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This issue is dedicated to the memory of Professor **Albert E. Kretschmer, Jr. (1925–2014)**, US American pasture agronomist, who made outstanding contributions to the science of tropical pastures and forages, mainly in Florida, USA. His friends and colleagues will not forget his infectious enthusiasm for pasture plant improvement. Al was a Fellow of the Tropical Grassland Society of Australia Inc. and member (1988-1991) of the Editorial Advisory Board of *Tropical Grasslands*.



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LivestockPlus – The sustainable intensification of forage-based agricultural systems to improve livelihoods and ecosystem services in the tropics*

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Abstract

As global demand for livestock products (such as meat, milk and eggs) is expected to double by 2050, necessary increases to future production must be reconciled with negative environmental impacts that livestock cause. This paper describes the LivestockPlus concept and demonstrates how the sowing of improved forages can lead to the sustainable intensification of mixed crop-forage-livestock-tree systems in the tropics by producing multiple social, economic and environmental benefits. Sustainable intensification not only improves the productivity of tropical forage-based systems but also reduces the ecological footprint of livestock production and generates a diversity of ecosystem services (ES) such as improved soil quality and reduced erosion, sedimentation and greenhouse gas (GHG) emissions. Integrating improved grass and legume forages into mixed production systems (crop-livestock, tree-livestock, crop-tree-livestock) can restore degraded lands and enhance system resilience to drought and waterlogging associated with climate change. When properly managed tropical forages accumulate large amounts of carbon in soil, fix atmospheric nitrogen (legumes), inhibit nitrification in soil and reduce nitrous oxide emissions (grasses), and reduce GHG emissions per unit livestock product.

The LivestockPlus concept is defined as the sustainable intensification of forage-based systems, which is based on 3 interrelated intensification processes: *genetic intensification* - the development and use of superior grass and legume

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*This concept and review paper was developed from active participation by and contributions from a large number of coauthors during an international workshop entitled “Pastures, climate change and sustainable intensification” held at CIAT, Cali, Colombia during 28–29 May 2013.

cultivars for increased livestock productivity; *ecological intensification* - the development and application of improved farm and natural resource management practices; and *socio-economic intensification* - the improvement of local and national institutions and policies, which enable refinements of technologies and support their enduring use. Increases in livestock productivity will require coordinated efforts to develop supportive government, non-government organization and private sector policies that foster investments and fair market compensation for both the products and ES provided. Effective research-for-development efforts that promote agricultural and environmental benefits of forage-based systems can contribute towards implementation of *LivestockPlus* across a variety of geographic, political and socio-economic contexts.

Resumen

De la misma manera que la demanda global de productos pecuarios (carne, leche, huevos) se duplicará para 2050, se espera que las producciones futuras tengan en cuenta los efectos ambientales negativos ocasionados por este sector. En este documento se describe el concepto *LivestockPlus* y se demuestra cómo en el trópico los forrajes mejorados pueden llevar a la intensificación sostenible de sistemas de producción mixta que integran forrajes/ganadería y cultivos y/o árboles, produciendo múltiples beneficios sociales, económicos y ambientales. La intensificación sostenible no sólo incrementa la productividad de los sistemas tropicales basados en forrajes, sino también reduce la huella ecológica de la producción pecuaria y genera una diversidad de servicios de ecosistema (ES, por sus siglas en inglés), como son el mejoramiento de la calidad del suelo, la reducción de la erosión y la sedimentación, y la mitigación de las emisiones de gases de efecto invernadero (GEI). La integración de gramíneas y leguminosas forrajeras mejoradas en los sistemas de producción mixta (agropastoril, silvopastoril y agrosilvopastoril) puede restaurar las tierras degradadas y aumentar la resiliencia de los sistemas a la sequía y el anegamiento asociados con el cambio climático. Si las prácticas de manejo son apropiadas, los forrajes tropicales acumulan grandes cantidades de carbono en el suelo, fijan el nitrógeno atmosférico (leguminosas), inhiben la nitrificación en el suelo y reducen las emisiones de óxido nitroso (gramíneas), y finalmente reducen las emisiones de GEI por unidad de producto pecuario.

El concepto *LivestockPlus* se define como la intensificación sostenible de los sistemas de producción basados en forrajes, con 3 procesos de intensificación interrelacionados como pilares: *intensificación genética* –el desarrollo y el uso de cultivares superiores de gramíneas y leguminosas para aumentar la productividad pecuaria; *intensificación ecológica* –el desarrollo y la aplicación de mejores prácticas agrícolas y de manejo de recursos naturales; e *intensificación socioeconómica* –el mejoramiento de las instituciones y políticas locales y nacionales, que permiten refinar las tecnologías y facilitan su uso duradero. Los aumentos en la productividad ganadera requerirán esfuerzos coordinados para desarrollar políticas de apoyo de los gobiernos, organizaciones no-gubernamentales y el sector privado para estimular inversiones y una compensación justa del mercado, tanto para los productos pecuarios como los servicios ecosistémicos proporcionados. Los esfuerzos efectivos de investigación para el desarrollo que promuevan los beneficios que los sistemas de producción basados en forrajes proporcionan para la producción agropecuaria y el medioambiente, pueden ampliar la aplicación de *LivestockPlus* a través de una variedad de contextos geográficos, políticos y socioeconómicos.

Introduction

The need to increase livestock production

The world population is expected to be 9.6 billion by 2050 (UNDESA 2012). Thus, 70% more food will be required in 2050 than in 2000 (Bruinsma 2009). Increasing yields per unit area in current agricultural zones is expected to achieve 90% of the required gains, with expanded areas in sub-Saharan Africa and Latin America providing the remainder (FAO 2010). Globally, livestock derive fodder from two-thirds (4.9 Bha) of all agri-

cultural areas, comprising 3.4 Bha of grazing land and one-quarter of the area sown to crops (Foley et al. 2011). The world has 17 billion livestock (mainly cattle including buffaloes, sheep, goats, pigs and chickens, but also including lesser-known species such as guinea fowl, yaks and camels, which are important in some areas). Livestock, especially ruminants, have the ability to convert low-quality biomass into high-quality nutrient-dense foods (Smith et al. 2013a), and currently contribute 15% of total food energy, 25% of dietary protein and some micronutrients not readily available from plants for human consumption (FAO 2009).

Table 1. Actual demand for livestock products in developing and developed countries in 2002 and projections for 2050 (adapted from Rosegrant et al. 2009).

| Livestock product | Developing countries | | | Developed countries | | |
|-----------------------------|----------------------|------|----------------|---------------------|------|----------------|
| | 2002 | 2050 | Difference (%) | 2002 | 2050 | Difference (%) |
| Meat | | | | | | |
| Consumption per capita (kg) | 28 | 44 | 57 | 78 | 94 | 21 |
| Total consumption (Mt) | 137 | 326 | 138 | 102 | 126 | 24 |
| Milk | | | | | | |
| Consumption per capita (kg) | 44 | 78 | 77 | 202 | 216 | 7 |
| Total consumption (Mt) | 222 | 585 | 167 | 265 | 295 | 11 |

Global demand for meat, milk and eggs is expected to double by 2050, with the largest increases occurring in developing countries (Delgado et al. 2001; Herrero et al. 2009) (Table 1). Meat and milk consumption in developing countries has increased 3 times faster over the last 30 years than in developed countries (FAO 2009), with the largest increases occurring in East and Southeast Asia, along with Latin America and the Caribbean (LAC). Although greatest changes have occurred in developing countries with large populations and fast-growing economies such as China, India, Indonesia and Brazil (Pica-Ciamarra and Otte 2011), consumption of livestock products is expected to increase significantly in countries with smaller populations and economies (ILRI et al. 2011).

Of the 5 agricultural commodities with the highest global economic value, 4 (milk, beef, pork and chicken) come from livestock, which are an important global asset with an estimated value of at least USD 1.4 trillion. Further, the livestock sector and associated market chains employ 1.3 billion people worldwide and contribute to the livelihoods of some 600 million smallholder farmers (Thornton 2010). Despite substantial investment in agricultural technology and farm management, yield increases from the Green Revolution have slowed during the last 4 decades (Ray et al. 2012). Many productivity increases came with high environmental costs such as nutrient and pesticide contamination, soil salinization and water pollution, and future increases must be achieved by reducing agriculture's environmental footprint (Godfray et al. 2010). To meet these multiple and urgent challenges, a more comprehensive and coordinated research and development approach is needed.

Diverse crop-forage-livestock systems

Livestock production systems in developing countries involve varying degrees of grazing and/or feeding of cut forages and grain concentrates (Seré and Steinfeld 1996). The main focus of this paper is on forage-based

crop-livestock-tree¹ systems in developing countries in the tropics. Most of the meat and milk produced in the developing world and almost half of the global cereal output come from mixed crop-livestock systems (Herrero et al. 2010). Improved performance of both crops and animals is essential for sustainable intensification (McDermott et al. 2010). Integration of forage systems with cropping systems should help mitigate negative environmental impacts resulting from intensification of cropping systems and improve the quality of forage systems through periodic restoration (Lemaire et al. 2014).

Tropical forage-based livestock production systems differ regionally (Peters et al. 2013a). In LAC, cattle are raised largely on sown pastures with increasing attention to crop components, while in West Africa cattle, sheep and goats graze native pastures and crop residues. In tropical Asia, cut-and-carry systems and crop residues predominate. In Eastern, Central and Southern Africa, native and sown forages are often combined with crop residues for both grazing and cut-and-carry to feed cattle and small ruminants. We class all such systems (grazing, cut-and-carry, agropastoral and silvopastoral systems) that utilize tropical grasses and legumes for feeding livestock as "tropical forage-based systems".

The majority of tropical forage-based systems face challenging production conditions. Soils are mostly infertile with low soil organic matter, very low pH, high aluminum (Al) saturation and phosphorus (P) deficiency. Rainfall is often markedly seasonal with prolonged (4–6 months) dry seasons, followed by unreliable wet seasons, that can be accompanied by waterlogging. These abiotic stresses, together with some major pests and diseases, affect both the quantity and quality of feed produced, and thus limit livestock productivity, particularly in prolonged dry seasons. Given such challenging biophysical conditions, coupled with lack of, or unapplied government policies, poorly performing markets and few

¹When using this simplifying term we refer to integrated agricultural production systems that involve forage-based livestock, crops and/or trees (agropastoral, silvopastoral and agrosilvopastoral systems).

investment incentives, land used for livestock production is in varying stages of degradation (Macedo 1997; Miles et al. 2004). As pastures degrade, productivity and organic matter inputs decrease, non-palatable plant species invade, vegetative cover is reduced (thus increasing susceptibility to erosion), soils become compacted and more acidic, and microbial biomass decreases (Macedo 1997; Oliveira et al. 2004). Losses in soil organic matter could be associated with reduced soil aggregation, leading to a possible corresponding decline of organic P, with potentially significant implications for the efficient cycling of P in tropical soils (Fonte et al. 2014). Despite these limitations, developing countries have greater potential to increase livestock production through restoration of degraded lands than developed countries (Smith et al. 2008; Murgueitio et al. 2011). Thus, we focus on grasses and legumes selected because of their superior biomass production, nutritional quality and persistence relative to native or naturalized species, mainly grasses.

Livestock production and the environment

Livestock production is the world's largest system of land use (de Fraiture et al. 2007) and livestock consume about two-thirds of all dry matter produced by terrestrial plants in the food system (Wirsenius 2003). As a consequence, livestock production can have substantial negative effects on the environment, including global warming (Steinfeld et al. 2006a, 2006b; Herrero et al. 2013b), nitrogen (N) pollution (Bouwman et al. 2013), high water use and contamination of water resources (Herrero et al. 2012). In addition, reduction in biodiversity occurs when lands supporting native vegetation are converted to pastures (Alkemade et al. 2013).

It is recognized that forage-based systems provide a number of ecosystem services (ES) such as regulating water flows, reducing erosion and greenhouse gas (GHG) emissions (Cárdenas et al. 2007; Peters et al. 2013a, 2013b), and improving soil biota and quality (Velásquez et al. 2012; Rousseau et al. 2013; Lavelle et al. 2014), as well as cultural services by promoting traditional lifestyles. The relative importance of these diverse ES depends on priorities of landowners and other stakeholders affected by agricultural activities, which are ecosystem-specific.

It is well documented that livestock are a major contributor to GHG emissions, estimated at 7.1 Gt (billion metric tons) carbon dioxide (CO₂)-equivalent/yr (Ripple et al. 2014), representing 14.5% of all anthropogenic GHG emissions (Gerber et al. 2013). Beef and milk cattle account for 41% and 21%, respectively, of livestock's emissions, including: methane (CH₄) from enter-

ic fermentation and animal manures; CO₂ from land use and land-use changes; and nitrous oxide (N₂O) from manure and slurry management and emissions associated with agricultural activities, mainly N fertilization, to produce animal feed (Scholes et al. 2014). Intensity of GHG emissions differs among geographical regions and production systems, including the animal species and the products in question. These differences are mostly driven by feed conversion efficiency (the amount of feed consumed per unit of product), which improves with dietary quality in terms of digestibility and protein content (Herrero et al. 2013a). Sub-Saharan Africa (SSA) produces a high intensity of emissions by livestock (Herrero et al. 2013b), owing to low animal productivity from large areas of arid lands, where animals have low productive potential, and feed available is of low quality and often scarce (Hristov et al. 2013).

Improving the quantity and quality of forage produced will improve animal production and feed efficiency and reduce GHG emissions (particularly CH₄) per unit of animal product (Hristov et al. 2013), but may result in increased emissions at the farm level, if animal numbers are not kept constant or are not reduced (Latawiec et al. 2014). Sustainable intensification of forage-based agricultural systems should result in release of land for other environmentally-friendly uses (such as tree plantations, reconversion to forest vegetation).

About 39% of the total water used for agriculture is associated with livestock production (de Fraiture et al. 2007), most being used in growing feed (Herrero et al. 2012). Consequently, water scarcity is a major limitation to livestock production in the seasonally-dry tropics (Rockström et al. 2007). Climate change can further aggravate water shortage problems, adversely affecting a high proportion of smallholder crop-livestock systems in marginal environments.

Opinions differ on how best to address the negative environmental effects of livestock production. While Pelletier and Tyedmers (2010) argue that growth of the livestock sector should be curbed, Steinfeld and Gerber (2010) suggest that production technologies (land intensification) with low ecological footprint should be developed for the benefit of poor smallholder producers in developing countries. Despite these contrasting views, there is general agreement on the importance of reducing the environmental footprint of livestock. This poses development challenges to improve food security and alleviate poverty. As crop and livestock farming complement each other (Herrero et al. 2010), the use of both improved forages and improved animal breeds can yield the same amount of food from a smaller area or more food from a similar area (Eisler et al. 2014).

Eco-efficiency and sustainable intensification

Coordinated research, development and policy initiatives are needed to improve the productivity of crop-forage-livestock-tree systems. Two related paradigms in the development literature, *eco-efficiency* and *sustainable intensification*, can be used to describe general approaches that aim to optimize social, economic and environmental objectives. *Eco-efficiency* aims to achieve highly-productive agro-ecological systems, which have a small environmental footprint, while being economically viable and socially equitable (CIAT 2009; Keating et al. 2013). *Sustainable intensification* produces increased outputs with more efficient use of inputs, while reducing environmental damage and building resilience, natural capital and ES (The Montpellier Panel 2013). Although social equity is not an explicit aim of sustainable intensification, it occurs within the context of sustainable development.

Three related processes lie at the heart of sustainable intensification (The Montpellier Panel 2013): *Genetic intensification* is the development and use of superior grass and legume cultivars for increased livestock productivity. This should be coupled with the development and use of superior animal breeds (not considered in the context of this concept and review paper). *Ecological intensification* is the application of improved farm and natural resource management (NRM) practices. *Socio-economic intensification* involves the improvement of local and national institutions and policies, which enable technology adoption, and supports their enduring use. In addition, fair and efficient market access for goods and services associated with both inputs and outputs is essential (Figure 1).

LivestockPlus: Concept and principles

The LivestockPlus concept (Figure 2) was formulated to demonstrate how improved forages, when and if properly managed, could lead to the sustainable intensification of mixed crop-forage-livestock systems in the tropics, while recognizing the multiple social, economic and environmental objectives. While minimizing trade-offs, LivestockPlus emphasizes the synergism between soils, plants, animals, people and the environment. The aim is to produce additional meat and milk based on 4 principles:

1) Selected sown grasses and legumes are more productive per unit land area than native or naturalized forages, and produce higher quality feed and thus may contribute to releasing land for alternative uses;

- 2) Sown grasses and legumes in combination with crop residues improve resource-use efficiency at farm level and produce more milk and meat, particularly during the dry season;
- 3) Sown grasses and legumes, especially when integrated with crops and trees, enhance system productivity and resilience and improve livelihoods. They also generate ES, thereby reducing the environmental footprint per unit livestock product; and
- 4) Multiple actions are needed to create conditions that are essential for the adoption and widespread use of improved forage-based systems, including: genetic improvement of livestock to match improved feeding; changes to regional and national policies; and increases in human and social capital.

We consider that increasing consumer demands for livestock products can and should be met by increasing productivity within the same region, particularly in the tropics. Although productivity could be increased using grain-based diets, we favor intensifying forage-based systems, based on goals of economic viability, environmental sustainability and social equity, associated with eco-efficiency (Rao et al. 2014). To spark greater interest and adoption of improved forages, the concepts and benefits of LivestockPlus need to be communicated to the global community. This paper is an initial step in that process.

LivestockPlus: Sustainable intensification of forage-based systems

Genetic intensification to provide a wide range of forage/feed options

Forage grasses. Domestication of forage grasses started when livestock producers began to collect and intentionally sow elsewhere seeds of plants that they considered improved livestock performance. As with crop plants, most useful forage plants were domesticated long before they were studied scientifically (Boonman 1993), being selected for different purposes according to user needs and the plants' characteristics. Many tropical grass species are useful as sown forages, and some are widely commercialized (Cook et al. 2005). Over the last 50 years, many thousands of accessions of grasses were evaluated in agronomic trials in the tropics and subtropics, resulting in the release of a number of cultivars for use as forages to improve livestock production (Table 2).

A number of cultivars are widely used as pastures. For the semi-arid tropics and subtropics, more than 30 cultivars of *Cenchrus ciliaris* (now *Pennisetum ciliare*) are available; some are extensively used. While

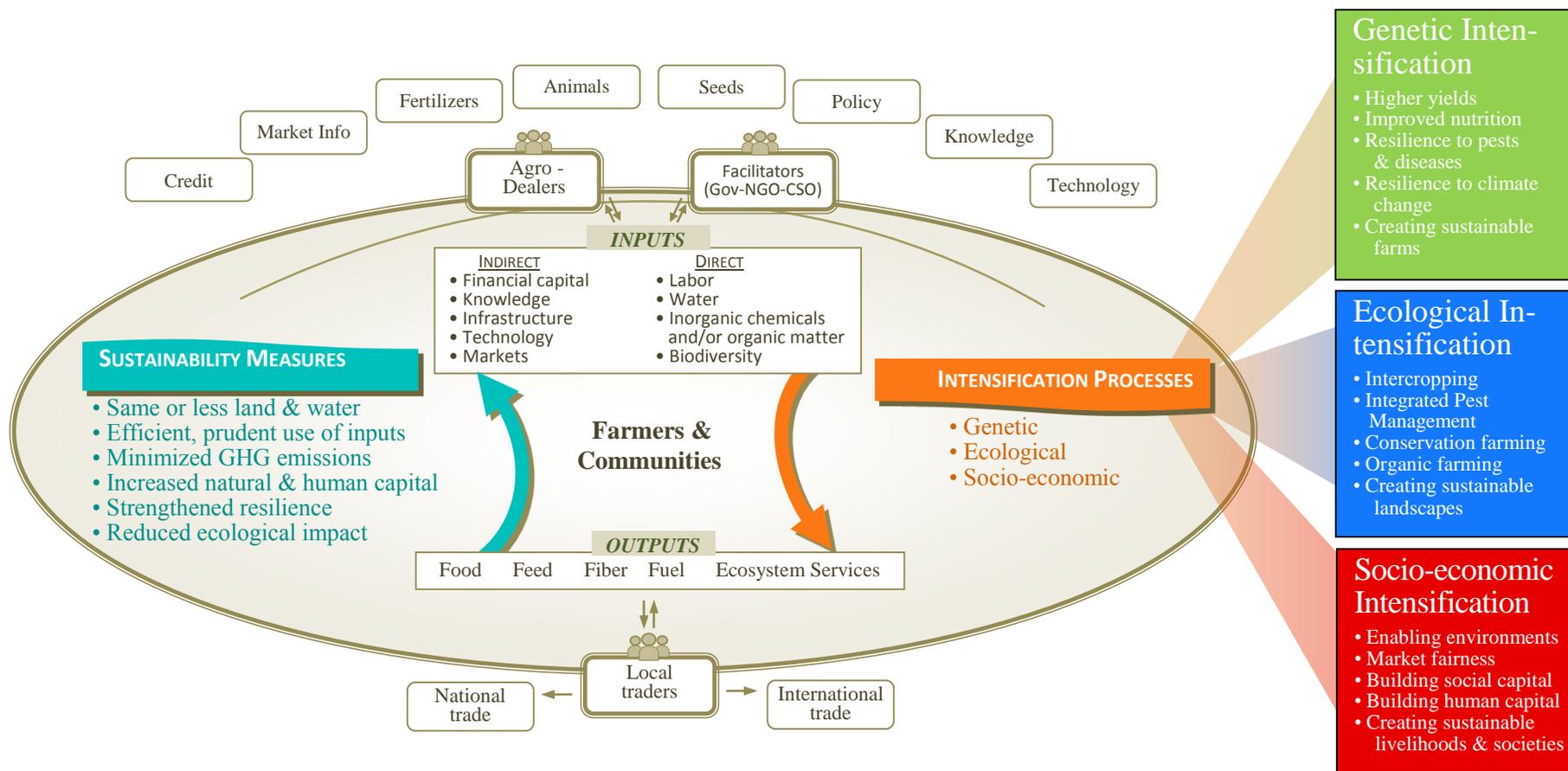


Figure 1. A sustainable intensification approach for improved forages to realize widespread social, economic and environmental benefits (modified from The Montpellier Panel 2013).

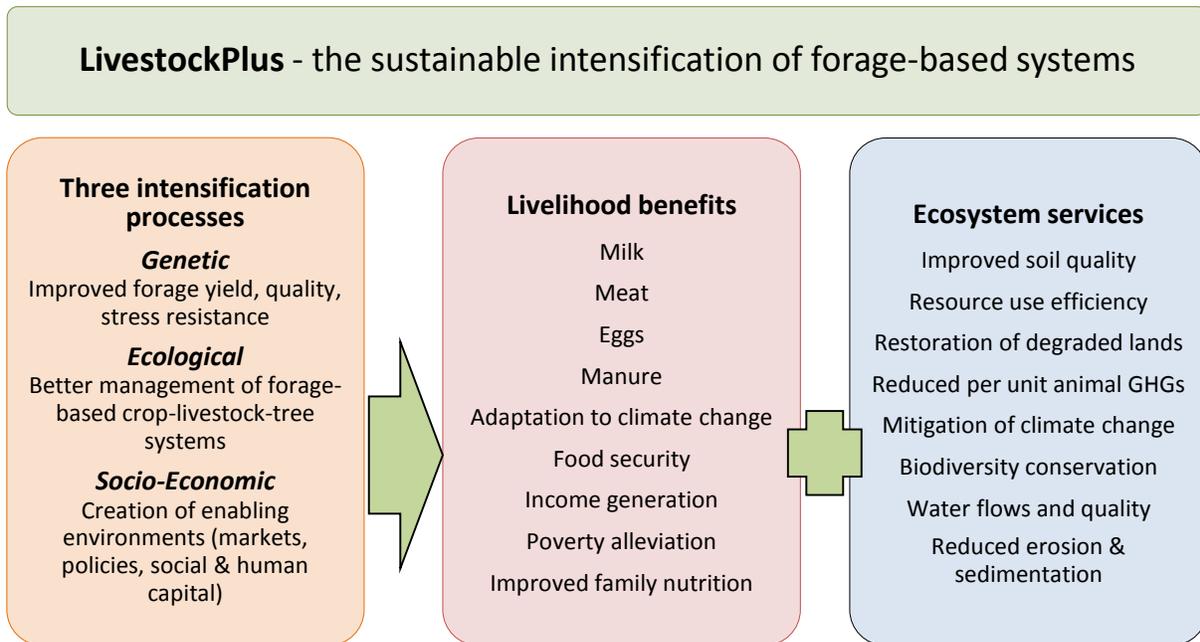


Figure 2. LivestockPlus: A concept to improve livelihoods and ecosystem services via the sustainable intensification of forage-based crop-livestock-tree systems.

Glenn Burton and colleagues achieved major genetic improvement in nutritive quality of bermudagrass (*Cynodon dactylon* and interspecific hybrids) at Tifton, GA, USA (Hill et al. 2001), the resulting cultivars are not widely grown in the lower-latitude tropics. Various cultivars of *Brachiaria* species, many of which are now accepted as *Urochloa* spp., have made an impressive contribution to animal production throughout the tropics, such as *B. brizantha* cvv. Marandu and Toledo; *B. humidicola* cvv. Tully and Llanero; *B. decumbens* cv. Basilisk; and *B. ruziziensis* cv. Kennedy (Miles et al. 2004). *Brachiaria* breeding at CIAT has produced the commercial cvv. Mulato, Mulato II, Cayman and Cobra. Guinea grass (*Panicum maximum*; now *Megathyrsus maximus*) is very productive on fertile soils in the humid and subhumid tropics and subtropics. Several accessions of *Paspalum* are adapted to wet sites. *Pennisetum purpureum* (napier grass or elephant grass) is widely used in cut-and-carry systems but available cultivars require fertilizer to sustain high yields and are subject to disease pressures (i.e. stunt disease) in Eastern Africa.

Breeding programs to improve temperate forage grasses began almost 100 years ago; in contrast, breeding of tropical forage grasses did not start until about 1960. The objectives of both plant breeding and germplasm selection were to identify or produce plants that were persistent and resistant to pests and diseases, with high yields of forage, high nutritive value and good seed yields and quality. Tolerance of acid soils, drought and waterlogging were also important; deep-rootedness was included to increase

drought tolerance and the ability to scavenge for soil nutrients in infertile soils. Characteristics that contribute to ES received little attention (Miles et al. 2004; Rao 2014), although deep-rootedness has now been shown to contribute to accumulation of C at depth in the soil (Fisher et al. 1994; 2007). In addition, feeding ruminants with high quality forage reduces the amount of methane emitted per unit of animal product (Herrero et al. 2013b), and some tropical forage grasses inhibit biological nitrification, which reduces N₂O emissions from the soil (Subbarao et al. 2009). Breeding and selection can increase the ES that forages provide only if there is genetic variation for the desired traits in the available germplasm.

Forage legumes. Forage legumes have: (1) symbiotic nitrogen fixation, contributing N to the system and having high protein concentrations; (2) deep taproots, which contribute to drought tolerance and increase the ability to scavenge for nutrients in infertile soils; (3) a diversity of chemical compounds, many of them anti-nutritive substances; and (4) great genetic, morphological, taxonomic and ecological diversity. Tropical forage legumes not only provide high-quality animal feed but also enhance soil fertility, improve soil structure and water infiltration, increase soil C accumulation and contribute to weed control and soil conservation (Thomas and Lascano 1995). In addition, most forage legumes contain phenols that can favorably modulate processes of biohydrogenation and methanogenesis (Waghorn et al. 2002; Jayanegara et al. 2011).

Table 2. A selection of important commercial forage grasses and legumes used in tropical livestock production systems (including crop-tree-livestock systems) and natural resource management.

| Species | Cultivar examples or (common name) | Current use | | | | | |
|--|---------------------------------------|----------------------|-------------------|---|-----------------------|---|--------------------------------|
| | | Livestock production | | | Livestock & NRM | Natural resource manage- ment (erosion and weed control, soil enhancement) | |
| | | Grazing | Cut & carry | Processing (e.g. hay & leaf meal/ pellets) | | Fodder banks, leys, improved fallows | Soil cover, green manure |
| Grasses | | | | | | | |
| <i>Brachiaria brizantha</i> | Marandu, Toledo | X ¹ | (x) | (x) | | (x) | |
| <i>Brachiaria decumbens</i> | Basilisk | X | (x) | (x) | | | |
| <i>Brachiaria humidicola</i> | Tully, Llanero | X | | (x) | | X | |
| <i>Brachiaria</i> hybrids | Mulato, Mulato II | X | (x) | (x) | | | |
| <i>Cenchrus ciliaris</i> | Biloela, Gayndah | X | | | | | |
| <i>Chloris gayana</i> | Callide, Katambora | X | | X | X | | |
| <i>Cynodon nlemfuensis</i> | (African Star grass) | X | | X | | | |
| <i>Digitaria eriantha</i> | (Pangola) | X | | X | | | |
| <i>Panicum maximum</i> | Mombasa, Tanzania | X | X | (x) | | (x) | |
| <i>Paspalum atratum</i> | Pojuca, Ubon | X | (x) | | | X | |
| <i>Pennisetum purpureum</i> | (Napier) | | X | | | X | |
| <i>Pennisetum</i> hybrids | (King grass) | | X | | | X | |
| Herbaceous legumes | | | | | | | |
| <i>Arachis pintoi</i> | Amarillo | X | | | | X | |
| <i>Calopogonium mucunoides</i> | (Calopo) | (x) | | | | X | |
| <i>Centrosema molle</i> | Common centro | X | | | | X | |
| <i>Centrosema pascuorum</i> | Cavalcade | X | | X | X | | |
| <i>Desmodium heterocarpon</i> subsp. <i>ovalifolium</i> | (Ovalifolium) | X | | | | X | |
| <i>Desmodium uncinatum</i> | (Silverleaf desmodium) | (x) | (x) | | | (x) | |
| <i>Lablab purpureus</i> | Rongai | (x) | X | X | | (x) | |
| <i>Macroptilium atropurpureum</i> | Sirat | X | | (x) | | (x) | |
| <i>Mucuna pruriens</i> | (Mucuna) | | (x) | | (x) | X | |
| <i>Pueraria phaseoloides</i> | (Tropical kudzu) | X | | | | X | |
| <i>Stylosanthes capitata</i> + <i>S. macrocephala</i> (mixture) | Estilosantes Campo Grande | X | | | (x) | | |
| <i>Stylosanthes guianensis</i> | CIAT 184, Cook | X | (x) | X | (x) | X | |
| <i>Stylosanthes hamata</i> | Verano | X | | | | X | |
| <i>Stylosanthes scabra</i> | Seca | X | (x) | | X | | |
| Shrub and tree legumes | | | | | | | |
| <i>Calliandra calothyrsus</i> | (Calliandra) | | X | | | X | |
| <i>Cratylia argentea</i> | (Cratylia) | X | X | (x) | | X | |
| <i>Flemingia macrophylla</i> | (Flemingia) | | | | | X | |
| <i>Gliricidia sepium</i> | (Gliricidia) | (x) | X | | | (x) | |
| <i>Leucaena leucocephala</i> | Cunningham, Tarramba | X | X | (x) | | X | |

¹X indicates major use; (x) indicates minor use.

