CSIRO PUBLISHING
Crop & Pasture Science, 2019, **70**, 68–76
https://doi.org/10.1071/CP18293

Methanogenic potential of commonly utilised South African subtropical and temperate grass species as influenced by nitrogen fertilisation

Cornelius J. L. du Toit[A,D], *Willem A. van Niekerk*[A], *Heinz H. Meissner*[B], *Lourens J. Erasmus*[A], *and Liesl Morey*[C]

[A]Department of Animal and Wildlife Sciences, University of Pretoria, Pretoria 0002, South Africa.
[B]No. 3 Die Hoewes, 276 von Willich Street, Centurion, 0157, South Africa.
[C]ARC-Biometry, ARC-Central Office, 1134 Park Street, Hatfield, 0087, South Africa.
[D]Corresponding author. Email: linde.dutoit@up.ac.za

Abstract. The aim of the study was to evaluate the effect of nitrogen (N) fertilisation on certain quality parameters and *in vitro* total gas and methane production of improved grass species commonly used as fodder species in South Africa. Treatments included seven grass species representing two photosynthetic pathways (C_3 and C_4) with three levels of N fertilisation (0, 50 and 100 kg N ha^{-1}). Plants were grown in a greenhouse and N was applied in a single application after a simulated defoliation. Sample material was harvested by hand after an 8-week regrowth period. Grass species and rate of N fertiliser both had effects ($P < 0.05$) on the nutritive value and *in vitro* organic matter digestibility of the selected species. Crude protein concentration increased ($P < 0.05$) and neutral detergent fibre concentration tended to decrease as the level of N fertilisation increased for both C_3 and C_4 species. Generally, no effect was found of N fertilisation on *in vitro* total gas or methane production; however, increasing the level of N fertiliser increased ($P < 0.05$) the methanogenic potential (*in vitro* methane/*in vitro* total gas production) of *D. glomerata*, *F. arundinacea* and *C. ciliaris* after a 24-h incubation period but no significant effects were reported after a 48-h incubation period.

Additional keywords: fermentation, greenhouse gas.

Received 21 June 2018, accepted 1 November 2018, published online 4 January 2019

Introduction

Understanding the effect of forage quality on the production of anthropogenic greenhouse gases from livestock is important for the development of mitigation strategies for agricultural systems (Beauchemin *et al*. 2008; Bhatta *et al*. 2017). The livestock sector is a significant source of greenhouse gas emissions in South Africa, contributing 60% of total agricultural CO_2-equivalent emissions (Meissner *et al*. 2013). Beef cattle, sheep and privately owned game enterprises rely mainly on extensive forage-based production systems and account for 85% of total livestock methane (CH_4) emissions in South Africa (Du Toit *et al*. 2013*a*, 2013*b*, 2013*c*).

One of the main factors contributing to the limited productivity of ruminant livestock in tropical and subtropical regions in developing countries is the poor nutritional conditions, characterised by highly lignified, low-digestibility feed from poor quality, nitrogen (N)-limited native rangeland and crop residues (Goel and Makkar 2012). Meissner *et al*. (1999) categorised roughage quality according to the digestible organic matter (OM) concentration as poor (<45%), low (45–55%), medium (55–70%) and high (>70%). Improving forage quality offered to ruminants through forage species selection, rangeland reinforcement through the introduction of more productive and nutritious species, and improved rangeland-management systems has the potential to reduce CH_4 emissions per unit animal product as a result of increased digestibility and reduced ruminal retention time of feed particles (Beauchemin *et al*. 2009; Banik *et al*. 2013). Benchaar *et al*. (2014) stated that a 15% reduction in CH_4 emissions could be achieved by increasing the digestibility of forages and a 7% reduction through increasing voluntary feed intake of livestock.

The influence of N fertilisation on improvement of forage quality and productivity has been investigated by several researchers (Valk *et al*. 1996; Rivera *et al*. 2017; Ullah *et al*. 2018). However, studies evaluating the effect of N fertilisation on the methanogenic potential (*in vitro* CH_4/*in vitro* total gas production, TGP) of tropical and subtropical grass species are not readily available. Nitrogen fertilisation can influence the pattern and rate of degradation in the rumen of crude protein (CP) and neutral detergent fibre (NDF) (Valk *et al*. 1996) by increasing the concentration of neutral detergent insoluble N and altering the protein : carbohydrate ratio (Valk *et al*. 1996), which could influence the methanogenic potential of forages.

Previous studies hypothesised that increased N fertilisation of forages would reduce fermentation gas production because of differences in the stoichiometry of fermentation of CP relative to carbohydrate, which will limit gas production and ruminal hydrogen (H^+) supply (Cone *et al*. 1999). Mathison *et al*. (1998) stated that increased nitrate levels in pastures can serve as an H^+ sink and reduce enteric CH_4 production from ruminants. In a study of perennial ryegrass cultivars, Lovett *et al*. (2004) reported a decrease in TGP and CH_4 production with increasing N-fertiliser application rates with short *in vitro* incubations.

In vitro techniques have been used by several researchers as a practical screening tool to predict plant digestibility, plant nutritive value, fermentation characteristics and methanogenic potential (CH_4 : TGP), taking into consideration the complex interaction between rumen microbes and feed particles (Lovett *et al*. 2006; Durmic *et al*. 2010; Banik *et al*. 2013; Durmic *et al*. 2017). Variability in these traits among accepted improved pasture species would allow for the selection of low-methanogenic pastures that do not compromise animal productivity. This would improve the ability of producers to reduce CH_4 emissions from livestock, reducing the carbon footprint of production systems and allowing more efficient and climate-friendly management without major changes in current production practices.

The aim of this study was to evaluate the influence of a range of N fertiliser application rates on the nutrient concentration, *in vitro* OM digestibility (IVOMD), *in vitro* TGP and *in vitro* CH_4 : TGP of commonly used improved subtropical (C_4) and temperate (C_3) grass species in South Africa.

