

FORAGE PRODUCTION AND DIVERSIFICATION FOR
CLIMATE-SMART TROPICAL AND TEMPERATE
SILVOPASTURES

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TROPICAL AND TEMPERATE SILVOPASTURES

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Dedicado a las comunidades de Pedasí y Los Asientos, Panamá.

Sin sus esfuerzos cotidianos nada hubiera sido posible.

Que los arboles crezcan, las lluvias vengan,
y que un medio ambiente sano alimente a todos.

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FORAGE PRODUCTION AND DIVERSIFICATION FOR CLIMATE-SMART TROPICAL AND TEMPERATE SILVOPASTURES

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ABSTRACT

Climate change creates much uncertainty for the future of animal agriculture, particularly due to an increase in summer droughts that result in the loss of range productivity. Silvopasture, the intentional integration of livestock, trees, and pasture on the same unit of land, is a promising option to diversify forage resources, compensate for losses caused by droughts during the summer forage gap, and in some cases, even increase agricultural production while maximizing conservation benefits. We examined three distinct silvopastures, two in the country of Panama and one in the state of Missouri, to better understand how the integration of woody perennial trees and shrubs affects forage productivity, availability, and nutritional value, particularly during the driest months. In Panama, we studied both recently established and mature silvopastures with the objective of assessing soil physical and chemical properties, plant water availability, and pasture dry matter production in different successional stages. In the mature silvopasture, we found that annual grass accumulation was greatest in open pastures, but was highest in silvopastures with moderately-spaced trees (~500 trees ha⁻¹) early in the dry season. In the recently established silvopasture, the simultaneous growth of grasses, trees, and fertilizer shrubs resulted in significant increases in soil fertility and marginally significant increases in grass production in plots that included the shrub *Leucaena leucocephala*. Further, cumulative forage production in plots with shrubs provided on average 9.66% more forage than plots without shrubs during the dry season. In Missouri, we assessed the feasibility of integrating the potential alternative forage shrub red mulberry (*Morus rubra* L.) into the understory of an existing silvopasture and found that seedling survival, growth, leaf productivity, and nutritive value could be optimized at an overstory planting density of ~123 trees ha⁻¹. High leaf mineral content and low fiber fractions during the late summer forage slump suggest that this species could serve as an effective supplemental forage to livestock in multi-strata silvopastures during drought conditions. Results of all three studies confirm that silvopasture has both competitive and facilitative influences on forage production, and that facilitative factors may be more prevalent during extreme drought.

Chapter 1

Silvopasture: How Raising Livestock with Trees Enhances Food Security in a Changing Climate

Abstract

Globally, the human population is exploding and is expected to reach 9.1 billion by 2050, resulting in a rapidly growing demand for animal products and continued natural resource degradation, all of which have profound effects on food security. This chapter draws on examples from across the globe to highlight the many contributions of silvopasture – the intensively managed integration of trees, forages, and livestock – to achieving food security. We focus on the production of forage, meat, and milk in silvopastoral systems as direct indicators of food supply as well as indirect indicators such as thermal stress in livestock, animal health, and habitat provisioning for pollinators. We address the problems of modern animal agriculture and how silvopasture may play a critical role in the sustainable intensification of livestock production systems. We then point out some important research needs and provide a rationale and brief outline for the major objectives of the three studies presented in this dissertation.

Key words: Silvopasture, agroforestry, food security, forage production, animal performance, animal welfare

Introduction

The Food-Climate Crisis

Agroforestry is often praised for the many environmental benefits it provides, such as carbon sequestration, enhancement of wildlife habitat, and reduction of toxic runoff into waterways. However, there remains an important and often overlooked consequence of the ecosystem services provided by agroforestry: food security. In a time when monocultures and chemicals of conventional agriculture prevail, there is growing concern about the future of food production, particularly in regards to soil loss and degradation, indiscriminate use of agrochemicals and their effects on native plants and pollinators, and the environmental and ethical abuses of industrial animal agriculture. Globally, the human population is rapidly increasing and is expected to reach 9.8 billion by 2050, urbanization is increasing, and incomes are rising. This has resulted in a rapidly growing demand for animal products and continued natural resource degradation, all of which have profound effects on food security (Delgado et al. 1999).

Although global grain production has more than doubled and global meat production has more than tripled over the last half-century (FAOSTAT 2010), food yield may need to increase by 50% or more in the next half century to keep up with demands (Godfray et al. 2010). Projected demands of meat and milk production are expected to grow at rates of 2.8 and 3.2% annually up to 2020 (Delgado et al. 1999). All the while, food producers are experiencing greater competition for land, water, and energy. Climate change is exacerbating consequences for animal production through its effects on forage and range productivity and heat related stress on the animal. Under climate change scenarios, water will become the main limiting factor to all livestock systems (Steinfeld

et al. 2006; de Fraiture et al. 2010) and extended droughts will become the norm. In the face of climate change, producing more food for a growing population while diminishing poverty and hunger is a daunting task, but a challenge that must be heeded. An even greater challenge is not only to increase productivity, but to do so while treading more lightly on the land (Cribb 2010).

Sustainable Livestock Production

Many decades of research have demonstrated that livestock management is critical for maintaining healthy pastures and optimal productivity (Gerrish 2004; Rayburn 2007). In 1959, farmer and scientist André Voisin coined the term *Rational Grazing* (Voison 1988), where he described the basic guidelines necessary for good grazing management: short periods of occupation followed by an ample recovery period. More recently, authors have built on these management guidelines with the introduction of terms such as *prescribed grazing* (USDA - NRCS 2010), *management intensive grazing* (MiG) (Gerrish 2004), *holistic planned grazing* (HPG), and *mob grazing* (Savory and Butterfield 2016). All of these terms apply the same key grazing principles proposed by Voisin, ultimately favoring important pasture species, improving soil health, and increasing forage productivity and nutritional quality (Flack 2016).

These sustainable livestock production methods can be implemented in open pasture or alternatively under dispersed tree cover in a silvopastoral setting. Silvopasture is an agroforestry practice where trees and livestock are combined with improved pasture plants and managed intensively, effectively integrating intensive animal husbandry, silviculture, and forage agronomy practices (Sharrow et al. 2009). The simultaneous

production of timber and livestock can increase the diversity of on-farm products, improve land-use efficiency, and provide better welfare for animals (Murgueitio et al. 2011; Calle et al. 2012b; Broom et al. 2013). Despite numerous accounts of silvopasture's ability to strike optimal balance between production and conservation (Ibrahim et al. 2010; Galindo et al. 2013; Jose et al. 2017), many producers remain skeptical, arguing that forage and animal productivity is too greatly reduced under tree cover (Fischer and Vasseur 2002; Garen et al. 2011).

In this paper, we review a number of studies from various regions of the world that highlight silvopasture's contribution to achieving food security. We focus on the production of forage, meat, and milk in silvopastoral systems as direct indicators of food supply as well as indirect indicators such as thermal stress in livestock, animal health, and habitat provisioning for pollinators. We conclude by addressing some of the problems of modern animal agriculture, why food security depends on it, and how silvopasture may play a critical role in the sustainable intensification of livestock production systems.

Silvopasture: A Contribution to Food Security

Forage Dry Matter Production

It is well-established that trees have both competitive (negative) and facilitative (positive) effects on other organisms around them (Jose et al. 2004; Jose et al. 2019). Canopy solar interception results in lower light transmittance, decreasing the photosynthetic rate of understory vegetation. Trees have been shown to compete vigorously for water and nutrients and can even emit allelopathic chemicals that impede the growth of surrounding vegetation. However, canopy interception can also provide

protection from desiccating winds, reduce soil surface temperature and soil evapotranspiration (Belsky et al. 1989; Belesky 2005), which can increase overall soil moisture content (Vetaas 1992). Some trees can fix atmospheric nitrogen and provide up to 650 kg N yr⁻¹, more than enough to fulfill crop N needs for sustained yield (Nygren et al. 2012). Leaf litter under trees has been shown to improve the physical properties of the surface soil, increasing soil nutrients and organic matter (Belsky 1994). As a result, the content of carbon, phosphorus, and nitrogen has been shown to gradually decline as a function from the distance of the trunk, resulting in significantly lower levels in the open ground than in sub-canopy soil (Belsky et al. 1989; Tiedemann and Klemmedson 2008).

Elevated nutrient levels can improve the forage quality of sub-canopy grasses, attracting grazers that return nutrients to the soil. This, combined with the trapping of wind and water-borne sediments by trees can contribute to an 'island of fertility' effect (Belsky et al. 1989; Dohn et al. 2013). Trees roots can also decrease the bulk density of the soil, creating the macro-porosity favorable to the infiltration of water, increasing water-holding capacity (Malmer et al. 2010). These benefits, combined with the selection of appropriate tree and forage species, can sometimes result in increased levels of productivity when compared with grass monocultures.

Tree canopy effects on the growth and nutritive value of understory forages depend on many factors, including forage type, local climate and topography, season, soil fertility and structure, and amount of photosynthetically active radiation (PAR). It is well known that shading has a more detrimental effect on warm-season (C4) grasses than it does on cool-season (C3) grasses (Kephart and Buxton 1993; Lin et al. 1998; Buergler et al. 2005). This is because the physiology of C4 grasses allows for greater biomass

accumulation per unit of photosynthetically active radiation (PAR) – or radiation use efficiency – than does the physiology of C3 species. The amount of rainfall appears to be important in determining forage production under shade. In xeric environments where water is the limiting factor, growth and development of many herbaceous species are facilitated by tree canopies through the improvement in moisture regimes (Joffre and Serge 1993), soil nutrients, and organic matter (Kellman 1979)). Several studies have demonstrated that under certain conditions, moderate shading can provide the optimal environment for grass growth and quality (Belsky 1994; Ibrahim et al. 2007; DeBruyne et al. 2011; Orefice et al. 2016). Hernández and Guenni (2008) concluded that *M. maximus* grasses benefited from a compensatory effect from trees that increased soil humidity and improved total forage biomass. Andrade et al. (2004) found that *M. maximus var. Massai* grass growing under artificial shade reached its highest dry matter accumulation rate under 30% shade cover in both the rainy and dry seasons. Moustakas et al. (2013) demonstrated that tree effects on grass biomass across a precipitation gradient in a subtropical savanna were facilitative in drier sites, with greater grass biomass observed beneath tree canopies than outside.

Conversely, many studies in temperate environments with more rainfall have shown that canopy coverage either maintains (DeBruyne et al. 2011) or reduces the quantity of understory forage produced (Feldhake et al. 2010; Orefice et al. 2016). In a study conducted in Appalachia, Neel and Belesky (2015) showed that hardwood silvopasture DM production was 60-70% that of open pasture in the spring and equal to only 40-60% of it in summer. Studying an alder and willow silvopasture in New Zealand,

Devkota and colleagues (2001) concluded that a 40 – 50% canopy closure would maintain pasture production at approximately two-thirds of that in unshaded pasture.

However, research in both temperate and tropical environments has suggested that silvopasture may extend forage longevity and provide more forage than conventional pastures during certain times of the year. Kallenbach (2009) compared the growth of cool season grasses in traditional open pastures to that of integrated pastures where silvopasture was used on only 25% of the total land area. Forage growth on integrated pastures outperformed that of traditional pastures early in the spring, mid-summer, and late-fall, all times when cool season grasses likely benefit from more moderate micro-

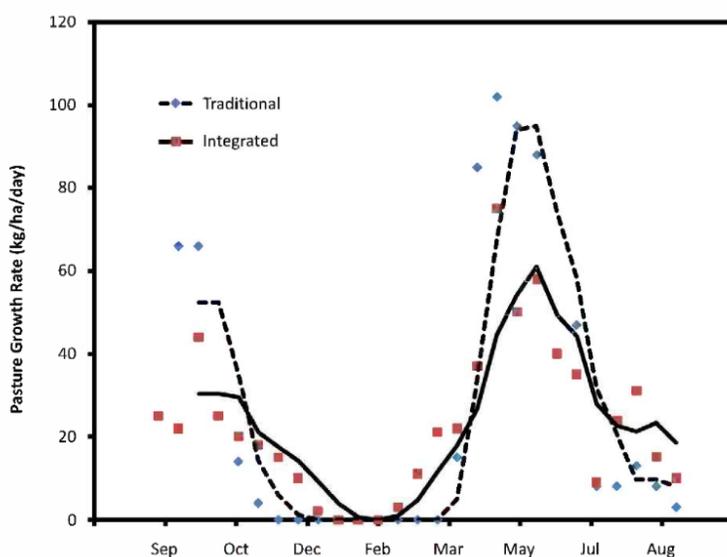


Fig. 1 Pasture growth rates in traditional (open) and integrated (25% of land area under silvopasture) pasture systems at the Horticulture and Agroforestry Research Center near New Franklin, MO. Source: (Kallenbach 2009)

climates in the understory (Fig. 1). Similarly, a study examining the growth of *M. maximus* in the understory of native tree plantations in Panama found that forage dry

matter (DM) accumulation was greatest under moderate tree coverage early in the dry season but produced more dry matter in open environments throughout the rainy season (Dibala and Jose, in preparation). These studies indicate that producers may achieve maximum gains by integrating silvopastures into larger open pasture operations and using them only during periods of relative scarcity.

Forage Nutritive Value

A large body of research indicates that forage nutritive value may increase when grown under tree canopies (Lin et al. 1998, 2001; Buergler et al. 2006; Feldhake et al. 2010; Neel and Belesky 2017; Orefice et al. 2017; Pang et al. 2019 a and b). Specifically, increases in crude protein (CP) content are commonly observed. This is likely due to adaptive mechanisms and changes in plant physiology such as elongation of the cell wall (Kephart and Buxton 1993) and increases in the specific leaf area and shoot:root ratio (Paciullo et al. 2017). The presence of nitrogen-fixing trees may also indirectly increase crude protein (CP) levels in forages through leaf decomposition, root exudation, and direct nutrient exchange (Sierra and Nygren 2006; Sierra et al. 2007; Jalonen et al. 2009a). Xavier and colleagues (2014) found that nitrogen recycled via the litter pathway in a silvopastoral system exceeded that in a monoculture by 34 kg ha⁻¹, concluding that the extra N recycled in the system – along with biological nitrogen fixation – would confer increases in quality and longevity of forage when compared to grass monocultures.

Typically, the structural carbohydrate metrics acid detergent fiber (ADF) and neutral detergent fiber (NDF) are either increased or unaffected by shade for most forage

species (Ladyman et al. 2003; Kallenbach et al. 2006; Sousa et al. 2010; Paciullo et al. 2011; Neel and Belesky 2015). However, there are a number of studies that report decreasing values with increased levels of shade (Kephart and Buxton 1993; Obispo et al. 2008; Medinilla-Salinas et al. 2013), indicating lower levels of lignification and overall higher digestibility.

It is well known that the nutritive value of a plant changes throughout its different stages of maturity, containing greater contents of total non-structural carbohydrates (TNC) in the early stages of growth and developing larger quantities of lignin and cellulose later in the season. This increase in lignification reduces digestibility and palatability of the plant, resulting in decreased animal intake. Thus, it is important for producers to manage livestock dynamically in response to temporal changes in both the quantity and quality of forages.

Tree Fodder Production and Nutritive Value

One way producers can respond to these changes is to rely on trees and shrubs to provide alternative and highly nutritious forage sources during critical periods. In the tropics, forage shrubs (also known as fodder shrubs) can be a strategic resource for farmers during the worst drought periods that often occur during the dry season. Some forage shrubs retain green foliage amidst even the harshest droughts due to their deep root systems that have specialized access to the water table. As the dry season progresses, forage shrubs have been shown to lose nutritive value, digestibility, and palatability at a slower rate than herbaceous forages (Talamuci and Pardini 1999), providing relatively

high quality supplemental forage to both ruminants and non-ruminants during times of scarcity.

A widely touted model of silvopasture that includes the use of native and non-native trees, shrubs, and herbaceous forages is known as intensive silvopasture. Intensive silvopastoral systems (ISS) include the planting of timber trees that are intercropped with high density ($\sim 10,000$ plants ha^{-1}) plantings of fodder shrubs and highly productive pasture grasses in a system that can be directly grazed by livestock (Murgueitio et al. 2011). Shrubs are periodically coppiced to encourage low, dense growth of the foliage. Cattle are provided permanent supplies of drinking water and rotated periodically with the use of electric fences to prevent overgrazing and to allow time for pastures to recover. ISS first began in Australia more than 40 years ago, but it is now becoming the technology of choice in Colombian and regional livestock sectors because they can help reduce the seasonality of plant and animal production, and therefore help to mitigate and adapt to climate change (Cardona et al. 2013).

There is compelling evidence that demonstrates its efficacy. An ISS using the shrubs *Leucaena leucocephala* and *Gliricidia sepium* combined with the grass *M. maximum* in the humid tropics of West Africa produced of over 20 tons of DM ha^{-1} of mixed tree-grass fodder (Atta-Krah and Reynolds 1989). Bacab-Pérez and Solorio-Sánchez (2011) compared forage availability and voluntary intake on two ISS ranches with a conventional ranch in Michoacán, Mexico, and found that the available forage in both ISS ranches was at least 2.6 times higher than that in the traditional ranch (17,290 and 18,851 versus 6,636 kg DM yr^{-1}). Furthermore, only 9% of the available *Leucaena* forage was rejected by cattle on both ISS farms (Table 1). Shelton and Dalzell (2007)

reported that *Leucaena*-grass pastures are the most productive, profitable, and sustainable beef production systems in northern Australia.

Table 1 Forage availability, refusal, and utilization efficiency at three farms in Michoacán, Mexico.

Farm	Forage	Edible forage (kg DM/Ha)	Rejection (kg DM/Ha)	Use (kg DM/Ha)	Use (%)
Los Huarinches	<i>L. leucocephala</i>	8,386	826	7,560	91
	<i>M. ximum</i>	8,904	4,655	4,249	48
	Total	17,290	5,481	11,809	68
El Aviador	<i>L. leucocephala</i>	9,156	826	8,330	91
	<i>M. ximum</i>	9,695	3,542	6,153	63
	Total	18,851	4,368	14,483	77
Conventional	<i>C. plectostachyus</i>	6,636	2,660	3,976	60

Source: Cardona et al. (2013); Originally modified from Bacab-Peréz and Solorio-Sánchez (2011)

The use of woody trees and shrubs for livestock fodder in temperate regions has been limited primarily due to a relatively limited plant selection and existing cultural and behavioral norms. Temperate regions lack the diversity of nutritious, nitrogen fixing woody plants capable of coppicing that exist in the tropics and trees that produce palatable fodder only do so during the growing season when highly preferred herbaceous forage is available, unless compromised by extreme weather. Cultural norms such as stockpiling and hay-baling are used instead of the cut-and-carry systems more commonplace in the tropics. However, researchers in temperate regions have explored the production and intake of densely planted forage shrubs and some species have shown particular promise. In North Carolina, black locust (*Robinia pseudoacacia*) fodder banks were highly preferred by meat goats with a mean dry herbage yield of 3,213 kg ha⁻¹ when

planted on a 50 cm spacing and coppiced at 50 cm (Addlestone et al. 1999). In New Zealand, full access to willow (*Salix* spp.) fodder banks was beneficial for ewe reproductive rates (Pitta et al. 2005). Other promising species for temperate intensive silvopastures include *Paulownia* (Mueller et al. 2001) and mulberry (*Morus* spp.) (Sánchez 2000).