In the 1930s in North Queensland, Australia, the presence of naturalized *Stylosanthes humilis* (then *S. sundai-ca*, “Townsville lucerne”) in natural pastures was observed to boost animal growth rates (McTaggart 1937), resulting in extensive research on the benefits of including adapted legumes in tropical grass pastures. The tech-

nology was subsequently taken up elsewhere in the tropics (Table 3). Selection from within large collections of germplasm identified cultivars of species in the genera *Centrosema*, *Desmodium*, *Leucaena* and *Stylosanthes* for use in tropical and subtropical Australia (Table 2). Only few cultivars were bred, e.g. *Macroptilium*

atropurpureum cv. Siratro (Hutton 1962) and *Centrosema pascuorum* cv. Cavalcade (Clements et al. 1986) in Australia and psyllid-tolerant *Leucaena* hybrids in Hawaii (Austin et al. 1998).

In tropical America, the focus was on legumes adapted to acid, infertile soils and biotic constraints. The most promising species identified were (Tables 2 and 3): *Arachis pintoi*, *Cratylia argentea*, *Desmodium heterocarpon* ssp. *ovalifolium* (“*D. ovalifolium*”), *Stylosanthes capitata* and *S. macrocephala*; the latter two were also released as a mixture in “Estilosantes Campo Grande” (Fernandes et al. 2005). Other species in the genera *Centrosema*, *Desmodium* and *Stylosanthes* also show promise but as yet there is little adoption by producers.

In general, the main constraints to increased use and impact of forage legumes are considered to be:

- 1) diseases and insect pests, e.g. anthracnose (caused by *Colletotrichum gloeosporioides*) in *Stylosanthes* and psyllids in *Leucaena leucocephala*;
- 2) anti-nutritive compounds, e.g. mimosine in *L. leucocephala* and tannins in *Flemingia macrophylla*;
- 3) lack of clear management guidelines that ensure persistence of an adequate proportion of legume in grass-legume associations; and
- 4) failure to meet, in some cases, farmer expectations of increased animal production due to low genetic potential of animals used.

In addition to improving livestock production (Table 3), forage legumes can have important impacts on the environment (see overview by Schultze-Kraft et al. 2014). As a consequence of N fixation, grass-legume pastures need no N fertilizer and so offer both economic and environmental benefits. Furthermore forage legumes improve soil quality and can increase the yield of subsequent crops, which is particularly important in small-holder crop-livestock systems. Deep-rooted legumes scavenge nutrients from deep in the soil and redistribute them at the soil surface in litter. Cover legumes reduce weed pressure, can control pests and protect soil from erosion (including loss of soil organic matter) by water and wind (see also Section “Ecological intensification to generate multi-dimensional benefits and to minimize trade-offs” below).

Crop residues as feed. Crop residues (CR) are an important strategic feed resource (Blümmel et al. 2012), totaling 3.8 Bt DM/yr worldwide, of which cereals contribute 74%, sugar crops 10%, legumes 8%, tubers 5% and oil crops 3% (Lal 2005). Cereal CR have low nutritive quality, but leguminous CR can be very nutritious. In contrast with forages, production costs for the CR are charged to the crop that produces them (Blümmel et al.

2009). While the nutritive quality of cereal CR for use as fodder can be improved by chemical, physical or biological treatments, there has been little uptake of these technologies.

The second generation of processes to produce bio-fuels focuses on hydrolyzing plant ligno-celluloses to sugars, which are then fermented to ethanol. If the process can be made cheap and efficient, hydrolyzing low-quality straw, stover and woody material for use as animal feed may be a viable option. The trade-offs would be whether to use the hydrolyzed material as animal feed or to make ethanol (Dixon et al. 2010).

Ecological intensification to generate multi-dimensional benefits and to minimize trade-offs

Benefits. Improved forage-based systems can produce a wide range of benefits (Figure 3). White et al. (2013) conducted a meta-analysis of 98 studies on the effects of improved forages and their management, using a “triple bottom-line” approach (Elkington 1997) to analyze social, economic and environmental changes along a generic forage-livestock value chain with links of input, production, transformation and marketing.

Improved forages provide *social benefits* by improving the welfare of individuals, households, communities and entire countries. Intermediate outcomes include increases or decreases in labor use of family members depending on the system. Increases in livestock production can improve food and nutritional security (Rosegrant et al. 2009). Other social benefits include enhanced capacity to participate in community organizations, which can lead to institutional and policy changes, with possible improved well-being and equity. Resilience of both the farm and the community is likely, particularly in integrated systems with diverse production and market risks.

Improved forages can generate a variety of *economic benefits*. At the farm level, changes in soil physical, chemical and biological properties can result in improved soil quality, increased water infiltration and reduced fertilizer requirements (Ayarza et al. 2007). Forages can allow higher land and animal productivity, resulting in a shift from subsistence-orientation to market-orientation. Traditional livestock products may give way to new value chains for special market niches, such as sale of fresh forage in Thailand (Nakamane et al. 2008), pasture seed in Bolivia (Pizarro and Sauma 2007), cheese in Central America (Holmann et al. 2004), concentrates from legume grains in Zimbabwe (Murungweni et al. 2004) and organic livestock products (Rahmann 2009).

Table 3. Effects of tropical legumes on cattle liveweight gain and milk yield.

| Pasture type | Country/ region | Climate/ ecosystem | Legume species | Grass alone | Grass with legume | Reference |
|---|--------------------------------------|---|--|--|--|-------------------------------------|
| A. Liveweight gain | | | | | | |
| Native (<i>Heteropogon contortus</i>) | Australia, Central Queensland | Dry subtropics | <i>Stylosanthes humilis</i> | 83 kg/an/yr | 121 kg/an/yr | Shaw and Mannetje (1970) |
| Native | Australia, Northern Territory | Dry tropics | <i>Centrosema pascuorum</i> ¹ | -183 g/an/d | 489 g/an/d | McCown et al. (1986) |
| <i>Urochloa mosambicensis</i> | Australia, Northern Queensland | Dry tropics | <i>Leucaena leucocephala</i> cv. Cunningham <i>L. diversifolia</i> | 381 g/an/d ² | 723 g/an/d ² 532 g/an/d ² | Jones et al. (1998) |
| <i>Brachiaria humidicola</i> | Venezuela | Humid tropics | <i>Desmodium ovalifolium</i> ³ | 336 g/an/d | 385 g/an/d | Chacón (2005) |
| <i>B. decumbens</i> | Colombia, Llanos | Subhumid (savanna) | <i>Pueraria phaseoloides</i> | 124 kg/an/yr | 174 kg/an/yr | Lascano and Estrada (1989) |
| <i>B. humidicola</i> | Colombia, Llanos | Subhumid (savanna) | <i>Arachis pintoi</i> | 61–115 kg/an/yr 230–288 kg/ha/yr | 89–151 kg/an/yr 302–390 kg/ha/yr | Lascano (1994) |
| <i>B. dictyoneura</i> ⁴ | | | | 106–124 kg/an/yr 248–369 kg/ha/yr | 124–166 kg/an/yr 332–459 kg/ha/yr | |
| <i>B. dictyoneura</i> ⁴ | Colombia, Llanos | Subhumid (savanna) | <i>Centrosema acutifolium</i> cv. Vichada <i>Stylosanthes capitata</i> | 191 g/an/d ⁵ | 456 g/an/d ⁵ 446 g/an/d ⁵ | Thomas and Lascano (1995) |
| <i>B. brizantha</i> | Mexico, Veracruz | Wet-dry tropics | <i>Cratylia argentea</i> | 580 g/an/d | 839 g/an/d | González- Arcia et al. (2012) |
| B. Milk yield (per cow/day) | | | | | | |
| Mixture of <i>B. humidicola</i> , <i>Hyparrhenia rufa</i> and <i>Cynodon dactylon</i> | Rwanda, Bugesera | Dry-subhumid (savanna), medi- um altitude | <i>Stylosanthes scabra</i> (leaf meal) | 0.98 L | 1.27 L (10% meal) 1.40 L (20% meal) 1.52 L (30% meal) | Mupenzi et al. (2009) |
| <i>B. decumbens</i> | Colombia, Cauca | Subhumid tropics (forest margin) | <i>Cratylia argentea</i> | 6.1 kg (cut & carry) 6.1 kg (grazing) | 6.7 kg (cut & carry) 7.5 kg (grazing) | Lascano et al. (2001) |
| <i>B. dictyoneura</i> ⁴ cv. Llanero <i>Andropogon gayanus</i> | Colombia, Cauca | Subhumid tropics (forest margin) | <i>Centrosema macrocarpum</i> <i>C. acutifolium</i> (CIAT 5568) <i>C. macrocarpum</i> <i>C. acutifolium</i> (CIAT 5568) | 8.1 kg 7.8 kg | 9.5 kg 10.0 kg 9.0 kg 8.1 kg | Lascano and Avila (1991) |
| <i>Cynodon nlemfuensis</i> | Costa Rica, Turrialba | Humid tropics (forest margin) | <i>Arachis pintoi</i> <i>Desmodium ovalifolium</i> ³ | 9.5 kg | 10.8 kg 9.4 kg | González et al. (1996) |

¹Supplementation as ley during the main dry season.²192 grazing days.³Now classified as *D. heterocarpon* subsp. *ovalifolium*.⁴Now classified as *B. humidicola*.⁵Means of 3 grazing cycles totalling 385 days; newly established pastures.

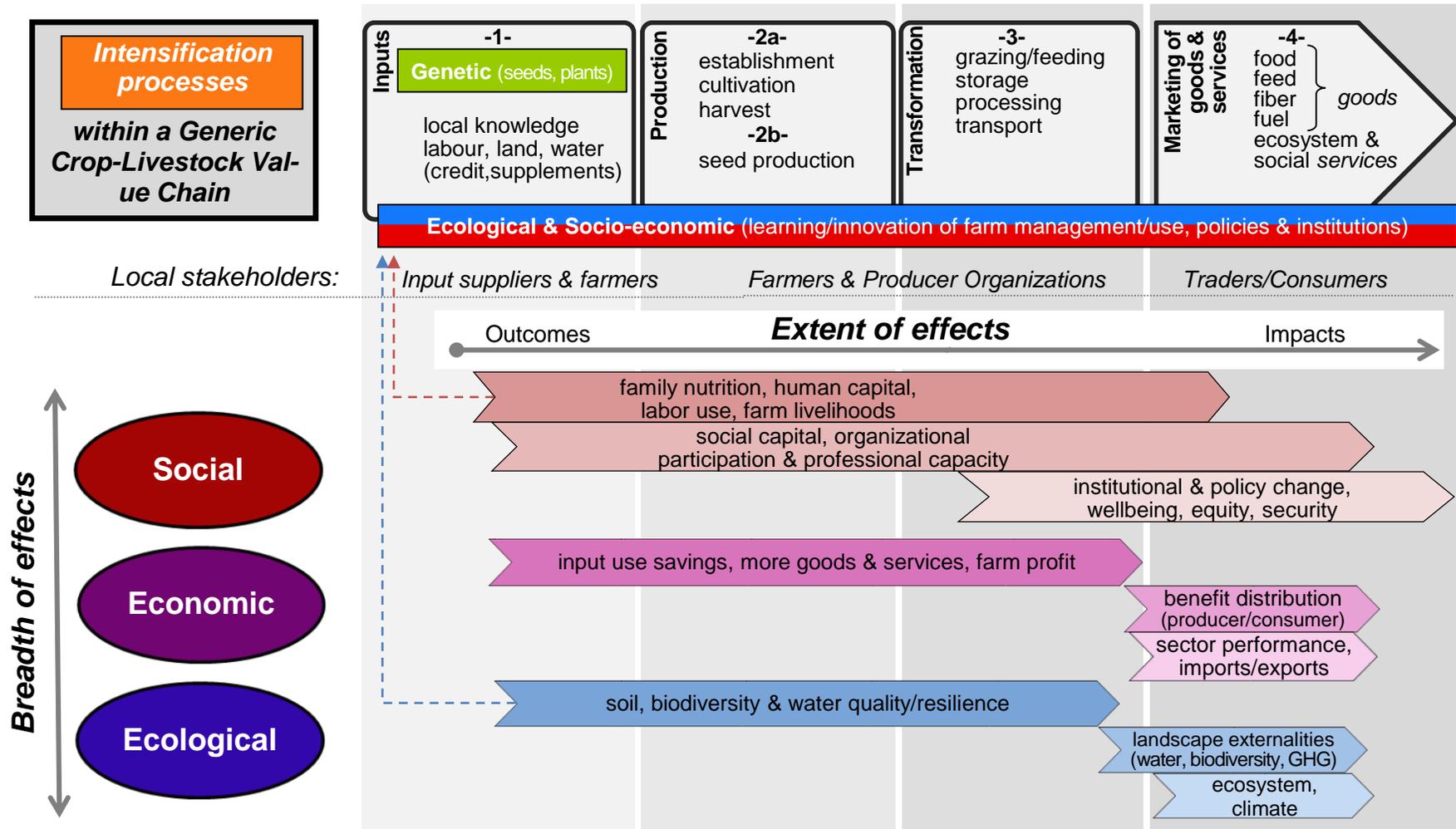


Figure 3. An array of effects generated by sustainable intensification processes of forages within a generic crop-livestock value chain (adapted from White et al. 2013).

Improved tropical forages can provide *environmental benefits* (Humphreys 1981; Schultze-Kraft and Peters 1997). At the farm level, forages adapted to biotic and abiotic stresses provide fast and complete soil cover that results in reduced erosion and weed infestation. Overall, plant production is more stable so that farms are more resilient to weather shocks.

Peters et al. (2013a) reviewed the potential of well-managed improved forages to mitigate GHG emissions, contrasting forage-based systems with feedlot systems, and concluded that the ecological footprint of forage-based systems was lower than that of feedlots. Live-stock-related interventions, including better management of crops and grassland and the restoration of degraded land and soils, can mitigate as much as 3.5 Bt CO₂-eq/yr. This represents about 75% of the global potential bio-physical mitigation (Smith et al. 2008). The potential of improved forages to accumulate C under adequate pasture and animal management is second only to forests (Fisher et al. 2007; Blanfort et al. 2012). A plausible 30% adoption rate of improved deep-rooted *Brachiaria* pastures in the Cerrados of Brazil would represent a mitigation potential of 29.8 Mt CO₂-eq/yr (Thornton and Herrero 2010).

The private sector is aware of these opportunities and is beginning to increase investments in both carbon credits and direct interventions in the supply chains, which provides scope for smallholders to trade mitigation credits to offset the costs of adapting their production systems and generate livelihood benefits. While credits are commonly traded in forestry systems, efforts are expanding to increase similar opportunities for silvopastoral systems (Banerjee et al. 2013; Nepstad et al. 2013).

Comparative analysis of GHG emissions from diverse production systems must include the environmental costs of feed production, including transport. Feedlot cattle produce fewer GHG emissions than forage-fed cattle per unit of beef produced, mainly due to better feed conversion (Casey and Holden 2006; Gerber et al. 2010). However, when we consider the GHG footprint of the grain they consume, forage cattle produce 15% lower total emissions per unit of beef (Pelletier et al. 2010).

Methane emissions. Although some compounds in forages such as tannins can reduce methane emissions by ruminants (Woodward et al. 2004), the most efficient strategy to achieve reduction in emissions is to increase productivity, which reduces methane emissions per unit livestock product. In this context, feeds with higher digestibility and nutrient content produce less methane per

unit of feed ingested (Oliveira et al. 2007). As an adjunct, the deep and vigorous root systems of forage grasses and legumes improve soil structure and aeration. In doing so, they create suitable environments for aerobic methanotrophs, which oxidize methane as a source of C and energy, making soils of forage-based systems important sinks for methane (Mosier et al. 2004).

Carbon accumulation. Well-managed grass and grass-legume pastures have a huge potential to accumulate C, with values comparable with forest systems (Peters et al. 2013b). However, pasture degradation can substantially reduce the carbon stored by forage-based systems (Amézquita et al. 2010). Including legumes with the grass (Fisher et al. 1994; Soussana et al. 2010) or including trees in agroforestry systems (Smith et al. 2008) can increase the C accumulated by forage-based systems. Moreover, forages that are well-adapted to edaphic and climatic stresses have a higher potential to accumulate C than field crops, which have lower net primary productivity, particularly in marginal conditions. Assad et al. (2013) estimated changes in soil C stocks in 3 major Brazilian biomes (Cerrado, Atlantic Forest and Pampa) due to land use change and found soil C stocks under pasture were 15% greater than under the native vegetation.

Nitrous oxide. JIRCAS, CIAT, Corpoica and the University of Hohenheim are researching mechanisms of biological nitrification inhibition (BNI) in forage grasses (Rao et al. 2014; Subbarao et al. 2015). Forages with high BNI capacity enhance N utilization, and reduce N₂O emissions to the atmosphere and nitrate leached to ground water. Research is in progress to quantify the residual effects of BNI on subsequent crop production (Moreta et al. 2014). *Brachiaria humidicola* has high BNI activity, and a few germplasm accessions of *B. humidicola* are also more suitable for temporarily waterlogged environments than the commercial cultivars (Cardoso et al. 2013).

Limitations. Negative impacts of improved forages include soil acidification by legume-only swards (Haynes 1983) and the potential invasiveness of exotic species (Richardson and Pysek 2012). At larger scales, the cumulative effects of increased farm productivity can reduce water flows and quality downstream. Whether off-farm environmental effects are beneficial or detrimental depends on the site-specific context and management practices (Quintero et al. 2009). A serious environmental concern is the potential destruction of

natural ecosystems, such as rainforests, by replacing them with improved pastures, with the concurrent loss of biodiversity at all levels (mainly when monospecific grass pastures replace native multi-species vegetation).

Life cycle assessment. Life cycle assessment (LCA) examines all processes of a production system to estimate all environmental impacts such as GHG emissions, land and energy use, or eutrophication and acidification of water bodies. The growing concern over the environmental footprint of livestock has led to the increased use of LCA, relating environmental impact to a unit of production such as kilograms of meat or milk (de Vries and de Boer 2010). The analysis covers on-farm (C accumulation and GHG emissions) and off-farm stages (fertilizer production, transport, processing and delivery, etc.) related to livestock production. For example, beef production in USA requires 28, 11 and 6 times more land, irrigation water and reactive nitrogen, respectively, and produces 5 times more GHG than the average of the other livestock categories of dairy, poultry, pork and eggs (Eshel et al. 2014). Correct analysis of LCA depends on: (1) boundary conditions; (2) use of the appropriate functional unit (e.g. liters milk corrected for protein and fat contents as opposed to liters fresh milk); and (3) accurate allocation of emissions between different products (e.g. dairy milk, other dairy products or dairy beef) (O'Mara 2012). Furthermore, since such results are highly dependent upon management practices and biophysical conditions, examples of LCA within developing country contexts are likely to reveal different estimates.

LCAs have given insights on environmental impacts of livestock production. For example, a study on milk production in Peru found that the environmental costs of growing crops to make feed concentrates were significant (Bartl et al. 2011). While examples from the tropics are lacking, a study of beef production in Canada concluded that mitigation practices to reduce GHG emissions should focus on reducing enteric CH₄ production from mature beef cows (Beauchemin et al. 2010). In a comparison of conventional and organic milk production in the Netherlands, conventional farms used more energy and caused more eutrophication, while organic farms had higher soil acidification and produced more ammonia, CH₄ and N₂O emissions (Thomassen et al. 2008). Some researchers have called for improvements in LCA methodology to account for indirect second-order effects. These include opportunity costs of livestock production relative to other uses, and further analysis of the competition for land between humans and animals (Garnett 2009; de Vries and de Boer 2010).

Trade-offs. Trade-offs occur when 2 or more competing objectives cannot be simultaneously satisfied in full, thereby resulting in conflict or compromise. The multi-scale and multi-dimensional nature of agroecosystems creates a variety of both trade-offs and synergies between production, livelihoods and environmental objectives. Trade-offs influence the potential acceptability, impact and sustainability of interventions. They must be carefully assessed to achieve the goals of balancing livestock production, livelihoods and environmental protection (Herrero et al. 2009; Smith et al. 2013b).

In many aspects of pasture management, farmers are faced with trade-offs, some of which are subtle, but nevertheless important. For example, removal of biomass from forages by grazing and cut-and-carry represents an export of nutrients from the soil to the animal. In grazed systems, losses are small, although redistribution of N within pure grass pastures becomes important at high stocking rates (Boddey et al. 2004). Where the forage is physically removed, nutrient balance can be negative, if manure is not returned or the loss is not compensated for by applying mineral fertilizers (Rufino et al. 2007). This is especially the case for grasses that have high nutrient demand.

In intercropped systems, forages compete with the main crops for nutrients and water (Zhiping et al. 2004), but give the farmer more options. Thus, intercropping with multi-purpose forages (e.g. for livestock feed and/or soil conservation/improvement) allows farmers to choose between options that generate different benefits. For example, the intercropped forages might be grazed by dairy cows to produce milk during the dry season, when price is highest. The forage legume *Canavalia brasiliensis* can be intercropped with maize to improve the productivity of the smallholder maize-bean-livestock system. A comparison of using *C. brasiliensis* as forage or green manure showed that the forage option generated more income in the short term, and in the longer term avoided the costs of feed supplements and leasing pasture land (Douxchamps et al. 2014).

Prudent management balances trade-offs in using a pasture resource by avoiding overgrazing or complete biomass removal and maintaining sufficient residue to ensure soil cover and rapid regrowth. In addition, livestock excrete about 80% of the N ingested (Rufino et al. 2007), so managing animal manure is a key issue (Douxchamps et al. 2014). In summary, managing the trade-offs with multi-purpose forages can help restore degraded lands and improve crop and livestock production.

Socio-economic intensification to promote wide-spread use of improved forages

Although many farmers and ranchers have adopted improved forages in countries throughout the tropics (White et al. 2013), substantial geographic areas continue to perform below their potential. Adoption of improved forages, much like other agricultural technologies, occurs when a series of conditions exist. These include: (1) superior performance benefits, with greater and more resilient forage yields, energy and nutrient production; (2) low training costs for extensionists and farmers; (3) low financial inputs for establishment and management; (4) effective communication/extension capacities available (public or private); and (5) access to markets for livestock products (Feder and Umali 1993; Shelton et al. 2005).

For areas with little adoption of improved forages, at least one of these conditions remains inadequate. In order to achieve widespread improvement in livelihoods and ES with improved forages, conditions 3–5 above must be met. Since local contexts and associated biophysical and socio-economic conditions differ greatly across the tropics, efforts to increase adoption of forages require different priority actions in different situations. While some situations may require relatively straightforward genetic and ecological (i.e. management) intensification, others will need substantial multi-faceted partnership efforts, including training, marketing and advocacy to change policy. Continued demonstration of the social, economic and environmental benefits of improved forages (Figure 3) can help achieve institutional change. It is important, however, to note that the contribution of improved forages is only one of many coordinated actions essential to achieve sustainable intensification of forage-based crop-livestock-tree systems.

In order for forages to realize their maximum contribution to livelihoods and ES throughout the tropics, 3 actions are needed: (1) changing mindsets and attitudes; (2) increasing opportunities for technology and market co-development amongst farmers, researchers and extensionists; and (3) improving coordination across public and private organizations for enabling vital policies and investments.

Action 1: Change mindsets and attitudes. Altering personal and professional behaviors is a complex undertaking and requires innovative policies and practical solutions at every level of society (Darnton et al. 2005). Sustainability implies new lifestyle choices, with changes to both production and consumption systems. Thus,

sustainable intensification is inherently about social transformation. Simple approaches that merely raise awareness need to expand into efforts that remove complex obstacles, which prevent changes in behavior (Robinson 2012). For example, some farmers in the tropics consider that forage plants are provided by nature and do not require active management, including the application of fertilizer (Peters et al. 2003). These attitudes may slowly change as extensive grazing lands become scarcer and consumer demands for livestock products increase incentives to invest in inputs that improve production. Nevertheless, efforts to publicize the multiple benefits of sustainably-intensified systems can help spur the adoption of improved forage management practices, both directly and indirectly.

Indirect effects occur by raising concerns and expectations of the general public, thereby influencing consumer preferences for sustainably-produced livestock products and associated ES. Social marketing strategies can promote sustainable behavior by making knowledge gained from psychological research relevant and accessible to those who design environmental programs (McKenzie-Mohr 2000). Analysis of social practices can provide better understanding of the underlying norms, values, identity, politics and consumption patterns, thereby revealing complex processes that lead to prevailing environmental practices (Barr et al. 2011). By going beyond advertising and publications, social marketing efforts extend into areas of community development, recruitment, training, and institution and infrastructure planning to achieve change (Robinson 2012).

Action 2: Increase opportunities for co-developing technologies and markets. Although the potential benefits from many improved forages may be known (Figure 3), their performance within specific farm contexts may not be. Scarce land, labor and rainfall are specific constraints that can limit the viability of forage options. Furthermore, crop-livestock systems in the tropics are diverse and dynamic, based on distinct agro-ecological and market conditions, resource endowments, land use, farm management and livelihood strategies. Thus, fitting the “most appropriate” improved forage into a particular context remains a persistent challenge (Byerlee and Collinson 1988; Giller et al. 2010).

Dialogue between farmers, extensionists, researchers and policymakers is needed to integrate forages into crop-livestock-tree systems. Processes of co-discovering and co-developing multiple benefits of forages reduce the gaps between research, development and implementation. For example, the Feed Assessment Tool (FEAST)

assists in formulating site-specific strategies and interventions for improved livestock feeding and production. It offers a systematic and rapid methodology to assess existing feed resources, constraints and opportunities (Duncan et al. 2012; Wassena et al. 2013).

The use of new organizational partnerships (public-public and public-private) and participatory research approaches helps farmers accumulate experience in inter-relating and negotiating with agro-dealers, local traders, consumers and government officials and increases trust and collaboration (Figure 1). Such activities, coupled with monitoring and evaluation and knowledge management and sharing can strengthen performance of both the links and associated connections along value chains (Peters et al. 2013a).

Action 3: Improve coordination across organizations for enabling vital policies and investments. Adoption of forage technology depends on the priorities and associated activities of a wide variety of organizations, including multiple levels of government (national-state-local), international bilateral agencies, non-government organizations (NGOs) with development and/or conservation objectives, producer and trade associations and community-based organizations. With so many types of stakeholders involved directly and indirectly in crop-forage-livestock activities, coordination is needed to avoid conflicting efforts and to achieve efficient, effective and equitable provision of services. Although past and current forage-livestock improvement programs often use an integrated approach (i.e. market development, improved feeding and management), attention is rarely paid to the genetic improvement of animals. To enhance adoption of improved high quality forages, there is a need to characterize and determine the most appropriate animal genotypes that will maximize economic benefits, and coordinate programs and policies. Three general types of government policy instruments (promotional, restrictive and supportive) can influence the adoption of crop-livestock-tree systems:

- Government incentives such as subsidized loans, subsidized credit, tax benefits and price subsidies can have a positive impact. Depending on the structuring and effectiveness of repayment mechanisms, the costs to the public can be minimal or neutral. For example, the state government of Mato Grosso do Sul in Brazil provides tax breaks to change livestock management practices (Bungenstab 2012). The Central American Bank for Economic Integration, funded by the Global Environment Facility, has developed green credits for supporting biodiversity, which take the form of loans to promote sustainable land use and good manure

management, both of which protect water sources (Guerrero Pineda 2012).

- Coercive or punitive measures by governments such as taxes, penalties and land use planning regulations can restrict farming and land use practices. Although these measures have long been a popular tool of the public sector to control environmental damage in developed countries, they have proven to be inefficient and ineffective in developing countries (Blackman 2010).
- Private-sector incentives, including payment for ecosystem services (PES) for C accumulation and storage, biodiversity conservation and watershed protection, are alternative approaches. While enabling both adaptation to, and mitigation of, climate change, improved livestock feeding can improve food security (Bryan et al. 2013). The value of these services can be made directly to providers, through PES or associated with the agricultural product via marketing and certification schemes (Pagiola et al. 2004; Wunder 2005; Van Noordwijk and Leimona 2010). Future opportunities to increase ES via improved forages are substantial, yet are predicated upon legal rights to land and resources, which require support of governments.

Since US\$21 billion was paid to developing countries by international sources in 2010 to generate ES (Sander and Cranford 2010), participating farmers and countries can generate substantial income by reducing emissions through livestock land use change (Havlik et al. 2014). For example, initiatives to reduce emissions from deforestation and forest degradation (REDD+), led by national governments, conservation NGOs and bilateral donors, focus on improved performance, sustainability and resilience of farms near forests. Economic analyses confirm that policies can encourage intensification of cattle ranching in Brazil and abate GHG emissions by sparing land from deforestation. A combination of revenue-neutral taxes and subsidies can help achieve these elements of sustainable intensification (Cohn et al. 2014; Strassburg et al. 2014).

Even without PES, farmers can increase incomes by differentiating their livestock products according to specific attributes such as animal breed, feed type, farm location or farm management practice. Formal certification assures consumers of the product quality, production attributes and validity of the associated price premium. The down-side is that establishing and implementing grades and standards increases producer costs and usually requires public and private sector involvement to support equitable participation in differentiated markets and monitor their performance (Alves-Pinto et al. 2013).

In the face of declining public funding for national agricultural research and extension agencies in many developing countries (Pardey et al. 1999), other organizations, including NGOs that specifically promote animal husbandry (e.g. Heifer International) and general rural development (e.g. CARE International, Catholic Relief Services, SNV-Netherlands), have assumed this role. As a result a blending of institutional responsibilities, while maintaining accountability, e.g. the mapping of expected outcomes from research and development (Earl et al. 2001) and the identification of impact pathways (Douthwaite et al. 2007), is needed to create inter-organizational dialogue.

Conclusions and future perspectives

LivestockPlus abides by the premises of sustainable intensification proposed by Garnett et al. (2013) of increasing food production through higher yields, while emphasizing food security and environmental sustainability. This concept proposes a practical pathway towards the goal of producing more livestock and crop products, with attention to livelihoods and ES for current and future generations.