Material and methods

Study area description

The experiment was conducted in a glass greenhouse at the Hatfield experimental farm at the University of Pretoria, South Africa. Seven grass species of current economic importance in South Africa were investigated (Table 1) in two groups: four species in the C_4 group and three species in the C_3 group. Three rates of N fertilisation commonly used in South African pasture production systems were evaluated: 0, 50, and 100 kg N ha^{-1}. All treatments were replicated three times in a randomised complete block design. Seeds were sourced from a commercial company and sown into 15-L pots in a controlled environment where the temperature and humidity ranges were 18–34°C and 30–68%, respectively. The pots were filled with 12 kg air-dried and sieved potting soil mixture comprising 20% clay, 23% silt and 57% sand. Soil samples were analysed at a commercial, accredited laboratory (Nvirotek Laboratories, Hartbeespoort, South Africa). Some physical and chemical characteristics of the soil were: bulk density 1.1 g cm^{-3}, Bray 1 phosphorus 300 mg kg^{-1}, potassium 2554 mg kg^{-1}, sodium 556 mg kg^{-1}, calcium 3650 mg kg^{-1}, magnesium 616 mg kg^{-1}, and pH(KCl) 5.63.

For each species, 10 seeds were planted per pot and allowed to germinate. Once established, the seedlings were thinned to three uniform seedlings per pot. All pots received a single dressing of N fertiliser as limestone ammonium nitrate (28% N) after the thinning process according to the experimental treatments. The pots were rotated once a week in the greenhouse to minimise the influence of environmental variation within the greenhouse. All pots were weighed and watered to 90% field capacity according to Pieterse *et al*. (1997). In order to prevent mineral loss, all pots were placed on a saucer and any leached water was returned to the pots 1 h after watering. For the remainder of the trial period, the pots were weighed every 3 days and watered to 90% field capacity.

Samples for analysis of nutritive value and *in vitro* fermentation were obtained from the second regrowth phase after an initial harvesting cycle in the establishment year. The initial growth period lasted for 6 weeks; thereafter, all pots were harvested and a soil core sample was taken from each pot by using a thin polyvinyl chloride pipe and analysed for mineral concentration, to ensure that all treatments had similar soil-nutrient composition before the N-fertiliser treatment was applied for the second regrowth phase. Both harvest cycles were done by hand at 5 cm above soil level. The second harvest was done after an 8-week regrowth period, when the C_4 species started to flower and the C_3 species were still vegetative. The harvested material was air-dried and ground to pass through a 1.0-mm screen. Material was stored at room temperature (20–25°C) in sealed containers for analysis.

Nutritive value

Plant samples were analysed for dry matter (DM), OM, CP, NDF, acid detergent fibre (ADF), acid detergent lignin (ADL) and IVOMD, and then metabolisable energy (ME) was estimated. The DM content was determined by drying samples for 24 h at 105°C in a forced-air oven, after which the samples were weighed then combusted at 450°C for 8 h in a muffle furnace to determine the OM concentration (AOAC 2000). Nitrogen concentration of samples was analysed by total

Table 1. Perennial grass species investigated, including common and scientific names, cultivar and photosynthetic pathway

Scientific name according to Gibbs Russel *et al*. (1991)

Common name	Scientific name	Cultivar	Photosynthetic pathway
Blue buffalo grass	*Cenchrus ciliaris*	cv. Molopo	C_4
Rhodes grass	*Chloris gayana*	cv. Katambora	C_4
Smuts finger grass	*Digitaria eriantha*	cv. Irene	C_4
Buffalo grass	*Panicum maximum*	cv. Gatton	C_4
Cocksfoot	*Dactylis glomerata*	cv. Cambria	C_3
Tall fescue	*Festuca arundinacea*	cv. Duramax	C_3
Perennial ryegrass	*Lolium perenne*	cv. Halo	C_3

combustion (AOAC 2000) on an FP-248 N and protein analyser (LECO Corporation, St. Joseph, MI, USA). NDF and ADF concentrations were determined using a 200/220 Fibre Analyser (ANKOM Technology, Fairport, NY, USA) based on the methods described by Van Soest *et al*. (1991). Sodium sulfite and heat-stable amylase were used in the analysis of NDF. The ADL concentration was determined according to Van Soest *et al*. (1991) through the solubilisation of cellulose with sulfuric acid in the ADF residue. The fibre fractions were expressed inclusive of residual ash. The ME was calculated from gross energy (GE) and IVOMD according to Minson (1990) and Robinson *et al*. (2004): ME (MJ kg^{-1} DM) = 0.81 ((GE × IVOMD)/100)

In vitro *digestibility, total gas, and methane production measurement*

The IVOMD was determined using the method of Tilley and Terry (1963) as modified by Engels and Van der Merwe (1967). Three rumen-cannulated Döhne Merino wethers were used as rumen-inoculum donors. The care, handling and maintenance of cannulated sheep were in accordance with animal welfare regulations of the Animal Ethics Committee of the University of Pretoria (EC018-14). The donor sheep were fed a diet consisting of 50% *Eragrostis curvula* hay and 50% *Medicago sativa* hay. Rumen fluid was collected 2 h after the morning feeding, pooled and filtered through two layers of cheese cloth. The rumen fluid was stored in a pre-warmed insulated thermos flask pre-filled with CO_2.

Samples for gas analysis were incubated in triplicate according to the procedure described by Theodorou *et al*. (1994). Dried plant sample (~400 mg) was weighed into 120-mL serum bottles. Filtered rumen fluid (15 mL) was mixed with anaerobic buffer–mineral solution (30 mL) prepared according to Goering and Van Soest (1988) with modifications suggested by Mould *et al*. (2005). After saturation with CO_2 the serum bottles were sealed with rubber stoppers and aluminium crimp seal caps. Possible gas build-up was equalised by inserting a hypodermic needle through the rubber stopper for ~5 s. Thereafter the sample bottles were placed in an incubator at 39°C with a rotary shaker set at 120 rpm. The incubation and gas-production measurements lasted for 48 h (Gemeda and Hassen 2014) and all measurements were corrected for blank gas production (gas production in buffered rumen fluid without sample). The system consisted of a digital data logger (Tracker 220 series indicators; Omega Engineering, Laval, QC, Canada) connected to a pressure transducer (PX4200-015GI; Omega Engineering). Gas pressure was measured at 0, 4, 12, 24, and 48 h by using the pressure transducer. After each pressure reading, a small gas sample (2 mL) was taken from the headspace with a Hamilton gas-tight syringe for immediate CH_4 analysis by gas chromatography (490 Micro gas chromatograph; Agilent, Santa Clara, CA, USA). The gas chromatograph was equipped with a 10-m stainless-steel Porapak-Q column and a thermal conductivity detector. The injector temperature was set at 45°C and the column temperature at 50°C, with a 30-ms injection time and static pressure of 80 kPa. Methane content (mL g^{-1} DM incubated) was calculated according to Banik *et al*. (2013).