Silvopastoral systems containing forage shrubs are effective at improving animal production because tree foliage is often of higher nutritional quality than grasses (Mueller et al. 2001). Sosa Rubio and others (2004) analyzed the nutritive value of 30 perennial woody species and found that 70% of them contained CP values of 12% or more. Studies in Greece have shown that the most common native woody plants have higher CP than grasses and forbs during the summer (Papachristou and Papanastasis 1994). In the case of tropical legumes, even the seeds are browsed, which can provide nutrients in excess of that required for digestion and metabolism, potentially correcting nutritional deficiencies in mature roughage (Aganga and Tshwenyane 2003).

The overall nutritive value of woody perennial forage often can be hindered by the presence of anti-nutritional compounds that have the ability to severely restrict nutrient utilization (Papanastasis et al. 2008). Secondary compounds such as condensed tannins, alkaloids, saponins, and oxalates are known to occur in many woody perennials and can have detrimental effects to the animal if consumed in high quantities. However, diets containing herbaceous forage with a high level of digestible CP have been shown to counteract the negative effects of tannins (Yiakoulaki 1995). Furthermore, tannins in low to moderate concentrations (20-40 g kg⁻¹ DM) can induce beneficial effects, which are associated with suppression of bloat in ruminants (Jones et al. 1973).

Tree Fruit Production

The more obvious food product of perennial trees and shrubs is fruit. In 1929, author J. Russell Smith exposed the masses to the agricultural wealth of trees in his seminal work *Tree Crops: A Permanent Agriculture* (Smith 1950). In this masterpiece, Smith expounds on the overlooked abundance of food for both humans and animals produced by woody perennials. He describes the fruiting patterns and yields of common trees like oak (*Quercus* spp.), hickory and pecan (*Carya* spp.), walnut (*Juglans* spp.), chestnut (*Castanea* spp.), persimmon (*Diospyros* spp.), carob (*Ceratonia siliqua*), mulberry (*Morus* spp.), and honeylocust (*Gliditsia triacanthos*). A litany of anecdotes from producers pepper the pages of this book, with statements like:

“I never weighed my pigs at the beginning and close of the mulberry season, but I think I can safely say that a pig weighing 100 pounds at the start would weight 200 pounds at the close” and,

“I let the cattle pick them (honeylocust pods) up where they can; and where they cannot graze, the beans are gathered and fed to them. My herd of heifers get a great part of their winter pasture from the honeylocust pods.”

Since then, many accounts like these have been corroborated with empirical evidence. Gold and Hanover (1993) noted that the edible seedpods from honeylocust trees can serve as supplemental feed for livestock over several months in autumn and winter when cool-season grass production is limited or negligible. In Virginia, whole-

ground honeylocust seedpods from the ‘Millwood’ cultivar had a nutritional profile comparable to that of ground whole-ear dent corn (*Zea mays* L.) or oat (*Avena sativa* L.) grain (Johnson et al. 2013). In that same study, mean DM yields of pod-bearing trees were 15.8, 4.8, and 14.7 kg tree⁻¹ in 2008, 2009, and 2010, respectively. In good years, a honeylocust crop can easily exceed 66 kg of cleaned seed per tree (Gold and Hanover 1993).

In the Mediterranean oak woodland known as the *dehesa*, Iberian pigs are raised extensively on acorns and grass during a two-month fattening period that coincides with the fruiting period of surrounding holm oak (*Quercus ilex* Lam. spp. *ballota*) and cork oak (*Quercus ilex* L.). In the managed *dehesa*, where mean tree density ranges between 30 and 50 trees ha⁻¹, the productivity of acorns is reported to be ten times higher than a dense *Quercus ilex* forest (Pulido 1999; Pulido et al. 2001). Although extremely variable, mean acorn yield was estimated to be 300 - 700 kg ha⁻¹; with yields of 8-14 kg tree⁻¹ for *Q. ilex* and 5-10 kg tree⁻¹ for *Q. suber* (Rodríguez-Estévez et al. 2007). Individual pigs can consume 7 – 10 kg of acorns day⁻¹, and generally will increase their weight from 100 to 160 kg (Nieto et al. 2002).

In Southeast Asia, livestock is raised under commercially important tree crops like coconut (*Cocos nucifera*), palm oil (*Elaeis guineensis*), and rubber (*Hevea brasiliensis*) on an estimated 210 million ha (Alexandratos 1995). The establishment of mixed pastures under coconuts in Sri Lanka resulted in increases of 17% and 11%, in nut and copra yields, respectively (Liyanage et al. 1993). Moreover, the nutrients from 73 kg of fresh manure and 30 l of urine palm⁻¹ year⁻¹ reduced the cost of fertilizing the coconuts by 69% (Devendra and Ibrahim 1999). Livestock can also help reduce the cost of weed

maintenance, as is the case with Chee and Faiz (1991), who reported a reduction of 20-40% in weeding costs due to regular grazing by cattle.

Animal Performance

Several important measurements of silvopasture's sustainable contribution to food security are livestock average daily gain (ADG), conception and reproductive rate, and stocking rate (animal units AU ha⁻¹). An increase in any of these metrics can translate into income generation for ranchers. Historically, most studies on silvopastoral systems in temperate regions have demonstrated either decreased or equal animal performance when compared to open pastures (Teklehaimanot et al. 2002; Kallenbach et al. 2006, 2010; Sharrow et al. 2009; Neel, J.P.S. and Belesky 2015). More recently, Pent and Fike (2018) compared average daily gains (ADGs) of lambs in black walnut (*J. nigra*) and honeylocust (*G. triacanthos*) silvopastures with open pasture of stockpiled tall fescue (*Schedonorus arundinaceus*) during the winter in Virginia. During the first three weeks of the trial, lambs did not consume honeylocust pods due to naivety, but after the fourth week, consumption of pods was so high that lamb ADG was significantly greater than that in plots without honeylocust. Future study is needed to determine whether honeylocust supports even greater lamb weight gains when there has been previous exposure to pods and higher quality herbaceous forages are available (Pent and Fike 2018). In a study previously described evaluating integrated silvopastures, Kallenbach (2009) reported that cows in silvopastures lost approximately 10% less weight over winter, reducing the need for supplementation by about 12%. Additionally, cows that

gave birth in the integrated treatment were 12% less likely to experience calving difficulty (Table 2).

Table 2 Performance of cow-calf pairs in a traditional (open) pasture system compared to those in an integrated system where both open and silvopastures were used. Source: (Kallenbach 2009)

Treatment	Cow body weight loss in winter (kg)	Calving difficulty (%)	Calf weaning weight (kg)
Traditional	105	15	270
Integrated	93	3	295
P value	0.02	0.04	<0.01

More examples of silvopasture's positive influence on animal performance can be found from the tropics. A silvopastoral system in Brazil including signal grass (*Brachiaria decumbens*) and the leguminous shrubs *G. sepium* and *Mimosa caesalpiniiifolia* planted at a density of 2500 plants ha⁻¹ produced similar livestock production per unit land area compared with signal grass in monoculture (de M. Costa et al. 2016). When considering additional income and ecosystem services provided by the woody components of the system, this is an important result.

Paciullo et al. (2011) evaluated dairy heifer performance in Brazilian silvopastures planted in *B. decumbens* grass with four species of 105 dispersed mature trees ha⁻¹ and drew comparisons with performance in similar *B. decumbens* open pasture. The authors concluded that a 13% increase in the CP content of *B. decumbens* in silvopasture compared with open pasture was sufficient to increase live weight gain of dairy heifers by 17% during the rainy season (Table 3). They posited that this increase in

annual average gain could contribute to a reduction in the age of first conception and, consequently, of the first calving event.

Another study at the EMBRAPA Dairy Cattle Center in Brazil concluded that Zebu-Friesian heifers grazing in a silvopasture planted in *B. decumbens* accompanied by *Acacia mangium*, *Mimosa artemisiana*, and *Eucalyptus grandis* at a density of 198 trees

Table 3 Average daily gain (g animal⁻¹) and gain per area (kg ha⁻¹), according to rearing systems and experimental year, in the rainy and dry seasons. Source: Paciullo et al. (2011)

Experimental year	Rainy season		Dry season	
	Silvopastoral	Monoculture	Silvopastoral	Monoculture
Average daily gain				
2004/2005	722Aa	624Ba	348ab	387a
2005/2006	647ab	563ab	298b	274b
2006/2007	628Ab	515Bb	420a	352ab
Gain per area				
2004/2005	298Aa	256Ba	88	97
2005/2006	242ab	230ab	75	68
2006/2007	258Ab	211Bb	105	89

Means followed by different letters, for each season of the year, capital in the row and the small in the column, are different ($P < 0.05$) by Tukey test.

ha⁻¹ had significantly greater live weight gain (LWG) five years after system establishment than those grazing *B. decumbens* monocultures (Xavier et al. 2014). Silvopasture-raised cattle averaged annual LWGs of 205 kg head⁻¹ while those in monocultures averaged 177 kg head⁻¹ yr⁻¹. This equates to a 16% increase in silvopasture-raised heifer annual LWG. The total annual animal intake was estimated to be 4.0 Mg DM ha⁻¹ in the silvopasture compared to 3.5 Mg ha⁻¹ in the grass monoculture. Forage DM annual mean in the monoculture was non-significantly greater than that in the silvopasture, but the authors could not determine whether this was due to shading or higher forage intake by heifers in the silvopasture (Fig. 2).

A study on sheep performance in Quintana Roo State, Mexico, analyzed five different feeding rations made up of various percentages of grasses and tree fodders and found that diets consisting of 75% or 100% tree fodder resulted in the greatest weight gains (Sosa Rubio et al. 2004). Similarly, sheep fed *Gliricidia* (Chadhocar and Kantharaju 1980) and *Brosimum alicastrum* leaves (Pérez et al. 1995) gained more weight than sheep grazing grass monocultures. In Bali, Indonesia, the production of a shrub layer creates a three strata forage system that has resulted in an increase in stocking rates by one animal ha^{-1} and an increase in live weight gain by $153 \text{ kg ha}^{-1} \text{ yr}^{-1}$ (Devendra 2012).

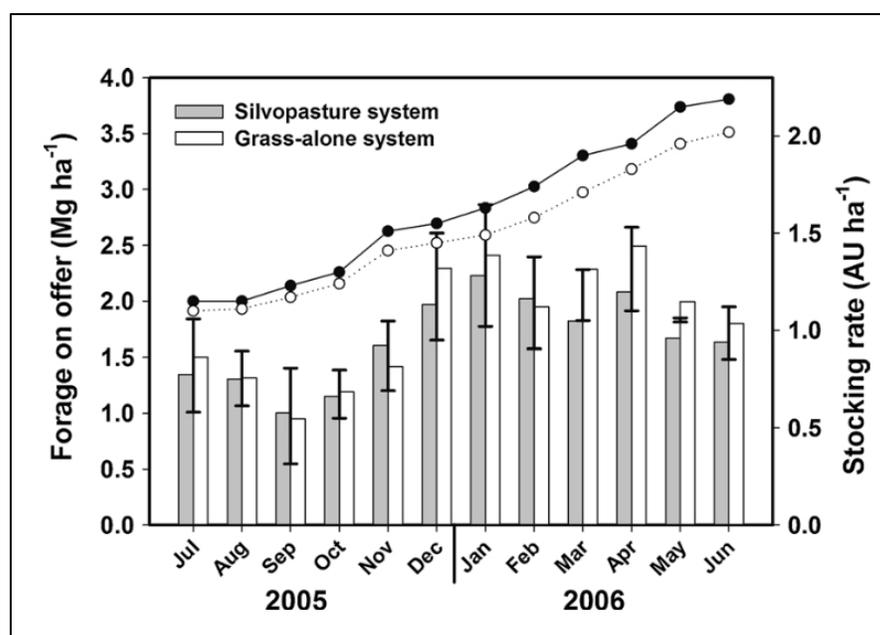


Fig. 2 Total dry matter yield of forage on offer and stocking rate of heifers (animal units ha^{-1}) from July 2005 to June 2006 in the silvopasture system and the *B. decumbens* monoculture. Dark bars and circles represent silvopasture; light bars and circles represent monoculture. Error bars represent least significant differences between means. One animal unit is equivalent to 450 kg of live weight. Source: (Xavier et al. 2014)

Yamamoto and others (2007) used data on herd, milk production, and land use from 74 farms in central Nicaragua to quantify the effects of silvopastoral systems on milk production. The data indicated that silvopastoral areas, especially pasturelands with moderate tree density (tree cover approximately 20%) have significant positive impacts on annual milk production, when overgrazing was avoided. The authors suggested that changing land use from low-density trees with natural pasture (LTNP) to moderate-density trees with conventional pasture (MDTC) using *B. brizantha* could see greatest improvements in production.

Research has shown that when installed and managed effectively, ISS can increase carrying capacity by as much as four-fold per hectare (4.3 heads ha⁻¹), milk production by as much as 130% to 16,000 l ha⁻¹ yr⁻¹, and meat production by as much as ten-fold (Table 4). These gains, largely due to better distributions of biomass throughout the year, have been shown to increase farm income by at least \$440 USD ha⁻¹ year⁻¹ while sustaining long-term system resiliency (Murgueitio et al. 2011; Calle et al. 2012a; Cardona et al. 2013). Meat quality of ISS stock rivals that of those fed in feedlots, in terms of slaughtering weight and age, fat thickness and color, meat color, and marbling score (Dalzell et al. 2006). Additionally, ISS has been shown to completely eliminate the use of chemical fertilizers from operations that once relied on the application of 400 kg urea ha⁻¹ year⁻¹ (Murgueitio et al. 2011).

Table 4 Production parameters of conventional and ISS farming systems in Australia, Mexico and Columbia. Source: (Cardona et al. 2013)

System	Country	Parameter			Reference
		Stocking rate (AU/Ha)	Live weight gain (g/an/d)	Meat production, kg/Ha/yr	
Conventional	Australia	1.5	411	225	Dalzell <i>et al.</i> , 2006
	Mexico	1 to 2.5	500	182-456	Solorio-Sánchez <i>et al.</i> , 2011
	Colombia	1.2	130	56.9	Córdoba <i>et al.</i> , 2010
ISS	Australia	3	822	910	Dalzell <i>et al.</i> , 2006
	Mexico	6	900	1,971	Solorio-Sánchez <i>et al.</i> , 2011
	Colombia	3.5 to 4.7	651-790	827-1,341	Córdoba <i>et al.</i> , 2010
		3.5	793-863	1,013 – 1,103	Mahecha <i>et al.</i> , 2011

Part of the reason productivity is so high in ISS is that they offer a diversity of forages to the animal. Evidence indicates that the contribution of legumes to the ruminant diet results in higher performance on mixed forages compared with those grazing on grass only (Tudsri and Prasanpanich 2001). This may be due to synergistic effects between grasses and roughage within the animal's gut. Carbohydrates are needed to supply energy for rumen microbial activity to efficiently digest and synthesize proteins. Thus, synchronous availability of total non-structural carbohydrates (TNC) and crude protein (CP) has been shown to be critical in the improvement of animal nutrition (Neel and Belesky 2015).

Another way to increase overall system productivity and output of silvopasture is to integrate a variety of livestock, either simultaneously or via the leader-follower grazing system. Manríquez-Mendoza and colleagues (2011) observed significantly greater annual meat production in a mixed-species silvopasture including both cattle and sheep

than for silvopastures grazed by either cattle or sheep. Leader-follower systems can often out-produce other grazing systems for total animal weight gain because each animal tends to consume its optimal foods first (Shepard 2013).

Thermal Stress

Thermal stress has been shown to be responsible for reductions in feed intake, average daily gain (ADG), and milk production in dairy cows and can be caused by changes in air temperature, relative humidity, wind speed, and solar radiation (Kendall et al. 2006). Symptoms of heat stress, such as increased respiration rate and body temperature, begin to occur at 30° C, and shade typically becomes beneficial to livestock when the temperature-humidity index (THI) is over 72 (Blackshaw and Blackshaw 1994). Thermal comfort is especially important for European or mixed European × Zebu cattle breeds, which are more sensitive to the high temperatures of the tropics than pure Zebu breeds (Kendall et al. 2006).

Several studies have shown that trees modify understory micro-climates, creating environments that can mitigate heat stress in animals (Tucker et al. 2008; Karki and Goodman 2015), increasing overall grazing time, average daily weight gain, and reproductive rates (Mitlöhner et al. 2001; Kallenbach 2009; Galindo et al. 2013). In turn, livestock have been shown to modify their behavior in the presence of trees, leading to more consistent and uniform grazing across the landscape (McIlvain and Shoop 1971; Karki and Goodman 2010). Trees can also protect animals against the dangers of extreme cold temperatures. Adequate wind protection from trees in Canada has been shown to reduce the effects of cold by more than half (Webster 1970).

Animal Health

Managed intensive rotational grazing and silvopasture can have direct impacts on animal health, helping to prevent the spread of parasites and disease. One of the most economically damaging and widespread ectoparasites affecting livestock production is the horn fly (*Hydrotaea irritans*), a Eurasian fly that relies on feces or vegetative refuse for reproduction, often causing irritation and transmitting disease in livestock (Giraldo et al. 2011; Broom et al. 2013). The continual animal movement paramount to rotational grazing lowers the rate at which livestock return to paddocks where dung patties have yet to fully decompose, reducing host-parasite interactions. Additionally, multi-species leader-follower systems can be used, where free range poultry follow livestock and actively forage on horn fly larvae developing in dung patties (Greg Judy, personal communication).

Silvopastures provide environments that are conducive to the establishment of beneficial insects, including many that help rapidly degrade cattle manure, further inhibiting the spread of the horn fly. In Colombia, Giraldo and others (2011) documented significantly greater numbers of dung beetles in ISS than in conventional pasture. The authors observed an inverse relationship between dung beetle and horn fly abundance in the two cattle raising systems, which they attributed to both plant cover and the contribution of plant litter provided by *L. leucocephala*. Plant litter favors not only the establishment of dung beetles but also of other beneficial fauna that can control pest populations and predatorial beetles (Giraldo et al. 2011). Silvopastures have been shown to support increased numbers of birds (McDermott and Rodewald 2014), ants (Rivera et al. 2013), and other beneficial predators that can lower populations of ticks and reduce

the incidence of diseases such as anaplasmosis, which has been shown to drop from 25 to < 5% (Yadav et al. 2019).

ISS contribute ample amounts of tree foliage to the diet, much of which contains condensed tannins, phenols, saponins, and other anti-nutritive secondary compounds that may have anti-parasitic effects. *Tithonia diversifolia*, a widely planted forage shrub in ISS throughout the tropics, appears to have promising effects on ruminal microbial ecology, reducing the methanogen and protozoa population and increasing the population of cellulolytic bacteria (Ruíz et al. 2014).

Still, there is some concern that silvopastoral environments could increase the presence of parasitic helminths. In southeastern Brazil, Costa and colleagues (2013) tested this hypothesis throughout a 6 month period and found no significant differences in overall weight, weight gain or helminth infestation between crossbred Holstein and Gir heifers grazed in silvopasture environments and traditional open pasture environments. In contradiction, Francisco et al. (2009) studied two groups of wild horses in Spain and concluded that silvopasture increased the presence of infection by gastrointestinal nematoda.

A relatively new area of research has examined livestock social interactions in silvopastoral systems as a diagnostic for social welfare. Améndola and others (2015) reported that heifers in an ISS maintained more stable social hierarchies and expressed more socio-positive behaviors, suggesting that animal welfare was enhanced.