The following questions are key to making the LivestockPlus concept operational:

- Can we reverse land degradation and improve GHG balance with well-managed forage-based landscapes in the subhumid and humid tropics?
- Is it possible to increase C accumulation and water use efficiency, while reducing GHG emissions per unit of livestock product?
- Are there synergies between crop and livestock production as they vary across regions?
- Where these synergies exist, how can they be exploited?
- How do market dynamics alter the magnitude of these synergies?
- How can LivestockPlus be implemented to promote inclusiveness and social equity and decrease existing gender gaps?

The LivestockPlus concept prioritizes the following action points for research-for-development topics:

Genetic intensification

- Develop stress-adapted and climate-resilient forage grasses and legumes.
- Develop forage grasses and legumes that contribute to reduced methanogenesis and increased polyunsaturated fatty acids with health implications for humans.

- Develop species and cultivar mixtures to improve functional biodiversity and to reduce land degradation.
- Improve interaction between forage researchers and livestock breeders and geneticists.

Ecological intensification

- Analyze the synergistic benefits and trade-offs from using crop residues with improved forages to overcome feed limitations, particularly in the dry season.
- Co-develop forage interventions for different farming systems, from extensive to semi-intensive, identifying suitable entry points for each system.
- Reduce yield gaps in milk and meat production by diversifying feed options.
- Contribute to reversing land degradation and mitigating GHG emissions.
- Assess in detail the potential of forage-based systems to accumulate C.
- Quantify differences between well-managed and degraded pastures in their capacity to accumulate C and determine the role of legumes and trees in further improving the potential for C accumulation.
- Develop methods to quantify ES as a basis for PES.
- Analyze trade-offs between forage productivity, forage quality and GHG emissions.
- Analyze trade-offs between C accumulation in soil, N₂O emission from soil and improvement of soil quality using grass-alone, grass-legume and grass-legume-tree associations.
- Develop decision support tools for use by policy makers, extensionists and farmers.

Socio-economic intensification

- Estimate the impacts of forage-based crop-livestock-tree systems as either trade-offs or win-win-win options for productivity, food and nutritional security and environmental benefits at different scales (from plot to farm to landscape to globe) and compare them with alternative scenarios.
- Assess direct economic benefits for farmers through product differentiation of environmentally-friendly products.
- Identify opportunities for rewarding farmers for ES.
- Identify the different social contexts in which forages are used and adjust actions accordingly.
- Change mindsets and attitudes of both producers and consumers on the importance and potential of improved land management with forage-based systems.

- Increase opportunities for technology and market co-development.
- Improve coordination across public and private organizations for enabling vital policies and investments.

The major outcomes of these actions will be achieved through site-specific research for development. Its target is to double livestock production on less land in the next 10 years in some regions of a few countries, where policies are favorable for adoption, freeing land for sustainable crop production and providing ES, including reduction of colonization pressure on unmodified ecosystems. Applying these interventions in resilient crop and livestock value chains will ensure economic gain and reduce poverty. They are expected to markedly increase the share of smallholder production linked to formal markets. Concerted research on the mitigation potential of forage-based systems to effect climate change can create a functional system of LivestockPlus in at least 5 countries within 5 or 6 years.

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Evaluation of new hybrid brachiaria lines in Thailand. 1. Forage production and quality

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Abstract

Forty-three new hybrid brachiaria lines were evaluated for forage accumulation and nutritive value in Northeast Thailand from 2006 to 2011 in experiments at 2 sites, using Mulato II hybrid brachiaria as a standard for comparison. The parameters evaluated were wet and dry season dry matter (DM) accumulation, leaf:stem ratio, crude protein (CP) concentration and fiber level [acid detergent fiber (ADF) and neutral detergent fiber (NDF)]. No lines consistently displayed superior dry season forage accumulation and leaf:stem ratio over Mulato II. In the wet seasons, 14 lines produced more DM than Mulato II but in only one wet season each. Mulato II produced forage with high leaf:stem ratio in all seasons. Many lines did have significantly higher CP concentrations and lower levels of ADF and NDF than Mulato II, but their forage accumulation and leaf:stem ratio were inferior. Four lines (BR02/1718, BR02/1752, BR02/1794 and BR02/0465) were granted Plant Variety Rights in 2011.

Resumen

En el período 2006–2011 en 2 sitios del noreste de Tailandia (Ubon Ratchathani y Amnart Charoen) fueron evaluadas por su producción de forraje y calidad nutritiva 43 líneas nuevas de híbridos de *Brachiaria*, incluyendo el cultivar (cv.) Mulato II como testigo, procedentes del CIAT. Los parámetros evaluados fueron producción de materia seca (MS) en épocas lluviosa y seca, relación hoja:tallo, concentración de proteína cruda (PC) y niveles de fibra detergente ácido (FDA) y fibra detergente neutro (FDN)]. En la época seca, ninguna de las líneas mostró en forma consistente una producción de MS y relación hoja:tallo superiores que cv. Mulato II. En las épocas lluviosas, 14 líneas produjeron más MS que Mulato II, pero sólo en una época lluviosa cada una. El cultivar Mulato II produjo forraje con alta relación hoja:tallo en todas las épocas. Varias de las líneas presentaron concentraciones de PC significativamente mayores y menores niveles de FDA y FDN que cv. Mulato II, pero su producción de forraje y la relación hoja:tallo fueron inferiores. Las líneas BR02/1718, BR02/1752, BR02/1794 y BR02/0465 alcanzaron la protección de obtención vegetal (*Plant Variety Rights*) en 2011.

Introduction

In 1988 CIAT (Centro Internacional de Agricultura Tropical) in Colombia and EMBRAPA (Empresa Brasileira de Pesquisa Agropecuária) in Brazil began breeding programs on hybridization of brachiaria grasses (Miles et al. 2004). Mulato hybrid brachiaria [*Brachiaria*

ruzizensis (now *Urochloa ruzizensis*) x *B. brizantha* (now *U. brizantha*)] was the first hybrid brachiaria cultivar released from the breeding program. Mulato was granted Plant Variety Rights in 2002 (Loch and Miles 2002) and released by the Mexican seed company Grupo Papalotla in 2004 (Miles et al. 2004). Mulato had high forage yields and forage nutritive value but produced very low seed yields (Hare et al. 2007a). Mulato II (*B. ruzizensis* x *B. decumbens* (now *U. decumbens*) x *B. brizantha*), the second hybrid brachiaria cultivar released, was granted Plant Variety Rights in 2004 (Loch and Miles 2004) and released by Grupo Papalotla

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in 2005 (Argel et al. 2007). Mulato II was similar to Mulato but demonstrated excellent drought tolerance and superior seed yields (Hare et al. 2007b; 2007c) and in 2005, Grupo Papalotla replaced Mulato with Mulato II.

From 2006 to 2011, further studies were conducted by Grupo Papalotla in Thailand on selected lines from the BR02 and BR06 hybrid brachiaria collections from CIAT, and 2 lines from the MX02 collection from Mexico, with the aim of identifying lines with overall superior forage attributes. BR02 and BR06 are the names of new hybrid progeny from the CIAT breeding program in spaced plant trials in Colombia between 2002 and 2006. MX02 is the name given to new hybrid progeny, from original BR0 progeny that were further evaluated by Grupo Papalotla in Mexico during 2002–2005. New cultivar selection in the Thailand experiments focused on dry matter yield, forage nutritive value, seed production, drought tolerance and persistence.

This paper describes these studies on forage accumulation and nutritive value of hybrid brachiaria lines. A second paper (Hare et al. 2015) describes research on the seed production of these same lines.

Materials and Methods

Experiment 1 – BR02 and MX02 collections

A field experiment was conducted at Ubon Ratchathani University, Thailand, (15° N, 104° E; 130 masl) from 2006 to 2008. The site was on an upland sandy low humic gley (Paleaquult) soil (Roi-et series) (Mitsuchi et al. 1986). Soil samples, taken at planting in May 2006, showed that the soil was acid (pH 4.6; water method), and low in organic matter (1.1%), N (0.04%), P (3.5 ppm; Bray II extraction method) and K (27.4 ppm). Prior to planting the experiment, the site had grown a

mixture of native grasses and legumes for many years. Thirteen hybrid brachiaria lines from the BR02 collection, 2 from the MX02 collection and cv. Mulato II (Table 3) were planted in June 2006 in a randomized complete block design with 4 replications; details of field crop management are presented in Table 1. Seedlings were grown in a nursery and transplanted into the field plots with 50 x 50 cm spacings (60 plants per plot). At each sampling cut, the forage in six 0.25 m² quadrats per plot was cut 5 cm from ground level and weighed fresh. A 300 g subsample was sorted into leaves and stems and dried at 70 °C for 48 h to determine dry weight. The dried subsamples were analyzed for total N (Kjeldahl method) in order to calculate crude protein (CP, %N x 6.25), acid detergent fiber (ADF, Van Soest method) and neutral detergent fiber (NDF, Van Soest method) concentrations. After each sampling cut, the remaining herbage in the plots was cut to 5 cm from ground level and removed.

Experiment 2 – BR06 collection

This study was conducted at a site, 90 km north of Ubon Ratchathani University, at the Amnart Charoen Livestock Development Centre, Amnart Charoen province, Northeast Thailand (15.5° N, 104.4° E; elevation 168 masl) from 2008 to 2011. The site was on an upland sandy reddish brown earth (Haplustalf) soil (Chatturat series) (Mitsuchi et al. 1986). Soil samples taken at planting in July 2008 showed that the soil was acid (pH 4.6; water method), very sandy (75% sand), and low in organic matter (0.4%), N (0.04%) and K (31 ppm), and adequate for P (25.2 ppm; Bray II extraction method). Prior to cultivation, the site had been growing Tanzania guinea grass (*Panicum maximum*, now *Megathyrsus maximus*) for 5 years.

Twenty-eight hybrid brachiaria lines from the BR06 collection, 4 from the BR02 collection (Table 7), and

Table 1. Field crop management during evaluation of hybrid brachiaria lines (Experiment 1).

| | |
|-----------------------|--|
| Field cultivation | Plowing x 2, disking x 1, harrowing x 1 |
| Plot size | 3 m x 5 m with 50 cm walkway around plots and 1 m between replications |
| Planting date | 1–5 Jun 2006 |
| Cleaning cut | 3 Aug 2006; all plots cut to 5 cm above ground level |
| Sampling harvests | |
| First wet season | 2006: 13 Sep & 2 Nov |
| First dry season | 2007: 10 Jan, 21 Mar & 30 Apr |
| Second wet season | 2007: 11 Jun, 26 Jul, 10 Sep & 29 Oct |
| Second dry season | 2008: 2 Jan & 25 Apr |
| Harvest quadrats/plot | Six 0.25 m ² random quadrats |
| Fertilizer | Nil at planting; 200 kg/ha NPK (15:15:15) after every harvest |

cvv. Mulato II, Toledo (*B. brizantha*) and Marandu (*B. brizantha*) were planted in a randomized complete block design with 4 replications in July 2008. Seedlings were grown in a nursery and transplanted into the field plots in 50 x 50 cm spacings (48 plants per plot). Details of crop management are presented in Table 2. Sampling and laboratory analyses were the same as for Trial 1.

Data from the experiments were subjected to analysis of variance, using the IRRISTAT program from the International Rice Research Institute (IRRI). Entry means were compared by Fisher's protected LSD ($P \leq 0.05$).

Results

Rainfall

Experiment 1 – BR02 and MX02 collections. Rainfall at Ubon Ratchathani in 2006 and 2007 was 9 and 11%, respectively, below the 13-yr mean (Figure 1). Rainfall in the dry seasons (Nov–Apr) was above average in 2006/2007 (33%) but nearly 90% below average in 2007/2008, with only 26 mm falling from November to April.

Table 2. Field crop management during evaluation of hybrid brachiaria lines (Experiment 2).

| | |
|-----------------------|--|
| Field cultivation | Plowing x 2, disking x 1, harrowing x 1 |
| Plot size | 3 m x 4 m with 50 cm walkway around plots and 1 m between replications |
| Planting date | 26–28 Jul 2008 |
| Cleaning cut | 8 Sep 2008; all plots cut to 5 cm above ground level |
| Sampling harvests | |
| First wet season | 2008: 20 Oct |
| First dry season | 2008: 18 Dec |
| | 2009: 28 Apr |
| Second wet season | 2009: 16 Jun, 3 Aug, 15 Sep & 29 Oct |
| Second dry season | 2010: 19 Jan & 27 Apr |
| Third wet season | 2010: 9 Jun, 4 Aug, 14 Sept & 26 Oct |
| Third dry season | 2011: 26 Apr |
| Harvest quadrats/plot | Six 0.25 m ² random quadrats |
| Fertilizer | Nil at planting; 200 kg/ha NPK (15:15:15) after every harvest |

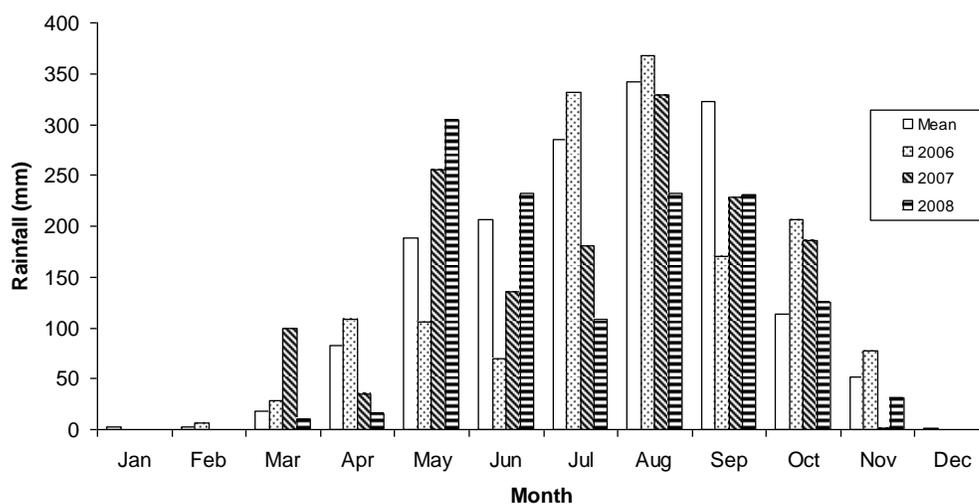


Figure 1. Rainfall at the Ubon Ratchathani University meteorological station, 1 km from the research site, during the experiment and the 13-yr mean (2000–2012).

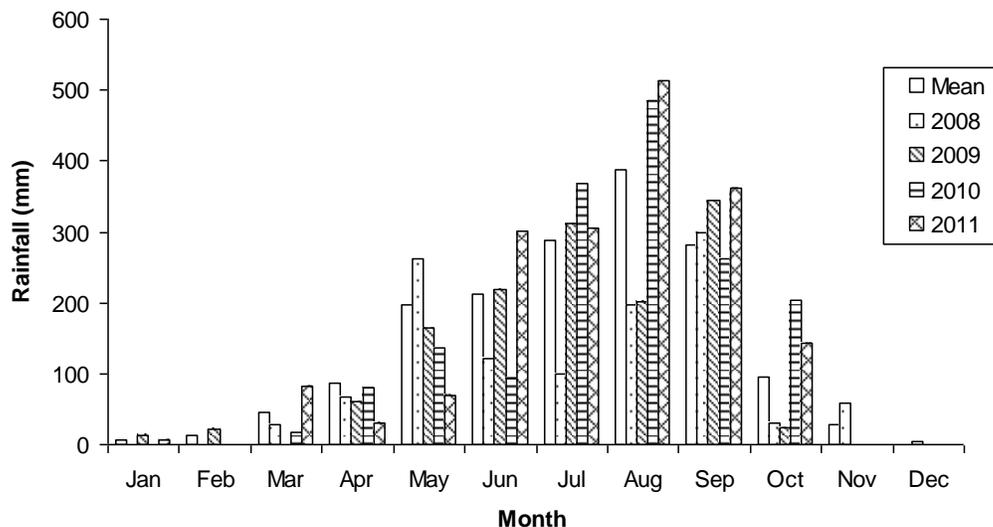


Figure 2. Rainfall at the Amnart Charoen meteorological station, 9 km from the research site, during the experiment and the 13-yr mean (2000–2012).

Experiment 2 – BR06 collection. Rainfall at Amnart Charoen in 2008 and 2009 was, respectively, 30 and 20% below the 13-yr mean, but 2010 rainfall was slightly above the mean (Figure 2). Rainfall in the dry seasons (Nov–Apr) during the experiment was 30% (2008/2009), 47% (2009/2010) and 74% (2010/2011) below the mean.

Forage accumulation and nutritive value

Experiment 1 – BR02 and MX02 collections. Only 2 lines accumulated more DM than Mulato II: BR02/1752 in the first dry season and second wet season and BR02/1718 in the second dry season (Table 3). BR02/0768 was the only line that produced a higher leaf proportion than Mulato II, which was in the second dry season.

Crude protein concentrations were higher in the dry season than in the wet season and higher in leaves than in stems (Table 4). BR02/1452 had higher CP concentrations than Mulato II in both seasons and plant parts.

ADF concentrations were lower in the dry season than in the wet season and in leaves than in stems (Table 4). BR02/1752, 1794 and 1452 had overall lower ADF concentrations than Mulato II, while BR02/0465 and MX02/1263 had higher ADF concentrations.

Lower NDF concentrations were found in the dry season than in the wet season and in leaves than in stems (Table 4). BR02/1752 had lower overall NDF concentrations, while BR02/0465 and MX02/1263 had higher NDF concentrations.

Experiment 2 – BR06 collection. Several BR06 lines accumulated significantly more DM than Mulato II: BR06/0206, 0387, 0423, 0850, 1348, 1366, 1388, 1454 and 2058 and Marandu in the first wet season; and BR06/0206, 1175, 1278, 1415 and 1454 in the third wet season (Table 5). In the second wet season, only Toledo accumulated more DM than Mulato II, while all BR06 lines, except for BR06/1000, 1278 and 1696, accumulated significantly less DM than Mulato II.

No lines or cultivars accumulated significantly more DM than Mulato II in the dry seasons (Table 5). In the first dry season, Mulato II accumulated significantly more DM than more than half the BR06 lines, BR02/1718 and BR02/1372. In the second dry season, DM accumulation was similar for all lines and cultivars, as it was in the third dry season, except for BR06/1433, 1567 and 1922, which produced significantly less DM than Mulato II.

No hybrid line or cultivar produced a higher leaf proportion than Mulato II throughout the trial at Amnart Charoen (Table 6). Mulato II produced a higher leaf proportion than all hybrids and other cultivars in all seasons, except: Marandu, BR06/1000, 1567, 1932 and 2020 in the first wet season; Toledo, BR02/1372, BR06/0012, 0387, 0531, 0584, 1000, 1175 and 1922 in the first dry season; BR06/0204 and 0423 and Toledo in the second wet season; and BR06/1922 in the second and third dry seasons.

Table 3. Dry matter accumulation and leaf percentages in forage DM of hybrid brachiaria lines in wet (May–October) and dry (November–April) seasons from 2006 to 2008 in Ubon Ratchathani, Thailand.

| Hybrid line/ cultivar | Dry matter accumulation (kg/ha) | | | | Leaf percentage (%) | | | |
|--------------------------|---------------------------------|--------|-------------|-------|---------------------|--------|-------------|--------|
| | First year | | Second year | | First year | | Second year | |
| | Wet | Dry | Wet | Dry | Wet | Dry | Wet | Dry |
| Mulato II | 4,460 | 3,280 | 10,570 | 2,320 | 69 | 85 | 72 | 81 |
| BR02/0465 | 3,720 | 3,240 | 11,590 | 2,480 | 66 | 77 | 66 | 78 |
| BR02/0768 | 3,680 | 3,910 | 10,650 | 2,240 | 68 | 87 | 74 | 88 |
| BR02/0771 | 3,770 | 3,580 | 10,870 | 2,330 | 70 | 80 | 71 | 83 |
| BR02/0799 | 3,650 | 2,460 | 10,730 | 2,520 | 64 | 85 | 63 | 80 |
| BR02/1245 | 3,900 | 3,370 | 10,400 | 2,170 | 62 | 78 | 64 | 80 |
| BR02/1372 | 3,910 | 3,960 | 8,720 | 2,460 | 60 | 76 | 66 | 78 |
| BR02/1452 | 2,680 | 2,320 | 7,670 | 1,930 | 70 | 80 | 67 | 80 |
| BR02/1485 | 4,920 | 3,410 | 11,790 | 2,560 | 60 | 81 | 65 | 79 |
| BR02/1718 | 4,790 | 3,710 | 10,390 | 2,930 | 51 | 77 | 67 | 76 |
| BR02/1728 | 3,810 | 3,510 | 10,560 | 2,160 | 63 | 79 | 56 | 81 |
| BR02/1747 | 4,230 | 3,220 | 10,540 | 2,280 | 51 | 77 | 56 | 76 |
| BR02/1752 | 4,200 | 3,990 | 12,600 | 2,160 | 60 | 78 | 55 | 79 |
| BR02/1794 | 3,700 | 3,300 | 11,150 | 2,500 | 61 | 78 | 55 | 77 |
| MX02/1263 | 5,010 | 3,230 | 10,860 | 2,110 | 63 | 81 | 66 | 80 |
| MX02/1423 | 4,150 | 3,190 | 10,570 | 2,050 | 56 | 77 | 56 | 73 |
| Mean | 4,040 | 3,360 | 10,640 | 2,230 | 62 | 80 | 64 | 79 |
| LSD (P<0.05) | 990 | 700 | 1,350 | 430 | 5.2 | 4.2 | 3.7 | 4.7 |
| F ratio | 2.78 | 3.48 | 5.73 | 2.62 | 10.06 | 5.39 | 22.56 | 4.11 |
| Probability | 0.004 | <0.001 | <0.001 | 0.006 | <0.001 | <0.001 | <0.001 | <0.001 |

Table 4. Average crude protein (CP), acid detergent fiber (ADF) and neutral detergent fiber (NDF) concentrations in stem (S) and leaf (L) of hybrid brachiaria lines in wet (May–October) and dry (November–April) seasons from 2006 to 2008 in Ubon Ratchathani, Thailand.

| Hybrid line/ cultivar | CP (%) | | | | ADF (%) | | | | NDF (%) | | | |
|--------------------------|--------|-------|-------|-------|---------|--------|--------|--------|---------|--------|--------|--------|
| | Wet | | Dry | | Wet | | Dry | | Wet | | Dry | |
| | S | L | S | L | S | L | S | L | S | L | S | L |
| Mulato II | 4.7 | 8.6 | 5.9 | 9.7 | 37.4 | 30.9 | 33.1 | 28.6 | 66.0 | 58.4 | 62.1 | 53.5 |
| BR02/0465 | 4.5 | 9.1 | 6.2 | 10.1 | 39.5 | 33.7 | 34.3 | 30.5 | 67.0 | 61.3 | 63.2 | 57.4 |
| BR02/0768 | 4.5 | 8.9 | 6.4 | 9.6 | 37.8 | 29.6 | 32.7 | 27.1 | 65.7 | 58.0 | 60.5 | 53.2 |
| BR02/0771 | 5.5 | 9.9 | 7.2 | 9.8 | 38.3 | 29.7 | 33.9 | 27.0 | 65.3 | 56.8 | 61.7 | 52.6 |
| BR02/0799 | 5.4 | 8.2 | 7.7 | 10.1 | 36.4 | 29.5 | 30.8 | 26.2 | 64.8 | 59.3 | 61.1 | 54.9 |
| BR02/1245 | 4.3 | 8.1 | 6.0 | 9.3 | 40.1 | 30.4 | 34.2 | 27.1 | 68.7 | 58.3 | 63.6 | 54.8 |
| BR02/1372 | 4.9 | 8.9 | 6.0 | 9.4 | 38.5 | 30.9 | 35.3 | 27.0 | 65.5 | 56.9 | 62.7 | 53.4 |
| BR02/1452 | 5.4 | 9.6 | 7.3 | 10.2 | 37.3 | 29.4 | 33.4 | 26.0 | 66.3 | 56.6 | 61.7 | 51.9 |
| BR02/1485 | 4.4 | 9.2 | 6.3 | 9.7 | 39.5 | 30.0 | 34.7 | 28.0 | 67.6 | 59.0 | 64.6 | 53.8 |
| BR02/1718 | 5.2 | 9.4 | 7.0 | 9.4 | 37.5 | 32.3 | 31.6 | 27.6 | 63.2 | 56.6 | 59.4 | 53.0 |
| BR02/1728 | 4.8 | 9.6 | 6.1 | 9.8 | 39.8 | 31.1 | 35.6 | 29.0 | 66.6 | 59.2 | 64.3 | 54.8 |
| BR02/1747 | 4.4 | 8.7 | 5.7 | 8.9 | 38.2 | 32.3 | 32.5 | 29.5 | 66.9 | 57.3 | 62.3 | 54.4 |
| BR02/1752 | 4.1 | 9.1 | 5.8 | 8.7 | 36.6 | 30.0 | 32.4 | 27.8 | 65.8 | 57.0 | 60.5 | 52.5 |
| BR02/1794 | 4.4 | 8.6 | 6.3 | 8.8 | 38.2 | 30.8 | 32.8 | 27.2 | 66.3 | 58.6 | 61.9 | 52.6 |
| MX02/1263 | 4.1 | 8.1 | 6.6 | 9.7 | 40.0 | 29.7 | 34.5 | 29.9 | 69.9 | 60.6 | 63.9 | 54.1 |
| MX02/1423 | 4.4 | 8.8 | 6.4 | 9.9 | 39.8 | 29.1 | 34.1 | 27.0 | 66.9 | 57.2 | 61.9 | 52.6 |
| Mean | 4.7 | 8.9 | 6.4 | 9.6 | 38.4 | 30.6 | 33.5 | 27.8 | 66.4 | 58.2 | 62.2 | 53.7 |
| LSD (P<0.05) | 0.59 | 0.90 | 0.90 | 0.81 | 0.93 | 0.82 | 0.79 | 0.76 | 1.00 | 0.91 | 0.85 | 0.73 |
| F ratio | 7.17 | 15.47 | 21.7 | 5.1 | 23.1 | 58.9 | 24.6 | 22.7 | 61.5 | 31.0 | 48.3 | 51.5 |
| Probability | <0.001 | 0.001 | 0.014 | 0.007 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 |

Table 5. Dry matter accumulation (kg/ha) of hybrid brachiaria lines and cultivars in wet (May–October) and dry (November–April) seasons from 2008 to 2011 in Amnart Charoen, Thailand.

| Hybrid line/ cultivar | First year 2008/2009 | | Second year 2009/2010 | | Third year 2010/2011 | |
|--------------------------|----------------------|--------|-----------------------|-------|----------------------|-------|
| | Wet | Dry | Wet | Dry | Wet | Dry |
| Mulato II | 1,990 | 4,390 | 9,770 | 1,280 | 5,940 | 1,070 |
| BR02/1794 | 2,670 | 3,680 | 8,790 | 1,150 | 5,300 | 930 |
| BR02/0465 | 1,490 | 4,030 | 10,730 | 1,540 | 5,870 | 1,180 |
| BR02/1718 | 2,390 | 3,490 | 9,340 | 1,300 | 5,430 | 1,210 |
| BR02/1372 | 2,370 | 3,140 | 7,390 | 1,410 | 5,330 | 1,170 |
| Marandu | 3,110 | 4,240 | 9,360 | 1,270 | 6,620 | 1,300 |
| Toledo | 2,800 | 4,340 | 11,690 | 940 | 5,970 | 1,140 |
| BR06/0012 | 2,260 | 3,270 | 7,040 | 1,200 | 6,120 | 890 |
| BR06/0204 | 2,290 | 3,110 | 6,930 | 1,160 | 6,000 | 650 |
| BR06/0206 | 2,990 | 3,870 | 8,330 | 1,240 | 7,510 | 810 |
| BR06/0387 | 2,870 | 3,070 | 6,560 | 1,300 | 6,260 | 850 |
| BR06/0405 | 2,680 | 3,390 | 7,480 | 1,200 | 6,280 | 890 |
| BR06/0423 | 3,290 | 3,740 | 7,710 | 1,450 | 6,580 | 1,280 |
| BR06/0531 | 2,630 | 4,050 | 8,520 | 1,510 | 6,860 | 1,130 |
| BR06/0584 | 2,610 | 3,660 | 7,640 | 1,160 | 6,190 | 790 |
| BR06/0850 | 2,890 | 3,940 | 8,590 | 1,020 | 6,530 | 690 |
| BR06/1000 | 2,330 | 3,210 | 8,730 | 1,630 | 7,690 | 780 |
| BR06/1132 | 2,460 | 3,430 | 7,190 | 970 | 6,360 | 760 |
| BR06/1175 | 2,480 | 3,330 | 7,680 | 1,270 | 7,180 | 860 |
| BR06/1254 | 2,820 | 3,200 | 7,980 | 1,240 | 6,770 | 1,010 |
| BR06/1278 | 2,860 | 3,910 | 8,820 | 1,880 | 7,650 | 1,440 |
| BR06/1348 | 3,090 | 4,390 | 8,460 | 1,200 | 6,860 | 740 |
| BR06/1366 | 3,030 | 3,410 | 8,040 | 1,180 | 6,700 | 990 |
| BR06/1388 | 2,890 | 3,910 | 7,250 | 1,140 | 5,960 | 790 |
| BR06/1415 | 2,760 | 3,430 | 7,460 | 1,050 | 7,790 | 810 |
| BR06/1433 | 2,800 | 3,410 | 6,930 | 790 | 6,270 | 580 |
| BR06/1454 | 2,960 | 3,480 | 8,150 | 1,230 | 7,870 | 900 |
| BR06/1567 | 1,940 | 3,660 | 7,110 | 860 | 4,430 | 610 |
| BR06/1696 | 2,750 | 3,660 | 8,820 | 1,740 | 6,320 | 1,090 |
| BR06/1832 | 2,820 | 3,750 | 6,810 | 1,340 | 5,160 | 1,170 |
| BR06/1922 | 2,740 | 2,970 | 6,710 | 880 | 4,090 | 540 |
| BR06/1932 | 2,470 | 3,450 | 8,410 | 1,420 | 6,060 | 1,010 |
| BR06/2020 | 2,380 | 3,740 | 8,190 | 1,260 | 6,100 | 810 |
| BR06/2058 | 2,970 | 3,300 | 8,210 | 1,150 | 4,820 | 900 |
| BR06/2204 | 2,420 | 3,240 | 7,280 | 1,280 | 4,910 | 1,040 |
| Mean | 2,640 | 3,610 | 8,120 | 1,250 | 6,220 | 940 |
| LSD (P<0.05) | 870 | 780 | 1,160 | ns | 1,190 | 420 |
| F ratio | 2.70 | 2.83 | 5.20 | 1.24 | 4.83 | 1.77 |
| Probability | <0.001 | <0.001 | <0.001 | 0.202 | <0.001 | 0.002 |

Crude protein concentrations in forage were higher in the dry season than the wet season and in leaves than in stems (Table 7). All BR06 lines had CP concentrations either significantly higher than or similar to those of Mulato II in both stems and leaves in the wet season and in stems in the dry season, while only BR06/1922 and 2058 had significantly higher dry season leaf CP levels than Mulato II.