Statistical analyses

The two groups of grass species (three species in the C_3 group and four in the C_4 group) were analysed separately. The data were subjected to analysis of variance with two factors (species and N application) and three block replications, using the GLM procedure in SAS version 9 (SAS Institute, Cary, NC, USA). The Shapiro–Wilk test was performed on the standardised residuals to test for deviations from normality (Shapiro and Wilk 1965). In cases where there were significant deviations from normality and it was due to skewness, outliers were removed until the distribution of the residuals was normal or symmetrical (Glass *et al*. 1972). Student's *t*-l.s.d. (least significant difference) was calculated at $P = 0.05$ to compare means of significant source effects.

Results

Forage quality

Both grass species and level of N fertilisation had significant ($P < 0.05$) effects on the nutritive value, IVOMD and *in vitro* gas-production characteristics of the grass species (Tables 2 and 3). Grass species × N fertilisation level interactions were significant

Table 2. Analysis of variance for forage quality factors (dry-matter basis) for selected subtropical (C_4) grass species

Sp, Species; N, nitrogen fertilisation; CV, coefficient of variation; CP, crude protein; NDF, neutral detergent fibre; ADF, acid detergent fibre; ADL, acid detergent lignin; IVOMD, *in vitro* organic matter digestibility; ME, metabolisable energy; TGP, *in vitro* total gas production; CH_4, *in vitro* methane production

Parameter	Sp	N	Sp × N	R^2	CV	Mean
Ash (%)	<0.001	0.119	0.190	0.81	7.05	11.60
CP (%)	<0.001	<0.001	0.881	0.76	11.50	7.09
NDF (%)	<0.001	0.002	<0.001	0.96	1.63	64.94
ADF (%)	<0.001	<0.001	0.017	0.94	2.38	36.23
ADL (%)	<0.001	0.504	0.676	0.72	12.26	4.70
IVOMD (%)	<0.001	<0.001	0.065	0.94	4.54	58.21
ME (MJ kg^{-1} DM)	<0.001	0.046	0.086	0.75	8.23	7.67
TGP 24 h (mL g^{-1} DM)	0.002	0.816	0.443	0.55	12.63	96.70
CH_4 24 h (mL g^{-1} DM)	<0.001	0.459	0.318	0.69	12.99	4.16
TGP 48 h (mL g^{-1} DM)	0.019	0.560	0.424	0.48	5.56	150.34
CH_4 48 h (mL g^{-1} DM)	0.018	0.725	0.663	0.48	11.69	10.37

for NDF and ADF concentrations in C_4 species (Table 2) and for IVOMD in C_3 species (Table 3).

Level of N fertilisation had no effect ($P > 0.05$) on *in vitro* gas-production parameters of either C_3 or C_4 species, except for CH_4 production of C_3 species after the 24-h incubation period (Table 3). *In vitro* gas production was not affected by species × level of N fertilisation interaction in either C_3 or C_4 species.

Nutritive analysis indicated that N fertilisation had an inconsistent effect on ash and NDF concentrations of both C_4 and C_3 species. Increasing N level decreased ($P < 0.05$) the ash concentration of *Cenchrus ciliaris* and *Chloris gayana* and the NDF concentration ($P < 0.05$) of *C. gayana* and *Panicum maximum* among the C_4 species, and decreased ($P < 0.05$) the ash and NDF concentrations of *Dactylis glomerata* among the C_3 species (Tables 4 and 5). No significant effect ($P > 0.05$) was shown on ADL concentration in either species group, whereas ADF concentration decreased ($P < 0.05$) in *C. gayana* and *P. maximum* and tended ($P < 0.10$) to decrease in other C_4 and C_3 species with increased N level. CP concentration increased ($P < 0.05$) with increasing N level across all C_4 and C_3 species except *Digitaria eriantha*. IVOMD increased in *C. ciliaris* and *D. eriantha* (Table 4) but decreased in *D. glomerata* (Table 5) as the level of N fertilisation was increased from 0 to 100 kg N ha^{-1}. ME concentration was not affected by level of N fertilisation in any of the grass species, although between-species differences were present across all N levels for C_4 species and at 100 kg N ha^{-1} for C_3 species.

Comparing C_4 species, *C. ciliaris* presented lower ($P < 0.05$) CP concentration and higher ($P < 0.05$) NDF, ADF and ADL concentrations across all N treatments than *P. maximum* and *D. eriantha*. *Panicum maximum* had higher ($P < 0.05$) IVOMD than the other C_4 species at 0 and 50 kg N ha^{-1} (Table 4).

There was less between-species variation for nutritive concentrations in the C_3 than the C4 species (Table 5). No differences were found for CP concentrations at 0 and 50 kg

Table 3. Analysis of variance for forage quality factors (dry-matter basis) for selected temperate (C_3) grass species
Sp, Species; N, nitrogen fertilisation; CV, coefficient of variation; CP, crude protein; NDF, neutral detergent fibre; ADF, acid detergent fibre; ADL, acid detergent lignin; IVOMD, *in vitro* organic matter digestibility; ME, metabolisable energy; TGP, *in vitro* total gas production; CH_4, *in vitro* methane production

Parameters	Sp	N	Sp × N	R^2	CV	Mean
Ash (%)	<0.001	0.182	0.122	0.77	6.97	14.36
CP (%)	0.036	<0.001	0.379	0.90	8.16	9.87
NDF (%)	<0.001	0.126	0.315	0.75	3.30	54.66
ADF (%)	<0.001	0.408	0.095	0.87	4.26	31.40
ADL (%)	0.034	0.118	0.334	0.56	14.86	3.72
IVOMD (%)	<0.001	<0.001	<0.001	0.97	3.03	72.07
ME (MJ kg^{-1} DM)	0.007	0.460	0.846	0.58	12.12	11.67
TGP 24 h (mL g^{-1} DM)	<0.001	0.149	0.327	0.77	10.96	113.10
CH_4 24 h (mL g^{-1} DM)	<0.001	0.018	0.089	0.75	15.48	4.81
TGP 48 h (mL g^{-1} DM)	<0.001	0.367	0.404	0.77	7.64	158.79
CH_4 48 h (mL g^{-1} DM)	<0.001	0.163	0.406	0.87	9.30	9.60

