. Fraydo. Forage production in perennial veldt grass cv. Mission was 11182 kg/ha with the hay treatment, which was more than double the 5353 kg/ha measured under frequent winter cutting.

Chicory was the only perennial grass or herb to produce significant forage in late spring and summer, with 44% or 7702 kg/ha produced in cv. Commander between 24/10/2013 and 22/01/2014. The next highest late spring and summer production was in tall wheat grass cv. Dundas, with 1889 kg/ha (or 23% of its total yield), and no other entry produced more than 750 kg/ha of late spring and summer biomass.

2.4 Discussion

This is the first time that the temporal distribution of feed value of a diverse range of grasses and legumes has been evaluated in Australia. This comprehensive evaluation of 120 species represented by 150 cultivars allows for the first time a detailed comparison of nutritive value of Australia's feedbase targeted at southern grazing systems. In this work we measured the temporal distribution of DMD (used to estimate ME), total nitrogen (used to estimate crude protein), ADF, NDF, hemicelluse and ash content on cumulative growth every three weeks to identify relationships between nutritive value and plant growth development stage. There are clear differences between species and genotypes within species in the digestibility at different times and the patterns of digestibility change over time. This was related to the capacity of plants to maintain digestibility at different growth stages and to differences in the rate of development of advancing growth stages over time. The results provide a reference point for estimating forage quality of species at a specific development stage under low moisture stress conditions (supplemented by irrigation).

Lucerne and chicory had excellent performance in this study, with the combination of high yield and quality delivering feed value that was superior to all other entries. Lucerne cv. SARDI 7 Series 2 produced 201 GJ of ME/ha, and chicory cv. Choice produced 180 GJ ME/ha under rainfed condition with sulla, the next highest legume, producing 144 GJ ME/ha (Table 7). The dry matter production advantages of lucerne and chicory over other forages has been demonstrated previously in comparative forage evaluations that have been done in medium to high rainfall environments by Dear *et al.* (2008), Li *et al.*(2008), Hayes *et al.* (2010) and Boschma *et al.* (2011).

Table 6a. Plant development (Dev) and forage yield (dry weight, DW) of perennial grasses and herbs under rainfed conditions at the Waite Institute (year 2)

var	Common name	Cut 9, 7/05/2013		Cut 10, 20/06/2013		Cut 11, 28/08/2013		Cut 12, 24/10/2013		Cut 12 Hay 24/10/2012		Cut 13, 17/12/2013			Cut 14, 22/01/2014				Cut 15, 27/02/2014			Total Frequent Yield	Total Hay Yield
		Dev	DW kg/ha	**Dev**	DW kg/ha	**Dev**	DW kg/ha	**Dev**	DW kg/ha	**Dev**	DW kg/ha	**Dev**	log DW	DW kg/ha	**Dev**	log DW	DW kg/ha	Burnt	**Dev**	log DW	DW kg/ha	kg/ha	kg/ha
Choice	chicory	1	2216	1	1314	1.109	625	2.0	5296	1.0	7343	2.0	8.41	4474	1.6	6.77	874	1	1.2	5.92	372	15803	16554
Commander	chicory	1	1734	0.986	1272	1.064	620	2.0	7027	1.0	7319	2.0	8.75	6336	1.5	6.64	766	1	1.2	6.33	559	17410	16469
Puna	chicory	1	2317	1.0	778	0.965	209	1.4	4421	1.0	4132	1.4	7.98	2928	1.2	6.95	1045	1	1.2	6.92	1016	13053	11506
Currie	cocksfoot	1	585	1.012	832	3.547	421	1.0	2907	3.0	5803	1.0	5.74	310	1.0	3.40	30	1	1.0	5.53	252	5416	7650
Howlong	cocksfoot	1	596	1.0	968	0.982	456	1.6	3492	1.0	5651	1.6	6.25	516	1.0	4.03	56	1	1.0	4.95	142	6522	7848
Megatas	cocksfoot	1	484	1.0	1021	0.927	172	1.7	2582	0.9	5933	1.7	6.13	460	1.0	3.48	32	1	1.1	3.98	54	5159	7916
Exceltas	coloured brome	1	460	2.197	689	2.702	96	1.4	2502	1.0	3837	1.4	6.25	519	1.0	3.65	38	1	1.0	1.19	3	4276	5423
Gala	grazing brome	1	573	1.0	1023	6.169	298	1.2	3096	5.0	6925	1.2	5.81	335	1.0	3.96	52	1	1.3	4.28	72	5471	8971
Nandu	grazing brome	1	780	0.9	1551	6.437	619	1.7	4768	5.0	8980	1.7	6.04	422	7.8	5.08	160	1	2.5	4.84	127	8507	11634
Uplands	Mediterranean cocksfoot	1	579	0.9	915	3.741	181	1.0	2956	2.7	6352	1.0	5.60	270	1.0	2.90	18	1	1.1	5.59	267	5092	8140
AberMagic HSG	perennial ryegrass	1	911	1.004	672	1.022	222	1.1	1908	1.0	3360	1.1	5.73	307	1.0	3.15	23	1	1.0	0.54	2	4172	5131
Banquet II	perennial ryegrass	1	806	1.075	790	1.048	521	1.3	2322	1.0	4128	1.3	6.20	493	1.0	4.09	60	1	1.0	5.15	172	5333	6485
Bealey	perennial ryegrass	1	856	1.007	879	1.025	583	1.4	2359	1.1	3830	1.4	6.09	441	1.0	3.86	48	1	0.9	4.73	113	5460	6252
Drylander	perennial ryegrass	1	632	1.008	923	1.073	150	1.1	2260	3.6	3742	1.1	5.71	303	1.0	3.05	21	1	0.9	5.07	159	4255	5638
Victorian	perennial ryegrass	1	564	1.1	634	1.033	116	1.0	2108	3.8	3047	1.0	5.15	172	1.0	2.81	17	5	1.1	4.26	71	3666	4554
Mission	perennial veldt grass	1	793	0.9	1216	5.911	95	4.8	2844	6.1	8659	4.8	5.96	386	1.0	4.09	60	1	1.0	4.13	62	5353	11182
Advanced AT	phalaris	1	1089	1.009	1875	1.992	663	1.8	3676	3.1	8609	1.8	5.66	286	4.5	4.12	62	1	1.1	4.64	103	7752	11773
Australian 2	phalaris	1	748	0.935	973	1.818	363	2.0	3464	2.8	5235	2.0	5.95	385	4.7	4.09	60	1	1.0	4.51	91	6198	7550
Holdfast GT	phalaris	1	859	1.0	1798	2.068	931	1.9	4026	3.4	11248	1.9	5.76	318	4.7	4.33	76	1	1.1	4.85	127	8142	14386
Lancelot	plantain	1	570	1.0	144	4.019	193	6.0	3108	4.0	4161	6.0	6.30	546	5.0	4.64	104	1	1.3	2.45	12	4621	5823
Tonic	plantain	1	1271	1.0	746	3.978	942	6.1	2715	4.0	5091	6.1	6.10	444	4.9	4.72	112	5	1.0	4.66	106	6330	7732
Matua	prarie grass	1	905	1.0	1368	6.313	486	1.2	3844	5.0	7873	1.2	6.32	554	6.9	5.03	152	1	2.3	4.93	139	7372	10895
Menemen	puccinellia	1	427	2.1	761	6.381	145	1.1	1331	5.0	4209	1.1	5.68	293	1.0	2.39	11	5	1.0	0.24	1	2759	5880
Trangie	ringed wallaby grass	1	339	1.9	488	7.048	245	7.3	3884	6.0	6689	7.3	5.52	250	8.0	3.97	53	1	1.6	4.40	82	5034	7540
Fraydo	tall fescue	1	1206	1.0	1859	1.384	1131	1.0	2862	5.0	9792	1.0	5.73	309	1.0	3.95	52	1	0.9	5.25	190	7675	13387
Quantum II	tall fescue	1	1145	1.0	1182	5.53	864	1.1	4386	5.0	6575	1.1	6.28	536	1.0	4.62	102	1	1.0	6.05	424	8260	9638
Resolute	tall fescue	1	1126	1.0	1303	1.293	982	1.0	3318	5.0	11153	1.0	5.75	314	1.0	3.42	31	1	1.0	5.50	244	7340	14074
Dundas	tall wheat grass	1	1009	0.984	1044	0.876	529	3.5	3976	1.1	7182	3.5	7.50	1803	2.0	4.45	86	1	1.0	5.21	183	8897	11272
SA 38843	timothy	1		1.1	197	0.97	36	1.0	183	1.0	0	1.0	3.53	34	1.0	1.78	6	5	1.0	0.15	1	518	173
Friend	wallaby grass	1		1.0	195	0.969	141	7.3	3076	1.0	3772	7.3	5.72	305	7.2	4.35	77	1	2.8	3.87	48	3682	4453
Fprob			<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001			<0.001	<0.001		<0.001	<0.001		0	<0.001	<0.001		<0.001	<0.001
sed			134	0.466	299	0.17	82.88	0.167	355.5	0.1	847.3	0.167	0.494		0.143	0.317		0	0.12	0.95		887	1112
5% lsd			255	0.885	568	0.323	157	0.317	675	0.2	1610	0.3173	0.938		0.271	0.603			0.23	1.81		1686	2113

Table 6b. Plant development (Dev) and forage yield (dry weight, DW) of perennial legumes under rainfed conditions at the Waite Institute (year 2)

Entry	Cut 9, 7/5/2013			Cut 10, 20/6/2013			Cut 11, 27/08/2013			Cut 12 23/10/2013		Cut 12 Hay 23/10/2013			Cut 13 17/12/2013			Cut 14, 22/01/2014				Cut 15,		27/02/2014	Frequent Cut	Hay Cut (kg/ha)
	Dev	ln DW	DW kg/ha	Dev	ln DW	DW kg/ha	Dev	ln DW	DW kg/ha	Dev	DW kg/ha	Dev	ln DW	DW kg/ha	Dev	ln DW	kg/ha	Dev	Burnt	ln DW	DW kg/ha	Dev	ln DW	DW kg/ha	DW kg/ha	DW kg/ha
Hytas	1	5.69	297	1	5.47	238	1	5.94	381	4.7	2848	5.0	8.9	7194	0.9	4.33	76	4.4	5	3.77	44	1.0	1.77	6	4083	6994
SA45714	1	5.77	319	4.2	1.96	7	1	0.05	1	2.4	0	2.5	4.3	72	4.0	3.90	49	5.6	5	3.30	27	1.1	0.10	1	562	956
Goldie	1	5.94	380	1	0.01	1	1	1.23	3	1.5	1336	1.0	7.3	1434	4.4	4.77	118	5.3	5	4.06	58	1.6	1.63	5	1971	2409
Lottas	1	5.75	314	1	-0.02	1	1	1.75	6	1.7	758	1.0	7.3	1544	4.7	6.10	447	4.9	1	5.00	148	1.6	2.36	11	1956	2958
SA45718	1	5.53	253	1	6.52	677	1	6.02	412	2.5	2700	1.6	8.2	3767	4.9	4.79	120	5.4	5	4.44	84	4.0	3.43	31	4522	5808
Kuratas	1	5.99	398	1	3.84	47	1	3.85	47	2.0	1163	2.6	8.0	3011	1.0	4.65	104	1.0	1	4.32	75	1.0	4.67	107	2328	4236
SA1231	1	6.27	530	1	6.37	582	1	5.70	298	1.0	2636	1.0	9.2	9672	1.0	6.41	607	1.0	1	5.07	159	1.0	3.41	30	4958	11171
Maku	1	5.80	329	1	1.40	4	1	5.09	163	1.7	1676	0.9	6.8	895	1.0	3.57	35	5.1	5	2.98	20	1.0	0.00	1	2373	1605
Canaritas	1	6.23	509	1	4.38	80	1	0.09	1	1.7	363	1.0	7.7	2292	1.5	5.10	164	1.2	1	4.97	145	1.0	1.44	4	1214	3278
Aurora	1	7.70	2217	1	7.23	1384	1	7.42	1661	2.1	3446	3.0	8.3	4048	2.1	8.41	4478	5.0	1	8.05	3131	4.3	7.33	1525	19993	18557
K202	1	7.67	2139	1	7.32	1512	1	7.62	2034	2.1	3792	3.0	8.6	5704	2.2	8.36	4268	5.0	1	7.59	1978	4.0	7.79	2409	18429	18254
S7S2	1	7.62	2028	1	7.34	1542	1	7.75	2310	2.1	3932	3.0	8.4	4286	2.3	8.53	5044	5.0	1	8.36	4256	4.3	7.80	2445	20922	20115
WL925HQ	1	7.59	1978	1	7.03	1129	1	7.66	2120	2.1	3655	3.0	8.6	5335	2.3	8.28	3932	5.1	1	8.25	3839	4.2	7.51	1817	18628	17887
SA38091	1	6.37	582	1	5.25	191	1	0.07	1	1.0	104	1.0	1.8	6	1.0	3.52	34	1.4	3	4.23	69	1.0	3.09	22	1136	1178
LosBanos	1	5.85	346	1	0.00	1	1	1.78	6	1.2	1869	1.0	8.1	3150	3.5	5.18	178	4.9	5	4.93	138	1.8	3.12	23	2719	4218
SA4966	1	7.04	1144	1	5.34	209	1	0.11	1	2.9	296	3.3	8.6	5636	2.6	4.27	72	3.0	1	5.12	167	1.8	2.50	12	2224	7357
Rubitas	1	5.91	367	1	5.95	385	1	6.44	629	2.4	4353	2.5	8.6	5394	1.5	4.96	142	4.8	5	4.81	122	1.0	0.01	1	6007	7481
Tuscan	1	5.87	355	1	5.55	258	1	5.33	207	1.6	3319	1.2	8.7	5985	3.4	5.89	360	5.6	5	5.34	208	1.2	0.84	2	4817	6841
SA41710	1	6.10	445	1	5.76	317	5	0.00	1	5.1	0	5.5	6.9	1030	1.0	5.24	189	1.0	1	5.16	175	1.0	1.40	4	1279	2289
Othello	1	6.23	505	1	5.82	337	1	3.44	31	3.1	3019	3.6	7.7	2130	5.0	6.90	995	5.1	1	5.62	275	1.7	4.55	94	5586	4525
Shoshone	1	6.33	562	1	5.02	151	1	0.00	1	2.9	1403	3.7	6.9	1004	5.0	6.59	731	4.9	1	4.93	139	1.6	4.27	71	3354	3239
Palestine	1	5.79	327	1	5.47	238	1	7.19	1325	1.0	2976	1.0	8.5	4949	2.0	4.97	143	5.8	5	4.26	71	4.8	0.01	1	4839	5871
Aokau	1	6.87	966	1	7.42	1674	1	6.43	618	3.8	5661	5.0	9.2	9897	1.3	3.47	32	1.0	1	3.66	39	1.0	0.00	1	8997	12609
Moonbi	1	6.98	1073	1	7.20	1337	1	6.03	417	4.0	4827	5.0	8.9	7187	1.4	3.49	33	0.9	1	3.55	35	1.0	0.08	1	7405	9665
Wilpena	1	7.31	1500	1	7.26	1424	1	6.44	628	3.9	4281	5.0	9.4	11968	1.5	3.52	34	1.1	1	3.42	31	1.0	0.00	1	8417	14957
Permatas	1	6.17	477	1	5.74	310	1.033	5.64	281	4.8	1396	5.0	7.7	2139	3.5	4.80	121	4.4	5	4.53	92	1.0	2.10	8	2782	3517
Tedera	1	6.08	437	1	1.55	5	1	0.09	1	0.9	-54	2.0	4.6	101	1.3	3.31	27	1.1	1	3.41	30	1.0	2.39	11	439	1406
Quest	1	6.08	437	1	6.39	595	1	7.19	1321	2.5	2220	2.9	8.5	4675	4.7	5.03	154	5.2	5	4.45	86	1.0	2.26	10	5036	6248
Storm	1	6.24	511	1	6.66	779	1	7.88	2649	2.9	1958	2.8	8.5	4779	5.3	5.26	193	5.9	5	4.92	137	1.0	1.41	4	5202	6691
Norgold	1	5.88	359	1	0.01	1	1	-0.63	1	1.5	2851	1.0	8.2	3771	1.9	6.38	589	2.2	1	5.07	159	1.9	5.85	346	4227	5092
F prob	0	<.001		<.001	<.001		<.001	<.001		<.001	<.001	<.001	<.001		<.001	<.001		<.001	<.001	<.001		<.001	<.001	<.001	<.001	<.001
LSD (5%)	0	0.35		0.08	1.76		0.02	1.55		0.3	712	0.3	0.3		0.4	1.03		0.4	0.65	0.94		0.24	2.27	0.00	1300	2770

Table 7. Metabolisable Energy (MJ/ha) and Crude Protein production (g/ha) of perennial legumes with hay cutting treatment (lsd refers to natural log (ln) of means only)

ME (MJ/ha)	7-May-13		20-Jun-13		27-Aug-13		8-Oct-13		17-Dec-13		22-Jan-14		27-Feb-14		Total
var	ln Mean	Mean	ln Mean	Mean	ln Mean	Mean	ln Mean	Mean	ln Mean	Mean	ln Mean	Mean	ln Mean	Mean	Mean
Canaritas	2.82	17	6.54	693	0	1	9.77	17413	0	1	0	1	4.32	75	18201
Norgold	8.03	3075	0.00	1	0	1	10.24	28029	8.33	4151	7.06	1162	7.75	2324	38743
Rubitas	8.23	3756	8.32	4097	8.85	6960	11.07	64473	0	1	0	1	0	1	79289
S 45718	2.63	14	8.77	6412	8.43	4592	10.52	37049	0	1	0	1	0	1	48070
SARDI 7 S2	9.91	20050	9.65	15460	9.86	19072	10.74	45982	10.76	46958	10.46	34752	9.89	19712	201986
SA1231	8.59	5388	8.66	5745	7.95	2833	11.33	83200	8.18	3572	0	1	6.62	747	101486
Shoshone	5.66	288	7.12	1233	0.06	1	9.23	10209	8.97	7895	7.39	1626	6.52	677	21929
Wilpena	9.68	16059	9.62	15123	8.94	7639	11.57	105873	0	1	0	1	0	1	144697
5% lsd							2.23								
CP (g/ha)	ln Mean	Mean	ln Mean	Mean	ln Mean	Mean	ln Mean	Mean	ln Mean	Mean	ln Mean	Mean	ln Mean	Mean	Mean
Canaritas	3.08	22	7.09	1204	0	1	10.24	27917	0	1	0	1	5.29	197	29343
Norgold	9.29	10829	0.00	1	0	1	11.44	92689	9.56	14228	8.1	3294	9.05	8544	129586
Rubitas	9.20	9897	9.26	10509	9.8	18398	11.71	121783	0	1	0	1	0	1	160590
S 45718	2.97	19	9.76	17379	9.4	12582	11.54	102744	0	1	0	1	0	1	132727
SARDI 7 S2	11.06	63577	10.75	46770	7.2	1366	11.67	116658	11.57	105345	11.23	75132	10.9	52052	460900
SA1231	9.58	14472	9.64	15367	8.8	6355	12.07	174033	8.76	6374	0	1	7.5	1808	218410
Shoshone	6.27	528	0.00	1	0	1	10.02	22471	9.62	15108	8.04	3093	7.4	1631	42833
Wilpena	10.51	36571	6.93	1019	9.76	17275	12.35	230960	0	1	0	1	0	1	285828
5% lsd							3.86								

Lucerne and chicory were the only entries to produce significant quantities of late spring and summer forage production (i.e. total of > 1500 kg/ha, Table 6a,b), extending the growing season of the pasture into summer by utilising stored soil moisture. The climate change predictions for southern Australia suggest the proportion of rainfall that occurs during summer will increase (Moore and Ghahamani 2013). In Australia, deep rooted summer active plants such as lucerne that can use subsoil moisture and respond quickly to summer rainfall are predicted to have the greatest effect in recovering from the negative impact of climate change on profitability and production (Ghahramani & Moore 2013). The high feed value of this production also presents opportunities to improve livestock production efficiency, and reduce methane emissions intensity. The feed value of lucerne and chicory was high enough to maintain livestock growth rates over summer, and reduce the time it takes for them to reach target live weights and carcass parameters for slaughter. This would reduce the amount of methane produced per kg of meat, but may be used to increase stocking rates and consequently result in higher methane production per hectare (Alcock and Hegarty, 2011). Alternatively, the forage may also be used to reduce the joining age of maiden ewes, and flush ewes at ovulation to increase lambing percentages, and these are both examples of where there is potential to reduce emissions intensity due to a decoupling of the relationship between production and emissions (Harrison *et al.,* 2014b). Increasing the number of lambs per ewe from 1.0 to 1.5 while holding stocking rates constant can reduce emissions intensity by 1.8 kg CO2-e/kg CFW+LWT and emissions per animal sold by 163 kg CO2-e/head (Harrison *et al.,* 2014b).

The seasonal timing of forage production has important implications for feed budgeting and system design. The capacity to have reliable on-farm summer production and grow-out weaners allows farmers to increase spring lambing and calving, which shifts late pregnancy and lactation to spring when high energy requirements are more easily met. Alternatively, species that have higher winter production and may be best placed to meet the demands of a traditional autumn lambing, include white clover cv. Storm (produced 39 kg/ha/day or 2649 kg/ha between 20 June and 28 August 2013), lucerne (1661−2310 kg/ha) and strawberry clover (1325 kg/ha). Perennial grasses had lower winter growth rates than expected, with Mediterranean tall fescue cvv. Fraydo (17 kg/ha/day or 1131 kg/ha between 20 June and 28 August 2013) and Resolute (982 kg/ha), plantain cv. Tonic (942 kg/ha), and phalaris cv. Holdfast GT (931 kg/ha) providing the highest production.

There were also plants, such as sulla, that have been identified as having the capacity to greatly improve hay or silage yields from spring forage production, which is likely to be an important component of managing seasons with increased climate variability. Sulla cv. Wilpena produced 11968 kg/ha in the hay treatment between 10 June 2013 and 8 October 2013 (in comparison to total of 4909 kg/ha in two cuts on 27 August 2013 and 23 October 2013 over a longer period). The capacity to harvest and conserve fodder during periods of high pasture production is much more cost effective than purchasing fodder or grain, particularly in seasons with low rainfall, and may result in the capacity to maintain higher livestock numbers during periods of drought. The large winter to spring forage yield from sulla in this experiment was not surprising, with yields over 20 t/ha being recorded previously in lower rainfall environments such as Booberowie in South Australia and Mooree in NSW (de Koning *et al.*, 2003). The result by de Koning *et al.* (2003), in relatively low rainfall environments, further demonstrates the potential of sulla to be a valuable fodder conservation option for medium to high rainfall environments in future years where the effects of climate change may be more critical.

Despite the high feed value potential of sulla, it is underutilised in Australia. The main reasons are attributable to high establishment costs (approximately $100 /ha sown at 5 kg/ha), the short 2−3 year lifespan of the plant, a poor understanding of its agronomy including management for recruitment and grazing, and a lack of registered herbicide options (despite good tolerance to glyphosate and metrabuzin). The poor winter yield under frequent cutting may have been associated with the interaction between cutting frequency and height for this species. Sulla developed a narrow, high growing point that was removed in the winter cut on 20 June 2013, and thus potentially needs to be cut more frequently to develop a low and broad crown, or less frequently to target winter production.

The cutting height used in this study may also be partly responsible for the low perennial grass yields, with cutting height potentially too high to maximise utilisation of these species. Other factors that are likely to be important include the timing and rate of nitrogen application, temporal changes in the availability of nitrogen, and the capacity of these plants to access soil nitrogen, in comparison to chicory, which had much higher forage yields. Crude protein was low (11.4%) in perennial ryegrass compared to chicory (20−23%, calculated from total nitrogen in supplementary file (Table 11a) on 28

August 2013, but equal or higher than chicory at other times in 2013/14 (average for rest of year 18% in chicory and 21% in perennial ryegrass).

The rankings for forage production in chicory and perennial grasses in this study were similar to rankings produced at Keith, SA, as part of a large multi-site evaluation of perennial grasses in southern Australia (Reed *et al.,* 2008), but in contrast to six other sites in that study where perennial ryegrass produced the highest yield (average 15.1 t/ha across 7 sites in southern Australia) and the performance of chicory was moderate. Keith is the closest of these sites to the Waite Institute in terms of geography and climate, and despite having a lighter soil texture, is most likely to have similar performance.

An alternative strategy to deal with increasing climate variability and its impact on feed availability in summer and autumn is to provide *in-situ* conservation of highly digestible forage produced from growth earlier in the season. Tedera is a new legume being developed with the aim of achieving *in-situ* conservation (Real *et al.,* 2014), and whilst this study confirmed its capacity to produce and maintain highly digestible forage over time under irrigation (Fig. 4b), yield (Table 6b) and persistence of tedera (Table 3a) under rainfed conditions in the second year were too low for this accession of tedera to be considered successful in this environment (439 kg/ha with frequent winter cutting and 1406 kg/ha with hay cut). Improving this species, and other perennial legumes, tolerance to abiotic stress is a key goal to improving resilience through drought and long term persistence in agricultural environments.

The perennial legumes had, on average, low ground cover at the end of the experiment. Only lucerne and Kennedia maintained > 70% ground cover at the end of the experiment. In contrast, the perennial grasses maintained very high levels of ground cover, with phalaris, cocksfoot, ryegrass, tall wheat grass, and tall fescue all having cultivars with around 90% ground cover at the end of the experiment, and brome, veldt and wallaby grass maintaining over 80% ground cover. The rankings of persistence for species and cultivars in this experiment are similar to that reported for grasses by Reed *et al.* (2008) and legumes by Li *et al.* (2008). The high ground cover of perennial ryegrass (74-89%), is also indicative of a low to moderate drought tolerance in the absence of grazing.

The native species in this experiment typically had lower yields and low nutritive value in comparison to introduced, domesticated and genetically improved grasses and legumes. An exception to the low nutrient value is in Timothy grass, which maintained digestibility above 65% as an immature plant in the first summer (Fig. 4a), and had 71% digestibility as a mature vegetative plant on 7 May 2013. Forage yield of Timothy grass cv. SA38843 however, was very low (total production 518 kg/ha in year 2). Wallaby and ringed wallaby grasses had lower digestibility in the reproductive growth stages (Fig. 4a), but moderate to high digestibility at the vegetative growth stage (64.5−67%, Table 5a) and yield (4453 and 7540 kg/ha respectively). The low digestibility in the reproductive phase is a potential adaptation to anti-herbivory to improve the success of seed production. The high feed value (yield and digestibility) of perennial veldt grass, a species that is naturalised on sandy soils in southern Australia, was a surprising result. Perennial veldt grass cv. Mission was one of the top performing perennial grasses, with 11182 kg/ha of production in the hay treatment (Fig. 5a), and relatively high digestibility at vegetative (73.5%) and reproductive growth stages (>60% DMD between 22 November 2012 and 20 February 2013).

2.5 Conclusion

We have demonstrated considerable variation for nutritive value and forage yield in temperate perennial legumes, grasses and herbs. Furthermore, we identify species such as lucerne and chicory that extend the growing season in a medium rainfall environment by using stored soil moisture to produce forage with high feed value, providing a cost effective option for growing out livestock and improving fertility in maternal stock. On-farm fodder conservation may also be used to improve the resilience of the farming system and reduce exposure to drought, and the underutilised species ‘sulla’ appears to be an excellent option for this strategy. The results of this study confirm that manipulating diet with high energy forage is a practical, and has the potential to be a cost effective solution to improve the efficiency of livestock production in these regions and reduce emissions intensity.

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SECTION 3: *In vitro* analysis of methanogenic traits

3.1 Abstract

We have used an *in vitro* batch fermentation system to examine the antimethanogenic properties of forty five forage annual and perennial grass and legume species, including 31 species of annuals and 14 species of perennials. All species were grown in South Australia and sampled multiple times in year 1 in vegetative and reproductive stage. A subset of species that had good agronomic, nutritional and/or bioactive properties were selected based on the year 1 results and were planted at two sites (SA and WA) and included species that do not inhibit methane directly, but had the potential to promote VFA production when fermented by rumen microbes. We sampledthis subset in the vegetative and reproductive stages of growth across two sites in two phenology stages and over two yearsto examine the variation in *in vitro*fementability traits due to site and year-to-year variation. We also used the *in vitro* batch system toscreen chicory samples to examine intraspecies variability, focussing on the most antimethanogenic accessions and the effects of different sampling times as well as the variation between those accessions at sampling time where the lowest antimethanogenic effect was observed. The top antimethanogenic plant *B. pelecinus* was examined further in an *in vitro* continuous fermentation system (RUSITEC) to confirm its effect over time and to help select doses/treatments for *in vivo* testing. There was variation in fermentation characteristics amongst the species and in relation to stage of growth. Of the annuals, *B. pelecinus*consistently produced significantly lower amounts of methane when fermented than any of the other species tested regardless of stage of growth, site and year.This antimethanogenic effect of *B. pelecinus* persisted when tested in the RUSITEC with different proportions of sub clover as substrate and did not affect DMD or VFA production at different levels of inclusion. The most effective mix with subclover wasa 50:50 ratio, where there was a significant reduction (50%) in methane production compared to subclover alone.Methane production on average tended to be lower during the vegetative stage than the reproductive stage but this was mainly seen in year 1 when plants were irrigated.Of the perennial species *D. hirsutum*, *D. glomerata* and *T. pratense*consistently produced less methane than the mean of other perennial species tested. There was a strong correlation between methane and VFA production in the vegetative stage of growth that was not apparent in samples taken during the reproductive stage of growth. Ranking according to methanogenic potential varied across stages and between years however, *D. hirstum/rectum* consistently ranked number 1*and L. corniculatus* and *T. pratense* were always amongst the top 5 plants, while *T. repens* and *M. sativa* were always amongst the five highest methanogenic plants. There was no clear pattern in ranking across years and stages of growth.We found that small but significant variability exists between the accessions and cultivars within chicory. The variance between the highest and the lowest entries was about 10% and these differences were not higher when these were tested at the cut where the most changes in methane were expected. Some accessions retained their low methanogenic potential across sampling times. The most widespread commercial accession, Choice, always ranked amongst the highest in terms of methanogenic potential.

