ISSN: 2346-3775

Tropical Grasslands -Forrajes Tropicales

Online Journal

Vol. 3 No. 3
September 2015

Published by:
Centro Internacional de Agricultura Tropical (CIAT), Cali, Colombia
In cooperation with:
• Chinese Academy of Tropical Agricultural Sciences (CATAS)
• Australian Centre for International Agricultural Research (ACIAR)

www.tropicalgrasslands.info

Editors

Management Committee

Editorial Board

Masahiko Hirata,
Faculty of Agriculture, University of Miyazaki,
Japan

Peter Horne,
Australian Centre for International Agricultural Research (ACIAR),
Australia

Johann Huguenin,
Sistemas Ganaderos en el Mediterráneo y las Zonas Tropicales, CIRAD,
France

Muhammad Ibrahim,
Instituto Interamericano de Cooperación para la Agricultura (IICA),
Belice

Asamoah Larbi,
International Institute of Tropical Agriculture (IITA),
Nigeria

Carlos E. Lascano,
Universidad Nacional de Colombia - Sede Bogotá,
Colombia

Robert Paterson,
Agricultural Consultant,
Spain

Bruce Pengelly,
Commonwealth Scientific and Industrial Research Organisation (CSIRO),
Australia

T. Reginald Preston,
University of Tropical Agriculture Foundation (UTA),
Colombia

Kenneth Quesenberry,
University of Florida,
USA

Max Shelton,
University of Queensland,
Australia

Werner Stür,
University of Queensland,
Australia

Cacilda B. do Valle,
Empresa Brasileira de Pesquisa Agropecuária (Embrapa),
Brazil

Principal Contacts

Rainer Schultze-Kraft
Centro Internacional de Agricultura Tropical (CIAT)
Colombia
Phone: +57 2 4450100 Ext. 3036
Email: r.schultzekraft@cgiar.org

Technical Support
Cristhian David Puerta R.
Centro Internacional de Agricultura Tropical (CIAT)
Colombia
Phone: +57 2 4450100 Ext. 3354
Email: c.d.puerta@cgiar.org

Table of Contents

Research Papers

[Forage intake, feeding behavior and bio-climatological indices of pasture grass, under the influence of trees, in a silvopastoral system] **129-141**

L.F Sousa, R.M. Maurício, D.S.C. Paciullo, S.R. Silveira, R.S. Ribeiro, L.H. Calsavara, G.R. Moreira

[Forage growth, yield and quality responses of Napier hybrid grass cultivars to three cutting intervals in the Himalayan foothills] **142-150**

Kesang Wangchuk, Krishna Rai, Harilal Nirola, Thukten, Chhoyten Dendup, Durba Mongar

[Performance of tropical legumes grown as understory of a eucalypt plantation in a seasonally dry area of the Brazilian Cerrado] **151-160**

Maria Luiza F. Nicodemo, Francisco H.D. de Souza, José Ricardo M. Pezzopane, João Carlos T. Mendes, Waldomiro Barioni Junior, Patrícia Tholon, Patrícia M. Santos

[Commonness and rarity pattern of plant species within *Terai* grassland of northeastern Uttar Pradesh, India] **161-186**

Sumit Srivastava, Ashish Dvivedi, R.P. Shukla

[Nutritive value and in situ rumen degradability of Marandu palisade grass at different locations within the pasture in a silvopastoral system with different babassu palm densities] **187-193**

Xerxes M. Tosta, Rosane C. Rodrigues, Sâmara S.C. Sanchês, Jocélio S. Araújo, Antônio da S. Lima Júnior, Clésio S. Costa, Francisco N. de S. Santos, Ana P.R. Jesus, Ivone R. da Silva, Francivaldo O. Costa, Francirose Shigaki, Sanayra da S. Mendes

[Esterase polymorphism for genetic diversity analysis of some accessions of a native forage grass, *Mesosetum chaseae* Luces, from the Brazilian Pantanal] **194-204**

Ana Clara S. Meirelles, Eliane R. Monteiro, Laura A.C. da Silva, Débora da Silva, Sandra A. Santos, Sandra A. de Oliveira-Collet, Claudete A. Mangolin, Maria de Fátima P.S. Machado

DOI: 10.17138/TGFT(3)129-141

Forage intake, feeding behavior and bio-climatological indices of pasture grass, under the influence of trees, in a silvopastoral system

L.F. SOUSA[1], R.M. MAURÍCIO[2], D.S.C. PACIULLO[3], S.R. SILVEIRA[2], R.S. RIBEIRO[2], L.H. CALSAVARA[2] AND G.R. MOREIRA[4]

[1]*Departamento de Zootecnia, Universidade Federal do Tocantins, Palmas, TO, Brazil.* http://ww1.uft.edu.br
[2]*Departamento de Engenharia de Biossistemas (DEPEB), Universidade Federal de São João Del-Rei, São João Del-Rei, MG, Brazil.* www.ufsj.edu.br/ppbe
[3]*Empresa Brasileira de Pesquisa Agropecuária, Embrapa Gado de Leite, Juiz de Fora, MG, Brazil.* www.embrapa.br/en/gado-de-leite
[4]*Departamento de Estatística e Informática, Universidade Federal Rural de Pernambuco, Recife, PE, Brazil.* www.deinfo.ufrpe.br

Keywords: Animal behavior, microclimate, shade, sheep.

Abstract

The aim of this study was to compare a silvopastoral system with a control (pasture only) in the Brazilian Cerrado. The silvopastoral system consisted of a tropical grass (*Brachiaria brizantha* cv. Marandu) pasture and trees (*Zeyheria tuberculosa*), while the control was a Marandu pasture without trees. Sheep intake, feeding behavior and microclimatic conditions were the variables evaluated. Temperatures within the silvopastoral system were lower than in the control (maximum temperature of 28 and 33.5 °C, temperature and humidity index of 74.0 and 79.2 for the silvopastoral system and control, respectively). There was increased dry matter intake (88.2 vs. 79.9 g DM/kg$^{0.75}$ LW/d, P<0.05), organic matter intake (89.6 vs. 81.1 g OM/kg$^{0.75}$ LW/d, P<0.05) and grazing time (572 vs. 288 min/d, P<0.05), and reduced total water intake (430 vs. 474 mL/kg$^{0.75}$ LW/d, P<0.05) and walking time (30 vs. 89 min/d, P<0.05) in grazing sheep in the silvopastoral system relative to the control. The results suggest that a silvopastoral system would provide a more favorable environment than a straight pasture for sheep performance in a tropical grazing situation.

Resumen

En el estudio se compararon un sistema silvopastoril con un sistema control de solo pastura en condiciones del Cerrado brasileño. El sistema silvopastoril consistió en una pastura de *Brachiaria brizantha* cv. Marandu con árboles de *Zeyheria tuberculosa*, mientras que el tratamiento control consistió en una pastura de cv. Marandu sin árboles. Como variables se midieron el consumo por ovejas, su comportamiento de pastoreo y las condiciones microclimáticas durante el tiempo de evaluaciones. Las temperaturas para el sistema silvopastoril fueron más bajas que en el control (temperatura máxima de 28 y 33.5 °C, e índice de temperatura-humedad de 74.0 y 79.2 para el sistema silvopastoril y el control, respectivamente). Las ovejas en pastoreo mostraron mayor consumo de materia seca (88.2 vs. 79.9 g MS/kg$^{0.75}$ peso vivo/día, P<0.05) y de materia orgánica (89.6 vs. 81.1 g MO/kg$^{0.75}$ peso vivo/día, P<0.05), mayor tiempo pastoreando (572 vs. 288 minutos/día, P<0.05), menor consumo total de agua (430 vs. 474 mL/kg$^{0.75}$ peso vivo/día, P<0.05) y menor tiempo caminando (30 vs. 89 minutos/día, P<0.05) en el sistema silvopastoril en comparación con el sistema control. Los resultados indican que para la producción ovina bajo condiciones tropicales un sistema silvopastoril puede proporcionar un entorno más favorable que un sistema de pastura sola.

Correspondence: Rogério M. Maurício, Departamento de Engenharia de Biossistemas (DEPEB), Universidade Federal de São João Del-Rei, Campus Dom Bosco, Pç Dom Helvécio, 74 – Dom Bosco, CEP 36301-160, São João Del-Rei, MG, Brazil.
E-mail: rogeriomauricio@ufsj.edu.br

Introduction

Silvopastoral systems (SPS), a combination of trees, forages and animals, are increasingly being adopted throughout the tropics as a sustainable alternative to straight pasture for animal production, consequently reducing the impacts of deforestation (Bocquier and Gonzalez-Garcia 2010; Maurício 2012) and increasing biodiversity (Dumont et al. 2013). In these systems, the dynamics of ecophysiological processes are different from those in traditional monoculture forage systems (Wilson et al. 1990). According to Maurício (2012), SPS require more complex management owing to the larger number of interactions between the different plant forms.

Trees in SPS take up nutrients from deeper layers in the soil and make them available to forage through the decomposition of leaves, twigs, flowers and fruits, which fall to the ground, thus improving soil fertility and, consequently, the quality and yield of the pasture (Reis et al. 2009). This can directly influence animal performance, since both quality and availability of nutrients are correlated with forage intake. Another positive aspect should be the reduction of environmental stress on animals due to the interaction between tree shade and animal behavior. The reduction of sunlight and ambient temperature provided by the tree shade should have an important microclimatic benefit for animals, resulting in increased forage intake and production (Paciullo et al. 2011) and reduction in the expenditure of metabolic energy to maintain homeostatic equilibrium (Forbes 1995).

The objective of this study was to compare the intake and feeding behavior of sheep and microclimatic conditions in a tropical grass [*Brachiaria brizantha* (now *Urochloa brizantha* (Hochst. ex A. Rich.) R.D. Webster) cv. Marandu] pasture, with and without the native tree species, bolsa-de-pastor [*Zeyheria tuberculosa* (Vell.) Bureau] during the rainy season in the Brazilian Cerrado ecosystem.

Materials and Methods

Location and treatments

The experiment was conducted in a silvopastoral system area on a private farm, in Lagoa Santa municipality, Minas Gerais, Brazil (19°35′36″ S, 43°51′56″ W; 747 masl). There were 2 system treatments: silvopastoral system (SPS; = grass plus trees) and monoculture (Mono; = grass only). According to the Brazilian soil classification, for both systems the soil is a red-yellow oxisol ('latossolo vermelho-amarelo'; Typic Acrustox − USDA classification) with 651 g/kg clay, 211 g/kg silt and 138 g/kg sand. The P level, in the 0–20 cm layer of both systems, was 1 mg/dm^3, while the pH level was 5.1 in the SPS and 4.9 in Mono (Reis et al. 2009). The silvopastoral system area on that farm (Figure 1) had been under development since 1984 by: managing the natural regeneration of the tree, *Zeyheria tuberculosa* (ZT); replacement of the grass *Hyparrhenia rufa* by Marandu; application, guided by soil analyses, of only modest rates of limestone and rock phosphate (1 and 0.5 tonne/ha, respectively); and no use of (soil organic matter deteriorating) fire as traditional pasture management tool.

Figure 1. Silvopastoral system with *Urochloa brizantha* cv. Marandu and the tree, *Zeyheria tuberculosa* (bolsa-de-pastor).

The tree species was selected for inclusion in the SPS owing to the quality of its wood, its fast growth, straight trunks, intermediate canopy density and resistance to cattle grazing. It is a very useful species for restoring degraded areas through natural regeneration, as its seeds are easily spread by wind. During the natural regeneration process prior to the commencement of the trial (June 2001), undesirable species were removed and at least 4 m were kept between ZT trees. At commencement, the trees were 15–23 m tall with a crown stem diameter of 40–60 cm. The density adopted was 160 trees/ha.

For the control treatment (Mono), an adjacent area, from which all trees were removed, was planted with Marandu using the same methodology. The total area, including the SPS and the pasture, consisted of approximately 2 ha.

The experiment consisted of: (1) assessment of forage grass production during 12 months and, in the rainy season when there was adequately high forage production, (2) a sheep intake and feeding behavior trial ("in vivo trial") during 12 days, coupled with (3) the assessment of relevant microclimatic variables.

Forage production

Within each treatment, 3 individual plots, with an area of 4 x 4 m (16 m^2) each, were randomly allocated and fenced with barbed wire to avoid interference from the sheep that were grazing in the surrounding area. At the beginning of the rainy season (November 25 in Year I), the Marandu stands on all plots were cut at 30 cm from ground level to make them uniform and a 30-d rest period was allowed for the stands to reach 60–80 cm in height as suggested for correct morphophysiological management (Costa et al. 2004). A single forage sample in each plot was harvested at 30 cm using the square method (1 m^2), thus simulating the correct management of Marandu (Johnson 1978). After cutting, forage samples were weighed, separated into green leaf and stem, dried in a forced-air oven at 55 °C, ground in a mill using a 1-mm mesh screen, and kept in plastic containers for further analyses. This procedure was performed during 7 growth periods over 12 months as described in Table 1.

Microclimatic variables

To obtain information about thermal comfort provided by the trees, the following microclimatic parameters were measured during the intake and feeding behavior trial (12 days): global radiation (GR), maximum and minimum air temperatures were measured daily with an alcohol thermometer taken at least 3.5 m from the tree stems and at 1.2 m above ground level, along with daily average rainfall. Temperature measurements from a dry/wet bulb thermometer and a black globe thermometer (Vernon's globe thermometer) were performed 6 times a day (07.00, 09.00, 11.00, 13.00, 15.00 and 19.00 h) during the entire period of the in vivo trial in order to establish:

A) The temperature and humidity index (THI) according to Kelly and Bond (1971):

$$THI = DBT - 0.55\ (1 - RH) \times (DBT - 58)$$

where:
THI = temperature and humidity index;
DBT = dry bulb temperature (°C); and
RH = relative humidity (%).

B) The black globe temperature and humidity index (BGTHI) according to Buffington et al. (1981):

$$BGTHI = TBG + (0.36 \times TDP) + 41.5$$

where:
TBG = temperature of black globe (°C); and
TDP = temperature of dew point (°C)
$TDP = (RH/100)^{0.125} \times [112 + (0.9 \times DBT)] + (0.1 \times DBT) - 112$

Intake

For the intake trial, 18 mongrel hair sheep (aged 2–4 years) were randomly assigned to 2 groups of 9 animals (4 males and 5 non-pregnant females) with mean weights of 27.6 ± 5.3 and 28.3 ± 4.9 kg and mean body scores of 2.00 and 2.03 (scale: 0 = extremely thin to 5 = extremely obese) for the SPS and Mono at commencement, respectively. On 25 November, the animals were de-wormed and after 15 days were allocated to the pasture treatments for an adaptation period of 45 days. A continuous stocking system was used at 9 animals/ha, with average initial pasture height of 50 cm during the late period of adaptation. A supply of green leaf dry matter of approximately 3–4 % of mean live weight was maintained. At the beginning of the in vivo trial, the stocking rate in both pastures was adjusted according to green leaf yield which could support the equivalent of up to 1.5 animal units (1 AU = 450 kg) per ha.

Table 1. Experimental harvest periods for forage.

Harvest	Season	Data	Days
P1	Rainy	24 Nov to 25 Dec - Year I	31
P2	Rainy	26 Dec to 25 Jan - Year II	31
P3	Rainy	26 Jan to 25 Feb - Year II	31
P4	Rainy	26 Feb to 28 Mar - Year II	31
P5	Transition rainy-dry	29 Mar to 28 Apr - Year II	31
P6	Dry	29 Apr to 28 Jul - Year II	92
P7	Transition dry-rainy	29 Jul to 23 Nov - Year II	118

For both treatments (SPS and Mono), the animals had access to potable water and a trough with a commercial mineral salt mixture, specific for sheep. Disappearances of water and salt on each treatment were recorded every second day. Estimates of evapotranspiration were made through "class A" tanks in both treatments, to adjust water intakes. Total water intake was calculated as free water intake (water disappearance less evapotranspiration) plus water intake from feed (moisture content of forage consumed).

Forage samples were collected daily by means of the simulated grazing technique described by Johnson (1978). After cutting, forage samples were weighed, predried in a forced-air oven at 55 °C, ground in a Willey mill equipped with a 1-mm mesh screen, and kept in plastic containers for later laboratory analyses. Dry matter (DM), ash, nitrogen, ether extract, lignin and acid detergent fiber (ADF) were analyzed according to AOAC (2009). Neutral detergent fiber (NDF) was assayed using the procedure described by Van Soest et al. (1991) without the use of α-amylase. NDF and ADF were expressed inclusive of residual ash. Acid detergent insoluble crude protein was assayed according to Licitra et al. (1996).

During the intake trial the animals weighed 35.2 ± 5.2 and 33.4 ± 6.3 kg and had mean body scores of 2.90 and 2.71 for the SPS and Mono, respectively. Intake was estimated 8 days after the adaptation period, using the external indicator LIPE®, for estimating digestibility and fecal production in ruminants (Ferreira et al. 2009), supplied as capsules introduced directly into the oesophagus of the animal for 5 consecutive days in a daily dose (0.25 g/animal/d) at 08.00 h. On the second day of administration of the external indicator LIPE®, simulated grazing (SG) according to Johnson (1978) was done at 08.00 and 17.00 h for each animal group (treatment) and the pooled forage used in the estimation of in vitro dry matter digestibility (IVDMD) and in vitro organic matter digestibility (IVOMD). On the third day, feces collecting (FC) began, directly from the animal's rectum, at the same time for 5 consecutive days. These samples were frozen at -20 °C and, at the end of all collections, a pooled sample was prepared for each animal for subsequent laboratory analyses. The schemes for delivery of the indicator, simulated grazing and collection of feces are described in Figure 2.

In the laboratory, samples were thawed at room temperature, dried at 55 °C for 72 h in a forced-air oven, ground to 1 mm (Willey type mill) and analyzed for DM, OM and ash following AOAC (2009) and for Klason lignin (Theander and Westerlund 1986). For the determination of LIPE® concentration, each sample was mixed with potassium bromide, pressed, pelleted and read by infrared spectroscopy (Boeriu et al. 2004).

Fecal production (FP) was calculated using the formulae described by Prigge et al. (1981):

$$\text{FP (kg DM/animal/d)} = \frac{\text{Indicator ingested (g/animal/d)}}{\text{Indicator in feces (g/kg DM)}}$$

$$\text{FP (kg OM/day)} = \frac{\text{Indicator ingested (g/animal/d)}}{\text{Indicator in feces (g/kg OM)}}$$

DM and OM intakes were obtained by the following equations:

DMI = [FP on a DM basis]/(1 - IVDMD/1000) + MSI;
OMI = [FP on an OM basis]/(1 - IVOMD/1000).

where:

DMI = total dry matter intake (kg DM/animal/d);
OMI = total organic matter intake (kg OM/animal/d);
IVDMD = in vitro dry matter digestibility (g DM/kg DM);
IVOMD = in vitro organic matter digestibility (g OM/kg OM); and
MSI = mineral salt intake (kg/animal/d).

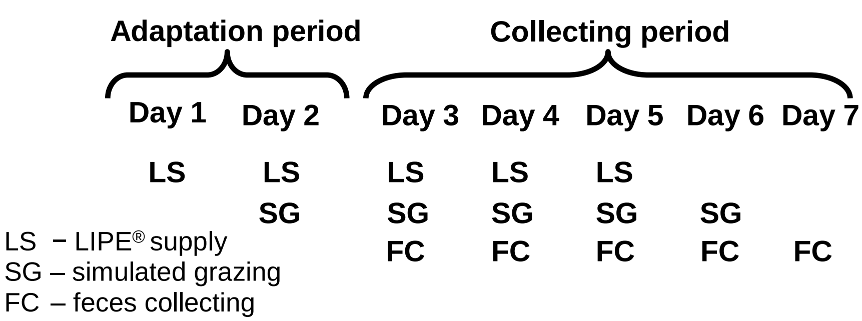


Figure 2. Procedure for delivery of the indicator, simulated grazing and collection of feces.

Feeding behavior

The feeding behavior trial began 5 days before the intake trial and 47 days after the beginning of the adaptation period, and continued for 3 consecutive days, using the same animals and grazing systems as for the intake trial. The behavior of each animal was observed and recorded every 10 minutes during 2 periods of 24 h with a day of rest between periods of observations, within the following ethological categories: grazing; ruminating (either standing or lying); resting; walking; drinking; and other. During these observations, the same microclimatic parameters used for the intake trial were also measured. Since management consisted of releasing the animals in the pasture at 06.00 h and sheltering them for the night at 19.00 h, night-time observations were made with the animals inside each paddock.

Statistical analyses

The statistical design used for all parameters related to the intake and behavior trials was completely randomized with 9 replicates (animals), and comparisons made by means of the F test at 5% probability. Regression analysis was done involving the microclimatic parameters DBT, RH, THI and BGTHI, whereas the equations were compared, across treatments, by the test for equality parameters and parallelism in non-linear regression models (Cohen 1983).

Results

Forage production

Total dry matter (TDM) production of Marandu during the rainy season (P1–P4) was similar (P>0.05) for both systems (Table 2). However, during the transition from rainy to dry (P5) and from dry to rainy (P7) and also during the dry season (P6), the SPS produced more forage (34%) than Mono (P<0.05). TDM production was affected (P<0.05) by harvest period (P1–P7) for both SPS and Mono treatments, with highest yields during the initial period at the start of the rainy season and lowest during the dry season (P<0.05). Green leaf production in both systems followed that of TDM during the harvest periods.

Table 2. Total forage DM production and green leaf DM production for silvopastoral (SPS) and monoculture (Mono) systems during the harvest periods.

System	Total forage DM production (t/ha)								
	Period							Total	
	P1**	P2**	P3**	P4	P5	P6	P7		
SPS	1.76aD	1.29aC	1.40aC	1.27aC	0.81bB	0.48bA	0.84bB	7.84	P^*_{System}
Mono	1.85aD	1.27aC	1.45aC	1.25aC	0.41aA	0.34aA	0.68aB	7.28	0.351824
Mean	1.81	1.28	1.43	1.26	0.61	0.41	0.76	1.08	$P^*_{Period\ x\ System}$
$P^*_{Periods}$				0.00014256					0.000854
System	**Green leaf DM production (t/ha)**								
	Period							Total	
	P1**	P2**	P3**	P4	P5	P6	P7		
SPS	1.35aD	0.98aC	1.05aC	0.92aC	0.43bB	0.31bA	0.48bB	5.53	P^*_{System}
Mono	1.44aE	0.99aD	1.12aD	0.94aD	0.16aB	0.09aA	0.27aC	5.00	0.27434
Mean	1.47	1.03	1.14	0.98	0.29	0.21	0.38	0.79	$P^*_{Period\ x\ System}$
$P^*_{Periods}$				0.0001232					0.001232

P1–P4 = rainy season; P5 = transition period (rainy-dry); P6 = dry period; and P7 = transition period (dry-rainy).
* Type 1 error probability.
** Intake trial periods.
Means within a column with different lower-case letters differ significantly at P<0.05 based on the Skott-Knott test.
Values within a row with different upper-case letters differ significantly at P<0.05 based on the Skott-Knott test.

Microclimatic variables

The daily average maximum temperature in Mono exceeded that in SPS (33.5 vs. 28.0 °C; Table 3), while mean minimum temperatures did not differ (21.0 vs. 20.5 °C). In the SPS, mean dry bulb temperature (DBT) under the tree canopy was lower than in the monoculture at all daily recordings except 19.00 h. The reverse was the case for relative humidity (Table 3), so that THI was greater in the monoculture only during the early part of the day, while BGTHI was greater in the monoculture for most of the day. Global radiation (Table 3) was higher in Mono than in SPS.

Regarding dry bulb temperature (DBT) (Table 4, Figure 3), data show that for monoculture, the day period with temperatures above the upper limit of the thermal comfort zone (TCZ) for sheep, which is 20–28 °C (Baêta and Souza 1997), was 4 h 55 min. The temperatures in SPS also exceeded TCZ, but were lower than those recorded for the monoculture.

Table 3. Microclimatic variables in silvopastoral (SPS) and monoculture (Mono) systems.

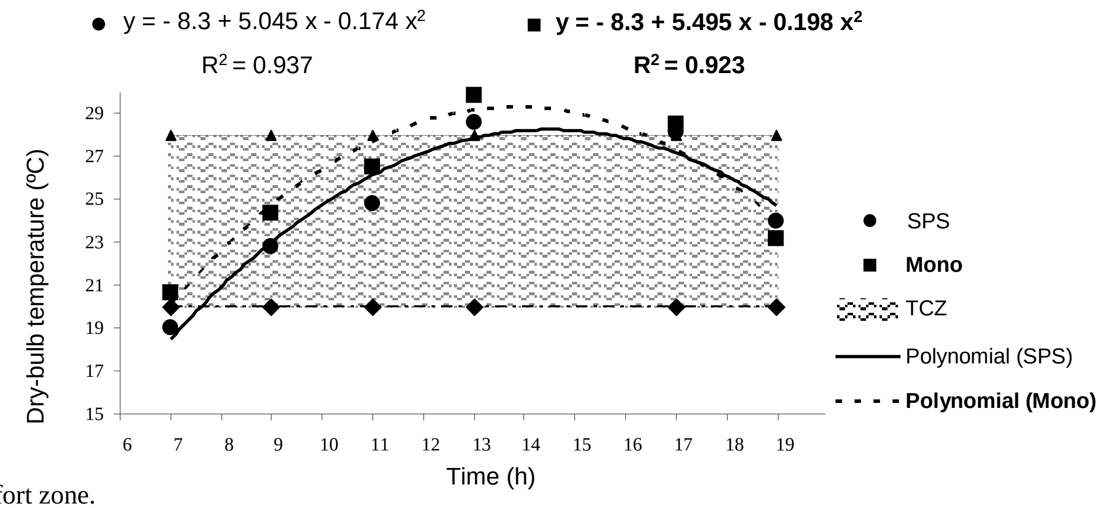
System and parameter[1]	Day	Time of day (h)						Mean
		07.00	09.00	11.00	13.00	17.00	19.00	
SPS								
Max. temp. (ºC)	28.0	-	-	-	-	-	-	-
Min. temp. (ºC)	21.0	-	-	-	-	-	-	-
Dry bulb temp. (ºC)		19.0	22.8	24.8	27.6	26.2	24.0	24.1
Rel. humidity (%)		100.0	84.5	75.5	60.6	56.1	80.6	76.2
THI		73.9	85.3	90.3	98.3	96.0	88.5	88.7
BGTHI		68.0	73.4	74.8	79.6	75.6	72.7	74.0
GR ($W/m^2/d$)		-	-	-	-	-	-	398.7
Mono								
Max. temp. (ºC)	33.5	-	-	-	-	-	-	-
Min. temp. (ºC)	20.5	-	-	-	-	-	-	-
Dry bulb temp. (ºC)		20.7	24.3	26.5	29.8	28.5	24.2	25.7
Rel. humidity (%)		94.8	78.0	65.1	50.9	52.8	85.8	71.3
THI		79.3	89.1	93.3	99.3	96.1	86.6	90.6
BGTHI		71.8	78.8	81.6	89.1	81.3	72.6	79.2
GR ($W/m^2/d$)		-	-	-	-	-	-	712.6

[1]THI = temperature and humidity index; BGTHI = black globe temperature and humidity index; GR = global radiation.

Table 4. Equations generated by regression analysis, relating microclimatic variable data in silvopastoral (SPS) and monoculture (Mono) systems.

System	Microclimatic variable[1]	Equation[2]	R^2
SPS	DBT	$y = 8.3 + 5.045x - 0.174x^2$ aA	0.94
Mono	DBT	$y = -8.7 + 5.495x - 0.198x^2$ bB	0.92
SPS	RH	$y = 219.7 - 21.95x + 0.755x^2$ bB	0.88
Mono	RH	$y = 246.4 - 28.26x + 1.025^2$ aA	0.89
SPS	THI	$y = 6.7 + 12.57x - 0.433x^2$ aA	0.98
Mono	THI	$y = 17.5 + 11.72x - 0.422x^2$ bB	0.97
SPS	BGTHI	$y = 34.4 + 6.316x - 0.230x^2$ aA	0.91
Mono	BGTHI	$y = 5.6 + 12.59x - 0.477x^2$ bB	0.92

[1]DBT = dry bulb temperature (ºC); RH = relative humidity (%); THI = temperature and humidity index; BGTHI = black globe temperature and humidity index; y = microclimatic variable; x = time of measurement (07.00, 09.00, 11.00, 13.00, 17.00 and 19.00 h).
[2]Equations relating to the same microclimatic variable followed by different upper-case letters are not parallel by the curve parallelism test (Cohen 1983) at 5% probability; equations relating to the same microclimatic variable followed by different lower-case letters differ by the curve identity test (Cohen 1983) at 5% probability.



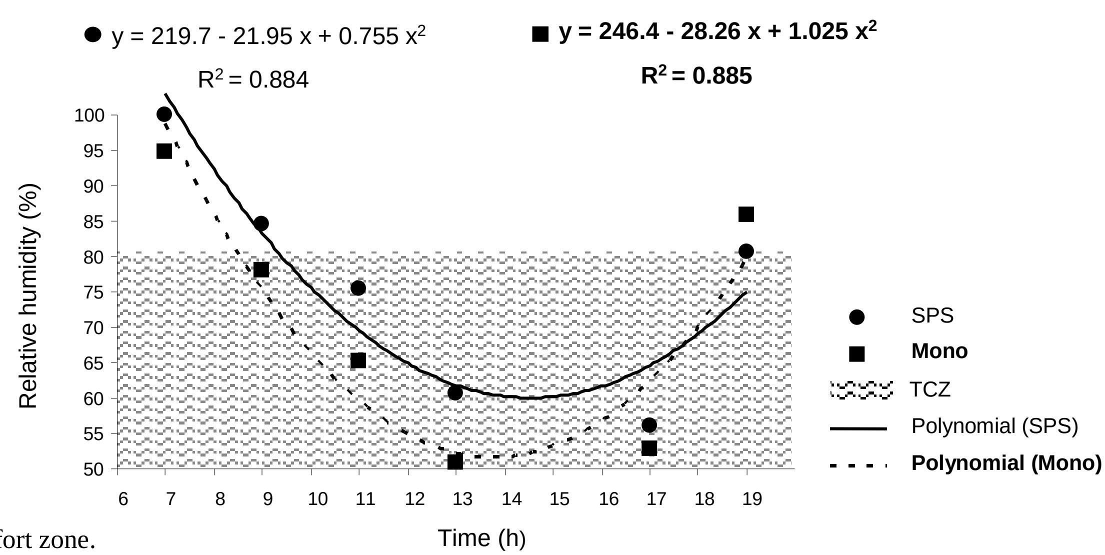
TCZ = thermal comfort zone.

Figure 3. Dry bulb temperature (DBT) in silvopastoral (SPS) and monoculture (Mono; in bold) systems during the day.

Relative humidity throughout the day, with the exception of 07.00 and 09.00 h for the SPS and 7.00 h for Mono (Table 4; Figure 4), was within the range of thermal comfort for the animals, which should be between 50 and 80% (Baêta and Souza 1997). Table 4 shows that there were significant differences (P<0.05) for RH between treatments and those were more pronounced in the period between 10.00 and 16.00 h (Figure 4).

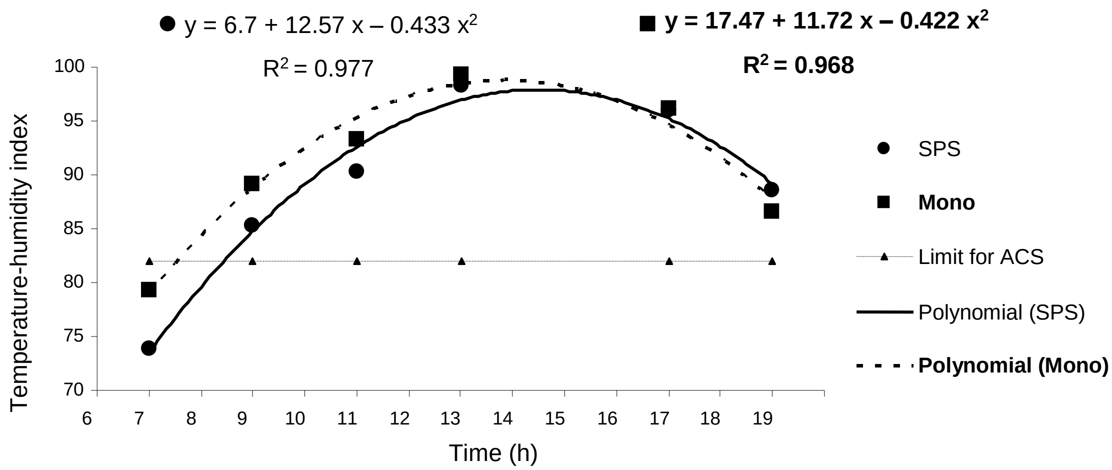
Results indicate that in both systems, THI was almost always within the "scale of extremely severe heat stress" (LPHSI 1990). This occurs because the trial was done in December, when this index reaches its highest values of the year. However, even under these conditions, SPS showed lower values (P<0.05) than those for the monoculture (Table 4; Figure 5).

For BGTHI, significant differences were observed (P<0.05) between the systems (Table 4), with a lower value in SPS than in Mono, which indicates milder microclimatic conditions in shaded pastures (Figure 6).



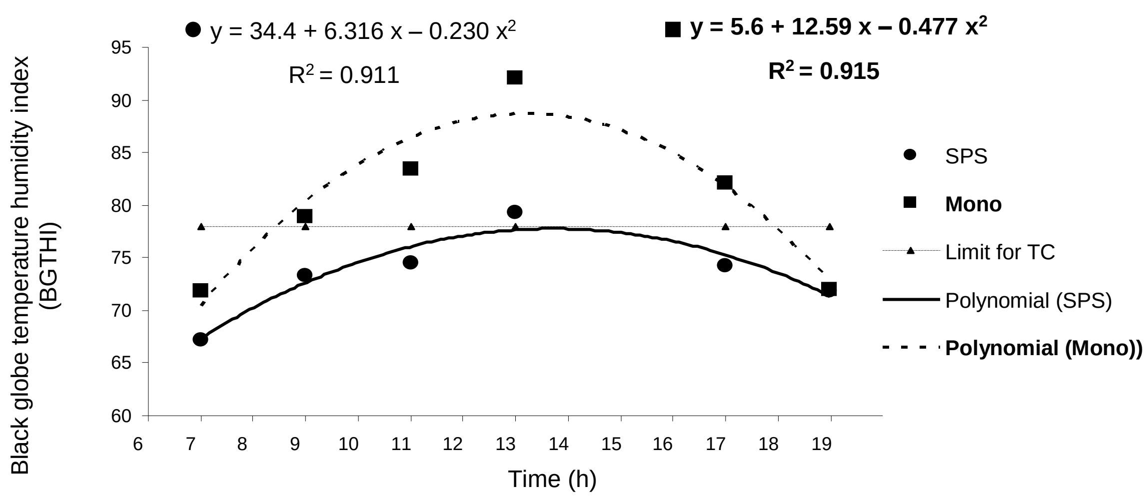
TCZ = thermal comfort zone.

Figure 4. Relative humidity (RH) in silvopastoral (SPS) and monoculture (Mono; in bold) systems during the day.



Limit for ACS = Limit below which there is absence of caloric stress.

Figure 5. Temperature humidity index (THI) for silvopastoral (SPS) and monoculture (Mono; in bold) systems during the day.



Limit for TC = Limit below which there is thermal comfort.

Figure 6. Black globe temperature humidity index (BGTHI) for the silvopastoral (SPS) and monoculture (Mono; in bold) systems during the day.

Intake

Nutritive value of Marandu grass samples was similar in both systems, except for DM content and for nitrogen fractions linked to the fibrous portion of the forage (Table 5). Both DMI and OMI by sheep in the SPS system were higher ($P<0.05$) than those in the monoculture (Table 6). Free water intake was lower and intake of water in the feed was higher ($P<0.05$) in the SPS (Table 6) than in Mono. Total water intake was 10.2 % higher ($P<0.05$) in the Mono than in the SPS.

Table 5. Chemical composition of samples from simulated grazing in silvopastoral (SPS) and monoculture (Mono) systems.

Parameter[1]	System	
	SPS	Mono
DM (g DM/kg fresh)	202.3	252.7
Organic matter (g/kg DM)	911.9	916.2
Ash (g/kg DM)	88.1	83.8
Crude protein (g/kg DM)	121.8	118.1
NDF (g/kg DM)	664.7	648.7
ADF (g/kg DM)	318.3	310.6
Lignin (g/kg DM)	25.6	23.0
Silica (g/kg DM)	2.5	4.8
INND (g/kg TN)	180.5	98.7
INAD (g/kg TN)	19.8	34.7
IVDMD (g DM/kg DM)	59.3	60.8
IVOMD (g OM/kg OM)	58.5	59.0

[1]DM = dry matter; INND = insoluble nitrogen in neutral detergent; INAD = insoluble nitrogen in acid detergent; TN = total nitrogen; IVDMD = in vitro dry matter digestibility; IVOMD = in vitro organic matter digestibility.

Table 6. Intake of dry matter, organic matter and water of sheep grazing in silvopastoral (SPS) and monoculture (Mono) systems.

Parameter	SPS	Mono	m.s.e.	P
DM intake				
(g DM/kg LW/d)	38.9	35.2	0.62	<0.001
(g DM/kg$^{0.75}$ LW/d)	88.2	79.9	1.14	<0.001
OM intake				
(g OM/kg LW/d)	37.3	34.6	0.68	<0.001
(g OM/kg$^{0.75}$ LW/d)	86.4	78.6	1.15	<0.001
Water intake (mL/kg$^{0.75}$ LW/d)				
Free water intake[1]	82	238		
Feed water intake[2]	348	236	4	<0.001
Total water intake	430	474	6	<0.021

m.s.e. = mean standard error.
[1]water used from drinking spouts.
[2]moisture in forage consumed.

Feeding behavior

Time periods spent grazing were longer (P<0.001) in the SPS than in Mono. On the other hand, animals spent more time walking (P<0.001), drinking (P=0.016) and resting (P=0.008) in Mono, than in SPS (Table 7). Time spent ruminating and in other activities did not differ (P>0.05) between systems.

Table 7. Feeding behavior parameters (min/d) for silvopastoral (SPS) and monoculture (Mono) systems.

Activity	SPS	Mono	m.s.e.	P
Grazing	572	288	43	<0.001
Ruminating	300	280	43	0.179
Resting	188	373	22	0.008
Walking	30	89	10	<0.001
Drinking	20	53	7	0.016
Other	330	357	8	0.217

Discussion

This study provides sound evidence that SPS provide advantages over a grass monoculture in this region in terms of pasture growth during dry periods and comfort of animals, resulting in improved feed intake and, probably, production. The study had some limitations, the main one being that we used animals as replicates inside single plots (i.e. areas). Ideally treatments should have been replicated in the experimental design with repetition over time. However, due to prohibitive costs, lack of funding, deficiency in labor and project size, the design of this experiment was not ideal. Nevertheless, inferences are valid and information provided met the objectives of this study.

Forage production

It was interesting that SPS produced 8% more forage grass during the year than the control despite the competition from trees and lower global radiation values. This advantage occurred during the transition periods and dry season (50% increase) with no difference during the wet season. In a tree-pasture system there is competition between the trees and the pasture for moisture and nutrients. In addition, shading can have negative impacts on DM production of pasture (Jackson and Ash 1998), which can be reflected in reduced animal production rates (Parsons et al. 1983). However, forage production is also dependent on other environmental parameters such as rainfall and temperature as well as soil moisture and soil fertility (Paciullo et al. 2011), which may counteract the negative effects of shade on DM production. It would seem that pastures in the silvopastoral system received a benefit from shading during the drier part of the year, possibly because of retention of soil moisture under the trees or better availability of nutrients as a result of recycling by the trees or a combination of these factors. In SPS, only 68% of the total pasture production occurred during the rainy season (P1–P4), while the control produced 75% of its growth during this period.