Habitat for Pollinators

Pollinator richness and density have been declining in recent years on a global scale (Thomann et al., 2013). Declines in wild bees and butterflies are linked to historical landscape modification (Burkle et al. 2013) and loss of key nesting and foraging sites (Baude et al. 2016). Pollinator decline threatens not only food security but could lead to the extinction of pollinator-dependent plants and ultimately the collapse of modern-day agriculture (Dubeux Junior et al. 2017). A report published by the Intergovernmental Science-Policy Platform for Biodiversity and Ecosystem Services identified agriculture as both a threat to pollinators and a potential solution to support them (Duvic-Paoli 2017). One key way to achieve this is through ‘ecological intensification,’ or the process of maintaining or enhancing agricultural productivity through the cultivation and management of beneficial biodiversity – a process not unlike silvopasture.

A study examining pollinator presence on two silvo-arable and four silvopastoral systems in the UK found that butterfly diversity was significantly higher on the agroforestry plots when compared to conventional pasture (Fig. 3). However, hoverfly and bumblebee abundance were higher in the silvoarable treatments but not for the silvopastoral treatments. The authors attributed this to strips of forbs and grasses retained in tree rows within the silvoarable plots. These so-called ‘pollination reservoirs’ have been shown to be crucial – even in small strips – to the provisioning of adequate pollinator habitat (Brosi et al. 2008). Moreover, planting insect-pollinated tree species may make silvopastures more attractive to pollinators (Varah et al. 2013).

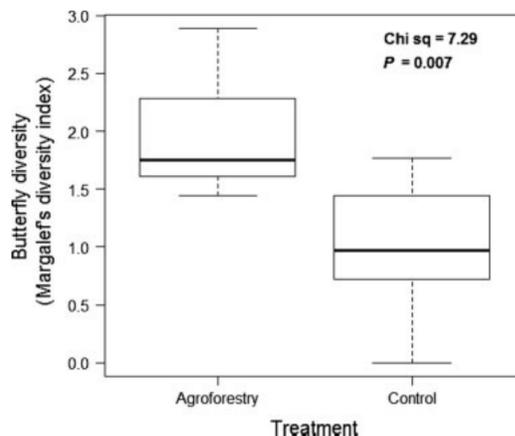


Fig. 3 Effects of treatment on butterfly diversity across all sites in the southern UK.
Source: (Varah et al. 2013)

Silvopasture in the Future

Silvopasture has been shown to be an effective strategy to ecologically intensify and increase food supply in livestock production systems, but it should not be promoted in isolation of other important food security considerations. In an eye-opening report, Steinfeld and colleagues (2006) claimed that the livestock sector emerges as one of the top two or three most significant contributors to the most serious environmental problems. With more than 20 billion domestic farm animals on the planet, they may be even more of a burden for the Earth's biosphere than the current 7.7 billion humans (Hahlbrock 2009). It is time we took a careful look at where and how livestock is being produced and whether or not they hinder or advance our aims to sustain the land in perpetuity (Janzen 2011).

Much of the world's increase in livestock production is occurring through intensive concentrated animal feeding operations (CAFOs), using feed produced on arable lands that could be growing food crops for humans (Pollen 2006). A large portion

of food energy in plant biomass is lost when it passes through animals, so that the number of people fed ha^{-1} of cropland declines when grain is diverted through livestock (Godfray et al. 2010). Stresses in which livestock are implicated include land use change, excretion of polluting nutrients, overuse of freshwater, inefficient use of energy, diverting food for use as feed and emission of greenhouse gasses (Janzen 2011). Thus, a worthy and prudent goal would be to decrease livestock product consumption and to be conscientious of where livestock products come from, if they are consumed.

With that said, many authors make cogent arguments for the role of animal agriculture (Janzen 2011; Hahn Niman 2014; Savory and Butterfield 2016). Livestock may compete with humans for food, but they also create protein from resources we cannot use directly – namely cellulose, from vast grasslands that cannot, or at least should not, be cultivated (Garnett 2009). Most grasslands have co-evolved with large ungulates and have even been shown to thrive best under periodic animal impact, restorative disturbances that naturally aerate and return nutrients to soils. Unlike arable cropland, perennial grasses are not regularly tilled, reducing erosion and sequestering large amounts of carbon to help mitigate climate change (Janzen 2004; Mbow et al. 2014). Carbon sequestration can be enhanced even further when combined with trees in silvopastoral systems (Udawatta and Jose 2012).

Animal agriculture is now widely engrained in the fabric of many cultures and societies. In fact, meat, milk and other animal products account for about a third of the protein consumed by humans globally and account for 40% of the global agricultural GDP (Steinfeld et al. 2006). This, combined with the growing stigma of affluence surrounding the consumption of meat is reason to believe that animal agriculture is here

to stay. Silvopasture is an age-old practice that – if adopted more widely – could augment the benefits and minimize the stresses of livestock production.

Establishing agroforestry on land that currently has low tree cover has been identified as one of the most promising strategies to raise food production without additional deforestation (Garrity et al. 2010). However, the establishment of silvopasture is often easier said than done. In many developing countries, a lack of land tenure makes farmers reluctant to invest in the long-term endeavor of establishing trees that may ultimately benefit others than themselves. Where land holdings are small, farmers are often unwilling or unable to spare land for agroforestry establishment, even if it promises higher long-term returns (Mbow et al. 2014). In the case of ISS, start-up costs can be relatively expensive and may be entirely prohibitive without the availability of subsidies (Murgueitio et al. 2011; Calle 2013) or incentive programs like payment for environmental services (PES) (Pagiola et al. 2005). ISS are also inherently complex, often requiring extensive capacity building, training, and deployment of new technologies through outreach and extension programs (Calle 2013).

National and regional policy-makers across the globe would be wise to address the obstacles facing landholding producers and create programs to facilitate the adoption and utilization of silvopasture. As climate change continues to intensify and jeopardize global food security, silvopasture should no longer be treated as an anomaly, practiced by the few; it should be widely recognized, supported, and promulgated for the effective food provisioning tool it is, expanding and facilitating green ranching opportunities to farmers around the world.

Rationale for the Current Study

In this chapter, we have highlighted a body of research that provides evidence for silvopasture's potential to enhance food security. However, there is still much skepticism among producers and silvopasture adoption rates remain low. There is a need for replicated on-farm demonstrations of low-input/high output silvopastures to help clarify existing doubts among producers. This could potentially encourage them to reconsider long-held, inaccurate belief systems that cattle and trees don't mix, particularly under climate change scenarios.

It is now abundantly clear that the sustainable intensification of meat production will be achieved in the long-term only with the development of working landscapes for the improved health and restoration of both ecosystems and livestock. Animal production must be attained with a minimum of external inputs. It should revolve around the planning of agricultural systems where ecological interactions and synergies between biological components replace human inputs (Cardona et al. 2013). However, there is still much to be learned about these inherently complex systems.

Recently, there has been a surge in research on the benefits of ISS (see Broom et al. 2013 for an overview). Additionally, much attention has been paid to the autoecology of neotropical timber species with implications for native species reforestation (Hall et al. 2011a). However, few studies have documented seedling growth and interspecific interactions among components in silvopastoral reforestation systems (Riedel et al. 2012). Additionally, little is known about potentially promising drought tolerant species – both native and non-native – and their interactive effects on these systems. Janzen (2011) claimed that the re-greening of ecosystems will most likely come by studying – not the

pieces such as cows, crops, soil, air, water – but their interactions. With the continuation of cattle ranching activities likely throughout much of the world, the integration of reforestation and conservation practices with ranching activities is particularly attractive and timely (Garen et al. 2011).

In the next three chapters, we present original data collected on the biophysical influences of trees on overall forage production in tropical and temperate low input systems. We examined three unique simulated silvopastures – two in the province of Los Santos, Panama and one in the state of Missouri, USA – to address the year-long competitive and facilitative effects of trees on neighboring grasses and shrubs. In chapter two, we present results on the production and nutritive quality of three commonly planted ‘improved’ African grasses for three light levels under mature mixed native species hardwood plantations in Panama. In chapter three, we investigate whether or not the planting of the ‘fertilizer shrubs’ *Leucaena leucocephala* and *Tithonia diversifolia* helps improve soil fertility and system forage productivity in newly established silvopastures in Panama. Results presented in the first two data chapters provide an assessment of important ecological interactions in simulated silvopastures at two different successional stages. In chapter four, we move to central Missouri, where we examined the initial growth, production, and nutritional value of the alternative forage shrub red mulberry (*Morus rubra* L.) for four light levels under a mature cherrybark oak (*Quercus pagoda*) plantation.

The specific objectives and hypotheses of each study are addressed in each chapter. All three studies provide important information on the cumulative effects of woody perennials on forage production of neighboring plants in three distinct

environments. Finally, we conclude with chapter five, a summary of the major findings and implications of this research.

Chapter 2

Tree Density Effects on Soil, Dry Matter Production and Nutritive Value of Understory *Megathyrus maximus* in a Seasonally Dry Tropical Silvopasture in Panama

Abstract

Panama's Azuero Peninsula experiences a five-month dry season that routinely subjects cattle to prolonged stress that can result in substantial losses in productivity. Silvopasture, an agroforestry practice combining forages, livestock, and trees, has been shown to optimize system productivity and mitigate the effects of environmental stress on livestock; however, few ranchers in Panama incorporate trees into pastures for long-held beliefs that trees and grass do not mix. We studied three cultivars of Guinea grass (*Megathyrus maximus* cv. Massai, cv. Mombaza, and cv. Tanzania) planted under open (O), moderate (M), and dense (D) tree coverage. We hypothesized that changes in soil chemical and physical properties, grass dry matter production, and nutritive content would be optimized in M in accordance with the stress gradient hypothesis, which predicts an increase in plant facilitation with increasing environmental stress. Results showed no beneficial changes in soil chemical properties due to higher tree densities, but bulk density decreased significantly with increasing tree cover. No differences existed for soil moisture between treatments. O produced significantly greater quantities of dry matter than M and D, and Massai produced significantly greater quantities of forage than Mombaza, but not compared to Tanzania. Nutritive value improved in the shade, with highest CP and lowest fiber (ADF and NDF) values reported for D and M. No differences in nutritive value were found among cultivars. Massai showed the greatest tolerance to drought, producing the most forage accumulation in the early part of the dry season, but only in M. Results partially support the hypothesis, suggesting that forage accumulation is greatest in M for Massai early in the dry season, but greatest in O over the year. This observed extension of the growing season under moderate tree densities could result in reduced animal weight loss between the months of January and April for this region. Research is needed on animal performance under similar tree densities to corroborate the findings reported here.

Key words: Tree-grass interactions, forage production, dry tropics, silvopastoral systems

Introduction

Background

Deforestation and forest degradation have resulted in the widespread loss of native forests across much of the world. One of the key drivers of tropical deforestation in the Neotropics is livestock production (Wassenaar et al. 2006). Of the 22 million hectares of forest lost between 1960 and 1995, 21 million hectares were then used for cattle production (Broom et al. 2013). Meanwhile, human demand for food and other products is increasing rapidly (Godfray et al. 2010). On Panama's Azuero Peninsula, cattle-ranching has been the dominant land use since the early part of the last century when the vast majority of the native dry tropical forests were converted to pasture (Janzen 1988, Griscom et al. 2011). Widespread deforestation resulted throughout the Azuero's eastern province of Los Santos due to government incentives for agricultural expansion, predominantly for cattle-ranching activities (Heckadon-Moreno 1984, 2009). Today, the deforestation rate on the Azuero Peninsula ranks among the highest in Panama (ANAM 2014). Over the past 30-40 years, high cattle stocking rates, repeated burning, and removal of native vegetation has degraded pasture lands, resulting in severe erosion and an overall decline in productivity throughout the region (Heckadon-Moreno 1984, Janzen 1988, Griscom et al. 2011).

Panama's Azuero Peninsula experiences a five-month dry season that routinely subjects cattle to prolonged stress that results in substantial weight loss and even death. Over the past three years, ranchers in the province of Los Santos have reported the deaths of over 500 cattle, with scores more losing over 115 kg, a weight that translates to an economic loss of \$150 per head (Cortez 2013). Climate change is expected to continue to

have far-reaching consequences for animal production through its effects on forage and range productivity and heat related stress on the animal. Under climate change scenarios, water will become the main limiting factor to all livestock systems (Steinfeld et al. 2006; de Fraiture et al. 2010). In fact, extended droughts have become increasingly commonplace on the Azuero Peninsula. This, coupled with a demand for animal products that is predicted to double in the first half of this century, is cause for concern (Godfray et al. 2010).

Silvopasture

In response to widespread deforestation in the 1980's, the Panamanian government began subsidizing landowners to plant non-native Teak (*Tectona grandis*) plantations all across the isthmus (Sloan 2008; Griscom and Ashton 2011a). Recent efforts have been made to reforest and restore native tree species because they are perceived to produce an increased diversity of socially sensitive goods and ecosystem services (Hall et al. 2011b). In Los Santos, where cattle ranching is the primary form of agriculture, a sensible first step in mitigating the harsh impacts of climate change could be to combine the goods and services of timber production and ranching with the use of silvopasture.

Silvopasture is an agroforestry practice where trees and livestock are deliberately combined with improved pasture plants to form a carefully designed system, integrating intensive animal husbandry, silviculture, and forage agronomy practices (Sharrow et al. 2009). Silvopasture can improve the balance between production and conservation, ensuring the availability of highly nutritious forage, reducing stress in cattle, capturing

and storing carbon, and favoring biodiversity by providing habitat that supports a diversity of plants and animals (Ibrahim et al. 2010; Galindo et al. 2013).

It is well-established that trees have both competitive (negative) and facilitative (positive) effects on the organisms around them (Jose et al. 2004; Jose et al. 2017). Canopy solar interception results in lower light transmittance, decreasing the photosynthetic rate of understory vegetation. Trees have been shown to compete vigorously for water and nutrients and can even emit allelopathic chemicals that impede the growth of surrounding vegetation. However, canopy interception can also provide protection from desiccating winds, reduce soil surface temperature and soil evapotranspiration (Belsky et al. 1989; Belesky 2005), which can increase overall soil moisture content (Vetaas 1992). Some trees can fix atmospheric Nitrogen and provide up to 650 kg N yr^{-1} , more than enough to fulfill crop N needs for sustained yield (Nygren et al. 2012). Leaf litter under trees has been shown to improve the physical properties of the surface soil, increasing soil nutrients and organic matter (Belsky 1994). As a result, the content of carbon, phosphorus, and nitrogen has been shown to gradually decline as a function from the distance of the trunk, resulting in significantly lower levels in the open ground than in sub-canopy soil (Belsky et al. 1989; Tiedemann and Klemmedson 2008).

Elevated nutrient levels can improve the forage quality of sub-canopy grasses, attracting grazers that return nutrients to the soil. This, combined with the trapping of wind and water-borne sediments by trees can contribute to an 'island of fertility' effect (Belsky et al. 1989; Dohn et al. 2013). Trees roots can also decrease the bulk density of the soil, creating the macro-porosity favorable to the infiltration of water, increasing water-holding capacity (Malmer et al. 2010). These benefits, combined with the selection

of appropriate tree and forage species, can sometimes result in increased levels of productivity when compared with monocultures.

A number of studies have been conducted on the genetic development and expansion of pasture grasses for use with intensive cattle production and silvopastoral systems. In response to various pathogens and pest outbreaks that have rendered common pasture grasses like Marandu (*Urochloa brizantha*) susceptible to mass die-off, research institutions have been selectively breeding, hybridizing, and experimenting with new grass varieties (Fernandes et al. 2014). More recently, attention has been paid to improving drought and shade tolerance among cultivars for use in silvopastoral systems.

Guinea grass (*Megathyrsus maximus* Jacq.) is one of the most important species for cattle production in tropical and subtropical regions (Paciullo et al. 2017) and, despite being a C₄ grass, many developed cultivars show medium levels of tolerance to shade (Santiago-Hernández et al. 2016). The genus *Megathyrsus* has an array of diversity, with most species standing erect, from 0.5 to 3.5 m high and is recommended for regions with annual rainfall from 800 to 1800 mm in well-drained soil with medium to high fertility (Muir and Jank, 2004). The cultivars Massai, Mombaza, and Tanzania, developed for increased productivity with simultaneous drought and shade tolerance characteristics, were released from the *M. maximus* breeding program at EMBRAPA (Brazilian Agricultural and Cattle Research Enterprise) and are currently among the most popular Guinea Grass cultivars in Latin America (Paciullo et al. 2017).

Ecological theory suggests that facilitation presides over competition along a gradient of increasing abiotic stress (Bertness and Callaway 1994; Brooker and Callaghan 1998; Dohn et al. 2013). The stress gradient hypothesis conjectures that neighboring

organisms are more likely to facilitate each other's growth and fitness under conditions of high environmental stress, such as high disturbance frequency and low resource availability (Bertness and Callaway 1994; Brooker and Callaghan 1998; Dohn et al. 2013). In xeric environments where water is the limiting factor, growth and development of many herbaceous species are facilitated by tree canopies through the improvement in moisture regimes (Joffre and Serge, 1993), soil nutrients, and organic matter (Kellman 1979). Moustakas et al. (2013) demonstrated that tree effects on grass biomass across a precipitation gradient in a subtropical savanna were facilitative in drier sites, with higher grass biomass observed beneath tree canopies than outside.

We expect that environmental conditions on our study site are such that the stress gradient hypothesis could be supported. The objectives of this study were to: 1) evaluate differences in dry matter production and nutritive value among three cultivars of *M. maximus* (cv. Massai, cv. Mombaza, and cv. Tanzania) under open (O), moderate (M), and dense (D) tree coverage, 2) quantify understory light levels with the use of hemispherical photography, and 3) test for differences in soil chemical and physical properties among treatments after the establishment of the grasses.

We hypothesized that dry matter production and nutritive quality would reach their peak in M. We further hypothesized that cation exchange capacity (CEC), base saturation (BS), and corresponding soil fertility indicators like organic matter and the exchangeable cations Mg, Ca, and K would be highest in D, but soil moisture would be highest for M during the dry season due to hydrological improvements under a sparse canopy.

Materials and Methods

Study site

This study was conducted near the Achotines Tuna Laboratory on privately-owned land in the dry tropical forest region (Holdridge 1967) of Los Santos Province on the Pacific side of Panama (Fig. 2; 7°15'30''N, 80°00'15''W). The area receives a long-term average of 1678 mm of rain per year, with an extended five-month dry season between late November and late April (Fig. 1). According to the Soil Atlas of Latin America, soils are classified as Dystric Cambisols (Cmdy) and are young and relatively nutrient rich, although degraded from decades of overgrazing (Gardi et al. 2015). Soil textures range from loam to clay loam (Table 1) with an average soil pH of 5.54 (\pm 0.17). The topography is undulating, with slopes ranging between 18 and 40% grade (Diogenes Ibarra, personal communication).

In 2005, twenty native tree species were planted on 31 hectares. Areas that remain to be reforested are now a mosaic of pastures of the African grasses Faragua (*Hyparrhenia rufa*) and Guinea grass (*M. maximus*), riparian forested zones, isolated trees, and live fences (Griscom et al. 2009). The land is currently being rotationally grazed by a herd of 30 Brahman cattle, which are sold and restocked annually. Study plots were established in and alongside a mixed native species tree plantation established in 2005 (11 years old; Fig. 3). The plantation was dominated by *Dalbergia retusa* Hemsl. (40% of the individuals), *Platymiscium pinnatum* (Jacq.) Dug. (18% of the individuals), *Swietenia macrophylla* King. (10% of the individuals), and *Diphysa americana* Benth. (10% of the individuals) (Table 2).