3.2 Methods

Testing *in vitro* fermentability and methanogenic potential of plant samples

Experimental design

Forty five forage species including 31 species of annuals and 14 species of perennials were grown in South Australia and collected in year 1 in vegetative and reproductive stage (SA YR 1). We then used a set of criteria to select the subset of species that had good agronomic, nutritional and/or bioactive properties and these were planted at two sites (SA and WA) and expanded the concept of 'antimethanogenic bioactivity' to include species that do not inhibit methane directly, but may promote VFA production when fermented by rumen microbes. We investigated annuals across two sites (G x E), as well as in two phenology stages and over two years.Ten annual priority species and all perennial species were replicated in SA in YR 2 to examine year-to-year variation (SA YR 2). In addition, the 10 annual priority species were grown in WA in two years and sampled in the vegetative and reproductive stages of growth to examine the variation in fementability traits due to site and year-to-year variation (WA YR 1 and WA YR 2).Finally, various chicory samples were collected to examine

intraspecies variability, focussing on the most antimethanogenic accessionsand the effects of different sampling times as well as the variation between those accessions at sampling time where the lowest antimethanogenic effect was observed. All plants were processed and tested using a batch *in vitro* fermentation assay (Durmic *et al.,* 2010).

The top antimethanogenic plant *B. pelecinus* was examined further in an*in vitro* continuous fermentation system (RUSITEC) to confirm the effect over time and in an open system, and to select doses/treatments for *in vivo* testing. Plant material was collected from biserrula during the reproductive stage of growth in 2013(SARDI) and from plots at UWA Shenton Park Research Station. Material was tested following the protocol of Li *et al.*(2013).

Partial Replicated Design was used to account for possible measurement errors, laboratory variations and variations in the field replicate samples. Data were analysed using ASReml model in the R statistical programme (Ihaka and Gentleman 1996) to obtain predicted values of all parameters in different batches using the lab control (LUC R2) as a covariate.

2.4 Results

The results are divided in 7parts as follows:

1. *In vitro* fermentability and methanogenic potential of 31 annual species (SA, YR 1)
2. *In vitro* fermentability and methanogenic potential of a subset ofannual speciesgrown in SA (SA YR 2)
3. *In vitro* fermentability and methanogenic potential of a subset ofannual species grown in WA (WA YR 1& YR 2)
4. *In vitro* fermentability and methanogenic potential of perennial species (SA, YR1 & YR 2)
5. Within species variation in *in vitro* methanogenic potential in chicory
6. Rusitec testing of top antimethanogenic plant *B. pelecinus*
7. The effect of plant processing on methanogenic potential in *B. pelecinus*

Sections 1-4 report on the main *in vitro* screening of plants across sites and phenology. The materials and methods for these are presented in the general methods section 3.2 above and the results are discussed in the general discussion. Sections 5 - 7 (chicory, rusitec and ‘plant form’ studies) had different objectives, material and methods, and as such are presented as separate experiments (mini papers), but are also discussed in the overall context of the general discussion.

1. *In vitro* fermentability and methanogenic potential of 31 annual species (SA, YR 1)

Information on *in vitro* fermentation profiles of 31 annual forage species in the vegetative stage of growthis presented in Table 1.1. There was limited variation between the measured parameters, except for one plant sample, i.*e Biserrula (Astragalus) pelecinus* subsp. *pelecinus*cv Casbah. This plant produced five to six times less methane (i.e. 6 mL/g DMi) compared to other plants (mean 28 mL/g DMi). In thisplant, the gas production (61 kPa) and acetate : propionate (1.3) were also reduced compared to other plants (mean 109 kPa and 2.2), with smallreduction in VFA (112 vs 131 mmol/L) and NH3 (268 vs 310 mg/L). Methane in other plants ranged between 23 mL/g DMi (*Trifolium subterraneum subsp. subterraneum*) and 33 mL/g DMi (*Melilotus siculus*).

Table 1.1. Fermentation profiles (predicted mean) amongst 31 annual pasture species grown in SA and collected duringthe vegetative stage of growth in YR 1. DMi - dry matter incubated.

Botanical name	Cultivar/ accession	Gas (kPa)	CH_4 (mL/gDMi)	VFA (mmol/L)	A:P	NH_3 (mg/L)
Astragalus (Biserrula) pelecinus subsp. pelecinus	Casbah	61	6	112	1.3	268
Astragalus hamosus	Ioman	121	32	136	2.2	297
Avena sativa	Winteroo	119	30	134	2.1	209
Brassica napus	43Y85	115	29	133	2.2	298
Brassica rapa	New York	105	25	127	2.2	379
Brassica tournefortii	SA 42783	107	24	126	2.4	354
Hordeum vulgare	Moby	119	31	139	2.1	221
Lolium multiflorum	Dargo	117	27	134	2.0	230
Lolium rigidum	Wimmera	113	28	130	2.1	283
Medicago arabica	SA 8774	113	31	131	2.2	239
Medicago littoralis	Angel	106	29	132	2.4	377
Medicago polymorpha	Scimitar	109	29	136	2.3	321
Medicago scutellata	Essex	106	28	131	2.3	411
Medicago truncatula	Caliph	109	32	126	2.3	382
Melilotus elegans	SA 37228	106	29	133	2.1	346
Melilotus siculus	SA 40002	115	33	132	2.2	356
Ononis alopecuroides	SA 8577	104	27	126	2.3	299
Ornithopus compressus	Santorini	102	31	124	2.4	284
Ornithopus sativus	Cadiz	106	31	126	2.4	306
Trifolium glanduliferum	Prima	114	31	133	2.4	325
Trifolium michelianum	Frontier	105	29	133	2.2	372
Trifolium purpureum	Paratta	111	30	132	2.4	316
Trifolium spumosum	Bartolo	108	27	128	2.5	355
Trifolium subterraneum subsp. brachycalycinum	Antas	109	25	133	2.3	260
Trifolium subterraneum subsp. subterraneum	Urana	102	23	130	2.4	208
Trifolium subterraneum var. yanninicum	Trikkala	104	24	127	2.3	247
Trigonella balansae	SA 5045	109	32	134	2.1	373
Trigonella coelesyriaca	SA 19767	115	32	137	2.2	277
Trigonella foenum-graecum	Wimmera Sungold	114	31	138	2.2	369
Triticum aestivum	Wedgetail	115	27	130	2.1	311
Vicia sativa	Languedoc	112	31	133	2.3	324
Mean		109	28	131	2.2	310
SEM		2.7	1.5	2.5	0.03	9.8

There was also limited variation between the parameters measured in these plants when collected at the reproductive stageof growth (Table 1.2). *B. pelecinus* again produced the lowest amounts of methane (5 mL/g DMi) compared to other plants (mean 37 mL/g DMi). The gas production (72 kPa) and acetate to propionate ratio (A:P; 1.4) in this plant was also lower compared to others (mean 97 kPa and 2.5), however there was no difference in the VFA concentrations (124 vs 123 mmol/L) or NH_3 (182 vs 188 mg/L). In other plants, methane was the lowest in *T. subterraneum var. yanninicum* (32 mL/g DMi) and the highest in *Trifolium spumosum* (46 mL/g DMi).

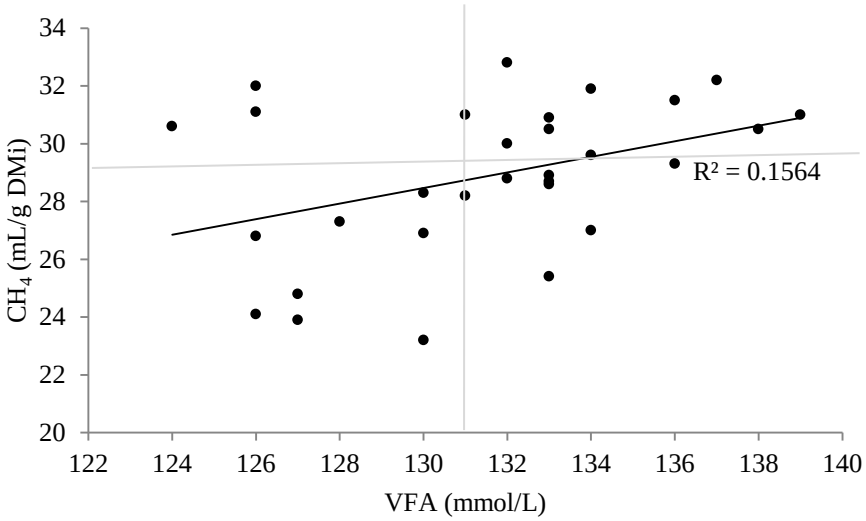
Table 1.2. Fermentation profiles (predicted mean)amongst 31 annual pasture species grown in SA and collected at the reproductive stage of growth in YR 1. DMi - dry matter incubated.

Botanical name	Cultivar/ accession	Gas (kPa)	CH_4 (mL/gDMi)	VFA (mmol/L)	A:P	NH_3 (mg/L)
Astragalus (Biserrula) pelecinus subsp. pelecinus	Casbah	72	5	124	1.4	182
Astragalus hamosus	Ioman	113	44	130	2.6	132
Avena sativa	Winteroo	88	34	115	2.4	89
Brassica napus	43Y85	89	34	121	2.8	308
Brassica rapa	New York	90	33	120	2.6	210
Brassica tournefortii	SA 42783	85	35	114	2.7	192
Hordeum vulgare	Moby	96	35	119	2.4	108
Lolium multiflorum	Dargo	90	37	117	2.4	121
Lolium rigidum	Wimmera	93	37	119	2.5	165
Medicago arabica	SA 8774	92	33	128	2.6	329
Medicago littoralis	Angel	94	40	120	2.5	215
Medicago polymorpha	Scimitar	96	39	127	2.6	293
Medicago scutellata	Essex	100	42	126	2.5	197
Medicago truncatula	Caliph	94	38	120	2.6	191
Melilotus elegans	SA 37228	95	37	118	2.5	167
Melilotus siculus	SA 40002	106	42	128	2.5	195
Ononis alopecuroides	SA 8577	98	41	124	2.7	175
Ornithopus compressus	Santorini	97	38	119	2.6	204
Ornithopus sativus	Cadiz	94	36	119	2.6	202
Trifolium glanduliferum	Prima	95	38	125	2.7	180
Trifolium michelianum	Frontier	101	41	128	2.5	164
Trifolium purpureum	Paratta	89	36	119	2.6	167
Trifolium spumosum	Bartolo	110	46	131	2.7	164
Trifolium subterraneum subsp. brachycalycinum	Antas	105	41	129	2.4	173
Trifolium subterraneum subsp. subterraneum	Urana	100	39	125	2.4	165
Trifolium subterraneum var. yanninicum	Trikkala	103	32	128	2.5	180
Trigonella balansae	SA 5045	96	40	124	2.4	238
Trigonella coelesyriaca	SA 19767	106	40	125	2.6	181
Trigonella foenum-graecum	Wimmera Sungold	104	44	124	2.5	194
Triticum aestivum	Wedgetail	98	39	127	2.4	128
Vicia sativa	Languedoc	104	42	126	2.8	230
Mean		97	37	123	2.5	188
SEM		1.4	1.2	0.8	0.04	9.3

There was no strong correlation between CH_4 and VFA production in any of the two growth stages tested (R^2 = 0.16 for vegetative, R^2 = 0.34 for reproductive stage, Fig. 1.1). Several plants in

vegetative stage and two in reproductive stage produced less methane and more VFA than the average values, but the plants were not the same across the stages.

a



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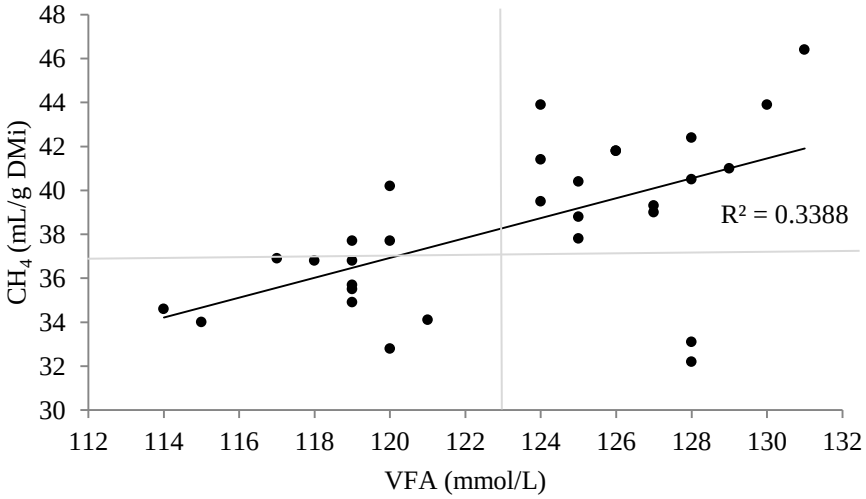


Fig. 1.1. Distribution of annuals in vegetative (a) and reproductive (b) stage according to their VFA and CH_4 production (excluding *Biserrula pelecinus*). DMi - dry matter incubated. Grey lines represent mean values for all plants.

In general, plants tendedto be more fermentable when in vegetative stage, as judged by higher averages for gas, VFA and NH_3 (109 kPa, 131 mmol/L and 310 mg/L) compared to reproductive stage (97 kPa, 123 mmol/L and 188 mg/L). However, on average, plants in the vegetative stage of growth produced less methane (average 28 mL/g DMi) than when they were in the reproductive stage of growth (37 mL/g DMi).

Selection of plants for further testing

Variability in the fermentation profiles was used toselect a subset of 15 species for testing at two different sites (SA and WA) in years 2 and 3. The selection criteria for choosingthe subsetof plants based on the *in vitro* results are listed in Table 1.3. Briefly, plants that reduced methane (high or moderate reduction), and/or promoted VFA, and/or promoted propionate, and/or reduced NH_3 were selected.

Table 1.3. Additional criteria from IVFT testing used for species inclusion for further testing

Species	Cultivar/ Accession	Criteria
Astragalus (Biserrula) pelecinus subsp. pelecinus	Casbah	Extremely low CH_4, VFA not reduced, propionate promoting
Avena sativa	Winteroo	Moderately low CH_4, promoting VFA, reduced NH3
Lolium multiflorum	Dargo	Moderately low CH_4, promoting VFA, reduced NH3
Brassica napus	43Y85	Moderately low CH_4, promoting VFA
Lolium rigidum	Wimmera	Moderately low CH_4, promoting VFA
Medicago arabica	SA 8774	Moderately low CH_4, promoting VFA
Medicago polymorpha	Scimitar	Moderately low CH_4, promoting VFA
Ornithopus compressus	Santorini	Moderately low CH_4, promoting VFA
Ornithopus sativus	Cadiz	Moderately low CH_4, promoting VFA
Trifolium spumosum	Bartolo	Moderately low CH_4, promoting VFA
Trifolium subterraneum subsp. subterraneum	Urana	Moderately low CH_4, promoting VFA
Trifolium subterraneum var. yanninicum	Trikkala	Moderately low CH_4, promoting VFA
Trigonella balansae	SA 5045	Moderately low CH_4, promoting VFA
Vicia sativa	Languedoc	Moderately low CH_4, promoting VFA
Hordeum vulgare	Moby	Reduced NH_3

2. *In vitro* fermentability and methanogenic potential of subset of annual species grown in SA (SA YR 2)

Results

Similar to the first year of the project,*B. pelecinus* again was the lowest methanogenic plant and produced 7-10 times less methane than any other plants tested (i.e. 4 and 6 mL/g DMi vs mean of 43 and 44 mL/g DMi, vegetative and reproductive stages, respectively, Table 2.1). It also had lower gas, and some small reduction in VFA and NH_3 values compared to the mean.

Table 2.1. Fermentation profiles (predicted mean) amongst subset of annual pasture species grown in SA and collected in vegetative and reproductive stage in YR 2 (SA YR 2). DMi - dry matter incubated

Botanical name	Cultivar/ Accession	Gas (kPa)	CH_4 (mL/g DMi)	VFA (mmol/L)	A:P	NH_3 (mg/L)
Vegetative stage						
Astralagus (Biserrula) pelecinus	Casbah	63	4	104	1.3	307
Avena sativa	Winteroo	121	50	116	2.1	176
Brassica rapa	New York	95	41	109	2.4	481
Lolium multiflorum	Dargo	104	43	116	2.3	450
Lolium rigidum	Wimmera Sungold	109	50	124	2.2	374

Botanical name	Cultivar/ Accession	Gas (kPa)	CH_4 (mL/g DMi)	VFA (mmol/L)	A:P	NH_3 (mg/L)
Medicago arabica	SA 8774	96	42	114	2.4	426
Medicago polymorpha	Scimitar	103	46	115	2.3	412
Melilotus siculus	SA 40002	110	50	125	2.1	454
Ornithopus compressus	Santorini	103	46	114	2.5	371
Ornithopus sativus	Cadiz	100	44	115	2.5	334
Trifolium michelianum	Frontier	104	46	119	2.2	437
Trifolium spumosum	Bartolo	105	49	115	2.6	386
Trifolium subterraneum subsp. brachycalycinum	Antas	103	42	111	2.4	287
Trifolium subterraneum subsp. subterraneum	Urana	104	47	117	2.6	319
Trigonella balansae	SA 5045	99	45	115	2.1	388
Vicia sativa	Languedoc	101	46	114	2.3	482
Mean		101	43	115	2.3	380
SEM		1.1	0.8	1.8	0.01	5.2
Reproductive stage						
Astralagus (Biserrula pelecinus)	Casbah	69	6	104	1.3	276
Avena sativa	Winteroo	102	45	102	2.2	142
Brassica rapa	New York	111	46	114	2.2	256
Lolium multiflorum	Dargo	111	44	111	2	130
Lolium rigidum	Wimmera Sungold	109	50	115	2.2	368
Medicago arabica	SA 8774	100	43	111	2.4	354
Medicago polymorpha	Scimitar	92	42	111	2.4	362
Melilotus siculus	SA 40002	111	50	116	2.3	295
Ornithopus compressus	Santorini	105	45	112	2.4	294
Ornithopus sativus	Cadiz	106	46	108	2.3	286
Trifolium michelianum	Frontier	110	48	113	2.4	261
Trifolium spumosum	Bartolo	114	51	115	2.5	316
Trifolium subterraneum subsp. brachycalycinum	Antas	115	49	116	2.2	240
Trifolium subterraneum subsp. subterraneum	Urana	107	47	115	2.3	298
Trigonella balansae	SA 5045	104	47	110	2.1	339
Vicia sativa	Languedoc	104	50	109	2.4	396
Mean		104	44	111	2.2	288
SEM		2.1	0.8	0.8	0.01	5.4

In terms of ranking according to methanogenic potential, there was no strong consistency across the stages of growth or between the years, except for *B. pelecinus* (consistently ranked 1, Fig. 2.2).*M. siculus, V. sativa* and*L.rigidum* were always ranked above 10, while*L. multiflorum, M. polymorpha* and *B. rapa* were always below 10. In some plant species, the vegetative stage was more methanogenic, while in the other species, the opposite occurred.

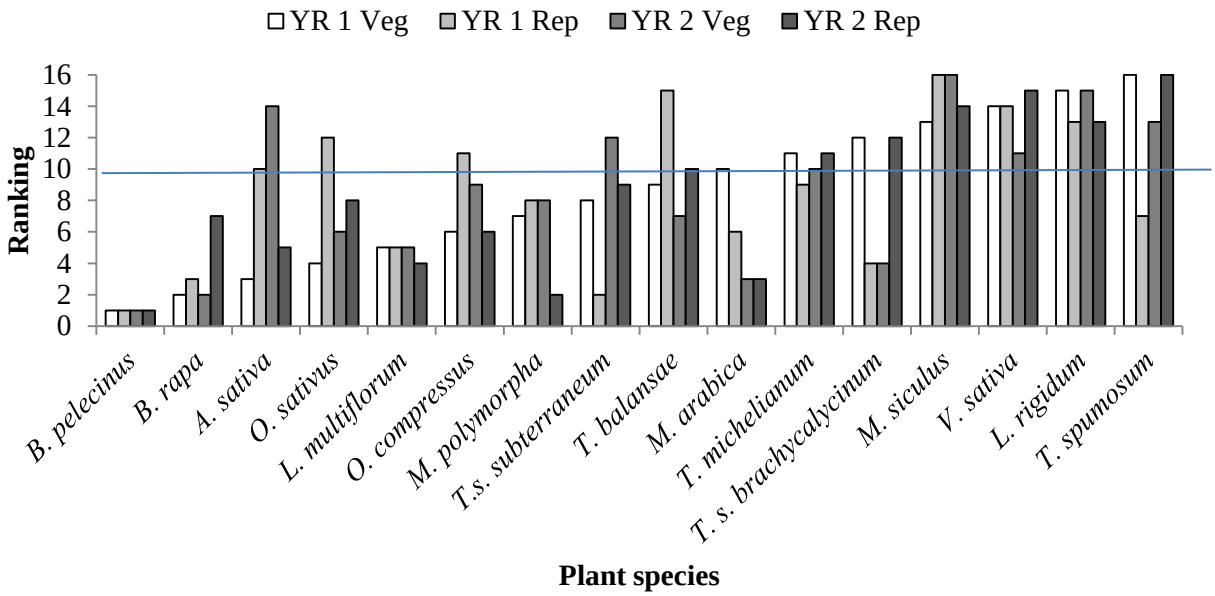


Fig. 2.2. Ranking of the annual plants according to their methanogenic potential, when collected in vegetative (Veg) and reproductive (Rep) stage, grown across 2 years (YR 1, YR 2) in SA

3. *In vitro* fermentability and methanogenic potential of subset of annual species grown in WA (YR 1 & YR 2)

Results

When plants were grown in WA, *B. pelecinus* was again the lowest methanogenic plant. In YR 1, it produced 5 times less methane than any other plants tested (i.e. 11 and 9 mL/g DMi vs mean of 45 mL/g DMi, (vegetative) and 41 (reproductive, Table 3.1). Similar to the fermentation trends in SA, this plant produced the lowest A:P (1.5 & 1.6, compared to mean of 2.0, vegetative/reproductive) and there was a reduction in total gas (61 kPa vs mean of 104 kPa), accompanied withasmall reduction in VFA (89 and 97 mmol/L vs mean of 104 and 106 mmol/L, vegetative/reproductive), but not in NH_3. The highest methanogenic plants were *T. balansae* (51 mL/g DMi) in vegetative and *M. siculus* (48 mL/g DMi) in reproductive stage.

Table 3.1. Fermentation profiles (predicted mean) amongst the subset of annual pasture species grown in WA and collected in vegetative and reproductive stages in YR 1 (WA YR 1). DMi - dry matter incubated

Botanical name	Cultivar/ Accession	Gas (kPa)	CH_4 (mL/g DMi)	VFA (mmol/L)	A:P	NH_3 (mg/L)
Vegetative stage						
Astralagus (Biserrula) pelecinus	Casbah	62	11	89	1.5	248
Avena sativa	Winteroo	106	45	105	2	106
Brassica rapa	New York	108	46	104	2.3	229
Lolium multiflorum	Dargo	116	48	108	2	115
Lolium rigidum	Wimmera Sungold	100	46	102	2.5	229
Medicago arabica	SA 8774	105	47	103	2.3	227
Medicago polymorpha	Scimitar	106	49	103	2.3	177
Melilotus siculus	SA 40002	107	50	104	2.3	260
Ornithopus compressus	Santorini	103	46	103	2.3	217

Botanical name	Cultivar/ Accession	Gas (kPa)	CH_4 (mL/g DMi)	VFA (mmol/L)	A:P	NH_3 (mg/L)
Ornithopus sativus	Cadiz	99	45	104	2.3	228
Trifolium michelianum	Frontier	108	44	104	2.2	198
Trifolium spumosum	Bartolo	110	47	109	2.3	216
Trifolium subterraneum subsp. brachycalycinum	Antas	108	47	106	2.2	217
Trifolium subterraneum subsp. subterraneum	Urana	110	47	104	2.3	143
Trigonella balansae	SA 5045	109	51	106	2.2	245
Vicia sativa	Languedoc	100	47	104	2.3	223
Mean		104	45	104	2.0	205
SEM		2.9	2.2	1.0	0.100	11
Reproductive stage						
Astralagus (Biserrula) pelecinus	Casbah	55	9	97	1.6	256
Avena sativa	Winteroo	94	45	100	2.5	141
Brassica rapa	New York	95	40	108	3.1	252
Lolium multiflorum	Dargo	97	41	105	2.4	164
Lolium rigidum	Wimmera Sungold	110	48	116	2.3	261
Medicago arabica	SA 8774	85	41	101	2.7	321
Medicago polymorpha	Scimitar	104	39	111	2.3	232
Melilotus siculus	SA 40002	102	48	116	2.5	302
Ornithopus compressus	Santorini	89	40	102	2.6	243
Ornithopus sativus	Cadiz	98	44	107	2.4	313
Trifolium michelianum	Frontier	96	43	108	2.7	197
Trifolium spumosum	Bartolo	97	46	98	2.9	257
Trifolium subterraneum subsp. brachycalycinum	Antas	95	43	104	2.5	260
Trifolium subterraneum subsp. subterraneum	Urana	99	44	105	2.5	250
Trigonella balansae	SA 5045	97	45	111	2.4	298
Vicia sativa	Languedoc	103	47	113	2.5	289
Mean		95	41	106	2.0	252
SEM		2.9	2.2	1.4	0.10	12

In YR 2, *B. pelecinus* was the most antimethanogenic plant, producing up to 6 times less methane (i.e. 8 and 7 mL/g DMi, vegetative/reproductive) than other plants (average 38 mL and 39 mL/g DMi, Table 3.2). It also produced less totalgas compared to the mean, as well as VFA, A:P, but not NH_3.

Table 3.2. Fermentation profiles amongst the subset of annual pasture species grown in WA and collected in vegetative and reproductive stage in YR 2 (WA YR 2). DMi - dry matter incubated. n/a - not analysed

Botanical name	Cultivar/ accession	Gas (kPa)	CH_4 (mL-g DMi)	VFA (mmol-L)	A:P	NH_3 (mg-L)
Vegetative						
Astralagus (Biserrula pelecinus)	Casbah	46	8	77	1.8	266
Avena sativa	Winteroo	91	42	89	3.0	160
Brassica rapa	New York	89	40	90	3.3	271
Lolium multiflorum	Dargo	95	41	92	2.9	208
Lolium rigidum	Wimmera Sungold	n/a	n/a	n/a	n/a	n/a
Medicago arabica	SA 8774	72	34	88	3.1	303
Medicago polymorpha	Scimitar	92	41	96	3.0	246
Melilotus siculus	SA 40002	88	39	86	3.0	252
Ornithopus compressus	Santorini	84	40	89	3.1	223
Ornithopus sativus	Cadiz	77	37	85	3.1	258
Trifolium michelianum	Frontier	96	46	96	3.0	221
Trifolium spumosum	Bartolo	91	44	92	3.2	224
Trifolium subterraneum subsp. brachycalycinum	Antas	84	40	87	3.0	221
Trifolium subterraneum subsp. subterraneum	Urana	75	37	81	3.2	229
Trigonella balansae	SA 5045	93	45	91	2.7	265
Vicia sativa	Languedoc	98	47	94	3.1	271
Mean		84	38	88	3.0	239
SEM		3.4	2.4	1.4	0.09	8.9
Reproductive						
Astralagus (Biserrula pelecinus)	Casbah	59	7	84	1.6	230
Avena sativa	Winteroo	97	40	92	2.5	74
Brassica rapa	New York	112	42	106	2.7	174
Lolium multiflorum	Dargo	105	40	90	2.5	88
Lolium rigidum	Wimmera Sungold	n/a	n/a	n/a	n/a	n/a
Medicago arabica	SA 8774	95	42	96	2.7	204
Medicago polymorpha	Scimitar	105	41	99	2.6	196
Melilotus siculus	SA 40002	100	42	100	2.6	204
Ornithopus compressus	Santorini	92	37	93	2.7	172

Botanical name	Cultivar/ accession	Gas (kPa)	CH_4 (mL-g DMi)	VFA (mmol-L)	A:P	NH_3 (mg-L)
Ornithopus sativus	Cadiz	89	36	91	2.7	203
Trifolium michelianum	Frontier	100	42	98	2.7	129
Trifolium spumosum	Bartolo	108	45	101	2.8	181
Trifolium subterraneum subsp. brachycalycinum	Antas	98	40	97	2.7	169
Trifolium subterraneum subsp. subterraneum	Urana	97	40	97	2.7	134
Trigonella balansae	SA 5045	103	44	97	2.5	209
Vicia sativa	Languedoc	104	44	101	2.8	189
Mean		98	39	96	2.6	177
SEM		3.3	2.5	1.5	0.08	9.8

When species were ranked according to methanogenic potential, only *B. pelecinus* was consistently low and retained its number 1 ranking (Fig.3.1). Amongst the other species, there were no clear trendsacross the stages of growth or between the years, however *V. sativa* was always ranked above 10, while*O. sativus*, *O. compressus* and*M. arabica*were always ranked below 10. Vegetative stage was more methanogenic in some, and not in the other species.

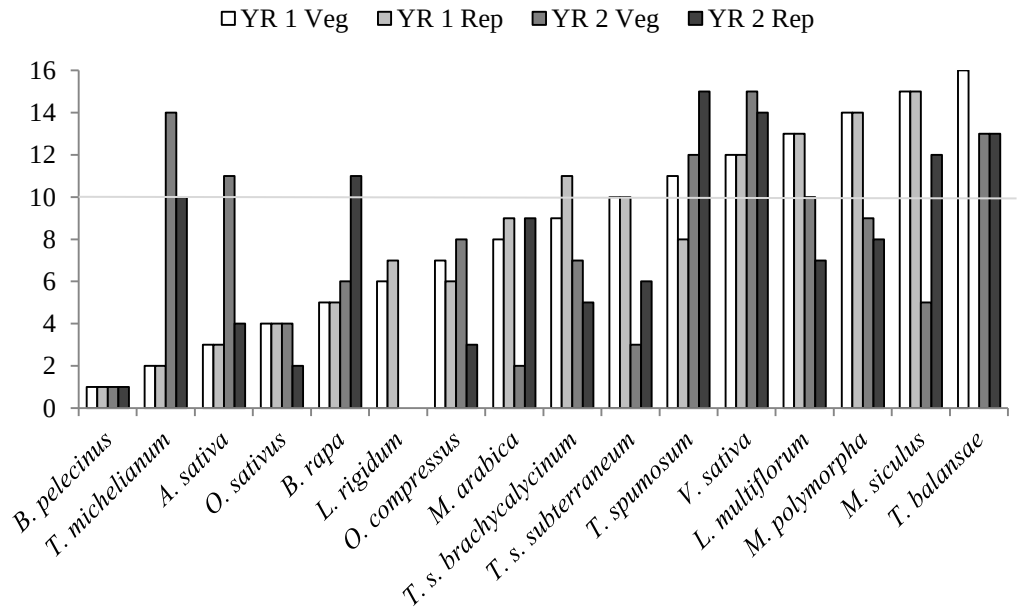


Fig. 3.1. Ranking of the annual plants according to their methanogenic potential, when collected in vegetative (Veg) and reproductive (Rep) stage, grown across 2 years (YR 1, Yr 2) in WA

Some differences were also observed when the subset were grown across different sites (Fig. 3.2). While means in SA were higher in YR 2 compared to YR 1, means in WA had the opposite trend. In SA in YR 1 (when plants were irrigated) there was also a greater difference between vegetative and reproductive stage, while these differences were less pronounced in other times and locations.