Microclimatic variables

The daily average maximum temperature (MaxTemp) in Mono (33.5 °C) was outside the thermal comfort zone (TCZ) for adult sheep in the tropics, which should be between 20 and 28 °C (Baêta and Souza 1997), while MaxTemp in SPS, which was 5.5 °C lower than in the control, remained within the TCZ (Table 3). Daily average minimum temperatures (MinTemp) remained within the TCZ for both systems. This suggests that sheep grazing on Marandu in monoculture would have suffered heat stress during the hottest hours of the day, as reported by Baumgard and Rhoads (2012). Shading imposed by the tree component would have attenuated the incident radiation and reduced air temperature, reducing the severity of microclimatic stresses experienced in the straight grassland ecosystem. According to Sousa et al. (2010), air dry bulb temperature (DBT) under the canopy of trees can be 2.3–9.5 °C lower than in grass monoculture, depending on time of day.

On the other hand, RH values in SPS were always higher than those observed in monoculture and were above those reported by Sousa et al. (2010). It is well documented that biotic components such as trees and canopy architecture affect relative humidity (Daly 1984; Qin et al. 2014). However, despite these differences, the values for RH were within the range of thermal comfort for sheep according to LPHSI (1990). High RH affects heat dissipation with long exposure and causes ethological changes in ruminants (Ungar 1996). However, when analyzed separately it is not considered a suitable parameter for assessing thermal comfort in sheep (Baêta and Souza 1997). Sheep can become acclimatized to high levels of humidity, which provides significant changes in thermal sensations, i.e. the perceptions of the physiological effects caused by temperature variations, making sheep suffer less from the effects of heat stress under these conditions (Andrade et al. 2007). Thus, higher humidity conditions in the SPS (Table 3) may have been offset by the adaptation of experimental animals.

According to LPHSI (1990), THI values for sheep above 86 should produce severe heat stress; that is, in both systems evaluated, only at 07.00 h would the animals not have suffered from some effects of tropical heat. Neiva et al. (2004), working in the northeast region of Brazil (05°43'02" S, 38°32'35" W), observed that, even under complete shading (stabled animals), the value of THI (81.1) was similar to that obtained at full sunshine (82.3) and thermal stress was observed in sheep under both sets of conditions. These values may be associated with tropical climatic conditions, which have no defined parameters, and therefore do not correlate with the parameters generated by the Livestock and Poultry Heat Stress Indices (LPHSI 1990). These findings suggest that, while SPS evaluated in this study increased the comfort of sheep over the control treatment, it might still have failed to eliminate environmental heat stress in grazing sheep (average THI 89.7). BGTHI values (Table 3) evaluated in the SPS were always lower than those measured in monoculture and were always below the critical value (BGTHI = 78), above which heat stress could cause various metabolic problems in cattle (Andrade et al. 2007). However, no critical value for sheep was found in the literature.

Intake

Differences in chemical composition, IVDMD and IVOMD of the forage grass in both systems (Table 5) were small and would not justify the superiority of 9.47% in DMI (g DM/kg$^{0.75}$ LW/d) and 9.43% in OMI (g OM/kg$^{0.75}$ LW/d) in the SPS relative to Mono (Table 6). Thus, the differences in intake probably occurred because of better microclimatic conditions in the SPS (Tables 3 and 4), since the better thermal comfort provided lesser restriction on metabolic and dietary thermogenesis (Forbes 1995). According to Baumer (1991), animals protected from the heat increase DMI and produce more meat and milk. Minson (1990) reported an average value for voluntary intake by sheep fed tropical grass (separated leaf and stem of 6 and 12 week regrowth) of the genera *Digitaria* and *Chloris* of 46 g DM/kg$^{0.75}$ LW/d. This value is much lower than that found in this study (80 g DM/kg$^{0.75}$ LW/d). The nutrient requirements of small ruminants (National Research Council 2007) specify intake values from 70 to 85 g DM/kg$^{0.75}$ LW/d for *Brachiaria decumbens and B. brizantha*. While intake values (% LW) of DM and OM in SPS were higher than in Mono, intakes in both systems were similar to those estimated by National Research Council (2007) for adult sheep with average weight of 30 kg and weight gain of 0.2 kg/d, i.e. 36.7 g DM/kg LW/d. Shading obviously benefited forage intake by animals in the SPS relative to those in full sunlight on monoculture (Table 6). Samarakoon et al. (1990) evaluated the effect of growing 2 subtropical grasses, *Stenotaphrum secundatum* and *Pennisetum clandestinum*, under artificial shade (50%) on DMI by sheep and found that shading reduced DMI. This was probably due to increasing levels of ADF in shaded grass with resultant decline in IVDMD and not due to any physiological effect of shade on sheep. In our study, pasture from SPS contained higher levels of fiber than in the control, and lower digestibility, but differences were small.

Total water intake by sheep on the control treatment was 11% higher than on the silvopastoral system, reflecting the greater heat stress suffered by those sheep. According to Baumer (1991), animals protected from the heat may reduce water intake by up to 20%. Interestingly, the higher moisture content in forage on SPS coupled with the higher feed intake on this treatment resulted in most of the water requirements of the shaded sheep being satisfied from the feed consumed (81%). This behavior is in accordance with the findings of Andrade et al. (2007), who suggest that grazing sheep under environmental conditions within the thermal comfort zone obtain 75–85 % of their water needs from green forage. Not only was moisture requirement of sheep on the monoculture higher than in SPS but a greater percentage was derived from free water supplies. This is an obvious advantage for the silvopastoral system of having to provide less free water to stock.

Feeding behavior

The greater amount of time spent by animals in the monoculture in walking, drinking and resting is indicative of behaviors that seek to mitigate thermal stress, by maintaining thermal homeostasis and reducing metabolic stress (Ashutosh et al. 2002). Forbes (1995) claims that sheep have the ethological habit of trading grazing time for walking in search of shade during the hottest hours of the day. However, this occurs not only for physiological reasons, such as heat stress, but also the ancestral instinct of protection and escape from predators (Ryder 1984).

Conclusions

This study has shown the benefits of a silvopastoral system for sheep production through reduction in heat stress, resulting in higher feed intake. It appears that silvopastoral systems could be more productive than straight grass systems for mongrel hair sheep under these environmental conditions and the increases in growth rates etc. should be documented. However, the extent to which these results can be extrapolated to cattle needs to be verified before benefits for bovines can be claimed.

Acknowledgments

The authors thank Prof. Lúcio Carlos Gonçalves for his constructive suggestions on the elaboration of this study, and the Departamento de Engenharia de Biossistemas (DEPEB-PPBE), FAPEMIG, CNPq, PVE-CAPES and CAPES-EMBRAPA, RumenGases.

References

Andrade IS; Souza BB; Pereira Filho JM; Silva AM. 2007. Parâmetros fisiológicos e desempenho de ovinos Santa Inês submetidos a diferentes tipos de sombreamento e a suplementação em pastejo. Ciência e Agrotecnologia 31:540–547. DOI: [10.1590/s1413-70542007000200039](10.1590/s1413-70542007000200039)

AOAC (Association of Official Analytical Chemists). 2009. Official Methods of Analysis. 15th Edn. AOAC, Arlington, VA, USA.

Asthutosh F; Dhanoa OP; Singh G. 2002. Changes in grazing behaviour of native and crossbred sheep in different seasons under semi-arid conditions. Tropical Animal Health and Production 34:399–404. DOI: 10.1023/A:1020044226250

Baêta FC; Souza C. 1997. Ambience in rural buildings: Thermal comfort. UFV Press, Universidade Federal de Viçosa, Viçosa, MG, Brazil.

Baumer M. 1991. Animal production, agroforestry and similar techniques. Agroforestry Abstracts 4:179–198.

Baumgard LH; Rhoads RP. 2012. Ruminant Nutrition Symposium: Ruminant production and metabolic responses to heat stress. Journal of Animal Science 90:1855–1865. DOI: 10.2527/jas.2011-4675

Bocquier F; González-García E. 2010. Sustainability of ruminant agriculture in the new context: Feeding strategies and features of animal adaptability into the necessary holistic approach. Animal 4:1258–1273. DOI: 10.1017/S1751731111001261

Boeriu CG; Bravo D; Gosselink RJA; Van Dam JEG. 2004. Characterisation of structure-dependent functional properties of lignin with infrared spectroscopy. Industrial Crops and Products 20:205–218. DOI: 10.1016/j.indcrop.2004.04.022

Buffington DE; Colazzo-Arocho A; Canton GH; Pitt D; Thatcher WW; Collier RJ. 1981. Black globe-humidity index (BGHI) as comfort equation for dairy cows. Transactions of the ASAE 24:711–714. DOI: 10.13031/2013.34325

Cohen A. 1983. Comparing regression coefficients across subsamples: A study of the statistical test. Sociological Methods and Research 12:77–94. DOI: 10.1177/0049124183012001003

Costa NL; Gonçalves CA; Oliveira JRC; Oliveira MAS; Magalhães JA. 2004. Resposta de *Brachiaria brizantha* cv. Marandu à regimes de corte. Comunicado técnico 279, Embrapa Rondônia, Porto Velho, RO, Brazil. http://goo.gl/hJGeYn

Daly JJ. 1984. Cattle need shade trees. Queensland Agricultural Journal 110:21–24.

Dumont B; Fortun-Lamothe L; Jouven M; Thomas M; Tichit M. 2013. Prospects from agroecology and industrial ecology for animal production in the 21st century. Animal 7:1028–1043. DOI: 10.1017/S1751731112002418

Ferreira MA; Valadares Filho SC; Marcondes MI; Paixão ML; Paulino MF; Valadares RFD. 2009. Evaluation of markers in ruminant trials: Digestibility. Brazilian Journal of Animal Science 38:1568–1573. http://goo.gl/Fj0G6X

Forbes JM. 1995. Voluntary food intake and diet selection in farm animals. CABI Publishing, Wallingford, UK.

Jackson J; Ash AJ. 1998. Tree-grass relationships in open eucalypt woodlands of northeastern Australia: Influence of trees on pasture productivity, forage quality and species distribution. Agroforestry Systems 40:159–176. DOI: 10.1023/A:1006067110870

Johnson AD. 1978. Sample preparation and chemical analysis of vegetation. In: Mannetje L't, ed. Measurement of grassland vegetation and animal production. Commonwealth Agricultural Bureaux, Aberystwyth, UK. p. 96–102.

Kelly CF; Bond TE. 1971. Bioclimatic factors and their measurements. In: Kelly FC; Bond TE, eds. A guide to environmental research on animals. National Academy of Sciences, Washington, DC, USA. p. 7–92.

Licitra G; Hernandez TM; Van Soest PJ. 1996. Standardization of procedures for nitrogen fractionation of ruminant feeds. Animal Feed Science and Technology 57:347–358. DOI: 10.1016/0377-8401(95)00837-3

LPHSI. 1990. Livestock and Poultry Heat Stress Indices. Agriculture Engineering Technology Guide, Clemson University, Clemson, SC, USA.

Maurício RM. 2012. Comment to "Pasture shade and farm management effects on cow productivity in the tropics" by Ainsworth Justin AW; Moe Stein R; Skarpe C (Agriculture, Ecosystems and Environment 155:105–110). Agriculture, Ecosystems and Environment 161:78−79. DOI: 10.1016/j.agee.2012.07.012

Minson DJ. 1990. Forage in ruminant nutrition. Academic Press, New York, USA.

National Research Council. 2007. Nutrient Requirements of Small Ruminants: Sheep, Goats, Cervids, and New World Camelids. National Academies Press, Washington, DC, USA.

Neiva JNM; Teixeira M; Turco SHN; Oliveira SMP; Moura AAAN. 2004. Effects of environmental stress on physiological parameters of feedlot sheep in the northeast of Brazil. Brazilian Journal of Animal Science 33:668–678. http://goo.gl/eHbJjr

Paciullo DSC; Castro CRR; Gomide CAM; Maurício RM; Pires MFA; Müller MD; Xavier DF. 2011. Performance of dairy heifers in a silvopastoral system. Livestock Science 141:166–172. DOI: 10.1017/S1751731114000767

Parsons AJ; Leafe EL; Collett B; Penning PD; Lewis J. 1983. The physiology of grass production under grazing. II. Photosynthesis, crop growth and animal intake of continuously-grazed swards. Journal of Applied Ecology 20:127–139. DOI: 10.2307/2403381

Prigge EC; Varga GA; Vicini JL; Reid RL. 1981. Comparison of ytterbium chloride and chromium sesquioxide as fecal indicators. Journal of Animal Science 53:1629–1633. DOI: 10.2134/jas1982.5361629x

Qin Z; Li Z; Cheng F; Chen J; Liang B. 2014. Influence of canopy structural characteristics on cooling and humidifying effects of *Populus tomentosa* community on calm sunny summer days. Landscape and Urban Planning 127:75–82. DOI: 10.1016/j.landurbplan.2014.04.006

Reis GL; Lana AMQ; Maurício RM; Lana RMQ; Machado RM; Borges I; Quinzeiro Neto T. 2009. Influence of trees on soil nutrient pools in a silvopastoral system in the Brazilian Savannah. Plant and Soil 323:11–16. DOI: 10.1007/s11104-009-0144-5

Ryder ML. 1984. Evolution of domesticated animals. In: Mason IL, ed. Sheep. Longman, New York, USA. p. 63–85.

Samarakoon SP; Shelton HM; Wilson JR. 1990. Voluntary feed intake by sheep and digestibility of shaded *Stenotaphrum secundatum*, *Axonopus compressus* and *Pennisetum clandestinum* herbage. Journal of Agricultural Science 114:143–150. DOI: 10.1017/S0021859600072129

Sousa LF; Maurício RM; Moreira GR; Gonçalves LC; Borges I; Pereira LGR. 2010. Nutritional evaluation of "Braquiarão" grass in association with "Aroeira" trees in a silvopastoral system. Agroforestry Systems 79:189–199. DOI: 10.1007/s10457-010-9297-8

Theander O; Westerlund EA. 1986. Studies on dietary fibre. 3. Improved procedures for analysis on dietary fibre. Journal of Agriculture and Food Chemistry 34:330–336. DOI: 10.1021/jf00068a045

Ungar ED. 1996. Ingestive behaviour. In: Hodgson J; Illius A, eds. The ecology and management of grazing systems. Oxford University Press, Oxford, UK. p. 185–218.

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

Wilson JR; Hill K; Cameron DM; Shelton HM. 1990. The growth of *Paspalum notatum* under the shade of a *Eucalyptus grandis* plantation canopy or in full sun. Tropical Grasslands 24:24–28. http://goo.gl/MlFwI9

(Received for publication 27 March 2014; accepted 19 May 2015)

DOI: 10.17138/TGFT(3)142-150

Forage growth, yield and quality responses of Napier hybrid grass cultivars to three cutting intervals in the Himalayan foothills

KESANG WANGCHUK[1], KRISHNA RAI[3], HARILAL NIROLA[2], THUKTEN[1], CHHOYTEN DENDUP[2] AND DURBA MONGAR[1]

[1]*Renewable Natural Resources Research and Development Center, Ministry of Agriculture and Forests, Bumthang, Bhutan.* www.moaf.gov.bt
[2]*Renewable Natural Resources Research and Development Center, Ministry of Agriculture and Forests, Yusipang, Bhutan.* www.moaf.gov.bt
[3]*National Jersey Breeding Center, Samtse, Bhutan*

Keywords: Bhutan, CO-3, crude protein, dry matter; Giant Napier, Pakchong-1.

Abstract

A 3 x 3 factorial study was conducted in the southern foothills of Bhutan to compare 3 cultivars of Napier hybrid grass (*Pennisetum purpureum* x *P. glaucum*: Pakchong-1, CO-3 and Giant Napier), at 3 cutting intervals (40, 60 and 80 days), in terms of forage growth, dry matter (DM) yield and crude protein (CP) concentration. The effects of cultivar x cutting interval were significant only on tiller number per plant and leaf:stem ratio (LSR). CO-3 consistently produced the highest tiller number per plant, leaves per plant and LSR, while Pakchong-1 produced the lowest. Pakchong-1 plants were taller, had bigger tillers and basal circumference and higher stem DM production than CO-3 and Giant. Leaf CP for all cultivars was about 17%, while stem CP concentration was lower for Pakchong-1 than for the other cultivars (3.6 vs. 5.3%, P<0.05). While 40-day cutting intervals produced high quality forage, yields suffered markedly and the best compromise between yield and quality of forage seemed to occur with 60-day cutting intervals. Pakchong-1 seems to have no marked advantages over CO-3 and Giant for livestock feed, and feeding studies would verify this. Its higher stem DM yields may be advantageous for biogas production and this aspect should be investigated.

Resumen

En el piedemonte de la cordillera del Himalaya al sur de Bután en un factorial de 3 x 3 se compararon 3 cultivares (cvs.) híbridos del pasto Napier (*Pennisetum purpureum* x *P. glaucum*): Pakchong-1, Giant Napier y CO-3, en intervalos de corte cada 40, 60 y 80 días. Las mediciones incluyeron crecimiento del forraje, producción de materia seca (MS) y concentración de proteína cruda (PC). Los efectos de cultivar x intervalo de corte fueron significativos sólo para el número de brotes por planta y la relación hoja:tallo. El cv. CO-3 produjo de manera sostenida el mayor número tanto de brotes como de hojas por planta y la mayor relación hoja:tallo, mientras el cv. Pakchong-1 presentó respectivamente los valores más bajos. En comparación con los cvs. CO-3 y Giant, las plantas de Pakchong-1 fueron más altas, con brotes más vigorosos y circunferencia basal mayor, y produjeron más MS de tallo. La concentración de PC en las hojas fue aproximadamente de 17% para todos los cultivares, mientras que la concentración de PC en los tallos fue menor para Pakchong-1 que para los otros cultivares (3.6 vs. 5.3%, P<0.05). Cuando el intervalo de corte fue de 40 días, el forraje fue de mayor calidad, pero los rendimientos se redujeron marcadamente. Los resultados indican que con intervalos de corte de 60 días se obtiene el mejor compromiso entre el rendimiento y la calidad del forraje. Para la alimentación del ganado, el cv. Pakchong-1 no parece tener ventajas marcadas sobre los cvs. CO-3 y Giant; estudios de producción animal deberían verificar esto. Sin embargo, sus rendimientos particularmente altos de MS de tallo pueden ser favorables para la producción de biogás y este aspecto debe ser investigado.

Correspondence: Kesang Wangchuk, Renewable Natural Resources Research and Development Center, Ministry of Agriculture, Bumthang, Bhutan.
Email: kesangwangchuk@rocketmail.com

Introduction

The pasture species Napier or elephant grass (*Pennisetum purpureum*) is widely distributed in tropical and subtropical regions of the world, and is highly productive in areas with good soil fertility and high rainfall, growing well up to 2,000 masl (Kumar 2013). Napier grass is often preferred by smallholders and, e.g. in east Africa, constitutes up to 80% of forage grown (Staal et al. 1987). This has been attributed to its wide range of adaptation, vigorous growth, high biomass productivity and deep root system to survive under drought conditions (Lowe et al. 2003; Anderson et al. 2008; Tessema 2008).

However, optimal management practices for Napier grass are not clear (Mutegi et al. 2008). Appropriate cutting management is essential for high production and quality of this species (Jorgensen et al. 2010; Tessema et al. 2010). Since the species is well suited for cut-and-carry systems (Bayer 1990), many studies have dealt with cutting management to optimize forage yield and quality of Napier grass (Bayble et al. 2007; Tessema et al. 2010; Rengsirikul et al. 2011). While Manyawu et al. (2003) reported a significant effect of growth stage on yield and quality and suggested a cutting interval of 6−7 weeks for optimum yield and quality of Napier grass, Tessema et al. (2010) obtained the highest crude protein (CP) concentration at about 13 weeks cutting interval. Ansah et al. (2010) recorded the highest CP concentration but lowest DM yield, when harvested at less than 9 weeks cutting interval.

These findings highlight the importance of optimum cutting interval and its varying effects on yield and quality. High cutting frequency reduces growth and development, whereas long intervals between harvests lead to accumulation of fiber and reduction in quality (Tessema et al. 2010). This is because Napier grass has high structural cell wall carbohydrates that increase rapidly with maturity causing decline in CP concentration and digestibility (Van Soest 1994). Studies also demonstrate that the effects of cutting interval on yield and quality vary with cultivars (Cuomo et al. 1996; Khairani et al. 2013), management practices and environmental conditions (Chaparro et al. 1996). Genotypic variation in growth characteristics of Napier grass has also been reported (Mwendia et al. 2006; Nyambati et al. 2010) and growth and morphological characteristics are correlated with DM yield and nutritional quality (Tudsri et al. 2002).

In the southern foothills of the Bhutan Himalaya, Napier grass is a promising and high-yielding fodder species, widely cultivated in smallholder systems and on commercial livestock farms. However, the number of cultivars in the field is limited and existing cultivars include Mott (dwarf type) and the hybrids CO-3 and Giant Napier. Recently, the hybrid cultivar Pakchong-1 was introduced from Thailand. Under good management, Pakchong-1 is known for fast growth with high forage yield, high CP concentration (16−18%) and wide range of adaption, and can be ratooned for up to 8 years (Kiyothong 2014). These features seem to compel the Department of Livestock to promote and encourage large scale multiplication of Pakchong-1 in the southern foothills. However, forage crops are highly area, location and season-specific (Tessema et al. 2010; Pandey and Roy 2011) and there is a need to verify if Pakchong-1 can outcompete the existing cultivars in terms of forage yield, quality and growth characteristics, under the wet subtropical conditions of the southern foothills. Therefore, the main objective of this study was to compare hybrid Napier grass cv. Pakchong-1 with cvv. CO-3 and Giant at 3 cutting intervals, in terms of forage growth characteristics, DM yield and quality. The cutting intervals were chosen in an attempt to identify the interval which might optimize forage yield and quality of these cultivars.

Materials and Methods

Study site

The field experiment was conducted from August 2013 to November 2014 at the National Jersey Breeding Center of Samtse district (27°02′ N, 89°05′ E; 260 masl) in southern Bhutan. The center has a total area of 124 ha and about 50% of the land is planted with improved subtropical pasture species (Ministry of Agriculture 2000). Topography is rugged. The mean maximum temperature of 35 °C is recorded in July and the mean minimum of 15 °C in January. Average annual rainfall is 900 mm and shows a unimodal distribution pattern, with the main precipitation from June to September, giving hot, wet summers and cold, dry winters. The growing season occurs between April and November. Rainfall recorded during the study period is presented in Figure 1.

Soil nutrient analysis

Soil is sandy loam with poor moisture holding capacity (Ministry of Agriculture 2000) and pastures are fertilized with effluents from the dairy sheds. Soils were sampled to a depth of 15 cm by collecting 10 samples of 300 g from the field prior to commencement of the experiment.

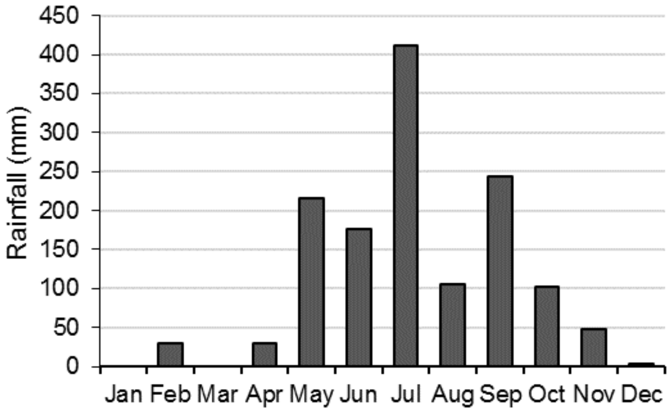


Figure 1. Monthly rainfall at study site in 2014.

The soil samples were dried at ambient temperature and analyzed at the National Soil Services Center at Semtokha, Bhutan. The methods used were micro-Kjeldahl digestion (Guebel et al. 1991) for total nitrogen (N), Bray and Kurtz method (Bray and Kurtz 1945) for available phosphorus (P) and the semi-micro leaching method (Blakemore et al. 1981) for exchangeable potassium (K^+). The laboratory results showed the following chemical composition: available P 133 mg/kg; available K 37.8 mg/kg; carbon 3.42%; nitrogen 0.22%; and C:N ratio 16.0.

Description of cultivars

Hybrid Napier grass cvv. Pakchong-1, CO-3 and Giant were used in this study. All 3 cultivars are derived from interspecific crosses between common elephant grass (*Pennisetum purpureum*) and pearl millet (*Pennisetum glaucum*).

Hybrid Napier cv. Pakchong-1 was developed by the Department of Livestock Development in Thailand. It is reported to grow over 3 m tall in less than 2 months, gives high yields and can be harvested after 45 days with a CP concentration of 16−18% (Kiyothong 2014).

Hybrid Napier cv. CO-3 was developed by the Tamil Nadu Agricultural University at Coimbatore, India and released for commercial cultivation in 1997 (Premaratne and Premalal 2006). It is characterized by profuse tillering, high yield potential, high CP concentration, quick regrowth capacity, high leaf:stem ratio, high palatability and freedom from pests and diseases (Premaratne and Premalal 2006).

Giant Napier was developed by the Indian Agricultural Research Institute (Patil and Joshi 1962). It is a robust plant with a vigorous root system, sometimes stoloniferous and with a creeping rhizome. It gives high yield especially under adequate fertilization and irrigation, but has high fiber content at maturity and poor seed production and is susceptible to frosts (Patil and Joshi 1962).

Experimental design and treatments

The study was established in August 2013. A 3 x 3 factorial experiment (3 cultivars − Napier grass cvv. Pakchong-1, CO-3 and Giant Napier grass x 3 cutting intervals – 40, 60 and 80 days) was laid out in 3 blocks (3 replications). Plot size for each cultivar was 25 m^2 (5 × 5 m). Twenty-five stem cuttings per cultivar per plot with 2 healthy nodes per cutting were planted on 8 August 2013 with a 1 x 1 m spacing. Plants along the borders of plots were excluded from measurement and were cut and forage disposed of at the time of field measurements. Only 10 plants per cultivar were sampled at each cutting interval. After each cut, cow dung slurry (prepared by mixing 0.2 kg fresh cow dung in 1 L water) was applied uniformly at the rate of 1 L/m^2. No chemical fertilizers were applied. Weeds were slashed at the time of cutting.

Measurement of morphological characteristics

Napier plants were allowed to establish well in 2013 and field measurements were carried out in 2014. Plants of all treatments were uniformly cut to a standard height of 5 cm on 20 March 2014. Harvest dates for the cutting treatments are presented in Table 1.

At the time of harvest, each plant constituted a bunch of tillers. Plant height, basal circumference at 10 cm above ground level and number of tillers per plant were recorded. The tallest tiller on each plant was used to measure height, tiller diameter and number of leaves per tiller. Diameter of the lowest node was measured with digital Vernier calipers. Total number of leaves was estimated from the tiller number per plant and leaf number per tiller.

Table 1. Harvest dates for cutting treatments in 2014.

Cutting interval	Harvest dates in 2014	Number of cuts
40 days	30 Apr, 10 Jun, 20 Jul, 30 Aug, 10 Oct, 20 Nov	6
60 days	20 May, 20 Jul, 20 Sep, 20 Nov	4
80 days	10 Jun, 30 Aug, 20 Nov	3

Forage dry matter (DM) production, leaf:stem ratio (LSR) and crude protein (CP) concentration

Individual plants were clipped at 5 cm from ground level and fresh stems and leaves of each of the 10 harvested plants were separated and weighed. After individual plant measurements, stems and leaves were bulked separately and subsamples (about 300 g for stems and leaves) taken for DM analysis. The subsamples were oven-dried at 60 °C for 48 h and leaf and stem DM % determined. On the basis of these DM % and fresh stem and leaf yields we estimated the stem and leaf DM yield of each plant. Leaf dry weight was divided by stem dry weight to estimate the leaf:stem ratio (LSR). Following DM estimation, the dried samples were processed and analyzed for total nitrogen (N) by the Kjeldahl method and CP was estimated as % N × 4.43. The conversion factor 6.25 was not used because we felt it would overestimate the plant protein concentration, and instead we used 4.43, a conversion factor reported to provide reasonably good estimates of the plant protein concentration (Yeoh and Wee 1994).

Data analysis

Data for the various harvests for each plot were averaged. The dataset was checked for outliers, followed by Shapiro Wilk's and Levene's tests for normality of data and homogeneity of variance, respectively. Pearson's correlation was run to find relationships between growth parameters. The Generalized Linear Model with a 2-factor ANOVA was used to compare the mean differences between cultivars subjected to the 3 cutting intervals. Measured variables with cutting dates were treated as response variables and cultivar as the explanatory variable. Tukey's LSD test was used to test the differences between means. Differences between means were considered significant if P values were less than 0.05. We analyzed the dataset in SPSS version 21 (Landau and Everitt 2004).

Results

Growth characteristics

The effects of cultivar and cutting interval on plant height, basal circumference, tiller number and diameter and leaf number are presented in Table 2. The only significant interaction between cultivar and cutting interval was for number of tillers per plant, so main effects only are presented. For cultivars, plant height followed the order Pakchong-1>Giant>CO-3, while basal circumference and tiller diameter for Pakchong-1 and Giant were similar and greater than for CO-3 ($P<0.05$). Cutting interval had significant effects on plant height and basal circumference, with height increasing progressively as cutting interval increased ($P<0.05$), while basal circumference at 60 and 80 day cutting exceeded that at 40 day cutting ($P<0.05$). Cultivar had a significant effect on number of leaves/tiller (Pakchong-1>CO-3, $P<0.05$) and leaves/plant (CO-3>Giant and Pakchong-1, $P<0.05$). Cutting interval had a significant ($P<0.05$) effect on leaves/plant (40 and 60>80 days).

There was a significant cultivar x cutting interval effect on the number of tillers per plant. While the number of tillers declined significantly for all cultivars with increasing cutting intervals, at all cutting intervals CO-3 gave the highest number of tillers and Pakchong-1 gave the least.

Table 2. Effects of cultivar and cutting interval on plant height, basal circumference, tiller number and diameter, and leaf numbers of 3 Napier hybrid cultivars. Means ± standard error followed by the same letter within columns are not different ($P>0.05$).

Treatment	Plant height (cm)	Basal circumference (cm)	Tillers per plant (no.)	Tiller diameter (mm)	Leaves per tiller (no.)	Leaves per plant (no.)
Cultivar						
CO-3	175 ± 5.6 c	110.0 ± 3.3 b	72.0 ± 3.2 a	4.0 ± 1.70 b	5.0 ± 0.8 b	360 ± 4.0 a
Giant	206 ± 5.6 b	141.0 ± 3.3 a	51.0 ± 3.3 b	13.8 ± 2.62 a	6.0 ± 0.6 ab	306 ± 3.6 b
Pakchong-1	244 ± 5.4 a	142.0 ± 3.2 a	40.0 ± 3.1 c	13.1 ± 2.52 a	7.0 ± 0.5 a	280 ± 3.5 b
Cutting interval						
40 days	151 ± 4.5 c	119.0 ± 2.7 b	63.0 ± 2.6 a	13.5 ± 2.45 a	6.0 ± 0.4 a	378 ± 2.4 a
60 days	218 ± 5.4 b	137.0 ± 3.2 a	57.0 ± 3.1 b	10.9 ± 2.27 a	7.0 ± 0.5 a	399 ± 3.5 a
80 days	256 ± 6.4 a	137.0 ± 3.8 a	43.0 ± 3.7 c	11.8 ± 3.21 a	6.0 ± 0.6 a	258 ± 3.6 b

Table 3. Effects of cultivar and cutting interval on leaf and stem DM production and leaf:stem ratio (LSR) of 3 Napier hybrid cultivars. Means ± standard error followed by the same letter within columns are not different (P>0.05).

Treatment	Leaf DM (kg/plant)	Stem DM (kg/plant)	Total DM (kg/plant)	LSR
Cultivar				
CO-3	0.33 ± 0.0 a	0.23 ± 0.0 b	0.56 ± 0.0 b	1.43 ± 0.2 a
Giant	0.34 ± 0.0 a	0.27 ± 0.0 ab	0.61 ± 0.0 a	1.26 ± 0.2 b
Pakchong-1	0.31 ± 0.0 a	0.33 ± 0.0 a	0.64 ± 0.0 a	0.94 ± 0.2 b
Cutting interval				
40 days	0.2 ± 0.0 b	0.04 ± 0.0 c	0.24 ± 0.0 c	5.00 ± 0.1 a
60 days	0.4 ± 0.0 a	0.35 ± 0.0 b	0.75 ± 0.0 b	1.14 ± 0.2 b
80 days	0.4 ± 0.0 a	0.43 ± 0.0 a	0.83 ± 0.0 a	0.93 ± 0.2 b

Forage DM production and LSR

The effects of cultivar and cutting interval on leaf and stem DM production and LSR are presented in Table 3. The only parameter for which there was a significant cultivar x cutting interval effect was LSR. LSR at 40 days cutting interval was greater for Giant and Pakchong-1 but declined sharply as the cutting intervals increased with the greatest effect for Pakchong-1 (P<0.05). The mean LSR values for Giant Napier were 4.50, 1.25 and 1.00 for 40, 60 and 80 days cutting intervals, respectively. Pakchong-1 gave mean LSR values of 4.80, 0.82 and 0.67 for 40, 60 and 80 days, respectively, while corresponding values for CO-3 were 3.75, 1.52 and 1.20.

Amongst the main effects, cultivar had a significant effect on stem DM production (Pakchong-1>CO-3, P<0.05) whereas cutting interval had a significant (P<0.05) effect on leaf DM/plant (60 and 80>40 days) and stem DM/plant (80>60>40 days).

Protein concentration

Cultivar had a significant effect on stem CP concentration with CO-3 and Giant exceeding Pakchong-1 (P<0.05) (Table 4). CP concentration of leaves declined dramatically with increase in cutting interval from 28.2% at 40 days to 8.8% at 80 days (P<0.05). Similarly CP concentration in whole plants declined from 40 to 80 days (P<0.05).

Correlation between growth parameters

As complementary information, correlations between growth parameters are presented in Table 5. While basal circumference was positively correlated with plant height, tiller number, leaf number and stem DM, plant height was positively correlated with leaf number and stem DM but negatively correlated with tiller number per plant. On the other hand, tiller number was negatively correlated with tiller diameter and stem DM but positively correlated with leaf number. Leaf DM was strongly and positively correlated with leaf:stem ratio.

Table 4. Effects of cultivar and cutting interval on CP concentration (% N x 4.43) of leaf and stem of 3 Napier hybrid cultivars. Means ± standard error followed by the same letter within columns are not different (P>0.05).

Treatment	Leaf	Stem	Whole plant (calculated)
Cultivar			
CO-3	16.5 ± 0.30 a	5.1 ± 0.35 a	10.8 ± 0.32 a
Giant	16.8 ± 0.30 a	5.6 ± 0.35 a	11.2 ± 0.32 a
Pakchong-1	17.2 ± 0.30 a	3.6 ± 0.35 b	10.4 ± 0.32 a
Cutting interval			
40 days	28.2 ± 0.32 a	-	28.2 ± 0.32 a
60 days	13.8 ± 0.26 b	5.2 ± 0.32 a	9.5 ± 0.29 b
80 days	8.8 ± 0.26 c	4.3 ± 0.26 a	6.6 ± 0.24 c

Table 5. Correlations between growth and production parameters of Napier grass.

Growth parameter	Basal circumference	Plant height	Tillers per plant	Tiller diameter	Leaves per plant	Leaf DM	Stem DM
Plant height (cm)	0.38**						
Tillers per plant (no.)	0.16*	-0.54**					
Tiller diameter (mm)	0.15	0.15	-0.32**				
Leaves per plant (no.)	0.34**	0.83***	0.75***	0.14			
DM leaf (kg/plant)	-0.03	-0.08	0.08	0.01	0.08		
DM stem (kg/plant)	0.44**	0.79***	-0.33***	0.01	0.07	-0.02	
LSR	-0.05	-0.11	0.09	-0.06	-0.09	0.99***	-0.05

Discussion

Growth characteristics of the 3 Napier grass cultivars responded to the varying defoliation regimes, demonstrating that defoliation impacts the morphological development and forage quality of Napier grass (Manyawu et al. 2003; Tessema 2005; Halim et al. 2013). The significantly taller plants, bigger tillers, bigger basal circumference and higher stem DM in Pakchong-1 highlight the vigorous growth of this cultivar and its adaptation to the wet subtropical conditions in the southern foothills of Bhutan. The bigger basal circumference and higher stem DM yield also suggest lower planting densities should be employed for this cultivar. Giant Napier appeared similar to Pakchong-1 in most morphological traits except for plant height.

The significant decline in tiller number with increasing cutting intervals for all 3 cultivars conforms with the reports of other studies that tillering is enhanced under frequent cutting (Clavero 1997; Onyeonagu and Asiegbu 2012). Zhang and Romo (1995) and Jones and Mott (1980) highlighted the need to focus on tillering and understanding tiller dynamics, when assessing the effects of different management strategies on population dynamics. Increased tillering is probably an adaptive feature to tolerate frequent defoliation by re-establishing lost photosynthetic area and maintaining basal area. Jones (1985) attributed increased tiller production to removal of apical dominance by defoliation and activation of basal buds, while low tiller production under long cutting intervals has been linked to higher mortality of tillers under reduced cutting frequency (Clavero 1997). Regardless of cutting regime CO-3 consistently produced the highest number of tillers, which demonstrates the profuse tillering capacity of this cultivar (Premaratne and Premalal 2006). High tiller production not only indicates stable productivity (Mukhtar 2006) but also is linked to better persistence after periods of unfavorable environmental conditions (Assuero and Tognetti 2010). Lafarge and Loiseau (2002) consider tiller production is a key factor in the resistance of grasslands to deterioration by ageing. Pakchong-1 consistently produced fewer but larger tillers.

While CO-3 had fewest leaves per tiller, its greater tillering ability resulted in more leaves per plant, which is a desirable attribute when producing forage for livestock. Despite this advantage, total leaf DM in all cultivars was similar. The leaf fraction determines pasture quality (Davison et al. 1981) and performance of animals is related to the amount of leaf in the diet (Tudsri et al. 2002).

The decrease in LSR with increase in cutting interval agrees with the reports of Smart et al. (2004) and Tessema et al. (2010) that LSR decreases with decrease in defoliation frequency. Decrease in LSR with longer cutting intervals is a function of the longer periods of physiological growth with reduced defoliation frequency stimulating stem growth at the expense of leaf production (Butt et al. 1993). Except when cut frequently, CO-3 produced the highest LSR, which is an important beneficial trait of this cultivar. CO-3 was the shortest among the cultivars tested and dwarf cultivars have been reported to produce a higher LSR over a wide range of maturities (Sukkagate et al. 1997), which is reflected in higher overall nutritive quality compared with taller varieties (Halim et al. 2013). Dwarf varieties also have higher tiller number, leaf area index and percentage of leaf than the normal and tall varieties of Napier grass. This result is of significance from the forage standpoint since LSR is an important factor affecting diet selection, quality and intake of forage (Smart et al. 2004). Since leaf DM yields of all cultivars were similar, it was the higher stem DM yields in Pakchong-1 and Giant that resulted in the lower LSR in these cultivars.

Leaf material for all 3 cultivars was of high quality with CP concentrations about 17%. While stems of all cultivars had lower CP concentrations than leaf, the mean values for total forage were still above the 1% N suggested by Milford and Minson (1966) as the figure below which feed intake is restricted.