Table 4. Effect of nitrogen fertilisation on chemical composition of improved subtropical C_4 grass species commonly used in South Africa
CP, Crude protein; NDF, neutral detergent fibre; ADF, acid detergent fibre; ADL, acid detergent lignin; IVOMD, *in vitro* organic matter digestibility; ME, metabolisable energy; MSE, mean square error; l.s.d., least significant difference; d.f., degrees of freedom. Within a column, means followed by the same letter are not significantly different ($P > 0.05$)

	N fert. (kg ha^{-1})	Ash	CP	NDF	ADF	ADL	IVOMD	ME (MJ kg^{-1} DM)
				(% of dry matter)				
C. ciliaris	0	10.58fg	5.16f	68.09bc	40.49b	5.78a	60.15b	8.26abc
	50	9.66gh	5.56ef	73.04a	42.82a	5.74a	58.14bc	7.62bcde
	100	8.95h	6.58cde	67.54bc	40.70b	5.89a	66.29a	8.73ab
C. gayana	0	13.07ab	6.00def	69.33b	35.53cd	4.59bc	55.27c	7.05de
	50	12.10abcde	7.14bcd	67.03c	34.88de	4.92ab	53.76cd	7.00de
	100	11.44def	7.66abc	66.88c	33.83ef	4.30bc	56.75bc	7.98abcd
D. eriantha	0	11.75bcdef	7.16bcd	59.75ef	35.02de	4.06bc	42.28e	5.61f
	50	11.05ef	8.37ab	59.13f	33.96ef	4.49bc	49.81d	7.46cde
	100	11.63cdef	8.35ab	60.17ef	33.68ef	4.36bc	50.25d	6.68ef
P. maximum	0	12.67abcd	6.77cde	64.84d	36.03cd	3.73c	66.02a	8.31abc
	50	12.97abc	7.60abc	64.64d	36.71c	4.04bc	67.53a	8.66ab
	100	13.37a	8.70a	61.49e	33.36f	4.49bc	70.23a	8.79a
l.s.d. ($P = 0.05$)		1.386	1.380	1.832	1.492	0.975	4.683	1.147
MSE (d.f.)		0.669 (22)	0.664 (22)	1.118 (21)	0.741 (21)	0.332 (22)	6.978 (20)	0.459 (22)

N ha^{-1} among C_3 species, but *Lolium perenne* had higher ($P < 0.05$) CP concentration than *D. glomerata* and *F. arundinacea* at 100 kg N ha^{-1}. *Dactylis glomerata* had higher ($P < 0.05$) NDF concentration across the different N treatments than *F. arundinacea* and *L. perenne*. Across all N treatments, *F. arundinacea* had the lowest ($P < 0.05$) ADF concentrations and *L. perenne* had the highest ($P < 0.05$) IVOMD (Table 5).

Forage in vitro *total gas and methane production potential*

For each of the C_4 species, there were no significant differences ($P > 0.05$) in any of the *in vitro* parameters (TGP, CH_4 and CH_4 : TGP) as the N-fertilisation level increased, after either the 24- or 48-h incubation period, except for *C. ciliaris*, which showed an increase ($P < 0.05$) in CH_4 : TGP at 100 kg N ha^{-1} after the 24-h incubation interval (Table 6).

Among C_4 grass species, *D. eriantha* and *C. gayana* had the lowest ($P < 0.05$) *in vitro* TGP and CH_4 production, respectively, in the control treatment (0 kg N ha^{-1}) after the 24-h incubation period. No differences were found in either TGP or CH_4 production at 50 kg N ha^{-1} after the 24-h incubation period. As the level of fertilisation increased to 100 kg N ha^{-1}, *C. ciliaris* and *P. maximum* produced higher ($P < 0.05$) *in vitro* CH_4 after the 24-h incubation period than *C. gayana* (Table 6).

Among the C_4 species after the 48-h incubation period, *P. maximum* and *C. ciliaris* had the highest ($P < 0.05$) TGP at 0 kg N ha^{-1}. *Chloris gayana* had TGP lower ($P < 0.05$) than *D. eriantha* and *P. maximum* but similar to *C. ciliaris* at 50 kg N ha^{-1}, and there were no differences between C_4 species for

Table 5. Effect of nitrogen fertilisation on the chemical composition of improved temperate C_3 grass species commonly used in South Africa

CP, Crude protein; NDF, neutral detergent fibre; ADF, acid detergent fibre; ADL, acid detergent lignin; IVOMD, *in vitro* organic matter digestibility; ME, metabolisable energy; MSE, mean square error; l.s.d., least significant difference; d.f., degrees of freedom. Within a column, means followed by the same letter are not significantly different ($P > 0.05$)

	N (kg ha^{-1})	Ash	CP	NDF	ADF	ADL	IVOMD	ME (MJ kg^{-1} DM)
				(% of dry matter)				
D. glomerata	0	15.41ab	8.12ef	58.81a	34.62a	3.90abc	53.30d	10.40b
	50	13.34c	9.69d	58.11a	34.52a	3.46bc	59.66c	10.75b
	100	13.42c	11.75b	54.92b	32.42ab	4.38ab	71.70b	10.49b
F. arundinacea	0	13.76bc	7.41f	53.20bcd	29.17c	4.55a	74.93b	12.60ab
	50	12.84c	9.27de	51.76cd	26.93c	3.48bc	73.34b	12.51ab
	100	12.52c	11.31bc	51.26d	27.73c	3.88abc	75.19b	13.82a
L. perenne	0	15.44ab	7.85f	54.48bc	31.56b	3.35c	81.22a	10.73b
	50	16.50a	10.13cd	54.62bc	33.03ab	3.24c	79.13a	11.71ab
	100	16.00a	13.27a	54.84bc	32.64ab	3.29c	80.15a	11.98ab
l.s.d. ($P = 0.05$)		1.732	1.394	3.118	2.314	0.958	3.787	2.447
MSE (d.f.)		1.002 (16)	0.648 (16)	3.246 (16)	1.788 (16)	0.306 (16)	4.788 (16)	1.999 (16)

Table 6. Effect of nitrogen fertilisation on *in vitro* total gas (TG) and methane production after incubation for 24 or 48 h of improved subtropical C_4 grass species commonly used in South Africa

l.s.d., Least significant difference; MSE, mean square error; d.f., degrees of freedom; Within a column, means followed by the same letter are not significantly different ($P > 0.05$)