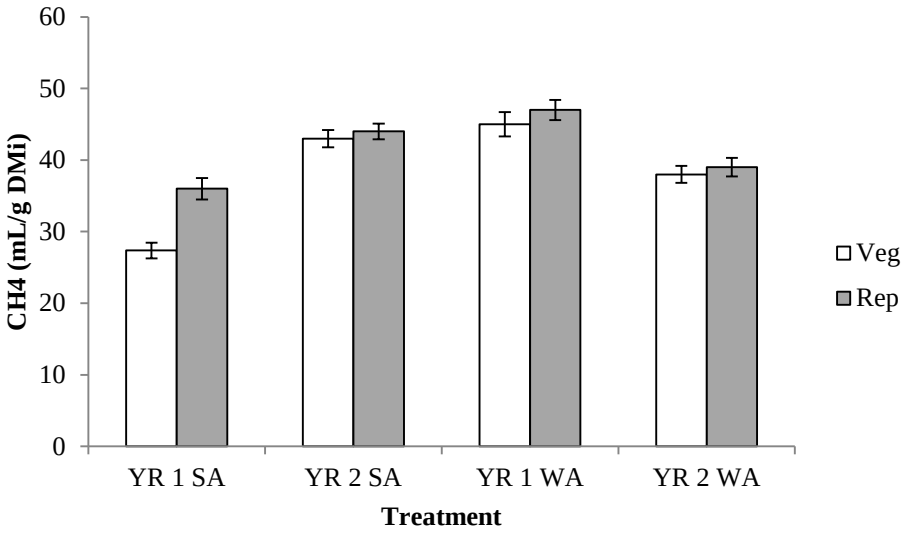


Fig. 3.2. Methane production (predicted mean ± SEM) across the subset of species examined across different stages and locations

4. *In vitro* fermentability and methanogenic potential of perennial species (SA, YR1 & YR 2)

Results

In YR 1, methane production varied from the highest in *M. sativa subsp. sativa* (30 mL/g DMi) and three plant species, namely *D. hirsutum* (16 mL/g DMi), *D. glomerata* (17 mL/g DMi) and *T. pratense* (18 mL/g DMi) produced less methane than the mean (23 mL/g DMi, Table 4.1). Two of these plants also produced lower gas, VFA and one reduced NH_3compared to the mean. Several other plants also had reduced gas, VFA, and NH_3, but all had similar A:P.

Table 4.1. Fermentation profiles (predicted mean) amongst 12 perennials forage species grown in SA and collected in reproductive stage in YR 1. DMi - dry matter incubated

Botanical name	Cultivar/ Accession	Gas (kPa)	CH_4 (mL/g DMi)	VFA (mmol-L)	A:P	NH_3 (mg-L)
Agropyron elongatum	Dundas	65	26	96	2.2	172
Cichorium intybus	Choice	72	25	105	2.3	155
Dactylis glomerata	Howlong	60	17	98	2.3	157
Dorycnium hirsutum	Canaritis	47	16	83	2.5	109
Hedysarum coronarium	Wilpena	74	24	98	2.4	128
Lotus corniculatus	Goldie	64	23	104	2.2	146
Medicago sativa subsp. sativa	S7S2	74	30	110	2.3	205
Onobrychis viciifolia	Shoshone	69	23	102	2.3	125
Phalaris aquatica	Advanced AT	67	25	102	2.0	138
Plantago lanceolata	Tonic	67	24	100	2.2	126
Trifolium pratense	Rubitas	66	18	106	2.3	213

Trifolium repens	Quest	70	24	107	2.1	191
Mean		66	23	101	2.3	155
SEM		1.3	1.6	1.6	0.01	2.9

There was no strong correlation between VFA and CH_4 production (R^2 = 0.36), and one plant, i.e. *Trifolium pratense* reduced CH_4 below the average, while sustaining VFA above the average values (Fig. 4.1).

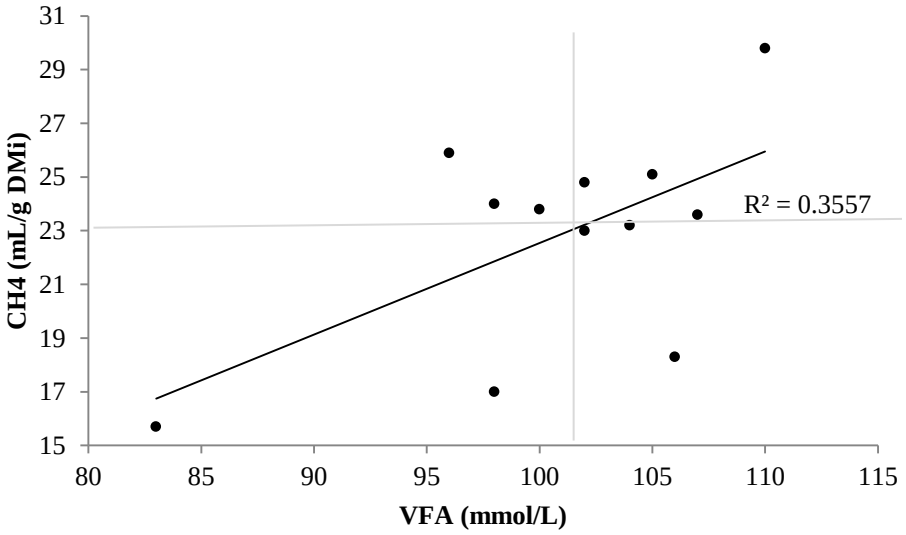


Fig. 4.1. Distribution of perennials in reproductive stage in YR 1 according to their VFA and CH_4 production. DMi - dry matter incubated. Grey lines represent mean values for all plants

In YR 2, in the vegetative stage, the lowest methanogenic species was still *D. hirstum*plus*D. rectum*(27 and 28 mL/g DMi) compared to the mean (36 mL/g DMi, Table 4.2). This effect was accompanied with some reduction in gas, VFA and NH_3, but an increase in A:P. In the reproductive stage, the overall methane across the species was higher (66 mL/g DMi) than in the vegetative stage, but *D. hirstum* maintained the lowest methane (47 mL/g DMi) amongst all perennial species tested.

Table 4.2. Fermentation profiles (predicted mean) amongst perennial pasture species grown in SA and collected in reproductive stage in YR 2. DMi - dry matter incubated.

Botanical name	Cultivar/ accession	Gas (kPa)	CH_4 (mL/g DMi)	VFA (mmol/L)	A:P	NH_3 (mg/L)
Vegetative						
Agropyron elongatum	Dundas	103	42	117	2.7	256
Cichorium intybus	Choice	101	37	117	2.8	234
Dactylis glomerata	Howlong	92	39	107	2.7	279
Dorycnium hirsutm	Canaritis	68	28	104	3.0	171
Dorycnium rectum	SA1231	81	27	107	3.0	173
Festuca arundinacea	Resolute	91	32	113	2.6	294
Hedysarum coronarium	Wilpena	102	39	114	2.6	237
Lolium perenne	AberMagic HSG	107	35	119	2.4	218
Lotus corniculatus	Goldie	100	34	119	2.6	196
Medicago sativa subsp. sativa	S7S2	92	39	119	2.7	330
Onobrychis viciifolia	Shoshone	90	36	110	2.6	178
Phalaris aquatica	Advanced AT	97	38	116	2.6	296

Botanical name	Cultivar/ accession	Gas (kPa)	CH_4 (mL/g DMi)	VFA (mmol/L)	A:P	NH_3 (mg/L)
Plantago lanceolata	Tonic	95	33	115	2.7	157
Trifolium pratense	Rubitas	98	37	117	2.7	302
Trifolium repens	Quest	97	42	122	2.6	272
Mean		94	36	114	2.7	240
SEM		2.4	1.1	1.3	0.04	13.9
Reproductive						
Agropyron elongatum	Dundas	26	65	96	2.2	172
Cichorium intybus	Choice	25	72	105	2.3	155
Dactylis glomerata	Howlong	17	60	98	2.3	157
Dorycnium hirsutm	Canaritis	16	47	83	2.5	160
Dorycnium rectum	SA1231	n/a	n/a	n/a	n/a	n/a
Festuca arundinacea	Resolute	n/a	n/a	n/a	n/a	n/a
Hedysarum coronarium	Wilpena	24	74	98	2.4	128
Lolium perenne	AberMagic HSG	n/a	n/a	n/a	n/a	n/a
Lotus corniculatus	Goldie	23	64	104	2.2	146
Medicago sativa subsp. sativa	S7S2	30	74	110	2.3	205
Onobrychis viciifolia	Shoshone	23	69	102	2.3	125
Phalaris aquatica	Advanced AT	25	67	102	2.0	138
Plantago lanceolata	Tonic	24	67	100	2.2	126
Trifolium pratense	Rubitas	18	66	106	2.3	213
Trifolium repens	Quest	24	70	107	2.1	191
Mean		23	66	101	2	160
SEM		1.1	2.0	1.8	0.03	8.4

n/a - not assessed due to lack of material due to poor growth

In the vegetative stage, CH_4 was not strongly correlated to VFA (R^2 = 0.29, Fig.4.2), but in the reproductive stage this correlation became strong (R^2 = 0.82, Fig.4.3). Three plants in vegetative and two in reproductive stage of growth reduced methane below the mean, while supporting VFA, with *L. corniculatus* maintaining the same effect across the stages.

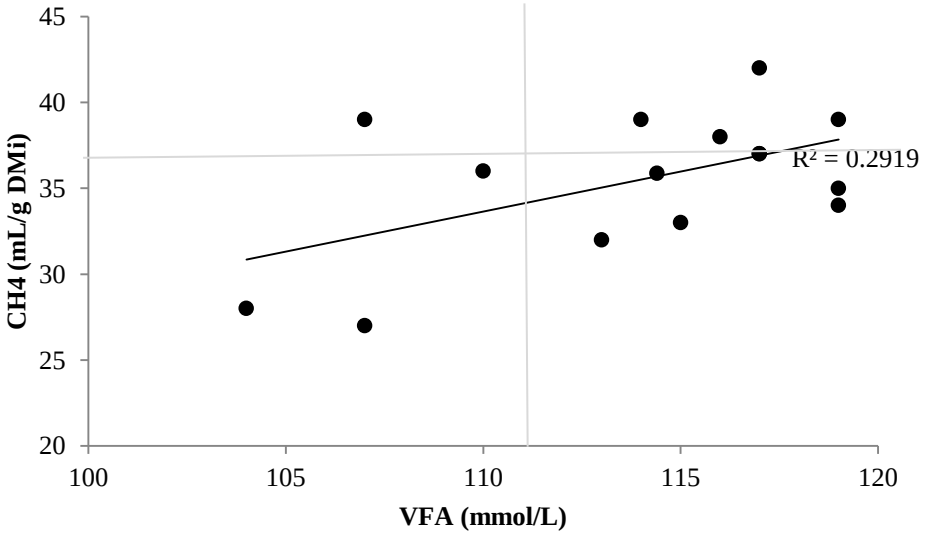


Fig. 4.2. Distribution of perennials in vegetative stage in YR 2 according to their VFA and CH4 production. DMi - dry matter incubated. Grey lines represent mean values for all plants

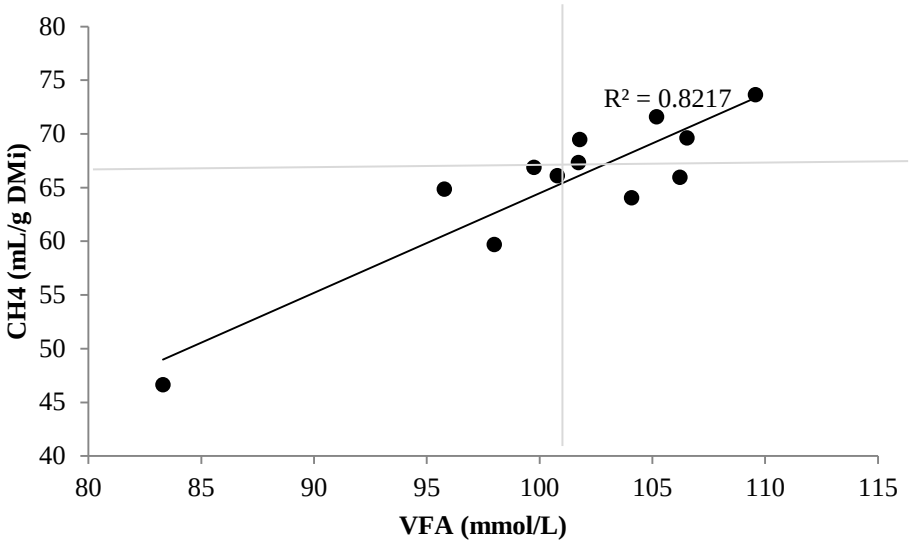


Fig. 4.3. Distribution of perennials in reproductive stage in YR 2 according to their VFA and CH_4 production. DMi - dry matter incubated. Grey lines represent mean values for all plants

Ranking according to methanogenic potential varied across stages and between years (Fig. 4.4). However, *D. hirstum/rectum* were consistently ranked number 1. *L. corniculatus* and *T. pratense* were always amongst the top 5 plants, while *T. repens* and *M. sativa* were always amongst the highest five methanogenic plants. There was no clear pattern in ranking across the years and stages.

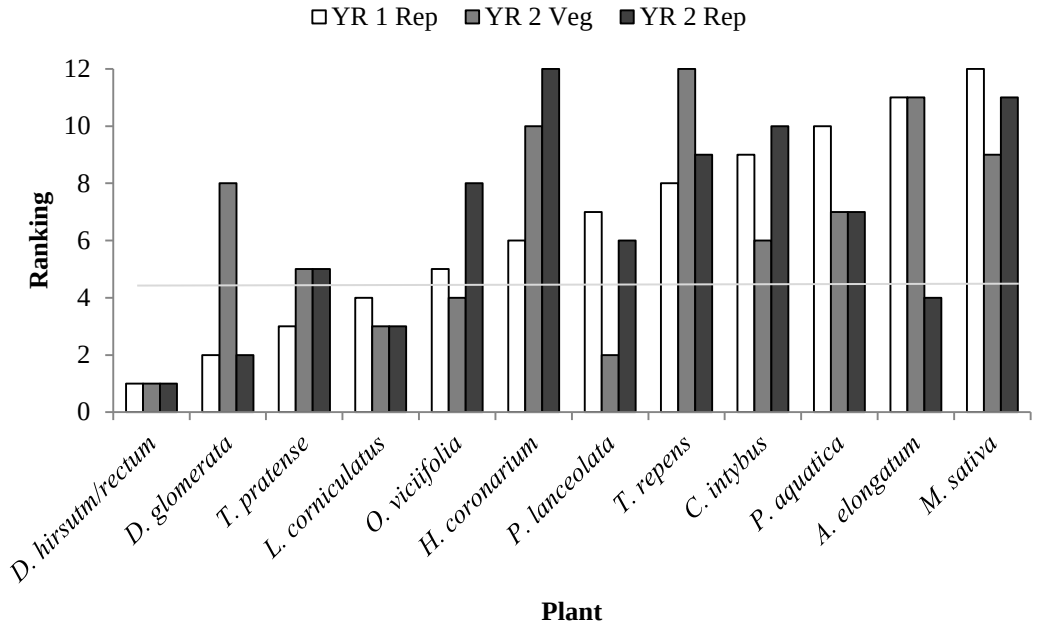


Fig. 4.4. Ranking of the perennial plants according to their methanogenic potential, when collected in vegetative and reproductive stage, grown across 2 years in WA.

5. Within species variation in *in vitro* methanogenic potential in chicory

Background

There is seasonal variability in agronomic, nutritive and methanogenic values of chicory (see Section 5). Chicory has been also examined as a bioactive plantunder B. CCH 6530 and, in preliminary testing as a crude extract, it has shown promising results in terms of having direct anti-methanogenic effects. Given the economic significance of chicory (Barry 1998, Li *et al.,* 2005) and a spectrum of samples available for testing within the ELLE we used chicory to investigate the within species variability, as affected by cultivar/accession in this pasture species.

The 3 objectives of the *in vitro* screening component of the chicory study were to examine 1) variability between the *in vitro* methane production of cultivars/accessions in spring to identify the lowest methanogenic cultivars/accession; 2) variability within cultivar Puna as affected by different sampling times; 3) variability between cultivars/accessions in summer.

Materials and methods

Plant material for this experiment was obtained from the experimentsreported in Section 5(objectives 1 and 3) and Section 2 (objective 2) and processed as described in general methods. Spaced plants of cultivars and accessions were grown and collected in spring of 2013 for objective 1 (cut 1,collected on 3 October 2013,) and in summer for objective 3 (cut 5,collected on 18 February 2014, objective 3). For objective 2, we collected samples throughout the year (cuts 9-15, collected on 5 March 2013, 24 June 2013, 28 August 2013, 24 October 2013, 17 December 2013, 22 January 2014 and 27 February 2014 respectively) from swards of Puna. At the time of collection, each plant sample was given a plant development score (i.e. 1 - vegetative only, 10 - open flower).

Results

Variation between entries in spring (Objective 1)

In spring there was some small but significant variation in methane production between the entries of chicory (Table 5.2). The lowest producing entries were 45310 (43.6 mL/g DMi) and Puna (44.5 mL/g DMi) and the highest was 39441 (48.7 mL/g DMi). The difference between the highest methanogenic entry and the two lowest ones was 10.5% and 8.5%. Most (76%) plants were in vegetative growth (score 1),while the remaining had growth scores of 1.5 and 2.

Table 5.2. *In vitro* methane production (mL/g DMi) of selected entries of chicory at spring (cut 1). DMi - dry matter incubated

Entry	No of plants tested	CH_4 (mL/g DMi)		SEM
45310	1	44	d	0.7
Puna	3	45	d	1.0
42961	3	45	cd	0.9
39415	3	45	bcd	0.5
38955	2	46	abcd	1.0
Commander	2	46	abcd	1.4
42588	3	46	abcd	1.0
39000	1	46	abcd	0.8
Puna II	3	46	abcd	0.4
41976	2	47	abcd	0.7
Le Lacerta	3	47	abcd	1.2
Grouse	3	48	abc	0.5
Choice	2	49	ab	0.4
39441	3	49	a	2.6

Variability within Puna in relation to different sampling times (Objective 2)

In this experiment we examined the methanogenic potential of this accession along several cutting times (cut 9-15) to determine how methane production changes throuhgout the year.

Results

There was asmall (10%) but significant difference in methane production between the highest (cut 11, 28 August) and the lowest (cut 13, 17 December)cuts of Puna (Table 5.3). Plant development scores were 1 for cuts 9-12, 1.4 for cut 13, 1.2 for cut 14 and 1.1 for cut 15.

Table 5.3*In vitro* methane production(mL/g DMi) of selected cuts of Puna. DMi - dry matter incubated

Cut	CH_4 (mL/g DMi)		SEM
9	42	ab	1.0
10	42	ab	0.8
11	43	a	1.1
12	42	ab	0.5
13	39	c	0.4
14	41	ab	0.4
15	41	bc	0.5

Variability between accessions in summer (Objective 3)

The late summer cut (cut 5) in a Chicory spaced trial was selected for addressing this objective because it is the time where large variation in digestible dry matter was reported (Section 5) and the time of the year when chicory will be utilised from a farming system viewpoint (ie. summer).

Results

There were small but significant differences between accessions at this sampling time (Table 5.4). The lowest methane was recorded with accession 39415 and 42588 (30 mL/g DMi), while the highest was with 38955 (35 mL/g DMi).The lowest ones produced only 15% less methane than the highest one. There was a general trend for cultivars/accessions with high development scores to produce lower methane.

The difference between the highest methanogenic entry and the two lowest ones was 10.5% and 8.5%.

Table 5.4. *In vitro* methane production (mL/g DMi) of selected entries in summer (cut 5).

Cultivar /Accession	Plant development score	CH_4 (mL/g DMi)		SEM
39415	4.3	30	e	0.9
42588	6.0	30	e	0.4
42221	6.0	31	cde	0.7
42961	5.7	31	de	0.3
Commander	6.0	31	cde	0.6
Grouse	6.0	31	de	0.6
Le Lacerta	5.8	31	de	0.3
39441	6.0	32	cde	0.4
39000	6.0	32	bcde	1.5
45310	1.0	33	abcd	1.4
Choice	4.0	33	bc	0.8
Puna	3.5	33	ab	0.4
Puna II	2.7	34	ab	0.6
38955	4.5	35	a	1.1

While the 'antimethanogenic ranking' between the cuts remained the same in some, it changed in others (Fig. 5.1). The accessions that remained consistently low were 39415 and 42961.

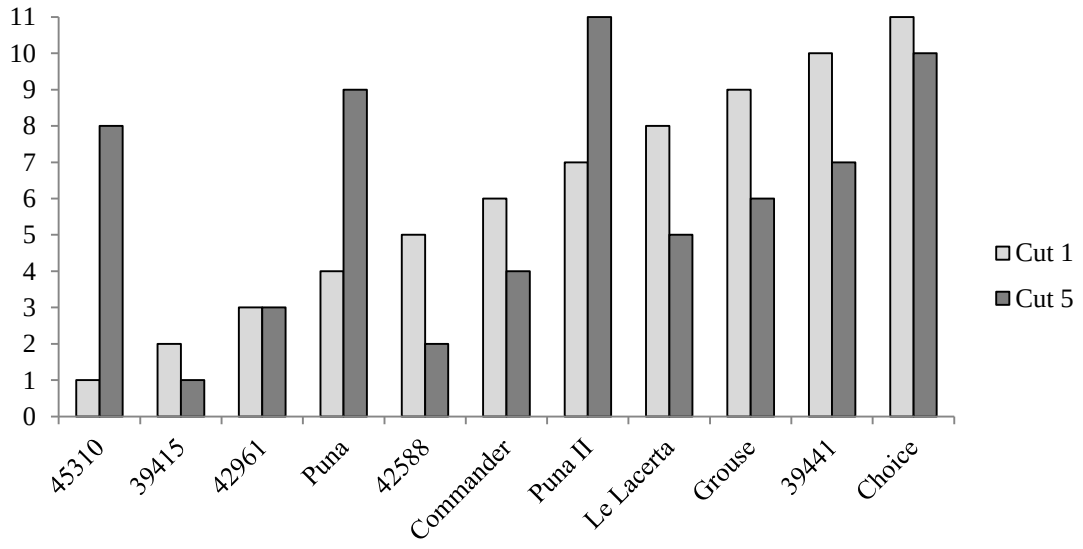


Fig. 5.1. Methanogenic ranking in selected accessions/cultivars of chicory across two cutting times

Discussion

Our results indicate that small but significant variability exists between cultivars/accessions/within chicory. The variance between the highest and the lowest entries was about 10%for both spring and summer. The difference was also observed within cultivar Puna when sampled at different times. The lowest methanogenic plant sample cut was 17 December, which was about 10% lower than the highest one, 28 August. While some accessions retained their lower methanogenic potential throughout sampling times, others did not. Some of the most promising accessions from this

perspective are 39415 and 42961, as they retained their low methanogenic potential across spring and summer.In summer methane production decreased as flowering developed. It is interesting that the most widespread commercial accession, Choice, always ranked amongst the highest in terms of methanogenic potential and it is possible to select for those that may result in less methane when fermented in the rumen.

6. Rusitec testing of top antimethanogenic plant *B. pelecinus*

Background

There is growing research interest in *Biserrula pelecinus* (biserrula) because of its low methanogenic potential when fermented by rumen microbes. Preliminary findings obtained from a glasshouse study (Banik *et al.,* 2013) have now been confirmed in the current project with field-grown samples collected at different growth stages and grown under various conditions.

Testing plants using a continuous culture fermentation system (RUSITEC) can be a useful provisional step between *in vitro*batch culture and *in vivo*animal experimentation to confirm the bioactivity and the persistency of microbial fermentation and methane production of any particular bioactive plant species. Further, this legume is likely to be grazed in a plant mixture rather than as a monoculture, due to some limitations in agronomic properties, palatability, and possible photosensitivity effects in animals when grazed at particular times. Testing using a RUSITEC may provide confirmation of the effect of plant mixes containing *B. pelecinus*.

The aim of the current study was to i) confirm the antimethanogenic effect of biserrula in the RUSITEC system when mixed with different proportions of subclover (a common pasture legume with good NV but produces higher levels of methane than biserrula); ii) examine the mechanism, the extent and the persistency of the effect; and iii) to establish an appropriate and safe amount of biserrula to test *in vivo*. We hypothesised that the antimethanogenic effect of biserrula would be persistent and dose-related, when mixed with the common pasture legume subclover in a RUSITECsystem.

Experimental design

The continuous fermentation *in vitro*was carried out using a RUSITEC, following the protocol of Li *et al.*(2013). The experiment was completely randomized design, with five treatments and three replicates per treatment. Four levels of biserrula were tested in a mix with subclover as a substrate (Table 6.1). The total fermentation period lasted 18 days, with a 4 day period of stabilization on a common substrate (oaten chaff), followed by 4 days of introduction of treatments and 10 days of incubation with the treatments and measurements. In the measurement period (day 9 – 18), gas samples were collected daily to measure methane production and total gas produced over 24 hrs, while fermentation liquid was collected for VFA, $NH_3$and pH analyses of the liquid. Fermentation liquid was also collected for HPLC analyses for B. CCH 6530. Residues within feed bags from all 10 days were collected to estimate dry matter digestibility (DMD), neutral detergent fiber (NDF), acid detergent fibre (ADF) and crude protein (CP) digestibility.

Plant material

Plants *B. pelecinus* cv. Casbah (biserrula) and *T. subterraneum* L. Dalkeith (subclover) were collected from a trial plot (0.2 ha per treatment) at The University of Western Australia Shenton Park Field Station, Western Australia, in early November 2013 before flowering time. The plants (i.e. leaf and stems 2 cm from the ground) were randomly collected from the trial area. Samples from individual plants were pooled, freeze-dried and ground to pass through a 4 mm sieve. Materials were stored at room temperature (i.e. 22° C) in sealed bags until the experiment.

Treatments

Four levels of biserrula containing 0, 250, 500, 750 and 1000 g of biserrula per kg DM of substrate (subclover)were tested (Table 6.1). Each of five treatments had three replicates (n = 15 fermentation vessels) and all of them were randomly distributed in two water baths.

Results

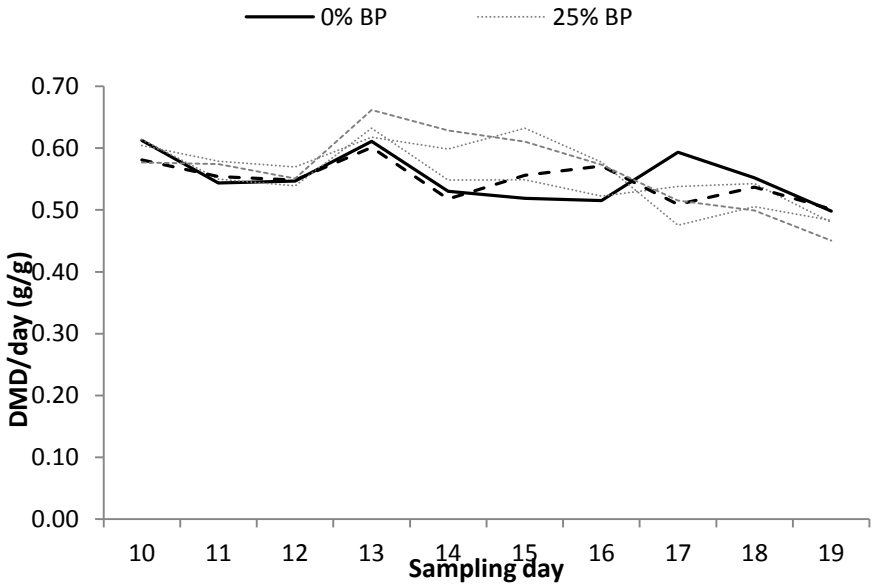
Across the whole measurement period (excluding the daily effect), there were no significant differences in DMD between the treatments (Table 6.1). When compared to 100% subclover ('control'), total gas was significantly reduced only with BP 500. This was the only treatment that differed to the others in methane production, having around 50% less methane compared to subclover on its own. The BP 750 and BP 100 had numerically lower values, but they were not statistically significant. When compared to subcloveron its own, the concentrations of VFA were not affected in any of the treatments containing biserrula. There was no clear dose-response of biserrula levels on any of the parameters measured.