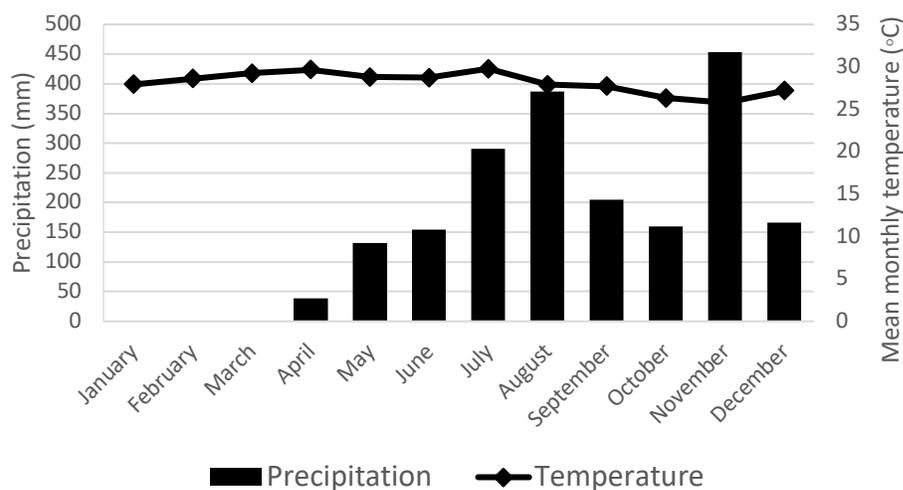


Fig. 1 Precipitation and mean monthly temperature near Playa Venao, Panama in 2016
(A portion of the data was provided by (ETESA 2018))

Table 1 Soil texture determined from three sampling depths (0-5, 5-10, and 10-15 cm) from one location within each of the three tree density treatments, moderate (M), dense (D), and open (O).

Soil depth	Soil Texture (%)			Texture class
	Sand	Silt	Clay	
Profile 1 (M)				
0-5	47.5	36	16.5	Loam
5-10	43	34	23	Loam
10-15	39	33	28	Clay Loam
Profile 2 (D)				
0-5	35.5	38	26.5	Loam
5-10	33	36.5	30.5	Clay Loam
10-15	29	38	33	Clay Loam
Profile 3 (O)				
0-5	48	37	15	Loam
5-10	46	33	21	Clay Loam
10-15	45	33	22	Clay Loam

Table 2 Tree species and their respective representation in the 2005 plantation at the IDB Forestal Ranch, near Playa Venao, Panama. Source: (Griscom and Ashton 2011b)

Species	Successional status	Attributes	Representation
<i>Dalbergia retusa</i> Hemsl.	Long-lived Pioneer	Nitrogen-fixing, Timber	40%
<i>Swietenia macrophylla</i> King.	Long-lived Pioneer	Fast growth, Timber	10%
<i>Cedrela odorata</i> L.	Pioneer	Fast growth, Timber	5%
<i>Tabebuia rosa</i> (Bertol) DC.	Long-lived Pioneer	Fast growth, Timber	5%
<i>Platymiscium pinnatum</i> (Jacq) Dug.	Pioneer	Nitrogen-fixing, Timber	18%
<i>Manilkara zapota</i> (Pittier) Gilly.	Late-successional	Wildlife, NTFP	7%
<i>Calycophyllum candidissimum</i> (Vahl) DC.	Late-successional	Fast growth, Timber	1%
<i>Diphysa americana</i> Benth.	Long-lived Pioneer	Nitrogen-fixing, Timber	10%
<i>Cordia alliodora</i> (Ruiz & Pav.) Oken.	Long-lived Pioneer	Fast growth, Timber	1%
<i>Tabebuia guayacan</i> (Seem) Hemsl.	Long-lived Pioneer	Fast growth, Timber	2%
<i>Albizia saman</i> (Jacq) Merr.	Long-lived Pioneer	Nitrogen-fixing, Timber	1%

A geometrical thinning was done in May of 2016 to establish an even distribution of shading in the moderately shaded treatment (M), where plantation overstory basal area was reduced from 13.45 m² ha⁻¹ (Dense shade, D) to 6.44 m² ha⁻¹ (Moderate shade, M) (Table 3). This 48% reduction of basal area corresponds to stocking levels that permit understory light levels between 35 and 50% of that found in the open (Garrett et al. 2004; Orefice et al. 2017). Open pasture treatments (O) were established directly adjacent to the plantation with similar edaphic and topographic conditions, pre-determined by sampling and analysis of the soils. Plots were fenced to avoid the entry of cattle and cleared of all vegetation with machetes. Roundup® herbicide (Glyphosate) was applied evenly to all plots prior to the broadcast seeding of grass at a seed rate of 8 kg ha⁻¹ of pure and viable seed (PVS). No fertilizer was applied to any of the plots at any time.

Table 3 Overstory composition in a mixed species native tree plantation established in 2005 at the IDB Forestal Ranch, near Playa Venao, Panama. Trees were geometrically thinned to approximate a 50% reduction in basal area.

Measurement	Treatment	
	Dense Shade (D)	Moderate Shade (M)
Trees (ha ⁻¹)	931 (55)	503.15 (76.65)
Diameter at breast height (cm)	12.43 (3.14)	11.90 (2.78)
Basal diameter (cm)	19.32 (4.98)	17.62 (4.32)
Height (m)	8.12 (1.36)	7.82 (1.45)
Basal area (m ² ha ⁻¹)	13.45 (0.56)	6.44 (0.66)
Mean quadratic Diameter (cm)	13.52 (0.216)	13.03 (1.24)
Relative Density	58% (2.4%)	28% (2.4%)

Means are reported with standard error in parentheses, n = 9. Relative density is the reported basal area of a stand in relationship to the maximum basal area reported for that forest type and size class. Source: (Ewel 1977)



Fig. 2 Two photos from the mixed native species plantation established in 2005 at the IDB ranch near Playa Venao, Panama, illuminating stark differences between the rainy (left) and dry (right) seasons.

Experimental Design

A completely randomized split-plot design was used with tree density as the main plot factor and grass type as the split-plot factor. Tree density consisted of open (O), moderately shaded (M), and densely shaded (D) treatments and grass type was one of three randomly selected *M. maximus* cultivars: cv. Massai, cv. Mombaza, and cv. Tanzania. Each main plot factor was replicated three times in 1/3 ha plots, each of which was divided into four equal-sized quadrants planted with one of the three randomly selected grass varieties. The remaining quadrant was not planted. Each plot was given a 6m buffer to prevent outside influence from neighboring treatments (Fig. 3).

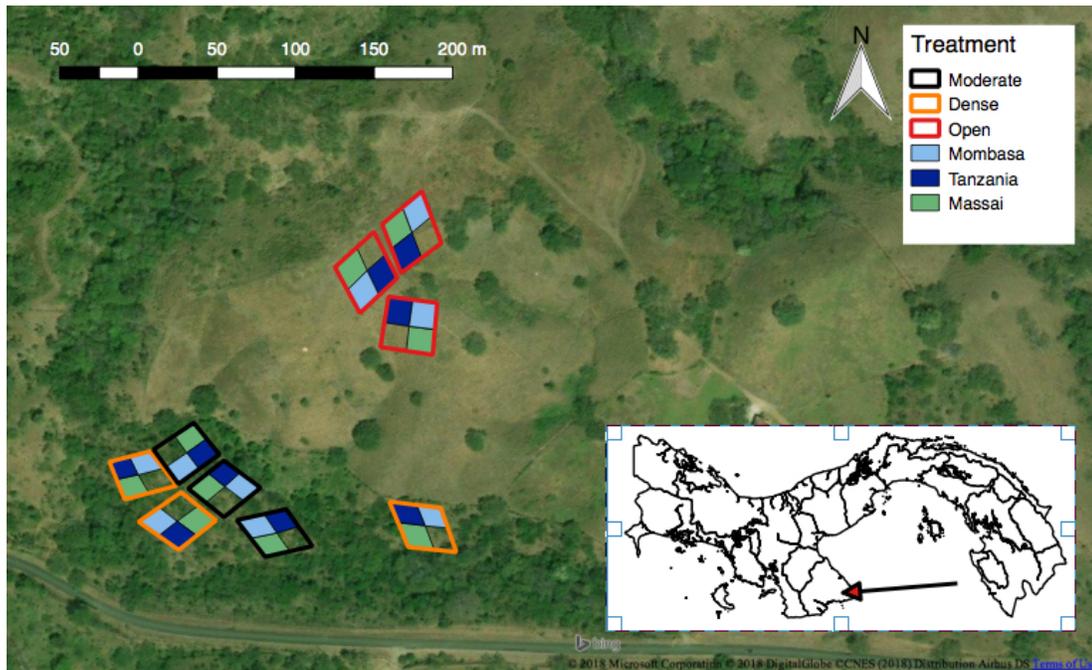


Fig. 3 Completely randomized layout with a split plot design for *M. maximus* (varieties Massai, Mombasa, and Tanzania) at the IDB Forestal ranch in Los Santos, Panama.

Understory Light Environment

Light quantity was determined by measuring solar transmission radiation with hemispherical photography (Fig. 4). Upward hemispherical images were photographed during the dry season (April) and wet season (November) of 2016 using a Cannon EOS Rebel with an Opteka Vortex Fisheye Lens. Images were taken 1 m above the ground on a tripod equipped with a Delta-T devices mount used for horizontal leveling and north-south orientation of the camera. Measurements were taken at dawn to maximize light evenness. Three photographs were taken at the middle and corners of each plot with automatic exposure compensation and bracketing at -2.0, -1.0, and 0. This was done to mitigate the effects of over-exposure that can result from using the automatic exposure in

dense canopies. Images were analyzed with Hemiview software (Delta-T Devices Ltd., Cambridge, U.K.), which classifies pixels into two classes: open sky and obstructed sky. The software calculates the global site factor (GSF), which is the sum of direct and diffuse radiation (excluding reflected radiation) entering through canopy openings as a proportion of the amount of radiation that would fall on the same point given no overhead obstructions over the course of a year (Griscom et al. 2009). Values range from 0 to 1, which 0 being no radiation (complete sky obstruction) and 1 being the radiation for an open location, where the sky is completely visible (Rich et al. 1999).

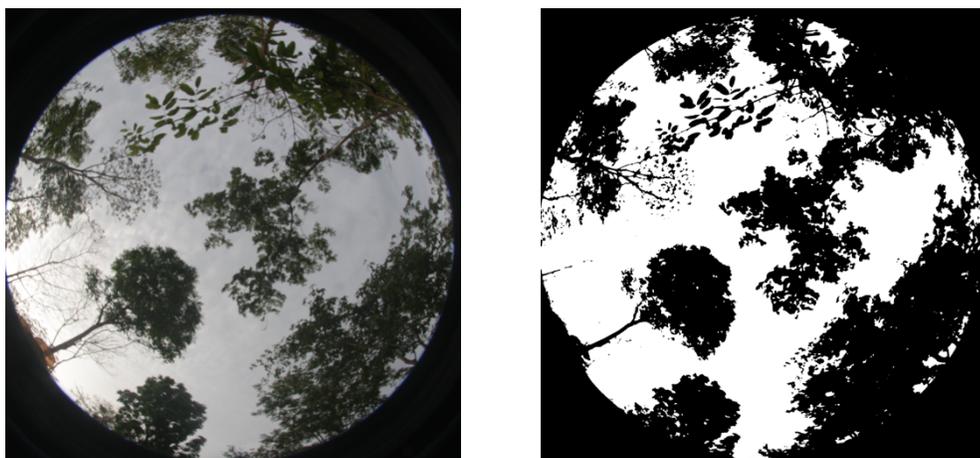


Fig. 4 Images of the canopy captured with hemispherical photography were analyzed with Hemiview software to generate Global Site Factor (GSF) to quantify understory light levels.

Soil Properties

Soil samples were collected preliminarily to confirm that all plots were relatively homogeneous and again one year later after grass was established. Six randomly distributed soil samples (200g) were collected at 0 -15 cm depth with an auger from each plot and analyzed for chemical and physical properties at the Smithsonian Tropical

Research Institute (STRI) in Panama City, Panamá. Inductively coupled plasma optical emission spectroscopy (ICP-OES) was used with a BaCl_2 solution to determine exchangeable Ca, Mg, K, Mn, Fe, Al, Zn, B, and the Mehlich-3 extraction (Mehlich 1984) technique was used to estimate P. Base saturation was calculated by summing the exchangeable cations. Effective Cation exchange capacity (ECEC, cmol (+) kg^{-1}) was calculated by summing the basic and acidic exchangeable cations and Base Saturation (BS, %) was calculated by dividing the Total Exchangeable Bases (TEB) by the ECEC and multiplying by 100. Another set of samples were sent to the soil testing laboratory at the University of Missouri where they were analyzed for organic matter (%), total carbon (TC, %), and total nitrogen (TN, %). Total carbon and nitrogen were determined by dry combustion using a CHN analyzer and organic matter was estimated by loss on ignition.

Bulk density was measured using a metal cylinder with a volume of 100 cm^3 . Three depths, 0-5 cm, 5-10 cm, and 10-15 cm, were sampled and sent to the laboratory where they were weighed wet, dried at 105° C , and weighed again to determine gravimetric soil moisture and bulk density. Texture was determined using particle size analysis.

Soil Volumetric Water Content (VWC)

Soil moisture was evaluated once every two months using the Time-Domain-Reflectometry method and a TDR meter (Field Scout™ TDR Soil Moisture Meter 100, using settings for high clay soil). Soil moisture, also known as volumetric water content (VWC), is the ratio of the volume of water to the total volume of soil. This was measured

at four depths (3.8, 7.5, 12, and 20 cm) systematically at twelve locations across each plot for the months of January, March, May, July, September, and November in 2016.

Grass Dry Matter Production and Nutritive Value

Improved forages were broadcast seeded on June 5, 2016. Four measurements were taken to represent two sampling seasons: wet (November 5 and December 5, 2016), and dry (February 5 and April 5, 2017). At the end of each sampling period, grass within each plot was cut to a uniform height of 20 cm above the soil and was left to grow for 30 days and 60 days to resemble rotational stocking during the wet and dry seasons, respectively. Eight locations were systematically distributed across each plot where grasses were sampled in square grids (0.5m x 0.5m) within a 3 m edge buffer to minimize potential edge effects. Grass wet matter was evaluated by cutting the grass to 20 cm in height with hedge shears, putting clippings into a burlap sack and immediately weighing (Fernandes et al. 2014). Samples were quartered to extract a 400 g subsample that was sent to the Cooleche Laboratory (Cooperativa de S/M de Productores de Leche) in Concepción, Panama. Samples were dried at 60°C for 48 hours to weight constancy before determination of dry biomass weight. Samples were then ground with a hammer mill to pass a 1 mm screen and analyzed with wet chemistry techniques to determine dry matter percent crude protein (CP, %), acid detergent fiber (ADF, %), neutral detergent fiber (NDF, %), total digestible nutrients (TDN, %), and relative feed value (RFV).

Data Analysis

Shade effects on grass dry matter yield (kg ha^{-1}) and nutritive value were analyzed with a two-way analysis of variance (ANOVA) using a mixed model with a split plot in space and time (Steel and Torrie 1980). The split plot design of this experiment considered treatments (O, M, and D) main plots, cultivar as sub-plots, and sampling dates as sub-sub-plots (Steel and Torrie 1980). Sampling dates were analyzed as repeated measures. For light quantity (GSF, %) and soil chemical and physical properties, two-way ANOVAs were conducted on treatments, with sampling dates analyzed as repeated measures. Bulk density and volumetric soil moisture were also analyzed with a mixed model using a split plot in space and time. Treatment (O, M, and D), soil depth, sampling date, and their interactions were considered fixed effects while the replicate, treatment nested under replicate, and sampling date nested under treatment and replicate were considered random effects.

All variables were tested for normality, \log_{10} transformed when necessary, and analyzed using the MIXED procedure in SAS (SAS Institute 2010). Pairwise comparisons were performed using the Tukey-adjusted least squares method (LSMeans) and main effects and all interactions were considered significant when $\alpha = 0.05$.

Results

Forage Dry Matter Production

Overall, O yielded 28.14% and 52.08% more forage than M and D, respectively. Significant differences existed between treatments O ($2730.75 \text{ kg ha}^{-1}$), M ($1962.25 \text{ kg ha}^{-1}$) and D ($1308.44 \text{ kg ha}^{-1}$) ($P = 0.0003$). Forage biomass of Massai ($2041.89 \text{ kg ha}^{-1}$)

was significantly greater than Mombaza (1963.45 kg ha⁻¹) but not for Tanzania (1996.10 kg ha⁻¹) (P = 0.0112). Mean dry matter yield was significantly different for all months (P = 0.0001) with November yielding the most (3845.77 kg ha⁻¹) and April yielding the least (153.57 kg ha⁻¹). There was a significant month*treatment interaction (P = 0.0001), although no difference existed between treatments for the month of April. When seasons were analyzed, there was significantly more dry matter produced during the wet season (P = 0.0001) than during the dry season. There was a treatment*cultivar*month interaction (P = 0.0001), with significantly greater dry matter yield for Massai in M than for D and O during the month of February (P = 0.0003 and P = 0.0409, respectively; Fig. 5). Means and standard errors of these variables are presented by treatment and season in Table 4 and dry matter yield harvest⁻¹ is shown in Figure 6.

Forage Nutritive Value

Crude protein (CP) levels were significantly affected by treatment (P = 0.0138) and season (P = 0.0129). Shade increased overall crude protein levels, with means of 7.76, 8.82, and 9.62% in O, M, and D, respectively. Pairwise comparisons showed that significant differences existed between D and O (P = 0.0055) and M and O (P = 0.036). Grasses sampled during the dry season had higher overall CP content than grasses sampled during the rainy season (P = 0.0165). There were no significant differences in CP content between the three cultivars (P = 0.0690), however, Tanzania had consistently numerically greater CP levels than Mombaza and Massai (Table 5). There were no significant interactions.

Table 4 Dry matter production (kg ha⁻¹) for the *M. megathyrus* cultivars Massai, Mombaza, and Tanzania under open (O), moderate (M), and dense (D) tree density treatments for the wet and dry season of 2016-2017.

Treatment	Wet Season					
	Massai		Mombaza		Tanzania	
	Nov	Dec	Nov	Dec	Nov	Dec
D	2576 (392) Aa1	2091 (450) Ab1	2562 (232) Aa1	2053 (388) Ab1	2620 (272) Aa2	2001 (365) Ab2
M	3992 (497) Ba1	2905 (425) Bb1	3576 (165) Ba2	2782 (163) Bb2	3738(180) Ba2	2854 (202) Bb2
O	5293 (296) Ca1	4652 (390) Cb1	5086 (162) Ca2	4624 (342) Cb1	5169 (187) Ca2	4634.43 (390) Cb1
	Dry Season					
	Massai		Mombaza		Tanzania	
	Feb	Apr	Feb	Apr	Feb	Apr
D	1124 (123) Ac1	135 (29) Ad1	1051 (188) Ac2	123 (48) Ad2	1127 (91) Ac1	128 (55) Ad2
M	1479 (158) Bc1	175 (75) Ad1	1497 (124) Bc1	151 (70) Ad1	1548 (92) Bc2	168 (49) Ad1
O	936 (86) Cc1	172 (80) Ad1	931 (144) Cc1	152 (52) Ad1	944 (150) Cc1	174 (55) Ad1

Uppercase letters represent treatments, lowercase letters represent sampling dates, and numbers represent grass cultivar. Entries that share a letter or number are not significantly different from one another at $P < 0.05$.