ADF and NDF concentrations varied between seasons, plant parts and hybrid lines (Table 7). Dry

season concentrations were lower than in the wet and leaf concentrations were lower than in stems. Most BR06 hybrid lines had significantly lower dry season leaf ADF and leaf and stem NDF concentrations than Mulato II, while some had lower leaf and stem ADF concentrations in the wet season. While leaf NDF concentrations in the wet season in the BR06 hybrid lines were generally lower than in Mulato II, stem NDF concentrations were generally higher in the hybrids.

Table 6. Leaf proportion (%) of forage DM of hybrid brachiaria lines and cultivars in wet (May–October) and dry (November–April) seasons from 2008 to 2011 in Amnart Charoen, Thailand.

| Hybrid line/ cultivar | First year 2008/2009 | | Second year 2009/2010 | | Third year 2010/2011 | |
|--------------------------|----------------------|--------|-----------------------|--------|----------------------|--------|
| | Wet | Dry | Wet | Dry | Wet | Dry |
| Mulato II | 66 | 74 | 70 | 83 | 72 | 88 |
| BR02/1794 | 52 | 70 | 54 | 75 | 56 | 81 |
| BR02/0465 | 56 | 67 | 65 | 73 | 65 | 78 |
| BR02/1718 | 38 | 69 | 64 | 73 | 67 | 76 |
| BR02/1372 | 43 | 72 | 67 | 76 | 68 | 81 |
| Marandu | 60 | 74 | 63 | 77 | 64 | 80 |
| Toledo | 64 | 73 | 68 | 76 | 69 | 78 |
| BR06/0012 | 58 | 75 | 64 | 76 | 65 | 77 |
| BR06/0204 | 61 | 60 | 69 | 69 | 64 | 77 |
| BR06/0206 | 49 | 67 | 63 | 71 | 64 | 75 |
| BR06/0387 | 52 | 72 | 66 | 75 | 63 | 79 |
| BR06/0405 | 33 | 69 | 55 | 73 | 61 | 78 |
| BR06/0423 | 58 | 70 | 68 | 72 | 66 | 73 |
| BR06/0531 | 58 | 72 | 66 | 76 | 67 | 76 |
| BR06/0584 | 54 | 72 | 65 | 72 | 64 | 73 |
| BR06/0850 | 52 | 62 | 51 | 65 | 54 | 68 |
| BR06/1000 | 64 | 75 | 62 | 77 | 64 | 79 |
| BR06/1132 | 40 | 61 | 61 | 68 | 63 | 76 |
| BR06/1175 | 53 | 75 | 57 | 77 | 62 | 80 |
| BR06/1254 | 57 | 68 | 65 | 74 | 65 | 80 |
| BR06/1278 | 58 | 70 | 61 | 72 | 66 | 73 |
| BR06/1348 | 50 | 65 | 64 | 68 | 62 | 71 |
| BR06/1366 | 28 | 62 | 55 | 67 | 61 | 71 |
| BR06/1388 | 40 | 66 | 54 | 66 | 57 | 67 |
| BR06/1415 | 57 | 68 | 61 | 70 | 61 | 72 |
| BR06/1433 | 45 | 69 | 59 | 72 | 64 | 74 |
| BR06/1454 | 51 | 69 | 56 | 70 | 62 | 72 |
| BR06/1567 | 62 | 69 | 65 | 72 | 63 | 76 |
| BR06/1696 | 55 | 69 | 62 | 72 | 59 | 75 |
| BR06/1832 | 52 | 65 | 60 | 67 | 58 | 68 |
| BR06/1922 | 54 | 76 | 62 | 81 | 65 | 86 |
| BR06/1932 | 60 | 72 | 65 | 74 | 63 | 75 |
| BR06/2020 | 60 | 66 | 58 | 71 | 60 | 75 |
| BR06/2058 | 41 | 70 | 66 | 74 | 64 | 78 |
| BR06/2204 | 58 | 70 | 66 | 72 | 67 | 76 |
| Mean | 53 | 69 | 62 | 74 | 63 | 76 |
| LSD (P<0.05) | 7.2 | 3.8 | 2.9 | 4.3 | 2.6 | 4.8 |
| F ratio | 12.15 | 8.95 | 20.18 | 7.89 | 15.77 | 6.91 |
| Probability | <0.001 | <0.001 | <0.001 | <0.001 | 0.110 | <0.001 |

Table 7. Average wet season (May–October) and dry season (November–April) crude protein (CP), acid detergent fiber (ADF), and neutral detergent fiber (NDF) concentrations in stem (S) and leaf (L) of hybrid brachiaria lines and cultivars from 2008 to 2011 in Amnart Charoen, Thailand.

| Hybrid line/ cultivar | CP (%) | | | | ADF (%) | | | | NDF (%) | | | |
|--------------------------|--------|--------|--------|--------|---------|--------|--------|--------|---------|--------|--------|--------|
| | Wet | | Dry | | Wet | | Dry | | Wet | | Dry | |
| | S | L | S | L | S | L | S | L | S | L | S | L |
| Mulato II | 6.2 | 8.3 | 7.0 | 11.8 | 36.9 | 31.2 | 33.7 | 28.0 | 64.6 | 59.8 | 60.4 | 52.1 |
| BR02/1794 | 6.0 | 7.8 | 7.0 | 9.4 | 38.2 | 31.0 | 32.4 | 28.2 | 65.7 | 59.6 | 56.9 | 51.6 |
| BR02/0465 | 5.5 | 8.5 | 7.7 | 11.9 | 39.9 | 34.3 | 36.3 | 30.9 | 66.8 | 64.7 | 63.3 | 56.5 |
| BR02/1718 | 6.3 | 8.5 | 7.4 | 10.6 | 37.7 | 31.6 | 33.3 | 28.6 | 63.0 | 58.7 | 58.0 | 51.5 |
| BR02/1372 | 7.1 | 8.8 | 7.1 | 9.8 | 38.5 | 30.9 | 35.5 | 28.2 | 64.3 | 58.7 | 61.9 | 50.2 |
| Marandu | 5.5 | 8.5 | 6.4 | 9.8 | 38.3 | 32.9 | 33.8 | 30.4 | 66.5 | 63.6 | 61.4 | 57.5 |
| Toledo | 4.4 | 6.8 | 5.5 | 8.4 | 39.3 | 34.5 | 35.0 | 32. | 68.0 | 64.8 | 61.6 | 58.7 |
| BR06/0012 | 7.4 | 9.0 | 8.9 | 9.7 | 39.6 | 33.1 | 34.0 | 31.0 | 65.7 | 60.2 | 59.6 | 53.1 |
| BR06/0204 | 7.9 | 9.1 | 7.7 | 10.7 | 36.3 | 31.1 | 32.7 | 27.5 | 62.5 | 58.5 | 60.0 | 49.9 |
| BR06/0206 | 7.0 | 9.1 | 8.2 | 11.3 | 39.0 | 31.5 | 33.7 | 27.2 | 65.9 | 60.2 | 59.6 | 50.9 |
| BR06/0387 | 7.7 | 9.3 | 10.9 | 11.4 | 39.5 | 31.0 | 30.7 | 27.9 | 65.8 | 58.6 | 52.2 | 48.5 |
| BR06/0405 | 7.2 | 8.8 | 8.2 | 11.1 | 39.5 | 31.7 | 33.7 | 27.8 | 66.3 | 58.5 | 56.5 | 49.1 |
| BR06/0423 | 7.0 | 8.3 | 8.0 | 9.8 | 37.0 | 31.1 | 32.5 | 28.0 | 66.3 | 58.5 | 56.9 | 50.1 |
| BR06/0531 | 6.3 | 8.6 | 7.0 | 10.7 | 38.0 | 30.4 | 33.9 | 27.6 | 65.5 | 57.9 | 56.6 | 47.9 |
| BR06/0584 | 7.5 | 8.1 | 10.1 | 10.7 | 37.7 | 31.3 | 30.9 | 27.9 | 65.4 | 60.5 | 57.7 | 51.1 |
| BR06/0850 | 6.0 | 8.3 | 7.5 | 10.8 | 39.6 | 30.5 | 34.2 | 26.7 | 69.0 | 60.7 | 62.9 | 51.2 |
| BR06/1000 | 6.6 | 9.2 | 7.9 | 10.6 | 38.0 | 30.8 | 33.0 | 27.6 | 65.4 | 57.0 | 58.7 | 48.3 |
| BR06/1132 | 6.5 | 8.6 | 6.8 | 10.5 | 37.7 | 31.3 | 33.2 | 28.1 | 65.4 | 59.8 | 61.1 | 51.0 |
| BR06/1175 | 6.0 | 9.2 | 8.5 | 10.9 | 37.7 | 31.3 | 31.3 | 27.2 | 65.0 | 58.3 | 57.8 | 49.8 |
| BR06/1254 | 7.2 | 8.1 | 10.9 | 12.0 | 37.8 | 30.6 | 31.8 | 26.1 | 64.4 | 59.1 | 57.4 | 49.1 |
| BR06/1278 | 6.6 | 8.9 | 8.6 | 11.7 | 39.3 | 31.3 | 33.3 | 27.6 | 64.8 | 58.0 | 58.2 | 48.8 |
| BR06/1348 | 7.3 | 8.5 | 7.1 | 10.8 | 38.1 | 30.5 | 34.2 | 27.2 | 64.5 | 58.4 | 60.2 | 49.3 |
| BR06/1366 | 6.6 | 8.9 | 8.8 | 11.8 | 39.0 | 31.5 | 34.1 | 27.7 | 66.9 | 59.5 | 58.8 | 49.9 |
| BR06/1388 | 6.6 | 8.8 | 8.6 | 10.5 | 40.1 | 31.3 | 34.2 | 27.5 | 67.5 | 58.8 | 59.5 | 51.0 |
| BR06/1415 | 6.7 | 8.0 | 7.4 | 11.0 | 38.5 | 30.9 | 34.3 | 27.6 | 67.7 | 60.5 | 60.4 | 51.8 |
| BR06/1433 | 8.5 | 9.3 | 8.3 | 11.8 | 38.9 | 30.7 | 33.5 | 26.2 | 65.3 | 57.4 | 55.8 | 49.0 |
| BR06/1454 | 7.6 | 9.2 | 9.5 | 11.7 | 38.0 | 29.3 | 34.2 | 25.6 | 64.2 | 56.0 | 57.4 | 48.2 |
| BR06/1567 | 7.2 | 7.9 | 8.2 | 10.6 | 38.8 | 32.6 | 32.5 | 27.7 | 65.5 | 60.1 | 58.2 | 51.9 |
| BR06/1696 | 6.8 | 7.9 | 7.5 | 10.1 | 39.0 | 30.6 | 34.2 | 28.1 | 65.5 | 57.3 | 59.0 | 51.0 |
| BR06/1832 | 7.2 | 8.1 | 8.8 | 11.4 | 38.1 | 30.3 | 34.1 | 28.0 | 66.0 | 59.4 | 60.3 | 51.5 |
| BR06/1922 | 7.4 | 8.4 | 9.5 | 13.2 | 37.6 | 29.5 | 31.7 | 25.6 | 65.1 | 57.3 | 59.1 | 48.2 |
| BR06/1932 | 7.0 | 8.4 | 7.5 | 10.8 | 38.0 | 30.1 | 32.4 | 26.3 | 65.3 | 58.8 | 58.8 | 50.7 |
| BR06/2020 | 7.6 | 8.9 | 9.0 | 10.9 | 37.2 | 29.4 | 33.3 | 27.0 | 63.9 | 56.0 | 59.6 | 49.8 |
| BR06/2058 | 7.1 | 8.8 | 7.6 | 13.1 | 37.0 | 29.3 | 31.8 | 24.3 | 65.0 | 57.1 | 58.7 | 47.6 |
| BR06/2204 | 7.4 | 8.6 | 7.8 | 11.1 | 36.5 | 29.2 | 32.6 | 25.9 | 64.2 | 59.1 | 58.4 | 50.3 |
| Mean | 6.8 | 8.6 | 8.1 | 10.9 | 38.5 | 31.1 | 33.3 | 27.7 | 65.5 | 59.2 | 58.9 | 50.8 |
| LSD (P<0.05) | 0.41 | 0.42 | 0.62 | 0.60 | 0.31 | 0.23 | 0.32 | 0.27 | 0.35 | 0.25 | 0.66 | 0.46 |
| F ratio | 31.5 | 16.2 | 27.8 | 20.6 | 127.6 | 394.5 | 117.4 | 271.3 | 312.4 | 606.4 | 84.6 | 238.5 |
| Probability | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 |

Discussion

This study suggests that some of the hybrid lines tested might have advantages over Mulato II in terms of wet season DM accumulation, but there was little evidence of any superiority in terms of DM accumulation during the dry season, when extra forage might be most needed. While, in some seasons, some BR02 and BR06 hybrid lines produced more forage than Mulato II, particularly in the wet season, only 2 hybrid lines produced significantly more DM than Mulato II in the dry season; BR02/1752 and BR02/1718 in one dry season each at the Ubon Ratchathani site. At the Amnart Charoen site, none of the BR06 lines accumulated more DM than Mulato II in the dry, but several did accumulate significantly more DM than Mulato II in the first and third wet seasons.

Forage accumulation was inconsistent, as nine BR06 lines produced, on average, 50% more DM than Mulato II in the first wet season and 5 lines produced, on average, 28% more DM than Mulato II in the third wet season. By contrast, in the second wet season at the Amnart Charoen site, 25 of the twenty-eight BR06 lines produced significantly less DM (28% less) than Mulato II. The superior DM accumulation of BR02/1752 over Mulato II (12,600 vs. 10,600 kg/ha) in the second wet season at the Ubon Ratchathani site was at variance with other reports (Hare et al. 2013a; Vendramini et al. 2014), where BR02/1752 displayed no forage production advantages over Mulato II.

A distinct advantage of Mulato II in these experiments was the superior leaf proportion in forage compared with the other hybrid lines, averaging 66–72% in the wet season and 74–88% in the dry season. The high production of lush, soft green leaves and low stem DM has always made Mulato II an attractive forage for livestock (Argel et al. 2007; Hare et al. 2009).

Concentrations of CP in both leaves and stems were not high in both experiments, and were particularly low (4.3–5.5%) in stems in the wet season in Experiment 1 (Table 4). Interestingly, many of the BR06 lines had superior stem CP concentrations to Mulato II but similar leaf CP concentrations. Compared with other studies on hybrid brachiaria grasses, CP concentrations in these experiments were generally inferior. In Florida, CP concentrations in whole plants (leaf and stem) of Mulato II averaged 13% in one study, 10% in a second (Vendramini et al. 2012) and 11.4% in a third (Vendramini et al. 2014). In cutting trials in Thailand, Mulato II, BR02/1752 and BR02/1794 produced high CP concentrations (8.8–9.4% in stems and 12.6–13.2% in leaves), only when cut every 30 days (Hare et al. 2013a). How-

ever, occasionally, there have been instances of high CP concentrations in hybrid brachiarias. In seed-production trials in Thailand, Mulato and Mulato II forage cut at seed-crop closing produced leaf CP levels up to 17.5% (Hare et al. 2007b). In those trials 200 kg/ha NPK (15:15:15) was applied monthly. In Florida, Mulato pastures, that received 150 kg N/ha in 3 applications (April, June and August), contained CP levels up to 17.2% and averaged 13.8% over 2 years (Inyang et al. 2010). However, such results are not common, and hybrid brachiarias appear to average about 7–11% CP in leaves in Thailand.

Many BR06 lines and BR02/1752 had lower fiber (ADF and NDF) percentages than Mulato II, particularly in the wet season. All hybrid lines and cultivars tested in these trials produced far lower fiber levels than *Panicum maximum* cultivars grown in adjacent trials at the same site (Hare et al. 2013b).

The higher CP concentrations and lower fiber levels than Mulato II in many BR06 lines make them appear attractive, but none produced more dry season forage than Mulato II and they generally had poorer leaf:stem ratios than Mulato II. Several did produce more wet season forage than Mulato II in some seasons but this was not consistent. In addition, their seed yields tended to be erratic and low compared with Mulato II (Hare et al. 2015). More studies need to be conducted on these lines before they could be considered superior to Mulato II and likely to warrant release as a cultivar.

Two BR02 lines have already been released as cultivars. The first was BR02/1752, which was granted Plant Variety Rights in 2011 (Loch et al. 2011b) and released as cv. Cayman by Grupo Papatotla in 2012 (Pizarro et al. 2013). In the current studies Cayman produced more DM than Mulato II in only one wet season and one dry season, and had significantly lower leaf production than Mulato II. The superior leaf production of Mulato II compared with Cayman was also found in another study at Ubon Ratchathani University (Hare et al. 2013a). The nutritive value of Cayman compared with Mulato II was variable, with overall lower CP concentrations but also lower fiber levels. However, Cayman had higher stem CP concentrations and consistently lower fiber levels than Mulato II in a separate study at Ubon Ratchathani University (Hare et al. 2013a). The main factor that justified Cayman's release as a cultivar was its superior waterlogging tolerance compared with Mulato II (Pizarro et al. 2013). It produced a mass of adventitious roots (1,065/plant) following 55 days of waterlogging compared with only 4/plant for Mulato II (Pizarro and Hare 2014). While Cayman's tolerance of waterlogging is lower than that of *B. humidicola* (now *Urochloa*

humidicola), Cayman has superior nutritional value to *B. humidicola*. There is a strong demand for high quality waterlogging-tolerant forage cultivars.

BR02/1794 was granted Plant Variety Rights in 2011 (Loch et al. 2011c) and released by Grupo Papalotla as cv. Cobra in 2014 (E. Stern pers. comm.). In this study, while Cobra had similar DM production to Mulato II, it had significantly lower leaf proportion, particularly in the wet season, where leaf:stem ratio, averaged across both experiments, was 55:45 for Cobra and 70:30 for Mulato II (data not shown). Two main attributes have justified Cobra's release: The first is its strong upright nature, which is ideal for cut-and-carry forage systems. Secondly, its seed production is superior to that of Mulato II, as in 4 of 5 seed harvests, Cobra produced significantly more seed than Mulato II (Hare et al. 2015).

Two other lines, BR02/1718 and BR02/0465, have been granted Plant Variety Rights (Loch et al. 2011a; 2011d) but have not been released as cultivars. BR02/1718 had similar DM production to Mulato II in all seasons but lower leaf production. BR02/0465 produced more DM in the wet season than Mulato II but had lower leaf production. Both lines produced significantly higher seed yields than Mulato II in some seasons (Hare et al. 2015).

While 43 hybrid brachiaria lines were evaluated in this study from 2005 to 2011, only 2 lines, BR02/1752 (cv. Cayman) and BR02/1794 (cv. Cobra), had some attributes superior to those of Mulato II, i.e. upright habit and waterlogging tolerance, that warranted their release as named cultivars. They were not equal to Mulato II in terms of DM yield and nutritive value in this study. While some other lines showed greater DM production in some wet seasons, the superiority displayed in these studies would not justify their being considered for release. Further studies would need to be done before such a decision could be made.

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Evaluation of new hybrid brachiaria lines in Thailand. 2. Seed production

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Keywords: Cayman, Cobra, Mulato II, seed yields, seed yield components.

Abstract

Forty-three new hybrid brachiaria lines bred at CIAT, Colombia, were evaluated for seed production in Northeast Thailand between 2006 and 2010 in 2 experiments at 2 sites, Ubon Ratchathani and Amnart Charoen. These lines were compared with Mulato II hybrid brachiaria. From the BR02 collection, 4 lines, BR02/1718, BR02/1752, BR02/1794 and BR02/0465, were granted Plant Variety Rights in 2011. BR02/1794 produced more seed than Mulato II on most occasions, including both harvests at Ubon Ratchathani and 2 of 3 harvests at Amnart Charoen. The next best yielding lines were BR02/1718 and BR02/0465, which produced more seed than Mulato II in 1 of 2 harvests at Ubon Ratchathani and 2 of 3 harvests at Amnart Charoen. Seed-set (percentage of cleaned seed to spikelets) was generally very low in all hybrid lines (1–12%). The reasons for low seed-set in hybrid brachiaria grasses are discussed, including: being a common defect in newly formed apomictic forage hybrids; previous selection for seed yield not being rigorous enough; and insufficient selection at latitudes and sites where commercial brachiaria seed production is practiced.

Resumen

En el período 2006–11 en 2 sitios del noreste de Tailandia (Ubon Ratchathani y Amnart Charoen) fueron evaluadas por su producción de semilla 43 líneas nuevas de híbridos de *Brachiaria*, incluyendo el cultivar (cv.) Mulato II como testigo, procedentes del CIAT. La línea BR02/1794 produjo más semilla que cv. Mulato II en 2 cosechas realizadas en Ubon Ratchathani y en 2 de las 3 cosechas en Amnart Charoen. Otras líneas con buenos rendimientos de semilla fueron BR02/1718 y BR02/0465 que produjeron más semilla que cv. Mulato II en una de las 2 cosechas en Ubon Ratchathani y 2 de 3 cosechas en Amnart Charoen. La formación de semilla (porcentaje del número de semillas limpias en relación con el número de espiguillas formadas) fue, en general, muy baja en todas las líneas, con un valor entre 1 y 12%. Se analizan las posibles razones de este bajo porcentaje en los híbridos de *Brachiaria* evaluados, entre ellas, un defecto común en híbridos apomícticos recién formados, falta de rigor en las etapas previas de selección para producción de semilla, y fallas en la selección de las latitudes y los sitios de producción comercial de semilla de *Brachiaria*. Las líneas BR02/1718, BR02/1752, BR02/1794 y BR02/0465 alcanzaron la protección de obtención vegetal (*Plant Variety Rights*) en 2011.

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Introduction

Mulato II [*Brachiaria ruziziensis* (now *Urochloa ruziziensis*) x *B. decumbens* (now *U. decumbens*) x *B. brizantha* (now *U. brizantha*)] was the second hybrid brachiaria cultivar released from the hybridization programs begun in 1988 at the Centro Internacional de Agricultura Tropical (CIAT) in Cali, Colombia (Argel et al. 2007). Even though Mulato II produced 60% higher seed yields than Mulato (*Brachiaria ruziziensis* x *B. brizantha*), which was the first hybrid brachiaria released (Hare et al. 2007a), Mulato II seed yields of 232–258 kg/ha were still very low compared with yields from other commercial brachiaria cultivars (not hybrids) elsewhere. In order to compete in price internationally with commercial brachiaria cultivars from Brazil and Australia, commercial seed yields from hybrid brachiaras must be at least 600–700 kg/ha. Commercial seed yields average 650–700 kg/ha in Brazil for cv. Marandu (*B. brizantha*) and cv. Basilisk (*B. decumbens*) (Souza 1999). In Australia, seed yields of Basilisk have reached 1,000 kg/ha (Hopkinson and Clifford 1993). Seed of these commercial brachiaria species is almost half the price of hybrid brachiaria seed. The high price of hybrid brachiaria seed is a reflection of low seed yields and represents a significant barrier to farmer uptake.

From 2006 to 2011, studies were conducted in Thailand on hybrid brachiaria collections from CIAT. The first paper of these studies reported on forage production and quality (Hare et al. 2015), while this paper focuses on seed production.

Materials and Methods

Two experiments were conducted with the aim of selecting lines that had higher seed yields than Mulato II.

Experiment 1. BR02 and MX02 collections

The first experiment was conducted at Ubon Ratchathani University, Thailand, (15° N, 104° E; 130 masl) during 2006 and 2007 alongside a forage biomass experiment. The site was on an upland sandy low humic gley soil that was acid (pH 4.6) and low in organic matter (1.1%), N (0.04%), P (3.5 ppm) and K (27.4 ppm). The mean rainfall was 1,620 mm, with a dominant dry season from November to April (Figure 1). The site is further described in the first paper on forage production (Hare et al. 2015). Thirteen hybrid brachiaria lines from the BR02 collection and 2 from the MX02 collection (Hare et al. 2015) were planted in a randomized complete block design with 3 replicates in June 2006. Seedlings were grown in a nursery and transplanted into the field plots using 50 x 50 cm spacings (48 plants per plot). Details of field crop management are presented in Table 1. Two seed harvests were conducted in 2006 and 2007.

Experiment 2. BR06 collection

This experiment was conducted at one site at the Amnart Charoen Livestock Development Centre, Amnart Charoen province, Northeast Thailand (15.5° N, 104.4° E; 168 masl) from 2008 to 2010 (3 harvests of each plot) alongside the forage trial. The site was on an upland sandy reddish brown earth with a mean rainfall of 1,640 mm, and a dominant dry season from November to April (Figure 2). Soil samples taken at planting in July 2008 showed that the soil was acid (pH 4.6), sandy (75%), and low in organic matter (0.4%), N (0.04%), and K (31 ppm), and adequate for P (25.2 ppm). The site is described further in the first paper on forage production (Hare et al. 2015).

Table 1. Field crop management of hybrid brachiaria lines during evaluation in Ubon Ratchathani, Thailand (Experiment 1).

| | |
|---------------------------|--|
| Field cultivation | Plowing x 2, disking x 1, harrowing x 1 |
| Plot size | 3 m x 4 m with 50 cm walkway around plots and 1 m between replications |
| Sowing date | 1–3 Jun 2006 |
| Cleaning and closing cuts | 2006: 3 Aug 2007: 27 Apr & 24 Jul All plots cut to 5 cm above ground level |
| Fertilizer | 2006: 3 Aug 200 kg/ha NPK (15:15:15); 7 Sep & 3 Oct 46 kg N/ha as urea 2007: 24 Jul 46 kg N/ha as urea; 28 Aug urea (46 kg N/ha), double superphosphate (18 kg P/ha), potash (52 kg K/ha), gypsum (17 kg S/ha); 5 Oct urea (46 kg N/ha) |

Twenty-eight hybrid brachiaria lines from the BR06 collection (Hare et al. 2015), 4 from the BR02 collection, Mulato II, Toledo (*B. brizantha*) and Marandu (*B. brizantha*) were planted in July 2008 in a randomized complete block design with 4 replications. Seedlings were grown in a nursery and transplanted during 26–28 July 2008 into the field plots in 80 x 50 cm spacings (32 plants per plot). Seed harvests were conducted in 2008, 2009 and 2010. Details of field crop management are presented in Table 2.

For both experiments all inflorescences in 3 m of the middle 2 rows were counted once a week. Twenty inflorescences were taken from just outside this area for reproductive analysis at peak anthesis (Table 3). All racemes were counted on each inflorescence and spikelets were counted on 3 racemes per inflorescence, selected from the top, middle and bottom of each inflorescence. At peak anthesis, nylon bags were tied over each seed head of 10 plants (5 plants/row in the above middle 2 rows) to collect the seed. The seed was allowed to fall naturally into the bags and collected once at the end of the season and cleaned through hand screens and a small seed blower to 99% pure seed. Settings were adjusted according to seed weights of each line. Following cleaning, seed yields were corrected to 10% seed moisture content. One thousand seed

weights (TSW) were calculated by drying 4 lots of 100 seeds per plot and correcting to 10% seed moisture.

Data from the experiments were subjected to analysis of variance, using the IRRISTAT program from the International Rice Research Institute (IRRI). Entry means were compared using Fisher's protected LSD ($P \leq 0.05$) procedure.

Results

Rainfall

Experiment 1. BR02 and MX02 collections. Rainfall for the experimental period is shown in Figure 1. The critical period of rainfall for seed production in Thailand is the period from July to October, when the plants establish, develop, and initiate and elongate inflorescences and seed is set and matures. The medium-term mean (13 years) rainfall at Ubon Ratchathani for this period is 917 mm, and in 2007 rainfall closely approximated the mean, but in 2006, rainfall during this critical period was 17% higher. October and November are important months for seed maturity and harvest. In 2006 and 2007, rainfall during these months exceeded the mean, by a factor of 1 in 2006 and 0.5 in 2007.

Table 2. Field crop management of hybrid brachiaria lines during evaluation in Amnart Charoen, Thailand (Experiment 2).

| | |
|---|--|
| Field cultivation | Plowing x 2, disking x 1, harrowing x 1 |
| Plot size | 3.2 m x 4 m with 50 cm walkway around plots and 1 m between replications |
| Sowing date | 26–28 Jul 2008 |
| Cleaning cuts | 2008: No cuts before harvest 2009: 13 Jan & 13 May 2010: 28 Apr & 16 Jun |
| Closing cuts ¹ | All plots cut to 5 cm above ground level 2008 & 2009: No closing cuts from sowing 2 Jul (first group ²), 28 Jul (second group), 8 Sep (third group) 2010: 20 Jul (first group), 10 Aug (second group) |
| Fertilizer (amounts of fertilizer applied based on experience of soils in the region) | 2008: 8 Sep NPK (15:15:15) 200 kg/ha 2009: 13 May, 2 Jul (first group), 28 Jul (second group), 8 Sep (third group) NPK (15:15:15) 200 kg/ha 13 Aug (first group), 28 Sep (second group), 19 Oct (third group) 46 kg N/ha as urea 2010: 28 Apr, 16 Jun, 20 Jul (first group), 10 Aug (second group) NPK (15:15:15) 200 kg/ha 31 Aug (first group), 21 Sep (second group) 46 kg N/ha as urea |

¹Closing cuts, 5 cm above ground level, were implemented about 90 days before peak anthesis (recorded in the first year in 2008) to avoid seed head lodging prior to anthesis.

²Groups are recorded in Table 3.