Table 6.1: Fermentative parameters of selected treatments in the RUSITEC. Significance – within the same column, values not sharing the same superscript differ significantly (P < 0.05)

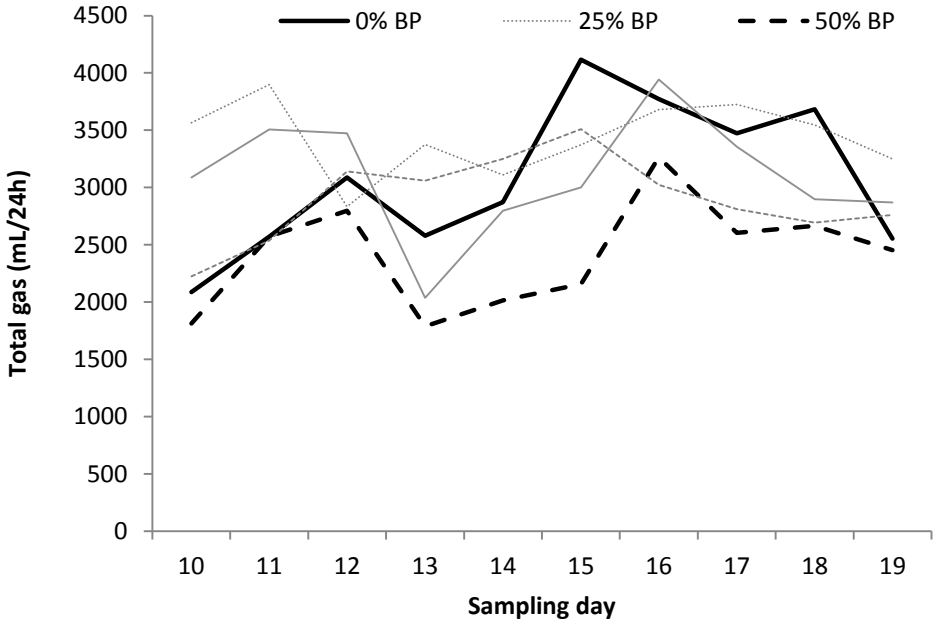
Treatment	Substrate composition	DMD (g/g)	Gas (mL/24h)	CH_4 (mL/24h)	VFA (mmol/L)
Control	Subclover	0.55	3080^{b}	107^{ab}	63^{ab}
BP 250	0.75 subclover : 0.25biserrula	0.55	3436^{a}	129^{a}	69^{a}
BP 500	0.50 subclover :0.50biserrula	0.55	2413^{c}	58^{c}	67^{ab}
BP 750	0.25 subclover : 0.75 biserrula	0.56	2901^{b}	96^{b}	59^{b}
BP 100	Biserrula	0.56	3097^{ab}	93^{b}	64^{ab}
SEM		0.03	403	24	8.3

Daily DMD, total gas, methane and VFA production are presented in Fig. 6.1. There was a notable day-to-day variability in gas and methane and more detailed statistical analysis is underway to address these differences. However, it appears that BP 500 was still consistently the lowest in terms of methane production (except for the last day when all treatments seem to start to converge).

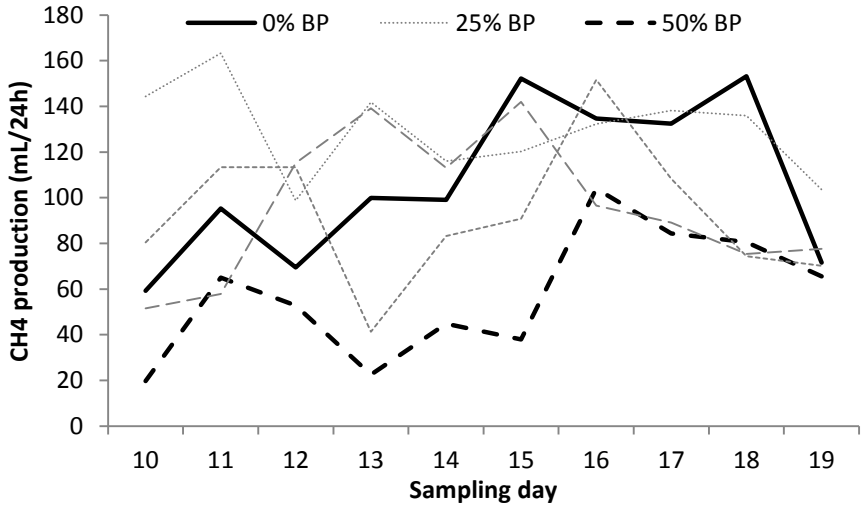
a.


0% BP
25% BP
0.70
0.60
0.50
0.40
0.30
0.20
0.10
0.00
DMD/day (g/g)
10
11
12
13
14
15
16
17
18
19
Sampling day

b.


0% BP
25% BP
50% BP
4500
4000
3500
3000
2500
2000
1500
1000
500
0
Total gas (mL/24h)
10
11
12
13
14
15
16
17
18
19
Sampling day

c.



d.

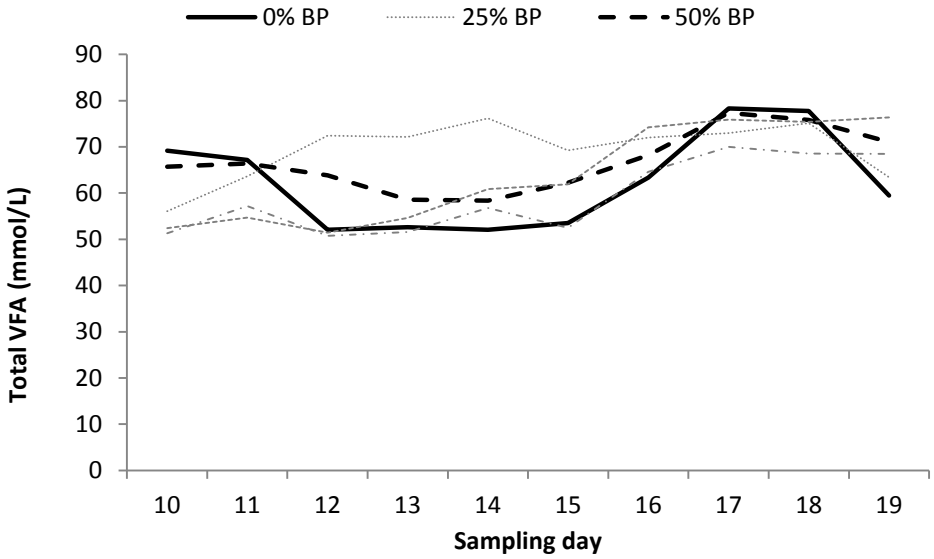


Fig. 6.1. Daily dry matter digestibility (DMD) (a), total gas (b), methane (c) and total VFA production (d) in fermentation vessels in RUSITEC system for different biserrula treatments (from Day 10 – 19). SEM_{10} presented in Table 6.1.

Discussion

When biserrula was mixed with subclover in a 50:50 ratio, there was a significant reduction (50%) in methane production compared to subclover alone. This level of inclusion did not affect DMD or VFA, but had some inhibitory effect on overall gas production. However this inhibition was only 20% and could potentially be attributed solely to the reduction in methane proportion of the gas. This is consistent with what we have observed in batch fermentations, reported previously. The effect was immediate (i.e. occurred straight after the addition of the plant) and persistent throughout the whole measurement period (10 days). In all other biserrula treatments, there was some reduction in methane with higher doses of biserrula, but they were not significant. The gas, DMD and VFA in all were also comparable to that of subclover. Batch testing of biserrula and French seredella mixes

revealed a similar trend i.e. the highest antimethanogenic effect in a plant mix was observed in a 50:50 ratio (Joy *et al.,* 2014). It appears that there is an optimal dose that is producing the most effectiveoutcome.

Our resultsalso confirmed that, when mixed with a methanogenic forage legume and fermented in the RUSITEC, the antimethanogenic effect of biserrula occurs and persists over 10 days. We also confirmed our *in vitro*batch findings that biserrula does not affect overall fermentation (as judged by DMD and VFA when compared to subclover). Finally, we have identified a dose that can significantly reduce methane production of subclover, without impeding overall fermentability. This dose can be recommended for further *in vivo* studies using subclover as base diet.These results imply that using biserrula as a component of the diet may provide a strategy to mitigate methane production from some more common grazing forages.

7. The effect of plant processing on methanogenic potential in *B. pelecinus*

Background

In the *in vivo* trial (see Section 6 of this final report), there was a limited effect when *B. pelecinus* was fed as hay. In our previous reports, *B. pelecinus* was consistently antimethanogenic, when collected as fresh materialand freeze-dried. The objective of this study was to examine if the methanogenic activity of *B. pelecinus* (highly antimethanogenic) and a highly methanogenic pasture *O. sativus* is affected by plant processing.

Materials and methods

We have tested opportunistic samples collected as hay from a commercial source in 2014 and fed to animals in the ELLE *in vivo* trial and samples in the same year collected fresh from plots at UWA Ridgefield farm (linked to the FTRG 2 Innovative systems project), WA and freeze-dried.

Results

There was a significant difference between hay and fresh-freeze dried material in both pasture species (Fig. 7.1). Hay from *B. pelecinus* produced 70% more methane than the same plant collected fresh and freeze dried. Biserrula hay was still significantly lower than hay from *O. sativus*, but produced30% less methane compared to fresh-freeze dried*B. pelecinus,*whichproduced 80% less methane than the fresh *O. sativus*.

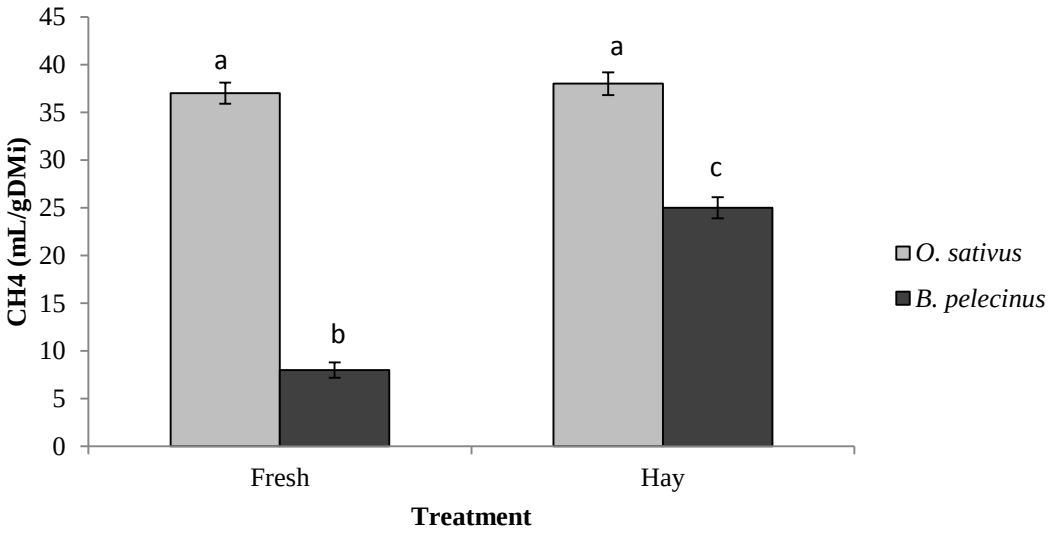


Fig. 7.1. Methane (mean ± SEM) produced (mL/g DMi) during *in vitro* fermentation of different treatments. DMi - dry matter incubated

Conclusion

It appears that the activity of *B. pelecinus* is affected by the type of processing, potentially also by location where the plant was grown or a combination of these factors. However, previously, we have demonstrated that (fresh) *B. pelecinus* maintains low methanogenic activity when grown at different locations in SA and WA. Further, the antimethanogenic activity of *B. pelecinus*, grown at the same location and fed as fresh pasture translated*in vivo*(P. Hutton, pers. comm. FtRG2 project), so it is most likely that the processing of the plant is the main driver of this effect. The plant secondary compounds (PSC) in this plant appear to be thermolabile or volatile, as the drying of this pasture is having an effect on its bioactivity. This may also explain limited activity observed*in vivo* when fed as hay (see Section 6).

2.6 Discussion

In this project we have confirmed that there is variability in methanogenic potential amongst an extensive selection of annual and perennial grass and legume species suitable for southern grazing systems. *B. pelecinus* consistently produced low methane, five to six times less than the other annual species tested. This plant also reduced microbial gas, but sustained VFA concentrations, which is consistent with our previous findings with this plant (Banik *et al.,* 2013). *B.pelecinus*also reduced A:P ratios to half of what was observed with other annual species. This is also consistent with what we have reported earlier, and has significance in livestock production, as propionate is the preferred VFA because it directs energy towards gluconeogenesis. It is possible that the mechanism of methane reduction in this plant is redirecting hydrogen from methane towards propionate production, but more research is needed to confirm this.

Amongst other annual species, there was some variability in methane production, for example the highest methanogenic plant (*Melilotus siculus*) produced 30% more methane than the lowest one (*Trifolium subterraneum subsp. subterraneum)*. It is therefore possible to select from some of the mainstream plants that do not have some of thelimitations of*B. pelecinus*, and develop strategies to moderate methane. Further, plant breeding may progress towards developing cultivars with more enhanced antimethanogenic properties. The variability was replicated across the two sites, SA and WA and between two plant phenological stages, vegetative and reproductive. However, only a limited number of plants retained their low or high methanogenic potentialacross these variables, while others varied. Variability in plant composition and in particular PSC has been reported in the literature (Dement *et al.,* 1974, Gebrehiwot *et al.,* 2001, 2002). These in turn may affect the microbial populations and methane production in the rumen.

It is encouraging that the methanogenic potential across all samples was not strongly linked to VFA, implying that it is possible to select candidates that reduce methane without compromising energy production from microbial fermentation. This in an important objective when developing mitigation strategies, and several plants from the current study qualify under these conditions.

There was some shift in fermentability between vegetative and reproductive stages in the annual species. With few exceptions, plants in the vegetative stage of growth were more fermentable (i.e. produced more gas, VFA and NH_3) to those in the reproductive stage of growth. Conversely, mean values for methane and A:P ratio were lower in the reproductive stage. This is comparable to findings of Navarro-Villa*et al.* (2011) who found that more mature plant material was less methanogenic than immature herbage from the primary growth.

Amongst the perennials, there was also variability in methanogenic potential, with three plants (*D. hirsutum, D. glomerata* and *T. pratense*) producing up to 50% less methane than the highest methanogenic plant, *M. sativa subsp. sativa*. However, two of these also caused about 20-25% reduction in VFA, while *T. pratense* sustained VFA. This effect is highly desirable and this plant may be one to consider advancing further. The mechanism of the antimethanogenic effect in these plants is not clear, but in the first two it is likely to be a direct antimicrobial effect and/or poor fermentability of primary chemical compounds in the plant, while the effect in *T. pratense* appeared to be more selective towards methanogenesis. Nutritive values and plant secondary compound analyses will clarify this further, but this plant is rich in phenolic compounds that have antimicrobial properties

against rumen hyperammonia producing HAP bacteria (Kagan and Flythe 2010, 2012) and it is possible that these compounds also have an effect on methanogens.

Studies onwithin species variability in methane were conducted using chicory. Our results show that the variation in methanogenic potential was small but significant in relation to accession andalso plant age (time when cut). This aligns with our previous reports on variability between accessions of biserrula (Banik *et al.*, 2013b). Some of the most promising accessions in this respect have been identified (accession 39415 and 42961), as they retained their low methanogenic potential across sampling times. It should be noted that the variability recorded in the current study may not be large in biological terms, but it provides a basis for further plant breeding within this species to produce cultivars having low methanogenic potential as a breeding objective.

Our resultsfrom the RUSITEC study showedthat the antimethanogenic effect of *B. pelecinus* persists over 10 days in an open fermentation system and that the effect was immediate (i.e. occurred straight after the addition of the plant). These results also confirmed our batch *in vitro* findings that biserrula does not affect overall fermentation (as judged by DMD and VFA comparable to subclover). Finally, we have found that biserrula maintains antimethanogenic properties when mixed with a methanogenic forage legume and we have identified a dose that can significantly reduce methane production of subclover, without impeding overall fermentability. The inclusion of biserrula at 50% of the diet subclover produced the most effective outcome and this dose can be recommended for further *in vivo* studies using subclover as a base diet.

Importantly, it appears as though the bioactivity of biserrula may be affected by drying and that fresh material is more active against methane production than when it is in the form of hay. Our preliminary results also suggest that testing fresh material that has been freeze dried better reflects the antimethanogenic activity of fresh materialand oven drying better reflects the effects of hay, but a more thorough examination of this is required.

Our results imply that using biserrula as a component of the diet may provide a strategy to mitigate methane production from some more common grazing forages.Several plants with direct antimethanogenic effect were identified, namely *B. pelecinus*, *D. hirsutum, D. glomerata* and *T. pratense,* with the level of inhibition between 50% and 80% when compared to the more commonly used forage species. Neither *B. pelecinus*nor*T.pratense* inhibited microbial activity and *B. pelecinus* promoted propionate production. Amongst other plants that were not 'directly antimethanogenic', it is possible to select for the ones that maintain or promote fermentation, hence 'indirectly antimethanogenic'. We have also provided some initial data that could be used for plant breeding in chicory, targeting low methanogenic potential.

There are clearly opportunities to select plant species that are less methanogenic for our southern grazing systems and have the potential to maintain or improve livestock productivity. Biserrula is clearly the most antimethanogenic plant that we screened and we have demonstrated that it could be used in a mixed sward and maintain its antimethanogenic capacity. The information we have generated needs to be used to design more efficient pasture based systems and be considered in breeding programs.

Section 4 Broad NIRS calibrations to predict nutritional value of 109 species across the southern feedbase of Australia

4.1 Abstract

Near infrared reflectance spectroscopy (NIRS) is used by livestock industries to predict the nutritive characteristics of feeds consumed by livestock. Once calibration equations are developed between reflectance and measured nutritional traits, NIRS is both rapid and inexpensive. The aim of this activity was to investigate the feasibility of developing broad NIRS calibrations for the southern feedbase of Australia.

A total of 4385 samples from 109 species of annual and perennial legumes, grasses and forbs were grown in common plots at two locations over 3 seasons. Plots were sampled across all growth stages and biomass was dried, ground before an NIR scan. A quarter of these samples were subject to laboratory analysis of dry matter digestibility (DMD), total nitrogen (N), neutral detergent fibre (NDF), acid detergent fibre (ADF) and organic matter (OM). Despite the large variation in the taxonomy and life history of the samples, the development of broad calibrations across the sample range was successful. When predicting samples from the collection that were not included in equation development, the statistics of prediction were; total N - r^2 0.96, RPD 5.3, *in vitro*DMD - r^2 0.93, RPD 3.7, ADF - r^2 0.93, RPD 3.9 and NDF - r^2 0.95, RPD 4.3. The prediction errors were considerably lower for annual species than perennial species.

We also investigated the possibility of calibrations to predict fermentability of the samples during batch culture in rumen fluid, as well as the by-products of fermentation; methane, ammonia and volatile fatty acids. With only limited samples (n=170), we could predict total methane (r^2 0.89, RPD 3.1), but not the other traits.

In conclusion, we have been successful in developing a tool to predict nutritional value of samples from the feedbase of southern Australia, although it could benefit from inclusion of more spatial and temporal diversity. The base collection of 1086 plant samples with both chemistry and matching spectra provide a powerful tool for generating new calibrations with the need for minimal additional chemistry. The data generated during this project are being used to compare nutritional value of species over time and investigate opportunities to improve productivity and reduce methane emissions intensity from sheep in southern Australia.

4.2 Introduction

Near infrared reflectance spectroscopy (NIRS) is used by livestock industries to predict the nutritional characteristics of feeds consumed by livestock. The method relies on the development of mathematical relationships between measured traits and light absorption properties in the NIR region (wavelength range 700 – 3000 nanometres). Once calibration equations are developed, NIRS is rapid, inexpensive, non-destructive and can predict a large range of traits at the same time (Deaville and Flinn, 2000). It is therefore a powerful tool within plant improvement programs for identifying plant species and genotypes within species with improved nutritive traits. NIRS also allows industry to conduct rapid assessment of the nutritional value of feeds and pastures, therefore informing feed purchasing decisions and grazing management. By providing a tool to improve quality of the feedbase and influence management, NIRS calibrations can improve productivity, profitability and reduce methane emissions intensity from ruminant industries.

There have been a number of studies exploring how much diversity is required to develop robust calibrations. Shenk and Westerhau found that if enough samples are utilised, broad multi-forage species calibrations can be nearly as accurate as those for single species (Shenk and Westerhaus, 1993). In southern Chile, a calibration was successfully developed for mixed swards, comprising 8 perennial grass and legume species (Lobos et al 2013). In the Chilean study 297 samples were scanned and subject to chemistry. It is suggested that for agricultural products, calibrations based on fewer than 50 samples are seldom satisfactory and that 150 or more samples were necessary for broad-based or ‘open’ populations. (Deaville and Flinn, 2000).

The aim of this project was to investigate the feasibility of developing broad NIRS calibrations for predicting nutritional value of the southern feedbase of Australia. Using samples from 109 forage

species we tested the hypothesis that it would be possible to develop a global calibration across a diverse range of forage species. An additional component of the work explored development of calibration equations for the prediction of by-products from *in vitro* fermentation over 24 hours, including total methane, ammonia and volatile fatty acids.

4.3 Methods

We utilised 4385 samples from 154 varieties (109 species) of forage and pasture species that were grown in common garden plots in 2 locations (Umbrae in South Australia and Brookton in Western Australia). The diversity of the sample base included commercialised and experimental material, with 60 species of annual legume, 38 species of perennial legume, 20 species of annual grasses, 49 species of perennial grasses, 11 species of annual forbs and 6 species of perennial forbs (Tables 1a and 1b). To capture the possible diversity in nutritional profiles, plants were sampled across all growth stages (approximately every 3-6 weeks).

Plot management

The primary field site was located in the Australian Pastures Genebank field nursery, at the Waite Institute, in South Australia. The fine sandy loam at this site is a red-brown earth of the non-sodic Urrbrae series. The upper 0.10 m contains 18% clay, increasing to 32% in the A2 horizon and soil pH (in $CaCl_2$) was 6.2. The site was rain fed from until summer when it was irrigated by sprinklers to mean Adelaide rainfall on a monthly basis. The experimental site was split into 5 experimental units within the same paddock for ease of management; (1) annual legumes, (2) annual grasses and forbs (3) annual grasses, (4) perennial grasses and forbs and (5) chicory breeding plots. Each entry within the experimental cohort was replicated across 3 plots and material from each plot was analysed separately. More information is available in the accompanying papers (Sections 1, 2 and 5 of this report).

The field site in Western Australia was located on a commercial farm near Brookton. At this site, a range of 16 annual legumes, forbs and grasses were grown across two consecutive seasons (Tables 1a and 1b list the species). Plots were sampled every 3 weeks through the growing season and for 2 months after senescence. The site was planted on 14 June 2013 and again 28 May 2014 in adjacent paddocks. The soil had a pH level ($CaCl_2$) of 4.6, organic carbon of 1.09%, texture of 1.5 and the colour was LTBR. The site was not irrigated.

Sample processing

The 4385 samples were collected between June 2012 and December 2014. Samples were either immediately frozen and later freeze dried or placed in a paper bag then oven dried at 60^{o}C. After drying, samples were ground to pass through a 1 mm screen using either a Cyclotech or Retsch Twister mill grinder. A preliminary study was conducted with samples that were halved and ground in each of the grinders. This revealed that there was little bias associated with the type of grinder. Samples were then scanned by NIRS and throughout the project a subset was set aside for chemical analysis. Across the 3 year project a total of 1086 samples were subject to the full range of nutritional analyses (wet chemistry).

Table 1a Annual and perennial legume species and accessions included in the dataset

	Genus	Variety
Perennial legumes	Clover	Hytas alsike
		Kuratas
		caucaisian
		Palestine
		strawberry
		Permatas talish
		Quest white
		Rubitas red
		Storm white
		Tuscan red
	Cullen	SA 4966
	Dorycnium	SA 1231
		Canaritas
	Kenedia	SA 41710
	Lotus	Goldie
		LosBanos
		Lottas
		Maku
		SA 45718
		SA 45714
	Lucerne	Aurora
		K202
		S7S2
		WL925HQ
	Melilotus	Norgold
	Sainfoin	Othello
		Shoshone
	Sulla	Aokau
		Moonbi
		Wilpena
	Tedera	
	Vetch (milk)	SA 38091
Annual legumes	Melilotus	Jota
		SA 37228
		*Messina SA 40002
	Ononis	SA 8577
	Serradella	*Cadiz
		Jebala
		*Santorini
	Trigonella	SA 19767
		SA 32999
		*SA 5045
	Vetch	Ioman
		*Languedoc
		Namoi
		Popany
Annual legumes (cont.)	Biserrula	*Casbah
		*Antas
	Clover	subterranean
		*Bartolo bladder
		Blaza crimson
		Cefalu arrowleaf
		Clare subterranean
		Denmark subterranean
		*Frontier balansa
		Gosse subterranean
		Lightening Persian
		Memphis berseem
		Paratta purple
		*Prima gland
		Rubitas red
		SA 15896 ball
		SA 20009 Moroccan
		SA 36400 spike
		SARDI Persian
		SARDI rose
		Sothis eastern star
		Tas 1698 striated
		Tas 2129 Lappa
		Tas1630/1807 cluster
		Tas511/348 Difuse
		Trikkala subterranean
		*Urana subterranean
	Fenugreek	Might
		SA 32200
		SA 32202
		*Wimmera sungold
	Hedysarium	SA 32504
	Lathyrus	Ceora
		ITA 8a
	Lotus	ornithopoides
	Medic	Angel strand
		Bindaroo button
		Caliph barrel
		Essex snail
		Herald strand
		Highlander rotata
		Orion sphere
		Paraponto gama
		SA 32612 phrygia
		SA 36809 spotted
		*SA 8774 spotted
		*Scimitar burr
		Tornafield disk

*All samples were grown at the primary research site is South Australia. Entries marked with an * were also grown at the field site in Western Australia*

Table 1b Annual and perennial grass and forb species and accessions included in the dataset

Lifecycle	Genus	Variety
Perennial grasses	Brome	Exceltas
		Gala
		Nandu
	Cocksfoot	Currie
		Howlong
		Megatas
		Uplands
	Fescue	Fraydo
		Quantum II
		Resolute
	Phalaris	Advanced AT
		Australian 2
		Holdfast GT
	Prairie grass	Matua
	Puccinellia	Menemen
	Ryegrass	AberMagic HSG
		Banquet II
		Bealey
		Drylander
		Victorian
	Timothy grass	SA 38843
	Velt grass	Mission
	Wallaby grass	Friend
		Trangie
	Wheatgrass	Dundas
Annual grasses	Barley	*Moby
	Fesper	
	Oat	*Winteroo
	Ryecorn	Southern Green
	Ryegrass	Concord
		*Dargo
		Eclipse
		Feast ll
		Maverick GII
		Progrow
		Safeguard
		Sungrazer
		Tama
		Turbo
		*Wimmera
		Zoom
	Triticale	Crackerjack2
	Wheat	Wedgetail
Perennial forbs	Chicory	Choice
		Commander
		Puna
		39441s
		42588s
		45310s
		C20-3a
	Plantain	Lancelot
		Tonic
Annual forbs	Canola	43Y85
		Hyola 50
		Taurus
	Chia	Black
		White
	Kale	Kestrel
	Quinoa	SA 45507
	Rape	Titan
		Winfred
	Turnip (forage)	Hunter
		*New York
	Wild turnip	SA 42783

*All samples were grown at the primary research site is South Australia. Entries marked with an * were also grown at the field site in Western Australia*

NIRS scanning and mathematical treatments

Spectra were collected using a Unity Spectrastar 2500X- rotating top window system (Unity Scientific). The spectrum file data from the Spectrastar was converted to a multifile for the chemometric software package Ucal (Unity Scientific) used to generate predictions.

NIR spectra can be influenced by particle size, light scatter and path-length variation and for this reason pretreatment of data can improve calibration accuracy (Deaville and Flinn 2000). During the project we tested a range of pretreatment options including standard normal variate detrending and derivatization with different derivative gap and smoothing. From this the best performing equations were selected. No wave specification trims were utilised and the entire available spectra from 680 nm to 2500 nm was employed. Outlier limits were left at default settings; T limit = 2.5, GD limit = 3.0 and neighbourhood size = 0.20. Partial least squares regression was used to develop the calibrations.

Preliminary calibration equations (year 1, SA site only)

In 2012, an initial cohort of 113 samples from the SA site was subject to chemistry. One hundred samples were used to develop the calibration and 13 were set aside for immediate validation. A further 44 samples were then selected (based on global and neighbourhood H values and diversity within the collection) and subjected to wet chemistry as an independent validation set to test the predictions. After assessing the predictive ability of this initial calibration, both validation sets were then used to expand the calibration database to establish a base calibration to predict the remaining cohort of samples from 2012.

Calibration equations in year 2 (SA and WA site)

During the 2013 season, the base 2012 calibration was used to predict incoming samples. Samples for wet chemistry were identified using global H and neighbourhood H values (selection of samples with high global H and neighbourhood H values), new species and the new WA site of collection

Table 2. Numbers and types of samples scanned and subject to chemistry.

		Broad calibration (samples from SA site)		Broad calibration (samples from WA site)	
		Scanned	Chemistry	Scanned	Chemistry
Annual	Legume	1175	408	300	77
	Forb	207	59	32	14
	Grass	374	101	73	12
Perennial	Legume	626	131		
	Forb	1111	136		
	Grass	487	88		

Chemistry on each sample included; total N, DMD, ADF, NDF, OM and total C.

Final calibration equations

A dataset of 910 spectra with chemistry that included samples across all groupings on both WA and SA sites for 2012, 2013 and 2014 was used to develop the mature calibration. Approximately half the dataset (n=460) was used to develop the calibration and the remaining half for independent validation (n=450). The final calibration equations were developed using partial least square regression with standard normal variates detrending and scatter correction. Wavelength bands from 680 – 2500 nm and a mathematical treatment of 1,6,6 (the first digit is the number of the derivative, the second digit is the gap over which the derivative is calculated, the third digit is the number of data points in the first smoothing). Cross validation was used to calculate the final standard error of cross validation (SECV).

In vitro gas fermentation calibration equations

A subset of 187 samples that had been subject to *in vitro* gas fermentationwas chosen from the South Australian cohort. A calibration was attempted for total methane (CH_4) produced during fermentation and both ammonia and total volatile fatty acids in the remaining rumen liquor. Seventeen samples were randomly selected and kept aside for an independent validation set.

Assessing predictive ability of equations

The performance of calibration equations was assessed using a number of criteria. Initially the r^2 value, 1-vr, SECV and RPD (relative percent difference) values. We then examined the r^2 and RPD values for validation samples (that were excluded from calibration development). RPD tests the strength of the relationship between a constituents values and the error of the NIR predicted results and was calculated by;

$$\mathbf{RPD = 1 / (1 - r^2)^{0.5}}$$ (Williams 2014)

The larger the RPD value the greater its strength. We have adopted the forage RPD guide of Williams (2014) who suggested RPD values of 0.0–1.9 are very poor and not recommended for forage testing; RPD values of 2.0–2.4 are poor and only of use for rough screening; RPD values of 2.5–2.9 offer a fair screening potential; RPD values of 3.0–3.4 are good (quality control); RPD values of 3.5–4.0 are very good (suited to process control) and RPD values of 4.1+ are deemed excellent.