In conformity with the report of Tessema et al. (2010), we found that a short cutting interval of 40 days seriously reduced DM yields of all cultivars. While protein concentration was very high, this would scarcely compensate for the greatly reduced forage production. On the contrary, except for Pakchong-1, a long interval of 80 days resulted in taller plants susceptible to lodging during strong winds. A general decline in CP concentration with increasing cutting interval corresponds with the results of other studies showing decline in CP with advancing phenological stages (Manyawu et al. 2003; Khaled et al. 2005; Peiretti et al. 2015). We consider that an intermediate cutting interval of 60 days appears optimal for the subtropical conditions of Bhutan. This is supported by our results revealing high leaf DM production, and acceptable CP concentration and LSR at 60 days cutting interval. Ansah et al. (2010) obtained the highest CP concentration when Napier grass was harvested at less than 9 weeks cutting interval but DM yields were low, while Van Man and Wiktorsson (2003) achieved the best balance between DM yield and forage quality of Napier grass at 8 weeks cutting interval.

It seems that Pakchong-1 is not superior to Giant Napier and CO-3, which is at variance with the higher CP concentration of 16−18% for Pakchong-1 reported from Thailand (Kiyothong 2014). There are two possible explanations for the contrast in results between southern Bhutan and Thailand. Firstly, the study site had only moderate levels of soil N, which may have affected CP concentration, since CP in forages is positively correlated with soil N (Mohammad et al. 1988; Singh et al. 2000). Secondly, climatic conditions differ greatly between the 2 countries. Thailand experiences a hot, humid tropical climate for most of the year, while the climate of the southern foothills of Bhutan is hot and humid in summer but cold and dry in winter. While Pakchong-1 might be ideally suited to a tropical climate, e.g. in Thailand, it may not perform to its full potential in the southern foothills of Bhutan.

Conclusion

While all 3 Napier hybrid grass cultivars performed well in the southern foothills of Bhutan, they varied in terms of growth characteristics, forage yield and quality. Cultivar CO-3 was superior to Pakchong-1 and Giant Napier in terms of leafiness and tiller production but Pakchong-1 had faster growth rate, bigger tillers and higher overall DM production. While Kiyothong (2014) suggested cutting as often as every 45 days to produce material of 16-18% CP, DM yields would suffer markedly under such a regime. From the forage standpoint under the environmental conditions of the southern foothills of Bhutan, Pakchong-1 Napier appears to have no real advantages over CO-3 and Giant Napier. However, there might be situations where attributes of Pakchong-1 could be beneficial for dairy farmers.

Pakchong-1's fast regrowth and high DM yield might be advantageous in conserving soil and providing early fodder. In the southern foothills, biogas production is practiced to meet energy needs of rural households. Under this scenario, the high DM yield and high stem production of Pakchong-1 could be important attributes for methane gas production. This aspect should be investigated.

Acknowledgments

The authors gratefully acknowledge the funding support of the Royal Government of Bhutan to undertake the field experiment. The authors also thank the National Jersey Breeding Center, Samtse, for providing the farm area for conducting the experiment.

References

Anderson WF; Dien BS; Brandon SK; Peterson JD. 2008. Assessment of Bermuda grass and bunch grasses as feed stocks for conversion to ethanol. Applied Biochemistry and Biotechnology 145:13–21. DOI: 10.1007/s12010-007-8041-y

Ansah T; Osafo ELK; Hanne HH. 2010. Herbage yield and chemical composition of four varieties of Napier (*Pennisetum purpureum*) grass harvested at three different days after planting. Agriculture and Biology Journal of North America 1:923–929. DOI: 10.5251/abjna.2010.1.5.923.929

Assuero SG; Tognetti JA. 2010. Tillering regulation by endogenous and environmental factors and its agricultural management. The Americas Journal of Plant Science and Biotechnology 4:35–48. http://goo.gl/0JnnCJ

Bayble T; Melaku S; Prasad NK. 2007. Effects of cutting dates on nutritive value of Napier (*Pennisetum purpureum*) grass planted sole and in association with Desmodium (*Desmodium intortum*) or Lablab (*Lablab purpureus*). Livestock Research for Rural Development 19, article # 11. www.lrrd.org/lrrd19/1/bayb19011.htm

Bayer W. 1990. Napier grass − A promising fodder for smallholder livestock production in the tropics. Plant Research for Development 31:103–111.

Blakemore LC; Searle PL; Daly BK. 1981. A. Methods for chemical analysis of soils. New Zealand Soil Bureau Scientific Report 10A. Revised Edn. Department of Scientific and Industrial Research, Wellington, New Zealand. DOI: 10.7931/DL1-SBSR-10A

Bray RM; Kurtz LT. 1945. Determination of total, organic and available forms of phosphorus in soils. Soil Science 59: 39–46. DOI: 10.1097/00010694-194501000-00006

Butt NM; Gary B; Donart MG; Southward RD; Noror M. 1993. Effects of defoliation on plant growth of Napier grass. Tropical Science 33:111–120.

Chaparro CJ; Sollenberger LE; Quesenberry KH. 1996. Light interception, reserve status and persistence of clipped Mott elephant grass swards. Crop Science 36:649–655. DOI: 10.2135/cropsci1996.0011183x003600030022x

Clavero LT. 1997. Tiller dynamics of dwarf elephant grass (*Pennisetum purpureum* cv. Mott) under defoliation. In: Proceedings of the XVIII International Grassland Congress, Winnipeg and Saskatoon, Canada, 1997. Session 22, p. 31–32.

Cuomo GJ; Blouin DC; Beatty JF. 1996. Forage potential of dwarf Napier grass and a pearl millet x Napier grass hybrid. Agronomy Journal 88:434–438. DOI: 10.2134/agronj1996.00021962008800030012x

Davison TM; Cowan RT; O'Rourke PK. 1981. Management practices for tropical grasses and their effects on pasture and milk production. Australian Journal of Experimental Agriculture and Animal Husbandry 21:196–202. DOI: 10.1071/EA9810196

Guebel DV; Nudel BC; Giulietti AM. 1991. A simple and rapid micro-Kjeldahl method for total nitrogen analysis. Biotechnology Techniques 6:427–430. DOI: 10.1007/bf00155487

Halim RA; Shampazuraini S; Idris AB. 2013. Yield and nutritive quality of nine Napier grass varieties in Malaysia. Malaysian Journal of Animal Science 16:37–44.

Jones CA. 1985. C4 grasses and cereals: Growth, development and stress response. Wiley and Sons, New York, USA.

Jones RM; Mott JJ. 1980. Population dynamics of grazed pastures. Tropical Grasslands 14:218–224. http://goo.gl/BHxtVw

Jorgensen ST; Pookpakdi A; Tudsri S; Stolen O; Ortiz R; Christiansen JL. 2010. Cultivar-by-cutting height interactions in Napier grass (*Pennisetum purpureum* Schumach) grown in a tropical rain-fed environment. Acta Agriculturae Scandinavica 60:199–210. DOI: 10.1080/09064710902817954

Khairani L; Ishii Y; Idota S; Utamy RF; Nishiwaki A. 2013. Variation in growth attributes, dry matter yield and quality among 6 genotypes of Napier grass used for biomass in year of establishment in Southern Kyushu, Japan. Asian Journal of Agricultural Research 7:15–25. DOI: 10.3923/ajar.2013.15.25

Khaled RAH; Duru M; Cruz P. 2005. Are leaf traits suitable for assessing the feeding value of native grass species? In: O'Mara FP; Wilkins RJ; Mannetje L 't; Lovett DK; Rogers PAM; Boland TM, eds. Proceedings of the XX International Grassland Congress, Dublin, 2005. Wageningen Academic Publishers, The Netherlands.

Kiyothong K. 2014. Miracle grass seen to boost local dairy production. www.pinoyfeeds.com/Super-napier.html

Kumar V. 2013. Napier grass (Elephant grass) variety. http://goo.gl/tYvmea

Lafarge M; Loiseau P. 2002. Tiller density and stand structure of tall fescue swards differing in age and nitrogen level. European Journal of Agronomy 17:209–219. DOI: 10.1016/s1161-0301(02)00011-4

Landau S; Everitt BS. 2004. A Handbook of statistical analyses using SPSS. Chapman & Hall/CRC, CRC Press LLC, Boca Raton, FL, USA.

Lowe AJ; Thorpe W; Teale A; Hanson J. 2003. Characterization of germplasm accessions of Napier grass (*Pennisetum purpureum* and *P. purpureum* x *P. glaucum* hybrids) and comparison with farm clones using RAPD. Genetic Resources and Crop Evolution 50:121–137.

Manyawu GJ; Chakoma C; Sibanda S; Mutisi C; Chakoma IC. 2003. The effect of harvesting interval on herbage yield and nutritive value of Napier Grass and hybrid *Pennisetums*. Asian-Australian Journal of Animal Science 16:996–1002. DOI: 10.5713/ajas.2003.996

Milford R; Minson DJ. 1966. Intake of tropical pasture species. Proceedings of the IX International Grassland Congress, São Paulo, Brazil, 1965. p. 81–82.

Ministry of Agriculture. 2000. Central Farms − Status, Review and Recommendations. Thimphu, Bhutan.

Mohammad N; Butt NM; Qamar IA. 1988. Effect of nitrogen fertilization and harvesting intervals on the yield and nutritive value of Napier grass. Pakistan Journal of Agriculture Science 9:478–482.

Mukhtar M. 2006. Dry matter productivity of the dwarf and normal elephant grasses as affected by the planting density and cutting frequency. Indonesian Journal of Animal and Veterinary Sciences 11:198–205. http://goo.gl/d5hpyL

Mutegi JK; Mugendi DK; Verchot L; Kung'u JB. 2008. Combining Napier grass with leguminous shrubs in contour hedgerows controls soil erosion without competing with crops. Agroforestry Systems 74:37–49. DOI: 10.1007/s10457-008-9152-3

Mwendia SW; Wanyoike M; Nguguna JGM; Wahome RG; Mwangi DM. 2006. Evaluation of Napier grass cultivars for resistance to Napier head smut. In: Proceedings, 10th KARI Biennial Scientific Conference. KARI, Nairobi, Kenya. p. 85–97.

Nyambati EM; Muyekho FN; Onginjo E; Lusweti CM. 2010. Production, characterization and nutritional quality of Napier grass (*Pennisetum purpureum* Schum.) cultivars in Western Kenya. African Journal of Plant Science 4:496–502.

Onyeonagu CC; Asiegbu JE. 2012. Influence of cutting frequency and fertilizer-N application on tiller production and herbage yield distribution over time in a guinea grass (*Panicum maximum*) sown pasture. African Journal of Biotechnology 11:7170–7185.

Pandey KC; Roy AK. 2011. Forage Crops Varieties. Indian Grassland and Fodder Research Institute (IGFRI), Jhansi, Uttar Pradesh, India.

Patil DB; Joshi AB. 1962. Pusa Giant Napier. Indian Farming 18:7–8.

Peiretti PG; Gai F; Tassone S. 2015. Nutritional value and fatty acid profile of Niger (*Guizotia abyssinica*) plant during its growth cycle. Livestock Research for Rural Development 27, article # 18. www.lrrd.org/lrrd27/1/peir27018.htm

Premaratne S; Premalal GGC. 2006. Hybrid Napier (*Pennisetum perpureum* x *Pennisetum americarnum*) var. CO-3: A resourceful fodder grass for dairy development in Sri Lanka. The Journal of Agricultural Sciences 2:22–33. http://goo.gl/2ciiZo

Rengsirikul K; Ishii Y; Kangvansaichol K; Pripanapong P; Sripichitt P; Punsuvon V; Vaithanomsat P; Nakamanee G; Tudsri S. 2011. Effects of inter-cutting interval on biomass yield, growth components and chemical composition of Napier grass (*Pennisetum purpureum* Schumach) cultivars as bioenergy crops in Thailand. Grassland Science 57:135–141. DOI: 10.1111/j.1744-697x.2011.00220.x

Singh D; Singh V; Joshi YP. 2000. Effect of nitrogen fertilization and cutting intervals on yield and quality of Napier Bajra hybrid. Journal of Range Management & Agroforestry 21:128–134.

Smart AJ; Schacht WH; Moser LE; Volesky JD. 2004. Prediction of leaf/stem ratio using near-infrared reflectance spectroscopy (NIRS): A Technical Note. In: Agronomy & Horticulture Faculty Publications, Vol. 39. http://goo.gl/QFvzF9
Staal S; Chege L; Kenyanjui M; Kimari A; Lukuyu B; Njubi D; Owango M; Tanner J; Thorpe W; Wambugu M. 1987. A cross sectional survey of Kiambu District for the identification of target groups of smallholder dairy producers. KARI/ILRI collaborative project research report, Nairobi, Kenya.
Sukkagate S; Tudsri S; Pookpakdi A. 1997. Effect of close cutting on the yield and quality of four tropical grass pastures. In: Proceedings, 32nd Kasetsart University Annual Conference, 3−5 February 1997, Kasetsart University, Bangkok, Thailand. p. 104–112.
Tessema Z. 2005. Variation in growth, yield, chemical composition and *in vitro* dry matter digestibility of Napier grass accessions (*Pennisetum purpureum*). Tropical Science 45:67–73. DOI: 10.1002/ts.51
Tessema Z. 2008. Effect of plant density on morphological characteristics, yield and chemical composition of Napier grass (*Pennisetum purpureum* (L.) Schumach). East African Journal of Sciences 2:55–61. DOI: 10.4314/eajsci.v2i1.40365
Tessema ZK; Mihret J; Solomon M. 2010. Effect of defoliation frequency and cutting height on growth, dry-matter yield and nutritive value of Napier grass (*Pennisetum purpureum* (L.) Schumach). Grass and Forage Science 65:421–430. DOI: 10.1111/j.1365-2494.2010.00761.x
Tudsri S; Ishii Y; Numaguchi H; Prasanpanich S. 2002. The effect of cutting interval on the growth of *Leucaena leucocephala* and three associated grasses in Thailand. Tropical Grasslands 36:90–96. http://goo.gl/FXEbNU
Van Man N; Wiktorsson H. 2003. Forage yield, nutritive value, feed intake and digestibility of three grass species as affected by harvest frequency. Tropical Grasslands 37:101–110. http://goo.gl/65RETA
Van Soest PJ. 1994. Nutritional ecology of the ruminant. Comstock Publishing Associates, Division of Cornell University Press, Ithaca, NY, USA.
Yeoh HH; Wee YC. 1994. Leaf protein contents and nitrogen-to-protein conversion factors for 90 plant species. Food Chemistry 49:245–250. DOI: 10.1016/0308-8146(94)90167-8
Zhang J; Romo JT. 1995. Impacts of defoliation on tiller production and survival in northern wheatgrass. Journal of Range Management 48:115–120. DOI: 10.2307/4002796

(Received for publication 31 January 2015; accepted 10 August 2015)

Tropical Grasslands – Forrajes Tropicales (2015) Volume 3, 151−160

DOI: 10.17138/TGFT(3)151-160

Performance of tropical legumes grown as understory of a eucalypt plantation in a seasonally dry area of the Brazilian Cerrado

MARIA LUIZA F. NICODEMO[1], FRANCISCO H.D. DE SOUZA[1], JOSE RICARDO M. PEZZOPANE[1], JOÃO CARLOS T. MENDES[2], WALDOMIRO BARIONI JUNIOR[1], PATRÍCIA THOLON[1] AND PATRÍCIA M. SANTOS[1]

[1]*Empresa Brasileira de Pesquisa Agropecuária, Embrapa Pecuária Sudeste, São Carlos, SP, Brazil.* www.embrapa.br/pecuaria-sudeste
[2]*Universidade de São Paulo, Escuela Superior de Agricultura 'Luiz de Queiroz', Departamento de Ciências Florestais, Piracicaba, SP, Brazil.* www.esalq.usp.br

Keywords: Dry matter production, forage quality, shade, silvopastoral system.

Abstract

Nine tropical legumes were grown outside the canopy and in the understory of an 8-year-old *Eucalyptus grandis* stand in order to assess their seasonal production and forage quality for 4 evaluation periods. Incident photosynthetically active radiation in the understory was 18% of that outside the canopy. In the understory, production of *Lablab purpureus*, *Centrosema schiedeanum*, *Clitoria ternatea*, *Pueraria phaseoloides*, *Alysicarpus vaginalis*, *Aeschynomene villosa*, Estilosantes Campo Grande (*Stylosanthes capitata* + *S. macrocephala*), *Calopogonium mucunoides* and *Arachis pintoi* was <1 kg/ha/d for most samples. Even considering this low production, the large area available for animal production in forest plantations might justify the interest in legumes because of their high nutritive value. *Lablab purpureus* produced the greatest amount of dry matter in the understory in the establishment phase (12.1 kg/ha/d), but did not persist. It could be a suitable candidate for a cover legume species mixture to provide early growth. *Centrosema schiedeanum* developed rapidly and showed a high capacity for ground cover (>70%) and persistence, and had high nitrogen concentration, thus demonstrating good potential for protecting soils and promoting nutrient cycling in forest plantations. Another species with potential is *A. pintoi*, which established slowly but towards the end of the experiment showed moderate to high understory ground cover.

Resumen

Con el fin de determinar la producción estacional y la calidad de forraje durante 4 períodos de evaluación, en condiciones de sequía estacional del Cerrado brasileño se cultivaron 9 leguminosas tropicales bajo y fuera del dosel de una plantación de *Eucalyptus grandis* de 8 años de edad. Bajo el dosel, la radiación fotosintéticamente activa incidente estaba reducida al 18% de la radiación fuera del dosel. La producción de materia seca (MS) de *Lablab purpureus, Centrosema schiedeanum, Clitoria ternatea, Pueraria phaseoloides, Alysicarpus vaginalis, Aeschynomene villosa*, Estilosantes Campo Grande (*Stylosanthes capitata* + *S. macrocephala*), *Calopogonium mucunoides* y *Arachis pintoi* fue <1 kg/ha por día para la mayoría de las muestras bajo el dosel. Incluso teniendo en cuenta esta baja producción, la gran superficie de plantaciones forestales disponible para la producción animal podría justificar el interés en leguminosas forrajeras debido a su alto valor nutritivo. En la fase de establecimiento, *L. purpureus* produjo la mayor cantidad de MS bajo el dosel (12.1 kg/ha por día), pero no fue persistente. Esta especie podría ser un candidato para una mezcla de especies de leguminosas de cobertura, para proporcionar una cobertura y producción tempranas. *Centrosema schiedeanum* presentó un desarrollo rápido y mostró una alta cobertura del suelo (>70%) y de persistencia con alta concentración de nitrógeno, indicando un buen potencial para proteger el suelo y promover el reciclaje de nutrientes en plantaciones forestales. *Arachis pintoi* es otra especie con alto potencial de cultivo bajo dosel; aunque su establecimiento fue lento, al final del experimento mostró moderada a alta cobertura del suelo.

Correspondence: M.L. Franceschi Nicodemo, Embrapa Pecuária Sudeste, Caixa Postal 339, São Carlos CEP 13560-970, SP, Brazil.
Email: marialuiza.nicodemo@embrapa.br

Introduction

Plantation forests are estimated to cover 264 million ha worldwide, an area that increased at a rate of 5 million ha/yr between 2000 and 2010 (FAO 2010). Raising animals in the understory of forest plantations can help control weeds, reduce the risk of forest fires, and increase land use efficiency and economic returns. It is also a potential strategy for producing animal protein in a more sustainable way. Grasses frequently dominate the understory of tropical forest plantations, but support only low stocking rates (Baggio and Schreiner 1988; Schreiner 1988). Leguminous plants can also occur in the understory (Tajuddin 1986; Sophanodora and Tudsri 1991), where they supply forage of high nutritional value and improve nitrogen cycling.

Dense shading has been associated with low dry matter (DM) production in tropical legumes (Andrade et al. 2004; Gobbi et al. 2009). Legumes show a variety of responses to shading. For example, species such as *Lablab purpureus* (Bazill 1987), *Arachis pintoi* (Ferreira et al. 2008), *Clitoria ternatea* and *Calopogonium mucunoides* (Congdon and Addison 2003), *Centrosema pubescens* (now: *C. molle*) (Stür 1991) and *Pueraria phaseoloides* (Wong et al. 1985; Congdon and Addison 2003) are considered shade-tolerant, in contrast to *Aeschynomene villosa* (Congdon and Addison 2003) and *Stylosanthes* spp. (Bazill 1987) that are shade-intolerant. *Alysicarpus vaginalis* has been reported to grow well in moderate shade (Wong et al. 1985).

In general, plant performance is affected by climate (precipitation, temperature, day-length), soils (pH, fertility, texture, drainage) and management regime (grazing, cutting, fertilization) as well as by the degree of shading (Stür 1991). Therefore, species recommended for shaded conditions in the wet tropics may differ from those in the dry tropics (Congdon and Addison 2003).

In the tropics, plant stresses caused by shading in forest understory may be worsened by seasonal drought (Valladares and Niinemets 2008). The interaction of these two factors may be significant, as shown in an experiment with *A. pintoi* cv. Belmonte (Andrade et al. 2004). There, in the rainy season the species produced 59.2 kg DM/ha/d in full sun but only 22.4 kg/ha/d under 70% artificial shade. In the dry season, it produced just 3.2 kg/ha/d under 70% shade (14% of that in rainy season), whereas in full sun its production was 23.0 kg/ha/d. Tolerance of one type of stress may be reduced by another simultaneous source of stress (Valladares and Niinemets 2008). In this case, plant strategies for capturing light more efficiently (i.e. investment in above-ground biomass) and controlling water balance (i.e. investment in root biomass, or senescence) are in conflict. Trees affect soil fertility, microclimate, resource (water, nutrients and light) availability and utilization, and pest and disease incidence (Rao et al. 1998), all factors that may interfere with plant growth.

Although the growth and production potential of leguminous forage plants in shade has been examined in various studies (e.g. Congdon and Addison 2003; Andrade et al. 2004), some of these studies were conducted in pots, with irrigation and artificial shading (Ferreira et al. 2008; Azevedo et al. 2009), and others in irrigated plots (Stür 1991). The objective of our study was to assess the growth, persistence and quality of tropical legumes in the understory of an adult stand of eucalyptus subjected to seasonal drought, with the aim of identifying their potential for forage production under such conditions.

Material and Methods

The experiment was carried out at the Forest Experimental Station of the University of São Paulo in Anhembi, São Paulo state, Brazil (22°40' S, 48°10' W; 455 masl). Climate at the site is Cwa (Köppen), with hot rainy summers and cold dry winters. The dry season extends from April to September. Mean annual rainfall is 1,100 mm, annual water deficit is 25 mm, and mean annual temperature is 20.9 °C. Mean temperatures range from 17.1 °C in the coldest month to 23.7 °C in the hottest month. The soil is a nutrient-poor Typic Hapludox (LVAd – dystrophic Red-Yellow Latosol), composed of 18% clay, 9% silt and 73% sand. Nine herbaceous legume species were selected for the study, based on known shade tolerance and on seed availability, and were distributed in 4 blocks outside the canopy and 4 blocks in the understory of an 8-year-old eucalyptus stand, for a total of 72 plots. The stand of *Eucalyptus grandis* was planted at a spacing of 3 x 2 m and was later thinned to 40% for a final density of 1,000 trees/ha in its 7th year of growth. An area nearby, felled, was used for outside-canopy evaluations, replicating the understory design.

The species were *Aeschynomene villosa* (Australian commercial seed mix "Villomix"), *Alysicarpus vaginalis* 'common', *Arachis pintoi* cv. Mandovi, *Calopogonium mucunoides* 'common', *Centrosema schiedeanum* (now: *C. pubescens*) cv. Belalto, *Clitoria ternatea* 'common', *Lablab purpureus* cv. Rongai, Estilosantes Campo Grande (*Stylosanthes capitata* + *S. macrocephala*) and *Pueraria phaseoloides* 'common'. *Aeschynomene villosa* and Estilosantes Campo Grande are considered to be fairly shade-intolerant and were included for comparison with species expected to be better adapted to understory conditions. Seeds were inoculated with Rhizobium strains of known effectiveness and sown in December 2008 at a density intended to produce 40 seedlings/m^2. Each plot was 2.5 x 5 m and consisted of 5-m rows at 0.5-m distance. In the understory evaluation, the plots were located between tree lines. The area designed for outside-canopy evaluation was distanced at least 10 m from the trees. Root competition might have occurred to some extent (Sudmeyer et al. 2004).

Soil samples collected at 0−20 cm depth were analyzed according to Raij et al. (2001) and showed the following results: P (extracted by ion-exchange resin), 9 mg/dm^3; organic matter, 20 g/dm^3; pH ($CaCl_2$), 3.7; exchangeable cations in mmol$_c$/dm^3: K, 1.2; Ca, 2; Mg, 1; H+Al, 64; Al, 62; sum of bases, 4; cation exchange capacity, 68; base saturation 6%; Al^{3+} saturation, 94%; and sulfur and microelements, in mg/dm^3: S-SO_4^{2-}, 10; B, 0.49; Cu, 1.0; Fe, 88; Mn, 8.8; and Zn, 0.5. In October 2008 the soil was amended with agricultural lime (3 t/ha) and the following per-plot doses, applied to the planted rows: 80 g KCl, 400 g simple superphosphate (SSP) and 40 g FTE (Fritted Trace Elements) BR15. Based on a subsequent soil analysis in August 2009, 2.4 g boric acid, 40.2 g KCl, 119.6 g SSP and 3,200 g dolomitic limestone (90% effective calcium carbonate) were added to each plot.

Data were collected in the following assessment periods: establishment (December 2008−March 2009, 89 days); 2009 dry season (March−October 2009, 226 days); 2009/10 rainy season (November 2009−May 2010, 182 days); and 2010 dry season (June−October 2010, 150 days). Forage production was assessed on the basis of two 1-m^2 samples per plot for the establishment phase and dry-season periods, and 0.25-m^2 samples in the rainy season. Cutting height was 10 cm above soil surface for *A. villosa*, *A. vaginalis*, *C. ternatea*, *L. purpureus* and Estilosantes Campo Grande, and 5 cm for *A. pintoi*, *C. mucunoides*, *C. schiedeanum* and *P. phaseoloides*. Sampling was done at the end of each of the aforementioned periods and subsequently remaining forage was cut and removed. After oven-drying at 60 ºC until constant weight, samples were analyzed for crude protein (CP) concentration (AOAC 1990) and in vitro dry matter digestibility (IVDMD) (Tilley and Terry 1963). To express forage production, the forage dry matter (DM) accumulation rate (kg/ha/d) was calculated by dividing DM production by the number of days of the respective regrowth period. Ground cover was assessed by 3 independent observers via a subjective scale (0% - no cover; 100% - ground totally covered) at the end of the establishment and the dry-season periods.

Photosynthetically active radiation (PAR) was measured monthly at 8 random points in the outside-canopy plots and at 8 points in the understory plots between 11:00 and 12:00 h with a ceptometer (AccuPAR LP-80, Decagon) read at each point in 4 cardinal directions. Weather data (mean temperature and rainfall) from the Anhembi Forest Experimental Station were used to calculate the 10-day sequential climatological water balance (Thornthwaite and Mather 1955) during the experimental period, considering a soil water holding capacity of 100 mm (Figure 1).

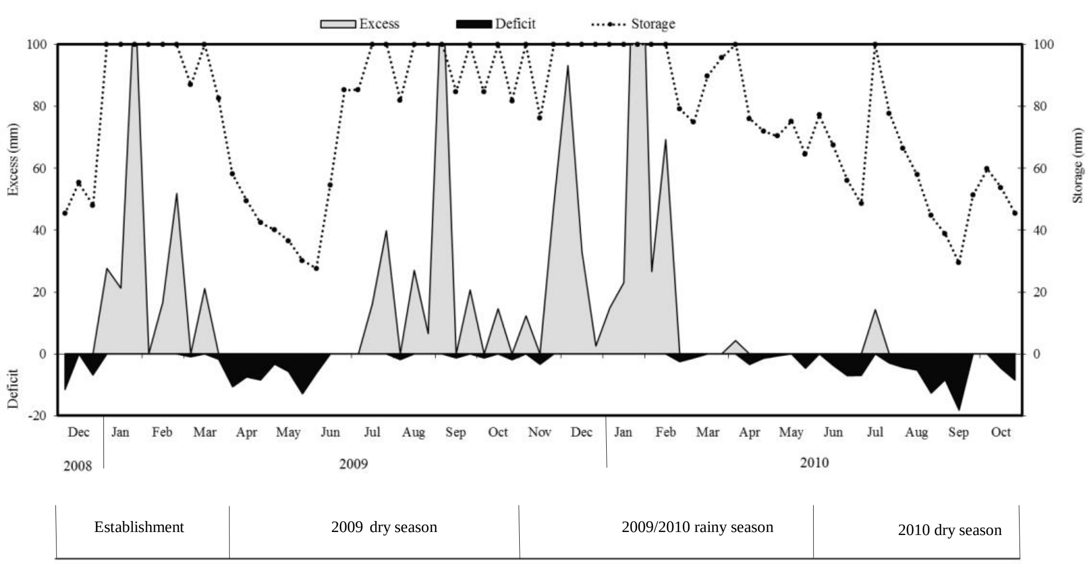


Figure 1. Water balance diagram for the period December 2008−October 2010 at Anhembi, São Paulo, Brazil.

Data analysis for the variables ground cover, DM accumulation rate, CP and IVDMD was performed independently for outside canopy and understory. A complete randomized block design was used with 4 replications, in a split-plot design with the species as plots and the evaluation period as subplot, based on Sampaio (1998), considering the effects of block (b), species (e) and period (p) according to the model:

$$Y_{ijk} = \mu + b_i + e_j + (b*e)_{ij} + p_k + (p*e)_{kj} + error_{ijk}$$

where: i=1,…,4 ; j=1,…,9 ; and k=1,…,4.

The interaction (b*e) compounds the error to test the effects of block and species.

Ground cover, DM accumulation rate, CP and IVDMD data were subjected to analysis of variance using the GLM procedure (SAS 9.1, SAS Institute, Cary, NC, USA). For multiple comparisons of means and interactions, LSMEANS was adopted, with significance of 5%.

Results

PAR incidence in understory plots was, on average, 18% of radiation in full sun (231 vs. 1,279 μmol/m^2/s). DM accumulation rate was low in the understory (Table 1) and in 20 of 36 observations, forage accumulation rates of <1 kg/ha/d were recorded. Only *L. purpureus* in the establishment period and Estilosantes Campo Grande in the rainy season yielded >5 kg/ha/d. Relative DM production (= production in understory relative to production outside canopy; calculations not presented) of DM was low and did not reach 40%.

Ground cover was <50% in 17 of the 27 observations made in understory (Table 2), including all assessments of *A. vaginalis*, *A. villosa* and Estilosantes Campo Grande. *Centrosema schiedeanum* cover exceeded 70% in all outside-canopy and in-understory observations. Although *A. pintoi* was slow to establish, it remained at 53% cover in understory at the end of the 2010 dry season, exceeding all except *C. schiedeanum* (P<0.05).

Table 1. Dry matter accumulation rate (kg/ha/d) in understory and outside canopy at the end of 4 assessment periods: establishment (89 days), 2009 dry season (226 days), 2009/10 rainy season (182 days) and 2010 dry season (150 days).

Species	Understory				Outside canopy			
	Establishment	2009 Dry	2009/10 Rainy	2010 Dry	Establishment	2009 Dry	2009/10 Rainy	2010 Dry
Lablab purpureus	12.1a[1]	2.2ab	0.9def	0.0b	35.5a	5.3d	11.2cd	0.0b
Centrosema schiedeanum	3.2b	3.3a	1.3cde	1.2a	9.1d	11.9bc	11.7cd	8.9a
Clitoria ternatea	2.1c	0.3de	2.3c	0.0b	11.2d	2.8d	21.4b	0.4b
Pueraria phaseoloides	0.0d	1.7bc	3.1b	0.2ab	2.6ef	12.6b	17.1bc	6.7a
Alysicarpus vaginalis	0.0d	0.0e	0.0f	0.0b	19.0c	0.0d	15.1bc	0.0b
Aeschynomene villosa	0.7d	0.3de	0.3ef	0.0b	6.8de	5.3d	19.5b	0.0b
Estilosantes Campo Grande	0.0d	1.6bc	5.3a	0.2ab	0.0f	21.5a	46.0a	5.3ab
Calopogonium mucunoides	2.5bc	0.7cde	1.5cd	0.0b	25.0b	3.4d	8.1d	0.2b
Arachis pintoi	0.9d	1.1cd	2.2c	0.4ab	0.3ef	5.8cd	9.3d	3.3ab

[1]Means within sites and columns followed by the same letter are not significantly different (P>0.05).

Table 2. Ground cover (%) in understory and outside canopy at the end of 3 assessment periods: establishment (89 days), 2009 dry season (226 days) and 2010 dry season (150 days).

Species	Understory			Outside canopy		
	Establishment	2009 Dry	2010 Dry	Establishment	2009 Dry	2010 Dry
Lablab purpureus	97.5a[1]	75.8b	0.0c	100.0a	72.5b	0.7c
Centrosema schiedeanum	73.3b	97.8a	85.7a	72.5b	100.0a	99.9a
Clitoria ternatea	59.6c	15.6d	1.9c	73.8b	72.9b	14.8c
Pueraria phaseoloides	29.3ef	73.2b	6.5c	33.3d	100.0a	95.2a
Alysicarpus vaginalis	17.5fg	0.3e	0.0c	75.0b	18.0c	8.5c
Aeschynomene villosa	42.1d	18.2d	0.0c	54.4c	71.5b	4.6c
Estilosantes Campo Grande	0.9g	36.7c	7.4c	7.9e	88.2ab	76.0b
Calopogonium mucunoides	59.2c	31.1c	0.0c	94.2a	75.8b	11.2c
Arachis pintoi	33.3de	91.1a	53.3b	15.4de	79.6b	98.9a

[1]Means within sites and columns followed by the same letter are not significantly different (P>0.05).

CP concentrations varied from 12.8 to 30.5% in legumes grown in the understory and from 8.2 to 27.5% in those grown outside the canopy (Table 3). The difference, in favor of in-understory plants, ranged between 5 and 20% between sites.

IVDMD varied from 42.3 to 69.4% in legumes grown in the understory and from 26.3 to 70.1% in those grown outside canopy (Table 4). There were no consistent differences between the 2 sites. *Alysicarpus vaginalis*, which exhibited the lowest digestibility, did not persist in the understory. *Lablab purpureus* (47.2−62.5%) and *A. pintoi* (58.2−70.1%) had the highest digestibility values (P<0.05) overall.

Discussion

Eucalyptus grandis intercepted 82% of incident radiation, even without a fully closed canopy. This degree of shading is similar to that found in adult rubber plantations in Southeast Asia (Chong et al. 1997).

Forage production and cover

In the establishment phase, *L. purpureus* produced 12.1 kg/ha/d and achieved 97.5% cover, producing the best results in understory. Relative DM production of this species (34%) was similar to that reported for the most productive tropical legumes (25.7−38.5% for the seasonally dry tropics) under 84% artificial shade (Congdon and Addison 2003). The species' quick establishment may be related to the large size of its seeds (Kolawolea and Kangab 1997). While it helps control weeds and protect the soil, *L. purpureus* did not regenerate to maintain its impressive early performance. As 2009 was an exceptionally wet year (Figure 1), with 666 mm of rainfall between April and October, the low production observed in *L. purpureus* was not due to drought stress but was probably related to the low cutting height adopted, and mainly to the species' life cycle as a short-lived herb (Aganga and Tshwenyane 2003).

Table 3. Crude protein (% dry matter) concentration in understory and outside canopy at the end of 4 assessment periods: establishment (89 days), 2009 dry season (226 days), 2009/10 rainy season (182 days) and 2010 dry season (150 days).

Species	Understory				Outside canopy			
	Establishment	2009 Dry	2009/10 Rainy	2010 Dry	Establishment	2009 Dry	2009/10 Rainy	2010 Dry
Lablab purpureus	20.8c[1]	20.1bc	22.0ab	-	15.4c	19.3d	14.4c	-
Centrosema schiedeanum	26.4ab	22.9ab	24.7a	28.4a	22.0a	26.1a	23.2a	25.6ab
Clitoria ternatea	27.8a	24.4a	16.2c	-	24.4a	25.2ab	14.1c	27.5a
Pueraria phaseoloides	-	21.3ab	18.8bc	22.5b	18.3bc	18.9d	20.3ab	21.8c
Alysicarpus vaginalis	-	-	-	-	17.3bc	-	8.2d	-
Aeschynomene villosa	30.5a	22.1ab	15.8cd	-	22.9a	15.5e	13.6c	-
Estilosantes Campo Grande	-	17.8c	12.8d	18.2b	-	14.8f	11.7c	17.6d
Calopogonium mucunoides	22.7c	21.4ab	20.9b	-	18.4b	22.4bc	19.5b	17.4d
Arachis pintoi	23.2bc	20.9bc	16.5c	27.2a	-	20.1cd	14.6c	25.6ab

[1]Means within sites and columns followed by the same letter are not significantly different (P>0.05).

Table 4. In vitro dry matter digestibility (% dry matter) in understory and outside canopy at the end of 4 assessment periods: establishment (89 days), 2009 dry season (226 days), 2009/2010 rainy season (182 days) and 2010 dry season (150 days).

Species	Understory				Outside canopy			
	Establishment	2009 Dry	2009/2010 Rainy	2010 Dry	Establishment	2009 Dry	2009/2010 Rainy	2010 Dry
Lablab purpureus	62.0b[1]	60.8ab	59.7a	-	62.5a	55.0ab	47.2bc	-
Centrosema schiedeanum	54.2c	48.4c	48.3b	56.0b	56.1a	38.9c	45.3bc	48.3cd
Clitoria ternatea	62.1ab	59.5ab	48.4b	-	60.9a	54.4ab	39.8c	58.1b
Pueraria phaseoloides	-	50.1c	52.6b	56.5b	53.9a	50.9b	53.1ab	55.7bc
Alysicarpus vaginalis	-	-	-	-	59.5a	-	26.3d	-
Aeschynomene villosa	69.4a	60.7ab	47.5bc	-	59.5a	36.4c	42.5c	-
Estilosantes Campo Grande	-	58.1b	51.3b	56.0b	-	48.1b	41.7c	53.9bc
Calopogonium mucunoides	50.5c	44.4c	42.3c	-	49.0b	37.7c	40.0c	38.8d
Arachis pintoi	65.1ab	64.1a	59.6a	69.0a	-	61.5a	58.2a	70.1a

[1]Means within sites and columns followed by the same letter are not significantly different (P>0.05).

The second highest production observed throughout the evaluation in understory was 5.3 kg/ha/d by Estilosantes Campo Grande. This rate of herbage accumulation, however, could support just 0.29 animal units (AU)/ha/yr, assuming that 1 AU = 450 kg live weight (LW), DM consumption = 2% of LW, and grazing efficiency = 50%. A low carrying capacity in understory has also been reported for mature rubber plantations in Southeast Asia planted with *Calopogonium caeruleum* and *Pueraria phaseoloides* (Chong et al. 1997). The highest carrying capacity in that study was 2 sheep/ha, with a LW gain of 100 g/hd/d; forage availability ranged from 200 to 800 kg/ha, which compares well with the yields observed in our study. Even considering the low productivity of the legumes, it might be expected that the large potential for animal production under the vast areas of forest plantations would increase the interest in high quality forages. However, under Brazilian conditions, plantation owners are mainly interested in tree production and prefer a maximum number of trees to achieve high timber yields rather than improve light conditions for understory forage production.