Table 3. Dates for peak anthesis for hybrid brachiaria lines in Ubon Ratchathani (Experiment 1) and Amnart Charoen (Experiment 2), Thailand.

| Experiment | Peak anthesis date and hybrid line/cultivar |
|--------------|--|
| Experiment 1 | |
| 2006 | Oct 24: BR02/0779; MX02/1423; Nov 11: BR02/1372, 1794; Nov 20: BR02/1484, 1718, 1728, 1747, MX02/1263; |
| 2007 | Dec 12: BR02/0465, 0768, 0771, 1245, 1452, 1752, Mulato II Oct 10–17: BR02/0779, 1372, 1728, 1794, MX02/1423; Oct 24: BR02/0465, 1718, 1752; Nov 1: BR02/0768, 0771, 1452, 1485, 1747, 1752; Nov 8: MX02/1263, Mulato II; No flowering: BR02/1245 |
| Experiment 2 | |
| 2008 | Sep 25–Oct 3: BR06/0405, 1366, 1388, 1433, 1454, BR02/1372; Oct 24: BR06/0206, 0387, 0423, 1000, 1132, 1175, 1415, 1696, 1832, 2058, BR02/0465, 1718, 1794; Nov 1–8: Mulato II, Marandu; Nov 14–21: BR06/0012, 0204, 0584, 0850, 1278, 1348, 1567, 1922, 1932, 2020, 2204; Dec 4: Toledo; No flowering: BR06/0531, 1254 |
| 2009 | ¹ *Sep 15: BR06/0405, 1366, 1388, 1433, 1454, 1922, BR02/1794, 1372; **Sep 28–Oct 8: BR06/0206, 0387, 0423, 0531, 0850, 1175, 1278, 1415; **Oct 19: BR06/0012, 1000, 1132, 1567, 1696, 1832, 1932, 2020, 2058, BR02/0465, 1718; **Nov 15: BR06/0204, 1348, 1584, 2204, Mulato II, Marandu; No flowering: **BR06/1254, ***Toledo |
| 2010 | *Sep 21–28: BR06/0206, 0405, 0850, 1132, 1175, 1278, 1366, 1388, 1415, 1433, 1454, 1922, 1932, 2020, BR02/1794, 1372; **Oct 19: BR06/0204, 1000, 1348, 1696, 1832, 2058, 2204, BR02/0465, 1718; **Nov 2: BR06/0012, 0387, 0423, 0531, 1567; **Nov 23: BR06/0584, Mulato II, Marandu; **Dec 20: Toledo; No flowering: **BR06/1254 |

¹Groups for closing cuts and fertilizer application: * First group, ** Second group, *** Third group.

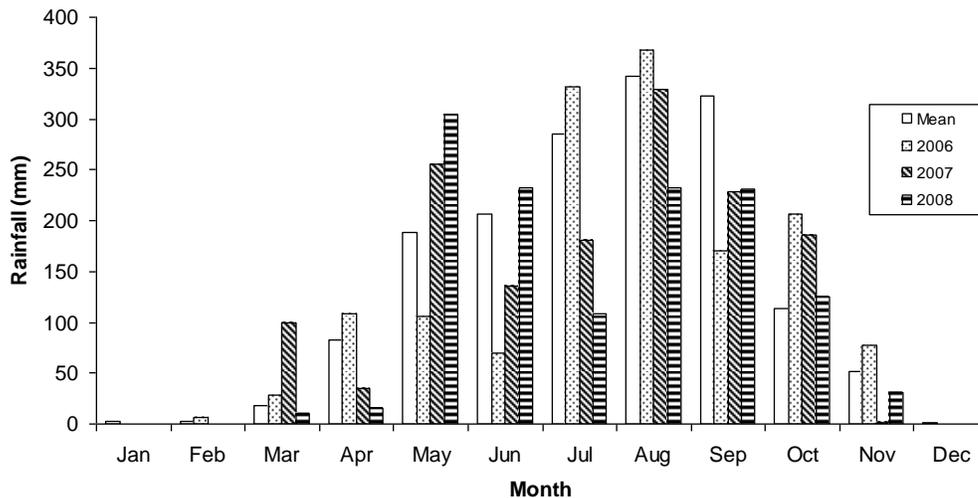


Figure 1. Rainfall at the Ubon Ratchathani University meteorological station, 1 km from the research site, during the experiment and the 13-yr mean (2000–2012).

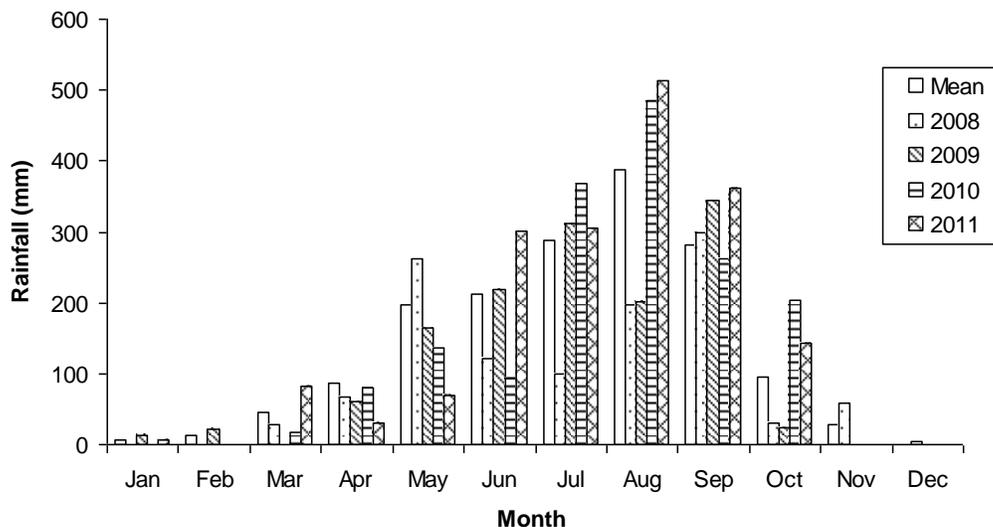


Figure 2. Rainfall at the Amnart Charoen meteorological station, 9 km from the research site, during the experiment and the 13-yr mean (2000–2012).

Experiment 2. BR06 collection. Rainfall for this experiment is shown in Figure 2. Rainfall for the July–September period in 2008 and 2009 was 38 and 22%, respectively, lower than the medium-term mean (13 years), while in 2010, it was 25% higher than the mean. For the October–November period, rainfall in 2008 and 2009 was 40 and 80%, respectively, lower than the medium-term mean. In 2010, rainfall for the same period was 62% higher than the mean but with no rain at all during November.

Seed production

Experiment 1. BR02 and MX02 collections. Seed yields ranged from 12 to 282 kg/ha and one line produced no seed at all in the second year. Hybrid brachiaria line BR06/1794 produced significantly higher seed yields than all other lines in both years, except for BR02/1718 and BR02/0465 in the second year (Table 4). BR02/1718 and BR02/0465 also produced higher seed yields than Mulato II in the second year but not in the first year. One line, BR02/1245, failed to produce any seed in the second year.

Mulato II produced significantly lower numbers of inflorescences per m² than many of the other hybrid lines, which were also significantly lower than the overall mean (Table 4). Lower numbers of inflorescences per

m² were produced in the first year compared with the second year.

Racemes per inflorescence, spikelets per raceme and TSW were lower in the second year than in the first year (Table 4). There was large variability in spikelet numbers among the lines, ranging from 24 to 48. BR02/0465 produced significantly heavier seed (10.3–10.5 g per 1,000 seeds) than all other lines (Table 4). Three lines (BR02/1485, 1747 and 1794) had significantly higher TSW than Mulato II at both harvests.

Experiment 2. BR06 collection. Seed yields ranged from 6 to 659 kg/ha with 2 lines producing no seed in some years and 1 line producing no seed at any harvest (Table 5). In the first year (2008), BR02/1794 and Marandu produced significantly more seed than the other hybrid lines, including Mulato II. The majority of the BR06 lines had lower seed yields in 2008 than Mulato II, Marandu and Toledo and the BR02 lines, except for BR02/1372, which produced low seed yields at every harvest. In the second year (2009), BR02/0465 produced a significantly higher seed yield than the other hybrid lines and cultivars. Seed yields of many of the BR06 lines improved, with BR06/1278 producing similar seed yields to Mulato II, and BR06/0423 and BR06/1000 producing, respectively, 412 and 400 kg/ha. In the third year, seed yields of nearly all cultivars and

lines declined significantly, except for BR06/1000 and 2058, which produced a little over 200 kg/ha (Table 5). BR06/1254 failed to produce seed at any harvest.

The majority of the BR06 lines produced significantly higher numbers of inflorescences (300–400/m²) in the first year than the cultivars and the BR02 lines (Table 5). In the second year, inflorescence numbers increased compared with numbers in the first year for most lines and cultivars, with a similar range (300–600/m²) for BR02 and BR06 lines and Mulato II. In the third year, there was a substantial decrease in inflorescence numbers for all lines and cultivars, particularly for Mulato II and Marandu. Toledo produced very few inflorescences in Years 1 and 3, and no inflorescences at all in Year 2.

Racemes per inflorescence declined with age, averaging 4.7 in the first year, 3.8 in the second year and 3.4 in the third year (Table 5). Overall, the majority of the

BR06 lines produced fewer racemes per inflorescence than Mulato II. Five BR06 lines (0204, 0584, 1132, 1348 and 1696) produced numbers of racemes similar to or higher than Mulato II at each harvest.

Spikelet numbers per raceme were similar in the first and second seed harvests, 38 and 39, respectively, but declined to 34.6 at the third seed harvest (Table 5). Several BR06 lines produced more than 40 spikelets per raceme at each seed harvest, significantly higher than Mulato II and most BR02 lines.

BR02/0465 produced significantly heavier seed than all other lines and cultivars at all harvests, except for Toledo at the first harvest (Table 5). BR02/1794 and BR02/1718 produced significantly heavier seed than Mulato II and all 3 produced significantly heavier seed than all BR06 lines, except for BR06/0531, at the second and third harvests (Table 5).

Table 4. Seed yield and components of seed yield at peak anthesis of hybrid brachiaria lines during 2006 and 2007 in Ubon Ratchathani, Thailand (Experiment 1).

| Hybrid line/ cultivar | Seed yield (kg/ha) | | Inflorescences ¹ (no./m ²) | | Racemes/ inflorescence ¹ (no.) | | Spikelets/ raceme ¹ (no.) | | TSW ³ (g) | |
|--------------------------|-----------------------|----------------|--|--------|---|--------|--|--------|-------------------------|--------|
| | 2006 | 2007 | 2006 | 2007 | 2006 | 2007 | 2006 | 2007 | 2006 | 2007 |
| Mulato II | 116 | 166 | 128 | 280 | 5.1 | 4.3 | 35.4 | 32.8 | 8.2 | 8.3 |
| BR02/0465 | 87 | 244 | 230 | 352 | 5.6 | 4.4 | 36.0 | 26.4 | 10.3 | 10.5 |
| BR02/0768 | 124 | 121 | 628 | 886 | 3.7 | 3.2 | 28.1 | 20.2 | 7.1 | 7.1 |
| BR02/0771 | 94 | 58 | 533 | 454 | 4.6 | 3.5 | 32.3 | 24.3 | 7.4 | 7.6 |
| BR02/0799 | 49 | 130 | 423 | 776 | 3.1 | 3.3 | 35.3 | 32.9 | 6.8 | 7.8 |
| BR02/1245 | 74 | - ² | 173 | - | 4.1 | - | 31.1 | - | 9.6 | - |
| BR02/1372 | 20 | 23 | 509 | 468 | 3.6 | 3.1 | 48.4 | 36.7 | 6.7 | 6.6 |
| BR02/1452 | 161 | 65 | 282 | 343 | 3.6 | 3.3 | 35.2 | 24.1 | 8.3 | 8.2 |
| BR02/1485 | 59 | 50 | 278 | 413 | 4.2 | 3.1 | 36.9 | 25.4 | 9.3 | 9.3 |
| BR02/1718 | 94 | 249 | 352 | 721 | 5.8 | 4.0 | 38.2 | 39.2 | 8.9 | 8.6 |
| BR02/1728 | 85 | 73 | 306 | 257 | 4.0 | 4.5 | 35.3 | 32.0 | 7.8 | 7.1 |
| BR02/1747 | 87 | 89 | 306 | 458 | 5.1 | 4.8 | 42.3 | 27.8 | 9.3 | 9.2 |
| BR02/1752 | 118 | 155 | 346 | 351 | 3.9 | 3.4 | 40.2 | 30.0 | 8.7 | 9.0 |
| BR02/1794 | 282 | 272 | 380 | 488 | 4.7 | 4.2 | 45.7 | 30.6 | 8.9 | 9.1 |
| MX02/1263 | 154 | 12 | 483 | 181 | 4.6 | 3.4 | 41.0 | 27.6 | 8.8 | 8.9 |
| MX02/1423 | 18 | 30 | 435 | 682 | 3.0 | 2.9 | 45.8 | 33.4 | 6.7 | 7.6 |
| Mean | 103 | 109 | 362 | 444 | 4.3 | 3.5 | 37.9 | 27.7 | 8.3 | 7.8 |
| LSD (P≤0.05) | 74 | 54 | 199 | 133 | 0.5 | 0.4 | 2.5 | 2.2 | 0.6 | 0.5 |
| F ratio | 6.94 | 13.19 | 3.83 | 35.03 | 27.9 | 81.5 | 42.7 | 135.8 | 35.2 | 194.3 |
| Probability | <0.001 | <0.001 | 0.001 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 |

¹Counted at peak anthesis. ²Failed to produce inflorescences. ³One-thousand-seed weight.

Table 5. Seed yield and components of seed yield at peak anthesis of hybrid brachiaria lines during 2008–2010 in Amnart Charoen, Thailand (Experiment 2).

| Hybrid line/ cultivar | Seed yield (kg/ha) | | | Inflorescences ¹ (no./m ²) | | | Racemes/ inflorescence ¹ (no.) | | | Spikelets/ raceme ¹ (no.) | | | TSW ³ (g) | | |
|--------------------------|-----------------------|----------------|--------|--|--------|--------|---|--------|--------|--|--------|--------|-------------------------|--------|--------|
| | 2008 | 2009 | 2010 | 2008 | 2009 | 2010 | 2008 | 2009 | 2010 | 2008 | 2009 | 2010 | 2008 | 2009 | 2010 |
| Mulato II | 256 | 497 | 19 | 111 | 361 | 19 | 5.8 | 4.5 | 3.8 | 37.9 | 32.7 | 29.1 | 8.1 | 7.9 | 7.6 |
| BR02/1794 | 370 | 206 | 95 | 231 | 348 | 132 | 5.7 | 3.7 | 3.9 | 36.7 | 42.5 | 36.6 | 9.4 | 8.4 | 8.8 |
| BR02/0465 | 276 | 659 | 140 | 149 | 338 | 128 | 5.3 | 4.3 | 3.7 | 35.9 | 37.9 | 30.5 | 10.2 | 11.1 | 10.4 |
| BR02/1718 | 309 | 492 | 151 | 221 | 528 | 105 | 6.0 | 3.1 | 4.3 | 37.5 | 36.7 | 34.2 | 8.8 | 8.3 | 8.0 |
| BR02/1372 | 64 | 11 | 6 | 199 | 463 | 229 | 3.9 | 5.3 | 3.1 | 51.5 | 39.6 | 32.9 | 7.6 | 6.8 | 7.1 |
| Marandu | 344 | 334 | 103 | 94 | 152 | 23 | 4.8 | 3.9 | 3.3 | 47.0 | 38.3 | 32.7 | 9.0 | 8.4 | 8.4 |
| Toledo | 256 | - ² | 36 | 48 | - | 23 | 6.5 | - | 4.0 | 35.5 | - | 29.2 | 11.4 | - | 9.6 |
| BR06/0012 | 47 | 58 | 22 | 263 | 376 | 64 | 3.3 | 2.7 | 2.6 | 53.4 | 45.7 | 28.8 | 8.2 | 7.4 | 7.0 |
| BR06/0204 | 18 | 67 | 51 | 64 | 185 | 126 | 6.7 | 6.0 | 3.7 | 31.8 | 35.3 | 53.2 | 6.7 | 6.7 | 7.3 |
| BR06/0206 | 23 | 73 | 19 | 298 | 302 | 135 | 3.0 | 2.8 | 2.6 | 58.5 | 49.0 | 39.2 | 7.1 | 7.2 | 6.9 |
| BR06/0387 | 176 | 264 | 143 | 407 | 575 | 271 | 4.7 | 3.1 | 2.8 | 41.8 | 41.7 | 27.2 | 6.9 | 7.1 | 7.1 |
| BR06/0405 | 34 | 29 | 31 | 307 | 475 | 250 | 3.5 | 3.2 | 3.3 | 41.1 | 34.3 | 33.0 | 6.1 | 5.8 | 6.0 |
| BR06/0423 | 218 | 412 | 58 | 266 | 356 | 41 | 5.8 | 4.6 | 4.2 | 38.5 | 37.8 | 36.3 | 7.1 | 7.1 | 6.6 |
| BR06/0531 | - ² | 141 | 54 | - | 280 | 137 | - | 3.2 | 2.9 | - | 38.4 | 30.6 | - | 8.2 | 8.1 |
| BR06/0584 | 45 | 32 | 5 | 121 | 121 | 22 | 5.4 | 4.8 | 3.6 | 20.3 | 20.8 | 18.4 | 6.8 | 6.3 | 6.2 |
| BR06/0850 | 148 | 204 | 42 | 188 | 275 | 95 | 4.5 | 3.8 | 3.0 | 24.9 | 48.3 | 44.6 | 6.0 | 5.6 | 5.2 |
| BR06/1000 | 126 | 400 | 202 | 220 | 317 | 215 | 4.9 | 4.3 | 3.2 | 30.0 | 48.8 | 37.9 | 7.7 | 7.8 | 8.1 |
| BR06/1132 | 114 | 220 | 41 | 281 | 421 | 102 | 6.6 | 4.9 | 4.3 | 30.6 | 31.7 | 31.7 | 6.8 | 6.9 | 6.6 |
| BR06/1175 | 169 | 320 | 64 | 303 | 327 | 195 | 3.6 | 3.4 | 3.0 | 50.0 | 46.6 | 35.7 | 7.9 | 7.7 | 6.8 |
| BR06/1254 | - ² | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| BR06/1278 | 137 | 483 | 72 | 223 | 262 | 100 | 3.3 | 3.2 | 3.0 | 57.5 | 55.0 | 46.4 | 7.8 | 7.6 | 6.9 |
| BR06/1348 | 112 | 88 | 93 | 207 | 456 | 124 | 5.8 | 4.1 | 4.1 | 28.3 | 27.6 | 33.9 | 6.0 | 5.2 | 6.0 |
| BR06/1366 | 172 | 71 | 65 | 339 | 324 | 216 | 3.5 | 3.4 | 3.1 | 58.7 | 50.7 | 43.8 | 6.8 | 6.0 | 6.3 |
| BR06/1388 | 76 | 6 | 12 | 468 | 421 | 145 | 3.3 | 3.2 | 3.3 | 42.8 | 35.0 | 35.4 | 5.7 | 5.4 | 5.6 |
| BR06/1415 | 90 | 134 | 29 | 462 | 416 | 136 | 3.7 | 4.2 | 4.0 | 50.3 | 40.3 | 35.3 | 6.7 | 5.7 | 5.5 |
| BR06/1433 | 95 | 13 | 11 | 444 | 634 | 272 | 3.1 | 2.2 | 2.2 | 41.7 | 31.8 | 26.1 | 6.2 | 5.5 | 6.1 |
| BR06/1454 | 164 | 28 | 17 | 367 | 447 | 231 | 3.3 | 3.2 | 2.9 | 53.8 | 50.6 | 43.7 | 6.9 | 6.4 | 6.5 |
| BR06/1567 | 57 | 71 | 28 | 368 | 359 | 69 | 4.0 | 3.8 | 3.4 | 19.3 | 22.9 | 20.1 | 6.6 | 6.0 | 6.2 |
| BR06/1696 | 200 | 228 | 106 | 388 | 394 | 265 | 7.2 | 5.8 | 4.2 | 47.3 | 37.2 | 28.6 | 7.8 | 7.5 | 6.7 |
| BR06/1832 | 121 | 214 | 65 | 336 | 442 | 73 | 5.2 | 4.2 | 3.3 | 34.2 | 34.6 | 33.4 | 7.3 | 7.1 | 7.8 |
| BR06/1922 | 140 | 59 | 38 | 379 | 316 | 131 | 3.7 | 3.4 | 3.1 | 25.4 | 35.1 | 33.2 | 6.6 | 6.0 | 6.5 |
| BR06/1932 | 46 | 269 | 39 | 171 | 318 | 122 | 4.7 | 2.9 | 3.0 | 26.8 | 50.9 | 48.2 | 7.0 | 7.2 | 6.8 |
| BR06/2020 | 58 | 161 | 56 | 220 | 333 | 176 | 4.2 | 3.6 | 3.4 | 31.7 | 44.5 | 41.4 | 7.0 | 6.9 | 6.1 |
| BR06/2058 | 223 | 315 | 205 | 395 | 532 | 155 | 6.0 | 4.5 | 3.6 | 30.9 | 33.8 | 32.0 | 7.1 | 7.2 | 7.1 |
| BR06/2204 | 52 | 99 | 85 | 234 | 342 | 117 | 3.8 | 3.5 | 3.0 | 32.7 | 33.9 | 34.5 | 7.2 | 6.6 | 7.6 |
| Mean | 135 | 191 | 63 | 251 | 349 | 133 | 4.4 | 3.6 | 3.3 | 37.0 | 36.9 | 33.7 | 7.0 | 6.6 | 6.8 |
| LSD (P<0.05) | 59 | 60 | 32 | 62. | 69 | 42 | 0.4 | 0.3 | 0.3 | 2.5 | 3.6 | 3.0 | 0.4 | 0.3 | 0.4 |
| F ratio | 21.7 | 66.5 | 22.2 | 33.0 | 32.1 | 26.6 | 129.6 | 113.5 | 63.1 | 247.9 | 89.0 | 77.4 | 220.8 | 270.9 | 121.6 |
| Probability | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 |

¹Counted at peak anthesis. ²Failed to produce inflorescences. ³One-thousand-seed weight.

Discussion

The main aims of the experiments were to identify hybrid brachiaria lines with seed yields higher than Mulato II and equal to or better than commercial seed yields of over 600 kg/ha produced by commercial brachiaria species in Australia and Brazil (Hopkinson and Clifford 1993; Souza 1999). These aims were partially achieved. While BR02/0465 was the only hybrid line that produced in excess of 600 kg/ha (659 kg/ha in the second harvest at Amnart Charoen), a number of lines produced more seed than Mulato II. The best overall seed producer, BR02/1794 (95–370 kg/ha), produced significantly more seed than Mulato II (19–497 kg/ha) in both harvests at Ubon Ratchathani and 2 of 3 harvests at Amnart Charoen. The next best lines (BR02/1718 and BR02/0465) produced more seed than Mulato II in 1 of 2 harvests at Ubon Ratchathani and 2 of 3 harvests at Amnart Charoen. BR02/1794 and BR02/0465 produced heavier seed (TSW) than Mulato II at every harvest.

The 3 lines above all reached peak flowering earlier than Mulato II (Table 3). BR02/1794 usually flowers earliest (late September–early October). Peak flowering of BR02/1718 and BR02/0465 is usually 2 to 3 weeks later in mid-October, while peak flowering of Mulato II is nearly always in the second week of November in Northeast Thailand.

Different flowering times can strongly influence seed production. If late October–early November is particularly dry, late-flowering species can fail to set seed on sandy soils with low soil moisture retention. This appeared to be the case with Mulato II in the third year at Amnart Charoen (2010). Heavy rainfall in the first half of October benefited the earlier-flowering lines, but with no rain falling from late October onwards, Mulato II produced only 19 inflorescences per m² and only 19 kg/ha of seed was harvested.

The seed yields from the BR06 lines overall were disappointing. It was only when Mulato II failed to produce a large number of inflorescences at the third seed harvest at Amnart Charoen, that the BR06 lines produced more seed than Mulato II. However, these third-harvest seed yields were also extremely low, averaging only 60 kg/ha.

Our experience with seed production of hybrid brachiaria grasses in Thailand is that seed yields decline with age, even though adequate levels of soil N are maintained by applying fertilizer. At the Ubon Ratchathani site seed yields from the first and second seed harvests were similar but at Amnart Charoen, seed yields were higher at the second harvest than at the first

and very low for nearly all lines at the third harvest. The decline in seed yield over years in many tropical grass species is considered to be caused by larger tillers in older stands providing nutritional support for weaker tillers (low-yielding or sterile) to the detriment of their own seed development and the long-term productivity of the stand (Loch et al. 1999), though for the brachiaria lines we have no data to support this hypothesis. Farmers in Thailand have found that seed yields from second-year hybrid brachiaria grass seed crops were less than half those of first-year seed crops. In order to get satisfactory seed yields, (300–400 kg/ha of clean seed), they treat hybrid brachiaria grass seed crops as annuals and replant every year, as they do with all other tropical grass seed crops (Hare 2014).

We consider that cleaned seed yields from commercial operations must be above 600 kg/ha for the seed prices of the hybrid brachiaria cultivars to become competitive with other commercial cultivars of brachiaria species. Some farmers in Thailand can produce more than 600 kg/ha of Mulato II seed by ground-sweeping but the majority produce only about 385 kg/ha (Hare 2014). Farmers in Northern Laos currently average 250 kg/ha of Mulato II seed from hand-knocking seed from seedheads (Hare 2014). In our experiments, we have at times produced 500 kg/ha of clean seed (98–99% purity by weight) (Hare et al. 2007b) by catching the seed in bags tied over the seedheads, but these occasions have been extremely rare. Commercial seed production of Mulato II is still very erratic.

Another factor which adds to the cost of hybrid brachiaria seed production is acid-scarification. This results in a loss of seed weight of 15–20% from scarifying off the glume, lemma and palea around each seed, light and empty seed, and small amounts of viable seed. Even though some viable seed is lost, without acid-scarification, germination of the seed fails to exceed 30% (Hare 2014).

Nearly all hybrid lines produced sufficient numbers of inflorescences, racemes and spikelets to indicate a potential for useful seed yields. In the trials at Ubon Ratchathani, most hybrid lines produced 300–500 inflorescences/m² and at Amnart Charoen, the BR06 lines produced 300–500 inflorescences/m² in the first and second years. Mulato II produced fewer inflorescences than the new lines at both sites. Inflorescence numbers have nearly always been the main indicator of whether a forage plant has the potential to produce seed. However, with hybrid brachiaria grasses, it appears that seed-set is the most determining factor of seed yields. By seed harvest there seems to be a massive failure of seed-set,

caryopsis maturation or both, with the cleaned seed coming from fewer than 10% of spikelets. In other brachiaria species it is not uncommon for abscission to precede maturation in a high proportion of spikelets (Hopkinson et al. 1996), but in the hybrid brachiaria grasses it appears to be an extremely high proportion.

Previous studies have shown seed yields of Mulato II are generally very low, with fewer than 2% of the spikelets formed producing viable seed (Hare et al. 2007a). There is speculation that this low seed-set is caused by pollen sterility, as Risso-Pascotto et al. (2005) found that more than 65% of pollen grains in brachiaria interspecific hybrids (*B. ruziziensis* x *B. brizantha*) were sterile and this sterility was genetic. Miles and Hare (2007) suggested this poor seed-set may be a common defect of newly formed apomictic forage grass hybrids. They referred to failures of buffel grass hybrids, which produced erratic and usually poor seed yields, leading to high seed prices. A hybrid-derived apomictic bahiagrass clone was not released because of concerns of low seed yields (Miles and Hare 2007).

In these current studies, we did not examine the percentage of spikelets that formed a caryopsis to calculate biological seed-set, but rather calculated economical seed-set, which is the ratio between realized and potential seed yield (Elgersma 1985). Mulato II seed-set (percentage of the number of cleaned seed to formed spikelets) ranged from 1.6 to 3.8% at Ubon Ratchathani and from 11.8 to 12.9% at Amnart Charoen. BR02/1794, which had superior seed yields to Mulato II, averaged 4.3% seed-set across both sites and seed-set for BR02/1752 ranged from 1.1 to 3.6% across both sites. Values for BR02/1718 were 1.8–3.1% at Ubon Ratchathani, and 7.0–12.2% at Amnart Charoen. Similarly, BR02/0465 had seed-set of 1.6–3.8% at Ubon Ratchathani, rising to 9.3–10.8% at Amnart Charoen.

The superior seed-set at Amnart Charoen compared with Ubon Ratchathani is interesting. Amnart Charoen is farther north than Ubon Ratchathani (15.5° vs. 15° N) and at a slightly higher elevation (168 vs. 130 masl). Grof (1968) showed that Basilisk signalgrass could set good seed yields and these seed yields were enhanced in drier upland regions in tropical latitudes (Loch et al. 1999). Basilisk seed production in Australia is predominantly on the Atherton Tablelands at lower latitudes but at elevations of 600–900 masl. In Brazil, successful seed production of Basilisk signalgrass and cv. Marandu is in the higher tropical latitudes (20 and 22° S) and at elevations of 700–1,000 masl (Souza 1999). The slightly higher elevation at Amnart Charoen compared with Ubon Ratchathani may have compensated for insuffi-

cient latitude and encouraged greater seed-set. Ferguson et al. (1983) showed that, at similar latitudes in South America (15–19° S), the site with the highest elevation (1,000 masl) produced the highest seed yields of signalgrass, even though it had the lowest latitude (15° S).

Under commercial conditions in Thailand, we have produced hybrid brachiaria seed in Ubon Ratchathani, Amnart Charoen, Mukdahan and Roi-et provinces. It is only in the more northerly province, Roi-et (16.8° N; 160 masl), that farmers still continue with Mulato II seed production (Hare 2014). In the other provinces seed yields are too low and erratic to be economical and farmers have ceased production. Roi-et farmers, however, found that BR02/1752 seed yields (100–200 kg/ha) were too low to interest them; thus BR02/1752 seed production is limited to Northern Laos (19–21° N; 700–1,200 masl), where farmers find the seed yields satisfactory (200–300 kg/ha) under their low-input management (Hare 2014). We have also commenced seed production of BR02/1794 in these Northern Laos provinces.

While 43 hybrid Brachiaria lines were evaluated for seed yield between 2005 and 2010, only 3 lines, BR02/1794, BR02/1718 and BR02/0465, displayed a potential for seed yields greater than or equal to Mulato II. BR02/1752 had seed yields similar to or slightly lower than Mulato II, though in another study at Ubon Ratchathani, BR02/1752 and BR02/1794 produced significantly higher seed yields than Mulato II (Bouathong et al. 2011).

In considering the commercial release of hybrid brachiaria lines as named cultivars, forage production and quality (Hare et al. 2015) and seed production were important considerations, together with the waterlogging tolerance of BR02/1752, released as cv. Cayman (Pizarro et al. 2013), and the upright nature of BR02/1794 for cut-and-carry forage, released as cv. Cobra (E. Stern pers. comm.).

Further research is needed to verify the influence of elevation and latitude on flowering and seed-set in hybrid brachiaria grasses. In future breeding of new hybrids, there must be more rigorous selection for seed production characteristics at latitudes and sites typical of where commercial brachiaria seed production occurs.

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Productive performance of three tropical legumes for protein banks in the dry tropics of Colima, Mexico

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Keywords: *Clitoria ternatea*, dry matter yield, forage quality, *Lablab purpureus*, *Mucuna pruriens*.