Wet chemistry

In vitro dry matter digestibility (DMD), adjusted to predict *in vivo* digestibility, was determined in duplicate using a modified pepsin-cellulase technique described by Clarke *et al.* (1982). Modifications include different sample weight (600 mg), the use of ANKOM Technology F57 filter bags, sealed plastic boxes as incubation vessels (Tupperware liquid tight Clear Mate rectangle containers, L22.4 cm x W15 cm x H3.5 cm, with 16 samples per tray) and use of an orbital mixer incubator (set at 48^{o}C and 2RPMs). Duplicate samples of seven AFIA standards (AFIA 2007) with known *in vivo* DMD are included in each batch to allow raw laboratory values to be adjusted to predict *in vivo* digestibility using linear regression (see Fig. 1). The average standard error of the standards across the runs was 0.261%.

For the method, duplicate samples (600 mg) are weighed into the Ankom bags and heat sealed. 16 bags were prepared and placed into the incubation containers and covered by a light weight stainless steel mesh (2.5 mm x 2.7 mm x 0.7 mm) that has been cut to fit inside trays to allow movement but keep samples submerged. 300 ml of a freshly prepared pepsin solution was added to each incubation container (8.2 g pepsin in 1.23 L of 0.125N HCl). Trays were sealed and placed in a Ratek Orbital mixer incubator set on 48^{o}C at 2RPMs.

Following the acid-pepsin digestion phase the solution was drained from the trays and bags placed in a 5 litre plastic jar half filled with warm dH_2O and very gently swirled and drained through a lid with sieve insert. This was repeated and then a final gentle rinse was done using 500 mL of acetate buffer made up at a concentration of 4.1 g anhydrous sodium acetate, 2.9 mL acetic acid (conc.) per litre dH_2O. The buffer was drained and samples arranged in clean trays for the second stage. The same buffer was used to make up a working cellulase solution: 8.0 g Cellulase (Onozuka FA) in 1.20 L acetate buffer.

Trays were then returned to the incubator for the second phase of 48 hours digestion at 48^{o}C and 2RPMs. After the treatment time trays were removed to a 5 litre plastic jar half filled with warm dH_2O and very gently swirled and drained through a lid with sieve insert. Sample bags were then removed to an oven tray and dried in a drying oven at 90^{o}C for 48 hours then weighed to determine how much material was digested. Where presented, the energy value of the sample (MJ/kg at maintenance level of feeding) was estimated by the equation: M/D = (0.172*DMD) - 1.707 (Standing Committee on Agriculture, 1990).

Concentrations of neutral detergent fibre (NDF) and acid detergent fibre (ADF) of the shrub material were measured sequentially, according to operating instructions, using an Ankom 200/220 Fibre Analyser (Ankom® Tech. Co., Fairport, NY, USA). Duplicate samples were analysed for each diet. The difference between NDF and ADF was deemed to be hemicellulose. An oaten hay samples was

included in each of the 103 fibre analysis runs during the project. The QC had NDF of 30.19 ± 0.1137 % DM and ADF of 19.71 ± 0.0665 %DM. Total ash was measured on duplicate samples according to the methods of Faichney and White (1983). Total nitrogen and carbon was determined by combustion using a Leco CN628 N Analyser (Sweeney and Rexroad 1987).

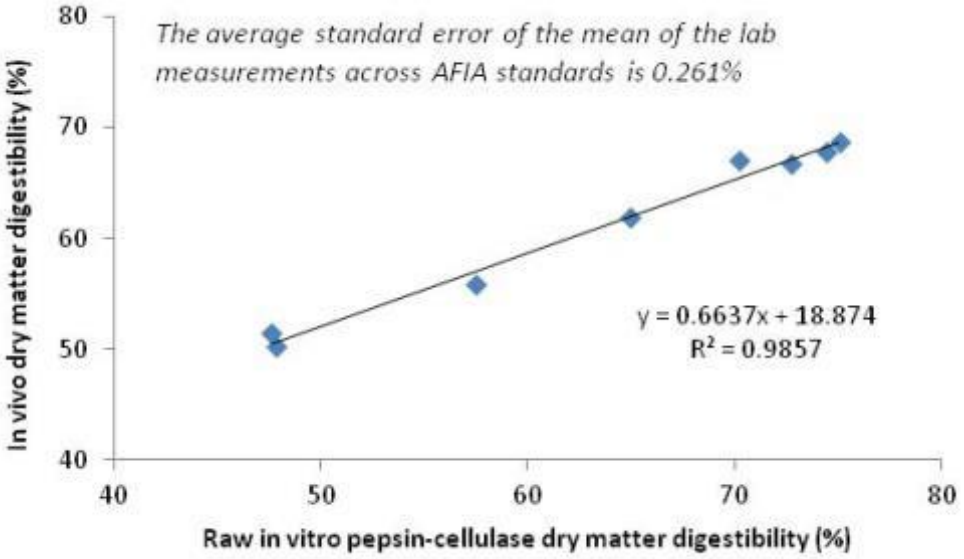


Fig 1. Mean predictions of AFIA standards across the project. Each *in vitro* mean was calculated from the data from 24 DMD laboratory runs throughout the project.

Plants were analysed for *in vitro* fermentability and methanogenic potential using an *in vitro* batch fermentation system (Durmic *et al.,* 2010). In each batch fermentation, five controls, including a negative batch control (rumen fluid only), positive batch control (oaten chaff + rumen fluid) and 3 AFIA pasture plant standards (lucerne, medic and vetch) were included in each run to identify differences in rumen fluid between runs. All samples were analysed over 7 separate batches, run on different days. Raw data for each sample (representing a plot in the field) was used for the prediction rather than analysed and adjusted means.

4.4 Results

Performance of multispecies calibrations

Table 3 presents the performance statistics for the multi-species equations. The calibration was generally successful as evidenced by the validation statistics. Total nitrogen was predicted with an RPD of 5.3, falling into the excellent category of Williams (2014). The mean error of prediction was 0.17 % (equating to about 1% CP). Throughout the project, N was the constituent that was predicted with the lowest errors.

Predictions of NDF were also excellent with an RPD of 4.3 and an error of 3.5 % units. The ADF predictions were very good with an RPD of 3.9 and error of 2.1 % units. DMD also fell into the very good category of Williams (2014) with an RPD of 3.7 and an error of 2.6 % units. Ability to predict OM seemed to decline markedly after the first year, with an RPD of 2.2 and an error of 0.85% units. We could not predict total C accurately using NIRS, probably due to a lack of diversity in total C between samples. The predictive power was much better in the first year across all traits, when samples from only the South Australian site were included.

Table 3 Performance statistics of the mixed species calibrations

Calibration	Trait	r^2	1-VR	SECV	RPD	r^2 (val)	RPD (val)
Year 1	NDF	0.961	0.947	2.90	5.1	0.959	4.9
	ADF	0.971	0.959	1.70	5.9	0.982	7.5
	DMD	0.967	0.947	2.40	5.5	0.971	5.9
	OM	0.914	0.863	1.20	3.4	0.976	6.5
	N	0.984	0.977	0.14	7.9	0.997	18.3
	C	0.891	0.835	0.67	3.0	0.853	2.6
Year 1&2	NDF	0.948	0.920	3.30	4.4	0.913	3.4
	ADF	0.966	0.948	1.80	5.4	0.926	3.7
	DMD	0.936	0.906	2.60	4.0	0.916	3.5
	OM	0.913	0.869	1.30	3.4	0.751	2.0
	N	0.976	0.959	0.18	6.5	0.968	5.6
	C	0.856	0.749	1.10	2.6	0.610	1.6
Final	NDF	0.941	0.918	3.50	4.1	0.945	4.3
Years 1-3	ADF	0.957	0.935	2.10	4.8	0.933	3.9
	DMD	0.937	0.916	2.60	4.0	0.926	3.7
	OM	0.905	0.851	0.02	3.2	0.794	2.2
	N	0.977	0.967	0.17	6.6	0.964	5.3
	C	0.713	0.634	0.71	1.9	0.495	1.4
Methane	CH_4	0.889	0.849	3.50	3.0	0.908	3.1
	NH_3	0.858	0.774	36.10	2.6	0.761	1.4
	VFA	0.839	0.783	5.00	2.4	0.800	1.3

Performance of the multispecies calibration across groups or individual species

Using the validation set, we investigated errors of prediction for each of the following groups; annual grasses, annual legumes, perennial grasses, perennial legumes and forbs. Table 4 presents the r^2 values and estimated RPD values derived from a regression of laboratory against predicted values using the final multispecies equations. Graphs for ADF and DMD are shown in Figs 2 and 3 respectively. Across all groups, prediction of total N was excellent. The equations performed best for the annual grasses and legumes and the forbs (mixed annual and perennial forbs). With the exception of OM, the RPD values indicate that we generated predictions for the annuals and forbs that are excellent (RPD > 4.1). The calibration resulted in very good predictions of ADF and DMD for the perennial grasses but for NDF only yielded a rough screening tool. For perennial legumes, ADF, DMD and OM performed very well but NDF was poor.

Table 4 Validation of global calibration with samples split into groups

Trait	Annual grasses		Annual legumes		Perennial grasses		Perennial legumes		Forbs	
	r^2	RPD	r^2	RPD	r2	RPD	r^2	RPD	r^2	RPD
NDF	0.951	4.5	0.955	4.7	0.818	2.3	0.767	2.1	0.980	7.1
ADF	0.956	4.8	0.973	6.1	0.927	3.7	0.880	2.9	0.983	7.7
DMD	0.964	5.3	0.959	4.9	0.927	3.7	0.887	3.0	0.981	7.3
OM	0.920	3.5	0.907	3.3	0.790	2.2	0.930	3.8	0.880	2.9
N	0.986	8.5	0.980	7.1	0.987	8.8	0.966	5.4	0.984	7.9

These groups are broken down further in Fig. 4 where we present measured versus predicted values for 5 randomly selected individual species. They include Biserrula (an annual legume), canola (an annual forb), Italian ryegrass (an annual grass), sainfoin (a perennial legume) and tall wheatgrass (a perennial grass). For all species the r^2 values were above 0.92 and it is clear from the graph, that NIR offers a good screening tool even within species.

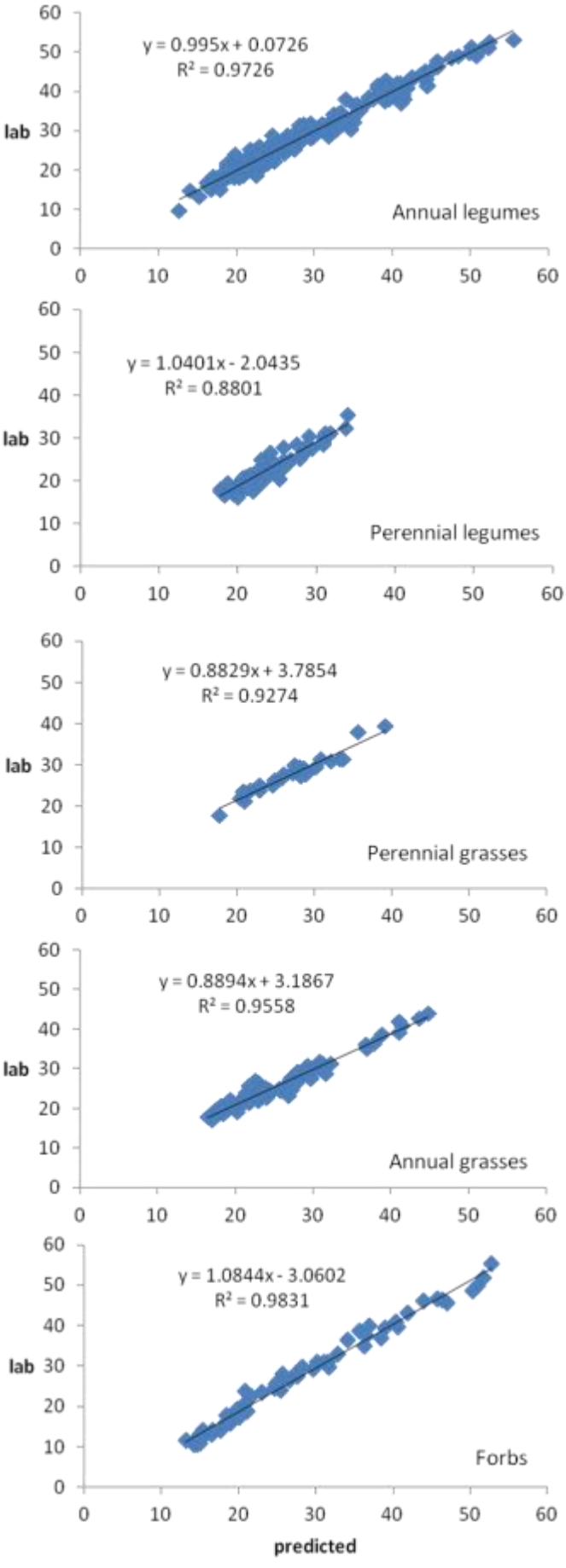


Fig 2. NIRS predicted plotted against measured acid detergent fibre for validation samples that were not used in calibration development

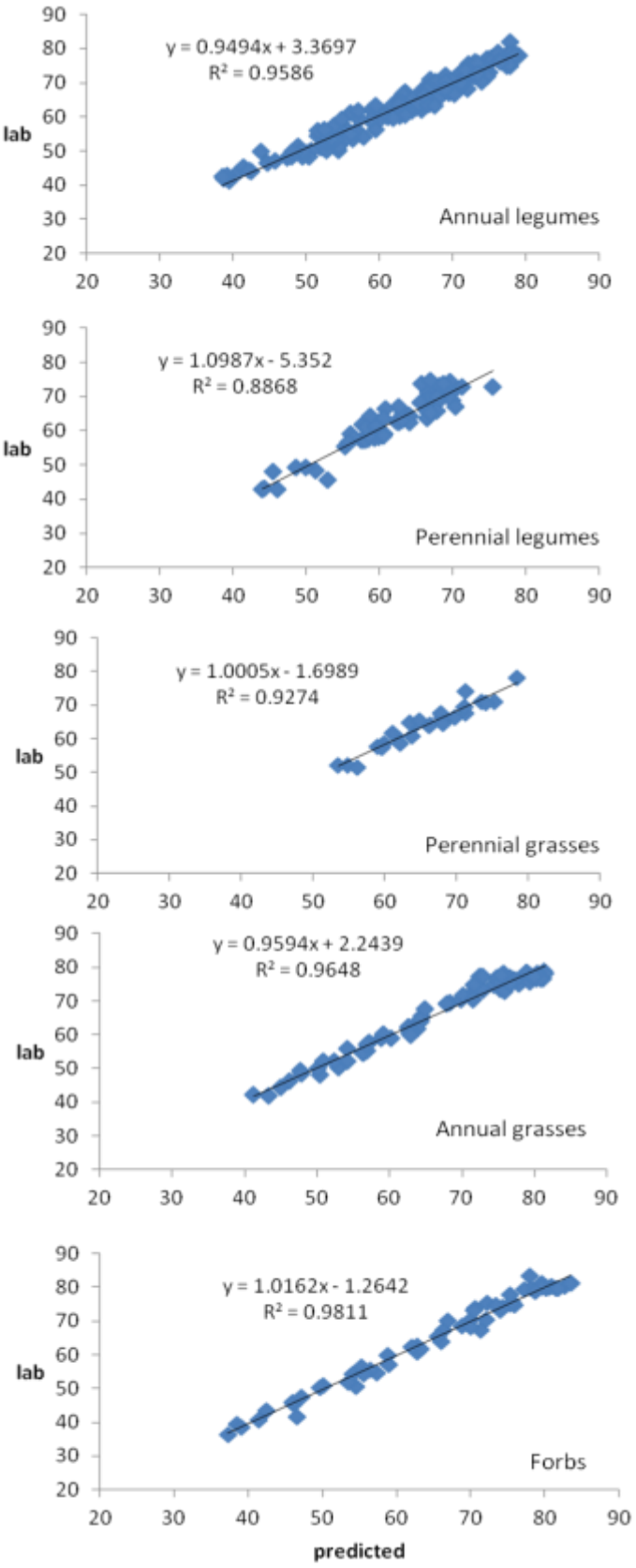


Fig 3. NIRS predicted plotted against measured dry matter digestibility for validation samples that were not used in calibration development

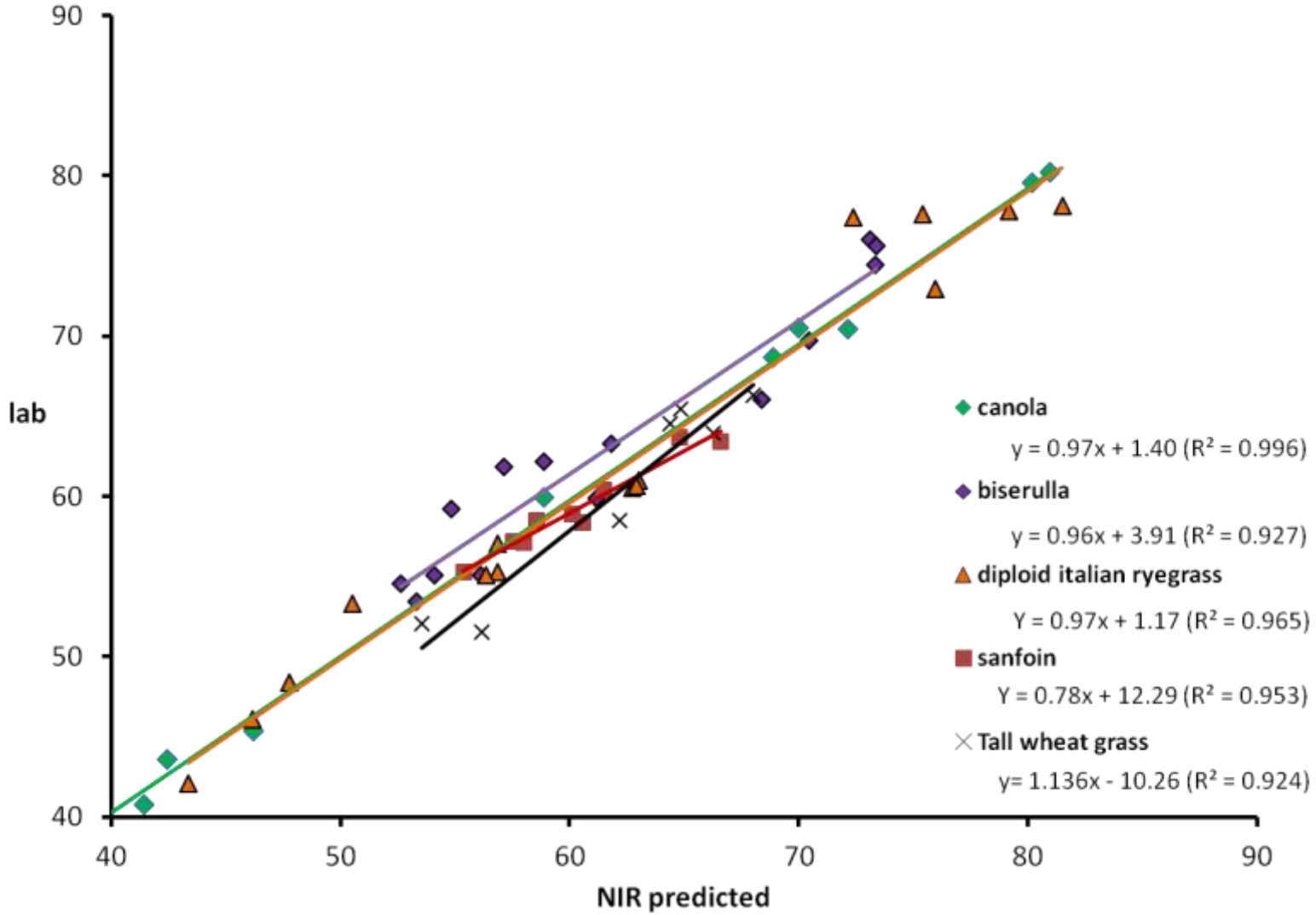


Fig 4. NIRS predicted plotted against measured dry matter digestibility for samples from 5 randomly selected species not used in calibration developmen

Performance of methane calibrations

While we developed a good calibration for predicting total methane produced during 24h fermentation in rumen fluid (RPD 3.1 secv 0.85 ml), we did not have success with ammonia or total VFA's (Table 3, Fig 6).

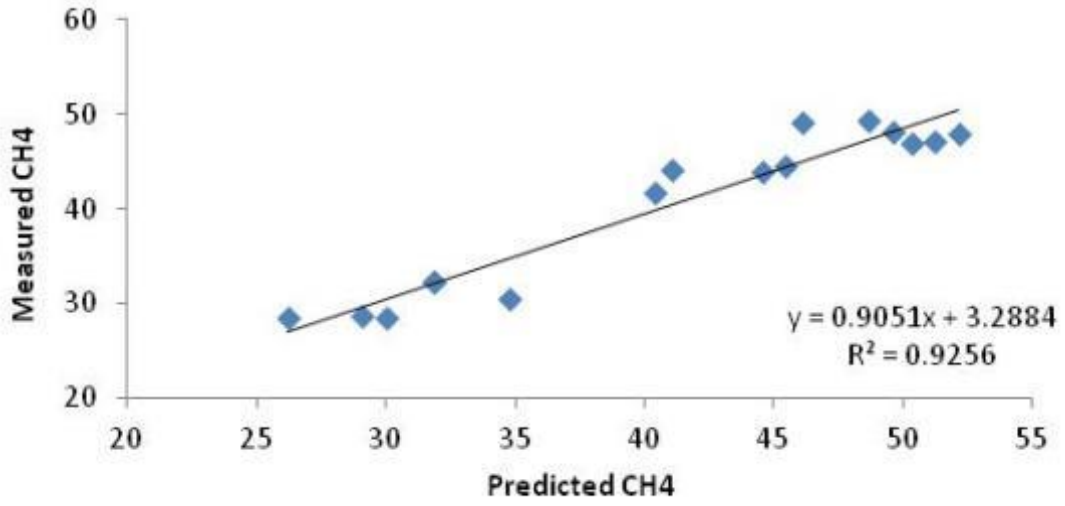


Fig 6. NIRS predicted values plotted against measured values of methane produced during 24 hour batch culture with rumen fluid

4.5 Discussion

The data presented in this paper suggest that a large, robust NIRS calibration for nutritional value of the southern feedbase of Australia is feasible, thus supporting our hypothesis. While the literature would suggest that increasing diversity leads to stronger calibrations (Shenk and Westerhaus, 1993), the diversity between species in this data set was much larger than others reported in the literature with inclusion of 109 species across a number of plant families. In the first year, with only 180 samples with matched NIR spectra and chemistry, we generated excellent calibrations. This is close to the recommended number of 150 samples as suggested by Deaville and Flinn (2000). Andueza *et al.* (2011) explored the development of species specific, family specific and global calibrations using samples of 5 temperate grasses and two legumes. They found that for CP, errors were similar.However for OMD increasing diversity led to greater errors of prediction.

The accuracy of predictions from the calibrations declined after the first year as we increased the diversity of the sample range with a second season in South Australia and a new site in Western Australia. The samples added in the second two years of the study were all from species that were already represented in the first year calibration. This highlights the need to include spatial and temporal diversity within the dataset if calibrations are to be used beyond the reference sample collection sites. Calibration populations must encompass all sources of variation likely to be found in future unknown samples of similar material (Windham *et al.,* 1989, Deaville and Flinn 2000). If this calibration is to be developed further, we would seek to build spatial diversity by including samples from other sites in southern Australia. For our final calibration, we split the reference samples into two groups for calibration and validation. Stronger calibrations may arise from utilising the majority of samples for calibration and fewer for validation.

These calibrations represent a useful tool for livestock industries in Southern Australia as they are likely to encompass nearly all of the species that could appear in monocultures or mixed swards across all of their lifecycles. We have developed a calibration that encompasses for all of the major pasture and forage varieties, including crops that are grazed and emerging

pasture species. Inexpensive and rapid prediction of the nutritional value of pastures assists producers to optimise grazing management and growth rates of young stock. This may lead to increased profitability and reduced methane emissions intensity if animals reach slaughter weight faster with less feed inputs. Development of accurate calibrations can be very useful in plant breeding and selection programs where large numbers of plants require assessment of their nutritional value. Using these equations we assessed chicory plants in a breeding experiment. By the 5th cut, the best accession had DMD of 70 % and CP of 15.7% while the poorest had DMD of 58.9% and CP of 11.9%. These differences have a profound impact on the potential productivity of sheep. Using Grazfeed to estimate intake and growth rates of mature ewes, the better accession would be consumed at a rate of 1.15 kg DM/day and lead to approximately 50 g of growth. Intake of the poorer quality accession would be less than 1 kg DM/day and the dry ewe will just maintain liveweight (Peck *et al.*, Section 5 of this report). The NIRS database also provides an opportunity for producers to measure improvements in the feedbase (or estimate total methane outputs from the feedbase) for future carbon reduction schemes. Although not tested in this experiment, NIRS may be used to estimate pasture composition in mixed swards using both feed and faeces (Coates *et al.*, 1987; Chataigner *et al.*, 2010). This could also support carbon reduction schemes if calibrations are developed to estimate species with known antimethanogenic potential (eg *Biserrula*) or quantity of antimethanogenic compounds such as nitrate in the sward.

A critical factor leading to success of this work has been the quality of the laboratory data behind the calibration (Deaville and Flinn 2000). Not all differences between NIRS predictions and reference values can be ascribed to NIRS prediction error (Coates 2002) as the error sources of the reference method are incorporated into the model (Murray, 1993). By using a single, highly trained laboratory operator and adoption of a range of quality control samples, we managed to keep lab errors to a minimum (*in vitro* DMD 0.23 %), NDF (0.11 % and ADF (0.7%). While it would be better to develop calibrations with samples of known *in vivo* digestibility, these samples are expensive to generate and it is difficult to produce samples at the extreme ends of the spectrum due to animal welfare concerns with very poor or very fermentable diets. We feel that our approach, by using a broad range of samples with known *in vivo* digestibility to calibrate our laboratory *in vitro* digestibility, is a good compromise. Laboratory calibration samples can be matched to the samples of interest to improve the prediction of DMD, for example we utilise the AFIA standards for temperate forages and our own forage shrub standards for native saltbushes. Our inability to develop good predictions for NDF in perennial legumes is a good example of the importance of sound laboratory work. We find that replicate samples during NDF runs have much greater variances for perennial legumes than for annual legumes or grasses. This variance is associated with the method and is not discernible after the subsequent ADF phase. Addressing this laboratory analysis issue is critical if we desire better calibrations for NDF in perennial legumes.

The current data set with over 1000 samples with matching scans and chemistry provides an excellent platform for future refinement or generation of calibrations for new traits. For future forage breeding or selection programs, use of multivariate statistics allows for narrowing of the database to select reference samples that are similar to the target species as a starting point for a new calibration. Equally, the large diversity can be used to develop sample-specific PLS models that are developed for each prediction sample (Schenk *et al.,* 1997). All reference samples from this study have been vacuum sealed and stored in dry conditions at 4^{o}C. This allows inclusion of other nutritional traits of interest in the future, examples could include; water soluble carbohydrates, aspects of rumen fluid fermentation kinetics, protein degradation rates, minerals (to date Ca, P, K and Mg have been predicted with some degree of success) or plant secondary compounds such as pectin (perennial legumes), oxalates (perennial grasses) or nitrates.

Use of hand held or machine mounted spectroscopy can allow for near real-time estimation of nutritional traits and reduce labour as samples may not need to be dried and ground. Use of mobile devises to send NIR data to remote laboratories is increasing. Ongoing research is investigating the tradeoffs in the use of fresh and unground material.

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Section 5 Intraspecies diversity of yield, persistence, nutritive value and *in vitro* methane production using forage chicory

5.1 Abstract

Forage chicory is a summer active perennial pasture that can provide a range of benefits to livestock producers in southern Australia. However current chicory cultivars have limitations in Australia and several researchers have recommended that breeding of chicory for Australian conditions be undertaken. In the current study, we measured the herbage production, nutritive value and *in vitro* methane production over one year on individual spaced plants of six chicory cultivars, nine wild accessions and genotypes that have been selected for persistence and grazing tolerance. We studied spaced plants to determine how much variation exists for these key traits within and between chicory populations. Puna, Puna II, Choice and several wild accessions had greater persistence than cultivars Commander, Grouse and Le Lacerta. The cultivars also differed in seasonal dry matter (DM) distribution, with Choice being the only cultivar to provide high yields throughout the year. The wild accessions were low yielding, but genotypes selected from the wild cohort were high yielding. High nutritional value of chicory was confirmed with mean dry matter digestibility of 75.1, 63.7 and 72.4 % in spring, summer and autumn respectively. A large amount of variation between and within cultivars, accessions and selections was found for all nutritional parameters, however the differences in their methanogenic potential were limited. Plant breeders will be able to exploit this variation for developing new chicory cultivar/s for Australian conditions which will increase the value of this species to a broader range of livestock producers.

5.2 Introduction

Chicory (*Cichorium intybus* L.) is a component of natural grasslands in many parts of the world, but has only a relatively recent history as a forage crop and as a component of perennial pastures (Li and Kemp 2005). Grasslands Puna was the world's first forage cultivar when released in New Zealand in 1985, and since then has been widely grown around the world. Chicory is highly productive, especially in the warmer six months of the year (Kemp *et al.,*2002, Ward *et al.,*2013) and it is more productive than lucerne on acidic soils (Li *et al.,*2008). Chicory is a deep rooted summer active perennial herb that can reduce ground water recharge when out of season summer rainfall occurs and hence reduce the risk of dry land salinity (Li *et al.,*2008, Reed *et al.,*2008). Climate change predictions for southern Australia is for lengthening of the dry summer period but with the proportion of rainfall during summer increasing (Moore and Ghahramani 2013) and hence chicory is likely to become more important to farming systems in southern Australia.