Tropical C4 plants need more light for photosynthesis than C3 plants, owing to the greater energetic cost of their CO_2-concentrating apparatus (Sage and McKown 2006). Some C4 plant species, however, can adapt to shaded environments given their phenotypic plasticity. Studies conducted in Brazil have shown that some tropical grasses (mostly C4) maintained vigorous growth rates and moderate production even in heavy shade: in the Cerrado biome between January and May (under 442 mm of total rainfall), *Panicum maximum* (now: *Megathyrsus maximus*) cv. Tanzânia, produced between 7 and 25 kg/ha/d in the understory of a 5.4-year-old *Eucalyptus urophylla* stand, which intercepted 68% of incident light (Andrade et al. 2001). In the wet tropics *Urochloa brizantha* cv. Marandu produced 37 kg/ha/d in the rainy season under a mature rubber plantation (Costa et al. 1999) and *U. decumbens* cv. Basilisk grown in 70% artificial shade produced between 24 and 64 kg/ha/d over 3 harvests, while the legume *Arachis pintoi* cv. Amarillo produced 16 and 21 kg/ha/d over 2 harvests (Gobbi et al. 2009). These results reflect the greater yield potential of grasses for grazing under heavy shade, compared with leguminous forages. The latter, however, could play a significant role for livestock production in forest plantations owing to their superior nutritive value.

The ground cover produced by *C. schiedeanum* in this study (Table 2) corroborates the potential of *Centrosema* spp. as cover crops in plantations of perennial tree species (Chee and Wong 1990). This species developed rapidly and showed a high capacity for ground cover as well as persistence under cutting. It maintained >70% ground cover throughout in both understory and outside the canopy. At the end of the 2010 dry season, ground cover was 86% in understory and 100% outside the canopy, higher than that of any of the other legumes tested ($P<0.05$). Stür (1991) ranked *Centrosema pubescens*, a closely related species (now: *C. molle*), among the 13 most productive of 84 legumes studied in irrigated plots under 80% artificial shade. Although *Centrosema* species are climbing vines that could smother small trees (Congdon and Addison 2003), such behavior was not observed in our study.

DM production of *A. pintoi* was low, varying from 0.4 to 2.2 kg/ha/d in understory. Andrade et al. (2004) reported that shade-tolerant species such as *A. pintoi* can maintain similar DM production in sunlight and under low to moderate shade (up to 30−50%), while Stür (1991) found that production was not maintained in heavy shade (i.e. 80%). Andrade and Valentim (1999) reported that *A. pintoi* BRA-031143 persisted when 70% of incident light was intercepted, but DM production decreased markedly as increasing levels of shading were imposed. In the Amazonian state of Acre, Brazil, with mean annual rainfall of 1,900 mm and a marked dry season, *A. pintoi* cv. Belmonte produced 22.4 kg/ha/d in the rainy season and 3.2 kg/ha/d in the dry season under 70% shade (Andrade et al. 2004), which highlights the association of shading and dry season stress. Part of the difference in performance between that study and ours may be attributable to the high temperatures in Acre and the use of artificial shade, which minimizes competition with trees for water and nutrients and reduces evapotranspiration (Andrade et al. 2004). Despite its low yields and slow establishment *A. pintoi* proved capable of providing moderate to high levels of ground cover, remaining at 53% at the end of the experiment (Table 2). This supports the findings of Congdon and Addison (2003) that this species presents high potential as a soil cover crop. In comparison, the other species tested (except *C. schiedeanum*) achieved <10% ground cover at the end of the study.

Although Estilosantes Campo Grande produced up to 46 kg/ha/d outside canopy, it developed slowly, having covered <10% of ground surface (Table 2) at the end of the establishment phase. Mostly, it showed low DM production and ground cover (Tables 1 and 2) in the understory. These results are in contrast with those of Azevedo et al. (2009) who grew Estilosantes Campo Grande in irrigated pots under 75% artificial shade; he made a single harvest and found DM yield was greater under deep shade than in full sun. Differences in moisture availability in the two situations might explain the difference.

All 4 species of *Stylosanthes* tested by Bazill (1987) showed poor adaptation to shade provided by a *Pinus caribaea* forest in Costa Rica. However, adaptation to shade varies with species and accessions (Stür 1991), and Estilosantes Campo Grande produced 5.3 kg/ha/d at the end of the 2009/10 rainy season, more than any other species ($P<0.05$).

Nutritive value

The nutritive value of forages is often expressed in terms of their crude protein (CP) concentration and digestibility; both are closely associated with DM consumption. Low CP concentration (<7%) decreases rumen fermentation and microbial synthesis of protein, slowing rate of passage of digesta and limiting DM intake (Minson 1990). Low digestibility limits the intake potential and energy availability of forage crops for animal production (Jung and Allen 1995). Legumes show higher CP concentration and more stable year-round values than grasses (Barcellos et al. 2008).

Crude protein. In our study, all species but one (*C. mucunoides*) presented particularly high CP values at the end of the 2010 dry season (Table 3), due to reasons we cannot ascertain. CP concentrations of the legumes we studied are close to those reported in the literature (Minson and Wilson 1980; Araujo Filho et al. 1994; Costa et al. 2009; Heinritz et al. 2012) with the exception of *A. vaginalis*, which showed a very low value after 180 days of rainy season growth (Table 3). This is probably a reflection of the early maturity of *A. vaginalis*, which seeded abundantly during the wet season, and disappeared in the subsequent dry season (Table 1). The decrease in the CP concentration of this species may reflect the development of fibrous structures as the plants matured and reproduction proceeded.

In the present study, CP values at the understory site were 5−20% higher than outside the canopy. This confirms reports in the literature (Lin et al. 2001; Congdon and Addison 2003) that protein concentrations are higher in shade-grown legumes, which can be attributed to increased rates of mineralization, litter degradation, and nitrogen cycling in the wetter, cooler conditions of a forest understory. In this context, fast decomposing legume litter may play an important role, particularly in plantations of *Eucalyptus* trees whose litter is known to decompose slowly for several reasons, among them a high C:N ratio (Balieiro et al. 2004). Microbial decomposition demands N; hence this activity may benefit from higher foliar N content of associated legumes (Forrester et al. 2006). As an example, Balieiro et al. (2004) studied litter decomposition in a mixed stand of the leguminous tree *Pseudosamanea guachapele* and *Eucalyptus grandis*. They reported an 11% increase in soil N deposition by the leguminous tree, associated with a shorter litter residence time (23%) than in eucalyptus, and increased N mineralization.

Digestibility. IVDMD values in understory were 51.2−59.8 % (Table 4), similar to those reported in the literature for *L. purpureus* (Aganga and Tshwenyane 2003), *C. ternatea* (Minson and Wilson 1980) and *P. phaseoloides* (Abaunza et al. 1991). No information on IVDMD values was found for *A. villosa* and Estilosantes Campo Grande. For *C. pubescens* (closely related to *C. schiedeanum*), Abaunza et al. (1991) reported an IVDMD of 52.2%, while Minson and Wilson (1980) reported 60−70% for *A. pintoi*. In our experiment, similar values were found for these species 89 days after planting, while values for *A. vaginalis* and *C. mucunoides* were lower. The low digestibility of *C. mucunoides* has been attributed to its dense epidermal hairs (Minson and Wilson 1980). *Alysicarpus vaginalis* was poorly adapted to the conditions of the experimental site and its quick maturation may have contributed to low IVDMD.

General considerations and research needs

The low DM production obtained by the legumes in this experiment should not be considered the only criterion in assessing their potential usefulness in forest plantations. Even at low animal stocking rates the better-adapted species may provide additional income when applied over large areas. Other potential benefits of legumes include faster decomposition of litter (see discussion above), reduction of forest fire risk, erosion and weed control, and nitrogen fixation for improved growth of the tree component in a silvopastoral system.

Among the research topics that could be suggested as a result of this study, 2 are highlighted:

(1) Studies of genetic variation within a species may permit the identification of genotypes that are adapted to heavy shade, even in species that this study found to be poorly adapted to such conditions.

(2) Similar to the common practice in SE Asian rubber and oil palm plantations (Jalani et al. 1998) of sowing

mixtures of cover legume species primarily for weed control (e.g. *Pueraria phaseoloides* + *Centrosema pubescens* (now: *C. molle*) + *Calopogonium mucunoides* + *Calopogonium caeruleum)*, the potential of species mixtures for understory livestock production should be researched. The benefit of such mixtures is due to increased diversity with potential substitutory and/or complementary effects. In the case of the legumes tested in this study, a mixture of *L. purpureus* and *C. schiedeanum* may provide a useful understory pasture. The inclusion of shade-tolerant tropical grasses could bring further benefits.

Conclusions

Three of the species tested show potential for use as understory forage legumes in forestry plantations: *Lablab purpureus* establishes quickly and has high initial DM production both outside canopy and in understory; however, being an annual, it does not persist and must be replanted. *Centrosema schiedeanum* shows rapid development, a high capacity for ground cover, and good persistence, and can also protect soils in tree plantations. While *Arachis pintoi* was slow to establish, it is capable of providing moderate to high ground cover in the understory subject to seasonal drought. These legumes can all increase the nutritive value of plantation understory vegetation for grazing, even though their DM production was comparatively low.

Acknowledgments

We thank Fapesp for financial support, Embrapa Agrobiologia, as well as Sementes Piraí and Sementes Safrasul. We are grateful to Prof. Dr. José Leonardo de Moraes Gonçalves, Dr. Gustavo Maia Souza, Dra. Patrícia Perondi Anchão Oliveira and Dr. Alfredo Ribeiro de Freitas for their suggestions and to the staff of the Anhembi Forest Experimental Station for logistical support.

References

Abaunza MA; Lascano C; Giraldo H; Toledo JM. 1991. Valor nutritivo y aceptabilidad de gramíneas y leguminosas forrajeras tropicales en suelos ácidos. Pasturas Tropicales 13(2):2−9. http://goo.gl/X99B8p

Aganga AA; Tshwenyane SO. 2003. Lucerne, lablab and *Leucaena leucocephala* forages: Production and utilization for livestock production. Pakistan Journal of Nutrition 2:46−53. DOI: 10.3923/pjn.2003.46.53

Andrade CMS; Valentim JF. 1999. Adaptação produtividade e persistência de *Arachis pintoi* submetido a diferentes níveis de sombreamento. Revista Brasileira de Zootecnia 28:439−445. DOI: 10.1590/s1516-35981999000300001

Andrade CMS; Garcia R; Couto L; Pereira OG. 2001. Fatores limitantes ao crescimento do capim-tanzânia em um sistema agrossilvipastoril com eucalipto, na região dos cerrados de Minas Gerais. Revista Brasileira de Zootecnia 30:1178−1185. DOI: 10.1590/s1516-35982001000500007

Andrade CMS; Valentim JF; Carneiro J; Vaz FA. 2004. Crescimento de gramíneas e leguminosas forrageiras tropicais sob sombreamento. Pesquisa Agropecuária Brasileira 39:263−270. DOI: 10.1590/s0100-204x2004000300009

AOAC. 1990. Official methods of analysis. 15th Edn. Association of Official Analytical Chemists (AOAC), Arlington, VA, USA. http://goo.gl/gawF2Y

Araújo Filho JA; Gadelha JA; Silva NL; Pereira RMA. 1994. Efeito da altura e intervalo de corte na produção de forragem de cunhã (*Clitoria ternatea* L.). Pesquisa Agropecuária Brasileira 29:979−982. http://goo.gl/a5EhUc

Azevedo BC; Souto SM; Dias PF; Alves A; Vieira MS; Matta PM. 2009. Efeito do sombreamento sobre o estabelecimento de Estilosantes Campo Grande. Asociación Latinoamericana de Producción Animal 17:97−102. www.bioline.org.br/pdf?la09014

Baggio AJ; Schreiner HG. 1988. Análise de um sistema silvipastoril com *Pinus elliottii* e gado de corte. Boletim de Pesquisa Florestal 16:19−30. https://goo.gl/3upWr9

Balieiro FC; Franco AA; Pereira MG; Campello EFC; Dias LE; Faria SM; Alves BJR. 2004. Dinâmica da serapilheira e transferência de nitrogênio ao solo, em plantios de *Pseudosamanea guachapele* e *Eucalyptus grandis*. Pesquisa Agropecuária Brasileira 39:597−601. DOI: http://dx.doi.org/10.1590/s0100-204x2004000600012

Barcellos AO; Ramos AKB; Vilela L; Martha Jr. GB. 2008. Sustentabilidade da produção animal baseada em pastagens consorciadas e no emprego de leguminosas exclusivas, na forma de banco de proteína, nos trópicos brasileiros. Revista Brasileira de Zootecnia [online] 37:51−67. DOI: 10.1590/S1516-35982008001300008

Bazill JAE. 1987. Evaluation of tropical forage legumes under *Pinus caribaea* var. *hondurensis* in Turrialba, Costa Rica. Agroforestry Systems 5:97−108. DOI: 10.1007/bf00047515

Chee YK; Wong CC. 1990. *Centrosema* in plantation agriculture. In: Schultze-Kraft R; Clements RJ, eds. *Centrosema*: Biology, agronomy, and utilization. Centro Internacional de Agricultura Tropical (CIAT), Cali, Colombia. p. 321−342.

Chong DT; Tajuddin I; Samat AMS; Stür WW; Shelton HM. 1997. Stocking rate effects on sheep and forage productivity under rubber in Malaysia. Journal of Agricultural Science 128:339−346. DOI: 10.1017/s0021859696004236

Congdon RA; Addison H. 2003. Optimising nutrition for productive and sustainable farm forestry systems: Pasture legumes under shade. Report. Rural and Industries Research

Development Corporation, Barton, ACT, Australia. http://researchonline.jcu.edu.au/192/

Costa NL; Townsend CR; Magalhães JA; Pereira RGA. 1999. Desempenho agronômico de gramíneas forrageiras sob sombreamento de seringal adulto. Pasturas Tropicales 21(2):65−68. http://goo.gl/yvyk6f

Costa NLC; Bendahan AB; Gianluppi V; Ribeiro PSM; Braga RM. 2009. *Calopogonium mucunoides*: Características agronômicas, produtividade e manejo. Comunicado Técnico 25. Embrapa Roraima, Boa Vista, RR, Brazil. http://goo.gl/NtcxD7

FAO (Food and Agriculture Organization of the United Nations). 2010. Global Forest Resources Assessment 2010. FAO Forestry Paper 163. Rome, Italy. www.fao.org/docrep/013/i1757e/i1757e.pdf

Ferreira DJ; Dias PF; Manhães Souto S. 2008. Com-portamento na sombra de acessos de amendoim forrageiro (*Arachis* spp.) recomendados para região da Baixada Fluminense. Archivos Latinoamericanos de Producción Animal 16:41−47. http://goo.gl/Oe3oQK

Forrester DI; Bauhus J; Cowie AL; Vanclay JK. 2006. Mixed-species plantations of Eucalyptus with nitrogen-fixing trees: A review. Forest Ecology and Management 233:211−230. DOI: 10.1016/j.foreco.2006.05.012

Gobbi KF; Garcia R; Garcez Neto AF; Pereira OG; Ventrella MC; Rocha GC. 2009. Características morfológicas, estruturais e produtividade de capim braquiária e do amendoim forrageiro submetidos ao sombreamento. Revista Brasileira de Zootecnia 38:1645−1654. DOI: 10.1590/s1516-35982009000900002

Heinritz SN; Hoedtke S; Martens SD; Peters M; Zeyner A. 2012 Evaluation of ten tropical legume forages for their potential as pig feed supplement. Livestock Research for Rural Development 24, Article #7. www.lrrd.org/lrrd24/1/hein24007.htm

Jalani BS; Chan K; Darus A. 1998. Legumes and ground cover in oil palm plantations in Southeast Asia. In: Pietrosémoli S; Hernández J, eds. Seminario Internacional: Cobertura de leguminosas en cultivos permanentes. Compendio. Santa Bárbara, 1−2 Octubre 1998. La Universidad del Zulia (LUZ), Maracaibo, Venezuela. p. 75−105.

Jung HG; Allen MS. 1995. Characteristics of plant cell walls affecting intake and digestibility of forages by ruminants. Journal of Animal Science 73:2774−2790. https://goo.gl/XdGMcc

Kolawolea GO; Kangab BT. 1997. Effect of seed size and phosphorus fertilization on growth of selected legumes. Communications in Soil Science and Plant Analysis 28:13−14. DOI: 10.1080/00103629709369868

Lin CH; McGraw RL; George MF; Garrett HE. 2001. Nutritive quality and morphological development under partial shade of some forage species with agroforestry potential. Agroforestry Systems 53:269−281. DOI: 10.1023/A:1013323409839

Minson DJ. 1990. Forage in Ruminant Nutrition. Academic Press, New York, USA.

Minson DJ; Wilson JR. 1980. Comparative digestibility of tropical and temperate forage − a contrast between grasses and legumes. Journal of the Australian Institute of Agricultural Science 46:247−249.

Raij B van; Andrade JC de; Cantarella H; Quaggio JA. 2001. Análise química para avaliação da fertilidade de solos tropicais. Instituto Agronômico de Campinas, Campinas, SP, Brazil.

Rao M; Nair P; Ong CK. 1998. Biophysical interactions in tropical agroforestry systems. Agroforestry Systems 38:3−50. DOI: 10.1007/978-94-015-9008-2_1

Sage RF; McKown AD. 2006. Is C4 photosynthesis less phenotypically plastic than C3 photosynthesis? Journal of Experimental Botany 57:303−317. DOI: 10.1093/jxb/erj040

Sampaio IBM. 1998. Estatística aplicada à experimentação animal. Fundação de Ensino e Pesquisa em Medicina Veterinária e Zootecnia, Belo Horizonte, MG, Brazil.

Schreiner HG. 1988. Viabilidade de um sistema silvipastoril em solos de areia quartzosa no Estado de São Paulo. Boletim de Pesquisa Florestal 17:33−38. http://goo.gl/fCoetv

Sophanodora P; Tudsri S. 1991. Integration of forages for cattle and goats into plantation systems in Thailand. In: Shelton HM; Stür WW, eds. Proceedings of Workshop on Forages for Plantation Crops. Sanur Beach, Bali, Indonesia 27−29 June 1990. ACIAR Proceedings No. 32. Australian Centre for International Agricultural Research, Canberra, Australia. p. 147−150. http://goo.gl/ezs1ak

Stür WW. 1991. Screening forage species for shade tolerance − a preliminary report. In: Shelton HM; Stür WW, eds. Proceedings of Workshop on Forages for Plantation Crops. Sanur Beach, Bali, Indonesia, 27−29 June 1990. ACIAR Proceedings No. 32. Australian Centre for International Agricultural Research, Canberra, Australia. p. 58−63. http://goo.gl/ezs1ak

Sudmeyer RA; Speijers J; Nicholas DB. 2004. Root distribution of *Pinus pinaster*, *P. radiata*, *Eucalyptus globulus* and *E. kochii* and associated soil chemistry in agricultural land adjacent to tree lines. Tree Physiology 24:1333–1346. DOI: 10.1093/treephys/24.12.1333

Tajuddin I. 1986. Integration of animals in rubber plantations. Agroforestry Systems 4:55−66. DOI: 10.1007/bf01834702

Thornthwaite CW; Mather JR. 1955. The water balance. Publications in Climatology 8:1−86.

Tilley JMA; Terry RA. 1963. A two-stage technique for the *in vitro* digestion of forage crops. Grass and Forage Science 18:104−111. DOI: 10.1111/j.1365-2494.1963.tb00335.x

Valladares F; Niinemets U. 2008. Shade tolerance, a key plant feature of complex nature and consequences. Annual Review of Ecology and Systematics 39:237−257. DOI: 10.1146/annurev.ecolsys.39.110707.173506

Wong CC; Mohd Sharudin MA; Rahim H. 1985. Shade tolerance potential of some tropical forages for integration with plantations. 2. Legumes. MARDI Research Bulletin 13:249–269. http://goo.gl/wia6Ek

(Received for publication 24 September 2014; accepted 1 August 2015)

DOI: 10.17138/TGFT(3)161-186

Commonness and rarity pattern of plant species within *Terai* grassland of northeastern Uttar Pradesh, India

SUMIT SRIVASTAVA, ASHISH DVIVEDI AND R.P. SHUKLA

Plant Ecology Laboratory, Department of Botany, D.D.U. Gorakhpur University, Gorakhpur, India.
www.ddugu.edu.in/department.php/Botany

Keywords: Community structure, frequency of occurrence, habit groups, habitat fragmentation, native grassland.

Abstract

We investigated the frequency of occurrence of plant species in grassy landscapes in northeastern Uttar Pradesh, India. Using random quadrats, field assessment was undertaken at 11 sites to sample an area of 333.75 ha, at an overall sampling density of 0.01%. A total of 287 plant species belonging to 183 genera and 53 families was recorded. Of these, 254 species were commonly distributed and 33 species exhibited localized occurrences; according to the rarity classes of Rabinowitz, the latter were classified as rare. One hundred and sixty-five species had large population sizes and 122 species exhibited small population sizes. The most common species, which exhibited high frequency and abundance, were predominantly from Poaceae, Cyperaceae, Papilionaceae, Asteraceae, Scrophulariaceae and Euphorbiaceae. Some species, which are known to have narrow geographical distributions, were locally abundant. Rare species showed restricted as well as localized distributions and were typically sampled at low population densities. The rare occurrences of once frequent and widespread species probably reflect acute fragmentation and shrinkage of specialized habitats as a result of intense cultural activities. Several species are to be considered as threatened. Studies on the status of rare plant species and the processes threatening their survival are urgently required.

Resumen

En ecosistemas de pastizales nativos en el noreste de Uttar Pradesh, India, se midió la frecuencia de aparición de especies vegetales. Las muestras fueron tomadas utilizando el método de cuadrados al azar en 11 sitios de estudio con 31 sitios de muestreo. El área total muestreada fue de 333.75 ha, con una densidad total de muestreo del 0.01%. En total se registraron 287 especies pertenecientes a 183 géneros y 53 familias. De éstas, 254 especies tenían una distribución común mientras 33 especies aparecían con baja frecuencia y en forma localizada; según las clases de rareza de Rabinowitz; estas últimas fueron clasificadas como raras. En poblaciones grandes ocurrieron 165 especies y en poblaciones pequeñas 122 especies. Las especies más comunes, con alta frecuencia y abundancia, fueron predominantemente de las familias Poaceae, Cyperaceae, Papilionaceae, Asteraceae, Scrophulariaceae y Euphorbiaceae. Algunas especies con una distribución geográfica reducida eran localmente abundantes. Especies clasificadas como raras presentaron tanto distribuciones restringidas como localizadas y en general ocurrían a bajas densidades poblacionales. Las ocurrencias raras de especies que antes eran frecuentes y de distribución amplia, probablemente reflejan una aguda fragmentación y reducción de los hábitats especializados, como consecuencia de intensas actividades agrícolas. Varias especies deben considerarse amenazadas y se sugieren estudios sobre los procesos que amenazan su supervivencia.

Introduction

The structure of plant communities has been conventionally analyzed worldwide (Mueller-Dombois and Ellenberg 1974; Singh and Yadava 1974). The impact of disturbance, described by Pickett and Whyte (1985) as a discrete event along the passage of time that modifies landscape, ecosystem, community and population structure, on the structure and composition of various grassland communities has been observed by various workers (Friedel 1997; Wilsey and Polley 2003), with over-exploitation of species and degradation of natural habitats reported to be the major threats to plant species. While excessive removal of plants from the wild, loss of habitat by deforestation and heavy grazing pressure in

Correspondence: R.P. Shukla, Plant Ecology Laboratory, Department of Botany, D.D.U. Gorakhpur University, Gorakhpur – 273-009, India.
E-mail: drrpshukla@rediffmail.com

pastures generally threaten the survival of species (Nayar and Sastry 1988), habitat specificity, narrow range of distribution, land use change, introduction of non-native species, habitat fragmentation and degradation of populations, population bottlenecks and genetic drift can also play a part (Weekley and Race 2001; Oostermeijer et al. 2003; Kala 2005).

Many authors have identified and classified the common and rare species of trees and shrubs (Hubbell and Foster 1986; Rabinowitz et al. 1986; Pitman et al. 1999) and emphasized the importance of this process for the conservation of biodiversity in the ecosystem. Information about the frequency of herbaceous plant species, however, is scarce. Daniels et al. (1995) suggested that endemism, elevation, vegetation, habitat and microhabitat specialization influence the relative abundance of plant species, while Pitman et al. (1999) suggest that herbaceous species have smaller geographical ranges than trees. Recently, Magurran and Henderson (2003) and Ulrich and Ollik (2004) proposed the use of composite models to study species abundance to improve ecological understanding of community structuring between common and rare species and predicted high rates of local extinction of rare species.

While a number of workers have studied various phyto-sociological characteristics of grassland communities in different parts of India (Bharucha and Ferreria 1941; Gupta and Sharma 1973) as well as community composition and productivity patterns (Dabadghao and Shankarnarayan 1973; Singh and Yadava 1974; Nautiyal et al. 1997), the so-called *Terai* grasslands of the plains of northeastern Uttar Pradesh (U.P.) have received little attention, especially in terms of diversity patterns and the loss and gain of species in recent times. They are a very important source of fodder for livestock and represent about 1,280 km^2 of the total regional area (Semwal 2005).

The present study was conducted to examine the composition, diversity and frequency patterns of plant species in the regional grassy landscape across the tract between the Sarju River and the foothills of the Himalayas. This region is known to be floristically rich with considerable habitat diversity (Ansari et al. 2006). The empirical relationship between microclimatic conditions of various sites and diversity patterns of respective plant communities was analyzed. The patterns of rarity within the regional flora have been described by using the database of rare species in India as well as the world (Rabinowitz 1981; Rabinowitz et al. 1986; IUCN 2001) in order to emphasize the need to conserve grassland vegetation and their species.

Materials and Methods

Study area

The *Terai* region is a belt of marshy grasslands, savannas, and forests located south of the outer Himalaya foothills, the Siwalik Hills, and north of the Indo-Gangetic Plain. These plains of northeastern Uttar Pradesh (U.P.) cover 14 districts and occupy 45,760 km^2. While the climax vegetation is forest and some patches of forest still remain, most of the area has been subjected to recurrent disturbance in the form of clearing, grazing, trampling and burning, resulting in agricultural fields and grassy landscapes, with many rivers, rivulets, nallahs, lakes and ponds. Abandoned arable land reverts to grassland as a result of secondary succession and tends to be stable under the influence of biotic disturbances such as fire, grazing and cutting practices. The regional plain slopes gently from northwest to southeast, and the landscape presents a mosaic of plant communities with varying amounts of grasses and forbs of contrasting life-forms. Composition of the grasslands, developed and maintained by various cultural practices, varies primarily according to the type of soil and available moisture within the upper layer.

Climate

The climate of the region is typically tropical monsoonal with 3 distinct seasons, viz. summer (March to mid-June), monsoon (mid-June to mid-October) and winter (mid-October to February). Average annual rainfall is about 1,814 mm for the entire study region, with 87% occurring during the wet summer and monsoon seasons. The number of rainy days per annum is 51 ± 3.2 and mean relative humidity is about 87% in the morning and 74% in the evening. The eastern *Terai* plains receive more rainfall over a longer period and possess much richer plant biodiversity than western and southern districts of the state. Mean maximum temperatures during wet summer, winter and dry summer seasons are 35.2, 27.0 and 39.3 ºC and mean minimum temperatures are 26.2, 12.1 and 24.2 ºC, respectively (based on climatic data for 2000–2005).

Soil

The soil of the region is part of the trans-Sarju Plain and comprises Gangetic alluvium brought down by rivers like Ghaghara, Rapti, Rohin and Gandak from the Himalayas in the north. The texture is sandy loam and pH is about neutral. In the northern area there are a few elevated mounds, locally called *dhus*, which range in size from a few hundred m^2 to 4–5 km^2 and have brown sandy soil.

Vegetation

The growing season extends from mid-June to mid-September, when most species flower and set seed. The general grassland vegetation of northeastern Uttar Pradesh is interspersed with patches of forest, old fields, open pasture, uplands (mounds or *dhus*), lowlands, orchards, playgrounds and human settlements.

Methods

We started this study in June 2011 with a general survey of the vegetation and habitat conditions over a vast stretch of grassy landscape of *Terai* of northeastern Uttar Pradesh, encompassing more than 11 districts and covering about 128,076 ha of a total 36,015 km^2 geographical area (Figure 1). Finally, 31 locations, showing marked differences in habitat conditions, were selected and sampled during August 2011 to March 2014 (Annexes Ia and Ib). Differences in habitat conditions appeared mainly in the degree of light exposure, soil moisture and soil texture as related to topography and disturbance in the form of fire, grazing, cutting and trampling. Twenty 50 x 50 cm quadrats were randomly laid out at each location, giving a total of 620 quadrats across 333 ha of the region.

The population densities of species occupying each quadrat were recorded, and total vegetal area of individuals of each species was measured through the chart-quadrat method. Based on these values, various phytosociological and diversity indices were derived through conventional methods (Mueller-Dombois and Ellenberg 1974; Magurran 2004). The simple indices were: frequency, density, vegetative cover, their relative values and *Importance Value Index* (IVI). Several other indices like *Simpson's Dominance Index* ($Cd = \sum p_i^2$), *Shannon's Diversity Index* ($\bar{H} = -\sum p_i \ln p_i$), *Pielou's Evenness Index* ($E = \bar{H}/\ln S$), *Abundance:Frequency Ratio* (Withford 1948) and *Family Importance Value* (FIV = relative density + relative diversity + relative vegetative cover) were also derived (Mori et al. 1983) (p_i represents the proportional abundance of the *i*th species in the community; S is the species richness; relative diversity of a family is the number of species within the family expressed as a percentage of the total number of species within all families represented in the community). The dominance-diversity curve (species-individual curves or rank-abundance model) was plotted as a log normal distribution model (Magurran 1988).

Figure 1: Map showing the study sites and sampling locations (1−31) in the *Terai* grasslands, northeastern Uttar Pradesh, India.

Identification of common and rare species

Rabinowitz's classification scheme (Rabinowitz 1981; Rabinowitz et al. 1986) was used to categorize each of the threatened taxa into 1 of 7 types. They were defined on the basis of the size of their geographic range (wide vs. narrow), habitat specificity (broad vs. restricted) and population size (large, dominant vs. small, scattered). From the combination of these traits, 8 categories were formed to decide commonness vs. rarity of a given species (Tables 1 and 2). For all species one or more of the following 10 items was allocated as a threat to their survival: 1. urbanization and land development; 2. agricultural activities; 3. logging and associated harvest activities; 4. mining and associated habitat destruction; 5. livestock grazing, especially unsustainable and inappropriate grazing; 6. trampling for recreation, resulting in deleterious changes in community composition; 7. over-harvesting for horticultural use; 8. excessive collection for various purposes like medicine; 9. natural disasters such as catastrophic floods and other less-common or miscellaneous threats like fodder, thatch collection etc.; and 10. invasion by alien species (Srivastava et al. 2014).

Results

The grassy landscape of northeastern Uttar Pradesh is represented by contiguous, small to fairly large patches of grassland vegetation ranging from 1 ha to around 80 ha in size.

Composition of taxa

The grassland ground-layer vegetation was comprised of grasses and sedges, collectively referred to as graminoids, and forbs or non-grass herbs. A total of 287 species was recorded, belonging to 183 genera and 53 families; among them, 27 species and 5 families have not yet been identified. Dicotyledons made up 94.5% of families, 74.1% of genera and 69.7% of species, while the remainder were monocotyledons.

Habit groups

The life-span data of different grassland species showed that about 90% of species were annuals and the remainder were perennials. The percentage of plant species in different habit groups was: herbs 87%, climbers 10% and shrubs 3%. Most species (>61%) were erect annuals, 29% were prostrate annuals and the remainder were perennials. Among climbers, 39% were annual twiners, 32% tendril-bearing and 29% perennial twiners. There was fairly high species diversity (H = 3.74) and quite low dominance (Cd = 0.058). The mean number of species per genus was 1.62, with 5.42 per family. The total density was 811.5 individuals/m^2 and evenness was 0.661.

Species richness

Among the 6 dominant families, Poaceae was the most common (17.2% of species), followed by Cyperaceae (11.2%), Papilionaceae (10.5%), Asteraceae (9.8%), Scrophulariaceae (4.9%) and Euphorbiaceae (3.5%). The remaining 44% of species represented 46 families, with 20 families represented by only 1 species, 3 by 2 species, 2 by 3 species, 8 by 4 species, 2 by 5 species, 1 by 6 species, 2 by 7 species, 2 by 9 species and 6 families by more than 10 species. In terms of genera, Poaceae, Asteraceae and Papilionaceae were dominant and were represented by 38, 19 and 16 genera, respectively.

Table 1. Rarity of plant species based on geographic range, habitat specificity and local population size (after Rabinowitz 1981).

Geographic range	Wide		Narrow	
Habitat specificity	Broad	Restricted	Broad	Restricted
Abundance somewhere, large population	Common	Predictable	Unlikely	Endemics
Abundance everywhere, small population	Sparse		Non-existent	

Table 2. Trait combinations for commonness/rarity of regional rare taxa.

1	Wide geographic range, broad habitat specificity, large population size
2	Wide geographic range, broad habitat specificity, small population size
3	Wide geographic range, restricted habitat specificity, large population size
4	Wide geographic range, restricted habitat specificity, small population size
5	Narrow geographic range, broad habitat specificity, large population size
6	Narrow geographic range, broad habitat specificity, small population size
7	Narrow geographic range, restricted habitat specificity, large population size
8	Narrow geographic range, restricted habitat specificity, small population size

The distribution of common and rare plant species among major families (Figure 2) shows that the foremost families in the regional grasslands shared the maximum number of rare plant species. For instance, Papilionaceae had 5 species, followed by Poaceae and Asteraceae (4 species each) and Lamiaceae with 3 species. Scrophulariaceae, Caesalpiniaceae and Boraginaceae families were represented by 2 species each and 12 families by a single species only. The family Apiaceae is economically and medicinally very important but contains the least number of species in the region. Four families, Lobeliaceae, Martyniaceae, Sphenocleaceae and Zygophyllaceae, had only 1 genus each with a single species, viz. *Lobelia alsinoides*, *Martynia annua*, *Sphenoclea zeylanica* and *Tribulus terrestris*, respectively. The local extinction of these very rare species would mean that the family would no longer be represented in this region.

Abundance distribution

Abundance is a quantitative indication of patchiness of species. Several common herbaceous species showed hyper-dispersion across grassland vegetation as evident from their density and abundance values. *Lindernia diffusa* had maximum average density and abundance. Species like *Desmodium triflorum, Evolvulus nummularis, Imperata cylindrica, Lindernia ciliata* and *Rungia repens* showed abundance values of >20 (Table 3).

The abundance:frequency (A:F) ratio provides a useful measure to show the degree of clumping or patchiness of species in restricted or localized areas. Table 4 shows the pattern of distribution of grassland species on the basis of A:F ratio, which was highest for erect annual herbs, followed by prostrate annual herbs, with the lowest value for prostrate perennial herbs. The erect annual

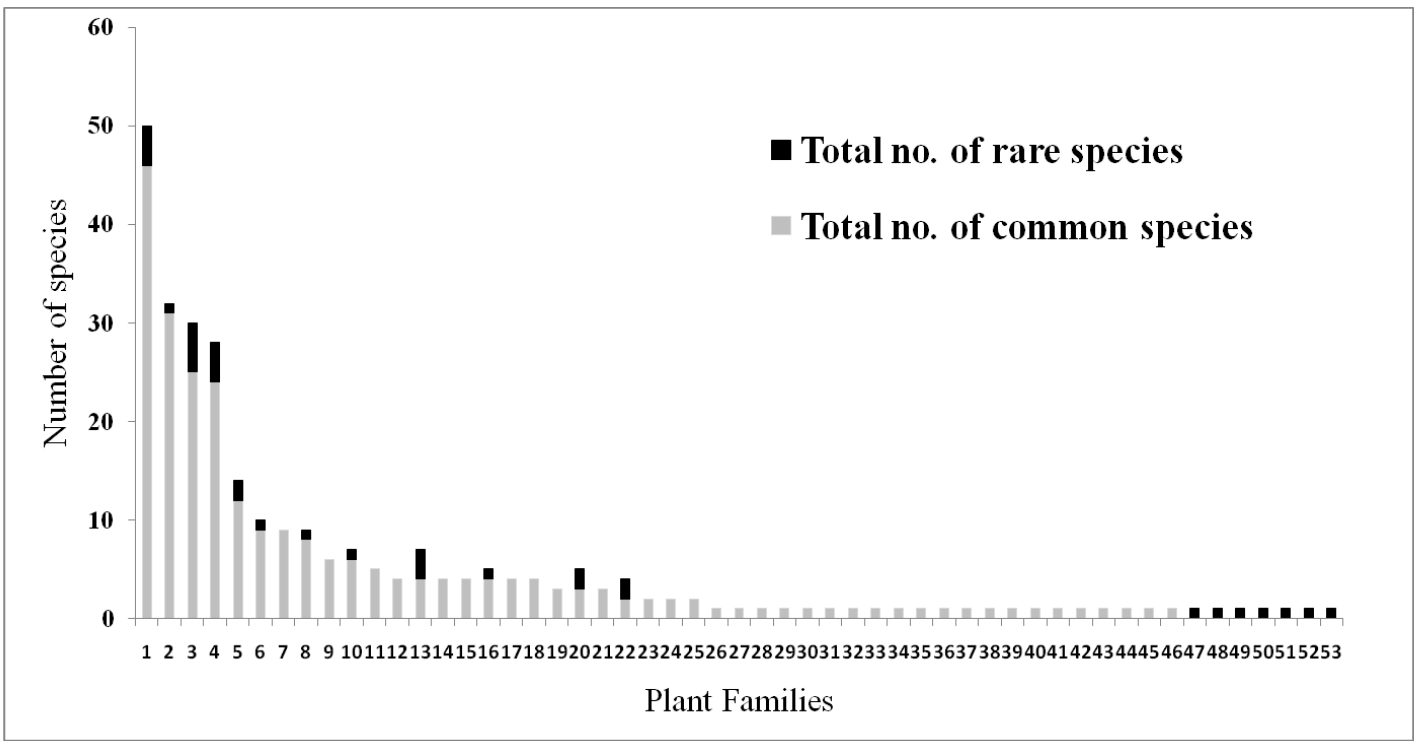


Figure 2. Distribution of plant species among different families of regional grassland vegetation in Uttar Pradesh. The sequence of families is in decreasing order of their species richness. 1. Poaceae, 2. Cyperaceae, 3. Papilionaceae, 4. Asteraceae, 5. Scrophulariaceae, 6. Euphorbiaceae, 7. Malvaceae, 8. Amaranthaceae, 9. Acanthaceae, 10. Convolvulaceae, 11. Unknown, 12. Cucurbitaceae, 13. Lamiaceae, 14. Onagraceae, 15. Polygonaceae, 16. Rubiaceae, 17. Tiliaceae, 18. Verbenaceae, 19. Asclepiadaceae, 20. Caesalpiniaceae, 21. Menispermaceae, 22. Boraginaceae, 23. Cuscutaceae, 24. Lythraceae, 25. Molluginaceae, 26. Aizoaceae, 27. Apocynaceae, 28. Basellaceae, 29. Bignoniaceae, 30. Cannabinaceae, 31. Capparidaceae, 32. Chenopodiaceae, 33. Commelinaceae, 34. Fumariaceae, 35. Moraceae, 36. Nyctanginaceae, 37. Oxalidaceae, 38. Papaveraceae, 39. Polygalaceae, 40. Portulacaceae, 41. Primulaceae, 42. Ranunculaceae, 43. Solanaceae, 44. Sterculiaceae, 45. Urticaceae, 46.Vitaceae, 47. Amaryllidaceae, 48. Apiaceae, 49. Lobeliaceae, 50. Martyniaceae, 51. Sphenocleaceae, 52. Violaceae, 53. Zygophyllaceae.

Table 3. Species showing maximum aggregation and hyper-dispersion (as evident from their density and abundance values) across the grassy landscape of northeastern Uttar Pradesh. (Based on 620 quadrats each of 0.25 m^2 size, laid across 11 districts.)