	N (kg ha^{-1})	24 h		48 h		CH_4 : TG	
		TG	CH_4	TG	CH_4	24 h	48 h
		(mL g^{-1} DM)					
C. ciliaris	0	105.28abc	4.68ab	154.59abc	11.00ab	0.044b	0.071ab
	50	98.31abcde	4.35bc	149.05abc	10.96ab	0.045b	0.074a
	100	102.86abcd	5.38a	153.29abc	10.90ab	0.053a	0.070abc
C. gayana	0	85.18cde	3.33d	136.70bc	9.52abc	0.039b	0.069abc
	50	82.20de	3.59cd	135.43c	9.82abc	0.043b	0.072ab
	100	89.20bcde	3.30d	145.84abc	9.06bc	0.037b	0.062c
D. eriantha	0	78.89e	3.50cd	135.73c	8.75c	0.044b	0.064bc
	50	101.79abcd	4.12bcd	160.67a	10.34abc	0.044b	0.064bc
	100	91.19abcde	4.07bcd	151.88abc	10.59abc	0.044b	0.069abc
P. maximum	0	110.44a	4.69ab	162.21a	11.32a	0.043b	0.069abc
	50	106.05ab	4.40bc	157.73ab	11.02ab	0.042b	0.070abc
	100	109.03ab	4.52ab	161.02a	11.18a	0.041b	0.069abc
l.s.d. ($P = 0.05$)		20.676	0.916	21.795	2.053	0.0074	0.0084
MSE (d.f.)		149.096 (22)	0.292 (22)	165.67 (22)	1.469 (22)	<0.0001 (22)	<0.0001 (22)

Table 7. Effect of nitrogen fertilisation on *in vitro* total gas (TG) and methane production after incubation for 24 or 48 h of improved temperate C_3 grass species commonly used in South Africa

l.s.d., Least significant difference; MSE, mean square error; d.f., degrees of freedom. Within a column, means followed by the same letter are not significantly different ($P > 0.05$)

	N (kg ha^{-1})	24 h		48 h		CH_4 : TG	
		TG	CH_4	TG	CH_4	24 h	48 h
D. glomerata	0	82.29e	3.29c	128.57c	6.95d	0.039bc	0.054de
	50	91.22de	3.86bc	133.86c	6.94d	0.041abc	0.051e
	100	105.67cd	4.87b	147.80bc	8.03cd	0.046a	0.054de
F. arundinacea	0	122.12abc	4.46bc	168.84a	9.46bc	0.036c	0.056cde
	50	133.83ab	6.18a	171.91a	10.05ab	0.046a	0.058cd
	100	139.22a	6.51a	180.18a	11.10a	0.047a	0.062bc
L. perenne	0	114.78bc	4.82b	166.39ab	11.42a	0.042ab	0069a
	50	119.21abc	4.74b	170.69a	11.33a	0.040bc	0.069a
	100	109.59cd	4.55bc	160.90ab	11.11a	0.042abc	0.067ab
l.s.d. ($P = 0.05$)		21.455	1.288	20.993	1.545	0.0061	0.0067
MSE (d.f.)		153.642 (16)	0.554 (16)	147.096 (16)	0.797 (16)	<0.0001 (16)	<0.0001 (16)

CH_4 production at this level of N fertiliser, after the 48-h incubation. No between-species differences ($P > 0.05$) were observed for C_4 species in the 100 kg N ha^{-1} treatment after the 48-h incubation (Table 6).

Considering the C_3 grass species evaluated, an increase in the level of N fertilisation increased ($P < 0.05$) *in vitro* CH_4 production after the 24-h incubation period for both *D. glomerata* and *F. arundinacea*, and after the 48-h incubation period for *F. arundinacea*. Level of N fertilisation had no effect on CH_4 : TGP for any of the C_3 species after the 48-h incubation, but after the 24-h incubation, increasing level of N fertiliser from 0 to 100 kg ha^{-1} increased ($P < 0.05$) CH_4 : TGP for *D. glomerata* and *F. arundinacea.* No effects were found on any of the *in vitro* gas production parameters among N treatments for *L. perenne* (Table 7).

After the 48-h incubation, *L. perenne* had a higher ($P < 0.05$) CH_4 : TGP than *D. glomerata* and *F. arundinacea* at both 0 and 50 kg N ha^{-1} and a higher CH_4 : TGP than *D. glomerata* at 100 kg N ha^{-1}. After the 24-h incubation, no differences among the C_3 species were found at 100 kg N ha^{-1}; however, *F. arundinacea* had a higher CH_4 : TGP ratio than *L. perenne* at 50 kg N ha^{-1}.

Discussion

The objective of the study was to elucidate the influence of N-fertiliser application levels on the nutrient concentration, *in vitro* digestibility, *in vitro* TGP and CH_4 production of commonly used, improved C_4 and C_3 grass species in South Africa.

Forage quality

Increasing the level of N fertilisation increased the CP concentration of the C_4 and C_3 grass species, with the exception of *D. eriantha*. These results agree with results reported by Morison *et al*. (1980), Valk *et al*. (1996) and Warner *et al*. (2015). The CP concentration reported in the present trial for both C_4 and C_3 species are lower than previously reported values for similar species (Pieterse *et al*. 1997; Johnson *et al*. 2001; Taute *et al*. 2002; Navarro-Villa *et al*. 2012; Banik *et al*. 2013). This might be due to differences in N-application rates and growth periods after N application among the studies, as well as to differences in growth phases harvested between the present study and previous reports. Wilman (1975) reported that the N concentrations of pastures peak at 10–14 days after N application and thereafter decrease over time. The effect of N application on forage CP concentration in the present study could have been reduced by the 8-week regrowth period employed before harvesting. The age of forage, whether from initial or subsequent cuttings, has a negative effect on forage quality by increasing fibre components as well as decreasing digestibility and/or CP concentration (Salon and Cherney 2000).

Increasing the level of N fertilisation decreased ($P < 0.05$) the NDF and ADF concentrations of *C. gayana* and *P. maximum* but had no effect ($P > 0.05$) on the fibre fractions of *C. ciliaris* and *D. eriantha* (Table 4). Similarly, *D. glomerata* showed a decrease ($P < 0.05$) in NDF concentration with increasing level of N fertilisation (Table 5). The fibre fraction of *F. arundinacea* showed a tendency to decrease with increasing N fertilisation, but level of fertilisation had no effect ($P > 0.05$) on the fibre fractions of *L. perenne*. The inconsistent influence of N fertilisation on NDF, ADF and ADL concentrations is similar to the findings of Minson (1990) and Valk *et al*. (1996) who reported that the physiological stage of development has a greater influence on fibre fractions of forage than does the level of N fertilisation. A similar inconsistent effect of N on forage fibre fraction was reported by Peyraud and Astigarraga (1998), who concluded that the nutrient-composition response of forages to N fertilisation is species-specific.