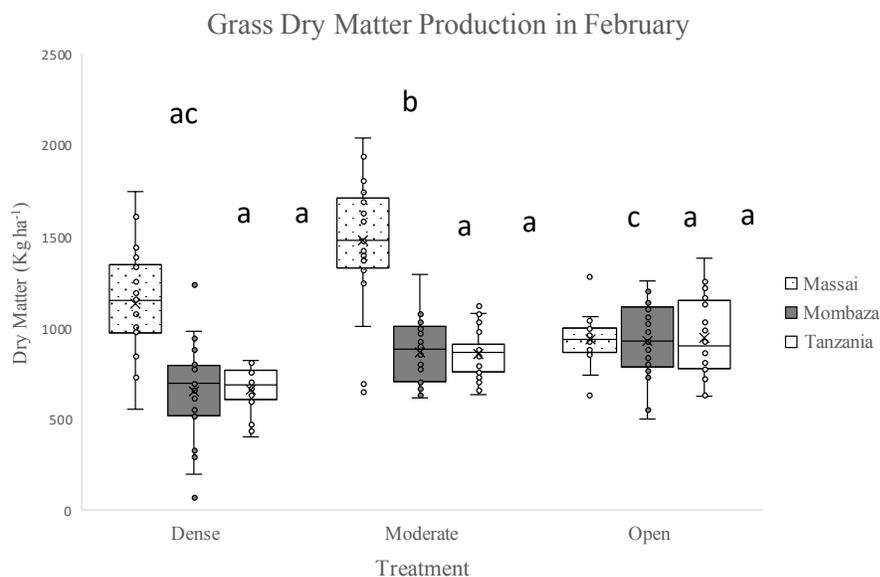


Fig. 5 Grass dry matter production for three cultivars of *M. maximus* in open (O), moderate (M), and dense (D) treatments for the month of February. Massai in M produced greater dry matter than both D and O. Entries that share a letter are not significantly different from one another at $P = 0.05$.

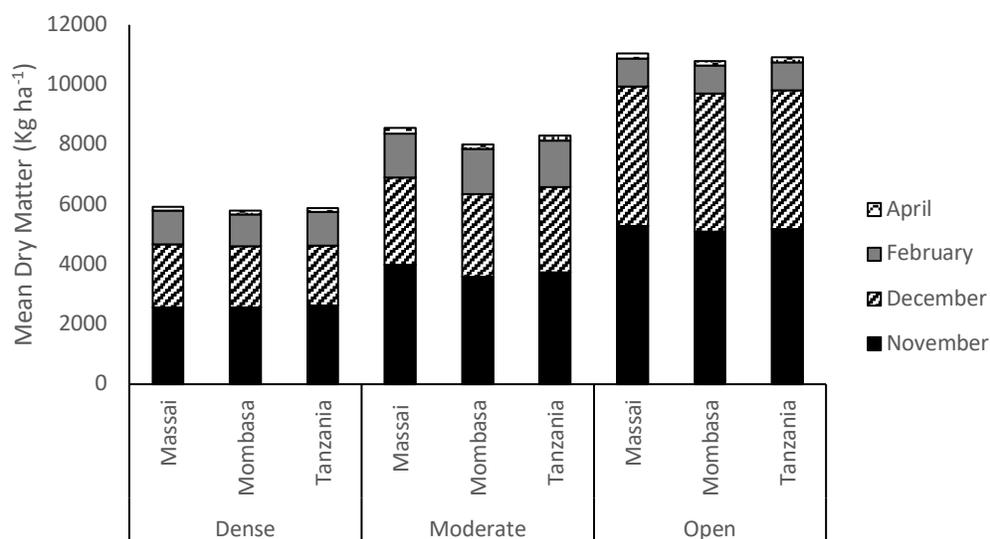


Fig. 6 Grass dry matter production for four sampling periods, two during the rainy season (November and December) and two during the dry season (February and April).

Acid detergent fiber (ADF) was significantly different for treatment ($P = 0.0064$), season ($P = 0.0001$), and cultivar ($P = 0.0092$). Grasses in O had significantly greater ADF concentrations than grasses in M ($P = 0.0037$) and D ($P = 0.0048$). Grasses sampled during the rainy season had higher ADF concentrations than grasses sampled during the dry season ($P = 0.0001$). Massai had significantly greater ADF concentrations than Tanzania ($P = 0.0024$), but did not differ from Mombaza ($P = 0.1068$). There was a significant treatment*season interaction for NDF ($P = 0.0115$). NDF of grasses growing in D and M was significantly greater during the rainy season, but did not differ between seasons in O ($P = 0.2644$).

Total Digestible Nutrients (TDN) and Relative Feed Value (RFV) were significantly affected by treatment ($P = 0.0059$; $P = 0.0051$), season ($P = 0.0001$; $P = 0.0001$), and cultivar ($P = 0.0074$; $P = 0.0117$). However, there were significant treatment*season interactions for both variables ($P = 0.0392$; $P = 0.0112$). TDN and RFV of grasses growing in D and M was significantly greater during the dry season, but did not differ between seasons in O ($P = 0.0984$; $P = 0.1489$).

Table 5 Crude protein (CP, %), acid detergent fiber (ADF, %), neutral detergent fiber (NDF, %), total digestible nutrients (TDN, %), and relative feed value (RFV) least squares means for the *M. megathyrsus* cultivars Massai, Mombaza, and Tanzania during the dry and rainy (bold) seasons under open (O), moderate (M), and dense (D) tree cover. Entries that share a letter are not significantly different from one another at $P < 0.05$.

Variable	Treatment				Cultivar				Season		
	O	M	D	ANOVA P-value	Massai	Mombasa	Tanzania	ANOVA P-value	Dry	Wet	ANOVA P-value
Crude Protein (%)	7.8A	8.8B	9.6B	0.0138	8.3a	8.8a	9.1a	0.069	9.1A	8.4B	0.0129
ADF (%)	44.6A	40.3B	40.6B	0.0064	43.0a	41.8a	40.7b	0.0092	39.4A	44.2B	< .0001
NDF (%)	64.8A	61.8B	62.0B	0.0064	63.6a	62.7a	62.2b	0.0305	61.2A	64.5B	< .0001
TDN (%)	57.1A	61.1B	60.9B	0.0059	58.6a	59.6ab	60.8b	0.0074	62.0A	57.4B	< .0001
RFV	77.9A	86.8B	86.3B	0.0051	81.4a	83.8ab	85.9b	0.0117	88.7A	78.7B	< .0001

Understory Light Environment

Treatments O, M, and D all differed significantly with respect to understory light levels ($P = 0.005$). In contrast, no significant difference was found between seasons ($P = 0.272$). Mean Global Site Factor values for the rainy season were 26.92, 48.09, and 98.53% for D, M, and O, respectively, and 29.50, 58.65, and 99.13% for the dry season, respectively (Fig. 7). This corresponds to a 51.19% and 72.68% reduction in GSF for M and D during the wet season and a 40.84% and 70.24% reduction in GSF for M and D during the dry season.

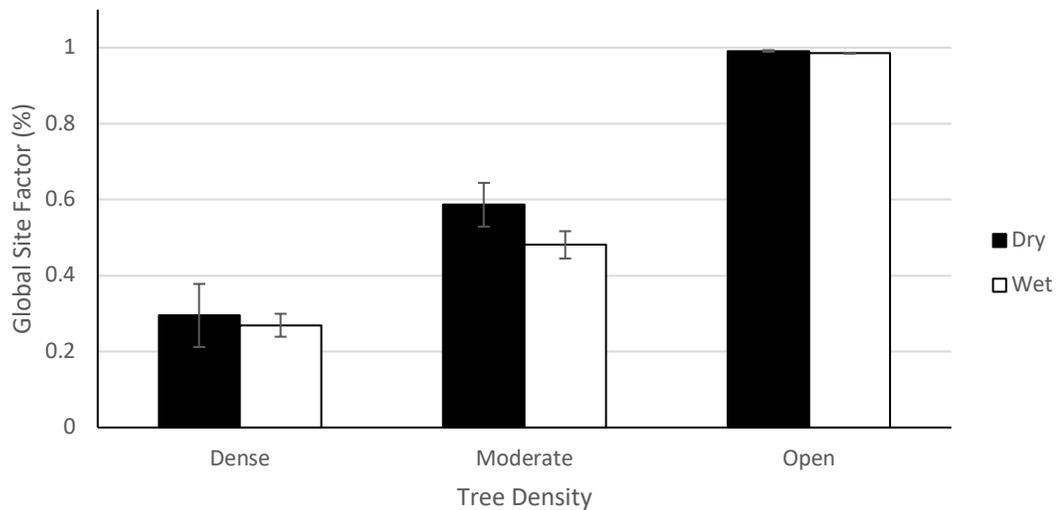


Fig. 7 Mean global site factor (GSF) measured under dense, moderate, and open tree coverage during the dry and wet seasons. Bars indicate \pm SE ($n = 9$)

Soil Properties

Analyses of soil chemical and physical properties demonstrated a relatively high soil fertility with an overall high base saturation (BS) of 99.83% ($\pm 0.28\%$), high cation exchange capacity (CEC) of 26.0 cmol(+)/kg (± 3.44 cmol(+)/kg), and a relatively low pH of 5.54 (± 0.17). Total Carbon (TC) and Total Nitrogen (TN) did not differ between treatments or years, and the overall C:N ratio was 10.82 (± 2.74). Overall soil organic matter (SOM) and bulk density was 3.22% (± 0.52) and 0.92 g/cm³ (± 0.09 g/cm³), respectively.

No significant differences existed between treatments in regards to CEC, BS, SOM, TC, and TN, however, it is worth noting that TN levels in M and D were numerically greater than O ($P = 0.2724$). The soil pH differed significantly in all three treatments ($P = 0.0088$), and there was a nearly significant increase between sampling periods (0.0806). Pairwise comparisons showed that O had significantly higher pH levels than M ($P = 0.0150$) and D ($P = 0.0109$). Bulk density differed between treatments ($P = 0.001$) and soil depth ($P = 0.0001$) (Fig. 8). O had significantly greater bulk density than M ($P = 0.008$) and D ($P = 0.0009$) while M was significantly greater than D ($P = 0.0173$). Bulk density increased with depth, with samples collected at 0-5 cm significantly less dense than those collected at 5-10 cm ($P = 0.0012$) and 10-15 cm ($P = 0.0001$).

Most soil nutrients did not differ significantly between treatments, however there were a few notable exceptions (Table 6). Exchangeable Potassium (K⁺) showed a marked increase in O when compared to both M and D ($P = 0.0349$). The least square means for dense, moderate and open treatments were 50.37, 51.16, and 66.63 mg kg⁻¹, respectively, with O containing significantly more K⁺ than D ($P = 0.0445$). Phosphorus (P) increased

non-significantly as tree density increased, with corresponding least square means of 1.807, 2.01, and 2.07 mg kg⁻¹ for O, M, and D, respectively.

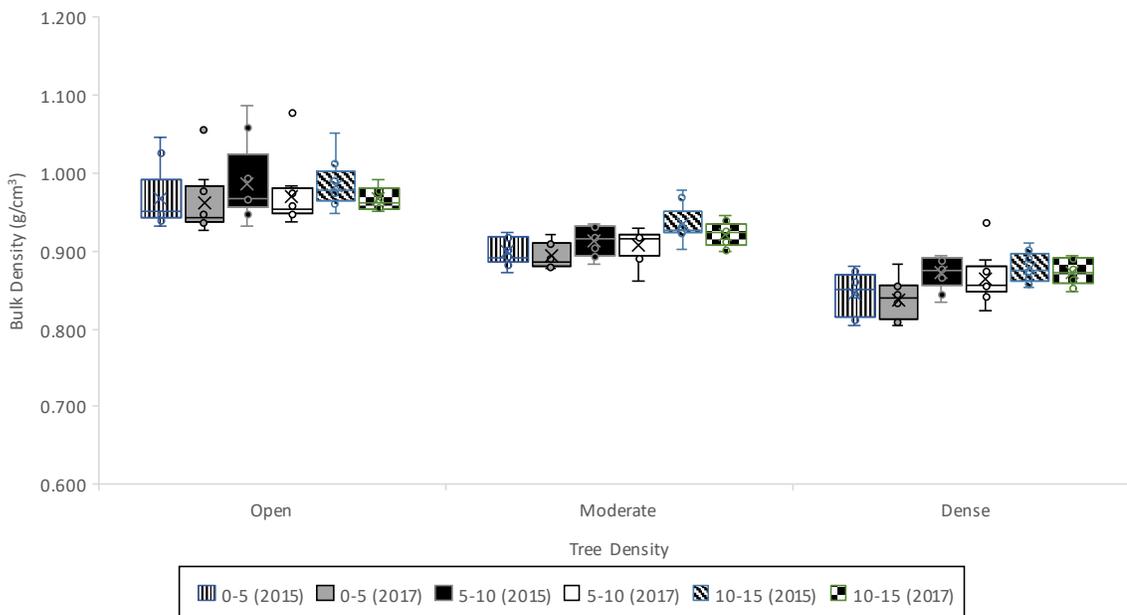


Fig. 8. Differences in bulk density (g cm⁻³) for three soil depths (0-5, 5-10, and 10-15 cm) in open (O), moderate (M), and dense (D) treatments for soils sampled in 2015 and 2017. Significant differences existed for depth and date in all three treatments. n = 9

There was a significant difference in sampling period for P, with 2017 concentrations greater than 2015 concentrations ($P = 0.0194$). CEC increased marginally as tree density decreased, with corresponding least square means of 26.48, 26.22, and 25.29 cmol (+) kg⁻¹, for O, M, and D, respectively.

Soil Volumetric Water Content

No significant differences existed between treatments for soil moisture ($P = 0.1641$), but there were clear differences between all six sampling periods ($P = 0.0001$). Soil moisture increased with depth, with deeper soil horizons containing more moisture than shallow horizons ($P = 0.0001$). The interaction between month and depth was highly significant ($P = 0.0001$). Several patterns were observed between treatments at different months and depths that are worth noting (Fig. 9). During the dry season (January and March), soil moisture increased linearly with soil depth, with lower horizons retaining numerically more moisture than upper horizons. At the onset of the rainy season in May, the soil moisture was greatest in the upper and lower horizons and driest in the middle horizons. During the wettest parts of the year, the highest moisture levels were found in the middle horizons, creating quadratic curves.

Table 6 Physical and chemical soil properties for soil collected in the rainy season of 2015 and 2017 within open (O), moderate (M), and dense (D) treatments. n = 9.

	Open (O)		Moderate (M)		Dense (D)	
	2015	2017	2015	2017	2015	2017
Texture	Loam	Loam	Loam	Loam	Clay Loam	Clay Loam
pH	5.61a	5.59a	5.32b	5.35b	5.34b	5.33b
B.D. (g/cm ³)	0.98 (0.04) a	0.97 (0.04) a	0.92 (0.02) b	0.91 (0.02) b	0.87 (0.03) c	0.86 (0.03) c
TC (%)	1.85 (0.41) a	1.98 (0.15) a	1.72 (0.34) a	1.78 (0.27) a	1.86 (0.30) a	1.86 (0.27) a
TN (%)	0.16 (0.02) a	0.17 (0.02) a	0.18 (0.03) a	0.19 (0.02) a	0.18 (0.04) a	0.18 (0.02) a
SOM (%)	3.23 (0.71) a	3.47 (0.25) a	3.01 (0.60) a	3.11 (0.47) a	3.25 (0.52) a	3.25 (0.48) a
CEC (cmol(+)/kg)	26.31 (3.02) a	26.64 (3.37) a	25.21 (4.86) a	27.23 (2.63) a	24.57 (2.71) a	26.01 (3.83) a
BS (%)	99.75 (0.16) a	99.88 (0.06) a	99.74 (0.16) a	99.92 (0.03) a	99.80 (0.15) a	99.87 (0.06) a
P (mg/kg)	1.79 (0.40) aA	1.83 (0.30) aA	1.92 (0.30) aA	2.10 (0.41) aB	1.84 (0.34) aA	2.31 (0.41) aB
K (mg/kg)	70.67 (8.59) a	62.60 (13.19) a	52.36 (12.70) b	49.95 (10.64) b	52.24 (9.61) b	48.51 (10.18) b
Ca (mg/kg)	5144.44 (395.03) a	5167.67 (286.49) a	5177.56 (394.00) a	5153.11 (338.80) a	5164.11 (353.68) a	5104.67 (389.61) a
Mg (mg/kg)	1420.67 (67.57) a	1414 (88.04) a	1369.11 (100.92) a	1393.89 (97.11) a	1384.44 (59.06) a	1403.78 (89.71) a
Mn (mg/kg)	54.60 (6.18) a	54.79 (7.64) a	54.36 (4.60) a	58.44 (4.07) a	54.60 (6.18) a	49.93 (9.40) a
B (mg/kg)	0.61 (0.47) a	0.84 (0.39) a	0.46 (0.31) a	0.95 (0.22) a	0.81 (0.47) a	0.83 (0.49) a
Fe (mg/kg)	146.83 (11.55) a	151.66 (12.57) a	153.68 (5.84) a	154.02 (9.72) a	154.09 (11.13) a	149.75 (10.76) a
Zn (mg/kg)	1.46 (0.34) a	1.40 (0.10) a	1.32 (0.21) a	1.42 (0.15) a	1.53 (0.13) a	1.43 (0.18) a
Cu (mg/kg)	4.59 (0.71) a	4.54 (0.49) a	4.23 (0.49) a	4.21 (0.40) a	4.04 (0.53) a	4.25 (0.59) a

Data are means (standard error). Means sharing the same letter within a row are not significantly different from one another at $P < 0.05$. Lower case letters represent treatment differences; upper case letters represent differences between sampling dates.