Species	Density	Abundance
Desmodium triflorum	58.2	20.5
Evolvulus nummularis	50.4	22.2
Imperata cylindrica	19.6	28.9
Lindernia ciliata	56.5	31.7
Lindernia diffusa	143.8	60.7
Rungia repens	62.6	28.8

Table 4. The range of abundance:frequency (A:F) ratio for species in different posture or growth habit groups across the grassy landscape of northeastern Uttar Pradesh.

Posture/Habit	Range of A:F ratio
Herbs	
Erect annuals	18.6–0.05
Prostrate annuals	17.2–0.20
Erect perennials	2.33–0.22
Prostrate perennials	0.90–0.14
Climbers	
Tendril annuals	3.37–0.59
Twining annuals	6.2–0.53
Twining perennials	6.2–0.27
Shrubs	
Erect annuals	2.23–0.16
Erect perennials	1.94–0.18

herbaceous species with A:F>10 were *Hemarthria compressa*, *Fimbristylis dichotoma*, *Cynoglossum lanceolatum* and *Zephyranthus citrina*. The lowest A:F ratio was shown by *Euphorbia hirta*.

Dominance-diversity curve

The dominance-diversity curves for the regional grassy landscape as an abstract community showed a log-normal distribution of individuals among constituent species (Figure 3). *Lindernia diffusa*, *Rungia repens*, *Desmodium triflorum* and *Lindernia ciliata*, had the highest number of individuals and formed the top end of the curve. On the other hand, the species which formed the tail end of the curve were *Amaranthus spinosus*, *Crotalaria retusa*, *Crotalaria pallida*, *Ludwigia adscendens* and *Solanum virginianum*. The latter species obviously showed rare occurrences.

Population status

Common species have a large range, wide habitat specificity and large populations. However, a few species have a large range and occur in a wide array of habitats, but in chronically small populations, and are considered quite rare (Rabinowitz et al. 1986). In our observations, a number of plant species, which had a large range and wide habitat specificity, apparently showed small populations due to sampling limitations. As these species

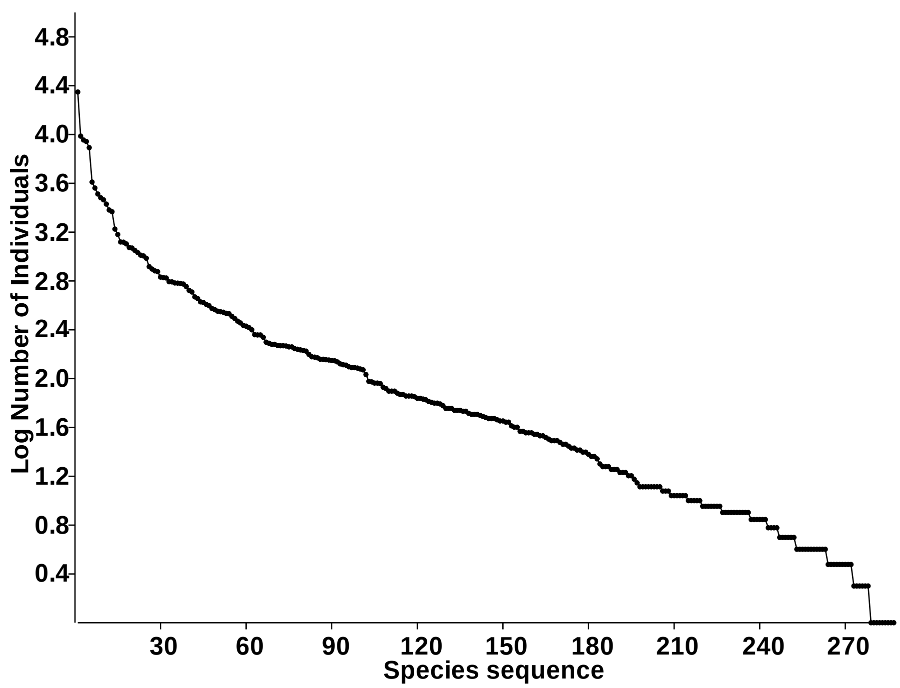


Figure 3. Rank-abundance model (dominance-diversity curve) of the grassy landscape of northeastern Uttar Pradesh.

Table 5. Number and status of rare and common plant species with different trait combinations across the grassy landscape vegetation of northeastern Uttar Pradesh.

Trait combinations	No.	Species	Status
Wide geographic range, broad habitat specificity, large population size	150	*Cynodon dactylon, Desmodium triflorum, Evolvulus nummularis, Imperata cylindrica, Lindernia ciliata, Lindernia diffusa, Rungia repens* and 143 more species	Common
Wide geographic range, broad habitat specificity, small population size	104	*Glinus oppositifolius, Dentella repens, Crotalaria prostrata, Blumea lacera, Ammania baccifera, Cissampelos pariera* and 98 more species	Common
Wide geographic range, restricted habitat specificity, large population size	2	*Centella asiatica, Evolvulus alsinoides*	Rare
Wide geographic range, restricted habitat specificity, small population size	2	*Euphorbia thymifolia, Hybanthus linearifolius*	Rare
Narrow geographic range, broad habitat specificity, small population size	2	*Leucas cephalotus, Leucas aspera*	Rare
Narrow geographic range, restricted habitat specificity, small population size	15	*Baccopa monnieri, Chamaecrista absus, Cyperus niveus, Eragrostis capensis, Eragrostis cilianensis, Heliotropium ovalifolium, Lobelia alsinoides, Martynia annua, Cullen corylifolium, Sphenoclea zeylanica, Cajanus scarabaeoides, Teramnus labialis, Tribulus terrestris, Vernonia* sp., *Zephyranthes citrina*	Rare
Narrow geographic range, broad habitat specificity, large population size	1	*Hygrophila auriculata*	Rare
Narrow geographic range, restricted habitat specificity, large population size	11	*Alternanthera pungens, Alysicarpus bupleurifolius, Senna pumila, Chrysanthellum indicum, Crotalaria calycina, Crotalaria pallida, Cynoglossum lanceolatum, Hemarthria compressa, Heteropogon contortus, Perotis indica, Spermacoce pusilla*	Rare

often showed vigorous growth in small patches, distributed across the whole of the range, they could not be considered rare, though their observed traits suggested so. Such an intriguing situation may be common-place in judging the population size of a species as determined by the limitations of method and extent of sampling. Thus, 6 of 8 combinations represent rare and 2 represent common species as evident through this study. Of the total 287 species, 150 showed large range, wide habitat specificity and large populations and 104 species showed large range, wide habitat specificity and small populations (Annex II). Both groups were considered common. The remaining 33 species within 6 different combinations were considered rare (Table 5). Populations of species with habit groups facing a combination of threats have declined within the region (Tables 6 and 7). Thirty-three species showed a maximum of 10 individuals per quadrat and were encountered in <1% of total quadrats observed.

Chrysanthellum indicum, *Evolvulus alsinoides* and *Spermacoce pusilla*, however, were recorded at only 1 of the 11 sites but there were numerous individuals at this site. Almost all species were exposed to the threats of urbanization and land development, habitat destruction, intense agricultural practices, unmanaged livestock grazing, trampling and other miscellaneous threats. The populations of some of the highly medicinal plants, such as *Hygrophila auriculata*, *Bacopa monnieri*, *Centella asiatica*, *Chrysanthellum indicum*, *Hybanthus linearifolius*, *Euphorbia thymifolia*, *Evolvulus alsinoides*, *Leucas aspera*, *L. cephalotus*, *Vernonia* sp. and *Tribulus*

Table 6. Types of threat reducing populations of various plant species to rarity and local extinction across grassy landscapes of northeastern Uttar Pradesh.

No.	Threat type
1	Urbanization and land development
2	Agricultural activities
3	Logging and associated harvest activities
4	Mining and associated habitat destruction
5	Livestock grazing, especially unsustainable and inappropriate grazing
6	Trampling by stock and humans
7	Over-harvesting for horticultural use
8	Excessive collection for various purposes like medicine and fodder
9	Natural disasters such as catastrophic floods and other miscellaneous threats
10	Invasion by alien plants

Table 7. Species by habit group facing combinations of threats (numbered as above**)** which have declined to the level of rarity within the regional grassy landscape in northeastern Uttar Pradesh.

Species	Habit	Combination of threats
Alternanthera pungens	Annual herb	1+4+6+8
Sphenoclea zeylanica	Annual herb	1+4+5+6
Alysicarpus bupleurifolius	Annual herb	1+4+5+6+8
Hygrophila auriculata	Perennial herb	1+2+4+8
Centella asiatica	Perennial herb	1+2+4+7+8+10
Tribulus terrestris	Annual herb	1+2+4+6+7+8+9
Eragrostis capensis, Eragrostis cilianensis, Hemarthria compressa, Zephyranthes citrina	Annual herb	1+2+4+5+6+8+9
Heliotropium ovalifolium, Lobelia alsinoides	Annual herb	1+2+4+5+6+8
Bacopa monnieri	Perennial herb	1+2+4+5+7+8+10
Leucas aspera, Leucas cephalotus	Annual herb	1+2+6+8+9
Chamaecrista absus	Annual herb	1+2+5+6+9
Euphorbia thymifolia	Annual herb	1+2+4+8+9
Vernonia sp.	Annual herb	1+2+3+4+5+8+9
Cajanus scarabaeoides, Crotalaria calycina, Crotalaria pallida, Cullen corylifolium, Cynoglossum lanceolatum, Cyperus niveus, Martynia annua, Perotis indica, Senna pumila, Spermacoce pusilla	Annual herb	1+2+4+5+8+9+10
Heteropogon contortus	Perennial herb	1+2+4+5+8+9+10
Teramnus labialis	Annual herb	1+2+4+5+8+9+10
Chrysanthellum indicum, Evolvulus alsinoides, Hybanthus linearifolius	Annual herb	1+2+3+4+6+7+8+9

terrestris have suffered seriously owing to voracious harvesting from the wild by untrained people. Some rare species of annual upland legumes, such as *Alysicarpus bupleurifolius, Alysicarpus longifolius, Senna pumila, Crotalaria calycina*, *Crotalaria pallida* and *Cullen corylifolium,* require a relatively longer time to produce seeds to complete their annual life cycle as compared with several non-legumes. The period of peak seed fall is often closely followed by high rainfall and moderate water-logging for about a week, and long submergence of fresh seeds may destroy the embryos, rendering them non-viable. On the other hand, the very rare and poor occurrences of some lowland therophytes, such as *Cyperus niveus, Lindernia pyxidaria, Lobelia alsinoides* and *Sphenoclea zeylanica*, may be attributed to severe overgrazing just before full blooming and the seed-setting stage. The second most important threat was invasive alien species. *Centella asiatica, Baccopa monnieri* and *Hygrophila auriculata* showed very poor populations under these conditions despite efficient modes of non-seed regeneration (Table 8), as invaders have a competitive advantage and readily suppress and replace the native species under stressful environments. Table 9 shows the specific habitats for rare plant species.

Table 8. Mode of regeneration and sprouting efficiency of different rare and most common (with asterisk) plant species of grassy landscapes of northeastern Uttar Pradesh.

Mode of regeneration	Species
Seed only	*Alysicarpus bupleurifolius, Chrysanthellum indicum, Crotalaria calycina, Crotalaria pallida, Cullen corylifolium, Cynoglossum lanceolatum, Cyperus niveus, Eragrostis capensis, Eragrostis cilianensis, Euphorbia thymifolia, Hemarthria compressa, Hybanthus linearifolius, Leucas aspera, Leucas cephalotus, Lindernia ciliata*, Lindernia diffusa*, Lobelia alsinoides, Martynia annua, Perotis indica, Rungia repens*, Senna pumila, Spermacoce pusilla, Sphenoclea zeylanica, Teramnus labialis, Tribulus terrestris*
Seed + Sprout	*Alternanthera pungens, Cajanus scarabaeoides, Heliotropium ovalifolium, Hygrophila auriculata*
Seed + Ramet	*Bacopa monnieri, Centella asiatica, Cynodon dactylon*, Evolvulus nummularis**
Seed + Rhizome + Storage roots	*Heteropogon contortus, Imperata cylindrica*, Zephyranthes citrina*
Seed + Sprout + Ramet	*Desmodium triflorum**

Table 9. Habitat specificity of rare plant species across the regional grassland vegetation of northeastern Uttar Pradesh. *Light:* O = Open, PS = Partial shade; *Soil moisture*: HM = High moisture, LM = Low moisture, AM = Average moisture; and *Soil texture*: S = Sandy, G = Gravel, C = Clay, CL = Clay-loam, SL = Sandy loam.

No.	Species	Family	Habitat specificity factors		
			Light	Soil moisture	Soil texture
1	*Alternanthera pungens*	Amaranthaceae	O	LM	G
2	*Alysicarpus bupleurifolius*	Papilionaceae	O	AM	C/CL
3	*Baccopa monnieri*	Scrophulariaceae	O	HM	C
4	*Cajanus scarabaeoides*	Papilionaceae	O	AM	CL
5	*Centella asiatica*	Apiaceae	O	HM	C
6	*Chamaecrista absus*	Caesalpiniaceae	PS	AM	CL
7	*Chrysanthellum indicum*	Asteraceae	O	AM	S/SL
8	*Crotalaria calycina*	Papilionaceae	O	LM	SL
9	*Crotalaria pallida*	Papilionaceae	O	LM	SL
10	*Cullen corylifolium*	Papilionaceae	PS	AM	SL
11	*Cynoglossum lanceolatum*	Boraginaceae	O	LM	SL
12	*Cyperus niveus*	Cyperaceae	O	AM	SL
13	*Eragrostis capensis*	Poaceae	O	AM	SL
14	*Eragrostis cilianensis*	Poaceae	O	AM	C/CL
15	*Euphorbia thymifolia*	Euphorbiaceae	O	LM	SL/G
16	*Evolvulus alsinoides*	Convolvulaceae	O	AM	SL/S
17	*Heliotropium ovalifolium*	Boraginaceae	O	HM	C
18	*Hemarthria compressa*	Poaceae	O	HM	C
19	*Heteropogon contortus*	Poaceae	O	LM	SL
20	*Hybanthus linearifolius*	Violaceae	O/PS	AM	SL/CL
21	*Hygrophila auriculata*	Lamiaceae	O	HM	C
22	*Leucas aspera*	Lamiaceae	O	LM	SL
23	*Leucas cephalotus*	Lamiaceae	O	AM	SL
24	*Lobelia alsinoides*	Lobeliaceae	PS	HM	CL
25	*Martynia annua*	Martyniaceae	O	LM	CL
26	*Perotis indica*	Poaceae	O	LM/AM	SL
27	*Senna pumila*	Caesalpiniaceae	PS	AM	CL
28	*Spermacoce pusilla*	Rubiaceae	O	AM	SL
29	*Sphenoclea zeylanica*	Sphenocleaceae	O	HM	C
30	*Teramnus labialis*	Papilionaceae	PS	AM	G
31	*Tribulus terrestris*	Zygophyllaceae	O	LM	S
32	*Vernonia* sp.	Asteraceae	O	LM	SL
33	*Zephyranthes citrina*	Amaryllidaceae	O/PS	AM	G & C

Discussion

Community structure and diversity pattern

This study shows that the *Terai* landscape contains very diverse assemblages and associations relative to semi-arid grasslands in other parts of India (Pandeya 1964; Dabadghao and Shankaranarayan 1973), but owing to continued alteration of the habitats through anthropogenic disturbance, the resulting landscape is gradually losing a number of plant species, which can flourish only in a narrow habitat range. The very little similarity and drastic differences among grassland patches, in terms of species richness and abundance, are probably due to the severity of a range of factors, including clipping, grazing, trampling, habitat fragmentation, water-logging, mining and transportation of soil. Singh and Joshi (1979) considered that high numbers of associations in hygrophilous grasslands could be due to different intensities of anthropogenic disturbance plus local variations in topography and soil depth. Locations found on elevated mounds or *'dhus'* supported a diverse assemblage of grasses and palatable herbs despite regular livestock grazing.

The grasslands in the study area showed much higher species richness than other vegetation types of the region, i.e. almost 3 times higher than that in adjacent forests (Pandey and Shukla 2003). Similar observations have been made in the protected grassland of Dudhwa National Park in *Terai* of Uttar Pradesh (Mathur et al. 2003). The reason for higher species diversity could be the variation in micro-habitat features and occurrence of a number of associations of grass species in the *Terai* grasslands (Shukla 2009).

Our analysis showed that annual herbs contributed most towards different phyto-sociological indices. While most herbaceous plant species can readily establish themselves, quickly produce herbage cover and improve soil fertility (Graham 1941), those which regenerate from seeds are less likely to persist in communities facing recurrent grazing and trampling, although forbs respond positively to disturbance (Belsky 1986). Prostrate perennial species, like *Cynodon dactylon, Desmodium triflorum, Evolvulus nummularis* and *Rungia repens,* showed marked dominance (density and IVI) across the region. They readily occupied the horizontal space created by disturbance through grazing and clipping, and often showed high seed production as well as ramet proliferation, even at sites facing regular disturbance. Multiple types of reproductive strategy can result in the dominance of species over an area (Harper and White 1974; Patrica et al. 2002). Moderate grazing also promotes rapid colonization of newly available space by these species, mainly through vegetative means.

The higher the abundance:frequency (A:F) ratio, the greater will be the tendency of species to clump, and vice-versa. Low values of both the abundance and frequency indicate rare occurrence of species, while very high values indicate dominance of species. A:F ratios of the most common prostrate perennial herbs like *Desmodium triflorum* and *Evolvulus nummularis*, were not high because both the abundance and frequency values of these species were quite high. The pattern of species abundance has been related to both growth pattern and habitat factors (Varghese and Menon 1999). Diversity is often considered to be a synthetic measure of a combination of structure, complexity and stability of a community (Hubbell and Foster 1983). Species distribution in a community is often non-random with dominant species being widely distributed, while subordinate species are generally locally distributed (Kolasa 1989). A moderate level of disturbance is, therefore, compatible with the maintenance of high biodiversity in the landscape. On the other hand, more severe disturbance through regular clipping causes greater dominance and low diversity, and the species of prostrate habit dominate the local communities (Gentry 1991).

The commonness between any two adjacent patches is proportional to the extent of their contact or boundary length (Cole and Hobbs 1994). Most of the adjacent patches of grassy landscape showed a number of common species, under genera like *Lindernia, Phyllanthus, Oldenlandia* and *Heliotropium*. The distribution of grassland species was largely random but several were also found locally aggregated on different spatial scales. A number of erect, prostrate and climber species showed aggregation in the form of compact to loose patches. Aggregation occurred due to either localized seed fall or vigorous vegetative proliferation through ramets. Pacala (1997) also reported that local or intra-specific aggregation is generated by limited seed dispersal, clonal growth and patchy environments.

The resource sharing and niche occupancy of species is frequently expressed by dominance-diversity curves (Whittaker 1975). Conditions like moderate grazing and reduced clipping and trampling allowed relatively greater numbers of species to share community resources, thus reducing the degree of dominance at the community level. A less steep and more flattened curve has also been reported by Raizada et al. (1998). However, some species depicted as rare by the curve, through quadrat sampling, were actually not so rare. They occurred in small patches but only on specified habitats with respect to soil moisture.

Disturbance may have positive effects on some species as reported earlier (Sundriyal et al. 1987) and that caused by herbivores may reduce the effect of competition (Grace and Jutila 1999). In comparison with other species in exposed communities, erect herbaceous species were dominant in terms of RD, RVC and IVI, especially in situations with low moisture and moderate disturbance. *Lindernia diffusa* occurred frequently and its dominance across the region may be due to frequent clipping and grazing. The periodic clipping inhibited the establishment of most of the upper strata species and promoted dominance of only a few prostrate species like *Rungia repens* and *Desmodium triflorum.*

Commonness of plant species

Forbs produce numerous fruits and seeds per plant. The seeds are usually small and often possess very hard seed coats, which can easily escape damage from trampling and digestion within the guts of birds, cattle and other animals. *Evolvulus nummularis* and *Desmodium triflorum* also regenerated efficiently through ramets and sprouts in addition to seeds. A few species like *Mukia maderaspatana*, *Ranunculus scleratus*, *Ruellia tuberosa* and *Soliva anthemifolia* were found in small loose patches with a good number of prostrate neighbors. Other species like *Boerhavia diffusa* and *Clerodendrum indicum* could directly build up a large phytomass from their root-stock and rhizome systems. Their dense vegetative cover suppresses the subordinate species and thus promotes the homogeneity of the community (Armesto and Pickett 1985).

Trampling had significant effects on the occurrence of some species. *Croton bonplandianum, Parthenium hysterophorus* and *Acalypha indica* were more common in areas facing frequent trampling, while species like *Tridax procumbens, Cyanthillium cinereum, Alysicarpus monilifer* and *Murdannia nudiflora* occurred when clipping and trampling were only occasional. With increased frequency of trampling and clipping, the occurrence of *Achyranthes aspera, Amaranthus viridis, Ruellia tuberosa* and *Gomphrena globosa* markedly increased. The combination of heavy trampling, grazing and clipping provided extreme conditions of biotic disturbance. Grasses, notably *Setaria glauca, Eragrostis amabilis* and *Cyperus rotundus*, and a few leguminous forbs, e.g. *Desmodium triflorum* and *Alysicarpus monilifer*, coped with such composite disturbance. About 10% of the common species showed ramet proliferation, while others displayed mass seed germination at suitable sites. Some prostrate species aggregated to form compact mats, which allowed few associates and low species richness of local communities. Erect species, on the other hand, allowed significant numbers of species among their aggregations. A number of new entrants produced propagules irrespective of their vitality level to the community. The dense vegetal cover of *Hyptis suaveolens* and *Parthenium hysterophorus* suppressed the growth of other species as evident at locality 8 (Marimata campus). Since these species were unpalatable and faced no clipping and little trampling owing to their height, they showed significant local dominance (Tripathi and Shukla 2007).

Rarity of plant species

A number of species, which showed low tolerance of disturbance and were totally absent from such sites (Table 6), occurred only in few localities and preferred sandy to loamy soils. Species such as *Centella asiatica* and *Hygrophila auriculata*, earlier frequent in or near water-logged areas, have also become quite rare during recent decades. On the other hand, a sizable number of species, which occupy quite specialized habitats, showed small population sizes within narrow distributional ranges (Table 6). Chiefly because of their medicinal importance, they have been over-exploited and are on the verge of local extinction. The population density of *Chamaecrista absus*, *Cyperus niveus*, *Leucas cephalotus*, *Leucas aspera*, *Lobelia alsinoides*, *Martynia annua* and *Cullen corylifolium* has declined markedly during the last few years. This indicates their specialized habitat requirements (Tripathi 1999). The land-use changes plus mining and transportation of soil for filling and construction purposes appear to be important threats. Voracious and unmanaged harvesting presents a major threat to the persistence of several valuable plant species of the region (Shukla 2009). Hubbell and Foster (1986) found that most rare species are specialists in terms of habitat or appropriate niches for regeneration. The fast expansion of townships and associated disturbances during the current era have caused major reduction in availability of specialized niches in the region.

The spread of viable seeds from parent plants can be restricted due to the absence of dispersal vectors, especially grazers. Several authors have highlighted the importance of migrating sheep and cattle for plant dispersal (Fischer et al. 1996; Poschlod et al. 1998). Although the fruits of *Tribulus terrestris* are dispersed far and wide through a hooking device, which attaches to the skin of cattle, the population of this species has shrunk rapidly during the last decade. A contributing factor could be the unavailability of suitable micro-sites for germination and growth of seeds. *Crotalaria calycina* and *Hybanthus linearifolius* are examples of species now classified as rare, as their population size has declined significantly during the last decade, despite production of sufficient viable seeds/fruits. These species prefer shaded and damp habitats around orchards, and availability of such habitats has declined markedly, mainly through increased urbanization and agricultural expansion. Metzger (2000) suggests that shade-tolerant species are more sensitive to habitat fragmentation than shade-intolerant species.

Species like *Hygrophila auriculata*, *Bacopa monnieri*, *Centella asiatica* and *Heliotropium ovalifolium* grow mostly on clayey soil subjected to some degree of water-logging. Currently, they have become infrequent across water-logged or lowland sites, despite their two-pronged regeneration strategy, i.e. through seeds as well as sprouts and ramets. With improvement of technologies and population explosion, humans have a major role in escalating extinction rates (Hopping et al. 2004). Species populations which are small in size and with restricted habitat requirements are more prone to extinction (Menges 1998; Butaye et al. 2005).

It was quite striking that some very rare species in the regional grassland were the only representatives of a particular genus, which, in turn, represented a single family. For example, Lobeliaceae, Martyniaceae, Sphenocleaceae and Zygophyllaceae were represented by *Lobelia alsinoides*, *Martynia annua*, *Sphenoclea zeylanica* and *Tribulus terrestris*, respectively. The loss of these single species would mean the loss or non-representation of a whole family. These species may be passing through their lag time, as often there is a lag time between when the species habitat is lost and when the species actually becomes extinct (Hopping et al. 2004). A great amount of evolutionary history and biological distinctiveness is lost when the last species of an entire genus or family becomes extinct (Kareiva and Marvier 2003). Levin and Levin (2004) strongly suggest concentrating efforts on saving such families and higher levels of taxonomic groups.

The survival and growth of several plant species into large populations indicated that habitat quality was not always worse for all species, especially invaders, which form one of the major threats to the survival and growth of several native species (Sekar 2012). The invaders colonize and successfully out-compete the native species, especially in open habitats with reduced competition, often created by grazing and man-made disturbance (Wu et al. 2004; Huang et al. 2009). The introduction and spread of invasive aliens may be facilitated by floods, changes in land-use pattern and environmental conditions caused by livestock grazing, timber harvest, agriculture and ornamental purposes (Srivastava et al. 2015). These alien species have the potential to damage or eliminate already feeble populations of rare taxa (Menges 1991).

Conservation strategy of grasses

Availability of viable seed is important for conservation of biodiversity and may determine the composition, structure and dynamics of present and future vegetation in different ecosystems. Grazing pressure in the *Terai* region is very intense and much of the seed-bearing parts of plants are removed. Further, developmental activities often create severe soil disturbance, and compaction and erosion have adverse impacts on the survival of grassland species (Godefroid and Koeda 2004a; 2004b). Disturbance complicates the relationship and may increase species richness by lowering dominance and presenting opportunities for some species to spread rapidly (Whitmore 1996; Gusson et al. 2009). The management of biodiversity at landscape levels involves the twin objectives of preservation and sustainable use of natural habitats.

Conclusion

The present data on abundance distribution and diversity pattern of plant species suggest that the acute fragmentation of the natural habitat of northeastern *Terai* has resulted in limited distribution of once very common and widely spread species. Fast shrinkage of grassland habitats due to urbanization, agricultural expansion, grazing, trampling, fire and the mining and transportation of soil for the brick industry and site filling have pushed many less-common but valuable plant species towards rarity and several of them face local extinction. It appears that many valuable species will be lost from these grasslands, if the current practices in the community are allowed to continue unchecked. The present observations emphasize the urgent need for studies on the status of existing rare plant species and identification of the most detrimental cultural practices threatening their survival before some important species are lost from these plant communities.

Acknowledgments

The authors thank Jeremy Russell-Smith for his meticulous correction and thorough review and also to an anonymous reviewer for his critical comments. The Agriculture Research Centre, Bahraich, U.P. provided free facility to analyze soil samples. The authors are also thankful to the Head, Department of Botany, D.D.U. Gorakhpur University, Gorakhpur, India for providing access to the departmental herbarium and other required facilities.

References

Ansari AA; Singh SK; Srivastava RC. 2006. Flora and vegetation of Madhaulia forest (U.P.). Oriental Enterprises, Dehradun, Uttarakhand, India.

Armesto JJ; Pickett STA. 1985. Experiments on disturbance in old-field plant communities: Impact on species richness and abundance. Ecology 66:230–240. DOI: 10.2307/1941323

Belsky AJ. 1986. Revegetation of artificial disturbances in grasslands of the Serengeti National Park, Tanzania: II. Five years of successional change. Journal of Ecology 74:937–951. DOI: 10.2307/2260225

Bharucha FR; Ferreria DB. 1941. The biological spectra of the Matheran and Mahabaleshwar flora. Journal of the Indian Botanical Society 20:195–211.

Butaye J; Adriaens D; Honnay O. 2005. Conservation and restoration of calcareous grasslands: A concise review of the effects of fragmentation and management on plant species. Biotechnology, Agronomy, Society and Environment 9:111–118. http://goo.gl/vulcsa

Cole PG; Hobbs RJ. 1994. Landscape heterogeneity indices: Problems of scale and applicability, with particular reference to animal habitat description. Pacific Conservation Biology 1:183–193. DOI: 10.1071/PC940183

Dabadghao PM; Shankarnarayan KA. 1973. The grass cover of India. Indian Council of Agricultural Research, New Delhi, India.

Daniels RJ; Kumar AN; Jayanthi M. 1995. Endemic, rare and threatened flowering plants of South India. Current Science 68:493–495. http://goo.gl/1siFnz

Fischer SF; Poschlod P; Beinlich B. 1996. Experimental studies on the dispersal of plants and animals on sheep in calcareous grasslands. Journal of Applied Ecology 33:1206–1222. DOI: 10.2307/2404699

Friedel MH. 1997. Discontinuous change in arid woodland and grassland vegetation along gradients of cattle grazing in central Australia. Journal of Arid Environment 37:145–164. DOI: 10.1006/jare.1997.0260

Gentry AH. 1991. The distribution and evolution of climbing plants. In: Putz FE; Mooney HA, eds. The biology of vines. Cambridge University Press, Cambridge, UK. p. 3–42.

Godefroid S; Koeda MN. 2004a. Interspecific variation in soil compaction sensitivity among forest floor species. Biological Conservation 119:207–217. DOI: 10.1016/j.biocon.2003.11.009

Godefroid S; Koeda MN. 2004b. The impact of forest paths upon adjacent vegetation: Effects of the path surfacing material on the species composition and soil compaction. Biological Conservation 119:405–419. DOI: 10.1016/j.biocon.2004.01.003

Grace JB; Jutila H. 1999. The relationship between species density and community biomass in grazed and ungrazed coastal meadows. Oikos 85:398–408. DOI: 10.2307/3546689

Graham EH. 1941. Legumes for erosion control and wildlife. USDA Miscellaneous Publication 412, US Department of Agriculture, Washington, DC, USA.

Grime JP. 1979. Plant strategies and vegetation processes. John Wiley & Sons Ltd., Chichester, UK.

Gupta RK; Sharma SK. 1973. Phytosociological changes in an enclosed area of Jodhpur. Journal of the Indian Botanical Society 52:1–11.

Gusson AE; Lopes SF; Dias Neto OC; Vale VS do; Oliveira AP de; Schiavini I. 2009. Características químicas do solo e estrutura de um fragmento de floresta estacional semidecidual em Ipiaçu, Minas Gerais, Brasil. Rodriguésia 60:403–414. http://www.jstor.org/stable/23499997

Harper JL; White J. 1974. The demography of plants. Annual Review of Ecology and Systematics 5:419–463. DOI: 10.1146/annurev.es.05.110174.002223

Hopping I; Mackro D; Delson L. 2004. Human effects on terrestrial biodiversity. The Traprock 3:1–5.

Huang QQ; Wu JM; Bai YY; Zhou L; Wang GX. 2009. Identifying the most noxious invasive plants in China: Role of geographical origin, life form and means of introduction. Biodiversity and Conservation 18:305–316. DOI: 10.1007/s10531-008-9485-2

Hubbell SP; Foster RB. 1983. Diversity of canopy trees in neo-tropical forest and implications to conservation. In: Sutton L; Whitmore TC; Chadwick C, eds. Tropical rain forest: Ecology and management. Blackwell Scientific Publications, Oxford, UK. p. 25–41.

Hubbell SP; Foster RB. 1986. Commonness and rarity in a neotropical rain forest: Implications for tropical tree conservation. In: Soulé ME, ed. Conservation Biology: The science of scarcity and diversity. Sinauer Associates, Sunderland, MA, USA. p. 205–231.

IUCN. 2001. IUCN Red List categories and criteria: Version 3.1. IUCN Species Survival Commission. International Union for Conservation of Nature, Gland, Switzerland and Cambridge, UK.

Kala CP. 2005. Indigenous uses, population density, and conservation of threatened medicinal plants in protected areas of the Indian Himalayas. Conservation Biology 19:368–378. DOI: 10.1111/j.1523-1739.2005.00602.x

Kareiva P; Marvier M. 2003. Conserving biodiversity cold spots. American Scientist 91:344–351. DOI: 10.1511/2003.4.344

Kolasa J. 1989. Ecological systems in hierarchical perspective: Breaks in community structure and other consequences. Ecology 70:36–47. DOI: 10.2307/1938410

Levin PS; Levin DA. 2004. The real biodiversity crisis. American Scientist 90:6–8. DOI: 10.1511/2002.1.6

Magurran AE. 1988. Ecological diversity and its measurement. University Press, Cambridge, UK.

Magurran AE. 2004. Measuring biological diversity. Blackwell Publishing, Oxford, UK.

Magurran AE; Henderson PA. 2003. Explaining the excess of rare species in natural species abundance distributions. Nature 422:714–716. DOI: 10.1038/nature01547

Mathur PK; Kumar H; Lehmkuhl JF. 2003. Terai grassland diversity, management and conservation perspectives. ENVIS Bulletin Grassland, Ecosystems and Agroforestry 1:1–28.

Menges ES. 1991. The application of minimum viable population theory to plants. In: Falk DA; Holsinger KE, eds. Genetics and conservation of rare plants. Oxford University Press, Oxford, UK. p. 47–61.

Menges ES. 1998. Evaluating extinction risks in plant populations. In: Fiedler PL; Kareiva PM, eds. Conservation biology for the coming decade. Chapman and Hall, New York, USA and London, UK. p. 49–65.

Metzger JP. 2000. Tree functional group richness and landscape structure in a Brazilian tropical fragmented landscape. Ecological Applications 10:1147–1161. DOI: 10. 1890/1051-0761(2000)010%5B1147:tfgral%5D2.0.co;2

Mori SA; Boom BM; Carvalino AM de; Santos TS dos. 1983. Ecological importance of Myrtaceae in an eastern Brazilian wet forest. Biotropica 15:68–70. DOI: 10.2307/2388002
Mueller-Dombois D; Ellenberg H. 1974. Aims and methods of vegetation ecology. John Wiley & Sons Ltd., New York, USA.
Nautiyal BP; Pandey N; Bhatt AB. 1997. Analysis of vegetation pattern in an alpine zone in North West Himalaya: A case study of Garhwal Himalaya with reference to diversity and distribution patterns. International Journal of Ecology and Environmental Science 23:49–65.
Nayar MP; Sastry ARK, eds. 1988. Red data book of Indian plants. Botanical Survey of India, Calcutta, India.
Oostermeijer JGB; Luijten SH; den Nijs JCM. 2003. Integrating demographic and genetic approaches in plant conservation. Biological Conservation 113:389–398. DOI: 10.1016/s0006-3207(03)00127-7
Pacala SW. 1997. Dynamics of plant communities. In: Crawley MJ, ed. Plant ecology. Blackwell Scientific, Oxford, UK. p. 532–555.
Pandeya SC. 1964. Ecology of grassland of Sagar, Madhya Pradesh: IIb. Composition of the associations open to grazing or occupying special habitat. Journal of the Indian Botanical Society 43:606–639.
Pandey SK; Shukla RP. 2003. Plant diversity in managed sal (*Shorea robusta* Gaertn.) forests of Gorakhpur, India: Species composition, regeneration and conservation. Biodiversity and Conservation 12:2295–2319. DOI: 10.1023/A:1024589230554
Patrica B; Emily L; Gerardo S; Christina S; Angeles I. 2002. Patterns of β-diversity in a Mexican tropical dry forest. Journal of Vegetation Science 13:145–158. DOI: 10.1111/j.1654-1103.2002.tb02034.x
Pickett STA; White PS, eds. 1985. The ecology of natural disturbance and patch dynamics. Academic Press, New York, USA.
Pitman NCA; Terborgh J; Silman MR; Nuñez PV. 1999. Tree species distributions in an upper Amazonian forest. Ecology 80:2651–2661. DOI: 10.1890/0012-9658(1999)_080 [2651:TSDIAU]2.0.CO;2
Poschlod P; Kiefer S; Tränke U; Fischer S; Bonn S. 1998. Plant species richness in calcareous grasslands as affected by dispersability in space and time. Applied Vegetation Science 1:75–91. DOI: 10.2307/1479087
Rabinowitz D. 1981. Seven forms of rarity. In: Synge H, ed. The biological aspects of rare plant conservation. John Wiley & Sons Ltd., New York, USA. p. 205–217.
Rabinowitz D; Cairns S; Dillon T. 1986. Seven forms of rarity and their frequency in the flora of the British Isles. In: Soulé ME, ed. Conservation biology: The science of scarcity and diversity. Sinauer, Sunderland, MA, USA. p. 182–204.
Raizada A; Joshi SP; Srivastava MM. 1998. Composition and vegetation diversity in an alpine grassland in the Garhwal Himalayas. Tropical Ecology 39:133–144.
Sekar KC. 2012. Invasive alien plants of Indian Himalayan region − diversity and implication. American Journal of Plant Sciences 3:177–184. DOI: 10.4236/ajps.2012.32021
Semwal RL. 2005. The Terai arc landscape in India: Securing protected areas in the face of global change. World Wide Fund for Nature (WWF)-India, New Delhi, India.
Shukla RP. 2009. Patterns of plant species diversity across Terai landscape in north-eastern Uttar Pradesh, India. Tropical Ecology 50:111–123.
Singh JS; Yadava PS. 1974. Seasonal variation in composition, plant biomass and net primary productivity of tropical grassland of Kurukshetra, India. Ecological Monographs 94:371–376. DOI: 10.2307/2937034
Singh JS; Joshi MC. 1979. Primary production. In: Coupland RT, ed. Analysis of grassland and their uses. International Biological Programme (IBP) Synthesis Series, Cambridge University Press, London, UK.
Srivastava S; Dvivedi A; Shukla RP. 2014. Invasive alien species of terrestrial vegetation of north-eastern Uttar Pradesh. International Journal of Forestry Research Volume 2014, ID 959875, 9 pages. DOI: 10.1155/2014/959875
Srivastava S; Dvivedi A; Shukla RP. 2015. *Solanum sisymbriifolium* Lam. (Solanaceae): A new invasive undershrub of the old-fields of northeastern Uttar Pradesh**.** Checklist: The Journal of Biodiversity Data. Volume 11, number 3, article 1643, 4 pages. DOI: 10.15560/11.3.1643
Sundriyal RC; Joshi AP; Dhasmana R. 1987. Phenology of high altitude plants at Tungnath in the Garhwal Himalaya. Tropical Ecology 28:289–299.
Tripathi SL. 1999. Plant diversity of grasslands of north-eastern U.P. with emphasis on populations of *Parthenium hysterophorus* L. Ph.D. Thesis. D.D.U. Gorakhpur University, Gorakhpur, India.
Tripathi SL; Shukla RP. 2007. Effect of clipping and grazing on various vegetational parameters of grassland communities of Gorakhpur, Uttar Pradesh. Tropical Ecology 48:61–70.
Ulrich W; Ollik M. 2004. Frequent and occasional species and the shape of relative-abundance distributions. Diversity and Distribution 10:263–269. DOI: 10.1111/j.1366-9516.2004.00082.x
Varghese AO; Menon ARR. 1999. Ecological niches and amplitudes of rare threatened and endemic trees of Peppara Wildlife Sanctuary. Current Science 76:1204–1208.
Weekley CW; Race T. 2001. The breeding system of *Zizyphus celata* Judd & D.W. Hall (Rhamnaceae), a rare, endemic plant of the Lake Wales Ridge, Florida, USA: Implications for recovery. Conservation Biology 100:207–213. DOI: 10.1016/S0006-3207%2801%2900024-6
Whitmore C. 1996. A review of some aspects of tropical rain forest seedling ecology with suggestions for further enquiry. In: Swaine MD, ed. The ecology of tropical forest seedlings. Man and the Biosphere Series Vol. 17, UNESCO, Paris, France and Parthenon Publishing Group, Inc. New York, USA. p. 3−39.