Abstract

The aim of this study was to evaluate the productive performance of mucuna (*Mucuna pruriens*), lablab (*Lablab purpureus*) and clitoria (*Clitoria ternatea*) for protein banks in Colima, Mexico, with irrigation used prior to the rainy season. Fifteen plots were allocated in a complete randomized block design with 5 replicates. Dry matter production, crude protein, calcium and phosphorus concentrations and leaf:stem ratio were evaluated. The highest dry matter production was recorded for clitoria and lablab (9.80 and 8.93 t/ha, respectively, over 240–260 days), while mucuna produced 5.5 t DM/ha in 120 days. Leaf production in clitoria (4.73 t/ha) exceeded that in lablab (3.23 t/ha) and mucuna (2.69 t/ha), while leaf:stem ratio was 0.94 for clitoria, 1.0 for mucuna and 0.58 for lablab. Crude protein concentrations in all species were high (21.7–27.8%) as were concentrations of Ca (1.17–1.64%) and P (0.38–0.67%). Use of the 3 forages is discussed. Studies in the absence of irrigation in a range of seasons would determine how relevant these findings are in those situations. Feeding studies with animals would provide additional information on which to decide the appropriate species to plant in different situations.

Resumen

El objetivo del estudio fue evaluar el desempeño productivo de las leguminosas frijol terciopelo (*Mucuna pruriens*), lablab (*Lablab purpureus*) y clitoria (*Clitoria ternatea*) cuando se utilizan como bancos de proteína con aplicación de riego controlado después de la época de lluvias en Colima, México. Las leguminosas fueron establecidas en un diseño experimental de bloques completos al azar con cinco repeticiones para un total de 15 parcelas. Se midieron la producción de materia seca (MS), los contenidos (%) de proteína cruda (PC), calcio (Ca) y fósforo (P), y la relación hoja:tallo. Clitoria, 240 días después de la siembra (dds), y lablab, 260 dds, mostraron las mayores producciones de MS (9.80 y 8.93 t/ha, respectivamente); mientras que mucuna, 120 dds, produjo 5.5 t/ha de MS. La producción de hoja de clitoria (4.73 t/ha) superó a la de lablab (3.23 t/ha) y a la de mucuna (2.69 t/ha). La relación hoja:tallo fue 0.94 en clitoria, 1.0 en mucuna y 0.58 en lablab. Las especies mostraron un alto contenido de PC entre 21.7 y 27.8%, Ca (1.17 y 1.64%) y P (0.38 y 0.67%). Se discute el uso de las 3 especies y se sugieren estudios adicionales sin aplicación de riego.

Introduction

The state of Colima is located in the seasonally dry tropical region of Mexico, which is characterized by frost-free temperatures and rainfall of about 900 mm/yr,

but also a pronounced seasonal arid pattern. This region provides a challenging environment for beef production owing to heat, disease and pest factors common to tropical areas, plus the added burden of a 7- to 8-month dry season, when forage quantity and quality are low (Peel et al. 2010).

Smallholder livestock production in the seasonally dry tropical areas is based on traditional dual-purpose systems (Macedo et al. 2003; Guevara et al. 2013),

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which provide only about 80 and 68% of the dry matter and protein requirements, respectively, of cattle. These systems have traditionally been based on feeding low-quality roughage sources and/or crop residues, mainly maize stover, during the dry season (Macedo et al. 2008; Guevara et al. 2013). The low protein concentration in these forages limits microbial activity in the rumen, resulting in depressed feed intake, low dry matter digestibility and suboptimal animal production, whether measured in terms of milk yield, draught power or growth rate (McDonald et al. 1996).

The integration of well-adapted protein bank legumes to supplement crop residues and grasses in animal production systems has the potential to improve forage quality in the dry season, and this strategy is being adopted much more widely by smallholders in many tropical countries (Pengelly et al. 2004; Rootman et al. 2004). In Zimbabwe, supplementing maize stover with lablab hay has significantly increased milk yields from 4–6 L/day to 6–17 L/day (Thorpe 1999). Milk yield and protein, lactose and non-fat solids from cows fed a ration with mucuna hay were similar to those from cows eating commercial feed concentrates (Murungweni et al. 2004).

Among the legume species being used or with potential as forage, lablab (*Lablab purpureus*) and mucuna (*Mucuna pruriens*) are annual legumes capable of producing large quantities of high-quality, above-ground biomass for livestock feed (Murungweni et al. 2004; Peters et al. 2010). In addition, clitoria (*Clitoria ternatea*) is a perennial climbing, strongly persistent, herbaceous legume with good potential under irrigation,

yielding good quality forage (Villanueva et al. 2004; Cabrera et al. 2010). These 3 legumes are some of those recommended for the seasonally dry tropical areas of Mexico. Since the rainy season is so short, irrigation is normally used either before or after the rainy season to ensure that crops grow satisfactorily.

This study aimed to evaluate the productive potential of the above legumes for protein banks in Colima, Mexico, when irrigation was used prior to onset of the rainy season.

Materials and Methods

The trial was carried out in Armería, Colima, Mexico (19°00'56'' N, 104°00'05'' W; 91 masl), where the climate is warm, subhumid with summer rains (Figure 1). The average annual temperature and rainfall are 26.5 °C and 790.8 mm, respectively (SEFOME 2012).

A complete randomized block design with 5 replications was used. Legumes were sown on 2 March 2007 in 9.60 m² plots, on a Eutric Regosol, at sowing rates of 15 kg/ha for mucuna (González 2007), 30 kg/ha for lablab (Martínez et al. 1987) and 20 kg/ha for clitoria (Villanueva et al. 2004). The area was irrigated prior to sowing, with drip irrigation applied every 10 days after sowing until the rains began (30 June 2007). Seeds were immersed for 5 minutes in water at 80 °C before sowing and urea, superphosphate and potassium chloride fertilizers were applied to the experimental plots to provide 100 kg N/ha, 80 kg P/ha and 80 kg K/ha. Weed control was done manually.

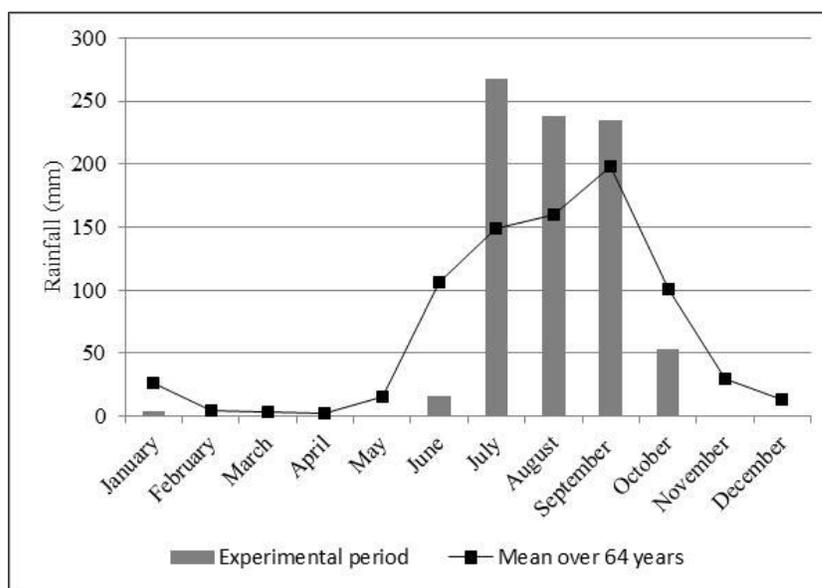


Figure 1. Rainfall during the study and the average of the last 64 years.

The legumes were harvested from an area of 4.80 m² in the center of the plot at a uniform height of 10 cm above ground. *Mucuna* (first harvest) and lablab (first and second harvests), were cut at 120 d of age, while clitoria was harvested at early flowering (10%), which occurred at 70 d for the first cut and at 47 d average for the 4 subsequent cuts.

At each harvest, an 800 g sample of fresh forage from each plot was selected, bagged and dried at 60 °C for 48 h for estimating dry matter production. Following drying, leaves and stems were separated and weighed. Leaf and stem yields and leaf:stem ratio were calculated. The dried samples were bulked over all harvests and mean crude protein, calcium and phosphorus concentrations were determined (Goering and Van Soest 1970; AOAC 1990). In the case of clitoria, the only one that behaved as a perennial, plant height, stem length and cover were evaluated before each harvest. To assess

plant cover, a 1 m² metal frame was used, with a single sample per plot. Plant height and length of stem were measured with a 1 m ruler on 5 randomly selected plants per plot.

The effects of legume and harvest on dependent variables were analyzed with ANOVA and Tukey's test (P=0.05) using the SAS general model procedure (SAS Institute Inc. 2009).

Results

Dry matter (DM) production of *mucuna* and lablab at the first harvest was greater than that of clitoria, while lablab and clitoria had greater total production than *mucuna* (P<0.05) (Table 1). Dry matter production of lablab decreased significantly from the first to the second harvest, while production of clitoria remained unchanged over the 5 harvests (Table 1).

Table 1. Dry matter production (t/ha) and leaf:stem ratio of 3 legumes in Colima, Mexico.

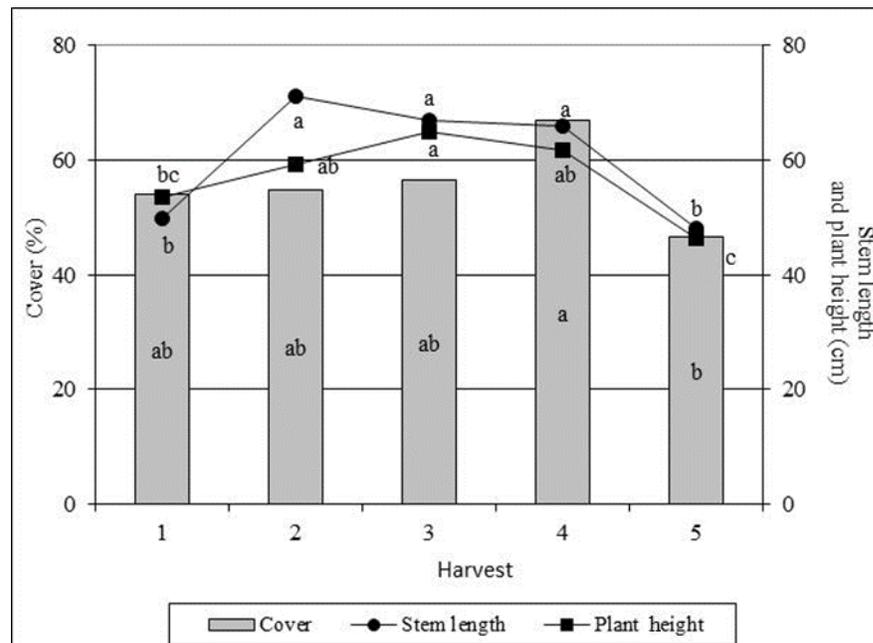
| | Harvest | | | | | Total | s.e. | Sig. level |
|--------------------------|---------|---------|--------|--------|--------|--------|------|------------|
| | 1 | 2 | 3 | 4 | 5 | | | |
| Whole plant | | | | | | | | |
| <i>Mucuna pruriens</i> | 5.50A† | | | | | 5.50B | | |
| <i>Lablab purpureus</i> | 5.89Aa† | 3.04Ab‡ | | | | 8.93A | 0.59 | 0.00 |
| <i>Clitoria ternatea</i> | 1.40Ba† | 2.15Aa† | 1.92a§ | 2.12a§ | 2.21a§ | 9.80A | 0.11 | 0.11 |
| s.e. | 0.65 | 0.21 | | | | 0.68 | | |
| Sig. level | 0.00 | 0.09 | | | | 0.01 | | |
| Leaf | | | | | | | | |
| <i>Mucuna pruriens</i> | 2.69A† | | | | | 2.69B | | |
| <i>Lablab purpureus</i> | 2.39Aa† | 0.84Ab‡ | | | | 3.23B | 0.28 | 0.00 |
| <i>Clitoria ternatea</i> | 0.71Ba† | 1.08Aa† | 0.84a§ | 1.03a§ | 1.07a§ | 4.73A | 0.05 | 0.07 |
| s.e. | 0.27 | 0.07 | | | | 0.30 | | |
| Sig. level | 0.00 | 0.24 | | | | 0.01 | | |
| Stem | | | | | | | | |
| <i>Mucuna pruriens</i> | 2.81A† | | | | | 2.81B | | |
| <i>Lablab purpureus</i> | 3.50Aa† | 2.21Aa‡ | | | | 5.71A | 0.37 | 0.17 |
| <i>Clitoria ternatea</i> | 0.69Ba† | 1.08Ba† | 1.08a§ | 1.09a§ | 1.14a§ | 5.08 A | 0.06 | 0.16 |
| s.e. | 0.40 | 0.21 | | | | 0.45 | | |
| Sig. level | 0.00 | 0.14 | | | | 0.00 | | |
| Leaf:stem ratio | | | | | | | | |
| <i>Mucuna pruriens</i> | 1.00AB† | | | | | 1.00 A | | |
| <i>Lablab purpureus</i> | 0.75Ba† | 0.38Bb‡ | | | | 0.58 B | 0.08 | 0.01 |
| <i>Clitoria ternatea</i> | 1.05Aa† | 1.07Aa† | 0.79a§ | 0.95a§ | 0.95a§ | 0.94 A | 0.04 | 0.10 |
| s.e. | 0.06 | 0.13 | | | | 0.06 | | |
| Sig. level | 0.04 | 0.01 | | | | 0.00 | | |

Values within columns and parameters followed by different upper-case letters and within rows followed by different lower-case letters are significantly different according to Tukey's test (P≤0.05).

†Irrigation; ‡Irrigation-rainy season; §Rainy season.

Table 2. Crude protein, calcium and phosphorus concentrations (%) of 3 legumes in Colima, Mexico.

| Species | Crude protein | Calcium | Phosphorus |
|--------------------------|---------------|---------|------------|
| <i>Mucuna pruriens</i> | 27.8 | 1.48 | 0.38 |
| <i>Lablab purpureus</i> | 21.6 | 1.64 | 0.67 |
| <i>Clitoria ternatea</i> | 22.1 | 1.17 | 0.41 |

**Figure 2.** Evolution of cover, stem length and plant height of clitoria (*Clitoria ternatea*) in Colima, Mexico. Different letters in columns or on lines denote significant differences ($P < 0.05$).

The production of leaf and stem of mucuna and lablab at the first harvest and stem production of lablab at the second harvest were greater than those from clitoria ($P < 0.05$) (Table 1). While total leaf production for clitoria was greater than for mucuna and lablab, lablab and clitoria produced more stem than mucuna ($P < 0.05$). As a result, leaf:stem ratio for mucuna and clitoria was greater than for lablab ($P < 0.05$). Leaf production and leaf:stem ratio of lablab decreased significantly from the first to the second harvest, while these parameters did not vary over the 5 harvests for clitoria (Table 1).

At 120 days of age, mucuna and lablab had average crude protein concentrations of 27.8 and 21.6%, respectively. Average concentrations of calcium and phosphorus in mucuna and lablab were 1.48 and 1.64%, and 0.38 and 0.67%, respectively. At 47 days of age, average concentrations of crude protein, calcium and phosphorus in clitoria were 22.1, 1.17 and 0.41%, respectively (Table 2).

Ground cover of clitoria was similar at the first 4 harvests, decreasing significantly at the final harvest, while height peaked at the 4th harvest. The stems showed maximum length from the second to the fourth harvest, and decreased towards the end of the study (Figure 2).

Discussion

This study has provided useful information on the potential of mucuna, lablab and clitoria as legumes for use as protein banks in the seasonally dry tropics of Mexico. All 3 legumes produced good yields of forage of high quality and could have a role in improving nutritional levels for ruminants, especially during the dry season. It is important to realize that the data in this study are for 1 year only and seedlings were irrigated for the 4 months until the rains started to ensure survival. Harvesting of mucuna, the first harvest of lablab and the first 2 harvests of clitoria occurred before the wet season started,

so the growth was produced by drip irrigation. Rainfall during the rainy season was well above the long-term mean, so yields obtained and survival of the species, especially clitoria, might be better than would be obtained under non-irrigated situations and in average or below average rainfall conditions.

The total DM yield of clitoria over the 5 harvests compared favorably with yields reported by Bakhshwain and Elfeel (2012) in Saudi Arabia, when it was drip-irrigated and heavily fertilized. Average DM production of clitoria under irrigation per harvest was lower than that reported in Sudan (2.95 t/ha) by Mohamed-Osman et al. (2013). While in our study DM production of clitoria remained unchanged over the 5 harvests, other authors in Mexico found that, owing to low rainfall and no irrigation, the greatest DM production occurred at the first harvest, with significant decreases subsequently (Carvajal and Lara 2005). A similar trend was observed in clitoria under irrigation (Bakhshwain and Elfeel 2012). In the present study, irrigation followed by adequate rainfall favored vigorous regrowth, while in other studies in the absence of irrigation, DM production varied significantly between the rainy and dry seasons (Sosa et al. 2008).

DM yield of the primary growth (first harvest) of lablab in this study was greater than the yields reported by Barnes (1996) in Ghana, who harvested less than 2.89 t/ha at 2 sites in consecutive years. In addition, total DM yield of lablab was greater than the 5.9 t/ha reported by Jingura et al. (2001) in Zimbabwe. However, Nworgu and Ajayi (2005) reported DM yields of 19.98–20.82 and 44.58–48.66 t/ha/yr in Nigeria¹, harvesting at 8 and 12 weeks, respectively. The DM yield of mucuna in this study was lower than the 8.2–11.6 t/ha reported by Kaizzi et al. (2004), but greater than the 2.61 t/ha of Jara (1997). Factors like soil moisture (rainfall), temperature, soil type, plant density, cutting height, cutting interval and fertilizer application affect DM production of legumes (Jingura et al. 2001; Njarui et al. 2004; Sosa et al. 2008; Ogedegbe et al. 2011).

The leaf fraction of forages generally has better nutritional value than more fibrous stems (Van Soest 1994). Since cattle select for the leaf fraction, the leaf:stem ratio is a very important parameter in determining the nutritional value of forages, including legumes (Hendricksen et al. 1981; Wood 1983). Legumes with high leaf:stem ratios would seem to be those of highest nutritional value (Norton and Poppi 1995). Clitoria maintained the same leaf:stem ratio throughout, in contrast with the results of Ramírez et al. (2003), who observed a decline

in this parameter with progressive harvests. Leaf:stem ratio of clitoria was significantly lower than values of up to 7.3 reported by Abusuwar and Omer (2011), who suggested that leaf:stem ratio increased with the addition of 50 kg triple superphosphate/ha before planting. At the first harvest, leaf:stem ratio for lablab was similar to that found by Murphy et al. (1999) in Honduras (0.76) in plants of similar age (117 days), but the overall value (0.58) was slightly lower than the bottom of the range (0.63–6.0) reported by Abusuwar and Omer (2011). As normally occurs in most forages, with maturity lablab showed a decrease in leafiness, resulting in a decrease in leaf:stem ratio. With regard to mucuna, leaf:stem ratio was significantly lower than that indicated (2.94) in a previous study (Nyambati and Sollenberger 2003).

The crude protein concentrations in clitoria, lablab and mucuna were much higher than the minimum requirement (7%) for maintenance of beef cattle (NRC 1984). Juma et al. (2006) reported crude protein concentrations in clitoria and mucuna of 21.8 and 18.0%, respectively, while Aganga and Autlwetse (2000) reported a crude protein concentration in lablab of 16.4%.

Calcium and phosphorus concentrations in the 3 legumes were higher than the suggested critical levels of 0.30% Ca and 0.25% P, necessary to meet ruminant requirements in the tropics (McDowell and Arthington 2005). Legumes are good sources of Ca, and are higher in Ca content than grasses.

In clitoria, length of stem was higher than that reported for 2 genotypes, blue (28.2 cm) and white (31.7 cm) in Venezuela by Suárez et al. (2012). The coverage and height of clitoria were better than observed in another Mexican study, in which plant coverage decreased from 63 to 11% and the height from 67 to 41 cm, from first to fourth harvest (Carvajal and Lara 2005). Meanwhile, Adjei and Fianu (1985) mentioned that clitoria coverage declined during the first year after planting, from almost 60% to less than 15%. These studies show that, despite clitoria being a perennial plant which could be expected to remain productive for perhaps 5 years (Pengelly and Conway 2000), it often performs as an annual. Its lack of persistence is often due to grazing management, soil type, weed competition, drought and cutting interval (Adjei and Fianu 1985; Peck et al. 2012). The fact that our crops were irrigated from before planting until the start of the rainy season and the rainy season was wetter than normal could indicate that the results obtained were the best that might be expected on this soil type in this region.

While all 3 species grew well and produced high yields of high quality forage, their various attributes make them suitable for use in different situations. One

¹Data presented without major methodological details.

needs to consider how the protein bank would function, i.e. would forage be harvested and stored for feeding livestock later in the year or would it be left to stand in the field for grazing off during the dry season? Issues like how well the species retain their leaves post maturity become important for stand-over forage. Owing to its perennial growth pattern, clitoria is more flexible in how the forage might be used. It could be used as green forage for several cuts, which could eliminate costs of hay making and storage. Some authors, e.g. Abreu et al. (2014), recommend feeding the forage fresh, either in a cut-and-carry system or under grazing. Annual legumes such as lablab and mucuna can provide a large quantity of forage within a short period, which can be conserved and used as hay for dry season livestock feeding. While this incurs additional costs for labor and storage, the area is freed up for growing other crops, especially under the conditions of our study, where growth of mucuna and most of the growth of lablab occurred before the start of the wet season. A major limitation for some producers with these annuals is that they might need replanting each year (Pengelly and Conway 2000).

Currently, it has been shown that using legume protein banks increases milk yield and weight gain, and improves household short-term income in tropical countries (Kabirizi et al. 2013; Nulik et al. 2013; Douxchamps et al. 2014).

Studies over a range of years in the absence of irrigation would provide a better understanding of how these legumes would perform under strictly rain-fed conditions.

Feeding studies with the forage produced by the 3 species would provide a sounder basis for decision making on which species to plant in different situations.

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Variations in soil properties, species composition, diversity and biomass of herbaceous species due to ruminant dung residue in a seasonally dry tropical environment of India

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Keywords: Animal manure, herbaceous vegetation, plant functional attributes, soil pH, species change

Abstract

Ruminants directly or indirectly influence nutrient cycling and vegetation structure in grassland ecosystems. We assessed the impact of natural cattle dung deposition on soil attributes and the resulting effects on species composition, species diversity and biomass of herbaceous vegetation in a natural grassland in the seasonally dry tropical environment of Banaras Hindu University, India. For this 72 plots of 1 × 1 m [12 locations × 2 treatments (dung residue and control) × 3 replicates] were selected in January 2013 and soil and vegetation samples collected. A total of 74 species belonging to 66 genera and 25 families were recorded. Principal Component Analysis (PCA) ordination revealed that the dung residue (DP) and control (CP) plots were distinctly different in terms of soil attributes and species composition. The *k*-dominance plot showed greater species diversity in DPs than CPs, with higher soil nutrients and moisture and lower soil pH in DPs than CPs. Similarly, DPs showed more herbaceous species and greater biomass than CPs. This trend can be explained by the positive responses of forbs, erect plants, annuals, large-statured, non-native and non-leguminous species to dung residue, while increased biomass can be partly due to cattle preferentially not grazing areas adjacent to a dung pat. Overall, the study showed that deposition of dung during grazing by cattle stimulates growth of pasture species and increases species diversity. Therefore cattle dung could be used as a sustainable alternative to chemical fertilizers to manage soil pH, species composition and diversity, and forage production in the seasonally dry tropical grasslands of India, which are nutrient- and moisture-limited.

Resumen

Los rumiantes directa o indirectamente influyen en el ciclo de nutrientes y en la estructura de la vegetación en los ecosistemas de pastizales. En el estudio se evaluó el impacto de la deposición natural de heces de bovinos en las características del suelo, la composición y diversidad de especies y en la biomasa de la vegetación herbácea de un pastizal nativo en ambiente tropical seco estacional de Banaras Hindu University, India. Para el efecto fueron seleccionadas 72 parcelas de 1 × 1 m [12 sitios x 2 tratamientos (residuo de heces y control) x 3 repeticiones]. Al comienzo del ensayo, en enero de 2013, se recolectaron muestras de suelo y vegetación. Se registraron un total de 74 especies pertenecientes a 66 géneros y 25 familias. Los Análisis de Componentes Principales (PCA) mostraron que las características de suelo y la composición de especies fueron diferentes entre los sitios con residuo de heces (DP) y el control (CP). La curva *k*-dominancia mostró una mayor diversidad de especies en las DPs que en las CPs, con niveles más altos de nutrientes y humedad en el suelo, y pH más bajo en DPs que en CPs. Del mismo modo, los DPs mostraron mayor número de especies herbáceas y mayor biomasa que los CPs. Esta tendencia se explica por las respuestas positivas de las especies herbáceas, erectas, anuales, de porte alto, no nativas y no leguminosas, a residuo de heces, mientras que el aumento de la biomasa puede deberse, en parte, a que el ganado prefiere no pastar en áreas adyacentes a residuos de heces. En general, el estudio mostró que la deposición de heces durante el pastoreo por el ganado bovino estimula el crecimiento de las especies y aumenta su diversidad. Por tanto las heces podrían ser utilizadas como una alternativa sostenible a los fertilizantes químicos para manejar el pH del suelo, la composición y diversidad de las especies y la producción de forraje en los pastizales tropicales en ecosistemas estacionales secos de la India, que presentan limitaciones de fertilidad y escasa humedad.

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Introduction

Grasslands occupy roughly 25% (33×10^6 km²) of the total land surface of the Earth (Shantz 1954) and about 18% of the total land area in India (Singh et al. 2006), the second most populous country globally. With the continuously growing human population, agricultural production per unit area has increased to fulfill the greater food requirements by increased use of N-based chemical fertilizers (Shukla et al. 1998). Usage of N-fertilizer has increased from 0.06 million tonnes in 1952 to 9.5 million tonnes in 1995, increasing the release of global warming gases into the atmosphere (Galloway et al. 2008; Zhou et al. 2010) and causing changes in soil, water and vegetation (Giles 2005). Therefore, an alternative to chemical N-fertilizer, which has the capacity to enhance forage production and species diversity with little or no negative effect on the environment, is needed.

The effects of dung on pasture ecosystems have been studied extensively with respect to nutrient cycling (Dickinson and Craig 1990) and species composition in temperate grasslands (MacDiarmid and Watkin 1971; Castle and MacDaid 1972). Such studies, with particular emphasis on biodiversity and biomass of plants, are lacking in tropical grasslands. We assumed that plants with different traits will respond differentially to dung residue and competitive interactions may be changed. Further, we hypothesized that dung residue may promote herbaceous biomass production and species diversity of certain plant species (Steinauer and Collins 1995), because moist dung is a nutrient-rich microhabitat that facilitates seed germination and seedling establishment of competitively superior species (Brown and Archer 1987).

The objectives of the present studies were to assess the effects of deposition of ruminant dung on soil and vegetation attributes in a seasonally dry tropical environment in India. Specifically, we examined the effects of ruminant dung deposition on: (1) community composition; (2) species diversity and biomass; and (3) diversity of plant functional groups in natural grasslands of Banaras Hindu University, Varanasi, India.

Material and Methods

Study sites

The study was conducted at 12 locations (INH - International Hostel; SUK - Sukanya; KAS - Kasturba; SNPG - Sarojani Nayadu; MMV - Mahila Maha Vidhyalay; BG - Botanical Garden; MB - Madhuban; MC - Meera

Colony; AG-1 - Agriculture Farm-1; AG-2 - Agriculture Farm-2; AG-3 - Agriculture Farm-3; and GB - Gandhi Bhawan) at the Banaras Hindu University (24°18' N, 83°03' E; 76 masl), Varanasi, India, during January–March 2013. The grassland studied is representative of the unmanaged rangelands in the region. The area is a part of the Indo-Gangetic Plains characterized by a tropical monsoon climate. The year is made up of a cold winter (November–February), a hot summer (April–June) and a warm rainy season (July–September). October and March are transitional months between rainy and winter, and winter and summer seasons, respectively. During the study period, mean maximum temperature was 25.9 °C (range 18–34.4 °C), while mean minimum temperature was 11.2 °C (range 4.9–16.6 °C). The soil is characterized as Banaras Type III, which is a well-drained, pale brown, silty loam (Buol et al. 2003). In general, the soil is moderately fertile, being low in available nitrogen and medium in available phosphorus and potassium with neutral to alkaline pH (Sagar et al. 2008).

Study design

For sampling, 12 locations were selected visually to represent the entire range of variations in terms of soil, vegetation and ruminant dung residue. Within each location, 3 homogeneous dung residue (DP) pats of one month age (because in the dry season dung completely disappears within 2 months; Holter 1979) and 3 adjacent control (CP) spots with no dung were selected. Around each pat and control spot, a plot of 1 × 1 m in size was established, because a single release of cattle excrement on soil roughly occupies this area (Haynes and Williams 1993). Cow and buffalo dung pats are easily decomposed and scattered by the activity of dung beetles to cover 1 m² area within a month (R. Sagar personal observation). Thus, a total of 72 plots (12 locations × 2 treatments × 3 replicates) were sampled.

Soil sampling and analysis

From each plot, 3 soil samples (0–10 cm depth) were randomly collected, using a corer of 100 cm³ capacity. These samples were mixed and gently homogenized. Large roots, fine roots, wood and litter were removed from the composite soil samples carefully and the soil sieved through a 2 mm mesh screen. One part of each sample was weighed and oven-dried at 105 °C to determine soil moisture content, bulk density and porosity, while a second portion was air-dried for analysis of soil

pH, total soil carbon (total-C), total soil phosphorus (total-P), total soil nitrogen (total-N: inorganic-N + organic-N), ammonium nitrogen ($\text{NH}_4^+\text{-N}$) and nitrate nitrogen ($\text{NO}_3^-\text{-N}$). The sum of $\text{NH}_4^+\text{-N}$ and $\text{NO}_3^-\text{-N}$ is referred to as mineral-N or inorganic-N.

Soil moisture was measured by the gravimetric method. Soil bulk density (g/cm^3) was determined by using the corer method (stainless steel cylinders with a volume of 100 cm^3) (Piper 1944) and was calculated as the dry weight of soil divided by the soil volume (Su and Zhao 2003). Soil porosity was calculated by subtracting the ratio of soil bulk density and particle density (ca. 2.65) from its maximum value of 1 (Sagar and Verma 2010). Soil pH was determined by using a glass electrode (1:2, soil:water ratio). Total soil-C was analyzed by the Walkley (1947) and total soil-N by the Jackson (1958) methods. $\text{NH}_4^+\text{-N}$ was determined by the phenate method (APHA 1985), $\text{NO}_3^-\text{-N}$ by the PDSA method (Jackson 1958) and organic-N by the Jackson (1958) method. Soil phosphorus was analyzed by Allen's method (Allen et al. 1974).