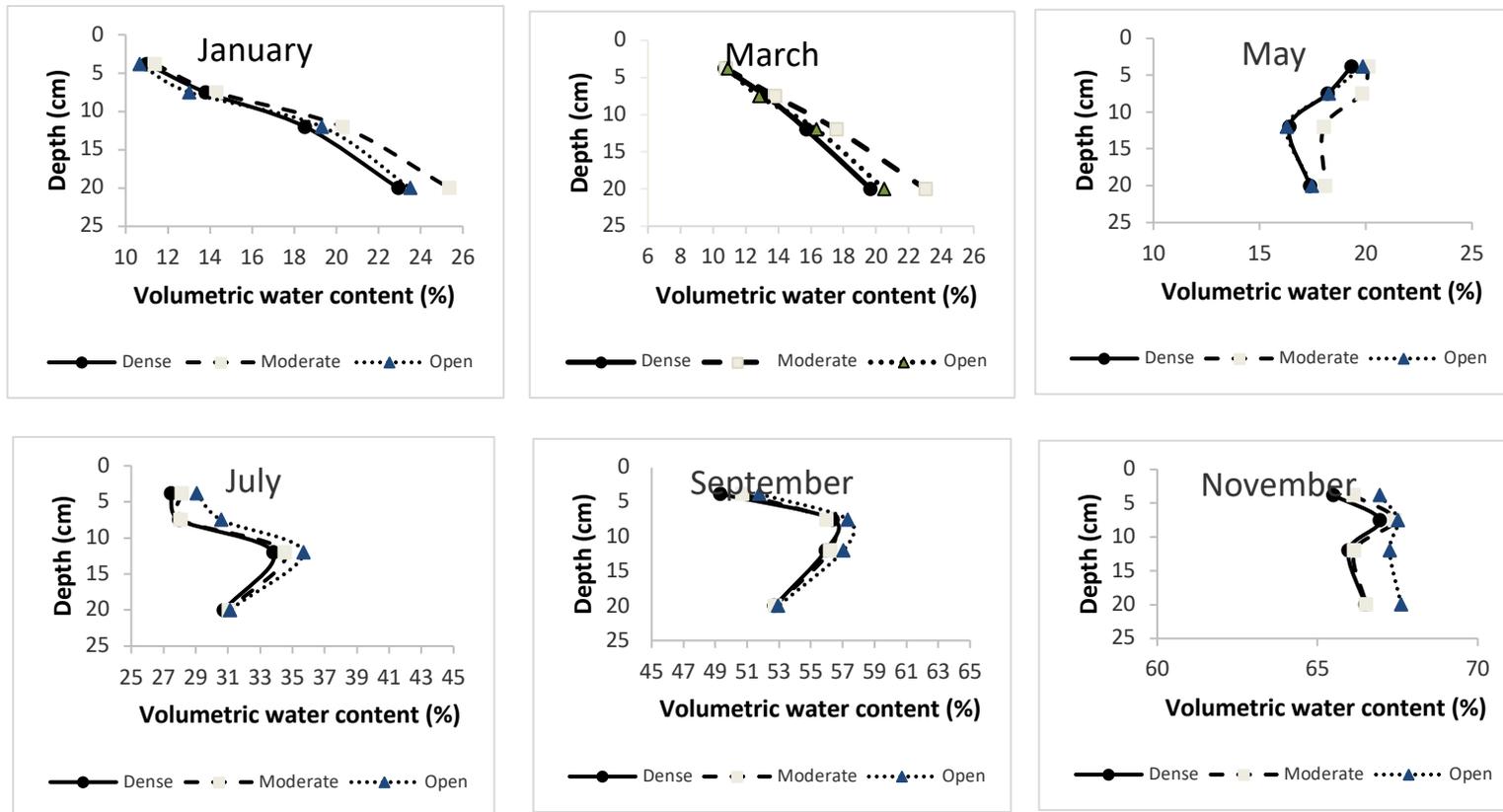


Fig. 9 Volumetric water content (%) measured during 6 months in 2016 in open (O), moderate (M), and dense (D) treatments. Although no significant differences existed between treatments, several interesting patterns can be observed. X-axes are plotted on different scales for ease of visualization.

Discussion

Forage Dry Matter Production

Treatment O produced significantly greater quantities of dry matter than M and D treatments. Forage yield declined by approximately 50% in each treatment along the shade gradient, demonstrating that the C4 grass *M. maximum* and its cultivars respond vigorously to increased solar radiation. A widely accepted ecological tenet is that generally, as light intensity decreases, photosynthesis slows and herbage production decreases (Lin et al. 2001; Feldhake et al. 2010). This is especially true for C4 species, whose physiology allows for greater biomass accumulation per unit of photosynthetically active radiation (PAR) – or radiation use efficiency – than does the physiology of C3 species. However, research examining the effects of tree cover on the productivity of *M. maximum* has drawn mixed conclusions. Obispo et al. (2008) studied the growth of *M. maximum* in open pastures in northern Venezuela and found significantly greater quantities of dry matter production when compared with low, medium, and high tree densities. A study conducted in Veracruz, Mexico, concluded that there was a marginal forage reduction of 30% under a canopy of *Gliricida sepium*, but there was no evidence for a significant negative effect of tree canopy on *M. maximum* mediated by a reduction in PAR (Medinilla-Salinas et al. 2013). Similarly, Viáfara et al. (1997) reported neutral effects of tree cover when grown under a sparse canopy of the nitrogen-fixing tree *Albizia saman* during the dry season in a tropical dry forest. Several studies have demonstrated that under certain conditions, moderate shading can provide optimal conditions for grass growth and quality (Belsky 1994; Ibrahim et al. 2007; DeBruyne et al. 2011; Orefice et al. 2016). Hernández and Guenni (2008) concluded that *M. maximum* grasses benefited

from a compensatory effect from trees that increased soil humidity and improved total forage biomass. Andrade et al. (2004) found that Massai grass growing under artificial shade reached its highest dry matter accumulation rate under 30% shade cover in both the rainy and dry seasons. The 48% reduction in basal area realized for the M treatment in our study allowed for as much as 50% shading in some areas, evidently too much shade for optimal production of this species. One important detail to note from our study is that when the trees were planted in 2005, they were planted on contour lines, resulting in plantations that look more like natural forests and less like evenly-spaced plantations. This resulted in a patchy distribution of sun flecks reaching the ground, with some areas receiving no radiation and others receiving much more. Planting trees more evenly across the landscape, at lower densities, could increase the overall forage yield.

Reported dry matter yields for *M. maximus* and its cultivars vary widely, with open pasture wet season estimates ranging from 2,400 kg ha⁻¹ (Brâncio et al. 2003) to as much as 20,900 kg ha⁻¹ for open pastures fertilized with 150 to 200 kg ha⁻¹ of nitrogen (Fernandes et al. 2014). These estimates depend greatly on site-specific climatic and edaphic conditions, management, and sampling protocol; thus, comparisons should be made cautiously. Our average open pasture wet season estimates for Massai, Mombaza, and Tanzania were 4972.76 ± 343.08, 4855.05 ± 252.01, and 4901.92 ± 288.59 kg ha⁻¹, respectively. Unsurprisingly, pasture productivity differed significantly between the wet and dry seasons, with the wet season producing on average 81, 89, and 88.6% more forage than the dry season for Massai, Mombaza, and Tanzania, respectively.

Our finding that Massai produced significantly more dry matter than Mombaza and Tanzania is not dissimilar from data presented elsewhere. Correa et al. (1998)

reported dry matter weights of 4,000, 3,600, and 3,300 kg ha⁻¹ at a cutting interval of 35 days for Massai, Mombaza, and Tanzania, respectively. Fernandes and colleagues (2014) evaluated the growth of 24 fertilized *M. maximus* genotypes over a two-year period and reported average dry matter yields of 16,750, 15,650, and 13,900 kg ha⁻¹ for Massai, Mombasa, and Tanzania, respectively. When different harvest periods and plant components are analyzed, cultivar comparisons are not as simple. Braga and colleagues (2014) found that Mombasa produced 855 kg ha⁻¹ more dry matter than Massai during the establishment phase, but produced 1427 kg ha⁻¹ less dry matter during the maintenance phase. In another study comparing the three varieties, Brâncio et al. (2003) reported the highest density of dry matter and leaf:stem ratio for Massai, but also found that it had the lowest leaf:dead material ratio. In that same study, Mombaza presented the poorest results, with the lowest leaf:stem ratio.

Although we did not find a significant difference in dry matter production between Mombaza and Tanzania, Tanzania consistently produced marginally greater dry matter than Mombaza. This finding contradicts the results of several other studies that conclude that Mombaza is more productive than Tanzania (Cecato et al. 2000). Santiago-Hernández et al. (2016) examined the growth of Mombaza and Tanzania growing in *Melia azedarach* L. plantations and found that Mombaza produced more forage biomass than Tanzania, with reported mean values of 4,683 and 4,279 kg ha⁻¹ harvest⁻¹, respectively. They also reported that total forage biomass was significantly greater during the rainy season than during the non-rainy season and that biomass declined significantly by 44% under full shade (1000 trees ha⁻¹ at 11.0 cm mean DBH).

Tanzania consistently produced numerically greater quantities of dry matter than Mombaza in all three treatments, so it is not likely due to differences in shade tolerance, but rather to some other factor. It is known that Mombaza and Tanzania are more demanding than Massai in terms of soil fertility (Brâncio et al. 2003) and it could be that Mombaza is even more nutrient-demanding than Tanzania. None of the treatments were ever fertilized over the course of the study, so the grasses had to rely solely on soil nutrient reserves. This is, however, speculation, and needs further research for confirmation.

Forage Nutritive Value

Our hypothesis predicting increased levels of CP in the shade was supported, indicating that trees may provide an added benefit to forage nutritive quality. Increased CP values for forages growing under shade is commonly reported in the literature (Lin et al. 1998, 2001; Feldhake et al. 2010; Neel and Belesky 2017; Orefice et al. 2017) and is likely due to adaptive mechanisms and changes in plant physiology such as elongation of the cell wall (Kephart and Buxton 1993), an increase in specific leaf area, and a decrease in the root:shoot ratio (Paciullo et al. 2017). The presence of nitrogen-fixing trees may also indirectly increase CP levels in forages through leaf decomposition, root exudation, and direct nutrient exchange (Sierra and Nygren 2006; Sierra et al. 2007; Jalonen et al. 2009^a).

In our study, the highest CP values ranged between 7.78 and 8.17 for the O treatment, 8.92 and 10.16 for the M treatment, and 9.12 and 10.65 for the D treatment. Generally, forage CP values between 11 and 12% are recommended for good

performance of beef cattle, and values become critical when they drop below 7% (Wright et al. 2010). A number of authors have reported much higher CP values for *M. maximus*. Viáfara and colleagues (1997) found CP values as high as 18% in Guinea grass growing under intermediate shade cover during the dry season. Obispo et al. (2008) reported CP values between 12 and 13.2% for Guinea grass growing under trees and values as high as 39.2% CP were reported for Guinea grass growing with the nitrogen-fixing tree *Gliricidia sepium* (Medinilla-Salinas et al. 2013).

From the ADF, NDF, TDN, and RFV results, we conclude that forage nutritive quality increased with increasing shade levels. Pooled mean ADF values for O, M, and D treatments were 43.20, 37.60, and 37.55%, respectively, while pooled NDF values for O, M, and D treatments were 64.01, 59.86, and 59.57%, respectively. In general, ADF and NDF show a high negative correlation with TDN and RFV, due to the presence of greater quantities of lignin and cellulose, which ultimately degrade the forage dry matter digestibility. Typically, ADF and NDF concentrations of most forage species are either increased or unaffected by shade (Ladyman et al. 2003; Kallenbach et al. 2006; Sousa et al. 2010; Paciullo et al. 2014; Neel and Belesky 2015). However, there are a number of studies that report decreasing values with increased levels of shade (Obispo et al. 2008; Medinilla-Salinas et al. 2013). The lower ADF and NDF values reported here could be due to grass developing more elongated sclerenchyma under shaded conditions (Paciullo et al. 2017).

We found that Massai had significantly greater ADF and NDF concentrations than Tanzania, but not Mombaza. This coincides with that reported by Brâncio et al. (2002), who claimed that Massai was a relatively low nutritive cultivar, with ADF values

around 44.2%, slightly higher than the 43.1% reported for Mombasa and Tanzania. Machado et al. (1998) claimed that Massai had above average ADF and NDF when compared to that of Mombasa and Tanzania, with respective values of 37.9, 37.1, and 36.0% for ADF and 75.5, 74.3, and 73.2% for NDF. This may reduce dry matter digestibility and animal productivity in Massai pastures when compared to other *Megathyrsus* cultivars (Brâncio et al. 2002; Euclides et al. 2014; Paciullo et al. 2017).

Season had a significant effect on all nutritive value measurements, with grasses sampled during the dry season showing higher CP, TDN, RFV, and lower ADF and NDF values than those sampled during the rainy season. This finding is supported by Santiago-Hernández et al. (2016), who found that overall, grass nutritional quality increased during the non-rainy season and under shade. Additionally, Wilson (1983) showed grasses that were drought-stressed tended to have higher in-vitro dry matter digestibility and overall nutritional quality. All three cultivars responded similarly to nutritive quality changes in regards to season, without any major differences between the three varieties.

Soil Properties

Certain locations on the Azuero Peninsula are known to have relatively fertile soils for Panama (Wishnie et al. 2007; Breugel et al. 2011; Griscom et al. 2011). Results of this research demonstrating high BS and relatively high CEC values corroborate this; however, overall low SOM and concentrations of essential nutrients such as P and N are indicative of a history of soil degradation, likely due to decades of extensive cattle ranching. Our hypothesis predicting significantly higher fertility levels under increasing tree cover was not supported, despite the presence of N-fixing trees in M and D.

The overall C:N ratio was similar to that which has been previously reported from grasslands and forests in Panama (Paul and Weber 2016) but neither TN nor TC differed significantly among treatments or years. This differs from the findings of Krieb and Pacala (2012), who compared soils collected from pasture and reforested areas on the Azuero Peninsula and found that both TC and TN were significantly greater under tree cover than in open pasture. Macedo et al. (2008) compared the soil fertility of an open pasture dominated by Guinea grass (*M. maximus*) with a thirteen-year old plantation dominated by fast-growing leguminous nitrogen-fixing trees and found that C and N stocks in the plantation recovered to levels found in nearby surrounding forest. Although significant differences did not exist between treatments, the authors concluded that reforested areas were in the process of recovery. Alternatively, our finding is supported by the work of Sandoval-Pérez et al. (2009) who found that C and N variables did not differ between primary forests, 26-year old pastures, and 26-year old secondary forests in Jalisco, Mexico.

Silvopastures, being a combination of both trees and grasses are inherently complex and there is still much to learn about how system components cycle and utilize nutrients. C₄ grasses and perennial woody species have different physiologies and have been shown to cycle nutrients differently. Reiners et al. (1994) suggested that reforested areas may not show an increase in total C or N because pasture grasses remaining on the land can contain reasonable amounts of carbon. In this same study, the authors reported greater nitrogen mineralization rates in forests than in open pastures, a finding that has been corroborated by other authors (Neill et al. 1995; Vitousek 2013). In our study, TN

did increase marginally with increasing levels of shade, so it is possible that with time, TN could eventually reach levels that are significantly different from open pasture.

The most drastic difference found between treatments in regards to the major cations was for exchangeable K^+ . Open pastures contained significantly greater concentrations of K^+ than either of the two forested treatments. This finding corroborates results presented by other authors, who studied changes in soil chemistry after conversion from forest to pasture (Reiners et al. 1994; Krieb and Pacala 2012), but contrasts with the results of other studies (Belsky et al. 1989; Casals et al. 2013). It is likely that K^+ is inextricably linked to the acidity of the soil, with greater K^+ concentrations occurring in less acidic environments due to greater exchange site availability at the soil particle surface. However, if this were the sole reason for such a difference, one would expect higher concentrations of alternative exchangeable bases in the open pasture, which we did not observe. It could be that relatively young, fast-growing tree species are highly demanding of K^+ in the dry tropical forest or that pasture grasses are more K^+ -efficient than woody perennials (Krieb and Pacala 2012).

The literature is contradictory in terms of the major base cations and their relationship to tree cover. Studies have shown that nutrients can both increase and decrease with tree cover and direct comparisons are of limited use due to the existence of a wide range of soil types, research methodologies, tree densities, and species compositions (Belsky et al. 1989). Although relatively low, concentrations of P increased significantly in our study from 2015 to 2017 in the M and D treatments. These results complement the findings of Casals and colleagues (2013), who found that the presence of scattered trees in seasonally dry tropical pastures in Nicaragua increased overall plant

available P, among other soil fertility parameters. Similarly, Krieb and Pacala (2012) reported higher cation concentrations in older reforested plots.

Soil pH was significantly lower in the M and D treatments than it was in the O treatment. This is in agreement with other studies (Reiners et al. 1994; Sandoval-Pérez et al. 2009) that report increasing acidity under trees, but it differs from the findings of Krieb and Pacala (2012), who reported the opposite trend for soils collected on the Azuero Peninsula. Reiners et al. (1994) explained that grasses cycle base cations more rapidly and generate fewer and less persistent organic acids than do forests. This, along with a long cultural history of burning pastures likely contribute to the lower pH observed in the O treatment. Burning pastures creates a base-rich ash deposition, perpetuating long-lasting increases in pH and base saturation (Reiners et al. 1994). Historically, all treatments were seasonally burned. Burning was discontinued in the plantation in 2005 upon tree planting while it was continued yearly in the open treatment to facilitate the establishment of maize. Frequent and periodic pasture burning may also be a reason for the aforementioned increases in exchangeable K and marginal increases in CEC in the O treatment.

Our results showing significant differences between treatments and soil depth for bulk density are widely supported in the literature (Belsky 1994; Staley et al. 2008; Orefice et al. 2017). Bulk density decrease is one of the most commonly recorded improvements in soil quality after reforestation of pastures (Li and Shao 2006), as tree roots create important macropores in the soil (Sandström 1998), and the removal of livestock often reduces compaction (Sharrow 2007). Our findings are similar to those presented by Orefice and colleagues (2017), who found that bulk density increased

significantly in open pasture and silvopasture and differed significantly from forest after two years of management. Bulk density also has important implications for soil moisture, in that it directly influences the rate and conversion of precipitation into plant available soil water and other physical properties such as porosity and hydraulic conductivity (Li and Shao 2006).

Soil Volumetric Water Content

As expected, there were strong differences between sampling periods, reflecting variable precipitation throughout the year. Our finding that soil moisture increased with depth did not come as a surprise, as superficial soil layers are directly exposed to atmospheric fluctuations, such as radiation, heat, wind, evaporation, litterfall, and bioturbation. Our hypothesis predicting greater moisture availability in the M treatment during the dry season was partially supported. During the month of February, Massai showed strong resilience to drought in moderate shade, accumulating more biomass than that of Tanzania and Mombasa for both O and D treatments (Fig. 6). Soil moisture levels have been shown to directly affect grass productivity and longevity in seasonally dry areas. In a study examining the growth of Massai under four artificial shade levels, Andrade et al. (2004) claimed that shaded treatments alleviated water stress during the dry period and decreased the seasonality of production of the forage grass. As shade level increased, the difference between the accumulation rate of dry matter between the wet and dry seasons decreased.

We noted several interesting patterns that are worth discussing. In the two driest months (January and March), all three treatments varied little in soil moisture at the

shallowest depths, but showed greater differences at the deepest depth (Fig. 7). In January, the M treatment had 2.42 and 1.86% more soil moisture than the D and O treatments at the 20 cm depth, respectively. In March, the M treatment had 3.36 and 2.53% more soil moisture, respectively, and in May, the M treatment had 0.71 and 0.61% more soil moisture than the D and O treatments, respectively. The M treatment also showed numerical differences at the 12 cm depth, with 1.64 and 1.76% more moisture than the D and O treatments at that depth, respectively. During the rainy months (July, September, November), soil moisture was marginally, yet consistently greater in the O treatment (Fig. 6). These differences are small, but biologically, in an environment where water is severely limited, they may prove to be important for plant production and longevity.

Hydrologic implications of changes in vegetation cover are controversial, and results vary widely with changing edaphic and climatic factors. A number of studies have shown strongly reduced streamflow and groundwater recharge after afforestation and reforestation has been scrutinized because of this (Calder et al. 2004; Farley et al. 2005). Some authors have touted the hydraulic benefits of planting trees and have encouraged their use in seasonally dry ranching systems to mitigate the effects of harsh seasonality (Vetaas 1992; Hernández and Guenni 2008). It cannot go without saying, however, that the hydraulic effects of trees are always site-specific, and there are circumstances where trees have a neutral (DeBruyne et al. 2011) or even negative effect (Wilson 1998) on water supply.