Increasing the level of N fertilisation increased IVOMD of two of the C_4 grass species, *C. ciliaris* and *D. eriantha*, but no significant effect was found for *C. gayana* and *P. maximum*. Similarly, Johnson *et al*. (2001) reported an increase in IVOMD for star grass (*Cynodon nlemfuensis*) fertilised with increasing levels of N, and Taute *et al*. (2002) reported no effect of N fertiliser on IVOMD of *P. maximum*. The IVOMD of C_3 species was not affected by level of N fertilisation, except in *D. glomerata*, which showed a decrease as level of N

fertilisation increased. These results are similar to those of Valk *et al*. (1996) and Lovett *et al*. (2004) for *L. perenne*. The decrease in the digestibility of *D. glomerata* can be explained by a slight increase in the lignin concentration with increased N fertilisation (Table 5). The increase in ADL concentration could be related to an increase in DM yield in response to N fertilisation, as reported by Lovett *et al*. (2004) and Cui *et al*. (2016), although DM yield was not recorded in the present study. Peyraud and Astigarraga (1998) also reported that N fertilisation increased the tiller : leaf ratio of forages, which could have a negative effect on forage digestibility. Nitrogen fertilisation can, however, have an indirect positive effect on digestibility by enabling earlier utilisation of grass forage. A higher level of N application allows for grass to be harvested at an earlier physiological age owing to increased growth response and yield (Peyraud and Astigarraga 1998). This could lead to increased intake and production from livestock and thus a reduced CH_4 intensity (CH_4 per unit product) of the pastures. However, these aspects of N fertilisation were not explored in the present study.

Gas production

Methane production from forages depends on both NDF concentration and forage digestibility, which are the two main drivers of H^+ production from carbohydrate fermentation in the rumen (Archimède *et al*. 2011). The gas production values reported in Table 6 are similar to gas production values reported by González Ronquillo *et al*. (1998) for C_4 grass species. In the present experiment, *C. ciliaris* and *P. maximum* produced the highest average *in vitro* CH_4 values across all N treatments after the 24- and 48-h incubation periods. This corresponds with higher NDF concentration and IVOMD (Table 4) of these species than of *D. eriantha* and *C. gayana*. These results correspond with those of Gemeda and Hassen (2014) and Doreau *et al*. (2016), who reported a positive correlation among CH_4 production, cell-wall contents and IVOMD of forages. *Digitaria eriantha* had the lowest average *in vitro* CH_4 production after the 24- and 48-h incubation periods and tended to have lower CH_4 : TGP than other C_4 species in the present trial. This could be attributed to the lower IVOMD ($P < 0.05$) of *D. eriantha*, which might have a negative effect on voluntary forage intake and subsequent CH_4 intensity (CH_4 per unit product).

The significant increase in 24-h CH_4 and CH_4 : TGP of *D. glomerata* and *F. arundinacea* corresponds with a significant increase in CP concentration as level of N fertilisation increases, and a reduction in fibre fraction of the species (Table 5). These results differ from data of Johnson and Johnson (1995) and Lovett *et al*. (2004), which indicated a decrease in CH_4 production when feed protein concentration increased. The increase in 24-h gas production could have been due to changes in the degradability of the CP and fibre fractions resulting from an increase in N fertiliser as reported by Valk *et al*. (1996). Crude protein levels above the threshold of 70 g kg DM^{-1}, as reported in the present study, are considered to enhance microbial multiplication in the rumen, thus improving fermentation (Njidda and Nasiru 2010). The negative correlation between NDF concentration and *in vitro* gas production reported by Njidda and Nasiru (2010) and Meale *et al*. (2012) was not observed in the present study. This might have been due to the relatively high IVOMD of the species reported here. Increasing N fertilisation from 0 to 100 kg N ha^{-1} had no effect on the *in vitro* TGP or CH_4 production of *L. perenne* after either incubation period. These results differ from those of Lovett *et al*. (2004), which showed a significant decrease in the *in vitro* gas and CH_4 production with increasing N-application levels to *L. perenne*. These differences might have been due to differences in the physiological age of the forages between the two trials.

In the present study, *D. glomerata* emerged as the C_3 species with the lowest CH_4 : TGP after the 48-h incubation, compared with *F. arundinacea* and *L. perenne*. Although this could be partly attributed to a reduction in CH_4 production, it might also indicate reduced overall ruminal fermentation potential. *Dactylis glomerata* had the lowest TGP after the 48-h incubation period. This reduced fermentation could be explained by the lower IVOMD of *D. glomerata*. This lower IVOMD in the present experiment could negatively influence DM intake through a reduced ruminal clearance rate, which could have negative implications for livestock productivity compared with *F. arundinacea* and *L. perenne* (Banik *et al*. 2013).

Conclusion

This study demonstrated significant differences in nutrient composition, digestibility, and *in vitro* gas-production characteristics among key South African improved pasture species. Although increasing N fertilisation levels affected the nutrient composition of the pasture species, the effect on *in vitro* CH_4 production per unit DM digested was limited. Between-species differences were found for 24-h *in vitro* CH_4 production in C_4 and C_3 species but these differences diminished after the 48-h incubation period. The data suggest that reductions in enteric CH_4 production are unlikely to be achieved through increased N-fertilisation levels alone. *Chloris gayana* and *D. glomerata* showed potential for reduced CH_4 output from C_4 and C_3 grass species, respectively, compared with the other species evaluated. However, these results are based on *in vitro* analysis and from hand-harvested samples grown in a greenhouse. There is a need for further assessment of fermentation characteristics and management practices of these species at various stages of maturity, and an *in vivo* evaluation is necessary before any species can be promoted as a low-methanogenic pasture.

Conflicts of interest

The authors declare no conflicts of interest.

Acknowledgements

This study forms part of a larger project jointly funded by the Red Meat Research and Development Trust of South Africa and the National Research Foundation of South Africa (TTK 14060668451).