Whittaker RH. 1975. Communities and Ecosystems. MacMillan Publishing Co., Inc., New York, USA.

Wilsey BJ; Polley HW. 2003. Effects of seed additions and grazing history on diversity and productivity of subhumid grasslands. Ecology 84:920–931. DOI: 10.1890/0012-9658(2003)084%5B0920:eosaag%5D2.0.co;2

Withford PB. 1948. Distribution of woodland plants in relation to succession and clonal growth. Ecology 30:199–208. DOI: 10.2307/1931186

Wu SH; Hsieh CF; Rejmánek M. 2004. Catalogue of the naturalized flora of Taiwan. Taiwania 49:16–31. http://ntur.lib.ntu.edu.tw/retrieve/168216/05.pdf

Annex Ia

Names and sampling areas of locations at the 11 study sites (districts). The habitat characteristics (see Annex Ib) at locations are expressed in terms of ***light regime*** (O - open, PS - partial shade); ***soil moisture*** (HM - high moisture, AM - average moisture, LM - low moisture); ***soil texture*** **(**SS - sandy soil, LS - loam soil, CL - clay soil, SLS - sandy-loam soil, CLS - clay-loam soil and GS - gravel soil); and ***disturbance regime*** (HD - high disturbance, MD - medium disturbance, LD - low disturbance).

Study site	Location	Habitat characteristics	Area (ha) of sampling sites
Bahraich	Acholaya (Stadium)	O,LM,SLS,LD	10.12
	Acholaya I	O,AM,SLS,MD	10.12
	Acholaya II	PS,HM,SLS,HD	11.33
	Acholaya III	O,LM,SLS,HD	14.17
	Acholaya Dhus	O,AM,SLS,MD	4.05
	Basahiya	O,LM,SS,MD	9.71
	Tikora (Parag Dairy)	O,HM,HD,CS	36.42
	Tikora (Mari Mata)	PS,AM,CLS,MD	2.02
Balrampur	Neel Palace	PS,AM,GS,LD	1.21
	Ranjeetpur	O,HM,CS,HD	8.09
	Hanuman Mandir	O,HM,CS,LD	2.83
	Rani Talab	O,LM,CLS,MD	1.21
Gonda	Sarayya Mafi	O,LM,LS,HD	80.94
Shravasti	Mahaet	O,LM,GS,LD	12.14
Basti	Ramauli	PS,AM,CLS,MD	3.24
Sant Kabir Nagar	Maghar	O,HM,CS,HD	11.33
Siddharth Nagar	Piperahawa	O,AM,CS,MD	38.45
Maharajganj	Chhapwa I	O,AM,CLS,MD	2.43
	Chhapwa II	PS,LM,SLS,LD	2.05
Gorakhpur	University Campus	O,AM,CLS,MD	6.07
	Airforce I	PS,AM,CLS,HD	7.29
	Airforce II	O,LM,CLS,MD	7.29
	Airforce III	PS,HM,CLS,HD	8.09
	Jagatbella	O,HM,CS,MD	12.14
Kushi Nagar	Sirshya I	O,LM,SLS,LD	4.86
	Sirshya II	O,LM,SLS,MD	2.43
	Sirshya III	PS,AM,SLS,HD	2.02
Deoria	Deoghat I	PS,LM,SLS,MD	6.07
	Deoghat II	PS,AM,SLS,HD	2.02
	Uska	PS,HM,CLS,MD	5.67
	Singahi	PS,HM,CLS,HD	5.20

Annex Ib

Habitat identification

The habitats of plant species were determined on the basis of characteristics that increase the heterogeneity of terrestrial vegetation: change in local topography; light regime; soil moisture; soil texture; and degree and type of anthropogenic disturbances.

Light regime: The degree of exposure was measured by an illuminometer (Kyoritsu-5200) at 10 random and nearly equidistant points on the floor of the vegetation during sunny days of November at mid-day. The light regime of open communities ranged from 70,000 to 75,000 lux and that of partially shaded communities was below 10,000 lux.

Soil moisture regime: The moisture content within the top 10 cm of soil was measured gravimetrically. More than 40% of soil moisture was treated as high moisture (HM), 20−40% as average moisture (AM) and <20% as low moisture (LM).

Soil texture: Soil texture refers to particle size composition and according to the proportions of sand, silt and clay, soils are classified, in general, as sandy, clayey, loamy, sandy-loam, silty-loam, clayey-loam and silty-clay soils. The physical characterization of soil at the study sites is based on analysis by the Agricultural Research Center, Bahraich (U.P.), India (Table A below). The silty-loam and silty-clay soils were termed as gravelly soil as they contained significant proportions of gravels and pebbles.

Disturbance: The anthropogenic disturbance factors (see also Table B below) operating at each sampling location were recorded. The intensity of each factor was scaled in the classes: 1 (absent or very little); 2 (occasional); and 3 (recurrent) (see Table C below).

Table A. Soil physical characteristics at the study locations within 11 districts across grassy landscapes of northeastern Uttar Pradesh.

Study site (district)	Sample no.	Location	Composition (%)				Texture
			Sand		Silt	Clay	
			Fine	Coarse			
Bahraich	1	Acholaya (Stadium)	40.2	31.2	16.8	11.8	Sandy-loam
	2	Acholaya I	42.0	30.6	16.2	11.2	Sandy-loam
	3	Acholaya II	40.4	30.2	17.0	12.4	Sandy-loam
	4	Acholaya III	40.4	32.0	16.8	10.8	Sandy-loam
	5	Acholaya Dhus	41.2	30.4	17.2	11.2	Sandy-loam
	6	Basahiya	44.7	35.8	8.3	11.2	Sandy
	7	Tikora (Parag Dairy)	4.0	2.8	9.8	83.4	Clay
	8	Tikora (Mari Mata)	18.2	3.6	43.8	34.4	Clay-loam
Balrampur	9	Neel Palace	14.2	26.2*	45.6	28.2	Gravel
	10	Ranjeetpur	2.8	2.0	9.0	86.2	Clay
	11	Hanuman Mandir	3.2	2.3	8.9	85.6	Clay
	12	Rani Talab	14.8	2.0	46.4	36.8	Clay-loam
Gonda	13	Sarayya Mafi	4.6	3.2	64.8	27.4	Loam
Shravasti	14	Mahaet	14..4	26*	45.6	28.2	Gravel
Basti	15	Ramauli	24.2	7.0	32.4	36.4	Clay-loam
Sant Kabir Nagar	16	Maghar	2.6	2.2	7.8	87.4	Clay
Siddharth Nagar	17	Piperahawa	3.8	2.5	9.9	83.8	Clay
Maharajganj	18	Chhapwa I	23.2	8.0	33.6	35.2	Clay-loam
	19	Chhapwa II	41.4	29.2	18.0	11.4	Sandy-loam
Gorakhpur	20	University Campus	19.6	2.0	44.8	33.6	Clay-loam
	21	Airforce I	19.2	2.4	44.4	34.0	Clay-loam
	22	Airforce II	18.6	2.2	42.8	36.4	Clay-loam
	23	Airforce III	13.8	3.0	46.8	36.4	Clay-loam
	24	Jagatbella	3.2	3.1	9.2	84.5	Clay
Kushinagar	25	Sirshya I	41.8	31.2	16.2	10.8	Sandy-loam
	26	Sirshya II	42.2	31.6	15.2	11.0	Sandy-loam
	27	Sirshya III	42.4	31.2	16.0	10.4	Sandy-loam
Deoria	28	Deoghat I	42.8	32.0	15.4	9.8	Sandy-loam
	29	Deoghat II	42.4	32.7	15.6	9.3	Sandy-loam
	30	Uska	23.2	8.0	30.4	38.4	Clay-loam
	31	Singahi	23.2	8.0	33.6	35.2	Clay-loam

*The coarse sand fraction also included gravels and pebbles.

Table B. Different types of disturbance classes at the study sites and their weightage score across grassy landscapes of north-eastern Uttar Pradesh.

Disturbance factor	Very little	Occasional	Recurrent
Agricultural practices	1	2	3
Fire	1	2	3
Forage removal	1	2	3
Fuel wood collection	1	2	3
Grazing	1	2	3
Habitat destruction	1	2	3
Horticulture	1	2	3
Invasion	1	2	3
Litter collection	1	2	3
Mining of soil	1	2	3
Root collection	1	2	3
Trampling	1	2	3
Flood	1	2	3

Table C. Disturbance levels based on weightage scores of various disturbances.

Disturbance level	Weightage score
Low	1 or 2 or 3
Medium	1 and 2 or 1 and 3
High	1 and 2 and 3

Annex II

Commonness and rarity of species of grassland vegetation across northeastern Uttar Pradesh. [Nomenclature according to The Plant List database (http://www.theplantlist.org)]. Habit: H = herb; C = climber; S = shrub; Posture: EA = erect annual; PA = prostrate annual; PerTw = perennial twiner; PPer = prostrate perennial; ATw = annual twiner; EPer = erect perennial; ATn = annual tendril.

Plant species	Family	Habit	Posture	Total no. individuals	Density ($/m^2$)	Population size	Habitat specialization	Geographical range
Acalypha ciliata Forssk.	Euphorbiaceae	H	EA	10	0.07	Large	Broad	Wide
Acalypha indica L.	Euphorbiaceae	H	EA	6	0.04	Large	Broad	Wide
Achyranthes aspera L.	Amaranthaceae	H	EA	63	0.41	Large	Broad	Wide
Aerva lanata (L.) Juss.	Amaranthaceae	H	EA	34	0.30	Small	Broad	Wide
Aeschynomene aspera L.	Papilionaceae	H	EA	7	0.05	Large	Broad	Wide
Aeschynomene indica L.	Papilionaceae	H	EA	3	0.02	Large	Broad	Wide
Ageratum conyzoides (L.) L.	Asteraceae	H	EA	32	0.21	Large	Broad	Wide
Ageratum houstonianum Mill.	Asteraceae	H	EA	357	2.30	Large	Broad	Wide
Alternanthera paronychioides A.St.-Hil.	Amaranthaceae	H	PA	148	0.95	Large	Broad	Wide
Alternanthera pungens Kunth	Amaranthaceae	H	PA	9	0.06	Large	Narrow	Narrow
Alternanthera sessilis (L.) R.Br. ex DC.	Amaranthaceae	H	PA	187	1.21	Large	Broad	Wide
Alysicarpus bupleurifolius (L.) DC.	Papilionaceae	H	EA	33	0.21	Large	Narrow	Narrow
Alysicarpus longifolius (Spreng.) Wight & Arn.	Papilionaceae	H	EA	14	0.09	Small	Broad	Wide
Alysicarpus monilifer (L.) DC.	Papilionaceae	H	PA	324	2.09	Large	Broad	Wide
Alysicarpus ovalifolius (Schum.) Leonard	Papilionaceae	H	EA	26	0.17	Small	Broad	Wide
Alysicarpus vaginalis (L.) DC.	Papilionaceae	H	PA	667	4.30	Large	Broad	Wide
Amaranthus spinosus L.	Amaranthaceae	H	EA	1	0.01	Large	Broad	Wide
Amaranthus viridis L.	Amaranthaceae	H	EA	57	0.37	Large	Broad	Wide
Ammannia auriculata Willd.	Lythraceae	H	EA	48	0.41	Small	Broad	Wide
Ammannia baccifera L.	Lythraceae	H	EA	72	0.46	Small	Broad	Wide
Anagallis arvensis L.	Primulaceae	H	EA	71	0.46	Large	Broad	Wide
Apluda mutica L.	Poaceae	H	EA	46	0.30	Small	Broad	Wide
Argemone mexicana L.	Papaveraceae	H	EA	7	0.05	Large	Broad	Wide
Bacopa monnieri (L.) Wettst.	Scrophulariaceae	H	EA	8	0.05	Small	Narrow	Narrow
Basella alba L.	Basellaceae	C	PerTw	3	0.02	Small	Broad	Wide
Blumea axillaris (Lam.) DC.	Asteraceae	H	EA	8	0.05	Large	Broad	Wide
Blumea eriantha DC.	Asteraceae	H	EA	35	0.23	Large	Broad	Wide
Blumea lacera (Burm.f.) DC.	Asteraceae	H	EA	8	0.05	Small	Broad	Wide
Blumea laciniata (Wall. ex Roxb.) DC.	Asteraceae	H	EA	26	0.17	Small	Broad	Wide
Boerhavia diffusa L.	Nyctaginaceae	H	PPer	120	0.77	Large	Broad	Wide
Bolboschoenus glaucus (Lam.) S.G.Sm.	Cyperaceae	H	EA	23	0.15	Small	Broad	Wide
Cajanus scarabaeoides (L.) Thouars	Papilionaceae	C	ATw	27	0.17	Small	Narrow	Narrow
Calotropis procera (Aiton) Druand.	Asclepiadaceae	S	EPer	36	0.23	Large	Broad	Wide
Cannabis sativa L.	Cannabinaceae	H	EA	1	0.01	Large	Broad	Wide

Continued

Plant species	Family	Habit	Posture	Total no. individuals	Density (/m^2)	Population size	Habitat specialization	Geographical range
Carex fedia Nees	Cyperaceae	H	EA	186	1.20	Large	Broad	Wide
Cayratia trifolia (L.) Domin	Vitaceae	C	ATn	16	0.10	Large	Broad	Wide
Ceesulia axillaris Roxb.	Asteraceae	H	EA	143	0.92	Large	Broad	Wide
Cenchrus biflorus Roxb.	Poaceae	H	EA	11	0.07	Large	Broad	Wide
Centella asiatica (L.) Urb.	Apiaceae	H	PA	17	0.11	large	Narrow	Wide
Chamaecrista absus (L.) H.S.Irwin & Barneby	Caesalpiniaceae	H	EA	7	0.05	Small	Narrow	Narrow
Chenopodium album L.	Chenopodiaceae	H	EA	24	0.15	Large	Broad	Wide
Chloris barbata Sw.	Poaceae	H	EA	467	3.01	Small	Broad	Wide
Chrysanthellum indicum DC.	Asteraceae	H	PA	595	3.84	Large	Narrow	Narrow
Chrysopogon aciculatus (Retz.) Trin.	Poaceae	H	EA	790	5.10	Large	Broad	Wide
Cissampelos pareira L.	Menispermaceae	C	PerTw	8	0.05	Small	Broad	Wide
Cleome viscosa L.	Capparidaceae	H	EA	7	0.05	Large	Broad	Wide
Clerodendrum indicum (L.) Kuntze	Verbenaceae	S	EPer	55	0.35	Small	Broad	Wide
Coccinia grandis (L.) Voigt	Cucurbitaceae	C	ATn	9	0.06	Large	Broad	Wide
Commelina benghalensis L.	Commelinaceae	H	PA	27	0.17	Large	Broad	Wide
Commelina diffusa Burm.f.	Commelinaceae	H	PA	45	0.29	Small	Broad	Wide
Corchorus aestuans L.	Tiliaceae	H	PA	83	0.54	Large	Broad	Wide
Corchorus fascicularis Lam.	Tiliaceae	H	EA	57	0.37	Large	Broad	Wide
Crotalaria calycina Schrank	Papilionaceae	H	EA	15	0.10	Large	Narrow	Narrow
Crotalaria medicaginea Lam.	Papilionaceae	H	EA	123	0.79	Large	Broad	Wide
Crotalaria pallida Aiton	Papilionaceae	H	PA	1	0.01	Small	Broad	Wide
Crotalaria prostrata Willd.	Papilionaceae	H	EA	512	3.30	Large	Narrow	Narrow
Crotalaria retusa L.	Papilionaceae	H	PA	1	0.01	Small	Broad	Wide
Crotalaria sp.	Papilionaceae	H	PA	79	0.51	Small	Broad	Wide
Croton bonplandianus Baill.	Euphorbiaceae	H	EA	69	0.45	Large	Broad	Wide
Cucumis melo L.	Cucurbitaceae	C	ATn	9	0.06	Large	Broad	Wide
Cullen corylifolium (L.) Medik.	Papilionaceae	H	EA	191	1.23	Small	Narrow	Narrow
Cuscuta chinensis Lam.	Cuscutaceae	C	ATw	1	0.01	Large	Broad	Wide
Cuscuta sp.	Cuscutaceae	C	ATw	50	0.32	Large	Broad	Wide
Cyanotis axillaris (L.) D.Don ex Sweet	Commelinaceae	H	EA	137	0.88	Small	Broad	Wide
Cyanthillium cinereum (L.) H.Rob.	Asteraceae	H	EA	1,077	6.95	Large	Broad	Wide
Cynodon dactylon (L.) Pers.	Poaceae	H	PPer	3,644	23.51	Large	Broad	Wide
Cynoglossum lanceolatum Forssk.	Boraginaceae	H	EA	123	0.79	Large	Narrow	Narrow
Cyperus alopecuroides Rottb.	Cyperaceae	H	EA	262	1.69	Large	Broad	Wide
Cyperus alulatus J.Kern	Cyperaceae	H	EA	47	0.30	Small	Broad	Wide
Cyperus capitatus Vand.	Cyperaceae	H	EA	568	3.66	Large	Broad	Wide
Cyperus castaneus Willd.	Cyperaceae	H	EA	130	0.84	Small	Broad	Wide
Cyperus compressus L.	Cyperaceae	H	EA	31	0.20	Large	Broad	Wide
Cyperus difformis L.	Cyperaceae	H	EA	51	0.33	Small	Broad	Wide

Continued

Plant species	Family	Habit	Posture	Total no. individuals	Density (/m^2)	Population size	Habitat specialization	Geographical range
Cyperus dubius Rottb.	Cyperaceae	H	EA	150	0.97	Large	Broad	Wide
Cyperus exaltatus Retz.	Cyperaceae	H	EA	13	0.08	Small	Broad	Wide
Cyperus michelianus (L.) Delile	Cyperaceae	H	PA	13	0.08	Large	Broad	Wide
Cyperus niveus Retz.	Cyperaceae	H	EA	2	0.01	Small	Narrow	Narrow
Cyperus rotundus L.	Cyperaceae	H	EA	44	0.28	Large	Broad	Wide
Cyperus sp. 1	Cyperaceae	H	EA	68	0.44	Large	Broad	Wide
Cyperus sp. 2	Cyperaceae	H	EA	269	1.74	Small	Broad	Wide
Cyperus sp. 3	Cyperaceae	H	EA	74	0.48	Large	Broad	Wide
Cyperus sp. 4	Cyperaceae	H	EA	28	0.18	Small	Broad	Wide
Cyperus trachysanthos Hook. & Arn.	Cyperaceae	H	EA	353	2.28	Large	Broad	Wide
Dactyloctenium aegyptium (L.) Willd.	Poaceae	H	EA	622	4.01	Large	Broad	Wide
Dentella repens (L.) R.Forst. & G.Forst.	Rubiaceae	H	PA	251	1.62	Small	Broad	Wide
Desmodium gangeticum (L.) DC.	Papilionaceae	H	EPer	52	0.34	Large	Broad	Wide
Desmodium triflorum (L.) DC.	Papilionaceae	H	PPer	9,015	58.16	Large	Broad	Wide
Desmostachya bipinnata (L.) Stapf	Poaceae	H	EPer	140	0.90	Large	Broad	Wide
Dichanthium annulatum (Forssk.) Stapf	Poaceae	H	EA	1,028	6.63	Large	Broad	Wide
Digera muricata (L.) Mart.	Amaranthaceae	H	EA	3	0.02	Small	Broad	Wide
Digitaria ciliaris (Retz.) Koeler	Poaceae	H	EA	218	1.41	Small	Broad	Wide
Echinochloa colona (L.) Link	Poaceae	H	EA	65	0.42	Large	Broad	Wide
Eclipta prostrata (L.) L.	Asteraceae	H	EA	49	0.32	Large	Broad	Wide
Elephantopus scaber L.	Asteraceae	H	PA	132	0.85	Large	Broad	Wide
Emilia sonchifolia (L.) DC. ex DC.	Asteraceae	H	EA	273	1.76	Small	Broad	Wide
Eragrostis amabilis (L.) Wight & Arn.	Poaceae	H	EA	2,330	15.03	Large	Broad	Wide
Eragrostis capensis (Thunb.) Trin.	Poaceae	H	EA	45	0.29	Small	Narrow	Narrow
Eragrostis cilianensis (All.) Janch.	Poaceae	H	EA	29	0.19	Small	Narrow	Narrow
Eragrostis minor Host	Poaceae	H	EA	420	2.71	Large	Broad	Wide
Eragrostis pilosa (L.) P.Beauv.	Poaceae	H	EA	92	0.59	Small	Broad	Wide
Eragrostis unioloides (Retz.) Nees ex Steud.	Poaceae	H	EA	969	6.25	Large	Broad	Wide
Erigeron bonariensis L.	Asteraceae	H	EA	12	0.08	Large	Broad	Wide
Euphorbia hirta L.	Euphorbiaceae	H	EA	54	0.35	Large	Broad	Wide
Euphorbia hypericifolia L.	Euphorbiaceae	H	EA	54	0.35	Large	Broad	Wide
Euphorbia thymifolia L.	Euphorbiaceae	H	PA	29	0.19	Small	Narrow	Wide
Evolvulus alsinoides (L.) L.	Convolvulaceae	H	PPer	763	4.92	large	Narrow	Wide
Evolvulus nummularius (L.) L.	Convolvulaceae	H	PPer	7,805	50.36	Large	Broad	Wide
Ficus heterophylla L.f.	Moraceae	S	EPer	5	0.03	Large	Broad	Wide
Fimbristylis aestivalis Vahl	Cyperaceae	H	EA	144	0.93	Large	Broad	Wide
Fimbristylis bisumbellata (Forssk.) Bubani	Cyperaceae	H	EA	2,406	15.52	Large	Broad	Wide
Fimbristylis cymosa R.Br.	Cyperaceae	H	EA	228	1.47	Small	Broad	Wide
Fimbristylis dichotoma (L.) Vahl	Cyperaceae	H	EA	3	0.02	Small	Broad	Wide

Continued

Plant species	Family	Habit	Posture	Total no. individuals	Density (/m^2)	Population size	Habitat specialization	Geographical range
Fimbristylis falcata (Vahl) Kunth	Cyperaceae	H	EA	406	2.62	Small	Broad	Wide
Fimbristylis ovata (Burm.f.) J.Kern	Cyperaceae	H	EA	1,187	7.66	Large	Broad	Wide
Fimbristylis quinquangularis (Vahl) Kunth	Cyperaceae	H	EA	608	3.92	Large	Broad	Wide
Fimbristylis schoenoides (Retz.) Vahl	Cyperaceae	H	EA	367	2.37	Large	Broad	Wide
Fimbristylis sp.	Cyperaceae	H	EA	60	0.39	Small	Broad	Wide
Fumaria indica (Hausskn.) Pugsley	Fumariaceae	H	EA	1	0.01	Large	Broad	Wide
Glinus lotoides L.	Molluginaceae	H	PA	16	0.10	Small	Broad	Wide
Glinus oppositifolius (L.) Aug.DC.	Molluginaceae	H	PA	37	0.24	Small	Broad	Wide
Gnaphalium polycaulon Pers.	Asteraceae	H	EA	142	0.95	Small	Broad	Wide
Gomphrena celosioides Mart.	Amaranthaceae	H	EA	40	0.26	Large	Broad	Wide
Grangea maderaspatana (L.) Poir.	Asteraceae	H	PA	72	0.46	Large	Broad	Wide
Heliotropium indicum L.	Boraginaceae	H	EA	13	0.08	Small	Broad	Wide
Heliotropium ovalifolium Forssk.	Boraginaceae	H	EA	12	0.08	Small	Narrow	Narrow
Heliotropium strigosum Willd.	Boraginaceae	H	PPer	2,917	18.82	Small	Broad	Wide
Hemarthria compressa (L.f.) R.Br.	Poaceae	H	EA	47	0.30	Large	Narrow	Narrow
Hemidesmus indicus (L.) R.Br. ex Schult.	Asclepiadaceae	C	PerTw	51	0.33	Small	Broad	Wide
Hemigraphis hirta (Vahl) T.Anderson	Acanthaceae	H	PA	229	1.48	Large	Broad	Wide
Heteropogon contortus (L.) P.Beauv. ex Roem. & Schult.	Poaceae	H	EPer	25	0.16	Large	Narrow	Narrow
Hybanthus linearifolius (Vahl) Urb.	Violaceae	H	EA	122	0.79	Small	Narrow	Wide
Hygrophila auriculata (Schumach.) Heine	Lamiaceae	H	EA	13	0.08	Large	Broad	Narrow
Hygrophila difformis Blume	Acanthaceae	H	EA	12	0.08	Small	Broad	Wide
Hyptis suaveolens (L.) Poit.	Lamiaceae	H	EA	186	1.20	Large	Broad	Wide
Ichnocarpus frutescens (L.) W.T.Aiton	Apocynaceae	C	PerTw	62	0.40	Large	Broad	Wide
Imperata cylindrica (L.) Raeusch.	Poaceae	H	EA	3,035	19.58	Large	Broad	Wide
Indigofera linifolia (L.f.) Retz.	Papilionaceae	H	PPer	376	2.43	Large	Broad	Wide
Indigofera linnaei Ali	Papilionaceae	H	PPer	47	0.30	Small	Broad	Wide
Ipomoea aquatica Forssk.	Convolvulaceae	C	ATw	2	0.01	Large	Broad	Wide
Ipomoea pes-tigridis L.	Convolvulaceae	C	ATw	5	0.03	Small	Broad	Wide
Ipomoea sp.	Convolvulaceae	C	ATw	4	0.03	Small	Broad	Wide
Jatropha curcas L.	Euphorbiaceae	S	Eper	11	0.07	Small	Broad	Wide
Kylinga brevifolia Rottb.	Cyperaceae	H	EA	1,012	6.53	Large	Broad	Wide
Lantana camara L.	Verbenaceae	S	EPer	5	0.03	Large	Broad	Wide
Laphangium luteoalbum (L.) Tzvelev	Asteraceae	H	EA	752	4.85	Small	Broad	Wide
Lathyrus aphaca L.	Papilionaceae	C	ATn	37	0.24	Small	Broad	Wide
Lathyrus odoratus L.	Papilionaceae	C	ATn	44	0.28	Small	Broad	Wide
Lathyrus sativus L.	Papilionaceae	C	ATn	13	0.08	Small	Broad	Wide
Launaea aspleniifolia (Willd.) Hook.f.	Asteraceae	H	EA	396	2.55	Small	Broad	Wide
Launaea nudicaulis (L.) Hook.f.	Asteraceae	H	EA	4	0.03	Small	Broad	Wide
Launaea procumbens (Roxb.) Ramayya & Rajagopal	Asteraceae	H	EA	6	0.04	Small	Broad	Wide

Continued

Plant species	Family	Habit	Posture	Total no. individuals	Density (/m^2)	Population size	Habitat specialization	Geographical range
Leucas aspera (Willd.) Link	Lamiaceae	H	EA	19	0.12	Small	Broad	Narrow
Leucas cephalotes (Roth) Spreng.	Lamiaceae	H	EA	51	0.33	Small	Broad	Narrow
Limnophylla sp. 1	Scrophulariaceae	H	EA	4	0.03	Small	Broad	Wide
Limnophylla sp. 2	Scrophulariaceae	H	EA	11	0.07	Small	Broad	Wide
Limnophylla sp. 3	Scrophulariaceae	H	EA	40	0.26	Small	Broad	Wide
Lindernia antipoda (L.) Alston	Scrophulariaceae	H	EA	182	1.17	Small	Broad	Wide
Lindernia brachiata (Link & Otto) Biswas	Scrophulariaceae	H	EA	22	0.14	Large	Broad	Wide
Lindernia ciliata (Colsm.) Pennell	Scrophulariaceae	H	EA	8,761	56.52	Large	Broad	Wide
Lindernia crustacea (L.) F.Muell.	Scrophulariaceae	H	EA	228	1.47	Small	Broad	Wide
Lindernia diffusa (L.) Wettst.	Scrophulariaceae	H	EA	22,288	143.79	Large	Broad	Wide
Lindernia procumbens (Krock.) Philcox	Scrophulariaceae	H	PA	108	0.70	Large	Broad	Wide
Lindernia pyxidaria All.	Scrophulariaceae	H	EA	158	1.02	Large	Narrow	Narrow
Lippia alba (Mill.) N.E.Br. ex Britton & P.Wilson	Verbenaceae	S	EA	9	0.06	Small	Broad	Wide
Lobelia alsinoides Lam.	Lobeliaceae	H	EA	141	0.91	Small	Narrow	Narrow
Ludwigia adscendens (L.) H.Hara	Onagraceae	H	PA	1	0.01	Small	Broad	Wide
Ludwigia octovalvis (Jacq.) P.Raven	Onagraceae	H	EA	10	0.06	Small	Broad	Wide
Ludwigia perennis L.	Onagraceae	H	EA	176	1.14	Small	Broad	Wide
Ludwigia prostrata Roxb.	Onagraceae	H	EA	9	0.06	Small	Broad	Wide
Malvastrum coromandelianum (L.) Garcke	Malvaceae	H	EA	34	0.30	Small	Broad	Wide
Martynia annua L.	Martyniaceae	H	EA	10	0.06	Small	Narrow	Narrow
Mazus pumilus (Burm.f.) Steenis	Scrophulariaceae	H	EA	129	0.83	Large	Broad	Wide
Mecardonia procumbens (Mill.) Small	Scrophulariaceae	H	EA	168	1.08	Small	Broad	Wide
Medicago polymorpha L.	Papilionaceae	H	EA	453	2.92	Small	Broad	Wide
Melochia corchorifolia L.	Sterculiaceae	H	EA	118	0.76	Large	Broad	Wide
Merremia sp.	Convolvulaceae	H	PA	17	0.11	Small	Broad	Wide
Momordica dioica Roxb. ex Willd.	Cucurbitaceae	C	ATn	3	0.02	Small	Broad	Wide
Mukia maderaspatana (L.) Roem.	Cucurbitaceae	C	ATn	4	0.03	Small	Broad	Wide
Murdannia nudiflora (L.) Brenan	Commelinaceae	H	PA	1,516	9.78	Large	Broad	Wide
Ocimum americanum L.	Lamiaceae	H	EA	55	0.35	Large	Broad	Wide
Oldenlandia biflora L.	Rubiaceae	H	PA	63	0.41	Large	Broad	Wide
Oldenlandia corymbosa L.	Rubiaceae	H	PA	1,315	8.48	Large	Broad	Wide
Oldenlandia tenelliflora (Blume) Kuntze	Rubiaceae	H	EA	827	5.35	Small	Broad	Wide
Operculina turpethum (L.) Silva Manso	Convolvulaceae	C	PerTw	8	0.05	Large	Broad	Wide
Oplismenus burmanni (Retz.) P.Beauv.	Poaceae	H	PA	529	3.41	Large	Broad	Wide
Oxalis corniculata L.	Oxalidaceae	H	PA	92	0.59	Large	Broad	Wide
Oxystelma secamone H.Karst.	Asclepiadaceae	C	ATw	2	0.01	Small	Broad	Wide
Panicum sumatrense Roth	Poaceae	H	EA	20	0.13	Small	Broad	Wide
Panicum antidotale Retz.	Poaceae	H	EA	74	0.48	Large	Broad	Wide
Panicum sp. 1	Poaceae	H	PA	125	0.81	Large	Broad	Wide

Continued

Plant species	Family	Habit	Posture	Total no. individuals	Density (/m^2)	Population size	Habitat specialization	Geographical range
Panicum maximum Jacq.	Poaceae	H	EA	296	1.91	Large	Broad	Wide
Panicum virgatum L.	Poaceae	H	EA	343	2.21	Small	Broad	Wide
Parthenium hysterophorus L.	Asteraceae	H	EA	350	2.26	Large	Broad	Wide
Paspalidium flavidum (Retz.) A.Camus	Poaceae	H	PA	1,311	8.46	Large	Broad	Wide
Paspalum distichum L.	Poaceae	H	EPer	672	4.34	Small	Broad	Wide
Paspalum scrobiculatum L.	Poaceae	H	EA	1,679	10.83	Large	Broad	Wide
Pennisetum glaucum (L.) R.Br.	Poaceae	H	EA	1,123	7.25	Large	Broad	Wide
Peristrophe bicalyculata (Retz.) Nees	Acanthaceae	H	EA	41	0.26	Large	Broad	Wide
Perotis indica (L.) Kuntze	Poaceae	H	PA	174	1.12	Large	Narrow	Narrow
Persicaria glabra (Willd.) M.Gómez	Polygonaceae	H	EA	8	0.05	Large	Broad	Wide
Persicaria lapathifolia (L.) Delarbre	Polygonaceae	H	EA	10	0.06	Large	Broad	Wide
Phyla nodiflora (L.) Greene	Verbenaceae	H	EA	85	0.55	Large	Broad	Wide
Phyllanthus niruri L.	Euphorbiaceae	H	EA	69	0.45	Large	Broad	Wide
Phyllanthus urinaria L.	Euphorbiaceae	H	EA	2,690	17.36	Small	Broad	Wide
Phyllanthus virgatus G.Forst.	Euphorbiaceae	H	EPer	1,173	7.57	Large	Broad	Wide
Physalis minima L.	Solanaceae	H	EA	7	0.05	Small	Broad	Wide
Physalis peruviana L.	Solanaceae	H	EA	3	0.02	Small	Broad	Wide
Polygala chinensis L.	Polygalaceae	H	EA	94	0.61	Small	Broad	Wide
Polygonum plebeium R.Br.	Polygonaceae	H	PA	286	1.85	Large	Broad	Wide
Portulaca oleracea L.	Portulacaceae	H	PA	64	0.41	Large	Broad	Wide
Pouzolzia zeylanica (L.) Benn.	Urticaceae	H	EA	19	0.12	Large	Broad	Wide
Pycreus pumilus (L.) Nees	Cyperaceae	H	EA	30	0.19	Small	Broad	Wide
Ranunculus sceleratus L.	Ranunculaceae	H	EA	4	0.03	Large	Broad	Wide
Rhynchospora colorata (L.) H.Pfeiff.	Cyperaceae	H	EA	55	0.35	Small	Broad	Wide
Ruellia tuberosa L.	Acanthaceae	H	EA	4	0.03	Large	Broad	Wide
Rumex hastatus D.Don.	Polygonaceae	H	EA	199	1.28	Large	Broad	Wide
Rungia repens (L.) Nees	Acanthaceae	H	PA	9,701	62.59	Large	Broad	Wide
Saccharum sp.	Poaceae	H	EA	425	2.74	Large	Broad	Wide
Saccharum spontaneum L.	Poaceae	H	EPer	76	0.49	Large	Broad	Wide
Sacciolepis indica (L.) Chase	Poaceae	H	EA	144	0.93	Large	Broad	Wide
Sacciolepis myosuroides (R.Br.) A.Camus	Poaceae	H	EA	79	0.51	Large	Broad	Wide
Salvia plebeia R.Br.	Lamiaceae	H	EA	11	0.07	Small	Broad	Wide
Salvia reptans Jacq.	Lamiaceae	H	EA	6	0.04	Small	Broad	Wide
Schoenoplectiella lateriflora (J.F.Gmel.) Lye	Cyperaceae	H	PA	13	0.08	Small	Broad	Wide
Schoenoplectiella mucronata (L.) J.Jung & H.K.Choi	Cyperaceae	H	EA	13	0.08	Small	Broad	Wide
Scoparia dulcis L.	Scrophulariaceae	H	EA	172	1.11	Large	Broad	Wide
Senna obtusifolia (L.) H.S. Irwin &Barneby	Caesalpiniaceae	H	EA	606	3.91	Large	Broad	Wide
Senna occidentalis (L.) Link	Caesalpiniaceae	H	EA	91	0.59	Large	Broad	Wide
Senna pumila (Lam.) K.Larsen	Caesalpiniaceae	H	PA	13	0.08	Large	Narrow	Narrow

Continued

Plant species	Family	Habit	Posture	Total no. individuals	Density (/m^2)	Population size	Habitat specialization	Geographical range
Senna tora (L.) Roxb.	Caesalpiniaceae	H	EA	4	0.03	Large	Broad	Wide
Sida acuta Burm.f.	Malvaceae	H	EA	191	1.23	Large	Broad	Wide
Sida cordata (Burm.f.) Borss.Waalk.	Malvaceae	H	EA	185	1.19	Large	Broad	Wide
Sida cordifolia L.	Malvaceae	H	EA	182	1.17	Large	Broad	Wide
Sida ovata Forssk.	Malvaceae	H	EA	7	0.05	Large	Broad	Wide
Sida rhombifolia L.	Malvaceae	H	EA	195	1.26	Large	Broad	Wide
Sida spinosa L.	Malvaceae	H	EA	18	0.12	Small	Broad	Wide
Solanum americanum Mill.	Solanaceae	H	EA	23	0.15	Large	Broad	Wide
Solanum virginianum L.	Solanaceae	H	EA	1	0.01	Large	Broad	Wide
Soliva anthemifolia (Juss.) Sweet	Asteraceae	H	PA	4	0.03	Small	Broad	Wide
Sonchus asper (L.) Hill	Asteraceae	H	EA	11	0.07	Large	Broad	Wide
Sonchus oleraceus (L.) L.	Asteraceae	H	EA	3	0.02	Small	Broad	Wide
Spermacoce pusilla Wall.	Rubiaceae	H	EA	4,076	26.30	Large	Narrow	Narrow
Sphenoclea zeylanica Gaertn.	Sphenocleaceae	H	EA	10	0.06	Small	Narrow	Narrow
Spilanthes acmella (L.) L.	Asteraceae	H	PA	31	0.20	Large	Broad	Wide
Tecoma capensis (Thunb.) Lindl.	Bignoniaceae	C	PerTw	3	0.02	Small	Broad	Wide
Tephrosia purpurea (L.) Pers.	Papilionaceae	H	EPer	6	0.04	Large	Broad	Wide
Teramnus labialis (L.f.) Spreng.	Papilionaceae	C	PerTw	8	0.05	Small	Narrow	Narrow
Tiliacora racemosa Colebr.	Menispermaceae	C	PerTw	1	0.01	Large	Broad	Wide
Tinospora sinensis (Lour.) Merr.	Menispermaceae	C	PerTw	9	0.06	Large	Broad	Wide
Trianthema portulacastrum L.	Aizoaceae	H	PA	8	0.05	Small	Broad	Wide
Tribulus terrestris L.	Zygophyllaceae	H	PA	5	0.03	Small	Narrow	Narrow
Tridax procumbens (L.) L.	Asteraceae	H	EA	603	3.89	Large	Broad	Wide
Triumfetta pentranda A.Rich.	Malvaceae	H	EA	31	0.20	Large	Broad	Wide
Triumfetta rhomboidea Jacq.	Malvaceae	S	EA	18	0.12	Large	Broad	Wide
Urena lobata L.	Malvaceae	S	EA	5	0.03	Large	Broad	Wide
Urena repanda Roxb. ex Sm.	Malvaceae	H	EA	35	0.23	Large	Broad	Wide
Vernonia sp.	Asteraceae	H	EA	9	0.06	Small	Narrow	Narrow
Xanthium strumarium L.	Asteraceae	S	EA	79	0.51	Large	Broad	Wide
Zephyranthes citrina Baker	Amaryllidaceae	H	EA	8	0.05	Small	Narrow	Narrow
Zornia gibbosa Span.	Papilionaceae	H	PA	3,255	21.00	Small	Broad	Wide
Samples in process of species identification:								
Unknown 3	Poaceae	H	EA	679	4.38	Small	Broad	Wide
Unknown 4	Poaceae	H	EA	1,270	8.19	Large	Broad	Wide
Unknown 5	Poaceae	H	EA	620	4	Large	Broad	Wide
Unknown 6	Poaceae	H	EA	36	0.23	Small	Broad	Wide
Unknown 7	Poaceae	H	EA	170	1.07	Small	Broad	Wide
Unknown 8	Poaceae	H	EA	57	0.37	Large	Broad	Wide
Unknown 9	Poaceae	H	EA	311	2.01	Small	Broad	Wide

Continued

Plant species	Family	Habit	Posture	Total no. individuals	Density (/m^2)	Population size	Habitat specialization	Geographical range
Unknown 10	Poaceae	H	EA	18	0.12	Small	Broad	Wide
Unknown 11	Poaceae	H	EA	340	2.19	Small	Broad	Wide
Unknown 12	Poaceae	H	EA	67	0.43	Large	Broad	Wide
Unknown 13	Poaceae	H	EA	11	0.07	Large	Broad	Wide
Unknown 14	Poaceae	H	EA	95	0.61	Small	Broad	Wide
Unknown 15	Poaceae	H	EA	19	0.12	Small	Broad	Wide
Unknown 16	Poaceae	H	EA	72	0.46	Large	Broad	Wide
Unknown 17	Poaceae	H	EA	36	0.23	Large	Broad	Wide
Unknown 18	Poaceae	H	EA	151	0.97	Large	Broad	Wide
Unknown 19	Unknown	H	EA	3	0.02	Small	Broad	Wide
Unknown 20	Papilionaceae	H	PA	25	0.16	Small	Broad	Wide
Unknown 21	Unknown	C	ATw	4	0.03	Small	Broad	Wide
Unknown 22	Papilionaceae	C	ATw	2	0.01	Small	Broad	Wide
Unknown 23	Papilionaceae	C	ATw	8	0.05	Large	Broad	Wide
Unknown 24	Asteraceae	H	EA	4	0.03	Large	Broad	Wide
Unknown 25	Papilionaceae	C	ATw	2	0.01	Large	Broad	Wide
Unknown 27	Acanthaceae	H	EA	17	0.11	Small	Broad	Wide
Unknown 28	Unknown	H	EA	5	0.03	Large	Broad	Wide
Unknown 29	Unknown	H	PA	4	0.03	Small	Broad	Wide
Unknown 30	Unknown	H	EA	2	0.01	Small	Broad	Wide

(Received for publication 31 January 2015; accepted 10 August 2015)

Tropical Grasslands – Forrajes Tropicales (2015) Volume 3, 187−193
DOI: 10.17138/TGFT(3)187-193

Nutritive value and in situ rumen degradability of Marandu palisade grass at different locations within the pasture in a silvo-pastoral system with different babassu palm densities

XERXES M. TOSTA, ROSANE C. RODRIGUES, SÂMARA S.C. SANCHÊS, JOCÉLIO S. ARAÚJO, ANTÔNIO DA S. LIMA JÚNIOR, CLÉSIO S. COSTA, FRANCISCO N. DE S. SANTOS, ANA P.R. JESUS, IVONE R. DA SILVA, FRANCIVALDO O. COSTA, FRANCIROSE SHIGAKI AND SANAYRA DA S. MENDES

Universidade Federal do Maranhão, Centro de Ciências Agrárias e Ambientais, Chapadinha, MA, Brazil.
www.ccaa.ufma.br

Keywords: Digestibility, fiber, Northeast Brazil, protein, tree-grass associations, *Urochloa brizantha*.