The nutrient concentration (kg/ha) at each location was calculated by multiplying soil bulk density (g/cm^3) by the determined nutrient value (mg/kg). Inputs of soil moisture, pH and nutrients at each location due to ruminant dung were calculated by subtracting the values of control plots (CPs) from the values of the dung residue plots (DPs).

Vegetation sampling and analyses

For each established $1 \times 1 \text{ m}$ plot, the numbers of individual plants were recorded by species and above-ground live biomass of each species was clipped at the soil surface. All samples were oven-dried at $80 \text{ }^\circ\text{C}$ to constant mass and weighed.

Six plant functional attributes pertaining to the various life forms (grasses, sedges and forbs), growth forms (erect, prostrate, procumbent and decumbent), life span (annual, biennial and perennial), relative height (tall, medium and short), N-fixing ability (leguminous forbs and non-leguminous forbs) and origin and distribution (native, non-native and cosmopolitan) were selected. We selected these traits because of their differentiating role of morphology, phenology, competitive ability and taxonomy (Diekmann and Falkengren-Grerup 2002). Species were classed as medium height if 45–90 cm tall, while those below and above this range were grouped as short and tall categories, respectively. Other traits were determined with the help of Flora of Raipur, Durg and Rajnandangaon (Verma et al. 1985) and Flora of the

upper Gangetic plain (Duthie 1903). The biomass of each functional attribute was computed by summing the biomass of all species in each category.

The Importance Value Index (IVI) of each herbaceous species for each location was calculated by summing the relative frequency, relative density and relative biomass (Mueller-Dombois and Ellenberg 1974). The alpha-diversity (H') and its components, i.e. species richness (number of species/ m^2), evenness (E ; distribution of importance values among the species), and beta diversity in terms of habitat heterogeneity (β) were calculated for each location. The following equations were used to calculate the species diversity indices:

$$H' = -\sum_{i=1}^s p_i \ln p_i \quad (\text{Shannon and Weaver 1949})$$

$$E = \frac{H'}{\ln S} \quad (\text{Pielou 1966})$$

$$\beta = \frac{Sc}{\bar{S}} \quad (\text{Whittaker 1972})$$

where:

p_i = the proportion of importance value belonging to species 'i'; S = number of species; Sc = total number of species in the pooled sample; and \bar{S} = average number of species per sample. The diversities of DPs and CPs were compared using the k -dominance plots in which percent cumulative importance values were plotted against log species rank (Platt et al. 1984).

Statistical analyses

Analysis of variance (ANOVA) procedures of SPSS package (SPSS 1997) were used to examine the effects of trait, treatment and location on the soil and vegetation parameters. Paired 't'-test was used to understand the notable variations in the means of soil and vegetation parameters between the treatments. A Tukey's HSD (honestly significant difference) test was used to determine the significance of differences in the soil and vegetation variables among the locations and the traits. The locations of DPs and CPs were ordinated by PCA, using PC-ORD software (McCune and Mefford 1999). Pearson correlation coefficient was established between the soil variables with the help of SPSS package (SPSS 1997). In addition, stepwise regression was used to find out the main soil variables to explain the variability in species and biomass in DPs and CPs with the help of SPSS software (SPSS 1997).

Results

Soil moisture, porosity, pH and nutrient concentrations

Across DP and CP locations, soil moisture, porosity, pH, $\text{NH}_4^+\text{-N}$, $\text{NO}_3^-\text{-N}$, mineral-N, organic-N and total-N ranged from 3.7 to 21%, 48 to 76%, 7.4 to 7.8, 0.9 to 5.4 kg/ha, 0.6 to 3.0 kg/ha, 1.6 to 8.4 kg/ha, 527 to 1,059 kg/ha and 529 to 1,064 kg/ha, respectively. The mean values for soil moisture ($t = 18.33$, $P \leq 0.0001$), porosity ($t = 12.86$, $P \leq 0.0001$), $\text{NH}_4^+\text{-N}$, $\text{NO}_3^-\text{-N}$, mineral-N, organic-N and total-N were significantly higher in DPs than in CPs (Tables 1–3). Contrastingly, the mean values for soil pH ($t = 17.44$, $P \leq 0.0001$) were higher in CPs than in DPs (Tables 1–3). ANOVA showed significant differences in these variables due to locations, treatments and location \times treatment (Table 3). Similarly, total-C, total-P and C:N ratio varied significantly due to location, treatment and location \times treatment (Table 3), with values approximately 2-fold greater in DPs than CPs (Tables 1 and 2). PCA ordination based on component soil attributes distinctly categorized DPs and CPs (Figure 1).

Pearson correlation analysis showed significant relationships between C:N ratio and $\text{NH}_4^+\text{-N}$ ($r = -0.58$, $P \leq 0.05$), $\text{NO}_3^-\text{-N}$ ($r = -0.59$, $P \leq 0.05$), mineral-N ($r = -0.68$, $P \leq 0.05$), organic-N ($r = -0.67$, $P \leq 0.05$), total-N ($r = -0.66$, $P \leq 0.05$), total-C ($r = 0.91$, $P \leq 0.001$) and total-P ($r = 0.67$, $P \leq 0.05$) in DPs, while in CPs, only total-C ($r = 0.97$, $P \leq 0.001$) and total-P ($r = -0.59$, $P \leq 0.05$) were significantly related with C:N ratio.

Nutrient inputs due to dung deposition

The subtraction of nutrient concentration of CPs from that of DPs is referred to here as nutrient input due to ruminant dung. ANOVA suggested that soil $\text{NH}_4^+\text{-N}$, $\text{NO}_3^-\text{-N}$, mineral-N, organic-N, total-N, total-C, total-P and C:N ratio contributed by ruminant dung varied substantially due to location (Table 3). Across the locations, the changes of these nutrients displayed the following ranges: 1.3–3.0, 0.9–1.9, 2.2–4.5, 124–502, 127–506, 3,928–10,718, 27–60 kg/ha and 0.1–12.2, respectively (Tables 1 and 2). Similarly, soil moisture (5.3–12.9%), porosity (0.0–14%) and pH (-0.09 to -0.33) inputs or outputs (depending on a particular case) also varied with the location (Table 3).

Table 1. Mean soil physico-chemical characteristics (\pm s.e.) of different off dung pat locations (CPs).

| Location | Moisture (%) | Porosity (%) | pH | $\text{NH}_4^+\text{-N}$ | $\text{NO}_3^-\text{-N}$ | Mineral-N | Organic-N | Total-N | Total-C | C:N ratio | Total-P (kg/ha) |
|----------|-----------------|---------------|-----------------|--------------------------|--------------------------|----------------|----------------|-----------------|-----------------|-----------------|-----------------|
| | | | | (kg/ha) | | | | | | | |
| INH | 3.7a (0.1) | 48a (2.2) | 7.8e (0.0) | 2.1b (0.1) | 1.3g (0.0) | 3.4c (0.2) | 719c (33) | 722cd (33) | 949a (33) | 1.3a (0.0) | 73ab (3) |
| KAS | 4.1ab (0.0) | 52ab (0.1) | 7.8de (0.0) | 1.9b (0.0) | 1.3e (0.0) | 3.1bc (0.0) | 744c (4) | 747cd (4) | 989a (2) | 1.3a (0.0) | 76ab (1) |
| SUK | 4.7cd (0.1) | 57bc (0.1) | 7.8bcd (0.0) | 1.8b (0.0) | 1.2e (0.0) | 2.9bc (0.0) | 684bc (3) | 687bcd (3) | 922a (3) | 1.3a (0.0) | 77b (0.15) |
| SNPG | 3.9a (0.1) | 51a (0.2) | 7.7e (0.0) | 1.9b (0.0) | 1.4fg (0.0) | 3.3c (0.0) | 731c (17) | 734cd (17) | 997a (11) | 1.4a (0.0) | 74ab (0.95) |
| MMV | 5.1def (0.1) | 62cd (2.3) | 7.7cde (0.0) | 3.0c (0.2) | 1.2e (0.1) | 4.2d (0.3) | 762c (43) | 766d (43) | 5,934d (365) | 7.7b (0.1) | 74ab (4.48) |
| BG | 5.5f (0.0) | 65de (0.2) | 7.7bcd (0.0) | 1.9b (0.0) | 0.8bc (0.0) | 2.7b (0.1) | 744c (9) | 747cd (9) | 5,549cd (33) | 7.4b (0.1) | 74ab (0.9) |
| MB | 5.3ef (0.1) | 64d (0.1) | 7.7bcd (0.0) | 2.9c (0.0) | 1.1de (0.0) | 4.0d (0.0) | 696bc (12) | 700bcd (12) | 4,999bc (30) | 7.2b (0.2) | 70ab (0.2) |
| MC | 4.9de (0.1) | 63d (1.3) | 7.7bcd (0.0) | 2.9c (0.1) | 1.1efg (0.0) | 4.1d (0.2) | 761c (29) | 765cd (29) | 5,770d (190) | 7.6b (0.1) | 74ab (2.9) |
| AG-1 | 5.3ef (0.1) | 69ef (0.2) | 7.6ab (0.0) | 1.3a (0.0) | 0.7ab (0.0) | 2.0a (0.0) | 527a (25) | 529a (25) | 6,022d (255) | 11.4cd (0.1) | 66ab (1.0) |
| AG-2 | 6.1g (0.1) | 70f (0.3) | 7.5a (0.0) | 0.9a (0.0) | 0.6a (0.0) | 1.6a (0.0) | 557bc (5) | 558ab (5) | 4,706b (55) | 8.4b (0.1) | 65a (0.8) |
| AG-3 | 5.3ef (0.2) | 65de (0.2) | 7.7bcd (0.1) | 2.1b (0.0) | 0.9cd (0.0) | 3.0bc (0.0) | 610abc (3) | 613abc (3) | 5,661cd (25) | 9.2bc (0.1) | 66ab (0.4) |
| GB | 4.4bc (0.2) | 48a (1.1) | 7.8de (0.0) | 3.1c (0.1) | 1.4g (0.0) | 4.5d (0.1) | 661abc (75) | 666abcd (75) | 8,519e (180) | 13.2d (1.6) | 73ab (3.68) |

INH = International Hostel, KAS = Kasturba, SUK = Sukanya, SNPG = Sarojani Nayadu, MMV = Mahila Maha Vidhyalay, BG = Botanical Garden, MB = Madhuban, MC = Meera Colony, AG-1 = Agriculture Farm-1, AG-2 = Agriculture Farm-2, AG-3 = Agriculture Farm-3 and GB = Gandhi Bhawan.

Table 2. Mean soil physico-chemical characteristics (\pm s.e.) of different dung pat locations (DPs).

| Location | Moisture (%) | Porosity (%) | pH | NH ₄ ⁺ -N | NO ₃ ⁻ -N | Mineral-N Organic-N Total-N | | | Total-C | C:N ratio | Total-P (kg/ha) |
|----------|-----------------|----------------|----------------|---------------------------------|---------------------------------|-----------------------------|----------------|----------------|--------------------|-----------------|-----------------|
| | | | | | | (kg/ha) | | | | | |
| INH | 9.0a (0.0) | 53a (0.1) | 7.6d (0.0) | 3.8ab (0.0) | 2.8de (0.0) | 6.5abc (0.0) | 843a (13) | 849a (13) | 9,632ab (38) | 11.4b (0.2) | 133f (0.6) |
| KAS | 10.1a (0.0) | 55a (0.1) | 7.6d (0.0) | 3.8abc (0.0) | 2.5bcd (0.0) | 6.3abc (0.0) | 906a (13) | 912a (13) | 11,226bcd (10) | 12.3bc (0.2) | 131f (0.6) |
| SUK | 12.2b (0.1) | 58ab (0.3) | 7.6d (0.0) | 3.8ab (0.0) | 2.5abcd (0.0) | 6.2ab (0.0) | 907a (11) | 913a (11) | 10,941abc (78) | 12.0bc (0.2) | 123cdef (1) |
| SNPG | 9.3a (0.0) | 54a (0.2) | 7.6d (0.0) | 3.8abcd (0.0) | 2.7cde (0.0) | 6.5abc (0.1) | 860a (16) | 867a (16) | 11,715cd (71) | 13.5bc (0.3) | 133f (0.8) |
| MMV | 14.7c (0.3) | 62bc (2.0) | 7.5c (0.0) | 5.4g (0.3) | 3.0e (0.2) | 8.4e (0.4) | 909a (44) | 917a (45) | 12,315cd (644) | 13.4bc (0.1) | 138f (7) |
| BG | 18.4fg (0.6) | 71ef (0.5) | 7.4a (0.0) | 4.4de (0.1) | 2.3ab (0.0) | 6.7bc (0.1) | 917ab (12) | 924ab (12) | 10,963bcd (167) | 11.9bc (0.1) | 106abc (1) |
| MB | 16.3de (0.3) | 68de (0.7) | 7.4ab (0.0) | 4.8efg (0.1) | 2.5abcd (0.1) | 7.3cd (0.2) | 916ab (28) | 923ab (28) | 11,126bcd (203) | 12.1bc (0.6) | 114cde (2) |
| MC | 15.8cd (0.2) | 65cd (1.2) | 7.5bc (0.0) | 5.3fg (0.2) | 2.7cde (0.1) | 8.0de (0.3) | 889a (32) | 897a (32) | 12,548d (429) | 14.0c (0.8) | 127def (4) |
| AG-1 | 19.5gh (0.3) | 74fg (0.2) | 7.4ab (0.0) | 4.3bcde (0.0) | 2.2a (0.0) | 6.4abc (0.0) | 892a (51) | 899a (51) | 10,785bcd (369) | 12.1bc (0.7) | 95ab (1) |
| AG-2 | 21.0h (0.6) | 76g (0.3) | 7.4a (0.0) | 3.6a (0.1) | 2.1a (0.0) | 5.7a (0.0) | 1,059b (21) | 1,064b (21) | 8,913a (91) | 8.4a (0.3) | 92a (2) |
| AG-3 | 17.5ef (0.3) | 69def (1.1) | 7.4ab (0.0) | 4.6ef (0.2) | 2.4abc (0.1) | 7.0bcd (0.3) | 883a (34) | 890a (34) | 10,726bc (366) | 12.1bc (0.6) | 110bcd (3) |
| GB | 14.5c (0.3) | 62bc (2.2) | 7.5bc (0.0) | 4.4cde (0.2) | 2.3ab (0.1) | 6.7bc (0.3) | 961ab (31) | 967ab (31) | 12,447cd (690) | 12.9bc (0.8) | 132f (6) |

Means within columns followed by different letters are significantly different at $P \leq 0.05$. INH = International Hostel, KAS = Kasturba, SUK = Sukanya, SNPG = Sarojani Nayadu, MMV = Mahila Maha Vidhyalay, BG = Botanical Garden, MB = Madhuban, MC = Meera Colony, AG-1 = Agriculture Farm-1, AG-2 = Agriculture Farm-2 AG-3 = Agriculture Farm-3 and GB = Gandhi Bhawan.

Table 3. Summary of ANOVA (F -values and degrees of freedom) of different soil and vegetation parameters due to location and treatment (DP and CP).

| Variable | Location | Treatment | Location \times Treatment |
|---------------------------------|---------------|--------------|-----------------------------|
| | $F_{11,48} =$ | $F_{1,48} =$ | $F_{11,48} =$ |
| Soil moisture | 215*** | 1,177*** | 109*** |
| Porosity | 111*** | 107*** | 5.97*** |
| pH | 49*** | 1,021*** | 8.46*** |
| NH ₄ ⁺ -N | 74*** | 2,768*** | 11.36*** |
| NO ₃ ⁻ -N | 40*** | 4,175*** | 8.63*** |
| Mineral-N | 50*** | 3,345*** | 9.38*** |
| Organic-N | 3.19** | 367*** | 7.73*** |
| Total-N | 3.25** | 377*** | 7.74*** |
| Total-C | 69*** | 3,965*** | 41.94*** |
| C:N ratio | 43.3*** | 902*** | 45.60*** |
| Total-P | 21.7*** | 1,636*** | 9.91*** |
| Richness | 26.48*** | 1,718*** | 5.37*** |
| Evenness | 3.42** | 22*** | 5.85*** |
| Shannon index | 23.17*** | 1,080*** | 7.35*** |
| Beta diversity | 8.5*** | 253*** | 2.91** |
| Biomass | 139*** | 761*** | 46.15*** |

** = $P \leq 0.001$; *** = $P \leq 0.0001$.

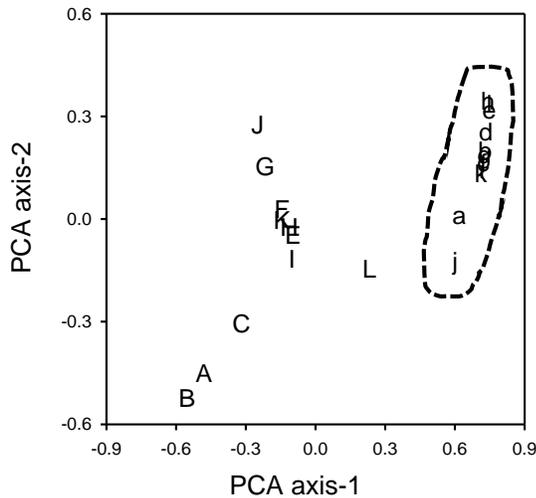


Figure 1. PCA ordination of different off dung pat (capital letters) and dung pat (small letters) locations (CPs resp. DPs) on the basis of nutrient concentrations. The letters within the dotted line represent the dung pat locations. In the ordination diagram A and a = International Hostel, B and b = Sukanya, C and c = Kasturba, D and d = Sarojani Nayadu, E and e = Mahila MahaVidhyalay, F and f = Botanical Garden, G and g = Gandhi Bhawan, H and h = Madhuban, I and i = Meera Colony, J and j = Agriculture Farm-1, K and k = Agriculture Farm-2, L and l = Agriculture Farm-3.

Species composition

A total of 74 species belonging to 66 genera and 25 families was recorded from seventy-two 1×1 m plots (Table 4). The families Asteraceae and Poaceae had the highest number of species (10), followed by Fabaceae (7) and Amaranthaceae (6), with 12 families being represented by a single species. The DPs had 72 species and CPs had 52 species. Twenty-three species were exclusively present in DPs, while only 2 species were restricted to CPs, and 49 species were common to both DPs and CPs (Table 4).

On the basis of biomass, *Cynodon dactylon* was the dominant species for both DPs and CPs. The second and third most common species in DPs were *Echinochloa crus-galli* and *Urena lobata*, respectively, while *Malvastrum tricuspidatum* was the second and *Oxalis corniculata* the third most common species in CPs (Table 4). PCA ordination based on component species of these 2 treatments also showed differences in species composition of DPs and CPs (Figure 2).

Species diversity and biomass

Across locations, the mean species number, evenness, Shannon index and beta diversity per plot varied from 3 to 17, 0.70 to 0.97, 1.05 to 2.62 and 1.07 to 3.14, respectively (Tables 5 and 6). ANOVA suggested that these diversity indices differed substantially due to location, treatment and location \times treatment (Table 3). Mean

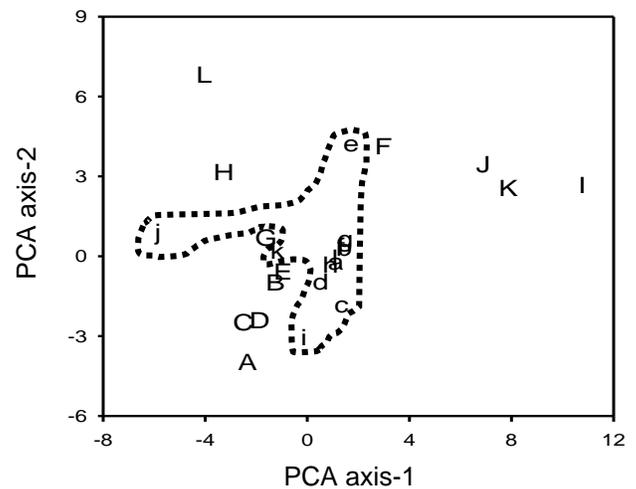


Figure 2. PCA ordination of different dung (capital letters) and off dung pat (small letters) locations (CPs resp. DPs) on the basis of relative biomass of herbaceous species. The letters within the dotted line represent the off dung pat locations. Stepwise regression showed that the soil phosphorus explained PCA axis-1 in CPs, while soil phosphorus and soil pH, respectively, explained PCA axes-1 and -2 in DPs. In the ordination diagram A and a = International Hostel, B and b = Sukanya, C and c = Kasturba, D and d = Sarojani Nayadu, E and e = Mahila MahaVidhyalay, F and f = Botanical Garden, G and g = Gandhi Bhawan, H and h = Madhuban, I and i = Meera Colony, J and j = Agriculture Farm-1, K and k = Agriculture Farm-2, L and l = Agriculture Farm-3.

values for species number and Shannon index were higher in DPs than in CPs. On the other hand, mean values for evenness and beta diversity were lower in DPs than in CPs (Tables 5 and 6). Thus, dung inputs by ruminants promoted species diversity and restricted the distribution of individuals among the species. The k -dominance plots for DPs and CPs are illustrated in Figure 3, in which the uppermost line (DPs) represented greater diversity than the bottom line (CPs).

On the basis of stepwise regression analysis, soil moisture explained 97% of the variation in species number and soil porosity explained 85% of the variation in Shannon index and 69% of the variation in beta diversity in CPs, while none of the soil variables explained the variability in species evenness. In contrast with these patterns, soil pH independently accounted for 85% of the variation in species number and, together with $\text{NO}_3\text{-N}$, explained 91% of the variation in species number in DPs. Similarly, soil pH also accounted for variation in Shannon index, while soil moisture accounted for variation in species evenness and beta diversity (Table 7). Linear regression analysis showed significant negative relationships between soil pH and species number in both DPs and CPs (Figure 4). Further, the higher determination coefficient (R^2) in DPs than in CPs (0.92 vs. 0.55) suggested that soil pH had a greater influence on species number in areas where dung was deposited.

Table 4. Species and biomass (g DM/ha) of herbaceous species of different dung pat and off dung pat locations (CPs resp. DPs).

| Species ¹ | Family | Dung pat (DP) | | Off dung pat (CP) | |
|---|----------------|---------------|---|-------------------|---|
| | | Biomass | Sites occupied | Biomass | Sites occupied |
| <i>Abutilon indicum</i> (L.) Sweet E,L,Pe,NLF,N | Malvaceae | 46 | BG, MC, AG-1, AG-2, AG-3 | 13 | MB |
| <i>Acalypha indica</i> L. ^{E,M,A,NLF,N} | Euphorbiaceae | 17 | INH, SUK, MMV, BG, MB | 4 | SNPG |
| <i>Achyranthes aspera</i> L. ^{E,L,Bi,NLF,N} | Amaranthaceae | 38 | INH, SUK, BG, MB, MC, GB | 8 | KAS, MB |
| <i>Aerva sanguinolenta</i> (L.) Blume E,L,Pe,NLF,N | Amaranthaceae | 13 | INH, SUK, MMV, MB, | 14 | MMV, BG, GB |
| <i>Aeschynomene indica</i> L. E,M,Bi,LF,NN | Fabaceae | 9 | AG-1,AG-2 | 0 | |
| <i>Ageratum conyzoides</i> L. E,L,A,NLF,NN | Asteraceae | 35 | SUK, MMV, BG, MB, MC, GB | 10 | AG-2 |
| <i>Alternanthera sessilis</i> (L.) R. Br. ex DC. ^{P,L,A,NLF,NN} | Amaranthaceae | 17 | BG, MB, MC, AG-1 | 24 | MMV, BG, GB |
| <i>Alysicarpus vaginalis</i> (L.) DC. De,L,Pe,LF,N | Fabaceae | 16 | AG-2, AG-3 | 4 | AG-2 |
| <i>Amaranthus spinosus</i> L. E,L,A,NLF,NN | Amaranthaceae | 33 | BG, AG-1, AG-2, AG-3 | 0 | |
| <i>Amaranthus viridis</i> L. ^{E,M,A,NLF,NN} | Amaranthaceae | 16 | BG, MB, MC, GB | 14 | BG, MB |
| <i>Ammannia baccifera</i> L. ^{E,L,A,NLF,N} | Lythraceae | 12 | GB | 54 | KAS, SUK, SNPG, MC, AG-1 |
| <i>Anagallis arvensis</i> L. ^{E,M,A,NLF,NN} | Primulaceae | 12 | AG-1, AG-3 | 0 | |
| <i>Argemone mexicana</i> L. ^{E,L,A,NLF,NN} | Papaveraceae | 22 | BG, MC, AG-1, AG-3 | 0 | |
| <i>Atylosia marmorata</i> R. Br. ex Benth. ^{P,L,A,LF,NN} | Fabaceae | 3 | MMV, BG, MB | 34 | BG, MB, AG-2, AG-3, GB |
| <i>Biophytum sensitivum</i> (L.) DC. E,L,A,NLF,NN | Oxalidaceae | 16 | AG-3 | 0 | |
| <i>Caesulia axillaris</i> Roxb. De,L,A,NLF,N | Asteraceae | 12 | MC, GB | 0 | |
| <i>Chenopodium album</i> L. ^{E,L,A,NLF,NN} | Amaranthaceae | 33 | BG, AG-1, AG-2, AG-3 | 0 | |
| <i>Commelina benghalensis</i> L. Pro,L,A,NLF,NN | Commelinaceae | 53 | MMV, BG, AG-1 | 0 | |
| <i>Convolvulus prostratus</i> Forssk. P,S,Pe,NLF,N | Convolvulaceae | 11 | INH, SUK, MMV, MB | 1 | MC |
| <i>Croton bonplandianus</i> Baill. E,M,Pe,NLF,NN | Euphorbiaceae | 12 | MC | 6 | MC |
| <i>Cynodon dactylon</i> (L.) Pers. P,S,Pe,G,COS | Poaceae | 194 | INH, SUK, SNPG, MMV, BG, MB, MC, AG-AG-2, AG-3, GB | 105 | INH, KAS, SUK, SNPG, MMV, BG, MB, MC |
| <i>Cyperus cyperoides</i> (L.) Kuntze P,M,Pe,Se,NN | Cyperaceae | 29 | KAS, SNPG, MB, MC, GB | 1 | MB |
| <i>Cyperus rotundus</i> L. ^{P,M,Pe,Se,COS} | Cyperaceae | 43 | INH, SUK, SNPG, MB, MC | 28 | INH, KAS, MMV, MC |
| <i>Dactyloctenium aegyptium</i> (L.) Willd. ^{De,S,A,G,NN} | Poaceae | 56 | INH, SUK, SNPG, MMV, BG, MB, MC, GB | 2 | MMV, MB |
| <i>Desmodium triflorum</i> (L.) DC. E,L,Pe,LF,NN | Fabaceae | 0 | | 34 | MMV, BG, MB, AG-1 |
| <i>Dichanthium annulatum</i> (Forssk.) Stapf ^{E,S,Pe,G,NN} | Poaceae | 77 | INH, SUK, SNPG, MMV, BG, AG- 1, AG-2, AG-3, GB | 9 | MB |
| <i>Digitaria ciliaris</i> (Retz.) Koeler De,S,A,G,NN | Poaceae | 48 | INH, KAS, SUK, SNPG, MMV, MC | 14 | AG-1, AG-3 |
| <i>Echinochloa colona</i> (L.) Link De,M,A,G,NN | Poaceae | 50 | INH, KAS, SNPG, AG-AG-2, AG-3 | 8 | AG-2 |
| <i>Echinochloa crus-galli</i> (L.) Beauv. ^{De,L,A,G,NN} | Poaceae | 134 | SNPG, MMV, BG, MB, MC, AG-1, AG-2, AG-3, GB | 0 | |

Continued

| Species ¹ | Family | Dung pat (DP) | | Off dung pat (CP) | |
|--|----------------|---------------|---|-------------------|--------------------------------------|
| | | Biomass | Sites occupied | Biomass | Sites occupied |
| <i>Eclipta alba</i> (L.) Hassk. P,S,A,NLF,NN | Asteraceae | 28 | INH, SUK, SNPG, MB, MC, GB | 10 | GB |
| <i>Eleusine indica</i> (L.) Gaertn. E,M,A,G,NN | Poaceae | 19 | INH, KAS, SUK, SNPG | 2 | MMV |
| <i>Eragrostis tenella</i> (L.) P. Beauv. ex Roem. & Schult. E,L,Pe,G,NN | Poaceae | 53 | INH, KAS, SUK, SNPG, MMV, MB, MC, AG-1, AG-2, AG-3 | 10 | SUK, AG-1 |
| <i>Euphorbia dracunculoides</i> Lam. E,L,Bi,NLF,NN | Euphorbiaceae | 34 | AG-1, AG-3 | 16 | AG-1, AG-2 |
| <i>Euphorbia hirta</i> L. Pr,L,A,NLF,NN | Euphorbiaceae | 62 | INH, KAS, SUK, SNPG, MMV, BG, MB, AG-1, AG-2, AG-3 | 5 | MMV |
| <i>Evolvulus nummularius</i> (L.) L. P,S,Pe,NLF,NN | Convolvulaceae | 22 | INH, MMV, BG, MB, GB | 7 | KAS, SUK, GB |
| <i>Evolvulus alsinoides</i> (L.) L. Pr,S,A,NLF,NN | Convolvulaceae | 42 | INH, KAS, SNPG, MMV | 1 | AG-2 |
| <i>Gomphrena celosioides</i> Mart. E,M,A,NLF,NN | Amaranthaceae | 49 | KAS, SNPG, MMV, MB, MC | 0 | |
| <i>Gnaphalium luteoalbum</i> L. De,M,A,NLF,NN | Asteraceae | 31 | KAS, SNPG, MMV, | 0 | |
| <i>Heliotropium indicum</i> L. E,M,A,NLF,NN | Boraginaceae | 16 | MB, MC, GB | 0 | |
| <i>Herpestis monnieri</i> (L.) Kunth E,M,Pe,NLF,N | Plantaginaceae | 20 | AG-1, AG-2, AG-3, GB | 8 | SUK, MC |
| <i>Hyptis suaveolens</i> (L.) Poit. P,L,A,NLF,NN | Lamiaceae | 19 | AG-1, AG-3, GB | 5 | GB |
| <i>Imperata cylindrica</i> (L.) P. Beauv. E,L,Pe,G,NN | Poaceae | 36 | AG-1, AG-2, AG-3 | 5 | BG |
| <i>Indigofera linifolia</i> (L. f.) Retz. Pr,M,A,LF,NN | Fabaceae | 13 | SNPG, AG-1, AG-2, AG-3 | 6 | AG-1 |
| <i>Lathyrus aphaca</i> L. E,M,A,LF,NN | Fabaceae | 8 | AG-2 | 3 | AG-2 |
| <i>Launaea procumbens</i> (Roxb.) Ramayya & Rajagopal P,S,Pe,NLF,N | Asteraceae | 38 | KAS, SNPG, MMV, BG, MB | 16 | MMV, AG-1, AG-2 |
| <i>Leucas aspera</i> (Willd.) Link E,M,A,NLF,NN | Lamiaceae | 33 | BG, MB, MC, GB | 1 | AG-1 |
| <i>Lindenbergia indica</i> (L.) Vatke E,L,A,NLF,N | Orobanchaceae | 12 | GB | 15 | MMV, BG |
| <i>Malvastrum tricuspidatum</i> (R. Br.) A. Gray E,L,Pe,NLF,NN | Malvaceae | 55 | SUK, SNPG, BG, MB, MC, AG-1, AG-2, AG-3 | 62 | INH, BG, MC, AG-1, 1GB |
| <i>Melilotus albus</i> Medik. E,L,Bi,LF,NN | Fabaceae | 28 | AG-1, AG-2 | 13 | AG-1, AG-2 |
| <i>Nicotiana alata</i> Link & Otto E,M,Pe,NLF,NN | Solanaceae | 2 | GB | 3 | MB |
| <i>Oldenlandia corymbosa</i> L. P,M,A,NLF,NN | Rubiaceae | 2 | KAS, SUK, SNPG | 12 | SNPG, AG-1, AG-2 |
| <i>Oplismenus compositus</i> (L.) P. Beauv. P,S,Pe,G,NN | Poaceae | 32 | KAS, SUK, SNPG, MMV, BG, AG- 1, AG-2, AG-3 | 19 | KAS, MMV, AG-3, GB |
| <i>Oxalis corniculata</i> L. Pr,S,Pe,NLF,NN | Oxalidaceae | 12 | AG-1, AG-3 | 55 | INH, KAS, SUK, SNPG, BG, MB, AG-1 |
| <i>Parthenium hysterophorus</i> L. E,L,Pe,NLF,NN | Asteraceae | 66 | MC, AG-2, AG-3, GB | 0 | |
| <i>Paspalidium flavidum</i> (Retz.) A. Camus P,L,Pe,G,NN | Poaceae | 0 | | 4 | AG-2 |
| <i>Peristrophe bicalyculata</i> (Retz.) Nees. E,L,Pe,NLF,NN | Acanthaceae | 6 | GB | 0 | |
| <i>Phyla nodiflora</i> (L.) Greene P,L,A,NLF,NN | Verbenaceae | 2 | MC | 0 | |