Chicory is also a high quality pasture (Barry 1998, Hayes *et al.,* 2010, Clark *et al.,*2013) that provides benefits to grazing animals in summer-autumn relative to other pastures (Michalk *et al.,*2003, Holst *et al.,*2006). Animal performance on chicory is similar to that on legumes and superior to grass-based pastures (Li and Kemp 2005). Unlike some other forage legumes, it does not cause bloat in cattle (Barry 1998) and cows grazing chicory in summer produce more milk than the cows grazing perennial grasses (Dairy Australia). In the central tablelands of New South Wales (Australia) chicory has the capacity to finish lambs consistently to market specification (Michalk *et al.,*2003, Holst *et al.,*2006). Another advantage of chicory is its capability to reduce methane emissions from ruminants grazing this forage (Waghorn *et al.,*2002, Roca *et al.,*2010). Methane is a potent greenhouse gas, with agriculture being the dominant source of anthropogenic methane emissions in Australia and ruminant livestock production contributes 52% of the total anthropogenic methane (Finn *et al.,*2015). Methane is also considered an energy loss in the animal, and diverting energy away from methane should result in an increase animal growth rate, productivity and efficiency (Johnson and Johnson, 1995).

Taking into account both nutritional and agronomic considerations, chicory is considered one of the best emerging plants for grazing livestock (Ramirez-Restrepo and Barry 2005). However chicory in Australia has poor persistence (Hayes *et al.,*2006, Clark *et al.,*2013, Li *et*

*al.,*2010, Ward *et al.,*2013) and it has been recommended that breeding of chicory for Australian conditions should be done (Dear *et al.,*2008, Li *et al.,*2010, Hayes *et al.,* 2010). Li *et al.,* (2010) evaluated the DM and persistence of cultivars and wild accessions in Australian conditions. However systematic studies that examine all traits (agronomy, nutritive) in a wide set of genotypes are lacking. The aim of this study was to examine diversity in DM, persistence, nutritive value and methane production between and within cultivars and wild accessions.

5.3 Materials and Methods

Location and soil type
The field site was located in the Australian Pastures Genebank field nursery, at the Waite Institute, in South Australia. The fine sandy loam at this site is a red-brown earth (Stace *et al.,* 1968) of the non-sodic Urrbrae series (Litchfield 1951). The upper 0.10 m contains 18% clay, increasing to 32% in the A2 horizon (Prescott 1931). Soil pH (in $CaCl_2$) was 6.2 and there was negligible calcium carbonate (Grace *et al.,*1995). The site was rain fed from planting until summer when it was irrigated by sprinkler to mean Adelaide rainfall on a monthly basis.

Germplasm
Table 1 lists the cultivars, accessions (wild), selections from cultivars and accessions used in the experiment, country of origin, number of plants grown and number of plants that died during the experiment. Selections are listed under the cultivar that they were selected from. Selections 1-7 were from a heavily grazed chicory trial at Turretfield South Australia and propagated by plants; selections 8-12 were from a rotationally grazed site in southeast South Australia and propagated by plants; selections 13-14 were from a heavily grazed site and propagated by seed. Selection criteria were persistence and production under grazing by sheep. Cultivars, accessions and selections 13-14 had seeds sown into jiffy pots while selections 1-12 were plants dug up from existing field experiments, leaves trimmed off and transplanted directly into the field. Plants were grown 60 cm apart in an 18 x 20 grid.

Table 1: List of cultivars, accessions and selections (S) from within cultivars, propagation method, country of origin, number of plants grown and number of plants that died during the experiment.

Group	propagation	Origin	plants grown	dead plants
Puna				
cv	seed	New Zealand	40	4
S1	plant		14	0
S8	plant		10	0
Puna II				
cv	seed	New Zealand	40	2
S2	plant		5	7
S9	plant		10	0
Choice				
cv	seed	New Zealand	40	7
S3	plant		18	11
S10	plant		9	3
Commander				
cv	seed	Italy	20	9
S4	plant		2	1
S11	plant		4	3
S13	seed		10	7
Grouse				
cv	seed	New Zealand	31	16
S5	plant		4	4
S12	plant		5	3
Le Lacerta				
cv	seed	Uruguay	20	13
wild				
39415		Italy	10	0
41976	seed	Azerbaijan	2	2
42211	seed	Azerbaijan	1	0
42588	seed	Australia	11	0
42961	seed	Australia	10	0
45310	seed	France	1	1
39441	seed	Afghanistan	10	2
S14 (39441)	seed		30	2
S6 (38955)	plant		2	1
S7 (39000)	plant		1	0

Plant sampling

On the 3 October 2013, 30 October 2013, 26 November 2013, 9 January 2014, 18 February 2014, 14 April 2014 and 2 June 2014 (cuts 1-7 respectively) each plant was scored for growth development stage (1-10, 1 leaf only, 10 open flower) and then hand cut with a set of shears to the crown of the plant. Fresh weight was recorded and 150-200 g subsamples were dried in an oven at 80°C for cuts 2, 3, 4, 6 and freeze dried for cuts 1, 5 and 7 to determine dry weight. Plants that died were recorded as 0 g drymatter.

Pasture Quality
Pasture quality was determined on each plant of cuts 1, 5 and 7 (spring, summer, autumn). After collection and weighing, biomass samples were immediately frozen for freeze drying. After drying, samples were ground to pass through a 1mm screen using a Retsch Twister mill grinder mill.

NIRS
Nutritional value was estimated using chemistry and near infrared spectroscopy (NIRS; see review by Deaville and Flynn, 2000). Samples were scanned by NIRS (Unity Spectrastar 2500X- rotating top window system, Unity Scientific) and nutritional traits were predicted calibrations generated using partial least squares regression with the chemometric software package Ucal (Unity Scientific). Subsets of samples were set aside for chemical analysis. The broad, multispecies NIRS calibration for DMD, total N, ADF, NDF and OM (ash) was built on over 1000 samples with matching spectra and chemistry (see Norman *et al.*, accompanying paper). Where a sample was analysed with chemistry (~10%), that data is used, otherwise data are from NIR predictions. Samples that did not fit within the calibration (as indicated by high global H and neighbourhood H values) were subject to chemistry.

The RPD statistic tests the strength of the relationship between a constituents values and the error of the NIR predicted results and was calculated by; RPD = 1 / $(1 - r^2)^{0.5}$ (Williams 2014).

The larger the RPD value the greater its strength. We have adopted the forage RPD guide of Williams (2014) who suggested RPD values of 0.0–1.9 are very poor and not recommended for forage testing; RPD values of 2.0–2.4 are poor and only of use for rough screening; RPD values of 2.5–2.9 offer a fair screening potential; RPD values of 3.0–3.4 are good (quality control); RPD values of 3.5–4.0 are very good (suited to process control) and RPD values of 4.1+ are deemed excellent.

Samples within this paper were predicted with the following accuracy; total N - r2 0.98, RPD 7.9, *in vitro* DMD - r2 0.98, RPD 7.7, OM (ash) - r2 0.88, RPD 2.9, ADF - r2 0.83, RPD 7.7 and NDF - r2 0.98, RPD 7.1. OM predictions are not as accurate as those for other traits, falling into the 'fair screening potential' category of Williams (2014) and data interpretation for this trait should proceed with a degree of caution.

Chemistry *In vitro* dry matter digestibility (DMD), adjusted to predict *in vivo* digestibility, was determined in duplicate using a modified pepsin-cellulase technique described by Clarke *et al.,* (1982). Modifications are outlined in Norman *et al.,* (accompanying paper) Duplicate samples of seven AFIA standards (AFIA 2007) with known *in vivo* DMD are included in each batch to allow raw laboratory values to be adjusted to predict *in vivo* digestibility using linear regression. The average standard error of the AFIA standards across the runs was 0.261%. The energy value of the sample (MJ/kg at maintenance level of feeding) was estimated by the equation: M/D = (0.172*DMD) - 1.707 (Standing Committee on Agriculture, 1990).

Concentrations of neutral detergent fibre (NDF) and acid detergent fibre (ADF) of the plant material were measured sequentially, according to operating instructions, using an Ankom 200/220 Fibre Analyser (Ankom® Tech. Co., Fairport, NY, USA). Duplicate samples were analysed for each diet. The difference between NDF and ADF was deemed to be hemicellulose. An oaten hay samples was included in each of the 103 fibre analysis runs during the project. The QC had NDF of 30.19 ± 0.1137 % DM and ADF of 19.71 ± 0.0665 %DM.

Total ash was measured on duplicate samples according to the methods of Faichney and White (1983). Total nitrogen and carbon was determined by combustion using a Leco CN628 N Analyser (Sweeney and Rexroad 1987). Where crude protein has been presented we have adopted the convention of CP = total N x 6.25.

In vitro methane and gas production
Plant material was processed and tested in the IVFT as described in Section 3 where all of the *in vitro* work undertaken in ELLE is reported in detail.

The methane production of a diverse subset of chicory plants from cut 1 (n=34) and cut 5 (n=35) was measured. The subset consisted of randomly selected plants from all cultivars and accessions propagated from seed (if there were no plants propagated from seed available we used plants propagated from plants). In addition to the samples above, we obtained chicory samples from earlier work within ELLE (reported by Humphries *et al.,* within this final report as the ELLE perennial pasture paper). Humphries *et al.,*grew a diverse range of perennial grasses and herbs (including chicory) in small swards (planted 11/8/2012) at the Waite institute. We obtained freeze dried samples of chicory cultivar Puna (sampled on 5th March 2013, 24th June 2013, 28th August 2013, 24th October 2013, 17th December 2013, 22nd January 2014 and 27th February 2014) and determined methane production.

Leaf Measurements
On the 9/1/2014 a representative mature leaf was cut off and the level of leaf incision scored (0 no incision and 5 incision to the midrib) and leaf area measured.

5.4 Results

This research was part of the NLMP and basic data will be available in the NLMP database.

Persistence
Puna, Puna II, Choice and many of the wild accessions had greater persistence than the other cultivars (table 1, Fig. 1). Selections had similar persistence as the cultivars/accessions that they we selected from. The plant deaths occurred from mid-summer onwards.

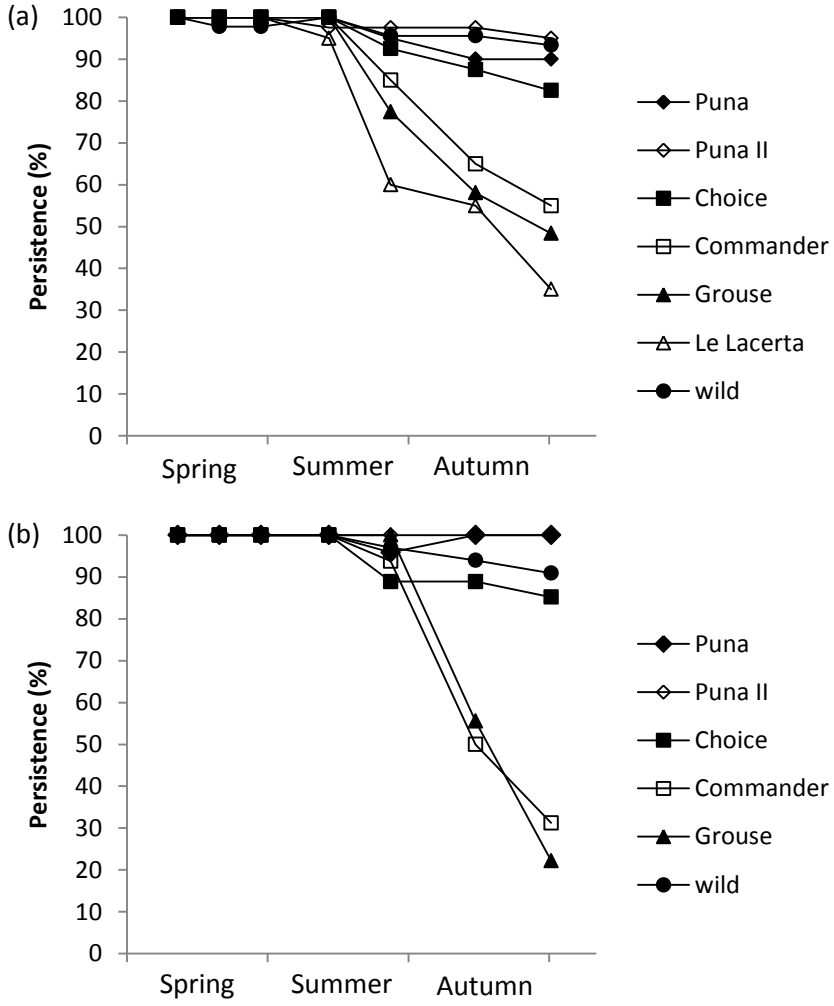


Fig. 1: Persistence of cultivars and wild accessions (top) and selections from within cultivars and wild accessions (bottom).

Plant development and DM production

Plant development score and DM production of cultivars, individual accessions and selections throughout the year are provided in table 2 and DM production of groups throughout the year is provided in Fig. 2. Choice produced high DM throughout the year and the highest DM overall; Puna and Puna II had low yield in the early cuts and high yields in later cuts; Commander, Grouse and Le Lacerta produced high DM in the early cuts and low yields in later cuts; Wild were low yielding throughout the year. The selections were higher yielding than the cultivars they were selected from,

Table 2: Plant development score (Dev) and DM (g/plant) produced from successive cuts of cultivars and selections from within cultivars (wild is accessions).

Group	Cut 1		Cut 2		Cut 3		Cut 4		Cut 5		Cut 6		Cut 7	
	Dev	DM	Dev	DM	Dev	DM	Dev	DM	Dev	DM	Dev	DM	Dev	DM
Puna														
cv	1	17	1.1	21	3	24	3.7	37	4.2	25	2.2	36	1	20
S1	1	31	1.2	29	3	27	4	42	3.9	28	1.9	35	1.1	20
S8	1	36	1.2	35	3	39	4.2	59	3.2	38	1.3	52	1.2	30
Puna II														
cv	1	18	1.3	19	3	19	3.2	31	3.2	25	1.9	38	1.1	22
S2	1	30	1.2	38	3	43	4.6	63	3.9	39	2.7	53	1.4	30
S9	1	34	1.5	31	3	28	4.7	42	3.5	24	1	33	1	22
Choice														
cv	1.2	36	1.7	43	3	36	4.4	59	4.8	30	2.7	34	1.3	21
S3	1	41	1.3	41	3	45	4.9	75	5.2	42	2.8	46	1.3	24
S10	1	62	1.1	55	3	47	4.7	77	3.9	38	1.9	47	1.3	26
Commander														
cv	1	38	1.4	48	3	41	4.8	65	6	18	5	19	2.9	8
S4	1	56	1	53	3	73	4	114	4.5	51	1	28	1	17
S11	1	91	1.6	65	3	46	4.8	91	6	33	5.8	18	2.5	6
S13	1.3	48	1.8	54	3	42	5.5	64	5.9	20	5.1	13	2	7
Grouse														
cv	1.4	44	2.2	56	3	36	5.5	48	5.9	15	5.3	14	3.2	5
S5	1.5	87	2.1	63	3	43	5.8	63	4.3	5	6	3	4	0
S12	1	47	1.7	40	3	37	5.3	52	5.8	21	4.9	13	1.5	3
Le Lacerta														
cv	1.2	54	2	51	3	32	5.5	46	6	8	5.9	7	3.5	2
wild														
39415	1	11	1	12	3	15	3.8	35	4.3	17	2	24	1.4	15
41976	1.5	73	2.3	88	3	45	5.8	73	6	26	6	17		
42211	1	38	2	48	3	54	6	53	6	16	6	20	3	3
42588	1.2	29	2.6	42	3	24	5.2	36	5.7	19	4.5	30	2.1	15
42961	1	8	1.1	13	3	19	5.9	20	5.9	9	5.5	13	1.5	6
45310	1.5	9	1.9	19	3	12	3.3	14	3.5	13	3.3	26	2	9
39441	1.4	12	2.3	21	3	14	5.3	16	5.4	9	5.3	15	3.9	8
S14 (39441)	1.2	41	1.8	43	3	38	5.3	66	5.7	30	4.2	35	2.1	20
S6 (38955)	1	32	1	32	3	43	5.2	98	4.5	37	1	26	1	8
S7 (39000)	1	29	3	52	3	46	6	64	6	47	5	38	1	16

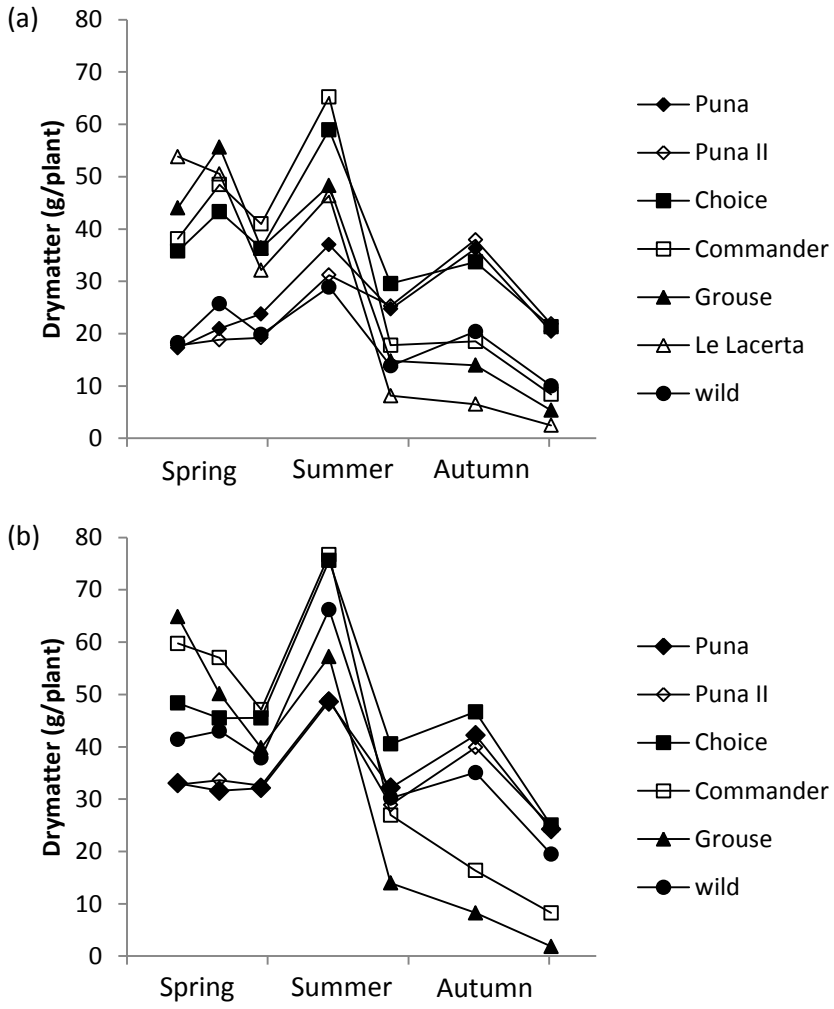


Fig. 2: Mean DM (g/plant) of different groups (a) cultivar/accession; (b) selections.

with the benefit diminishing over time. The higher early growth from selections is likely to be due to being propagated from plants, whereas cultivars were propagated from seed. Selections from wild accessions had the greatest genetic gain, for example S14, had much higher DM than SA39441 (note both propagated by seed) and had the highest total yield of those propagated by seed.

There was a large variation for DM production within each cultivar/accession and selection (Fig 3). The maximum DM is much higher than the median DM. For many of the selections the maximum yield and/or median yield are higher than that for the cultivars that they were selected from. Most of the selections were propagated by plants whereas the cultivars were propagated from seeds. However selections from SA39441 were propagated from seed and had plants with much higher DM than SA39441 (Fig 4). The variation within selections of SA39441 is much higher than in SA39441 itself.

Plant development was highest at cut 4, 5 and 6 (Table 2, Fig. 5) with lower levels recorded for Puna II and Puna for cut 5 and 6 and lower levels for Choice at cut 6. Similar to DM production large variation exists within cultivars, accessions and selections (data not presented).

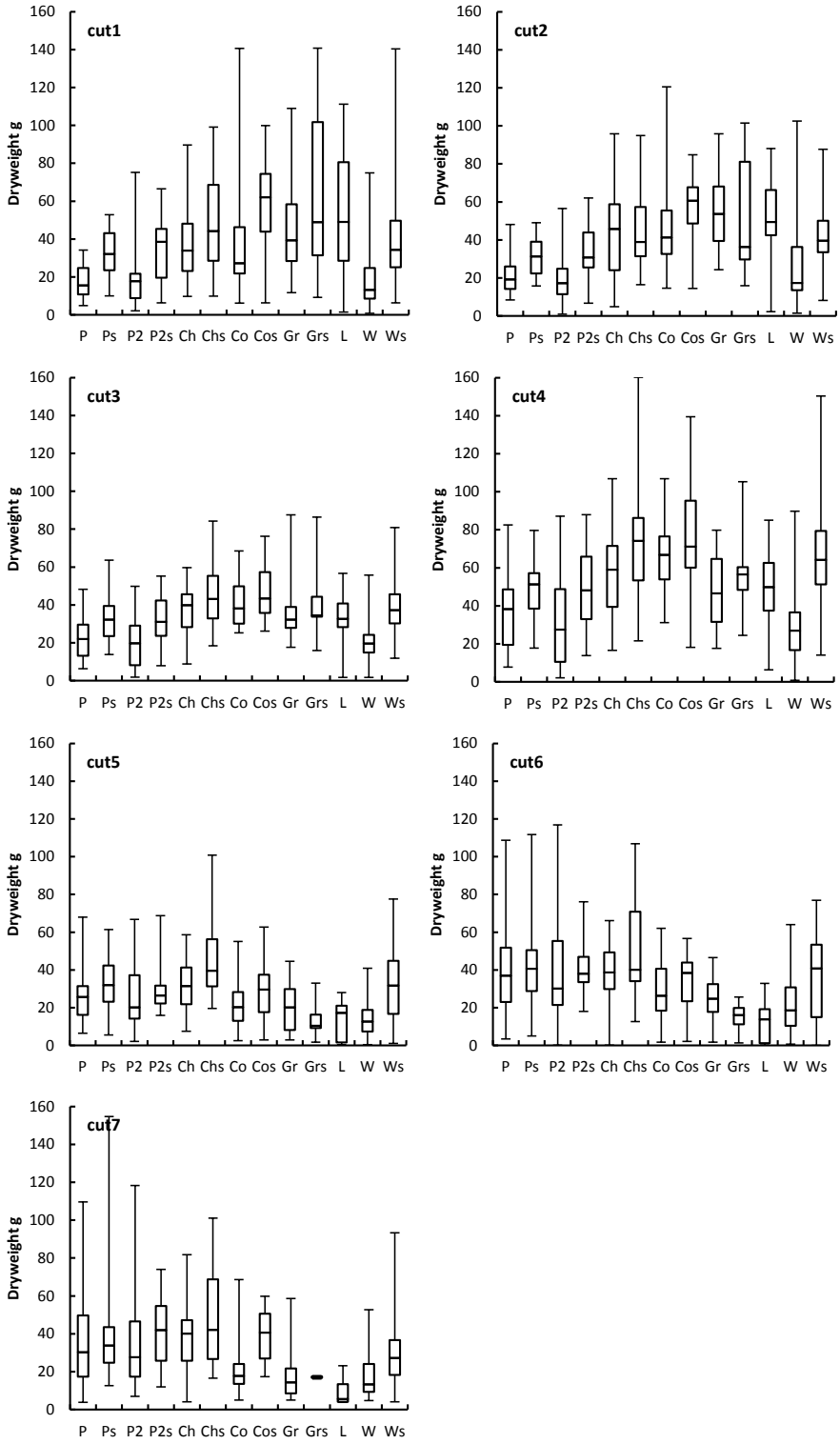


Fig. 3: Boxplots showing the variation in plant dry weight (g/plant) of different cultivars (P=Puna, P2 =Puna II, Ch=Choice, Co=commander, Gr=grouse, L=Lacerta, W=Wild accessions) and selections (s) from within cultivars.

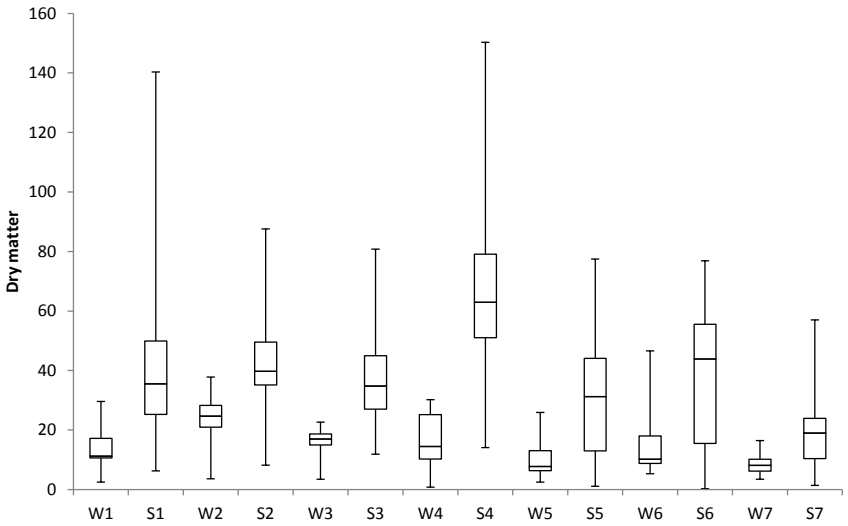


Fig. 4: Boxplots showing the variation in DM (g/plant) production of wild accession SA39441 (W) and selections (S) made from SA39441 for seven cutting times (1-7).

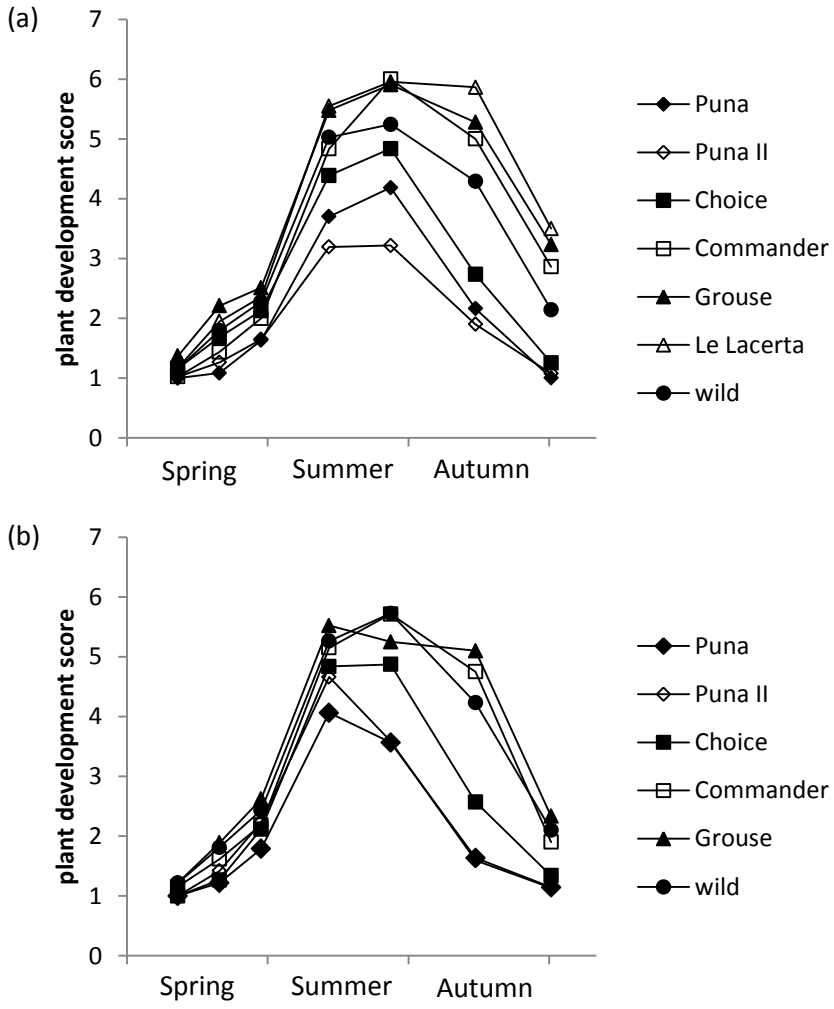


Fig. 5: Plant development scores, cultivars (a) and selections (b).

Table 3: Mean dry matter digestibility (DMD), crude protein (total N x 6.25), ash, neutral detergent fibre (NDF) and acid detergent fibre (ADF) content of chicory accessions at cuts 1, 5 and 7.

	DMD (%)			Crude Protein (% DM)			Ash (% DM)			NDF (% DM)			ADF (% DM)		
Group	cut 1	cut 5	cut 7	cut 1	cut 5	cut 7	cut 1	cut 5	cut 7	cut 1	cut 5	cut 7	cut 1	cut 5	cut 7
Puna cv	76	66.8	73.8	20.8	15.1	19.9	14.8	14.5	18	22	32.8	24	14.4	23.8	16.9
Puna sel	75.1	70.1	73.7	20.4	15.7	19.3	15.5	16.2	18.6	22.9	29.6	25.3	15.7	20.9	17.7
Puna II cv	75.7	68.8	73.7	20.4	15.8	20.1	15	15.2	18.3	22.2	30.6	25.1	14.5	21.6	17
Puna II sel	74.8	67.4	73.5	19.9	14.9	20	15.3	15.2	18.3	23.1	32.1	25.9	15.9	23.6	17.5
Choice cv	75.1	66	73.3	19.4	13.5	18.7	14.8	15.2	18.1	22.7	34.4	25.3	15.5	25.1	17.7
Choice sel	74.9	64.4	73.5	20	13.3	19.4	15.2	14.1	19.1	23.3	37.2	26.4	16	27.2	18.2
Comm. cv	75.7	58.9	70.4	19.3	11.9	17.5	14.4	12.2	18.6	22.1	45.2	33.6	14.1	31.4	21.3
Comm. sel	75	61.1	72.9	18.1	12.1	18.1	15.1	13.7	18.6	23	42.3	24.7	15.5	30.1	17.5
Grouse cv	75.2	59.2	71.8	18.3	11.9	18.1	14.2	12.5	18.9	22.5	43.9	30.2	15.3	31.5	20
Grouse sel	74.4	60	72.8	19.8	12.5	21	15.2	13.5	18.2	24.3	43.4	30.4	16.1	31	19.6
Lacerta cv	74.7	60.9	70.5	18.2	11.5	16.8	14.4	12.2	17.4	22.8	40.4	33.8	15.6	30.1	22.3
wild cv	74.3	59.9	71.4	20.7	12.3	19.8	15.7	11.9	18.8	25.4	43	30.8	16.6	31.1	20.2
wild sel	74.6	60.4	71.3	19.6	12.5	18.6	16.1	12.9	19.3	24.6	42.5	29.3	16.7	31	20.1
Grand mean	75.1	63.7	72.4	19.8	13.4	19.1	15.1	13.8	18.5	23.2	37.7	27.7	15.5	27.2	18.8
LSD	0.85	3.9	1.52	1.56	1.59	1.27	1.16	1.91	1.01	1.43	4.87	2.33	0.94	3.7	1.43
P value	***	***	***	***	***	***	***	***	***	***	***	***	***	***	***

Nutritional value
The nutritive value data is presented in table 3. Cut 5, which had more plants with high development score (Table 2, Fig 5), had lower DMD, CP, N, and higher ADF and NDF, than the other two cuts. Puna, Puna II and Choice had lower development scores in cut 5 and the quality measurements were different from the other cultivars in cut 5. ASH was high in cut 7 and a trend for Puna, Puna II and Choice to be high in cut 5.