Abstract

The objective of this study was to evaluate the nutritive value and in situ rumen degradability of grass collected from different locations within the pasture in a silvopastoral system with different densities of trees. The silvopastoral system consisted of *Urochloa* (syn. *Brachiaria*) *brizantha* cv. Marandu and the babassu palm, *Orbignya* sp. (now: *Attaleia speciosa*). We used a completely randomized design with a 3 x 3 factorial arrangement for nutritional value (3 differently shaded locations and 3 palm tree densities) and a 3 x 3 x 3 factorial arrangement for dry matter (DM) disappearance (3 locations, 3 palm densities and 3 incubation times). There was no effect of location within the pasture nor of palm tree density on the concentrations of NDF, ADF, lignin, cellulose and hemicellulose. However, location influenced the concentrations of crude protein (CP) and DM, with highest CP in material grown in full sunlight. At all densities, DM disappearance at 96 h for pasture grown in full sunlight exceeded that for pasture grown in full shade. These factors need to be compounded with the possible depressant effect of trees on DM production of pasture when considering the benefits of silvopastoral systems.

Resumen

En el estudio se evaluó el valor nutritivo y la degradabilidad ruminal (digestibilidad) in situ de *Urochloa brizantha* (sin. *Brachiaria brizantha*) cv. Marandu en un sistema silvopastoril con diferentes densidades de árboles de la palma babasú (*Orbignya* sp., ahora: *Attaleia speciosa*). El diseño experimental para el valor nutritivo de la gramínea fue completamente al azar con arreglo factorial 3 x 3 [3 ubicaciones (sitios de muestreo) dentro del sistema: pleno sol, parcialmente sombreado y sombra total; y 3 densidades de palma]; y un arreglo factorial 3 x 3 x 3 para degradabilidad de la materia seca (3 ubicaciones, 3 densidades y 3 tiempos de incubación: 6, 24 y 96 horas). No se encontraron efectos del sitio de muestreo dentro del sistema ni de la densidad de las palmas en los porcentajes de NDF (fibra detergente neutro), ADF (fibra detergente ácido), lignina, celulosa y hemicelulosa. No obstante el sitio de muestreo dentro del sistema influyó en las concentraciones de proteína cruda (PC) y materia seca (MS), siendo más alta la PC en la gramínea que creció a pleno sol. En todas las densidades, la degradabilidad de la MS a 96 horas para la gramínea a plena luz fue más alta que a la sombra total. Al considerarse los beneficios de los sistemas silvopastoriles, estos aspectos de valor nutritivo deben ser tenidos en cuenta, junto con un posible efecto negativo de los árboles en la producción de MS del pasto.

Correspondence: Rosane Cláudia Rodrigues, Universidade Federal do Maranhão, BR 222, km 04, Boa Vista, Chapadinha CEP 65500-000, MA, Brazil.
E-mail: rosanerodrig@gmail.com

Introduction

In Brazil, pasture consisting mostly of tropical grasses, is the main inexpensive feed source for ruminants that are able to convert fibrous biomass, which is of no nutritional value to humans, into valuable animal protein. Whereas livestock production takes place mainly in pasture-only systems, there is increasing interest in systems that include a crop and/or tree component (Paciullo et al. 2007).

Maranhão State, particularly, has a huge diversity of vegetation, ranging from Cerrado (center, south and east of the state) to Amazonian vegetation in the west. Despite this variability of vegetation, the native palm tree, babassu (*Orbignya* sp., now *Attaleia speciosa*), occurs in 80% of the state and has immense economic and social importance through the byproducts it yields (Matos et al. 2010).

Silvopastoral systems are common, where there is the intentional combination of trees, pasture and cattle, all managed in an integrated manner (Embrapa 2011). In this system, it is essential to understand the interactions of animals, forage plants and trees, aiming to plan intercropping to minimize the negative effects and enhance the gains related to the specific interactions of the various factors in the system (Andrade et al. 2001, 2004; Araújo et al. 2013). With respect to grazing in silvopastoral systems, Castro et al. (1999) emphasized the need for more information on the main tropical grasses such as *Brachiaria*, as chemical composition and digestibility are affected by low light.

In this context, knowledge of the nutritive value and digestibility of the forage at different locations within the pasture in silvopastoral systems with babassu palm can provide the basis for developing management strategies to increase animal production. Thus, the present study aimed to evaluate the nutritive value and in situ digestibility of Marandu grass at different locations within the pasture in a silvopastoral system. Given the economic and social importance of the native babassu palm in the region (Matos et al. 2010), we took advantage of availability of experimental paddocks with different tree densities to furthermore consider any additional effects, such as from competition for nutrients, soil moisture etc., on pasture quality.

Materials and Methods

Study site

The study was conducted at the Água Viva Farm, in the municipality of Matinha, Maranhão State (02º59'35" S, 45°06'25" W). The pasture species was *Brachiaria brizantha* (now: *Urochloa brizantha*) cv. Marandu and the tree species was the babassu palm, *Orbignya* sp. (now: *Attaleia speciosa*). This grass-tree system was already established on the property and was under grazing. The grass was collected from exclusion cages.

In the rainy period of 2012 (March–July), the pasture was mowed before application of dolomitic limestone (1 t/ha) and fertilization with nitrogen as urea (60 kg N/ha) and phosphorus as superphosphate (12 kg P/ha).

Experimental setup

Treatments consisted of 3 densities of babassu palm per hectare (low, medium and high; LDP, MDP and HDP) corresponding with 39, 72 and 92 trees per hectare, respectively, and 3 locations within the pasture: no interference from palm tree shade (full sunlight, WIS), intermediate positioning between unshaded and shaded locations (partial sun, MID) and under the palm trees in full shade (full shade, IWS), in a 3 x 3 factorial arrangement with 3 replications in a randomized design. For dry matter disappearance (DDM), we used a completely randomized design with a 3 x 3 x 3 factorial arrangement (3 densities of palm trees, 3 sampling sites and 3 incubation times).

The shaded (IWS) area was the area around the palm tree, i.e. the exclusion cage (0.5 x 0.5 m) was placed next to the base of the palm tree; in the MID area, at some point in the day the palm tree shadow was projected onto the exclusion cage; and at full sun (WIS), at no time was the palm tree shadow projected onto the exclusion cage.

Measurements

At the beginning of the experiment, pastures had an average height of 60 cm. Samples were collected from 28-day-old regrowth by cutting the grass to 5 cm above ground level, within exclusion cages placed at 28-day intervals in the paddocks according to the particular treatment, when the cages were relocated to a new spot. Samples were collected during the dry period of 2012 (November 2012–January 2013). Samples of each treatment were mixed and chemical analyses conducted. Data presented result from the average of the experimental period.

Samples were pooled in their respective treatments for chemical analysis. In the laboratory, samples were weighed and oven-dried at 55 °C to constant weight. Afterwards, samples were ground in a Wiley mill for determination of concentration of dry matter (DM), neutral detergent fiber (NDF), acid detergent fiber (ADF), lignin, crude protein (CP), cellulose and hemicellulose, according to Silva and Queiroz (2002).

For in situ degradability, and following the suggestions of Tomich and Sampaio (2004), one male sheep, castrated and cannulated in the rumen, with an average body weight of 60 kg, was fed the forage and mineral salt, with water ad libitum. Incubation times used were: 6, 24 and 96 hours, according to the methodology of Sampaio et al. (1995). For each incubation time, 18 nylon bags (each with 5 g of ground sample material) were inserted in the rumen, representing the 3 tree densities x 3 locations x 2 replications. Bags for the various incubation times were inserted progressively at the appropriate times and all were removed at the same time. Bags were 14 x 19 cm, with 50 µm porosity. After the incubation periods, bags were removed and immediately washed, oven-dried at 50 °C for 48 h, weighed and the residue analyzed for DM, CP, NDF and ADF (Silva and Queiroz 2002).

In order to determine the loss of material at time zero, bags containing samples were washed in cold water. After washing, the bags were subjected to the same procedures as incubated bags. In situ rumen degradability (“digestibility”) was measured as percentage of dry matter disappearance (DDM), calculated over each time interval by the proportion of forage that disappeared from the bags after incubation in the rumen.

To evaluate the parameters of DDM, we used the Brody model according to the equation of Orskov and McDonald (1979), modified by Sampaio (1988):

%Deg DM = A - Bexp (-c*Time)

where:

A = potential degradability of the forage, without time for colonization, i.e. if the Deg% at time zero was 0%;
B = percentage of degraded material deposited in the rumen without time for colonization; and
c = degradation rate constant of the material remaining in the rumen at any incubation time.

Effective DM degradability (DE) was calculated assuming 3 ruminal passage rates (2, 5 and 8%/h) through the equation described by Orskov and McDonald (1979):

DE = a' + (b'*c/c+k)

where:

a' = % disappearance at time zero (mean);
b' = A - a';
c = degradation rate constant; and k = passage rate.

Statistical analysis

Data were first subjected to normality (Cramér-von Mises test) and homogeneity of variances (Levene test). Data met the assumptions and were subjected to analysis of variance. Statistical analyses were run considering a significance level of 5%, using the GLM procedure of SAS 9.0 (2002).

Results

The chemical composition of forage samples is shown in Table 1. Location within the pasture and palm density had no significant (P>0.05) effects on NDF, ADF, lignin, cellulose and hemicellulose concentrations in the forage. Regarding CP, the only significant effect was that of location (P<0.05): regardless of palm tree density, pasture grown in full sunlight (WIS) showed the highest CP concentration and that in full shade (IWS) the lowest.

Significant interactions were detected between density of palm trees and location within the pasture (P<0.05) for DM percentage in forage. In general, pasture grown in full shade had higher DM concentration than that grown in either partial sun or full sunlight (P<0.05). For pasture grown in partial sun, the DM concentration was higher at the highest palm density (P<0.05).

At all densities of palm trees in the pasture and locations within the pasture, DDM increased progressively with time (P<0.05) (Table 2). This result is associated with the time necessary for the attachment of ruminal microorganisms to fiber particles in the rumen and for digestion to commence.

At each time of incubation and for each location in the pasture, DDM increased as the density of trees increased. However, the differences were inconsistent across treatments, being significant (P<0.05) only for WIS at 6 h incubation, IWS at 24 h and MID at 96 h (Table 2). In situ rumen degradability (Table 3) of all variables had a coefficient of determination (R^2) above 93%, which indicates that the non-linear model used by Sampaio et al. (1995) has satisfactorily fitted the data for DDM.

Table 1. Chemical composition (% DM) of Marandu palisade grass grown at different locations (WIS – full sunlight; MID − intermediate location between shade and sun; and IWS – full shade) within the pasture in silvopastoral systems with different densities of babassu palm (LDP – low; MDP – medium; and HDP – high).

Nutritive value	Location	Density of babassu palms			CV (%)	P-value Location	P-value Density	P-value Location x Density
		LDP	MDP	HDP				
Neutral detergent fiber (NDF)					2.97	0.62	0.23	0.29
	WIS	75.5	71.1	75.0				
	MID	74.4	72.6	73.1				
	IWS	72.4	73.3	72.9				
Acid detergent fiber (ADF)					4.69	0.59	0.44	0.92
	WIS	61.3	59.2	59.9				
	MID	60.8	57.8	58.9				
	IWS	58.9	58.9	58.7				
Lignin					17.70	0.91	0.25	0.55
	WIS	7.83	6.60	8.21				
	MID	7.84	6.63	8.18				
	IWS	7.94	7.91	7.30				
Cellulose					5.23	0.82	0.40	0.99
	WIS	53.4	51.8	51.7				
	MID	53.0	51.5	51.4				
	IWS	52.3	50.9	50.7				
Hemicellulose					11.58	0.86	0.57	0.24
	WIS	14.3	12.0	15.0				
	MID	13.6	14.8	14.2				
	IWS	13.5	14.4	14.8				
Crude protein					11.08	<0.0001	0.14	0.99
	WIS	5.54Aa[1]	5.98Aa	5.83Aa				
	MID	4.67Aab	5.25Aab	4.96Aab				
	IWS	3.94Ab	4.52Ab	4.08Ab				
Dry matter (% fresh weight)					16.28	<0.0001	0.04	0.02
	WIS	34.8Ab	30.8Ab	37.9Ab				
	MID	38.7Bb	31.2Bb	52.4Aa				
	IWS	58.4Aa	56.9Aa	56.9Aa				

[1]Means within rows followed by the same upper-case letter, and within columns followed by the same lower-case letter are not significantly different by Duncan's test at 5% probability.

Table 2. Mean values for dry matter disappearance (%) of Marandu palisade grass grown at different locations (WIS – full sunlight; MID − intermediate location between shade and sun; and IWS – full shade) within the pasture in silvopastoral systems with different densities of babassu palm (LDP – low; MDP – medium; and HDP − high). CV (%) = 7.78.

Location	Density of babassu palms	Incubation time		
		6 h	24 h	96 h
WIS	LDP	29.2Cβ[1]	47.7Bα	68.8Aα
MID		32.4Cα	48.3Bα	64.8Aβ
IWS		29.2Cα	43.3Bβ	65.0Aα
WIS	MDP	35.2Cαβ	50.7Bα	71.4Aα
MID		32.4Cα	49.4Bα	68.3Aαβ
IWS		31.0Cα	47.5Bαβ	67.4Aα
WIS	HDP	36.9Cα	51.9Bα	72.6Aα
MID		36.2Cα	51.7Bα	72.6Aα
IWS		32.8Cα	51.3Bα	69.1Aα

[1]Means within rows followed by the same upper-case letter (comparing times of incubation) and within columns followed by the same Greek letter (comparing densities within location and time of incubation) are not significantly different by Duncan's test at 5% probability.

Table 3. Parameters of in situ rumen degradability of dry matter of Marandu palisade grass grown at different locations (WIS – full sunlight; MID – intermediate location between shade and sun; and IWS – full shade) within the pasture in silvopastoral systems with different densities of babassu palm (LDP – low; MDP – medium; and HDP – high).

Location	Density of babassu palms	Potential degradability (A)	Rumen degradable fraction (B)	Degradation rate constant (c)	R^2	Effective dry matter degradability (DE) for passage rates (%/h) of:		
						2%	5%	8%
WIS	LDP	71.4	78.5	3.8	98.8	52.5	40.2	34.2
MID		66.7	73.0	4.7	98.3	51.3	40.1	34.2
IWS		70.3	72.7	2.7	98.6	50.4	40.0	35.4
WIS	MDP	71.8	56.4	2.5	97.8	46.4	33.7	28.2
MID		75.2	62.0	2.4	98.4	57.1	50.0	47.2
IWS		74.3	63.2	1.9	98.4	47.2	33.6	27.8
WIS	HDP	74.6	60.2	2.9	98.3	49.0	34.9	28.5
MID		72.8	60.9	3.2	98.2	51.5	39.0	33.2
IWS		71.4	64.6	3.6	98.2	50.8	37.9	31.7

Discussion

Location (sampling site) within the silvopastoral systems containing different densities of palm trees showed little influence on the nutritive value of Marandu palisade grass, except for crude protein concentration. In spite of the young age of the samples (28 d), all pasture produced had unexpectedly low CP concentration (3.9−6.0%) which was possibly influenced by the dry season. Pasture grown in full sunlight was of higher quality than that grown in full shade (5.8 vs. 4.2% CP, $P<0.05$). These values are lower than the level of 7% recommended for appropriate rumen fermentation. Azar (2011) evaluated the nutritional value of Marandu palisade grass in a silvopastoral system and observed a higher CP concentration in pasture in full sunlight grown in association with trees than in grass grown in a monoculture, in both rainy and dry periods. The pastures in that study were irrigated during the dry season. This suggests that recycling of nutrients by the trees might have made more nutrients available to the pasture. However, Paciullo et al. (2007), working with *B. decumbens*, in conditions of full sun and shade, found no interaction between light condition and the chemical composition of the plant.

Competition for moisture and nutrients between the pasture and palm trees would have played a part in production, especially during the dry season. During this time pasture around the babassu palms had the appearance of standing hay, indicating stress being experienced by the grass. The results point to the great competitive strength of babassu palm, because it occupies the same underground niche, exploring the same resources as the grass, and thus enters into direct competition with pasture. This was evidenced by the highest DM content of pasture in fully shaded positions.

We could find no general consensus in the literature on the effect of shading in relation to the nutritional value of tropical forages. However, some Brazilian reports dealing with Marandu grass agree that there is no major shade effect: Reis et al. (2013) evaluated the influence of nitrogen fertilization and artificial shading on chemical variables of Marandu grass and also found no effect of shading on the NDF concentration. Similar results were achieved by Lacerda et al. (2009) and Reis et al. (2011) in pastures of gamba grass (*Andropogon gayanus*) and Marandu with natural shading, respectively.

According to Van Soest (1965), concentrations of NDF above 55% may negatively influence voluntary intake and thus impair animal performance. Pasture from all locations in our study had NDF levels above 70%, indicating that voluntary intake would be affected and supplements would need to be fed in conjunction with the forage to achieve good animal performance.

Reis et al. (2013) found that shading and nitrogen fertilization caused a reduction in ADF concentration. In contrast, Sousa et al. (2010) registered the opposite result, with values for ADF being higher in shaded areas due to stem elongation and greater height of the canopy.

There is no consensus on the effect of shading on the lignin content in forages. Plants grown in shade tend to have higher lignin content compared with those experiencing no restriction of light, but shaded plants have lower physiological age, which can result in lower levels of lignin (Reis et al. 2013).

It is noteworthy that there was no significant effect of babassu palm density on any of the nutritive value factors analyzed, except for DM %. Apparently there were no major competitive effects.

Moreira et al. (2009) examined the in situ dry matter degradability of Marandu grass harvested from 2 silvopastoral systems containing the trees, *Zeyheria tuberculosa* and *Myracrodruon urundeuva*, in Cerrado and found no difference in DDM between forage grown in the shade of these trees and that grown in grass monoculture. The values we found for DDM at 96 hours digestion were similar to the 75.5 and 74.4% reported by Castro et al. (2009) for *B. brizantha* cv. Marandu harvested at 28 and 56 days of age, respectively. Similarly, Rodrigues et al. (2004) studied 3 accessions of *B. brizantha* aged 21 and 42 days and found average values for DM degradation of 77.4 and 76.3%, respectively.

Furthermore, for all situations the effective degradability values tended to decrease with the increase in passage rate. This would be due to the effects on action of ruminal microorganisms, which is affected by the time spent in the rumen, since the slower the passage rate of this fibrous material through the rumen the greater the action of microorganisms, thereby influencing the effective digestibility.

The effective degradability (DE) values of DM for the passage rate of 2%/h were lower than those reported by Rodrigues et al. (2004), who worked with 3 accessions of *B. brizantha* harvested at 21 days of age and found a DE around 56.7%, similar to the values reported by Moreira et al. (2009) with Marandu palisade grass grown in silvopastoral systems.

For the 5%/h passage rate, DE values of DM registered in our experiment were higher than those obtained by Moreira et al. (2009) for Marandu (34.9−37%) and lower than those reported by Castro et al. (2009) when working with *B. brizantha* harvested at 28 and 56 days of age (46.8 and 43.8%, respectively).

Conclusions

This study has shown that palm density in a silvopastoral system had little influence on nutritive value of the associated Marandu palisade grass, but shade-affected locations within the system had effects on protein concentration in the pasture. Since material grown in full sunlight had higher CP concentrations than that grown in full shade, forage quality could be expected to decline as trees matured and spread more shade over the pasture. This effect would need to be compounded with the reduced DM production of pasture in a silvopastoral system relative to monoculture, when assessing the benefits of these systems.

Acknowledgments

To FAPEMA (Fundação de Amparo à Pesquisa e ao Desenvolvimento Científico e Tecnológico do Maranhão) for financial support and to the Study and Research Group, FOPAMA, for their help in conducting the experiment in the field and assistance in the laboratory.

References

Andrade CMS; Garcia R; Couto L; Pereira OG. 2001. Fatores limitantes ao crescimento do capim-tanzânia em um sistema agrossilvipastoril com eucalipto, na região dos Cerrados de Minas Gerais. Revista Brasileira de Zootecnia 30:1178–1185. DOI: 10.1590/S1516-35982001000500007

Andrade CMS; Valentim JF; Carneiro JC; Vaz FA. 2004. Crescimento de gramíneas e leguminosas forrageiras tropicais sob sombreamento. Pesquisa Agropecuária Brasileira 39:263–270. DOI: 10.1590/S0100-204X2004000300009

Araújo RP; Almeida JCC; Araújo SAC; Ribeiro ET; Pádua FT; Carvalho CAB; Bonaparte TP; Dominicis BB; Lista FN. 2013. Produção e composição química de *Brachiaria decumbens* cv. Basilisk em sistema silvipastoril sob diferentes espaçamentos com *Eucalyptus urophylla* S.T. Blake. Revista Brasileira de Agropecuária Sustentável 3:90–98. www.rbas.com.br/artigos.php?id=5

Azar GS. 2011. Características do capim-Marandu e do solo em sistemas de monocultura e silvipastoril com coqueiros. Ph.D. Thesis. Universidade Federal do Piauí, Teresina, PI, Brazil.

Castro CRT; Garcia R; Carvalho MM; Couto L. 1999. Produção forrageira de gramíneas cultivadas sob luminosidade reduzida. Revista Brasileira de Zootecnia 28:919–927. DOI: 10.1590/S1516-35981999000500003

Castro CRT; Paciullo DSC; Gomide CAM; Muller MD; Nascimento Júnior ER. 2009. Características agronômicas, massa de forragem e valor nutritivo de *Brachiaria decumbens* em sistema silvipastoril. Pesquisa Florestal Brasileira 60:19–25. DOI: 10.4336/2009.pfb.60.19

Embrapa. 2011. Sustentabilidade da pecuária através de sistemas silvipastoris no estado do Pará. Empresa Brasileira de Pesquisa Agropecuária, Embrapa Amazônia Oriental, Belém, PA, Brazil.

Lacerda MSB; Alves AA; Oliveira ME; Rogério MCP; Carvalho TB; Veras VS. 2009. Composição bromatológica e produtividade do capim-andropógon em diferentes idades de rebrota em sistema silvipastoril. Acta Scientiarum. Animal Sciences 31:123–129. DOI: 10.4025/actascianimsci. v31i2.4549

Matos DGP; Bernal FSM; Farias Filho MS. 2010. A importância social e econômica do extrativismo do babaçu na microrregião de Itapecuru-mirim, estado do Maranhão. Anais do XVI Encontro Nacional de Geógrafos. Crise, práxis e autonomia: Espaços de resistência e de esperanças. Porto Alegre, RS, Brazil.

Moreira GR; Saliba EOS; Maurício RM; Sousa LF; Figueiredo MP; Gonçalves LC; Rodriguez MN. 2009. Avaliação da *Brachiaria brizantha* cv. marandu em sistemas silvipastoris. Arquivo Brasileiro de Medicina Veterinária e Zootecnia 61:706–713. DOI: 10.1590/S0102-09352009000300026

Orskov ER; McDonald I. 1979. The estimation of protein degradability in the rumen from incubation measurements weighted according to rate of passage. Journal of Agricultural Science 92:499–503. DOI: 10.1017/S0021859600063048

Paciullo DSC; Carvalho CAB; Aroeira LJM; Morenz MJF; Lopes FCF; Rossiello ROP. 2007. Morfofisiologia e valor nutritivo do capim-braquiária sob sombreamento natural e ao sol pleno. Pesquisa Agropecuária Brasileira 42:573–579. DOI: 10.1590/S0100-204X2007000400016

Reis GL; Lana AMQ; Maurício RM; Borges I; Moreira GHFA; Lana RMQ; Sousa LF; Neto TQ. 2011. Influence of a silvopastoral system on forage parameters in the Brazilian savanna. Revista Brasileira de Agropecuária Sustentável 1(1):179–190. http://goo.gl/zkUnFm

Reis GL; Lana AMQ; Emerenciano Neto JV; Lemos Filho JP; Borges I; Longo RM. 2013. Produção e composição bromatológica do capim-marandu, sob diferentes percentuais de sombreamento e doses de nitrogênio. Bioscience Journal 29:1606–1615. http://goo.gl/t6hhdP

Rodrigues ALP; Sampaio IBM; Carneiro JC; Tomich TR; Martins RGR. 2004. Degradabilidade *in situ* da matéria seca de forrageiras tropicais obtidas em diferentes épocas de corte. Arquivo Brasileiro de Medicina Veterinária e Zootecnia 56:658–664. DOI: 10.1590/S0102-09352004000500014

Sampaio IBM. 1988. Experimental designs and modeling techniques in the study of roughage degradation in rumen and growth of ruminants. Ph.D. Thesis. University of Reading, Berkshire, UK.

Sampaio IBM; Pike DJ; Owen E. 1995. Optimal design for studying dry matter degradation in the rumen. Arquivo Brasileiro de Medicina Veterinária e Zootecnia 47:373–383.

Silva DJ; Queiroz AC. 2002. Análise de alimentos: Métodos químicos e biológicos. 3rd Edn. Universidade Federal de Viçosa (UFV), Viçosa, MG, Brazil.

Sousa LF; Maurício RM; Moreira GR; Gonçalves LC; Borges I; Pereira GB. 2010. Nutritional evaluation of "Braquiarão" grass in association with "Aroeira" trees in a silvopastoral system. Agroforestry Systems 79:189–199. DOI: 10.1007/s10457-010-9297-8

Tomich TR; Sampaio IBM. 2004. A new strategy for the determination of forage degradability with an *in situ* technique through the use of one fistulated ruminant. The Journal of Agricultural Science 142:589‒593. DOI: 10.1017/S0021859604004654

Van Soest PJ. 1965. Symposium on factors influencing the voluntary intake of herbage by ruminants: Voluntary intake relation to chemical composition and digestibility. Journal of Animal Science 24:834–844. DOI: 10.2134/jas1965.243834x

(Received for publication 26 June 2014; accepted 14 August 2015)

DOI: 10.17138/TGFT(3)194-204

Esterase polymorphism for genetic diversity analysis of some accessions of a native forage grass, *Mesosetum chaseae* Luces, from the Brazilian Pantanal

ANA CLARA S. MEIRELLES[1], ELIANE R. MONTEIRO[2], LAURA A.C. DA SILVA[2], DÉBORA DA SILVA[2], SANDRA A. SANTOS[3], SANDRA A. DE OLIVEIRA-COLLET[4], CLAUDETE A. MANGOLIN[4] AND MARIA DE FÁTIMA P.S. MACHADO[4]

[1]*Postgraduate Program in Agronomy, Universidade Estadual de Maringá, Maringá, PR, Brazil.* www.dbc.uem.br
[2]*Postgraduate Program in Genetics and Breeding, Universidade Estadual de Maringá, Maringá, PR, Brazil.* www.dbc.uem.br
[3]*Empresa Brasileira de Pesquisa Agropecuária, Embrapa Pantanal, Corumbá, MS, Brazil.* www.embrapa.br/pantanal
[4]*Departamento de Biotecnologia, Genética e Biologia Celular, Universidade Estadual de Maringá, Maringá, PR, Brazil.* www.dbc.uem.br

Keywords: Genetic polymorphism, genetic resources, grama-do-cerrado, isoesterases, Poaceae.

Abstract

The aim of the present study was to estimate the genetic diversity within the samples of *Mesosetum chaseae* from the Embrapa Pantanal Germplasm Bank (BAG) and assess how they are genetically structured to guide proposals to: 1) identify native forages for further testing to measure their suitability for sowing in conjunction with or as an alternative to exotic forages, mainly *Urochloa humidicola*; and 2) improve the species *M. chaseae* with samples that are maintained in the BAG. Isozyme α- and β-esterases were analyzed in 10 accessions collected from different locations in the Nhecolândia sub-region of the Pantanal, and maintained in the BAG. Accessions A11, which showed the highest effective number of alleles, and A32 with the highest average values of expected and observed heterozygosity, were identified as warranting further study as possible options for sowing as pasture forages, as well as for use in recovering poor and degraded areas in the Pantanal region. A high level of population differentiation was detected among the 10 accessions, indicating that they form genetically structured populations and that all accessions are important samples of *M. chaseae*, which should be maintained in the BAG. Crosses between sample plants with the highest genetic distances are recommended to implement improvement plans with a prospect of broadening the genetic base of the species.

Resumen

El objetivo del estudio fue estimar la diversidad genética entre las muestras de *Mesosetum chaseae* del Banco de Germoplasma (BAG) de Embrapa Pantanal y evaluar cómo están estructuradas genéticamente con el objeto de guiar propuestas para: 1) identificar un forraje nativo para estudios de su potencial como especie complementaria o alternativa a forrajes exóticos, sobre todo *Urochloa humidicola*; y 2) mejorar la especie *M. chaseae* con base en muestras disponibles en el BAG Pantanal. En un total de 10 muestras recolectadas en diferentes localidades de la subregión Nhecolândia del Pantanal, se analizaron las isoenzimas α- y ß-esterasas. Se identificaron las accesiones A11, que mostró el número efectivo más alto de alelos; y A32, que presentó los más altos valores promedio de heterocigosidad esperada y observada, como promisorias para trabajos de investigación adicionales con el objeto de desarrollar nuevas opciones tanto para pasturas como para recuperar áreas degradadas en la región del Pantanal. Se identificó un alto nivel de diferenciación poblacional entre las accesiones, lo que indica que las 10 accesiones estudiadas forman poblaciones genéticamente estructuradas y que todas son muestras importantes de *M. chaseae*, que por tanto deben ser conservadas en el Banco de Germoplasma de Embrapa Pantanal. Se recomiendan cruzamientos entre las muestras con las distancias genéticas más altas para implementar planes de mejoramiento con miras a ampliar la base genética de la especie.

Correspondence: M. de F.P.S. Machado, Department of Biotechnology, Genetics and Cell Biology, State University of Maringá, Maringá CEP 87020-900, PR, Brazil.
E-mail: mfpsmachado@uem.br

Introduction

The Pantanal region, located in Mato Grosso do Sul State (MS), Central Brazilian Plateau, is the largest wetland in the world and one of the most diverse nature reserves on the planet; it is considered as a World Biosphere Reserve and Natural Heritage by UNESCO. This region is characterized by large pasture fields, with 95% of its area occupied by extensive cattle farms (Godoi Filho 1986). While many exotic species have been introduced to increase productivity, little effort has been made to understand and estimate the potential use of native species such as the grass *Mesosetum chaseae* Luces (Poaceae family). The most widely sown exotic forage has been the African grass *Urochloa humidicola* (syn. *Brachiaria humidicola*) due to its tolerance of poor soils, flooding and trampling, resulting in increases in carrying capacity of the land and farm profitability. However, monocultures in plant communities often result because of the exclusive use of this species, combined with its ability to spread in different landscapes of the Pantanal (Santos et al. 2011). Although scientific research has shown that the effect of *U. humidicola* on plant diversity is influenced by season and topography in areas experiencing seasonal flooding (Bao et al. 2015), the situation can be different in flood-free areas with soil fertility problems, mainly savannas (Pivello et al. 1999), because few plant species are adapted to such conditions, mainly native grasses (Santos et al. 2011).

Mesosetum chaseae is an important native forage plant in the Pantanal region, and is found mainly in flood-free and temporarily flooded grasslands, showing habitat plasticity and vegetative propagation in these environments (Santos et al. 2002; Alvarez et al. 2004). It is known as "grama-do-cerrado" (grass of the savanna) and is one of the most prominent forage species owing to its productivity (Santos 2001), acceptability by animals (Santos et al. 2002) and drought resistance (Santos et al. 2005). The "grama-do-cerrado" displays good tillering and grows well on poor soils. As it is prevalent under extreme climatic conditions (e.g. severe drought), this species has been considered to have potential for reclaiming degraded areas in the Pantanal. Annual aboveground dry matter production of 2,000-3,800 kg/ha has been recorded under good conditions (Santos et al. 2011), and Pinheiro et al. (2005) showed that *M. chaseae* retained forage quality during ripening and could be used for hay-making.

Studies on quality of seeds, cytogenetics and reproduction (Silva et al. 2011; 2012; 2013) represent the few investments that have been recently made for the characterization of *M. chaseae*. In the literature we found no records of molecular level studies. Investigations of genetic divergence at the molecular level of this species, however, are important to guide proposals to: 1) identify native forages for further testing to measure their suitability for sowing with or as an alternative to exotic forages, mainly *Urochloa humidicola*; and 2) implement plans for improvement of *M. chaseae* with samples maintained in the Germplasm Bank (Banco Activo de Germoplasma, BAG) of Embrapa Pantanal (Empresa Brasileira de Pesquisa Agropecuária in Corumbá, MS, Brazil). The current study aimed to make a start to document the genetic diversity of the species, and check how the accessions of *M. chaseae* from the BAG of Embrapa Pantanal are genetically structured to guide further testing in the field and future breeding programs. If suitable accessions can be identified it could provide a platform for promoting the conservation of biodiversity in pastures and the sustainability of native forage resources. Genetic polymorphism in loci for α- and β-esterase isozymes was analyzed to estimate genetic diversity and structure of the *M. chaseae* accessions.

Material and Methods

Samples of *M. chaseae* were collected from different locations in Nhecolândia, a sub-region of the Brazilian Pantanal, and are maintained in the germplasm collection at Embrapa Pantanal (Figure 1). Table 1 indicates the original collection sites according to the Universal Transverse Mercator (UTM) system.

Seeds from each *M. chaseae* accession were collected and distributed for germination in 500-mL pots containing sterile soil. The resulting plants were kept at room temperature, watered daily and used for the study. Leaves were collected from 10–30 plants of each accession; the plants were individually evaluated through electrophoresis. Leaf pieces (200 mg) from each plant were homogenized with a glass rod in an Eppendorf micro-centrifuge tube with the use of 60 μL extraction solution prepared with 1.0 M phosphate buffer pH 7.0, containing 5% PVP-40, 1.0 mM EDTA, 0.5% β-mercaptoethanol and 10% glycerol solution and maintained in a cold chamber. After homogenization, the samples were centrifuged at 25,000 rpm (48,200 x g) for 30 min, at 4 °C, in a Juan 23 MR*i* (Thermo Scientific, USA) centrifuge, and the supernatant was used for each sample.

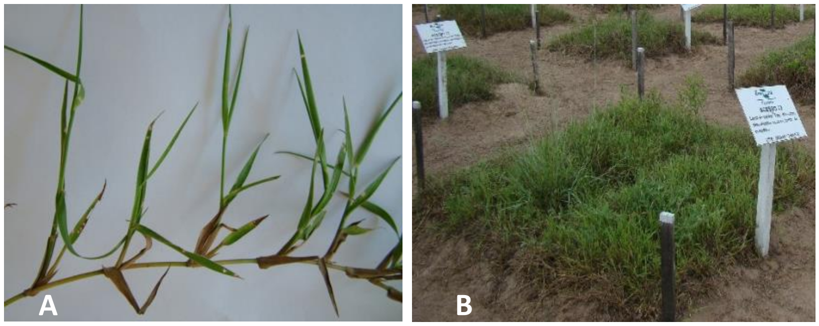


Figure 1. Tillers of *Mesosetum chaseae* (A) and accessions (B) maintained in the germplasm collection of Embrapa Pantanal, Nhumirim Farm, Nhecolândia sub-region of the Pantanal (Corumbá, MS, Brazil).

Table 1. Accessions of *Mesosetum chaseae* maintained in the germplasm collection of Embrapa Pantanal, location of the collection, habitat and geographic coordinates.

Accession	Location of the collection	Habitat	Geographic coordinates
A1	Nhumirim Farm wintering area 2	Non-flooded open grassland	19°00'04"S 56°37'09"W
A4	Campo Dora Farm wintering area 10	Seasonally flooded open grassland	18°58'55"S 56°40'55"W
A5	Chatelodo Farm wintering area 10	High open grassland	19°04'37"S 56°40'23"W
A8	Valdir's Field wintering area 10	Non-flooded savanna	18°56'12"S 56°36'56"W
A9	Valdir's Field wintering area 10	Edge of woodland	18°56'09"S 56°36'57"W
A11	Nhumirim Farm Reservation	Non-flooded open grassland	18°58'57"S 56°40'55"W
A17	Nhumirim Farm Reservation	Savanna grassland	19°04'26"S 56°40'19"W
A24	Nhumirim Farm Reservation	Non-flooded open grassland	18°58'03"S 56°37'36"W
A31	Nhumirim Farm Reservation	Savanna grassland	18°58'24"S 56°33'21"W
A32	Nhumirim Farm Reservation	Non-flooded open grassland	19°00'12"S 56°36'24"W

Polyacrylamide gels (12%) were used to analyze the esterase isozymes (EST; EC 3.1.1) according to the method of Frigo et al. (2009). The polyacrylamide gel was prepared with 0.375 M Tris-HCl, pH 8.8 as buffer. A 6.2-mL volume of acrylamide/bis-acrylamide solution (30 g acrylamide and 0.8 g bis-acrylamide dissolved in 100 mL of double-distilled water), 4.0 mL 1.5 M Tris-HCl, pH 8.0, 6.2 mL double-distilled water, 320 μL ammonium persulfate 2% and 16 μL TEMED [Tetramethylethylenediamine] were used to separate the gel. The stacking gel was prepared with 3.0 mL acrylamide/bis-acrylamide (5 g acrylamide and 0.25 g bis-acrylamide dissolved in 50 mL double-distilled water), 3.0 mL 0.24 M Tris-HCl, pH 6.8, 30 mL double-distilled water, 250 μL ammonium persulfate (2%) and 3 μL TEMED.