Continued

| Species ¹ | Family | Dung pat (DP) | | Off dung pat (CP) | |
|---|----------------|---------------|--|-------------------|------------------------------|
| | | Biomass | Sites occupied | Biomass | Sites occupied |
| <i>Portulaca oleracea</i> L. ^{P,S,A,NLF,NN} | Portulacaceae | 66 | INH, KAS, SUK, SNPG, MMV, MB | 0 | |
| <i>Ranunculus sceleratus</i> L. ^{E,L,A,NLF,NN} | Ranunculaceae | 32 | AG-1, AG-2, AG-3 | 0 | |
| <i>Rorippa dubia</i> (Pers.) H. Hara ^{E,M,A,NLF,N} | Brassicaceae | 28 | AG-1, AG-2, AG-3 | 14 | INH, SUK |
| <i>Ruellia tuberosa</i> L. ^{E,M,Bi,NLF,NN} | Acanthaceae | 4 | GB | 1 | MC |
| <i>Rumex dentatus</i> L. ^{E,L,Bi,NLF,N} | Polygonaceae | 25 | AG-1, AG-2, AG-3 | 0 | |
| <i>Rungia pectinata</i> (L.) Nees ^{Pr,M,A,NLF,N} | Acanthaceae | 4 | GB | 21 | INH, SUK, BG, AG-3 |
| <i>Rungia parviflora</i> Nees ^{Pr,M,A,NLF,NN} | Acanthaceae | 26 | KAS, SUK, SNPG, BG, MB, AG-1, AG-2, AG-3 | 0 | |
| <i>Scoparia dulcis</i> L. ^{E,M,Pe,NLF,NN} | Plantaginaceae | 22 | SUK, SNPG, MMV AG-3, GB | 1 | AG-3 |
| <i>Sida acuta</i> Burm. f. ^{E,L,Bi,NLF,NN} | Malvaceae | 57 | KAS, SUK, SNPG, GB | 47 | INH, KAS, SNPG, BG, MC, AG-3 |
| <i>Sida cordifolia</i> L. ^{E,S,Pe,NLF,NN} | Malvaceae | 121 | INH, KAS, SUK, SNPG, MMV, BG, MB, MC, GB | 0 | |
| <i>Solanum nigrum</i> L. ^{E,L,A,NLF,NN} | Solanaceae | 22 | MC, AG-2 | 17 | MC, AG-2, AG-3 |
| <i>Sonchus oleraceus</i> L. ^{E,L,A,NLF,NN} | Asteraceae | 47 | AG-1, AG-2, AG-3 | 21 | AG-1, AG-2, AG-3 |
| <i>Spilanthes acmella</i> (L.) L. ^{E,M,Pe,NLF,NN} | Asteraceae | 21 | BG, MB, MC, GB | 0 | |
| <i>Tridax procumbens</i> L. ^{Pr,M,Pe,NLF,NN} | Asteraceae | 16 | SUK, SNPG, MB, MC | 15 | SUK, SNPG |
| <i>Uraria picta</i> (Jacq.) Desv. ex DC. ^{E,L,Pe,LF,N} | Fabaceae | 20 | AG-2, AG-3 | 0 | |
| <i>Urena lobata</i> L. ^{E,L,Pe,NLF,NN} | Malvaceae | 162 | KAS, SNPG, MMV, BG, MB, MC, AG-1, AG-2, AG-3, GB | 0 | |
| <i>Vernonia cinerea</i> (L.) Less. ^{E,M,A,NLF,NN} | Asteraceae | 9 | SUK, MB, GB | 6 | MMV |

¹Nomenclature according to the Tropicos taxonomic database (www.tropicos.org).

Abbreviations used: E = Erect, P = Prostrate, De = Decumbent, Pr = Procumbent; L = Tall, M = Medium, S = Short height; A = Annual, Bi = Biennial, Pe = Perennial; G = Grasses, Se = Sedges, NLF = Non-leguminous forb, LF = leguminous forb; N = Native, NN = Non-native, COS = Cosmopolitan; INH = International Hostel, SUK = Sukanya, KAS = Kasturba, SNPG = Sarojani Nayadu, MMV = Mahila MahaVidhyalay, BG = Botanical Garden, GB = Gandhi Bhawan, MB = Madhuban, MC = Meera Colony, AG-1 = Agriculture Farm-1, AG-2 = Agriculture Farm-2 and AG-3 = Agriculture Farm-3.

Across species, locations and treatments, herbaceous biomass varied between 0.4 and 194 g/m² (Table 4) and from 14.6 to 93 g/m² across locations and treatments (Tables 5 and 6). ANOVA showed substantial variation in herbaceous biomass owing to location, treatment and coupling of location and treatment (Table 3). Step-wise regression suggested that soil moisture explained much of the variation in herbaceous biomass in both treatments (DP and CP), with greater values in DP than in CP (Tables 5–7). Thus, greater soil moisture availability together with soil nutrients provided greater biomass accumulation in this dry tropical grassland.

Plant functional attributes

ANOVA revealed significant variation in species number and biomass of plants with different functional attributes due to trait, location and treatment and their interactions (Table 8). The differences in mean species number and biomass among plants with different traits in DPs and CPs, analyzed by the HSD test, are presented in Table 9. Forbs plus erect, annual, tall, non-native and non-leguminous plants predominated in both DPs and CPs, while mean values for species number and biomass for plants with different traits were greater in DPs than in CPs (Table 9).

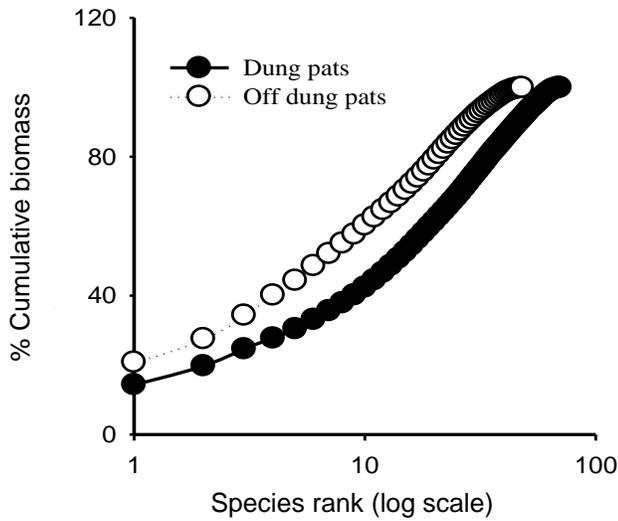


Figure 3. The k-dominance plot in which total percentage cumulative biomass is plotted against log species rank for dung and off dung pats (DPs resp. CPs).

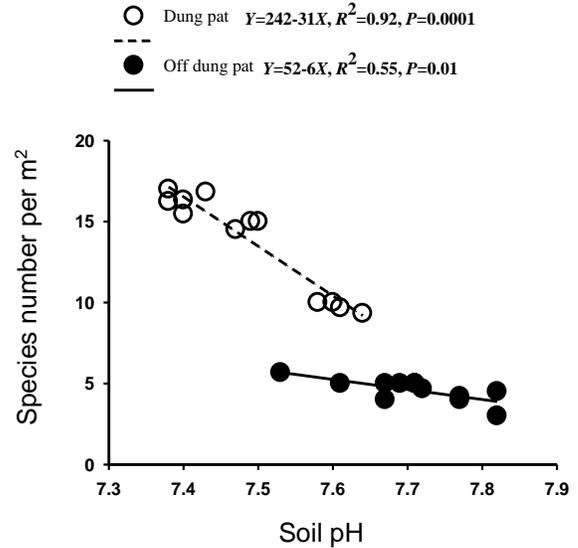


Figure 4. Linear relationships between soil pH (X) and species number (Y) at dung and off dung pat locations (CP resp. DP).

Table 5. Mean values for vegetation parameters (\pm s.e.) at different off dung pat locations (CPs).

| Location | Species number | Evenness | Shannon index | Beta diversity | Biomass (g DM/m ²) |
|----------|-------------------|------------------|-------------------|-------------------|--------------------------------|
| INH | 4.5ab (0.00) | 0.96ab (0.03) | 1.05a (0.04) | 1.76a (0.19) | 14.7a (0.13) |
| KAS | 4.2ab (0.33) | 0.89ab (0.04) | 1.14ab (0.06) | 1.80ab (0.10) | 17.0a (0.58) |
| SUK | 4.0ab (0.00) | 0.90ab (0.03) | 1.24abc (0.05) | 2.11ab (0.06) | 22.5bc (0.29) |
| SNPG | 3.0a (0.00) | 0.97b (0.00) | 1.07a (0.00) | 2.51abc (0.09) | 14.6a (0.15) |
| MMV | 4.7bc (0.33) | 0.96ab (0.01) | 1.40bc (0.07) | 2.32abc (0.05) | 24.8d (0.17) |
| BG | 5.0c (0.00) | 0.93ab (0.01) | 1.49c (0.02) | 2.63abc (0.13) | 28.3d (0.88) |
| MB | 5.0c (0.00) | 0.89ab (0.01) | 1.44bc (0.02) | 2.69abc (0.27) | 24.5c (0.29) |
| MC | 5.00c (0.00) | 0.78a (0.08) | 1.26abc (0.12) | 2.77bc (0.26) | 23.0bc (0.29) |
| AG-1 | 5.00c (0.00) | 0.92ab (0.02) | 1.48c (0.03) | 3.09c (0.31) | 29.0d (0.58) |
| AG-2 | 5.67d (0.33) | 0.79a (0.02) | 1.41bc (0.04) | 3.14c (0.14) | 30.0d (0.58) |
| AG-3 | 5.00c (0.00) | 0.91ab (0.04) | 1.46c (0.07) | 3.13c (0.32) | 24.0c (0.58) |
| GB | 4.00abc (0.58) | 0.85ab (0.05) | 1.14ab (0.08) | 2.27abc (0.08) | 21.2b (0.44) |

INH = International Hostel, KAS = Kasturba, SUK = Sukanya, SNPG = Sarojani Nayadu, MMV = Mahila Maha Vidhyalay, BG = Botanical Garden, MB = Madhuban, MC = Meera Colony, AG-1 = Agriculture Farm-1, AG-2 = Agriculture Farm-2, AG-3 = Agriculture Farm-3 and GB = Gandhi Bhawan.

Table 6. Mean values for vegetation parameters (\pm s.e.) at different dung pat locations (DPs).

| Location | Species number | Evenness | Shannon index | Beta diversity | Biomass (g DM/m ²) |
|----------|----------------|----------|---------------|----------------|--------------------------------|
| INH | 9.3a | 0.75ab | 1.69a | 1.18ab | 44a |
| | (0.33) | (0.01) | (0.04) | (0.10) | (1.4) |
| KAS | 10.0a | 0.70a | 1.63a | 1.46abc | 50a |
| | (0.00) | (0.02) | (0.05) | (0.08) | (0.3) |
| SUK | 10.0a | 0.82abcd | 1.90ab | 1.39abc | 53a |
| | (0.00) | (0.04) | (0.10) | (0.11) | (1.3) |
| SNPG | 9.7a | 0.84bcd | 1.92abc | 1.07a | 48a |
| | (0.33) | (0.00) | (0.04) | (0.07) | (0.3) |
| MMV | 15.0b | 0.83abcd | 2.25cd | 1.53abc | 68b |
| | (0.58) | (0.05) | (0.13) | (0.20) | (2.7) |
| BG | 17.0c | 0.81abcd | 2.27cde | 1.65abc | 88de |
| | (0.33) | (0.01) | (0.01) | (0.13) | (3.1) |
| MB | 16.33bc | 0.82abcd | 2.22bcd | 1.21ab | 77c |
| | (0.88) | (0.04) | (0.11) | (0.07) | (1.8) |
| MC | 15.00b | 0.80abcd | 2.16bcd | 1.29ab | 74bc |
| | (0.58) | (0.01) | (0.03) | (0.26) | (1.1) |
| AG-1 | 15.46b | 0.91cd | 2.49de | 1.59abc | 93e |
| | (1.00) | (0.01) | (0.05) | (0.08) | (2.7) |
| AG-2 | 16.24bc | 0.93d | 2.44de | 2.01c | 93e |
| | (1.45) | (0.01) | (0.08) | (0.10) | (1.6) |
| AG-3 | 16.82bc | 0.89cd | 2.33de | 1.79bc | 79cd |
| | (0.88) | (0.01) | (0.04) | (0.13) | (2.2) |
| GB | 14.50b | 0.92cd | 2.62e | 1.27ab | 74bc |
| | (0.58) | (0.01) | (0.05) | (0.11) | (1.1) |

Means within columns followed by different letters are significantly different at $P \leq 0.05$. INH = International Hostel, KAS = Kasturba, SUK = Sukanya, SNPG = Sarojani Nayadu, MMV = Mahila Maha Vidhyalay, BG = Botanical Garden, MB = Madhuban, MC = Meera Colony, AG-1 = Agriculture Farm-1, AG-2 = Agriculture Farm-2 AG-3 = Agriculture Farm-3 and GB = Gandhi Bhawan.

Table 7. Products of stepwise regressions between different soil and vegetation variables in off dung (CP) and dung pats (DP) for herbaceous vegetation.

| Off dung pat (CP) | | | | Dung pat (DP) | | | |
|-------------------|----------------------------------|----------------|---------------|---------------|---------------------------|----------------|---------------|
| Models | Regression equations | R ² | P | Models | Regression equations | R ² | P |
| 1 | $SR = -0.58 + 0.31M$ | 0.97 | ≤ 0.0001 | 1 | $SR = 38 - 4.7pH$ | 0.85 | ≤ 0.0001 |
| | | | | 2 | $SR = 44 - 5.7pH + 0.6Ni$ | 0.91 | ≤ 0.0001 |
| 1 | No relation | - | - | 1 | $E = 0.67 + 0.01M$ | 0.46 | ≤ 0.02 |
| 1 | $Sh = 0.15 + 0.02Por$ | 0.85 | ≤ 0.0001 | 1 | $Sh = 22.06 - 2.7pH$ | 0.70 | ≤ 0.001 |
| 1 | $\beta = -0.47 + 0.05Por$ | 0.69 | ≤ 0.001 | 1 | $\beta = 0.69 + 0.05M$ | 0.56 | ≤ 0.005 |
| 2 | $\beta = 4.45 + 0.03Por - 0.05P$ | 0.81 | ≤ 0.001 | | | | |
| 1 | $B = -11.07 + 7.0M$ | 0.82 | ≤ 0.0001 | 1 | $B = 5.87 + 4.32M$ | 0.97 | ≤ 0.0001 |

In the equations, *S*, *E*, *Sh*, β , *B*, *M*, *Por*, *P*, *pH* and *Ni* represent species number, evenness, Shannon index, beta diversity, biomass, soil moisture, soil porosity, soil phosphorus, soil pH and soil nitrate nitrogen, respectively.

Table 8. Summary of ANOVA on herbaceous species number and biomass of different trait categories.

| Sources | Dependent variables | Df | F | |
|------------------------------|---------------------|-----|--------------------|--------------------|
| | | | Species | Biomass |
| Trait | Life form | 2 | 525*** | 324*** |
| | Growth form | 3 | 303*** | 97*** |
| | Life span | 2 | 172*** | 105*** |
| | Height | 2 | 40*** | 34*** |
| | Nativity | 2 | 664*** | 339*** |
| | N-fixing ability | 1 | 231*** | 179*** |
| Location | Life form | 11 | 4.20*** | 4.71*** |
| | Growth form | 11 | 4.59*** | 2.97** |
| | Life span | 11 | 4.19*** | 4.77*** |
| | Height | 11 | 4.17*** | 3.59*** |
| | Nativity | 11 | 4.60*** | 5.32*** |
| | N-fixing ability | 11 | 3.02** | 2.41* |
| Treatment | Life form | 1 | 365*** | 236*** |
| | Growth form | 1 | 425*** | 143*** |
| | Life span | 1 | 299*** | 203*** |
| | Height | 1 | 364*** | 144*** |
| | Nativity | 1 | 427*** | 244*** |
| | N-fixing ability | 1 | 92*** | 70*** |
| Trait × Location | Life form | 22 | 7.15*** | 5.16*** |
| | Growth form | 33 | 6.90*** | 3.74*** |
| | Life span | 22 | 2.73*** | 2.41** |
| | Height | 22 | 10.85*** | 6.01*** |
| | Nativity | 22 | 6.00*** | 8.04*** |
| | N-fixing ability | 11 | 2.32* | 1.45 ^{NS} |
| Location × Treatment | Life form | 11 | 1.69 ^{NS} | 1.86* |
| | Growth form | 11 | 1.75 ^{NS} | 1.44 ^{NS} |
| | Life span | 11 | 1.94* | 2.49** |
| | Height | 11 | 1.82* | 1.31 ^{NS} |
| | Nativity | 11 | 2.00* | 2.28* |
| | N-fixing activity | 11 | 1.26 ^{NS} | 0.95 ^{NS} |
| Trait × Treatment | Life form | 2 | 111*** | 71*** |
| | Growth form | 3 | 97*** | 32*** |
| | Life span | 2 | 49*** | 29*** |
| | Height | 2 | 5.96** | 9.01*** |
| | Nativity | 2 | 270*** | 146*** |
| | N-fixing activity | 1 | 77*** | 49*** |
| Trait × Location × Treatment | Life form | 22 | 4.11*** | 2.63*** |
| | Growth form | 33 | 5.34*** | 2.42*** |
| | Life span | 22 | 1.97* | 0.86 ^{NS} |
| | Height | 22 | 4.48*** | 3.00*** |
| | Nativity | 22 | 2.39** | 2.59*** |
| | N-fixing activity | 11 | 1.90* | 1.64 ^{NS} |
| Error | Life form | 143 | | |
| | Growth form | 192 | | |
| | Life span | 143 | | |
| | Height | 144 | | |
| | Nativity | 144 | | |
| | N-fixing activity | 96 | | |

* = P≤0.01, ** = P≤0.001, *** = P≤0.0001 and ^{NS} = non-significant.

Table 9. Mean species number (per m²) and biomass (g DM/m²) (\pm s.e.) of plants with different functional traits in dung pat (DP) and off dung pat (CP) locations.

| Plant functional attribute | Trait | Off dung pats | | Dung pats | | % Increase/decrease | |
|----------------------------|----------------------|-----------------|------------------|------------------|-----------------|---------------------|---------|
| | | Species | Biomass | Species | Biomass | Species | Biomass |
| Life form | Grasses | 1.00b (0.12) | 6.19b (0.75) | 3.93b (0.28) | 24.10b (2) | 293 | 289 |
| | Sedges | 0.12a (0.06) | 0.61a (0.31) | 0.26a (0.11) | 1.13a (0.53) | 117 | 85 |
| | Forbs | 3.30c (0.21) | 16.00c (1.31) | 9.26c (0.55) | 44.85c (2) | 181 | 180 |
| Growth form | Erect | 2.12b (0.20) | 11.00b (1.25) | 8.00c (0.47) | 37.50c (3) | 277 | 241 |
| | Prostrate | 1.58b (0.19) | 7.09b (1.00) | 3.73b (0.24) | 22.00b (2) | 136 | 210 |
| | Procumbent | 0.47a (0.10) | 2.78a (0.62) | 1.08a (0.16) | 8.08a (1) | 130 | 191 |
| | Decumbent | 0.25a (0.07) | 1.93a (0.33) | 1.58a (0.15) | 3.50ab (1) | 132 | 81 |
| Life span | Annual | 1.81b (0.21) | 9.80b (1.21) | 7.19c (0.38) | 35.08b (2) | 297 | 257 |
| | Biennial | 0.36a (0.10) | 1.97a (0.61) | 0.80a (0.16) | 4.00a (1) | 122 | 103 |
| | Perennial | 2.25b (0.22) | 11.00b (1.29) | 5.40b (0.26) | 31.00b (2) | 140 | 182 |
| Height | Tall | 2.08c (0.20) | 10.80b (1.31) | 7.60b (0.54) | 45.00b (4) | 265 | 317 |
| | Medium | 0.97a (0.11) | 5.00a (0.9) | 3.02a (0.24) | 19.02a (1) | 211 | 280 |
| | Short | 1.37b (0.13) | 7.00a (1.20) | 2.77a (0.38) | 15.06a (2) | 102 | 115 |
| Nativity | Native | 0.94a (0.14) | 4.55a (0.72) | 1.42a (0.22) | 10.50a (1) | 51 | 131 |
| | Non-native | 2.90b (0.26) | 14.72b (1.40) | 11.00b (0.50) | 54.00b (4) | 279 | 267 |
| | Cosmopolitan | 0.58a (0.12) | 3.53a (0.76) | 0.97a (0.10) | 7.30a (2) | 67 | 107 |
| N-fixing activity | Leguminous forbs | 0.81a (0.12) | 6.00a (0.66) | 0.36a (0.37) | 4.90a (2) | -56 | -17 |
| | Non-leguminous forbs | 2.49b (0.18) | 10.00b (1.27) | 8.64b (0.58) | 39.95b (3) | 247 | 300 |

Within parameters, means within columns followed by different letters are significantly different ($P \leq 0.05$).

Discussion

Soil properties

While ruminants depend on grasslands, they control their structure and cycling of energy and nutrients (Augustine and McNaughton 1998) through foraging, trampling and dung and urine inputs, which are important sources of moisture and nutrients for plant establishment and growth on drier locations (Bakker et al. 2004; Williams and Haynes 2006). The greater soil moisture and porosity in DPs compared with CPs in this study may be due to

increased physical mixing of soil by micro- and macro-organisms (dung beetles, earthworms, termites, bacteria and fungi) in DPs (Lovell and Jarvis 1996; Williams and Haynes 2006). The study suggests that dung inputs to the soil by ruminants may improve physical properties, water infiltration and water holding capacity of the soil (Brouwer and Powell 1998).

The lower soil pH in DPs than in CPs may be due to the release of carbonic acids during decomposition of dung residues in the presence of adequate soil moisture and temperature (Rao et al. 2009; Verma et al. 2013). Williams and Haynes (2006) suggest that dung with suf-

ficient soil moisture absorbs greater solar heat, which raises the temperature of underlying soil, and accelerates the decomposition process of dung residue (Stanford et al. 1973), resulting in increased release of carbonic acids. Other studies have also suggested that application of ruminant manure to acidic soils increases the level of acidity (Bussink and Oenema 1998; Whalen et al. 2000).

The negative relationships of C:N ratio with $\text{NH}_4^+\text{-N}$, $\text{NO}_3^-\text{-N}$, mineral-N, total-N and total-C suggested greater rates of N and C mineralization in DPs compared with CPs. High levels of nutrients in dung residue (undigested food with lignified plant tissues and cell wall, gut microorganisms, secretions and cellular debris from the gut mucosa; Church 1969) might have determined the rates of C- and N-mineralization. It has been suggested that organic matter rich in N (low C:N ratio) has greater C- and N-mineralization than organic matter with low N concentration (Vourlitis et al. 2007; Rao et al. 2009; Verma et al. 2013). In temperate grazed grassland, the return of animal dung can contribute 260 kg N/ha (Schnyder et al. 2010) and 22,500 kg C/ha (Whitehead 1986) and in intensively managed systems may boost concentrations of these elements to a much higher level than in unmanaged grasslands (Lovell and Jarvis 1996). In the present study, the total-N (232 kg/ha) and total-C (6,860 kg/ha) contributed by ruminant dung residue to the soil were lower than the values reported by Schnyder et al. (2010) for N and by Whitehead (1986) for C. It reflected lower nutrient mineralization and C:N ratio in the tropics than in temperate grasslands. Differences in forage quality in the 2 different climates could explain the differences.

Composition, diversity and biomass of plant functional traits

Based on the diversity indices and *k*-dominance analyses, the study showed comparatively higher species diversity in DPs than in CPs. In different situations, other studies have also emphasized that diversity can be unequivocally compared only when the *k*-dominance plots from the locations to be compared do not overlap. In this circumstance, the lowest line will correspond to the most diverse community and the uppermost line will represent the least diversity (Sagar and Singh 2005; Sagar et al. 2012; Verma et al. 2013). This pattern can be explained because of cumulative effects of 2 mechanisms: (1) grazers might have added viable seeds of grasses, forbs and woody species via their digestive tracts to the soil; and (2) the dung might have provided sufficient moisture and nutrients for the germination and establishment of the deposited as well as remaining

seeds at the respective microsites. Seeds of grasses, forbs and woody species can remain viable even after passing through the digestive tracts of ruminants (Thomson et al. 1990; Gardener et al. 1993). It appears that the dung residue created favorable environmental conditions to the microsites for the germination of seeds and subsequent seedling establishment due to increased soil fertility, increased water holding capacity and reduced competition with existing species (Ocumpaugh et al. 1996). Further, nutrients from dung residue may either suppress or destroy some existing species and create gaps and resource availability for other species (Watt 1947; Coffin and Lauenroth 1988). Our experience and observations indicate that livestock preferentially graze areas with no dung in a pasture and avoid pasture adjacent to dung pats. Consequently, areas where dung is deposited carry a much higher biomass of pasture because of differential grazing pressure on dung and non-dung areas. Therefore, the apparent difference between the two plots (DPs and CPs) would over-estimate the increase in growth as a result of dung deposition.

Soil pH is an important attribute affecting species diversity because of its relationship with the availability of nutrients and toxic elements (Pausas and Austin 2001). In unmanaged grassland, Grime (1973) reported maximum species diversity at a range of soil pH of 6.1–6.5; species diversity declined as soils became more acidic or alkaline because few species were adapted to highly acidic or alkaline soils. Both low and high soil pH and nutrients can limit seed germination and plant performance (Van den Berg et al. 2005). In this alkaline soil, dung residue lowered soil pH and resulted in the accumulation of a larger number of species. Evidently, the negative relationships between soil pH and the parameters of species diversity in DPs promoted species diversity due to decreased soil pH as reported by Verma et al. (2013) in a nitrogen-amendment experiment.

In an N-deposition study, Lauenroth et al. (1978) reported variation in community structure mainly due to changes in several dominant groups. While factors like rooting depth, N-use efficiency and association with mycorrhizae can affect responses of plants to changed nutrient conditions (Ren et al. 2011), soil water and annual rainfall are vital factors which can interact with N to influence ecosystem functioning (Chen et al. 2011). When water and N were added separately to shortgrass steppe in North America, above-ground biomass increased by 250 and 100%, respectively, but the increase was 700% when water and N were added together (Lauenroth et al. 2008). In the present study, dung deposits increased the tall, erect, annual, non-native and non-leguminous forbs. This is not surprising as the native vegetation would

have evolved largely in the absence of additional nutrients. It has been reported that weedy and ruderal species are successful invaders in N-rich environments (Sharma et al. 2005; Gaertner et al. 2012); hence they dominated in DPs compared with CPs. According to Diekmann and Falkengren-Grerup (2002), tall species are typically favored by N-deposition at the cost of short species, and can overgrow and shade the short-statured species. Short-statured species are excluded due to light limitation (Stevens et al. 2006). In this study, most forbs were erect in growth habit and hence, adapted to compete for light, which may be a reason for the greater increment in species number and biomass of forbs in DPs than CPs. Nevertheless, N-fixing species normally compete for light less effectively than non-N-fixing species (Haynes 1980). Thus, the study suggests that the natural attributes of forbs allowed them to take advantage of the higher nutrient levels due to dung deposition. Similarly, with the help of a meta-analysis including data from 304 studies and 456 terrestrial plant species, Xia and Wan (2008) also reported 54% increase in the herbaceous biomass due to fertilizing with N.

Overall, the study revealed that the seasonally dry tropical grasslands, which experience relatively high soil pH and low soil moisture and nutrients, benefit from ruminant dung deposition, through reduction in soil pH, and increase in soil moisture and nutrients. These conditions favored seed germination and seedling establishment of opportunistic plants, which led to increased diversity and biomass of herbaceous species in the dry tropical pasture studies. While it is well known that application of ruminant dung can benefit a pasture by increasing dry matter yields, this study has shown that the species composition in available forage can be changed as well, which can also affect nutritional value, depending on the species' palatability. It is important to return dung to pastures or croplands, where animals are housed or placed in corrals at night, to ensure the sustainable use of the pastures/grasslands. The study suggested that dung could be a substitute for chemical fertilizers to increase soil nutrients and herbaceous species diversity. However, further study of diversity-productivity relationships is vital before a clear understanding of full benefits of fertilizing with dung is available to make recommendations for the sustainable management of seasonally dry tropical ecosystems.

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