References

AOAC (2000) ‘Official method of analysis.’ 17th edn. (Association of Official Analytical Chemists: Rockville, MD, USA)

Archimède H, Eugene M, Marie-Magdeleine C, Boval M, Martin C, Morgavi DP, Lecomte P, Doreau M (2011) Comparison of methane production between C3 and C4 grasses and legumes. *Animal Feed Science and Technology* **166–167**, 59–64. doi:10.1016/j.anifeedsci.2011.04.003

Banik BK, Durmic Z, Erskine W, Ghamkhar K, Revell C (2013) *In vitro* ruminal fermentation characteristics and methane production differ in selected key pasture species in Australia. *Crop & Pasture Science* **64**, 935–942. doi:10.1071/CP13149

Beauchemin KA, Kreuzer M, O'Mara F, McAllister TA (2008) Nutritional management for enteric methane abatement: a review. *Australian Journal of Experimental Agriculture* **48**, 21–27. doi:10.1071/EA07199

Beauchemin KA, McAllister TA, McGinn SM (2009) Dietary mitigation of enteric methane from cattle. *Perspectives in Agriculture, Veterinary Science, Nutrition and Natural Resources* **4**, 1–18. doi:10.1079/PAVSNNR20094035

Benchaar C, Hassanat F, Gervais R, Chouinard PY, Petit HV, Massè DI (2014) Methane production, digestion, ruminal fermentation, N balance, and milk production of cows fed corn silage- or barley silage-based diets. *Journal of Dairy Science* **97**, 961–974. doi:10.3168/jds.2013-7122

Bhatta R, Saravan M, Baruah L, Malik PK, Ssampath KT (2017) Nutrient composition, rate of fermentation and in vitro rumen output from tropical feedstuffs. *The Journal of Agricultural Science* **155**, 171–183. doi:10.1017/S0021859616000642

Cone JW, Van Gelder AH, Soliman IA, De Visser H, Van Vuuren AM (1999) Different techniques to study rumen fermentation characteristics of maturing grass and grass silage. *Journal of Dairy Science* **82**, 957–966. doi:10.3168/jds.S0022-0302(99)75315-4

Cui JH, Yang HJ, Yu CQ, Bai S, Song SS, Wu TT, Sun W, Shao XM, Jiang LS (2016) Effect of urea fertilisation on biomass yield, chemical composition, in vitro rumen digestibility and fermentation characteristics of forage oat straw in Tibet of China. *The Journal of Agricultural Science* **154**, 914–927. doi:10.1017/S0021859616000198

Doreau M, Benhissi H, Thior YE, Bois B, Genestoux L, Lecomte P, Morgavi DP, Ickowicz A (2016) Methanogenic potential of forages consumed throughout the year by cattle in a Sahelian pastoral area. *Animal Production Science* **56**, 613–618. doi:10.1071/AN15487

Du Toit CJL, Meissner HH, Van Niekerk WA (2013*a*) Direct methane and nitrous oxide emissions of South African dairy and beef cattle. *South African Journal of Animal Science* **43**, 320–339. doi:10.4314/sajas.v43i3.7

Du Toit CJL, Meissner HH, Van Niekerk WA (2013*b*) Direct greenhouse gas emissions of the game industry in South Africa. *South African Journal of Animal Science* **43**, 376–393. doi:10.4314/sajas.v43i3.10

Du Toit CJL, Van Niekerk WA, Meissner HH (2013*c*) Direct greenhouse gas emissions of the South African small stock sectors. *South African Journal of Animal Science* **43**, 340–361. doi:10.4314/sajas.v43i3.8

Durmic Z, Hutton P, Emms J, Hughes S, Vercoe P (2010) *In vitro* fermentation traits of Australian woody perennial plant species that may be considered as potential sources of feed for grazing ruminants. *Animal Feed Science and Technology* **160**, 98–109. doi:10.1016/j.anifeedsci.2010.07.006

Durmic Z, Ramirez-Restrepo CA, Gardiner C, O'Neil CJ, Hussein E, Vercoe PE (2017) Differences in the nutrient concentrations, *in vitro* methanogenic potential and other fermentative traits of tropical grasses and legumes for beef production systems in northern Australia. *Journal of the Science of Food and Agriculture* **97**, 4075–4086. doi:10.1002/jsfa.8274

Engels EAN, Van der Merwe FJ (1967) Application of an *in vitro* technique to South African forages with special reference to affect certain factors on the results. *South African Journal of Agricultural Science* **10**, 983–995.

Gemeda BS, Hassen A (2014) *In vitro* fermentation, digestibility and methane production of tropical perennial grass species. *Crop & Pasture Science* **65**, 479–488. doi:10.1071/CP13450

Gibbs Russel GE, Watson L, Koekermoer M, Smook L, Barker NP, Anderson HM, Dallwitz MJ (1991) 'Grasses of Southern Africa.' Memoir of the Botanical Survey of South Africa No. 58. (National Botanical Gardens: Pretoria, South Africa)

Glass GV, Peckham PD, Sanders JR (1972) Consequences of failure to meet assumptions underlying the fixed effects analyses of variance and covariance. *Review of Educational Research* **42**, 237–288. doi:10.3102/00346543042003237

Goel G, Makkar HPS (2012) Methane mitigation from ruminants using tannins and saponins, a status review. *Tropical Animal Health and Production* **44**, 729–739. doi:10.1007/s11250-011-9966-2

Goering HK, Van Soest PJ (1988) 'Forage fiber analysis, apparatus, reagents, procedures, and some applications.' Agriculture Handbook No. 379. (USDA-ARS: Washington, DC)

González Ronquillo M, Fondevila M, Barrios Urdaneta A, Newman Y (1998) *In vitro* gas production from buffel grass (*Cenchrus ciliaris* L.) fermentation in relation to the cutting level, the level of nitrogen fertilisation and the season of growth. *Animal Feed Science and Technology* **72**, 19–32. doi:10.1016/S0377-8401(97)00181-8

Johnson KA, Johnson DE (1995) Methane emissions from cattle. *Journal of Animal Science* **73**, 2483–2492. doi:10.2527/1995.7382483x

Johnson CR, Reiling BA, Mislevy P, Hall MB (2001) Effects of nitrogen fertilisation and harvest date on yield, digestibility, fibre and protein fraction of tropical grasses. *Journal of Animal Science* **79**, 2439–2448. doi:10.2527/2001.7992439x

Lovett D, Bortolozzo A, Conaghan P, O'Kiely P, O'Mara FP (2004) *In vitro* total and methane gas production as influenced by level of nitrogen application, season of harvest and perennial ryegrass cultivar. *Grass and Forage Science* **59**, 227–232. doi:10.1111/j.1365-2494.2004.00421.x