The soils identified in this study are finer textured and heavily-weathered, requiring continual organic input from leaves and bioturbation from roots to maintain

their structure and porosity (Malmer et al. 2010). When removed of vegetation, these soils become vulnerable to erosion and land degradation when exposed to intense rainfall, which ultimately results in reduced rates of infiltration, less groundwater recharge, and a loss of dry-season water sources (Sandström 1998). Immediately after harvest, water runs off the surface and results in short-term increased water yield, but unlike in temperate regions, semi-arid and arid regions experience little piston-flow recharge and much of the moist soil water returns to the atmosphere by evaporation (Bruijnzeel 2004). When this is coupled with increased compaction from tree harvesting skidders, roads, and ranching activities, infiltration is even further limited. Under these conditions, macropores created by tree roots become the only active mechanism for soil water recharge.

Although not significant, our results demonstrating marginally lower soil moisture concentrations for the D and O treatments indicate that soil moisture may be dependent on tree density. It could be that there is an optimal number of trees ha^{-1} to maximize soil water storage, beyond which they begin to deplete storage. This idea, known as *optimal tree cover theory*, was tested by Ilstedt et al. (2016) in a seasonally dry agroforestry parkland in West Africa. Using groundwater budgets calibrated with measurements of drainage and transpiration, the authors found that 16% of the annual rainfall percolating to a depth of 1.5 m occurred around the edge of the tree canopy. This number was reduced to 1.3% in open areas, 37 m away from the nearest tree. They also found that in open areas, the date at which the first draining water was recorded was positively related to the distance of the nearest tree, with areas closest to trees receiving water about 30 days earlier than those located furthest away. Based on these results, the authors recommended a maximum density of 16 trees ha^{-1} to maximize soil water recharge for

this site, however, if crowns are actively pruned groundwater recharge may remain positive for up to as many as 60 trees ha⁻¹ (Ilstedt et al. 2016).

The marginally greater amounts of moisture in deeper soil layers under moderate tree cover observed in this study during the driest months could be explained by mechanisms similar to those described by Ilstedt et al. (2007). The main positive hydraulic benefit of trees is increased infiltration rates, especially in soils that are prone to surface run-off. Trees that shed their leaves during the dry season, like the majority of those studied here, can provide further enhancement for groundwater recharge by contributing organic matter to the soil via litterfall and reducing transpiration rates during the dry season. Ilstedt et al. (2016) recorded no percolation under the canopy, presumably due to interception and transpiration, but drainage improved directly at the edge of the canopy. Maximizing the canopy-edge interface could positively affect groundwater recharge. In fact, Ilstedt and colleagues (2007) simulated different canopy cover densities and found that the largest tree to tree distance achieved 35% higher groundwater recharge than the average random distribution.

Another explanation for marginally lower moisture values in the O treatment during the dry season could be due to evapotranspiration caused by heavy winds and high temperatures (Lin 2010). Grasses in the M and D treatments were provided with shade that has been shown to lower average surface temperatures by as much as 3° C and evapotranspiration by 1.8 mm day⁻¹ in the tropics (Murgueitio et al. 2011, CIPAV unpublished data). Trees also provide a barrier from the harsh winds experienced on the Peninsula, which experiences average wind gusts of 36.7 km hour⁻¹ during the month of February (worldweatheronline.com). Our result showing that soil moisture was

marginally, yet consistently greater in the O treatment during the rainy season can be explained best by a lack of canopy interception. During the rainy season, trees in the M and D treatments with fully developed leaves likely intercepted raindrops, preventing them from reaching the forest floor. Contrarily, in the O treatment, raindrops fell directly on top of grasses and the soil surface.

Conclusions

It is evident that grass productivity and nutritive quality are influenced by a plethora of factors, including but not limited to climatic, edaphic, structural, and ecological factors, along with past and current land use management strategies. This study confirmed that all three cultivars of *M. maximus* responded vigorously to sunlight, significantly increasing dry matter production with increased levels of solar radiation. We also confirmed that the cultivar Massai produced significantly greater amounts of forage than Mombasa, but not for Tanzania. Interestingly, Massai showed the greatest tolerance to drought, producing the most dry matter forage accumulation in the early part of the dry season, but only for areas with moderate (M) tree density. This suggests that, during the early part of the dry season, microclimatic conditions for grass growth are optimal in areas with moderate densities of trees. Drought conditions cause grasses to mature earlier, becoming inadequate to meet demands for animal production. This observed extension of the growing season, coupled with thermal stress reduction provided by moderate tree cover (McIlvain and Shoop 1971; McArthur 1991; Murgueitio et al. 2011), could result in improved animal weight gains between the months of January and April at this study site. The use silvopasture during times of the year when it may be preferable to open

pasture has been recommended in combination with traditional systems for temperate ranching. Kallenbach (2009) showed that cattle provided access to silvopasture retained greater weights and weaned heavier calves than those in the open pasture. Research is needed on animal productivity in silvopasture studied here to determine if similar benefits can be realized in the tropics.

It is critical to note that productive swards of *M. maximus* will not be achieved without proper nutrient and livestock management, regardless of overstory tree density. Tropical grass growth is severely limited by N and P deficiency (Silveira et al. 2015), and results presented here show no indication – at least in the short-term – of beneficial changes in soil chemical properties due to higher tree densities. In fact, the highest soil fertility existed in the O treatment, likely due to historical burning and fertilizing that did not take place on the tree plantation. To increase overall forage production, nutritional value and overall livestock performance at the IDB Forestal ranch, we recommend either annual inputs through responsible N and P fertilization or the incorporation of nitrogen-fixing legumes such as *Arachis pintoi* and *Pueraria phaseoloides* (Andrade et al. 2004). We also recommend the continued use of managed intensive rotational grazing (MIRG), with paddock occupancy determined by a sward height of 70 cm (Euclides et al. 2014) as opposed to a fixed-length rest period. All *M. maximus* cultivars have a tendency towards excessive growth of stems when there is no control of sward height (Da Silva et al. 2009). Use of target sward heights in pre- and post-grazing helps to control the growth of stems that can be harmful to intake and cattle performance (Benvenuti et al. 2008).

Results from this study are intriguing, encouraging further research on the topic. However, these results should be interpreted with caution due to several limitations that

we could not avoid. Since the establishment of the plantation 15 years ago, land use and management of the O treatment has been different than that of the M and D treatments, resulting in different soil chemical and physical characteristics. We sampled soils previous to the study for all treatments and found that differences existed only for pH, bulk density, and K^+ , with similar values for all other nutrients. This compromises the homogeneity of all three treatments and makes it difficult to conclude that differences observed were due to the treatment and not to some other confounding variable. The use of adequate sample sizes and the utilization of a mixed model to account for random variation in the study were measures we took to account for these differences, decrease overall variation, and achieve replicable results. Nonetheless, caution should be heeded in their interpretation and they most certainly should not be extrapolated and applied to silvopastoral systems with different climates, soils, and ecological communities. Further research is needed to corroborate the results presented here, with greater emphasis placed on sparse tree canopies (10-30% shade) and how they affect animal production.

With climate change worsening and drought conditions becoming more prevalent and pervasive on the Azuero Peninsula, the integration of trees into ranching systems may serve as an important buffer and defense against severe losses in production. The same trees that ranchers in Los Santos once regarded as incompatible with cattle production may, in fact today, be an indispensable component during the dry season.

Chapter 3

Can Fodder Shrubs Act as Fertilizer? The Neighborhood Effect of Shrubs on Soil Fertility, Pasture Production, and *Albizia saman* Growth and Survival in Simulated Silvopastures in Panama

Abstract

The nutrients nitrogen (N) and phosphorus (P) limit the growth of pasture plants in many terrestrial ecosystems, and in developing countries, the use of fertilizers by small landholding farmers is often not economically feasible. Fertilizer trees are trees that can fix atmospherically available N and effectively cycle major nutrients. The use of fertilizer trees in ranching systems could serve to intensify agricultural production while maximizing conservation benefits. We examined the neighborhood effects of the fertilizer shrubs *Leucaena leucocephala* and *Tithonia diversifolia* on the native tree species *A. saman* and exotic grass *Megathyrus maximumus* var. Massai. We hypothesized that fertilizer trees would significantly increase important soil fertility indicators such as Cation Exchange Capacity (CEC), soil organic matter (SOM), major cations Ca, K, and Mg, and plant available N and P and decrease bulk density. We expected water availability to be limited, particularly during the dry season and in plots with shrubs. We also hypothesized that shrub plots would significantly increase the DM productivity of Massai due to nutrient provisioning. Lastly, we hypothesized that *A. saman* saplings surrounded by shrubs would grow significantly faster and have higher survival rates than those planted without neighbors. Two years after planting, bulk density was significantly reduced in all plots except for C, where the soil was left bare. There were significant increases in Ca, K, CEC and SOM in plots that included shrubs throughout the course of the study. Additionally, plant available N and P increased in plots containing *L. leucocephala* and *T. diversifolia* shrubs. There were no planting regime effects on soil water availability and *A. saman* sapling moisture stress. Massai grass DM production showed a marginally significant positive response to the association with *L. leucocephala*, and although not significant, was reduced in the presence of *T. diversifolia*. *A. saman* growth and survival was not affected by planting regime. cumulative forage production in plots with shrubs provided on average 10 % more forage than plots without shrubs during the dry season. Although the findings presented here indicate facilitation by *A. saman* and *L. leucocephala* on surrounding pasture growth, plantations are still in their early stages, and results should be heeded with caution. Combining grasses with shrubs and trees creates three distinct foraging layers that can help mitigate forage losses during severe droughts and provide nutritional diversity to livestock.

Key Words: Silvopasture, fodder shrubs, soil fertility, dry matter production, seedling growth

Introduction

The Green Revolution Problem

In many terrestrial ecosystems, water and nutrients, especially nitrogen (N) and phosphorus (P), limit the growth of plants. Consequently, these limiting factors also pose as major constraints to agricultural production (Matson 1997). Changing land-use practices have enabled commodity crop harvests to double in the past four decades, largely due to production gains resulting from “Green Revolution” technologies, including high-yielding cultivars, chemical fertilizers and pesticides, and mechanization and irrigation (Foley 2005). Thus, the use of synthetic inorganic fertilizers has increased dramatically, especially since the late 1960s.

In many developing countries of the world, the use of fertilizers by small landholding farmers is often not economically feasible, due to increasing prices, inaccessibility, and more frequent droughts (Garrity et al. 2010). Additionally, the over-application of nitrogen and phosphorus fertilizers have been a cause of concern for non-point source environmental pollution (Carpenter et al. 1998), human health issues, and increased greenhouse gas emissions such as nitrogen oxides (Nox), carbon dioxide, and methane (Snyder et al. 2009). As a result, progressive approaches have been taken throughout the world to incorporate naturally occurring facilitative plant interactions into farming systems to enhance productivity.

Organic matter is a well-known source of nutrients, however neither animal manure nor green biomass is usually found in adequate quantities to meet the high application rates (10-40 Mg ha⁻¹ yr⁻¹) required to satisfy the nutrient demand of commodity crops (Mafongoya et al. 2006). Arguably, the most renowned approach is

Evergreen Agriculture. Evergreen farming systems feature both perennial and annual species (trees and food crops) that are maintained and managed on the land throughout the entire year (Garrity et al. 2010). This practice contributes to integrated soil fertility management (ISFM), which maximizes fertilizer and organic resource use efficiency and crop productivity (Sanginga and Woomer 2009). This approach is also associated with reduced tillage, increased residue retention on the soil surface, minimal disturbance, and the incorporation of fertilizer trees.

The term ‘fertilizer tree’ is commonly used to refer to the utilization of N-fixing trees in agricultural production systems to improve the availability of N to crops or to rehabilitate degraded lands (Ajayi et al. 2011; Sileshi et al. 2014). Through biological nitrogen fixation, some trees can replenish soil N reserves by capturing atmospheric nitrogen and making it available in the soil (Nygren et al. 2012). There are additional trees that can recycle the soil’s phosphorus (P), calcium (Ca), magnesium (Mg) and potassium (K), but these macronutrients must be sourced externally when they are highly depleted from the soil. The appropriate selection and use of fertilizer trees in agroforestry systems could serve to intensify agricultural production while maximizing conservation benefits (Phalan et al. 2011; Tschardtke et al. 2012).

Integrating Fertilizer Trees into Prevailing Land-use Strategies

The threat of conventional agricultural expansion and the resulting native vegetation and soil loss is now predominant throughout many regions of the world. This, coupled with the ever-worsening effects of climate change, has resulted in a surge of interest in ecological restoration. In Panama, reforestation has been widely promoted and

implemented over the last two decades (Lamb et al. 2005; Wishnie et al. 2007; Breugel et al. 2011; Hall et al. 2011a, b). However, the land, labor, and financial resources required to establish tree plantations, coupled with a lack of economic returns within the first 5–10 years, are often insurmountable deterrents for landowners (Garen et al. 2009; Paul and Weber 2016). In order for reforestation to be accepted as a viable land use strategy in Latin America, it must be economically viable (Minnemeyer et al. 2011; Calle et al. 2012a). The planting of commercially valuable timber trees as a restoration strategy could be more widely accepted if simultaneously combined with either food and energy crops in “Taungya” agroforestry systems (Schlönvoigt and Beer 2001; Paul and Weber 2016) or with important multipurpose ‘fertilizer’ trees in silvopastoral systems (Plath et al. 2010).

Silvopastoral systems that combine timber trees and fertilizer shrubs with livestock and forages are considered to be a promising option for the restoration of native tree species in extensive pastures throughout cattle-ranching regions (Plath et al. 2010; Murgueitio et al. 2011; Riedel et al. 2012). Trees provide innumerable ecosystem services, including erosion control, pest control for crops or high value timber trees (Wagner et al. 1996; Hall et al. 2011a), and wildlife habitat. Trees and shrubs can also provide alternative and highly nutritious forage sources during critical periods of the year. In seasonally dry areas of the tropics, ranchers cannot rely on dependable year-long grass productivity. During droughts, grass productivity slows or ceases, and increasing the availability of highly palatable tree fodders is a cost-effective way for ranchers to bridge the dry-season fodder gap (Morrison et al. 1996). Moreover, the integration of fodder shrubs may improve and maintain soil fertility, while possibly providing natural

nutrient supplies to surrounding crops (Nygren and Leblanc 2009) or pasture grasses (Sierra et al. 2007; Daudin and Sierra 2008; Jalonen et al. 2009b).

Much like natural systems, productivity of an agroforestry system is ultimately the net result of negative and positive interactions among the components (Jose et al. 2004; Jose et al. 2019). In perennial intercropping systems, both aboveground and belowground competitive and facilitative forces act to determine plant survival, productivity, and overall yield in the first few years after plantation establishment (Jose et al. 2006; Kelty 2006). Intercropping might result in substantial silvicultural advantages, including improved microclimate, reduced soil evaporation and improved soil fertility (Vieira et al. 2009). Conversely, competition for light, water and soil nutrients could also serve to reduce or inhibit tree growth. Additionally, exposure to potential allelopathic chemicals or attracted pests could harm tree seedlings (Rao et al. 1998). As important as all of this is, relatively few studies have documented fertilizer tree effects on adjacent site fertility and neighborhood plant productivity in silvopastoral reforestation systems.

Objectives

The goal of this study was to examine the isolated and combined effects of the fertilizer shrubs *Leucaena leucocephala* and *Tithonia diversifolia* on soil physical and chemical properties, water availability, dry matter production of the improved grass *Megathyrus maximus* var. Massai (hereafter referred to as Massai), and the growth rate and survival of *Albizia saman* saplings in the first two years after silvopasture establishment. The objectives of this study were to: 1) establish silvopastures with plantings of *Albizia saman* surrounded by combinations of *L. leucocephala*, *T.*

diversifolia, and Massai on three farms in the province of Los Santos, Panama, 2) describe the differences in soil chemical and physical properties among planting regimes (treatments), 3) quantify differences in water availability by assessing soil volumetric water content (VWC) at four depths and plant moisture stress (PMS) of *A. saman* saplings, 4) evaluate treatment differences in grass dry matter (DM) production, and 5) examine differences in the growth rate and survival of *A. saman*.

We hypothesized that treatments including either of the fodder shrubs together or singly would increase soil fertility indicators such as soil pH, organic matter (OM), cation exchange capacity (CEC), base saturation (BS, %), macronutrients Mg, Ca, and K, and plant available N (nitrate and ammonium) and P due to N-fixation and P mobilization induced by the respective shrubs. We expected fertilizer trees to significantly decrease bulk density over the study. We hypothesized that soil VWC would increase proportionally with soil depth, that it would be greatest during the rainy season, and that it would be significantly lower for plots containing shrubs during the dry season only. We expected *A. saman* PMS to be significantly greater during the dry season as well as for seedlings located within plots containing shrubs due to competition. We hypothesized that plots with fertilizer shrubs would produce significantly more grass dry matter than those without them due to nutrient provisioning. Finally, we expected *A. saman* saplings to benefit from the presence of neighboring fertilizer shrubs, with significantly more saplings surviving and growing faster than those planted without shrubs.

Materials and Methods

Study site

An agroforestry field experiment was conducted between July 2015 and August 2017 at three farms on the Pacific side of Panama in the Province of Los Santos (7°15'30'' N, 80°00'15'' W) (Fig. 1). The study area receives an average of 1678 mm of rain per year, with an extended five-month dry season lasting from December until May (Fig. 2). 2015 – 2016 was a particularly dry year where the El Niño Southern Oscillation (ENSO) event resulted in the third longest dry season on record (173 days) for Panama, with over 90% of the country experiencing severe drought conditions (Bretfeld et al. 2018).

According to the Soil Atlas of Latin America (Gardi et al. 2015), soils are classified as Dystric Cambisols (Cmdy) in Los Asientos and Calabacito and Vertic Luvisols (LVvr) in Pedasi; they are young and relatively nutrient rich, although degraded from decades of overgrazing (Gardi et al. 2015). The topography is undulating, with slopes ranging between 18 and 40% grade (Diogenes Ibarra, personal communication).

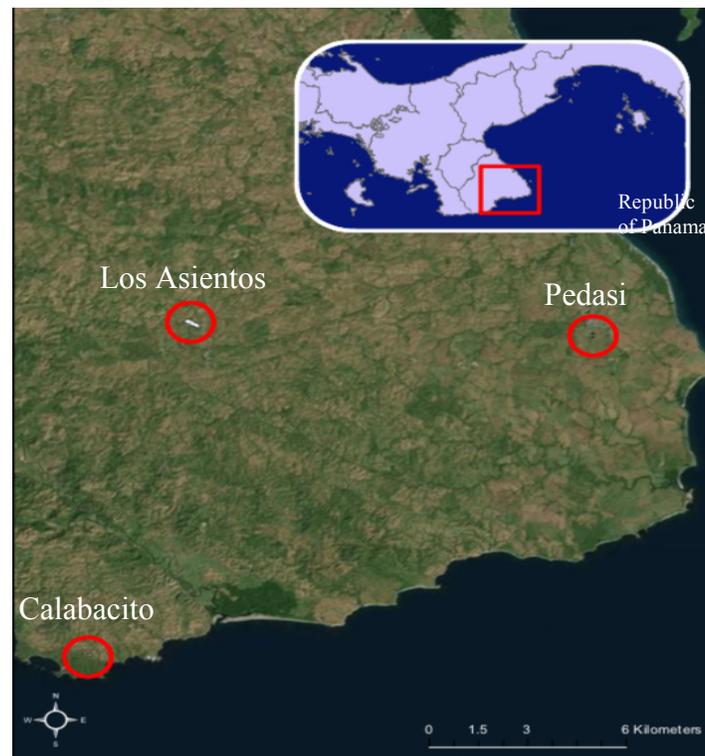


Fig. 1 Locations of the three study sites at the southeastern side of the Azuero Peninsula in the Province of Los Santos, Panama. Each study site was blocked and considered a replicate.