The electrophoresis (PAGE, polyacrylamide gel electrophoresis) was performed for 5 h at 4 °C, and a constant voltage of 200 V. The running buffer was 0.125 M Tris/0.0959 M glycine, pH 8.3.

The esterases were identified through procedures previously described by Sala et al. (2011). The gels were soaked for 30 min in 50 mL 0.1 M sodium phosphate, pH 6.2, at room temperature. Esterase activity was visualized by placing the gels for 1 h in a staining solution prepared with 50 mL of sodium phosphate solution, 30 mg of β-naphthyl acetate, 40 mg of α-naphthyl acetate, 60 mg of Fast Blue RR salt and 5 mL of N-propanol. The polyacrylamide gels were dried as described by Sala et al. (2011), and kept at room temperature for 60 min in a mixture of 7.5% acetic acid and 10% glycerol embedded in 5% gelatin. They were then placed between 2 sheets of wet cellophane paper stretched on an embroidering hoop and left to dry for 24–48 h.

The genetic variability in the *M. chaseae* samples was analyzed with POPGENE 1.32 Computer Software (Yeh et al. 1999) for the analysis of allele frequencies, mean observed heterozygosity (H_o) and expected heterozygosity (H_e) and mean number of alleles per locus (N_a), effective number of alleles per polymorphic locus (N_e), percentage of polymorphic loci (%P), χ^2 test for deviation from Hardy-Weinberg equilibrium. The *F* statistic of Sewall Wright (Wright 1965), the deficit of heterozygotes (F_{IS} and F_{IT}), and the genetic diversity between the 10 samples (F_{ST}) were also estimated using POPGENE 1.32. To explore the hierarchical partitioning of genetic variation within and among the samples, we performed an Analysis of Molecular Variance

(AMOVA, GenAlEx 6.2; Peakall and Smouse 2006). The genetic identity (Nei 1973) and distances among the *M. chaseae* samples were calculated by UPGMA grouping. Polymorphisms from α/β-esterase loci were also analyzed using STRUCTURE software 2.0 (Pritchard and Wen 2003), which evaluates the level of genetic admixture between the 160 *M. chaseae* plants. The genotypes were clustered, with the number of clusters (K) ranging from 2 to 10, and were tested using the admixture model with a burn-in period of 5,000 repeats followed by 50,000 Markov Chain Monte Carlo (MCMC) repeats, considering the presence and absence of alleles across the sample. The true number of populations (K) is often identified using the maximal value of Δ (K) returned by the software. The most probable number (K) of sub-populations was identified as described by Evanno et al. (2005). The display of the graphical output of the results STRUCTURE was taken as input data using the STRUCTURE HARVESTER, a website and software that are used to visualize STRUCTURE outputs and to implement the Evanno method (Earl and von Holdt 2012) to display a graphical representation.

Results

Native PAGE analysis for esterase isozymes in leaves of *M. chaseae* recorded with α-naphthyl acetate and β-naphthylacetate displayed α/β-esterases produced from 4 clearly defined loci and isozymes with undefined polymorphism on the fifth locus (Figure 2). The α/β-esterases were numbered in sequence, starting from the anode, according to their decreasing negative charge. The number of loci and alleles were not identified in the case of esterase isozymes with lesser anodic migration. Three alleles were detected at the *Est-1* locus, 5 alleles at the *Est-2* and *Est-3* loci, and 4 alleles at the *Est-4* locus from the leaves of the *M. chaseae* samples; allele variations were not clarified for the *Est-5* locus. Allele frequencies were analyzed for *Est-1*, *Est-2*, *Est-3* and *Est-4* loci; the estimated proportion of polymorphic loci in the *M. chaseae* accession is 100%.

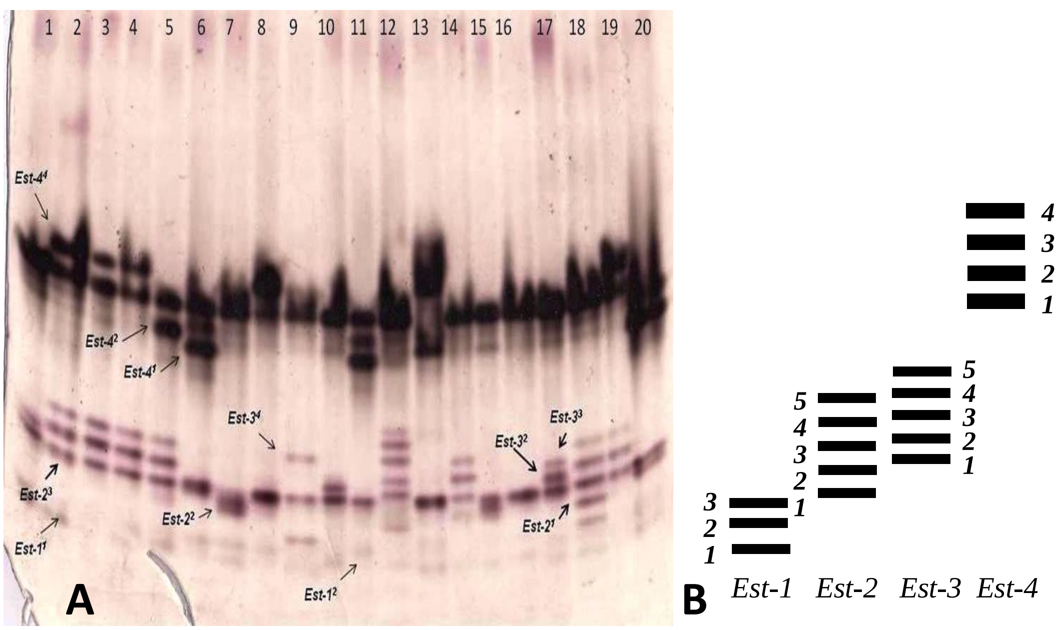


Figure 2. Polymorphism of α- and β-esterases detected in A4 (samples 1–10) and A8 (samples 11–20) accessions of *Mesosetum chaseae* produced from 4 esterase loci. Gel **A** with A4 samples: **1)** *Est-1*$^{1/2}$, *Est-2*$^{3/3}$, *Est-3*$^{1/2}$, *Est-4*$^{3/3}$; **2)** *Est-1*$^{1/1}$, *Est-2*$^{3/5}$, *Est-3*$^{4/4}$, *Est-4*$^{3/4}$; **3)** *Est-1*$^{1/2}$, *Est-2*$^{3/5}$, *Est-3*$^{4/4}$, *Est-4*$^{3/4}$; **4)** *Est-1*$^{1/2}$, *Est-2*$^{3/5}$, *Est-3*$^{4/4}$, *Est-4*$^{3/4}$; **5)** *Est-1*$^{2/2}$, *Est-2*$^{3/5}$, *Est-3*$^{4/4}$, *Est-4*$^{2/3}$; **6)** *Est-1*$^{1/2}$, *Est-2*$^{3/3}$, *Est-3*$^{4/4}$, *Est-4*$^{1/3}$; **7)** *Est-1*$^{1/2}$, *Est-2*$^{2/2}$, *Est-3*$^{1/1}$, *Est-4*$^{3/3}$; **8)** *Est-1*$^{2/2}$, *Est-2*$^{3/3}$, *Est-3*$^{1/1}$, *Est-4*$^{3/4}$; **9)** *Est-1*$^{1/3}$, *Est-2*$^{1/3}$, *Est-3*$^{4/4}$, *Est-4*$^{3/3}$; **10)** *Est-1*$^{1/1}$, *Est-2*$^{3/3}$, *Est-3*$^{2/2}$, *Est-4*$^{3/3}$. A8 samples: **11)** *Est-1*$^{2/2}$, *Est-2*$^{3/3}$, *Est-3*$^{1/1}$, *Est-4*$^{1/3}$; 1**2)** *Est-1*$^{1/1}$, *Est-2*$^{4/5}$, *Est-3*$^{4/5}$, *Est-4*$^{3/3}$; 1**3)** *Est-1*$^{1/2}$, *Est-2*$^{3/3}$, *Est-3*$^{1/1}$, *Est-4*$^{2/4}$; 1**4)** *Est-1*$^{1/1}$, *Est-2*$^{4/4}$, *Est-3*$^{3/4}$, *Est-4*$^{3/3}$; 1**5)** *Est-1*$^{1/1}$, *Est-2*$^{2/2}$, *Est-3*$^{1/1}$, *Est-4*$^{3/3}$; **16)** *Est-1*$^{1/2}$, *Est-2*$^{3/3}$, *Est-3*$^{1/1}$, *Est-4*$^{3/3}$; 1**7)** *Est-1*$^{1/2}$, *Est-2*$^{3/3}$, *Est-3*$^{2/3}$, *Est-4*$^{3/3}$; 1**8)** *Est-1*$^{1/3}$, *Est-2*$^{1/3}$, *Est-3*$^{3/4}$, *Est-4*$^{3/3}$; 1**9)** *Est-1*$^{1/2}$, *Est-2*$^{3/5}$, *Est-3*$^{4/4}$, *Est-4*$^{3/4}$; 2**0)** *Est-1*$^{1/3}$, *Est-2*$^{2/2}$, *Est-3*$^{1/1}$, *Est-4*$^{2/3}$. In **B**: the possible alleles in each *Est-1* (3), *Est-2* (5), *Est-3* (5) and *Est-4* (4) locus for α- and β-esterases detected in the 10 accessions of *M. chaseae*.

A comparison between the diversity parameters in the 10 accessions (Table 2) showed that the number of effective alleles is higher in A11 (N_e = 2.7383) than in the other *M. chaseae* accessions. The observed and expected mean heterozygosities were highest in accession A32 (H_o = 0.75; H_e = 0.5972). Departure from Hardy-Weinberg equilibrium was observed in 28 of 40 tests (70%) in 10 accessions, resulting from an excess of heterozygous samples at the *Est-1*, *Est-3* and *Est-4* loci (Table 3); the fixation index (F_{IS}) was negative at the *Est-1*, *Est-3* and *Est-4* loci. An excess of heterozygous plants (H_o>H_e) occurred in accessions A11, A17, A31 and A32 (Table 2). The deficit of heterozygous plants due to inbreeding within sub-populations was only 4.3% (F_{IS} = 0.0432) (Table 3).

A relatively high level of population differentiation was detected among the *M. chaseae* accessions (F_{ST} = 0.1693), which suggests that the 10 accessions form genetically structured populations (Table 3). The gene flow estimate calculated from F_{ST} (Nm) was also high (Nm = 1.2270) for the alleles from *Est-1*, *Est-2*, *Est-3* and *Est-4* loci. The UPGMA dendrogram obtained from the cluster analysis of Nei's (1978) unbiased genetic distance (Figure 3) reveals the formation of 2 main groups: 1 larger group was formed by 2 sub-groups comprising: i) accessions A1, A4, A5, A8 and A9, and ii) accessions A11 and A17; and the second group comprising accessions A31 and A32. Accession A24 formed an isolated group. Nei's identity (I) values ranged from 0.4839 (between accessions A9 and A11) to 0.9837 (between accessions A4 and A5) (Table 4). AMOVA showed higher genetic variation within (73%; Sum of Squares = 334,556; Variance Components = 2.23) than among (27%; Sum of Squares = 138,475; Variance Components = 0.834) the *M. chaseae* accessions.

The correlation between the genetic distance matrix and the cophenetic matrix calculated by the Mantel test (Rohlf 1989) was low (r^2 = 0.004 for 1,000 permutations), indicating that the level of genetic differentiation between frequencies of alleles in the *Est-1*, *Est-2*, *Est-3* and *Est-4* loci of the plants from the 10 *M. chaseae* accessions was not related to the geographical distance separating the plants.

The clustering of the 160 plants from 10 accessions according to a model-based Bayesian algorithm is shown in Figure 4. Each bar in the graph represents a plant and its inferred proportion of allele admixture. The colors represent 3 different clusters corresponding to differential allele proportions in each plant.

Table 2. Number of alleles (N_a) and number of effective alleles (N_e) per polymorphic locus, mean observed heterozygosity (H_o) and expected heterozygosity (H_e), and % of polymorphic α/β-esterase loci in the plants of 10 accessions of *Mesosetum chaseae* maintained in the germplasm collection of Embrapa Pantanal.

Accession	N	N_a	N_e	H_o	H_e	P%
A1	10	3.00	2.3837	0.5000	0.5188	100
A4	10	3.25	2.1450	0.4500	0.5150	100
A5	10	3.75	2.1900	0.4667	0.5004	100
A8	10	2.00	2.0983	0.4000	0.5075	100
A9	26	3.25	2.1605	0.3077	0.3937	75
A11	20	3.50	2.7383	0.5750	0.5394	100
A17	20	3.25	2.1799	0.5500	0.5151	100
A24	14	3.00	2.4541	0.3750	0.5364	100
A31	20	3.50	2.1843	0.5125	0.5000	100
A32	20	3.25	2.5453	0.7500	0.5972	100

Table 3. Number of alleles (N_a), number of effective alleles (N_e), mean observed heterozygosity (H_o) and expected heterozygosity (H_e) at *Est-1*, *Est-2*, *Est-3* and *Est-4* loci, fixation coefficients *F* (F_{IS}, F_{IT}, F_{ST}; Wright 1965) and gene flow (Nm), in plants of 10 accessions of *Mesosetum chaseae*.

Locus	N	N_a	N_e	H_o	H_e	F_{IS}	F_{IT}	F_{ST}	Nm*
Est-1	160	3.00	2.3311	0.3937	0.5753	-0.0409	0.2549	0.2842	0.6297
Est-2	159	5.00	4.2614	0.3648	0.7653	0.4744	0.5429	0.1314	1.6675
Est-3	159	5.00	2.9681	0.7484	0.6631	-0.2155	-0.0798	0.1117	1.9885
Est-4	160	4.00	1.8492	0.4750	0.4592	-0.2164	-0.0048	0.1740	1.1872
Mean	160	4.25	2.8525	0.4955	0.6147	0.0432	0.2052	0.1693	1.2270

*Nm = Gene flow [Fst = 1/(4Nm+1)]

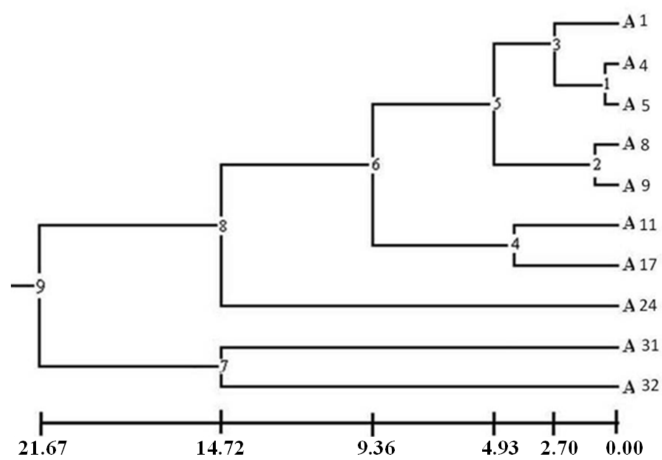


Figure 3. Similarity among the 10 accessions of *Mesosetum chaseae* from the Nhecolândia sub-region maintained in the germplasm collection of Embrapa Pantanal, based on UPGMA cluster analysis (Nei 1978) of the allele polymorphism at *Est-1*, *Est-2*, *Est-3* and *Est-4* loci.

Table 4. Dendrogram-based Nei's (1978) genetic distance: Nei's genetic identity (above diagonal) and genetic distance (below diagonal).

	A1	A4	A5	A8	A9	A11	A17	A24	A31	A32
A1	---	0.9391	0.9561	0.6756	0.8837	0.6069	0.8927	0.8602	0.9155	0.8393
A4	0.0628	---	0.9837	0.7030	0.9333	0.6234	0.9053	0.8489	0.9085	0.7195
A5	0.0449	0.0165	---	0.7931	0.8590	0.7105	0.8929	0.8143	0.9212	0.7885
A8	0.3922	0.3524	0.2318	---	0.5313	0.7450	0.7126	0.6096	0.6905	0.5621
A9	0.1236	0.0690	0.1520	0.6324	---	0.4839	0.8135	0.9207	0.8456	0.6898
A11	0.4993	0.4726	0.3418	0.2944	0.7260	---	0.8063	0.5026	0.8118	0.6542
A17	0.1135	0.0995	0.1133	0.3388	0.2064	0.2153	---	0.7174	0.9774	0.6611
A24	0.1506	0.1638	0.2055	0.4950	0.0827	0.6879	0.3321	---	0.7394	0.7660
A31	0.0883	0.0960	0.0820	0.3704	0.1677	0.2085	0.0229	0.3019	---	0.7442
A32	0.1752	0.3293	0.2376	0.5760	0.3714	0.4243	0.4139	0.2665	0.2954	---

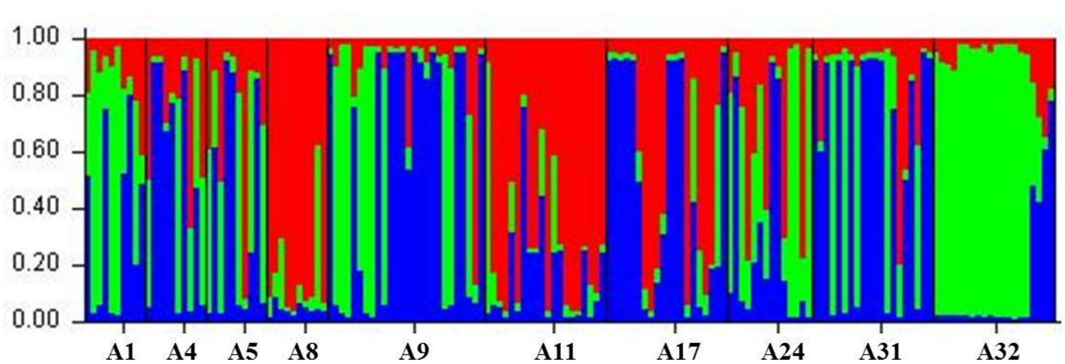


Figure 4. Bar plot-like population structure, based on polymorphism at the *Est-1*, *Est-2*, *Est-3* and *Est-4* loci for 10 accessions (A1, A4, A5, A8, A11, A17, A24, A31 and A32) of *Mesosetum chaseae* from the Nhecolândia sub-region maintained in the germplasm collection of Embrapa Pantanal within the K clusters. Each plant is represented by a single vertical bar broken in K colored segments (K = 3), with lengths proportional to each of the K inferred clusters. Each color represents the proportion of fragments for each individual, represented by a vertical bar.

Table 5. Proportion of the plants from 10 accessions of *Mesosetum chaseae* from the Nhecolândia sub-region maintained in the germplasm collection of Embrapa Pantanal in each group (K = 3), and number of plants per accession.

Accession	Group			Number of plants
	Red	Green	Blue	
A1	0.147	0.509	0.344	10
A4	0.261	0.255	0.484	10
A5	0.276	0.347	0.377	10
A8	0.835	0.120	0.046	10
A9	0.104	0.350	0.546	26
A11	0.720	0.110	0.170	20
A17	0.393	0.092	0.515	20
A24	0.296	0.492	0.275	14
A31	0.143	0.246	0.611	20
A32	0.081	0.788	0.132	20

The optimal K value determined by Bayesian analysis indicated that the plants were grouped into 3 clusters ($\Delta K2 = 0.00$; $\Delta K3 = 33.7349$; $\Delta K4 = 0.72333$; $\Delta K5 = 1.7489$; $\Delta K6 = 1.1321$; $\Delta K7 = 1.5338$; $\Delta K8 = 10.4170$; $\Delta K9 = 0.1642$; $\Delta K10 = 0.00$). The bar plot obtained for the K value (K = 3; $\Delta K = 33.7349$), and the results were consistent with the evidence of plants with a mixture of ancestral genome of either 2 or 3 groups, and plants with genome predominantly of 1 of the groups. At least 78% of the plants from accession A32 were in the green cluster and at least 72% of the plants of accessions A8 and A11 were in the red cluster. Plants from accessions A1, A9 and A31 contain predominantly a mixture of genome of the ancestral groups blue and green, those in A17 a mixture of ancestral groups blue and red and those in A4, A5 and A24 a mixture of genome of the groups blue, green and red (Table 5).

Bayesian analysis indicated that the most probable number of sub-populations is 3 in the *M. chaseae* accessions from the Nhecolândia sub-region kept in the germplasm collection at Embrapa Pantanal. In accession A32 there are predominantly plants ascending from one sub-population (green cluster), in accessions A8 and A11 there are predominantly plants ascending from another sub-population (red cluster), while in accessions A4, A5 and A24 there are plants ascending from the 3 sub-populations.

Discussion

The expected heterozygosity in *Est-1*, *Est-2*, *Est-3* and *Est-4* loci of *M. chaseae* ranged between 0.3937 and 0.6013 (plants from accessions A9 and A32). The mean H_e value (0.6153) of the plants from 10 accessions distributed across the Nhecolândia sub-region was high. Accession A11, showing the highest effective number of alleles ($N_e = 2.7383$), and A32 the highest value of mean expected and observed heterozygosity ($H_e = 0.5972$; $H_o = 0.7500$) present the highest genetic diversity. Accessions A11 and A32 may be, therefore, recommended for crossings to enlarge the genetic basis of *M. chaseae* in future breeding programs. In addition, these accessions should be planted in a range of environments to determine their suitability for increasing pasture areas planted to native species, as well as for recovering poor and degraded areas in the Pantanal region. Allendorf and Luikart (2007) suggested that the level of heterozygosity is an important indicator of the amount of adaptive genetic variation in a plant population. High heterozygosity implies a high chance for divergent selection. Populations with high heterozygosity may have been derived through different selective pressures throughout their geographical distribution and may persist under climatic or seasonal changes that may eliminate other populations. Studies of phenotypic characteristics in some samples of *M. chaseae* showed that accession A11 stands out for the large number of green leaves and a larger number of emerging leaves (Santos et al. 2007). High heterozygosity and production of green leaves are important features supporting the potential use of A11 for expansion of pastures and restoration of degraded areas, provided that it also has other desirable attributes such as high nutritional value, and adaptation to drought, mismanagement, disease and insect pests, which are yet to be evaluated.

High genetic diversity (H_e>0.50) was also detected in accessions A1, A4, A5, A8, A17, A24 and A31, indicating that these samples may have potential for use in breeding programs and warrant further study. High heterozygosity would indicate that the plant is likely to have a substantial amount of genetic variation to withstand different selective pressures. An excess of heterozygous plants (H_o>H_e) was observed in accessions A11, A17, A31 and A32. The deficit of heterozygotes (H_o<H_e) was higher in A24 than in A1, A4, A5, A8 and A9. Higher density and productivity, excellent coverage with a higher number of tillers/m^2, and fewer dry leaves have been described as phenotypic characteristics for accessions A5, A9 and A24 (Santos et al. 2007).

Analyses of the genetic divergence among all 10 accessions showed a global value (F_{ST}) of 0.1693. According to Wright (1978), values of F_{ST} ranging from 0.15 to 0.25 indicate high genetic differentiation between populations. On the other hand, the estimated mean level of genetic divergence among populations for Poaceae is higher than 0.20 (Hamrick and Godt 1996). Thus, in the case of *M. chaseae*, an outcrossing grass (Silva et al. 2013), the genetic differentiation among accessions may be considered as moderate

The genetic structure observed in the accessions of *M. chaseae* is also evident in the bar plot clustering of the 160 plants from the 10 accessions showing 3 sub-populations. The 3 probable sub-populations indicated by Bayesian analysis seem not associated with the original habitat of each accession. Plants of the green group (accession A32) originated from areas of non-flooded grassland and open grassland areas, while plants of the blue group originated from savanna (accessions A17 and A31) and the edge of the cordillera (accession A9). The genetically structured populations showing genetic diversity are all important samples of *M. chaseae*, which should be maintained in the Germplasm Bank of Embrapa Pantanal.

Despite the genetic structure observed in the accessions of *M. chaseae*, the analysis of polymorphic α/β-esterase loci has indicated a significant gene flow (Nm = 1.2265) between the plants of the 10 accessions, suggesting the occurrence of outcrossing between the samples (Govindaraju 1989). The Bayesian analysis showed a mixture of plants of 3 (green, blue and red) clusters in the 10 accessions. The reproductive system in *M. chaseae* may be the main vector for the suggestive exchange of alleles. In *M. chaseae*, reproduction (tested in protected flowers) indicated allogamy (Silva et al. 2013). In addition, the high gene flow may be explained by a mixture of samples among accessions due to the dispersion of seeds that may occur in certain areas exposed to occasional flooding periods.

The genetically structured accessions of *M. chaseae* showing high genetic diversity are according to their wide distribution in the Pantanal region, their habitat plasticity and vegetative development in these environments as shown by Santos et al. (2002) and Alvarez et al. (2004). *Mesosetum chaseae* specimens had a remarkably high number of alleles per locus and greater genetic diversity than those reported within populations of other grasses (Godt and Hamrick 1998). The combinations of characteristics such as: tropical region, regional range, perennial life form, sexual mode of reproduction, outcrossing breeding system, and seed dispersal mechanism, may explain the great genetic diversity in samples of *M. chaseae*. The low number of loci analyzed and the high genetic diversity generally ascribed to the esterase isozymes may also explain the high genetic diversity observed in our study compared with other grasses (Godt and Hamrick 1998).

In *Eragrostis curvula*, a valuable native forage grass in Africa, the esterase pattern proved useful as an additional criterion for identifying the individual taxa making up the so-called *Eragrostis curvula* complex (*E. curvula*, *E. conferta*, *E. robusta*, *E. chloromelas* and *E. lehmanniana*) and for evaluating their reciprocal relationships (Poverene and Curvetto 1989). Isozymes (including esterases) were used for genetic characterization of different samples of elephant grass (*Pennisetum purpureum*), a species of forage widely distributed in Brazil after its introduction to the country (Daher et al. 1997), although the values for heterozygosity were not presented. When the isozymes α- and β-esterase were used to analyze the forages *Brachiaria brizantha* (now: *Urochloa brizantha*), *B. plantaginea*, *B. humidicola* and *B decumbens* (now: *U. plantaginea*, *U. humidicola* and *U. decumbens*, respectively), a clear distinction between the 4 species was possible (Martins et al. 1999). Using the esterase system also made it possible to identify quickly and accurately the patterns of 7 cultivars of elephant grass (*Pennisetum purpureum*) and their hybrids with Pearl millet (*P. americanum*) from the germplasm bank of elephant grass (Freitas et al. 2000).

Higher genetic variability within than between samples also has been described for other forage species of the Poaceae family such as *Bromus auleticus*, using isozymes (esterases and peroxidases) and random amplified polymorphism of DNA (Yanaka et al. 2005). Since the year 2000, besides isozymes, DNA markers have also been used as tools to investigate the genetic diversity in forages. *Stylosanthes macrocephala*, a tropical forage legume native to Brazil, showed high polymorphism of random amplified DNA segments (RAPD), but the levels of heterozygosity and genetic divergence among accessions (F_{ST}) were not reported (Barros et al. 2005). Recently, the characterization of microsatellite markers in the grass, *Paspalum atratum*, has been considered a potential and promising tool for genetic diversity studies of *Paspalum* species (Cidade et al. 2010). Association of Single-Nucleotide Polymorphism markers with candidate genes in mapping of forage quality traits in perennial ryegrass was reported in Gill et al. (2012).

Estimates of heterozygosity are important indicators of the adaptive potential of populations for increasing grazing areas, and also for investigations for improvement of the species. Plants showing characteristics of interest (e.g. resistance to drought, greater leaf weight, early development) and high levels of heterozygosity are promising sources of inbred lines for breeding programs. As the levels of heterozygosity of the 10 accessions of *M. chaseae* have been estimated in our study, identification of features of interest for each sample would be necessary before mounting a breeding program for the species. The values of genetic identity (Nei 1973) and distances between the accessions estimated in our study (Table 4) are important information to assist in formulating a breeding program for the species. Crosses between accessions with the highest genetic distances (A9 and A11: 0.7260; A8 and A9: 0.6324; and A8 and A32: 0.5760) would broaden the genetic base of the species. Only 10 combinations among the accessions (A1 and A4, A1 and A5, A1 and A31, A4 and A5, A4 and A9, A4 and A17, A4 and A31, A5 and A31, A9 and A24 and A17 and A31) showed a very high level of genetic identity ($I>0.90$). Lower levels of genetic identity have been reported for other forage species: 0.0 to 0.857 and 0.2916 to 0.7142 in samples of *Pennisetum purpureum* (Daher et al. 1997; Freitas et al. 2000), and 0.0 to 0.50 in 16 samples of *Bromus auleticus* (Yanaka et al. 2005).

Analysis of the genetic polymorphism in loci for α- and β-esterase isozymes of *M. chaseae* accessions kept in the Germplasm Bank of Embrapa Pantanal was important for selecting accessions for further testing to determine their potential for use in recovering degraded areas, and for sowing as grazing pasture areas in the Pantanal region. The study has shown that, although the accessions presented a very high level of genetic identity ($I>0.90$), the samples form genetically structured populations ($0.15<Fst<0.25$). Field testing of accessions in a range of environments is warranted to determine if they possess other desirable attributes, e.g. persistence, drought-tolerance, high nutritive value, and disease- and pest-resistance, before they could be recommended for widespread sowing. Since there were no identical samples, all should be maintained in the Germplasm Bank of Embrapa Pantanal. Crossing plants from accessions with the highest genetic distances is recommended for the improvement of this species, with the prospect of broadening the genetic base and opening possibilities for the emergence of new characteristics of interest.

Acknowledgments

The authors thank CAPES (Coordenação de Aperfeiçoamento de Pessoal de Nível Superior, Brasília, DF, Brazil) for the financial support.

References

Allendorf FW; Luikart G. 2007. Conservation and the genetics of populations. Blackwell Publishing, Malden, MA, USA.

Alvarez JM; Rocha JF; Santos SA; Machado SR. 2004. Anatomia foliar de *Mesosetum chaseae* Luces (Poaceae) vegetando em diferentes ambientes com e sem influência de pastejo na subregião da Nhecolândia, Pantanal Sul-Mato-Grossense. In: Anais, IV Simpósio sobre Recursos Naturais e Socio-Econômicos do Pantanal, 23−26 November 2004, Corumbá, MS, Brazil (CD-ROM).

Barros AM; Faleiro FG; Karia CT; Shiratsuchi LS; Andrade RP de; Lopes GKB. 2005. Varia-bilidade genética e ecológica de *Stylosanthes macro-cephala* determinadas por RAPD e SIG. Pesquisa Agro-pecuária Brasileira 40:899–909. DOI: 10.1590/s0100-204x2005000900010

Cidade FW; Souza-Chies TT; Souza FHD; Batista LAR; Dall'Agnol M; Valls JFM; Zucchi MI; Souza AP. 2010. Microsatellite loci for *Paspalum atratum* (Poaceae) and cross-amplification in other species. American Journal of Botany 97:e107–e110. DOI: 10.3732/ajb.1000207

Daher RF; Moraes CF; Cruz CD; Pereira AV; Xavier DF. 1997. Diversidade morfológica e isoenzimática em capim-elefante (*Pennisetum purpureum* Schum.). Revista Brasileira de Zootecnia 26:255–264.

Earl DA; vonHoldt BM. 2012. STRUCTURE HARVESTER: A website and program for visualizing STRUCTURE output and implementing the Evanno method. Conservation Genetics Resources 4:359–361. DOI: 10.1007/s12686-011-9548-7

Evanno G; Regnaut S; Goudet J. 2005. Detecting the number of clusters of individuals using the software Structure: A simulation study. Molecular Ecology 14:2611–2620. DOI: 10.1111/j.1365-294x.2005.02553.x

Freitas NSA; Falcão TMMA; Burity HÁ; Tabosa JN; Silva MV. 2000. Caracterização e diversidade genética do capim-elefante e seus híbridos com milheto mediante padrões isoenzimáticos. Pesquisa Agropecuária Brasileira 35:1125–1133. DOI: 10.1590/s0100-204x2000000600008

Frigo MJ; Mangolin CA; Oliveira Junior RS; Machado MFPS. 2009. Esterase polymorphism for analysis of genetic diversity and structure of wild poinsettia (*Euphorbia heterophylla*) populations. Weed Science 57:54–60. DOI: 10.1614/ws-08-096.1

Gill G; Faville M; Bryant C; Rouillard J-M; Hanley Z. 2012**.** SNP discovery and candidate gene-based association mapping of forage quality traits in perennial ryegrass**.** In: Bushman BS, ed. Proceedings of the 7th International Symposium on the Molecular Breeding of Forage and Turf. p. 24.

Godoi Filho JD. 1986. Aspectos geológicos do Pantanal Mato-Grossense e de sua área de influência. In: Anais, I Simpósio sobre Recursos Naturais e Sócio-Econômicos do Pantanal, Corumbá, MS, Brazil. p. 63–76.

Godt MJW; Hamrick JL. 1998. Allozyme diversity in grasses. In: Cheplick GP, ed. Population biology of grasses. Cambridge University Press, Cambridge, UK. p. 11–29. DOI: 10.1017/cbo9780511525445.003

Govindaraju DR 1989. Variation in gene flow levels among predominantly self-pollinated plants. Journal of Evolutionary Biology 2:173–181. DOI: 10.1046/j.1420-9101.1989.2030173.x

Hamrick JL; Godt MJW. 1996. Effect of life history traits on genetic diversity in plant species. Philosophical Transactions of the Royal Society B 351:1291–1298. DOI: 10.1098/rstb.1996.0112

Martins CC; Martins D; Mori ES; Souza FHD; Ramos PRR. 1999. Eletroforese de isoenzimas de plântulas na identificação de espécies de *Brachiaria.* Planta Daninha 17:433–443. DOI: 10.1590/s0100-83581999000300010

Nei M. 1973. Analysis of gene diversity in subdivided populations. Proceedings of the National Academy of Sciences of the United States of America 70:3321–3323.

Nei M. 1978. Estimation of average heterozygosity and genetic distance from a small number of individuals. Genetics 89:583–590. DOI: 10.1073/pnas.70.12.3321

Peakall R; Smouse PE. 2006. GENALEX 6: Genetic analysis in Excel. Population genetic software for teaching and research. Molecular Ecology Notes 6:288–295. DOI: 10.1111/j.1471-8286.2005.01155.x

Pinheiro LC; Santos SA; Comastri Filho JA. 2005. Produção de forragem de pastagem com predominância de grama-do-cerrado submetida a duas épocas de vedação, no Pantanal. In: Sociedade Brasileira de Zootecnia, ed. Anais, VII Congresso Internacional de Zootecnia, Campo Grande, MS, Brazil (CD-ROM).

Poverene MM; Curvetto NR. 1989. Esterase isozyme variation in the *Eragrostis curvula* complex (Poaceae). Plant Systematics and Evolution 166:173–181. DOI: 10.1007/bf00935947

Pritchard JK; Wen W. 2003. Documentation for STRUCTURE software: Version 2. http://pritch.bsd.uchicago.edu

Rohlf FJ. 1989. NTSYS-Pc: Numerical Taxonomy and Multivariate Analysis System. Exeter Publishing Co, New York, USA.

Sala J; Mangolin CA; Franzoni J; Machado MFPS. 2011. Esterase polymorphism and the analysis of genetic diversity and structure in cactus populations descended from *Cereus peruvianus* plants regenerated in vitro. Biochemical Genetics 49:270–282. DOI: 10.1007/s10528-010-9405-5

Santos SA. 2001. Caracterização dos recursos forrageiros nativos da subregião da Nhecolândia, Pantanal, Mato-Grosso do Sul, Brasil. Ph.D. Thesis. Universidade Estadual Paulista, Botucatu, SP, Brazil.

Santos SA; Costa C; Souza GSE. 2002. Composição botânica da dieta de bovinos criados em pastagem nativa na sub-região da Nhecolândia, Pantanal, Brasil. Revista Brasileira de Zootecnia 31:1648–1662. DOI: 10.1590/s1516-35982002000700007

Santos SA; Comastre Filho JA; Cardoso EL. 2005. Identificação de espécies forrageiras nativas tolerantes à seca na sub-região da Nhecolândia, Pantanal. In: Anais, VI Congresso Brasileiro de Zootecnia, 2005, Campo Grande, MS, Brazil (CD-ROM).

Santos SA; Silva LAC; Abreu UGP; Jank L; Ogihara LK; Parrili M; Matsuhara AS. 2007. Avaliação de descritores fenotípicos de populações da Grama-do-cerrado no pico de seca, Pantanal. In: Resumos, Simpósio Internacional sobre Melhoramento de Forrageiras, Campo Grande, 2007. Embrapa Gado de Corte, Campo Grande, MS, Brazil (CD-ROM).

Santos SA; Desbiez A; Crispim SMA; Comastri Filho JA; Abreu UGP; Rodela LG. 2011. 3. Natural and cultivated pastures and their use by cattle. In: Junk WJ; Silva CJ; Cunha CN; Wantzen KM, eds. The Pantanal: Ecology, biodiversity and sustainable management of a large neotropical seasonal wetland. Pensoft Publishers, Sofia − Moscow. p. 327–353. DOI: 10.13140/2.1.3056.7367

Silva LAC; Santos SA; Costa C; Meirelles PRL; Comastri-Filho JA; Garcia JB. 2011. Perfilhamento de plantas e qualidade de sementes de *Mesosetum chaseae* Luces na sub-região da Nhecolândia, Pantanal, MS. Veterinária e Zootecnia (UNESP) 18:610–620.

Silva LAC; Pagliarini MS; Santos SA; Silva N; Souza VF. 2012. Chromosome number, microsporogenesis, microgametogenesis, and pollen viability in the Brazilian native grass *Mesosetum chaseae* (Poaceae). Genetics and Molecular Research 11:4100–4109. DOI: 10.4238/2012.september.12.1

Silva LAC; Pagliarini MS; Santos SA; Valle CB. 2013. Stigma receptivity, mode of reproduction, and mating system in *Mesosetum chaseae* Luces (Poaceae), a native grass of the Brazilian Pantanal. Genetics and Molecular Research 12:5038–5045. DOI: 10.4238/2013.october.25.2

Wright S. 1965. The interpretation of population structure by F-statistics with special regard to systems of mating. Evolution 19:395–420. DOI: 10.2307/2406450

Wright S. 1978. Variability within and among populations. University of Chicago Press, Chicago, IL, USA.

Yanaka FY; Dall´Agnol M; Schifino-Wittmann MT; Dias PMB; Gomes KE. 2005. Variabilidade genética em populações naturais de *Bromus auleticus* Trin. ex Nees (Poaceae) com base em isoenzimas e marcadores RAPD. Revista Brasileira de Zootecnia 34:1897–1904. DOI: 10.1590/s1516-35982005000600013

Yeh FC; Yang RC; Boyle T. 1999. POPGENE Version 1.31: Microsoft Window-based freeware for population genetic analysis. University of Alberta and Center for International Forestry Research.

(Received for publication 19 September 2014; accepted 31 July 2015)