Lovett D, McGilloway D, Bortolozzo A, Hawkins M, Callan J, Flynn B, O'Mara F (2006) *In vitro* fermentation patterns and methane production as influenced by cultivar and season of harvest of *Lolium perenne* L. *Grass and Forage Science* **61**, 9–21. doi:10.1111/j.1365-2494.2006.00500.x

Mathison GW, Okine EK, McAllister TA, Dong Y, Galbraith J, Dmytruk OIN (1998) Reducing methane emissions from ruminant animals. *Journal of Applied Animal Research* **14**, 1–28. doi:10.1080/09712119.1998.9706212

Meale SJ, Chaves AV, Baah J, McAllister TA (2012) Methane production of different forages in *in vitro* ruminal fermentation. *Asian-Australasian Journal of Animal Sciences* **25**, 86–91. doi:10.5713/ajas.2011.11249

Meissner HH, Zacharias PJK, O'Reagain PJ (1999) Forage quality (feed value). In 'Veld management in South Africa'. (Ed. NM Tainton) (University of Natal Press: Pietermaritzburg, South Africa)

Meissner HH, Scholtz MM, Engelbrecht FC (2013) Sustainability of the South African livestock sector towards 2050. Part 2: Challenges, changes and required implementations. *South African Journal of Animal Science* **43**, 298–319.

Minson DJ (1990) 'Forage in ruminant nutrition.' (Academic Press: San Diego, CA, USA)

Morison J, Jackson MV, Sparrow PE (1980) The response of perennial ryegrass to fertilizer nitrogen in relation to climate and soil. Technical Report No. 27: 71. Grassland Research Institute, Hurley, UK.

Mould FL, Morgan R, Kliem KE, Krystallidou E (2005) A review and simplification of the *in vitro* incubation medium. *Animal Feed Science and Technology* **123–124**, 155–172. doi:10.1016/j.anifeedsci.2005.05.002

Navarro-Villa A, O'Brien M, Lopez S, Boland TM, O'Kiely P (2012) *In vitro* rumen methane output of grasses and grass silages differing in fermentation characteristics using the gas-production technique (GPT). *Grass and Forage Science* **67**, 472–487.

Njidda AA, Nasiru A (2010) *In vitro* gas production and dry matter digestibility of tannin containing forages of a semi-arid region of

north-eastern Nigeria. *Pakistan Journal of Nutrition* **9**, 60–66. doi:10.3923/pjn.2010.60.66

Peyraud JL, Astigarraga L (1998) Review of the effect of nitrogen fertilisation on the chemical composition, intake, digestion and nutritive value of fresh herbage: consequences on animal nutrition and N balance. *Animal Feed Science and Technology* **72**, 235–259. doi:10.1016/S0377-8401(97)00191-0

Pieterse PA, Rethman NFG, Van Bosch J (1997) Production, water use efficiency and quality of four cultivars of *Panicum maximum* at different levels of nitrogen fertilisation. *Tropical Grasslands* **31**, 117–123.

Rivera JD, Gipson ML, Gipson RG, Lemus RW (2017) Effects of supplement or fertiliser on forage quality, and performance of stocker cattle grazing warm-season pastures. *Animal Production Science* **57**, 116–121. doi:10.1071/AN15197

Robinson PH, Givens DI, Getachew G (2004) Evaluation of NRC, UC Davis and ADAS approaches to estimate the metabolisable energy values of feeds at maintenance energy intake from equations utilizing chemical essays and in vitro determinations. *Animal Feed Science and Technology* **114**, 75–90. doi:10.1016/j.anifeedsci.2003.12.002

Salon PR, Cherney DJR (2000) Eastern gamagrass forage quality as influenced by harvest management. In 'Proceedings Second Eastern Native Grass Symposium'. (Eds JC Ritchie, JA Dickerson, CA Richie) pp. 298–304. (USDA-ARS and USDA-NRCS: Beltsville, MD, USA)

Shapiro SS, Wilk MB (1965) An analysis of variance test for normality (complete samples). *Biometrika* **52**, 591–611. doi:10.1093/biomet/52.3-4.591

Taute A, Van Niekerk WA, Rethman NFG, Coertze RJ (2002) An evaluation on nitrogen fertilised *Panicum maximum* cv. Gatton at different stages of maturity during autumn: 1. Dry matter yield and certain qualitative parameters. *South African Journal of Animal Science* **32**, 208–216.

Theodorou MK, Williams BA, Dhanoa MS, McAllen AB, France J (1994) A simple gas production method using pressure transducers to determine the fermentation kinetics of ruminant feed. *Animal Feed Science and Technology* **48**, 185–197. doi:10.1016/0377-8401(94)90171-6

Tilley JMA, Terry RA (1963) A two-stage technique for the *in vitro* digestion of forage crops. *Journal of the British Grassland Society* **18**, 104–111. doi:10.1111/j.1365-2494.1963.tb00335.x

Ullah MA, Hussain N, Schmeisky H, Rasheed M, Anwar M, Rana AS (2018) Fodder quality improvement through intercropping and fertilizer application. *Pakistan Journal of Agricultural Sciences* **55**, 549–554. doi:10.21162/PAKJAS/18.4579

Valk H, Kappers IE, Tamminga S (1996) *In sacco* degradation characteristics of organic matter, neutral detergent fibre and crude protein of fresh grass fertilised with different amounts of nitrogen. *Animal Feed Science and Technology* **63**, 63–87. doi:10.1016/S0377-8401(96)01024-3

Van Soest PJ, Robertson PJ, Lewis BA (1991) Methods for dietary fibre, neutral detergent fibre, and non-starch polysaccharides in relation to animal nutrition. *Journal of Dairy Science* **74**, 3583–3597. doi:10.3168/jds.S0022-0302(91)78551-2

Warner D, Podest SC, Hatew B, Klop G, van Laar H, Bannink A, Dijkstra J (2015) Effect of nitrogen fertilisation rate and regrowth interval of grass herbage on methane emission of zero-grazing lactating dairy cows. *Journal of Dairy Science* **98**, 3383–3393. doi:10.3168/jds.2014-9068

Wilman D (1975) Nitrogen and Italian ryegrass: 2. Growth up to 14 weeks: nitrogen, phosphorus and potassium content and yield. *Journal of the British Grassland Society* **30**, 243–249. doi:10.1111/j.1365-2494.1975.tb01384.x