The DMD of the cultivars and selections in the three seasons are presented as boxplots in Fig. 6. More variation was present in cut 5 than at the other two cuts. DMD decreased as plants progressed towards flowering, however there was variation at each plant development score (Fig 7). Puna, Puna II and Choice had lower development scores in summer than the other cultivars and this is why they have higher DMD in summer.

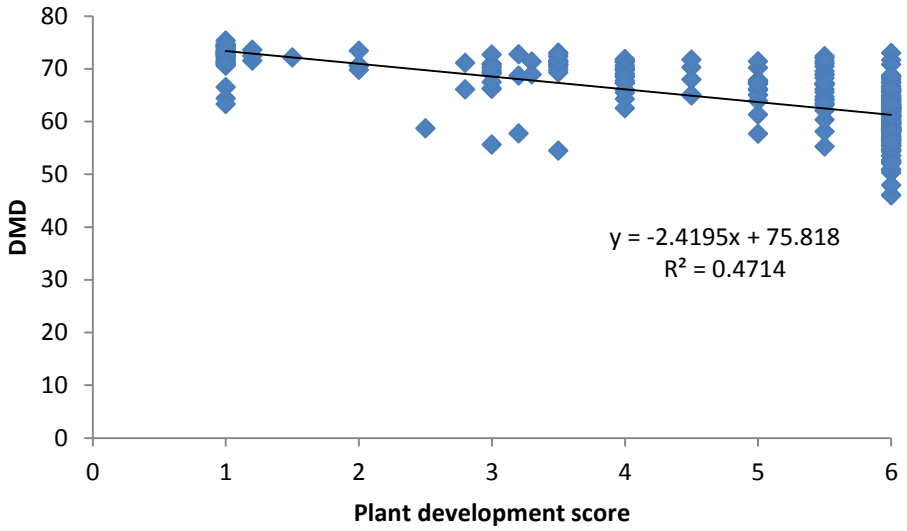


Fig. 7: DMD (%) plotted against plant development score for summer (cut 5).

Methanogenic potential
Methanogenic potential is also reported and discussed in more detail in Section 5 'Within species variation in *in vitro* methanogenic potential in chicory'. Briefly, methanogenic potential from Puna swards dropped in early summer and were similar at other times of the year (Fig 8). Plant development score were 1 (vegetative only) for the first four cuts and 1.4, 1.2 and 1.1 for cuts 5-7 respectively.

For spaced plants, methane production from cut 1 was greater than the methane production from cut 5 (Fig 9). Variation in methane production was similar for the two cutting times. For cut 5 methane production was correlated with pasture quality measurements but not in cut 1 (Fig 10).

Leaf Measurements
Choice, Commander, Grouse, Le Lacerta and their selections had lower levels of leaf incisions then Puna, Puna II and wild (Fig 11). High level of variation for leaf area was present for all groups, however Puna II had one individual with very large leaf size (140) and all others < 85 (i.e. similar to maximum for other groups). Level of leaf incision did not relate to DMD (Fig 12), other pasture quality or methane production (data not shown).

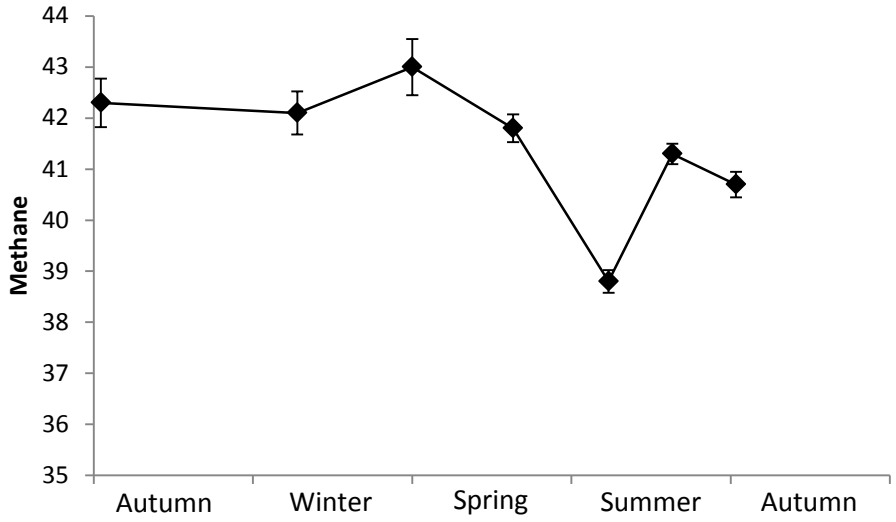


Fig. 8: Methane production (ml/gDMi) from swards of cultivar Puna throughout the year – error bars show SEM.

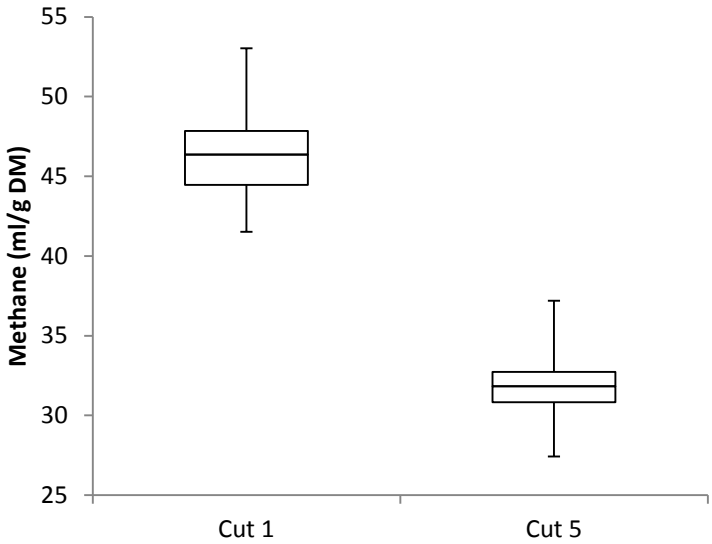
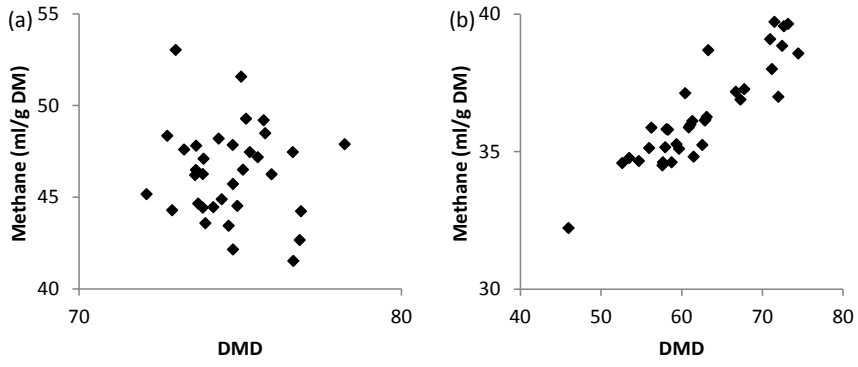


Fig. 9: Boxplots of methane production of diverse range of chicory plants sampled at cut 1 and cut 5.



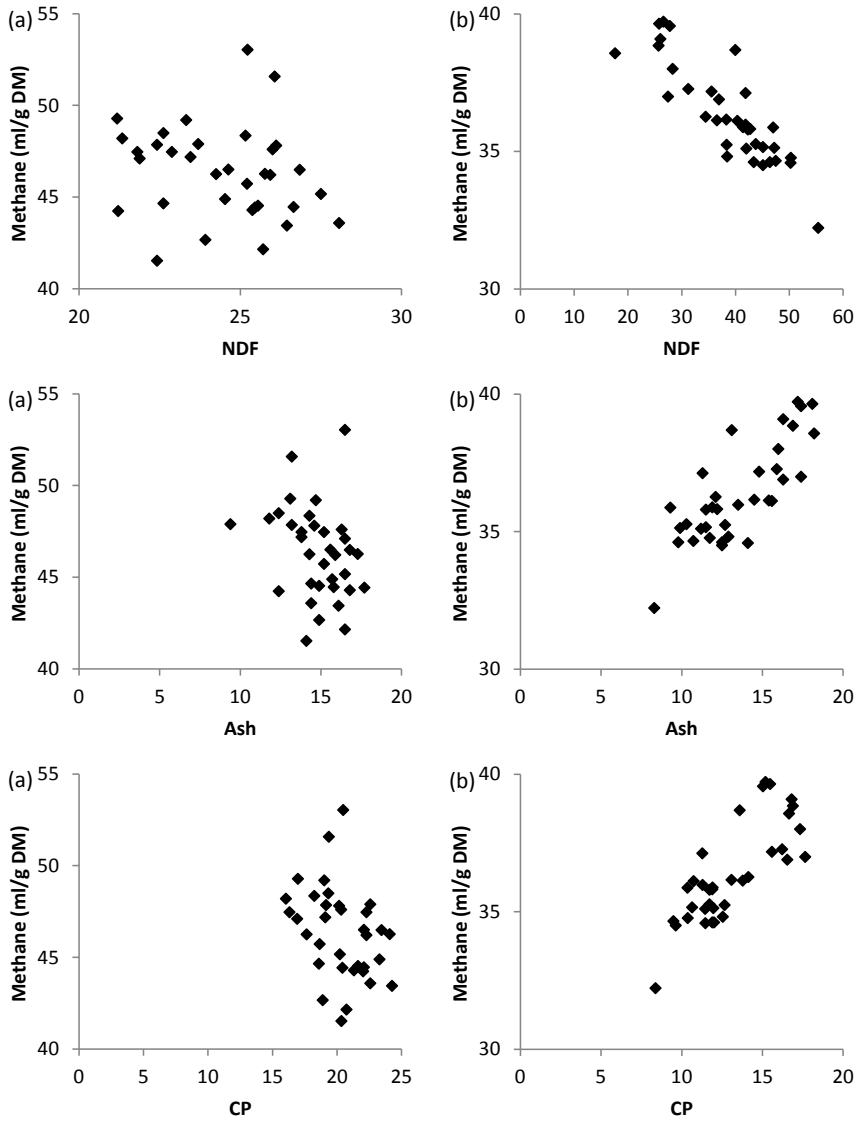


Fig. 10: Methane production (ml/g DMi) plotted against DMD, NDF, ASH, CP for cut 1 (a) and cut 5 (b).

5.5 Discussion

In this study we compared individual plants from a diverse range of chicory cultivars, accessions and selections for DM, persistence, pasture quality and methane production. A large amount of diversity for all these parameters existed between and within cultivars, accessions and selections. The diversity suggests that selections for a range of parameters could be made in order to develop a cultivar with improved feeding value in Australian conditions.

Persistence and DM

The persistence of chicory is increased if chicory has a short grazing period followed by a long rest period (Kemp *et al.,*2002, Alemseged *et al.,*2003, Dowling *et al.,*2006, Li *et al.,*2008). However even when grazed under short grazing long rest period, chicory in Australia has poor persistence and is the major shortfall of chicory (Hayes *et al.,*2006, Clark *et al.,*2013, Li *et al.,*2010, Ward *et al.,*2013) Though we grew chicory as spaced plants rather than in a sward, we had many plants die and found large differences in persistence between cultivars. Puna, Puna II, Choice and most of the wild accessions had better persistence than Commander, Grouse and Le Lacerta. These results are similar to what Li *et al.,* (2010) reported for their Hamilton (Victoria) site. We included wild accessions SA39441 and SA42961 that have been reported to have good persistence (Li *et al.,* 2010) and found this to be the case in our study. We also found wild accessions SA39415 and SA42588 to have good

persistence. SA42588 was collected from Renmark South Australia, which has dry hot summers and it is possible that it became naturalised because it was able to cope with these harsh conditions.

The cultivars Puna, Puna II and Choice had better persistence than Grouse, Commander and Le Lacerta, which supports the results of Li *et al.,* (2010). Puna II and Choice were developed by making selections from within Puna (Rumball 2003 a&b) and their close genetic relationship may explain why they all had good persistence. Choice and Puna II were selected for increased cool season vigour (Rumball 2003a&b). In this study, Choice but not Puna II had high production in the cooler seasons. The high production of Choice and its good persistence indicates that selection for both these traits together is possible.

Selections (for persistence under grazing) of Grouse and Commander had similar persistence as the cultivar they were selected from. However selections in this experiment were propagated by plant, which means they had different plant age to the cultivars grown from seed and this may have affected actual plant persistence. In order to see if selections from Grouse and Commander have better persistence than their parent cultivar needs to be tested by growing them all from seed.

Puna and Puna II had low DM production in spring and early summer but high DM in late summer and autumn. In contrast, Commander, Grouse and Le Lacerta had high DM in spring and early summer but low DM in late summer and autumn. Choice was the only cultivar to have high DM throughout. When farmers are choosing which cultivar to use they will need to take into account the value of the extra DM for the different seasons. Young *et al.,*(2010) report that the value of summer growth for later lambing flocks was ~$100/t DM in early summer and up to $200/t DM in late summer. This means that Choice is the most valuable cultivar as it had high yields in early summer, late summer and autumn.

Selections from cultivars had the same seasonal pattern of production as the cultivars they were selected from. However selections provided higher DM than the cultivars they were selected from especially in the early cuts. Most of the selections were propagated from plants rather than seed and this is probably why they had higher DM. Wild selections from SA39441 were propagated from seed and produced more DM than SA39441 and in fact the top yielding cultivar Choice propagated from seed. The SA39441 selections were grown from seed collected from 3 individual SA39441 plants selected for persistence and production under grazing. The superior growth of the offspring could be due to superior growth of maternal plant or from pollen from nearby cultivars or a combination of both. The performance of SA39441 selections indicates that you can increase persistence and DM together and that wild germplasm can be introgressed into cultivated germplasm for an overall benefit. The wild selections had a wide range of variation in DM production; several cycles of selection would reduce the number of low yielding plants.

Nutritive value

Nutritive value measurements support the reports of chicory having high feeding value given sufficient biomass (Barry 1998, Clark *et al.,*2013, Hayes *et al.,*2010). Overall, the nutritional value was lower in cut 5 than the other two cuts that measured pasture quality. Mature stems have lower pasture quality than immature stems, older leaves and younger leaves (Lee *et al.,*2015) and it is recommended that farmers graze their chicory before older stems develop. The higher plant development scores in cut 5 would explain why the pasture quality was lowest at this time. Foster *et al.,*(2002), Sanderson *et al.,*(2003) report there were few meaningful differences in nutritive value among cultivars Puna and Lacerta. For cut 5 we found Puna to have better nutritive values then Lacerta, but this can be explained by Puna having lower plant development scores than Lacerta.

Plants with higher development scores had lower DMD and crude protein and higher ADF – thus lower feeding value. However there was significant variation in DMD and CP at each plant development score. This indicates that it is feasible to select for higher feeding value amongst plants progressing to flower. We did not sort leaves from stems and suggests that this be considered when selecting plants at this time of year. Our results indicate that higher pasture quality in summer can be achieved by selecting plants with a delay in flowering and also by selecting high quality plants from those that are flowering.

For the first sampling date, DMD ranged from 74 % (Puna cv) to 76 % (a wild accession) and these accessions had CP levels of 21% and 19% respectively. Using the ruminant nutritional model

GrazFeed (Freer et al 1997), a mature sheep would consume approximately 1.2 kg DM/day of both accessions. A dry ewe would gain approximately 90 g of liveweight per day while a pregnant ewe (at 100 days gestation, carrying twins) would gain about 50 g of liveweight/day. The contrast is much greater when comparing the best and worst accessions at cut 5. The best accession (Puna sel.) had DMD of 70 % and CP of 15.7% while the poorest (Commander sel.) had 'bolted' resulting in DMD of 58.9% and CP of 11.9%. These differences have a profound impact on the potential productivity of sheep. Using Grazfeed to estimate intake and growth rates of mature and pregnant ewes (as above), the better accession would be consumed at a rate of 1.15 kg DM/day and lead to approximately 50g of growth for the dry ewe and 20 g for the pregnant ewe. Intake of the poorer quality accession would be less than 1 kg DM/day and the dry ewe will maintain liveweight while a pregnant one would lose about 70 g per day of liveweight without supplements. It is therefore very important to consider both biomass and nutritional value when deciding the best cultivars to select. Just multiplying estimated metabolisable energy by biomass yield may not capture the full benefit and voluntary feed intake and class of stock needs to be factored into the decision.

The development of an accurate and dedicated NIRS calibration to predict nutritional traits will provide a valuable tool for future chicory and breeding. Although data concerning the NIRS calibration is presented in an accompanying paper, we can report that we predicted the key nutritional traits with accuracy that allowed meaningful comparisons for plant breeding. The rapid and inexpensive nature of the technique allows for nutritional assessment at the single plant level and across multiple cuts.

Puna II and Choice are selections from Puna for high and low levels respectively of sesquiterpene lactones (Rumball *et al.,* 2003 a&b). These three cultivars had similar *in vitro* nutritive values which suggests that sesquiterpene lactones may not affect nutritive value of chicory, however simple enzymatic laboratory methods may not accurately represent what happens in a much more complex ruminant.

Chicory cultivars have been developed with more uniformed leaf shape. However we found levels of leaf incision did not affect DMD, other pasture quality measurements, methane production or DM. This suggests that selecting for more uniform leaf shape is cosmetic rather than a trait to provide a benefit to grazing animals.

Methane

A more detailed discussion of the *in vitro* methane production of chicory in relation to other pasture species is provided in Section 6, the focus here is on the relationship between methane and the plant production and nutritive value characteristics of the chicory samples tested. Importantly, chicory has nutritional characteristics that provide high quality, high intake and throughput diets that can result in good liveweight gain and potentially lower emissions intensity. Our results indicate that for cut 5, where there were clear relationships between methane production and nutritional characteristics, there is variation that provides some scope for improving DMD and reducing methane production (Fig 10).

Puna swards had the lowest methane per unit DM in early summer with 38.8 ml CH_4/g DM. This is higher than the mean of single plants sampled in late summer. However methane decreased as plant development scores increased and spaced plants with plant development score of one produced 39.0 ml CH_4/g DM, which is similar to that recorded in swards in early summer.

Methane production in cut 5 related to pasture quality measures but not in cut 1. In cut 5 we had more plants with high development scores and this is what influenced plant quality measurements and methane measurements. For cut 1 differences did not relate to pasture quality measurements. With more uniform plant development scores it is possible that variations in secondary compounds are affecting methane production.

Breeding

Breeding chicory for Australian conditions with a focus on breeding for persistence and drought tolerance has been recommended (Dear *et al.,* 2008, Hayes *et al.,* 2010, and Li *et al.,*2010). Li *et al.* (2010) looked at several wild accessions and report that some accessions demonstrated superior persistence than most cultivars. We found a further two accessions that demonstrated high persistence. We also had selections made from wild plants that had high DM production and

persistence. This indicates that you can use wild germplasm to contribute persistence to the gene-pool and with selection can overcome lower DM production present in accessions.

In this study we found large variation for DM production within each cultivar, accession and selection at each cut. This indicates that it should be possible to make improvements in DM production. Hayes *et al.* (2010) recommend breeding for increased winter DM production, however they used cultivar Puna, which has low winter production (Li *et al.,* 2010). Wild selections had high production in early cuts, which suggests that vigour in early seasons can be combined with persistence. The main benefit that chicory has over other perennial pastures, with the exception of lucerne, is its ability to produce DM in summer. In many locations in southern Australia, summer to autumn is a time when a feedgap exists (Moore *et al.,* 2009). With climate change, adding a summer active perennial is a profitable way of dealing with climate change for livestock enterprises in southern Australia; improved profit results from providing green forage in times of feed shortage rather than through increasing net primary productivity (Ghahramani and Moore 2013). In Australia, cropping is still predominately carried out in conjunction with livestock production (Bell and Moore 2012). In the mixed farming zone livestock are removed from the area of the farm that is allocated to grain crop production in late autumn and late autumn to early winter is a time of feed shortage on mixed farms. Therefore, early winter production can also be highly valued by farmers. Lucerne breeding has been able to increase winter activity while maintaining its ability to produce over summer and suggests this could also be achieved in chicory.

Our results demonstrate that variation exists within cultivars and wild accessions for DMD. DMD was most variable at the summer cut and when selecting for high DMD is most likely to deliver a benefit. Breeding for high DMD has the ability to increase livestock production without having to increase DM on offer.

Chicory is an outcrossing species as is the more widely grown perennial pasture legume lucerne. This suggests that many of the breeding techniques used to improve lucerne will be applicable to chicory breeding. Lucerne breeding programs in Australia have made improvements in persistence and suggest that improvements can be made for persistence in chicory. The results in this study demonstrated that there is a large amount of variation in persistence, DM production and DMD that could be used to improve chicory through breeding programs for livestock productivity and emissions intensity.

5.6 Conclusion

Chicory is a relatively new pasture species in Australia that, like the more widely grown lucerne, is summer active and can provide benefits relative to summer dormant perennials to the livestock industry and farming systems. In Australia, chicory has poor persistence and no chicory breeding for Australian conditions has occurred. Breeding a chicory cultivar adapted to Australian conditions would increase the value of this species to a broader range of livestock producers because the summer activity could improve livestock productivity and reduce methane emissions intensity. It may also assist producers with developing systems for climate adaptation by making use of more of the out of season summer rainfall that is associated with climate change. We have found large variation in persistence, DM production, pasture quality and methane production between and within chicory cultivars, accessions and selections that could be used to develop better cultivars. NIRS prediction equations have also been developed within the ELLE project, which is a tool that will allow large numbers of samples to be tested quickly and cheaply for nutritional value. Pasture breeders should be able to use this tool and in combination with the breeding techniques that have been applied with lucerne, to develop new chicory cultivar/s that are persistent, and have better nutritional characteristics with an extended growing season. These traits are all essential for improving the efficiency of livestock production and adapting to the more variable climate.

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Section 6 *In vivo* measurements of methane emissions from sheep fed two pasture species that have potential to reduce emissions and emissions intensity

6.1 Introduction

Methane production from ruminants contributes about 10% of Australia's national carbon emissions (55Mt CO_2^{-e})(Australian's National Greenhouse Accounts, 2014). It is the most prevalent source of agricultural greenhouse gas emissions and mitigation of this emission is therefore a priority. As methane is a loss of energy, reducing emissions may also lead to productivity increases. Methane production in ruminants is directly affected by their dietary intake and type of diet. In the rumen, it is mainly formed by methanogenic Archaea (methanogens) that utilize hydrogen and carbon dioxide during fermentation. Thus, dietary management is the most likely key to make an immediate impact on mitigating methane because it targets the source of the methane emissions (Beauchemin *et al.*, 2008). The majority of sheep and cattle in Australia graze extensive pastures during their lifetime. Therefore, manipulation of the pasture feedbase to improve metabolisable energy and/or directly reduce methane offers a promising and practical approach to reduce methane emissions per unit of animal product.

Recent *in vitro* batch culture and *in vivo* feeding studies have identified the potential of Biserrula (*Biserrula pelecinus* L.), an annual legume species, to reduce methane emission (Banik *et al.*, 2013, Hutton *et al.*, 2014). Thirty accessions of Biserrula were found to produce lower methane outputs when compared to subterranean clover (*Trifolium subterraneum* L.) and red clover (*Trifolium pratense* L.) in 24 h batch culture fermentation (Banik *et al.*, 2013). In an animal house study where sheep were fed 100% Biserrula, subterranean clover or French serradella (*Ornothopus sativas* Brot.), researchers found that there was no difference in CH_4 produced per unit dry matter (DM) intake, however, sheep fed Biserrula produced about half of the CH_4 per unit of digestible energy intake when compared to sheep fed the other species (Hutton *et al.*, 2014). Biserrula seems to have the potential to mitigate methane emissions from pasture systems.

Biserrula is a monotypic genus and was introduced to broaden the feed base with other Mediterranean native legumes in 1997 (Loi *et al.*, 2001). Pasture scientists collected Biserrula preferentially because it was predominantly found on acidic soils derived from granite in areas with low moisture-holding capacity, conditions analogous to areas of southern Australia (Loi *et al.*, 2015). Compared to other pasture legumes such as subterranean clover, Biserrula is deeper rooted and may therefore produce green feed for longer at the end of the annual growing season (Carr *et al.*, 1999). It also has smaller seeds than many other legume species and therefore higher seedbank survival after grazing (Russi *et al.*, 1992; Edward *et al.*, 1998). Very high seed dormancy allows Biserrula to maintain a seed bank over several years of crop rotations (Loi *et al.*, 1997, Loi *et al.*, 1999). Biserrulais less preferred by sheep than other legumes, forbs and grasses and therefore has value in cropping systems as sheep preferentially graze herbicide resistant weeds (Thomas *et al.*, 2014) With these agronomic benefits and antimethanogenic activity, Biserrula appears to be a promising pasture for grazing systems with lower carbon emissions. Unfortunately, Biserrula has been linked to photosensitisation in sheep (Hogg *et al.*, 2010). Therefore, it is essential to explore the antimethanogenic effect of Biserrula as part of a mixed diet.

In this experiment we have investigated the potential of Biserrula (cv Casbah) to reduce methane emissions from sheep grazing various proportions of Biserrula and French Serradella (cv Margarita) hay. By measuring methane production in respiration chamber and apparent digestibility in metabolism crates, we aim to determine the feeding value and antimethanogenic potential of Biserrula and French serradella over a 30 d feeding period. We are testing the hypothesis that sheep offered Biserrula hay will produce proportionally less methane per unit of ME intake than sheep offered Serradella hay. The experimental design incorporates 5 diets with varying levels of Biserrula and Serradella hay.

6.2 Materials and methods

The experiment was conducted with the approval from the CSIRO Centre for Environment and Life Sciences Animal Ethics Committee, in compliance with the Australian Code of Practice for Care and Use of Animals for Scientific Practices (National Health and Medical Research Council, 2013)

Plant materials

For a 30 day pre treatment phase, sheep were fed a commercial sheep pellet (Macco Feeds Australia, PO Box 53, Williams, WA 6391). Biserrula (cv Casbah) and French serradella (cv Margarita) were sourced from the same farm in Badgingarra, Western Australia. The plants were cut for hay, air dried in the field and baled before transport and commercial chaffing. The nutritional profiles of the pellets, Biserrula hay and Serradella hay are provided in Table 1.
Representative subsamples of the feeds (sampled through the experiment) were oven dried at 65oC for 48 h and ground to pass through a 1-mm screen using a cyclotech mill. Concentrations of organic matter (OM), in the samples were determined as described by (Faichney and White, 1983). Concentrations of neutral detergent fibre (NDF) and acid detergent fibre (ADF) of the samples were measured sequentially using an Ankom 200/220 Fibre analyser (Ankom® Tech. Co., Fairport, NY, USA)(AFIA, 2009). Total N was determined by combustion using a Leco CN628 N Analyser (Sweeney and Rexroad, 1987). Digestibility of dry matter and organic matter was determined using enzymatic digestion (pepsin:cellulase), calibrated with pasture samples with known *in vivo* digestibility (AFIA, 2007, Norman *et al.*, 2010).

Table 1 Chemical composition of Biserrula, Serradella and the pelleted diet fed to merino cross wethers (DM basis)*

	Chemical composition* (g/kg DM)			
	OM	CP	NDF	ADF
Biserrula	910	158	363	264
Serradella	920	150	447	349
Pellet	949	96	444	253

*DM = Dry matter, OM = organic matter, CP = crude protein, NDF = neutral detergent fibre, ADF = acid detergent fibre.

Animal management and feeding

Forty four merino cross wethers (14-months old) were transported to the Animal Research Facility at CSIRO Floreat, Western Australia and acclimatised to humans, the pelletted pre-treatment diet and the environment. Seventeen days after arrival the wethers were weighed and condition scored (Suited 1994), and put into individual pens in the animal house. The initial average live weight (LW) of the sheep was 50 ± 3.4 kg. Sheep were fed once daily at 0830h with free access to water. Individual animal LW and body condition score was measured at 14 d intervals during the experiment and feed intake was recorded daily.

During the adaptation period, sheep were fed 1.2 kg of the commercial sheep pellets for 14 d to acclimatize to the animal house conditions, which was 1.2 times the maintenance requirement for metabolisable energy (ME). After a pre-treatment measurement of methane (see details below), sheep were allocated to one of the five experimental groups in a randomized design with eight sheep per group. The treatment diets are: B0 (100% Serradella); B25 (25% Biserrula + 75% Serradella); B50 (50% Biserrula + 50% Serradella); B75 (75% Biserrula + 25% Serradella) and B100 (100% Biserrula). Allocation was stratified randomly based on live weight and condition score to minimise differences between groups. Each group was offered approximately 1.2 times maintenance level (1.2 kg of hay for each animal per day). Treatment diets were gradually introduced to sheep in a staggered manner over two weeks to ensure sheep were offered treatment diets over the same period of time when measuring methane emission.

Methane measurements

Sheep were introduced to the methane chambers on several occasions for 2 h to adapt to methane chambers and reduce stress prior to any methane measurement. On the day of methane

measurement, feed on offer was proportionally reduced to approximately 1.0 times maintenance to ensure consistent intakes for each measurement period (1 kg of pellets or 1 kg of hay) and reduce individual animal variability in feed intake. One independent measurement of individual animal methane production (g/kg DM intake) over 23 h was conducted after 14 d on the pelleted diet, followed by two repeated measurements of individual animal methane production after 30 d on treatment diets. Each round of methane measurement lasted for six days. Methane measurements were conducted using six open circuit respiration chambers as described by Klein and Wright (2006). In brief, each chamber was constructed of clear polycarbonate over an internal aluminium frame with an internal volume of 2.2 m^3 (interior: length 1.6 m, width 0.86 m and height 1.6 m). Chambers were fitted with an automatic water outlet, a feed bin and composite slotted flooring to allow urine and faeces to accumulate underneath within the chamber space. The mean flow rate of air through the chambers was 0.35 L/min being sourced from inside the air conditioned room containing the six chambers and maintained at 21°C and 90 % RH. Preconditioned air from the room was drawn into each chamber through a port (50 mm diam.) located at the front of each chamber and drawn out through a similar sized duct located on the top and at the rear of each chamber before passing through a flow meter (Elster American Meter AL-800) and exhausted outside of the room. Every 5 min, gas sample from the outlet of each chamber was collected and directly supplied to a gas chromatographer (Shimadzu GC-2010 Shimadzu Corporation, Kyoto, Japan), equipped with 2 flame ionisation detectors, 3.2 mm × 3.05 m stainless steel columns packed with molecular sieve 5A, 80/100 mesh (Alltech Associates Pty Ltd, Baulkham Hill, NSW), and 2 Valco valves fitted with 1.0 mL sample loops. The carrier gas was nitrogen at 400 kPa head pressure. The oven and detector temperatures were set isothermally at 150°C and 300°C. Sample injection, data acquisition, and calculation of CH_4 peak areas were achieved by Shimadzu GC solution Chromatography Data System, Version 2.3 (Shimadzu Corporation, Kyoto, Japan). The gas chromatograms were calibrated three times each day (0900, 1200 and 1530 h) using 100 mg/L CH_4 in a nitrogen Micromat-14 gas standard mix (BOC Gas, Perth, WA) and 10 mg/L CH_4 in a nitrogen standard mix (BOC Gas, Perth, WA). During CH_4 measurement, temperature, relative humidity, air pressure and carbon dioxide concentration in the chamber were monitored to ensure that test animals were comfortable and safe.

Sheep were brought into the respiration chambers at 0900 h and taken out of chamber at 0800 h the next day, which allowed one hour to clean the chambers and swap occupants. At least one sheep from each treatment was measured in the chambers each day. Sheep were fed immediately after introduction to the chamber. Feed intake per individual sheep while in the chamber was determined at the end of measurement. The output of methane obtained from each chamber was converted from 23 h CH_4 production to a g/g DM intake, g/g OM intake and g/unit ME for each animal. All sheep ate their full ration in the week before and during the chamber measurement.

Rumen fluid and blood sampling

After methanemeasurement, sheep were returned to their pen in the animal house and fed their treatment diet. After three hours of feeding, rumen fluid was collected using a stomach tube. The first 50 mL from each sample was discarded to minimize contamination by saliva, the second 50 mL of rumen fluid was used for measurement. The pH values were measured using portable pH meter and then two 3 mL sub-samples were mixed with 200 µL of 99% H_2SO_4 in separate sealed vials and immediately placed on ice. These samples were stored at -20°C to await VFA analysis. VFA analysis was carried out on an Agilent 7890A gas chromatograph fitted with a flame ionization detector (FID). The column used was a Nukol fused silica capillary column 30 m x 0.25 mm I.D. with film thickness of 0.25 µm from Supelco. FID temperature was 230°C, injector temperature was 240°C and the carrier gas was helium at a pressure of 15 psi. Sample supernatant (0.1 mL) was added to 1 mL internal standard and injected (1 µL) with a split ratio of 30:1, The oven temperature was a constant 150°C for a run time of 15 minutes. The internal standard was 3-methyl valeric acid. Calibration standard contained 60 mmol/L acetic, 20 mmol/L propionic, 6.67 mmol/L iso-butyric, 20 mmol/L butyric 10 mmol/L iso-valeric and valeric and 4 mmol/L hexanoic and was prepared freshly before use.

Jugular blood samples were collected into 10 mL lithium-heparin tubes (Becton, Dickinson and Company) for each sheep after first and second rumen fluid sampling. Samples were centrifuged (2000×g for 10 min) and split into plasma and red blood cell fractions. The plasma was frozen at -20°C and stored for analysis of liver, kidney and, muscle function, as well as for nutritional wellbeing (Olympus AU400, Auto Clinical Chemistry Analyser). The samples were analysed following the

manufacturer's specifications at the Animal Production Laboratory, Department of Agriculture and Food Western Australia, South Perth, Western Australia.

Feed digestibility

After two weeks on the treatment diets, a subset of 32 sheep were used to obtain a measurement of digestibility using individual metabolism crates across 6 days. Sheep were fed same diet daily at 1100h with free access to water. All faeces from each sheep were collected daily and weighed for six consecutive days. A sub-sample of the daily faecal output was retained (10%), weighed and oven-dried at 60°C for three days to determine total dry faecal output. The apparent digestibility of dry matter (DMD) was calculated as follows:

Dry matter digestibility (DMD%) = 100*[g DM intake – g DM faecal output]/g DM intake

The dried faecal samples were then ashed in a furnace at 550^0C to get the organic matter of faeces for calculation of organic matter digestibility (OMD). The apparent digestibility of organic matter (OMD), digestible organic content (DOMD) and metaboliable energy (ME) was calculated as follows:

Organic matter digestibility (OMD%) = 100*[g OM intake – g OM faecal output]/g OM intake

Digestible organic content (DOMD%) = 100*[g OM intake – g OM faecal output]/g DM intake.

Metabolisable energy (ME) = 0.169 OMD – 1.986

Total urine volume was measured and acidified urine collected for determination of nitrogen utilisation. Analyses are ongoing.

Statistical Analysis

All statistical analyses were performed using GenStat (16^{th} Edition, VSN International, 2014). The statistical analysis was conducted by fitting linear mixed models to CH4/DM, CH4/OM and CH4/unit ME,*in vivo* DMD, *in vivo* OMD, VFA production and live weight. Fixed factors were Biserrula inclusion rates; random factor (sheep), time effect (sampling time); and a covariate factor: (pre-treatment measurement. In the linear mixed model of CH_4/DM, CH_4/OM and CH_4/ME, the fixed model included terms for the adjusted covariate (adj_Covar CH_4), the chamber in which the measurements were made (Treat_CHB), the day on which the measurements were made (Treat_Day), a linear term for % Biserrula in the diet (linear_Bis), non-linear effects of Biserrula, i.e. those remaining after the linear effect is removed (TRT), and time effect (Time).
The covariate measurement for methane has been analysed using a regression model that included terms for the chamber in which the measurements were made and the day on which the measurements were made. The analysis revealed that there was a significant effect of chamber (in particular, one chamber had much higher errors than others). Therefore, an adjusted value for covariate CH_4 was calculated for each animal by removing effects of chamber. If the ANOVAs were significant, means were separated using least significant differences (LSD, $P < 0.05$).

The digestibility of dry matter, digestibility of organic matter and metabolic energy, live weight gain during the whole experiment were analysed using ANOVA (general linear model) with treatment as factors to compare the variation between treatments. When ANOVAs were significant, means were separated using least significant differences (LSD, $P < 0.05$).
The fixed linear model for live weight, feed intake and volatile fatty acids included terms for live weight (used as a covariate for feed intake and liveweight) or condition score in the covariate period, a linear term for % Biserrula in the diet (Biserrula), non-linear effects of Biserrula, i.e. those remaining after the linear effect is removed (TRT), whether the measurement was made on the first or second occasion (Time), Biserrula x Time and TRT x Time, with sheep as random effect. If ANOVAs were significant, means were separated using least significant differences (LSD, $P < 0.05$).

Two blood tests before treatment and after treatment were analysed using ANOVA with treatment and time as factors to compare the variation between treatments. All blood tests for individual animal were also compared to clinical health range to determine if any animal was unwell. When ANOVAs were significant, means were separated using least significant differences (LSD, $P < 0.05$).

6.3 Results

Feed intake and animal productivity

There was no difference between animals in treatment groups in either feed intake or live weight change during the whole experiment period, with an average 80 g/d live weight gain. The *in vivo* DMD, *in vivo* OMD, *in vivo* DOMD and metabolic energy increased when the inclusion rate of Biserrula in the diet increased (P < 0.001, Table 2 and Fig 1).

Table 2. Average live weight gain in sheep fed different treatment diets and digestibility of dry matter, digestibility of organic, and metabolic energy of treatment diets*

	B0	B25	B50	B70	B100	se	P
Live weight gain (g/d)	80	86	80	70	85	10.9	0.835
Digestibility of dry matter (DMD, g/kg)	626^{a}	640^{a}	679^{b}	690^{b}	702^{b}	0.006	<0.001
Digestibility of organic matter (OMD, g/kg)	637^{a}	651^{a}	684^{b}	694^{bc}	707^{c}	0.005	<0.001
Digestable organic content (DOMD, g/kg)	586^{a}	598^{a}	626^{b}	634^{bc}	646^{c}	0.005	<0.001
Metabolic energy (ME, MJ/kg DM intake)	8.78^{a}	9.02^{a}	9.57^{b}	9.74^{bc}	9.97^{c}	0.089	<0.001

*Means with different superscripts within a row differ significantly (P<0.05).

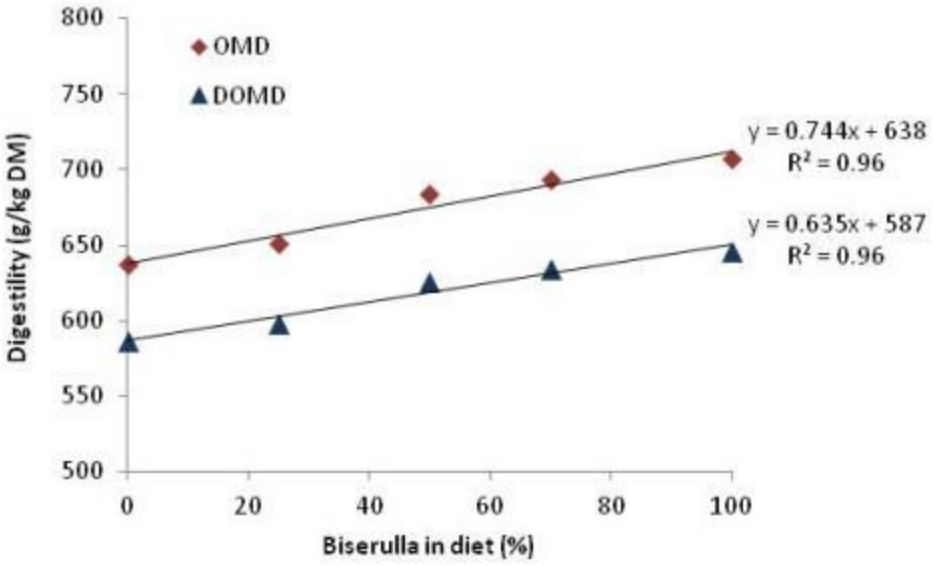

Fig 1. *In vivo* digestibility of organic matter (OMD) and digestible organic matter in the dry matter (DOMD) of Serradella hay based diets with varying levels of Biserrula substitution.

Methane production

The first time the data were analysed, there was a significant effect of chamber (P<0.001) on methane production (Table 3). There were no significant differences between the two repeated methane measurements in treatment period, no linear relationship between Biserrula in the diet and methane emissions or interactions between measurement time and Biserrula. However, the variance between residual values was much higher for one chamber ('F') than the other five chambers. On this basis (and after recommendation by the biometrician), a second analysis was carried out excluding chamber 'F' (Table 4). There were time effects of two methane measurement in treatment period (P =

0.031), but there was no significant treatment effect or interactions between measurement time and Biserrula. Analyses for CH_4/OM intake, CH_4 /ME followed the same pattern as for CH_4 /DM intake. There is a significant linear relation of Biserrula on CH_4/ME (P = 0.008) when chamber F is excluded.

Table 3. Adjusted means of methane production from five treatment diets and the significances of fixed effects for each chamber measurement*

Diet	CH_4 g/kg DM intake	CH_4 g/kg OM intake	CH_4 g/ME
B0	9.25	10.06	0.929
B25	8.61	9.38	0.834
B50	9.46	10.34	0.862
B75	9.52	10.42	0.859
B100	9.17	10.07	0.820
s.e.	0.628	0.687	0.058
adj_Covar_CH_4	0.088	0.086	0.082
Treat_CHB	<0.001	<0.001	<0.001
Time	0.089	0.089	0.083
Treat_Day	n.s.	n.s.	n.s.
Linear-Bis	n.s.	n.s.	n.s.
Treatment	n.s.	n.s.	n.s.
Time.Bis	n.s.	n.s.	n.s.
Time.Treatment	n.s.	n.s.	n.s.

* adj_Covar CH_4 = adjusted covariate, Treat_CHB = the chamber in which the measurements were made, Treat_Day = the day on which the measurements were made, Linear_Bis = a linear term for % Biserrula in the diet, Treatment = non-linear effects of Biserrula, i.e. those remaining after the linear effect is removed, Time = second or third chamber measurement; n.s. means P > 0.1.

Table 4. Adjusted means of methane production from five treatment diets excluding chamber F and the significances of fixed effects for each chamber measurement without chamber F*

Diet	CH_4 g/kg DM intake	CH_4 g/kg OM intake	CH_4 g/ME
B0	8.69	9.45	0.873
B25	8.60	9.37	0.830
B50	8.76	9.58	0.798
B75	8.38	9.18	0.758
B100	8.98	9.87	0.804
s.e.	0.228	0.249	0.023
adj_Covar_CH_4	<0.001	<0.001	<0.001
Treat_CHB	<0.001	<0.001	<0.001
Time	0.031	0.031	0.073
Treat_Day	n.s.	n.s.	0.057
Linear-Bis	n.s.	n.s.	0.008
Treatment	n.s.	n.s.	n.s.
Time.Bis	n.s.	n.s.	n.s.

*adj_Covar CH_4 = adjusted covariate, Treat_CHB = the chamber in which the measurements were made, Treat_Day = the day on which the measurements were made, Linear_Bis = a linear term for % Biserrula in the diet, Treatment = non-linear effects of Biserrula, i.e. those remaining after the linear effect is removed, Time = second or third chamber measurement; n.s. means P > 0.1.

Volatile fatty acids

There was a significant linear effect of the rate of Biserrula on concentrations of acetate, propionate and acetate/propionate ratio (Table 5). The concentration of acetate and acetate/propionate ratio was reduced when the proportion of Biserrula in the diet increased ($P < 0.001$). The propionate production increased when the proportion of Biserrula in the diet increased ($P < 0.001$). There was no difference with total VFA production. There was no Biserrula × sampling time interactions for any individual or the total VFAs.

Blood

Blood plasma samples were analysed for liver and kidney function including GGT (gamma-glutamyltransferase, indicator of liver damage), GLDH (glutamate dehydrogenase, indicator of liver damage), CK (creatine kinase, indicator of muscle damage), creatinine (indicator of kidney function), total bilirubin (liver function), direct bilirubin (liver function), ALT (alanine aminotransferase, an indicator of liver and muscle damage) and plasma urea (indicator of kidney health). All animals fitted within parameters deemed to be within the normal range. There was no significant difference of liver and kidney function from sheep fed different treatment diets. The values of albumin and globulin ratio, albumin, creatine kinase, haptoglobin, magnesium and urea from sheep after feeding the treatment diets were higher than before treatment diet ($P < 0.001$). The values of creatinine, glutamate-dehydrogenase, haematology, phosphate, total protein and conjugated bilirubin from sheep before treatment diet were higher than after treatment diet ($P < 0.005$).

Table 5. The molar proportions and the significance of fixed effect of individual volatile fatty acids in the rumen fluid of sheep fed five treatment diets with varying proportions of Biserrula*

Volatile fatty acids (mmol/L)	B0	B25	B50	B75	B100	se	Covariate	Time	Linear-Bis	TRT	Time. Bis	Time. TRT
Total VFA (mmol/L)	98.8	90.9	88.5	91.2	92.2	3.17	n.s.	0.066	n.s.	n.s.	n.s.	n.s.
VFA (mol/100mol)												
Acetate	69.5	68.1	65.4	63.5	61.8	1.16	n.s.	n.s.	<0.001	n.s.	n.s.	n.s.
Butyrate	8.54	8.73	9.31	9.68	9.29	0.65	n.s.	n.s.	n.s.	n.s.	n.s.	0.07
Propionate	18.9	20.6	22.6	23.9	25.7	1.3	n.s.	n.s.	<0.001	n.s.	n.s.	n.s.
Caproate	0.2	0.14	-	0.26	0.26	0.04	0.02	n.s.	0.087	n.s.	n.s.	n.s.
Valerate	1.75	1.77	1.66	1.77	1.36	0.15	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
Isobutyrate	0.6	0.54	0.63	0.62	0.6	0.04	0.04	n.s.	n.s.	n.s.	n.s.	n.s.
Iso valerate	0.44	0.46	0.53	0.54	0.63	0.05	n.s.	n.s.	0.005	n.s.	n.s.	n.s.
Acetate/Propionate	3.74	3.4	3	2.75	2.52	0.22	n.s.	n.s.	<0.001	n.s.	n.s.	n.s.

* Linear_Bis = a linear term for % Biserrula in the diet, Treatment = non-linear effects of Biserrula, i.e. those remaining after the linear effect is removed, Time = second or third chamber measurement, Time.Bis = interaction of chamber measurement and Biserrula; n.s. means P > 0.1.

Table 6. Blood plasma analysis from sheep fed five treatment diets*

	Treatment Diet						F prob		
	B0	B25	B50	B75	B100	se	Time	TRT	Time. TRT
Creatine kinase (U/L)	128	107	117	92	112	16.1	0.013	n.s.	n.s.
Alanine aminotransferase (U/L)	11.9	12.3	12.4	12.3	12.3	0.55	0.089	n.s.	n.s.
Gamma-glutamyltransferase (U/L)	52.0	48.8	52.7	48.7	53.0	2.46	n.s.	n.s.	n.s.
Glutamate-dehydrogenase (U/L)	19.9	17.3	18.6	11.1	20.6	3.84	0.003	n.s.	n.s.
Conjugated (Direct) Bilirubin (µmol.L)	0	0.187	0.187	0.062	0.150	0.05	0.002	n.s.	n.s.
Total Bilirubin (µmol.L)	2.62	2.63	2.94	2.81	2.80	0.13	n.s.	n.s.	n.s.
Urea (µmol.L)	4.2	3.9	3.8	4.0	4.3	0.33	<0.001	n.s.	n.s.
Creatinine (µmol.L)	101	99	91	103	103	2.37	<0.001	n.s.	n.s.
Calcium (mmol.L)	2.73	2.72	2.77	2.74	2.73	0.02	0.061	n.s.	n.s.
Magnesium (mmol.L)	0.94	0.97	0.95	0.90	0.94	0.02	<0.001	n.s.	n.s.
Phosphate (mmol.L)	2.06	1.91	1.99	1.85	1.85	0.08	0.02	n.s.	n.s.
Beta-hydroxybutyrate (mmol.L)	0.48	0.50	0.53	0.59	0.51	0.04	0.058	n.s.	n.s.
Cholesterol (mmol.L)	1.41	1.37	1.37	1.28	1.38	0.06	n.s.	n.s.	n.s.
Total Protein (g/L)	71.5	73.4	73.4	73.8	71.7	0.96	<0.001	n.s.	n.s.
Albumin (g/L)	35.3	35.0	35.5	34.5	35.3	0.38	<0.001	n.s.	n.s.
Iron (µmol.L)	22.9	24.7	22.6	20.6	22.6	1.58	n.s.	n.s.	n.s.
Albumin and globulin ratio	1.0	1.0	1.0	1.0	1.0	0.03	<0.001	n.s.	n.s.
Haematology (mg/dL)	25.6	25.5	22.1	22.1	24.0	3.00	0.028	n.s.	n.s.
Haptoglobin (mg/mL)	0.32	0.33	0.32	0.30	0.31	0.01	<0.001	n.s.	n.s.

*n.s. means P > 0.1.

6.4 Discussion

The methane yield expressed as g/MJ ME from sheep fed the legume hays decreased linearly as the proportion of Biserrula hay in the diet increased, supporting our hypothesis that Biserrula has the potential to reduce methane emissions from sheep although the differences were not as large as we anticipated. We found that the total methane output as g/kg DM intake and g/kg OM intake from sheep fed 100% Biserrula hay were very close to sheep fed 100% Serradella hay or a mixture of both. In this study the Biserrula hay had higher digestibility and therefore greater energy when compared with the Serradella hay. Within the context of the experiment and restricted feeding levels, the improved digestibility and lower methane yield per MJ/ME did not translate into differences in animal growth rate.

Our study differs from the *in vivo* study of methane production from Biserrula reported by Hutton *et al.* (2014) in several respects. In the study by Hutton *et al.* (2014), animals were fed freshly cut pasture material each day not hay. The methane production from sheep fed Biserrula was 10.82 g/kg DM intake and 0.57 g/MJ DE, compared to the 8.98 g/kg DM intake and 0.804 g/MJ ME (approximately 0.66 g/MJ DE) reported in our study. For Serradella, Hutton et al (2014) measured methane production of 13.49 g/kg DM intake and 1.17 g/MJ DE as compared to 8.69 g/kg DM and 0.873 g/MJ ME (approximately 0.72 g/M DE) in our study. In both experiments, there was no significant difference of CH_4 g/kg DM intake between Biserrula and Serradella. However, Hutton *et al.* (2014) found that CH_4 g/MJ DE of Biserrula was about 50% lower than Serradella (both fed fresh) and we found no significant differences between the 100% diets when they were fed as hay. It is worth noting that the DE values of Biserrula and Serradella of Hutton *et al.* (2014) were based on estimated values, which were higher compared with our measurement in metabolism crates.

It is well established that CH_4 g/kg DM intake decreases as feed intake increases (Hammond *et al.*, 2013). The feed intakes of sheep offered 100% Biserrula or 100% Serradella in both studies were approximately 1.2 times maintenance for ME and the studies differed in that we offered paddock dried hay and Hutton *et al.* (2014) fed fresh pasture. The variance of methane output of the same diet is likely to be affected by the time required to chew and reduce fibre size and passage rate of rumen, which will lead to a reduction of methane output if passage rate is high (Janssen, 2010). Fresh pasture, however, is more likely to be ingested with higher passage rate in the rumen, contrary to the fact that CH_4 g/kg DM intake of fresh Biserrula and Serradella were higher than dried hay. The growing environment can affect plant nutrition in various ways and possibly had an impact on the antimethanogenic effect of plant. The difference of methane production between our study and Hutton *et al.* (2014) is likely due to the growing location, as methane production of Biserrula and Serradella harvested from two field sites ranged from 3.1 to 6.1 mL/g DM and 18.5 to 37.5 mL/g DM in *in vitro* fermentation (Banik *et al.*, 2013). Future studies on comparisons of methane output and digestibility from fresh and dry Biserrula harvested at the same site will help understand these differences.

The digestibility of the Biserrula hay was 10-12% higher than the Serradella hay offered in this study, yet methane yield was unrelated to DM digestibility, supporting the survey of cattle studies by Johnson and Johnson (1995) who did not find any relationship between digestibility and methane output. The effect of diets was more apparent when methane yield was expressed as g/kg OM intake and g/MJ ME, showing a reduction of up to 8% methane when sheep consumed the more digestible Biserrula at the same intake. This negative relationship suggests that the effect on methanogensis in sheep fed Biserrula is mostly due to the diet being more rapidly digested and/or that there has been a direct bioactive effect of Biserrula, which has been reported *in vitro*.

The higher molar proportion of propionate and lower acetate/propionate ratio after feeding the diet containing higher Biserrula indicates a reduction in hydrogen availability for methane formation, which is in support of Moss *et al.* (2000) and Janssen (2010) that diverting hydrogen to an alternative hydrogen sink, such as, propionate, will lead to a drop in methane formation. The increased propionate production of sheep fed Biserrula diet also indicates that there should be a trend towards improved energy efficiency of the diet, but this was not apparent from the live weight changes in the sheep within the experiment. A longer feeding experiment and/or unrestricted feed on offer may have led to a different outcome. The maintenance of total VFA production and reduction in acetate concentration of Biserrula diet indicates that activity of fibre digestion was low, which corresponding with lower fibre content of Biserrula compared with Serradella. These fermentation characteristics of sheep fed Biserrula suggest that the effect on methane production may have been due to an influence on the fibre degrading organisms rather

than a direct effect on methanogens, but this remains uncertain until the analysis of the microbial profiles are completed.

Biserrula as part of a mixed annual pasture may have value for its high digestibility and therefore faster growth rates when biomass is not limiting, however, the opportunity to reduce carbon emissions remains unclear. Its tolerance of low rainfall and ability to remain green for longer during the growing season may benefit farmers through faster growth rates at the end of the season and faster turn-off of sheep. The faster turn-off of sheep means sheep produce less methane to reach target weights compared to sheep with average turn-off rates, and the emissions intensity on farm are improved.

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Significance to Australian agriculture

The key results from this project have been discussed within each of the 6 sections of the report as well as the significance of those results to Australian agriculture and the overall outcomes of the project. In this section we have provided a general overview of the outcomes of the project as well as the significance of the results to agriculture, how industry may use the results from this project and apply them to their systems, and how the results could be used to reduce greenhouse gas emissions and in the development of and ERF methodology.

In this project we hypothesized that there would be differences in the potential of pasture species to improve livestock production and reduce methane, and that these differences could be associated with temporal patterns of forage yield and feed quality, or more directly through manipulation of methanogenic bacteria in the rumen. Our results support the hypothesis becausethere were clear differences in the persistence and temporal pattern of biomass production and nutritive value amongst the species we examined. This variability in key traits that influence sheep production enable the identification of commercially-available and regionally-appropriate species with the potential to be used more widely by landholders to reduce methane emissions and emissions intensity. There were also some species that had antimethanogenic bioactivity.

In general, the results generated in this project provide quantitative information to producers to assist them in making better choices of pasture species for different regions. Our data suggest that there is variability in productivity and temporal patterns of nutritive value both within and between species that could be used by Australian producers to increase sheep productivity and reduce emissions intensity. There is less variability in the bioactivity of the plant species and their ability to reduce methane. However, there was one species in particular, Biserrula pelecinus, which stood out from the rest as being a species that could have a direct impact on methane emissions and production efficiency.Biserrula reduced methane in the laboratory as well as when it was fed to animals in the animal house and we have demonstrated that it could be used in a mixed sward and maintain its antimethanogenic capacity. There were other anitmethanogenic species (perennial), for example *D. hirsutum*, *D. glomerata* and *T. pratense,* which could be valuable in a system, but unlikely to be valuable as the sole source of the diet.So, there are clear opportunities to select plant species that are less methanogenic for our southern grazing systems and have the potential to maintain or improve livestock productivity.

Producers could use the quantitative information we have generated in this project to make better choices of the pasture species/cultivars they grow, and the mix they may use, andimprove the amount and quality of plant material available for livestock production. They could also extend the period of time this material is available throughout the year. We have identified some species that should be considered as candidates for selective breeding because they show promise, and useful variation, for biomass, nutritive value and fermentative traits that could help reduce emissions and emissions intensity. The benefit to producers of these particular pasture species is not immediate, but could be targeted within the pipeline species being developed for use in the future.

Another exciting outcome from the project has been the development of the NIR calibration equations. These calibrations represent a useful tool for livestock industries in Southern Australia as they are likely to encompass nearly all of the species that could appear in monocultures or mixed swards across all of their lifecycles. The current data set with over 1000 samples with matching scans and chemistry provides an excellent platform for future refinement or generation of calibrations for new traits. NIR is an inexpensive and rapid way to predict the nutritional value of pastures and could assist producers to optimise grazing management and growth rates of young stock. This may lead to increased profitability and reduced methane emissions intensity if animals reach slaughter weight faster with less feed inputs. Development of accurate calibrations can also be very useful in plant breeding and selection programs where large numbers of plants require assessment of their nutritional value. The NIRS database also provides an opportunity for producers to measure improvements in the feedbase (or estimate total methane outputs from the feedbase) for future carbon reduction schemes.

In completing the project we have established a comprehensive database of baseline measurements of biomass, nutritive value, *in vitro* fermentabilty and bioactivity across seasons, sites and phonological stage for the key commercially available and pipeline pasture species most suitable for southern Australian grazing systems.There is valuable information within the database that could aid modelling projects to guide management decisions on farm. This information may also assist producers with developing systems for climate adaptation by making use of more of the out of season summer rainfall that is associated with climate change. Currently, there is a interest in developing and ERF methodology that is based around new ‘systems’ for reducing emissions, and the database generated in this project will be a valuable asset for developing that methodology. The systems-based methodology would have a focus on designing more productive systems based around the choice and management of pasture species that provide more feed of higher quality at critical times for sheep production to improve production efficiency and reduce emissions intensity. The ‘systems’ methodology would also include pasture species, either in a mix or monoculture, which are known to have a direct effect on methane production. Our database provides quantitative information to inform the many aspects of this methodology.

Future research needs

In this project we have examined variability in growth, nutritional and bioactive characteristics of a comprehensive selection of pasture species suitable for temperate grazing systems in southern Australia. The database of information offers a lot for modelling projects but there is a need to plant a collection of a subset of the most promising species in a wider range of field sites across southern Australia not just SA and WA. We can use the data we have generated in this project to make informed choices about the subset of plants but they should then be established at multiple sites without irrigation and designed to get data on growth, nutritive value and bioactivity specifically designed to feed into modelling programs. The information that could be generated from this type of exercise would be invaluable for decision making on farm. The NIR calibration equations could be extended further with this additional information. The NIR calibration equations are so encouraging from this work that they should developed further regardless, so that they become a standard ‘tool’ for the industry. We also think that a project of a similar nature to ELLE should be undertaken for northern Australia and potentially the Asian Pacific rim. We have also identified plants that have favourable growth, nutritive value and bioactivity with potential to be ‘improved’ through breeding and this should be considered. We have confirmed that Biserrula is the most bioactive pasture species we’ve tested at any time, site or stage of growth and we should really be considering how best to integrate it into production systems, which will involve a combination of field base and animal house studies.

Publications

Papers in peer-reviewed journals and conference papers

Durmic, Z., Moate, P. J., Eckard, R., Revell, D. K., Williams, R., Vercoe, P. E., (2014) *In vitro* screening of selected feed additives, plant essential oils and plant extracts for rumen methane mitigation. J Sci Food Agric 94, 1191-1196.

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