All three sites were grazed extensively historically. Considered part of the dry tropical forest region (Holdridge 1967), the area is a mosaic of pastures dominated by African grasses with scattered *Guazuma ulmifolia* and *Cordia alliodora* trees (Griscom et al. 2011), forested riparian zones, and live fences. Common native trees found in remnant forests include *Anacardium exelsum*, *Enterolobium cyclocarpum*, *Bursera simarouba*, *Calycophyllum candidissimum*, and *Hura crepitans*, among others.

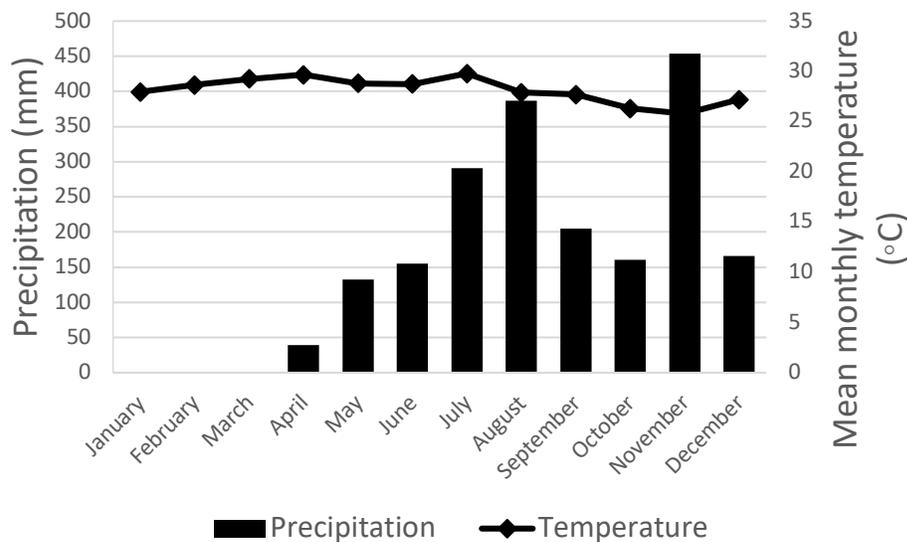


Fig. 2 Precipitation and mean monthly temperature near Playa Venao, Panama in 2016 (A portion of the data was provided by (ETESA 2018).

Confounding factors such as slope, aspect, landform, existing vegetation, and soil type were considered and limited as much as possible in the selection of plots. An effort was made to include ranchers who had expressed an interest in experimenting with silvopasture. Two of the three farms belonged to members of a community Silvopasture Association known as APASPE. Farms were located in Pedasi, Los Asientos, and Calabacito and will be referred to as such throughout.

Species Selections

Overstory Component: Albizia saman

A study looking at the tree planting preferences of landowners in Los Santos concluded that guachapalí de ganado (*Albizia saman*) was one of the most popular trees selected because of its relatively fast growth rate, ability to fix nitrogen, and its

production of edible pods, fodder, and shade for cattle (Garen et al. 2009). Although highly variable between trees and years, *A. saman* can produce up to 70,000 pods tree⁻¹, equating to a total of 272 kg tree⁻¹ year⁻¹ (Janzen 1977). Not only are *A. saman* pods purportedly relished by livestock, but they are produced during the dry season, when herbaceous forages become limited. There have been several reports of increased herbage growth beneath mature *A. saman* crowns, earning the tree the nickname, ‘raintree’, in that the tree was thought to mysteriously produce rain at night to enable this effect (Durr 2010). For the purposes of this experiment, we planted *A. saman* seedlings at high densities (3m x 3m) to encourage growth of straight boles and to minimize crown expansion. Post-experiment, trees will be thinned to a density of around 100 to 150 trees ha⁻¹ to promote pasture and shrub production.

Mid-story Component: Leucaena leucocephala and Tithonia diversifolia shrubs

Two of the most commonly recommended shrubs for tropical intensive silvopastoral systems are *Leucaena* (*Leucaena leucocephala*) and Mexican Sunflower (*Tithonia diversifolia*). These shrubs are periodically coppiced to encourage low, dense growth of the foliage and intermittently browsed by livestock in rotationally grazed paddocks.

L. leucocephala is native to Mexico and naturalized throughout Central and South America. It is extremely palatable to cattle and is tolerant of drought (Vandermeulen et al. 2018a). This species bears root nodules that are formed after infection of the roots with compatible strains of *rhizobium* bacteria, allowing it to fixate atmospheric nitrogen and possibly increase production in neighboring plants (Kadiata 1997). Shelton and

Dalzell (2007) claimed that nitrogen fixed by *Leucaena* returns to the ground and is used by surrounding grasses, increasing the quantity and quality of forage.

T. diversifolia, a shrub belonging to the *Asteraceae*, is native to Mexico and parts of Central America and is now widely distributed in Africa, Asia, and South America. It is a non-legume shrub that grows to a height of 1-3 m, responds well to coppicing, and quickly produces large quantities of biomass. The leaves of *T. diversifolia* have been shown to contain unusually high concentrations of nutrients (N, P, K, Ca, Mg) in comparison with most leguminous species used in agroforestry systems, making it the ideal candidate for the study of interspecific nutrient transfer via leaf decomposition (Jama et al. 2000; Partey et al. 2011). *T. diversifolia* does not fixate atmospheric N and does not constitute a net input of nutrients. Thus, if biomass is taken off the site, nutrients must be replenished from an external source.

Although the exact mechanism for why *T. diversifolia* acquires and accumulates nutrients is still largely unknown, some authors have speculated that high tissue nutrient concentrations of phosphorus (P) may be due to an intense association with vesicular arbuscular (VA) mycorrhizal fungi (Jama et al. 2000; Thor Smestad et al. 2002) and/or plant-moderated alterations to the rhizosphere via root exudation of organic anions (George et al. 2002). Because of this, *T. diversifolia* may have the potential to enhance P availability to neighboring plants because of its access to pools of P that are unavailable to other crops (Jama et al. 2000; George et al. 2001; Cobo et al. 2002). Despite the claims that these two species provide the backbone of intensive silvopastoral systems, their combined effects on soil fertility, neighboring plant growth, and water stress have yet to be assessed and are worthy of study.

Understory Component: African Guineagrass (Megathyrsus maximus var. Massai)

Guinea grass (*Megathyrsus maximus* Jacq.) is one of the most important species for cattle production in tropical and subtropical regions (Paciullo et al. 2017) and despite being a C4 grass, many developed cultivars show medium levels of tolerance to shade (Santiago-Hernández et al. 2016). The genus *Megathyrsus* has an array of diversity, with most species standing erect, from 0.5 to 3.5 m high and is recommended for regions with annual rainfall from 800 to 1800 mm in well-drained soil with medium to high fertility (Muir and Jank 2004). The cultivar Massai was developed for increased productivity with simultaneous drought and shade tolerance characteristics and is currently among the most popular Guinea Grass cultivars in Latin America (Paciullo et al. 2017).

There has been some concern that the aggressive nature of non-native improved pasture grasses may inhibit the growth of associated woody species. However, Andrade and colleagues (2008) suggested that improved grasses may help to stimulate root growth of adjacent trees in a water competitive environment. This root growth could be associated with an intensification of hydraulic redistribution of water and nutrients, contributing to the longevity of forage production in the dry season (Richards and Caldwell 1987)

Planting and Plot Maintenance

On August 13, 2015, we initiated the establishment of simulated three-strata silvopastoral systems at each of the three farms. *Albizia saman* seedlings (25 cm average height) were purchased from a local nursery and planted as the overstory component¹; *L.*

¹ Initially, *Albizia saman* and *Tabebuia guayacan* seedlings were planted on two separate side by side plots on each farm in a split-plot design. *T. guayacan* seedlings suffered high mortality the first dry season

leucocephala and *T. diversifolia* were simultaneously planted as the mid-story component; and Massai grasses were broadcast seeded on June 5, 2016 to establish the understory component (Fig. 3). *L. leucocephala* seedlings (25 cm in height) were also purchased from a local nursery, where they were inoculated with a liquid strain of *rhizobium* bacteria one week after seed-sowing around the root system of the plant (Forestier et al. 2001). Stem cuttings of *T. diversifolia* were taken from a nearby fodder bank and propagated vegetatively (Fig. 4).

We consider this a simulated silvopasture because all livestock were excluded from plots with fences for the entire two-year study, necessary as per on-farm trials conducted by Hall et al. (2011a). Livestock presence was simulated by periodic defoliation events, described in detail below.

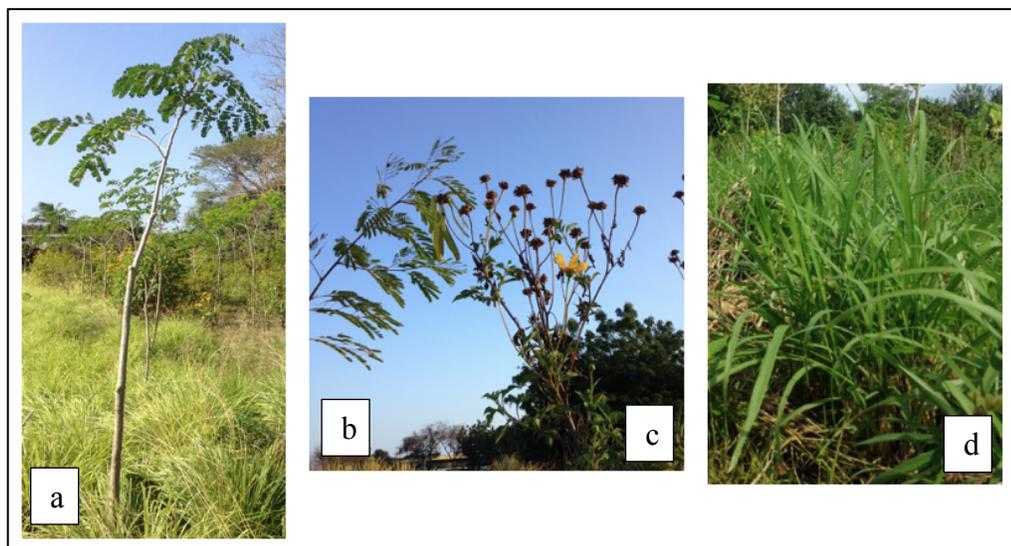


Fig. 3 The four components of the three-strata silvopastoral system established on three farms in the Los Santos, Panama: a) *Albizia saman* in the overstory, b) *Leucaena leucocephala* and c) *Tithonia diversifolia* in the mid-story, and d) Massai grasses in the understory.

and due to cost limitations were not replanted. Subsequently, all *T. guayacan* plots were abandoned. Please see Appendix B for data collected on *T. guayacan* seedlings one year after planting.



Fig. 4 Planting perennial plants for the establishment of silvopastoral systems in Los Santos, Panama. The photo on the bottom left shows several hundred *Tithonia diversifolia* stem cuttings ready for planting.

Experimental Design

A factorial randomized complete block design with three replications (sites) was used. Eight uniform 15m x 15m plots were established at each site. Twenty-five *Albizia saman* seedlings were planted on each of the eight plots on a 3m x 3m spacing and surrounded by varying combinations of plants. *L. leucocephala*, *T. diversifolia*, and *M. megathyrsus* var. *Massai* were either present or absent in each plot for a total of eight treatment combinations. The shrubs were planted in a triangular arrangement at a 45° angle one meter in front of each seedling in a variety of combinations (Fig. 5). In June of 2016, one year after perennial plant establishment, half of the plots (a, b, c, and g) were broadcast seeded with Massai in the following arrangements:

- a) *L. leucocephala* + *L. leucocephala* + *Massai* (2,222 *Leucaena* ha⁻¹) (L-M²)
- b) *T. diversifolia* + *T. diversifolia* + *Massai* (2,222 *Tithonia* ha⁻¹) (T-M)
- c) *L. leucocephala* + *T. diversifolia* + *Massai* (1,111 *Leucaena* and *Tithonia* ha⁻¹) (L-T-M)
- d) *L. leucocephala* + *L. leucocephala* – *Massai* (L)
- e) *T. diversifolia* + *T. diversifolia* – *Massai* (T)
- f) *L. leucocephala* + *T. diversifolia* – *Massai* (L-T)
- g) *Massai* (M)
- h) – *L. leucocephala* – *T. diversifolia* – *Massai*

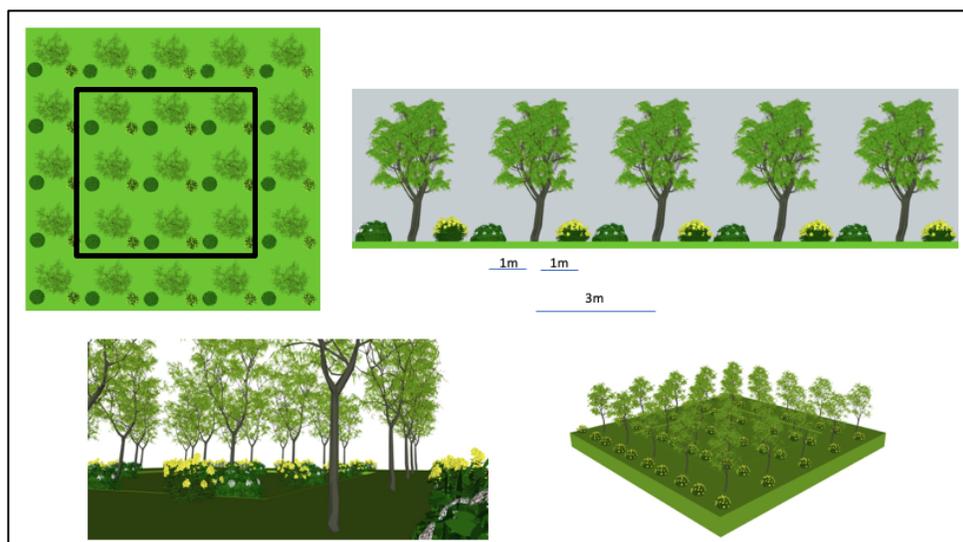


Fig. 5 Several renderings depicting the plantation design. Twenty-five *Albizia saman* seedlings were planted on a 3m x 3m spacing. Various combinations of the fertilizer shrubs *L. leucocephala* and *T. diversifolia* were planted in a triangular arrangement at a 45° angle one meter in front of each *A. saman*. *Massai* grasses were planted in four of the eight plots. The black box in the middle of the image to the upper left indicates the area wherein trees were sampled.

² These abbreviations will be used heretofore to denote respective treatments

Measurements were conducted only on the nine innermost plants of each plot, allowing for a 4.5m buffer between treatment plots.

Soil Physical and Chemical Properties

Bulk Density and Texture

To measure bulk density, one soil sample was taken in the center of each plot prior to planting in July of 2015 and again two years later after silvopasture establishment. Three depths (0-5 cm, 5-10 cm, and 10-15 cm) were sampled by hammering a metal cylinder with a volume of 500 cm³ into the soil until the square was level with the soil surface. Samples were sent to the soils laboratory at the Smithsonian Tropical Research Institute (STRI) in Panama City, Panama where they were weighed wet, dried at 105° C for 24 hours, and weighed again to determine bulk density as soil dry weight (g) volume⁻¹ (cm³). Texture was determined with particle size analysis.

Nutrients

Soil samples were collected prior to planting during the rainy season in July of 2015 and again two years later after plantation establishment. Six randomly distributed soil samples (200g) were collected at 0-15 cm depth with an augur from each plot, air-dried without sun or heat, and combined by taking an even amount from each sample. Soil samples (100 g) were sent to the soil testing laboratory at the Smithsonian Tropical Research Institute (STRI) in Panama City, Panamá. Inductively coupled plasma optical emission spectroscopy (ICP-OES) was used with a BaCl₂ solution to determine exchangeable Ca, Mg, K, Mn, Fe, Al, and Na and the Mehlich-3 extraction (Mehlich 1984) technique was used to estimate plant available P. Effective Cation exchange

capacity (ECEC, cmol (+) kg^{-1}) was calculated by summing the basic and acidic exchangeable cations and Base Saturation (BS, %) was calculated by dividing the Total Exchangeable Bases (TEB) by the ECEC and multiplying by 100. Another set of samples were sent to the soil testing laboratory at the University of Missouri where they were analyzed for organic matter (OM, %), total carbon (TC, %), and total nitrogen (TN, %), nitrate and ammonium. Total carbon and nitrogen were determined by dry combustion using a CHN analyzer and organic matter was estimated by loss on ignition. Nitrate and ammonium were extracted in 1 M KCl solution and determined by colorimetry with an autoanalyzer. Soil pH was determined using the wet method (1:1 ratio of water to soil) (Schofield and Taylor 1955).

Soil Volumetric Water Content (VWC)

Soil volumetric water content (VWC) was evaluated once every two months using the Time-Domain-Reflectometry method and a TDR meter (Field ScoutTM TDR Soil Moisture Meter 100). This was measured at four depths (3.8, 7.5, 12, and 20 cm) systematically at twelve locations across each plot for the months of January, March, May, July, September, and November in 2016. Samples were taken 0.5 m in front of all nine trees within each plot.

Plant Moisture Stress (PMS)

Sapling leaf water potential (Mpa) was measured with a Model 600 pressure chamber (1970s) from the PMS instrument company once during the months of January, March, May, July, September, and November 2016. Samples were taken from leaves of

lower branches of the nine central timber trees between the hours of 04:00 and 06:00. It is assumed that before dawn, leaves are in equilibrium with the soil water potential (Schultz 1996). Immediately before excision, leaves were placed in a plastic bag and the air was expelled. The leaf was clipped from the stem and transferred to the pressure chamber within the plastic bag which was attached by a paper clip (Turner and Long 1980). Five mm of the petiole extended externally to the chamber and a rate of nitrogen gas increase of 0.3 Mpa/minute was used. When maximum compensation pressure attainable within the chamber (4 Mpa) was insufficient to cause emergence of xylem sap at the cut surface, a water potential of > 4 Mpa was recorded.

Grass and Shrub Dry Matter Production

Grass

After grasses were fully established, four measurements were taken to represent two sampling seasons: wet (November 5 and December 5, 2016), and dry (February 5 and April 5, 2017). At the end of each sampling period, grass within each plot was cut to a uniform height of 20 cm above the soil and was left to grow for 30 days and 60 days to resemble rotational stocking during the wet and dry seasons, respectively. In each of the four plots containing grasses, grasses were sampled in square grids (0.5m x 0.5m) at eight systematically distributed locations. Grass wet matter was evaluated by cutting the grass to 20 cm in height with hedge shears, putting clippings into a burlap sack and immediately weighing (Fernandes et al. 2014). Samples were quartered to extract a 400 g subsample that was sent to the Cooleche Laboratory (Cooperativa de S/M de Productores

de Leche) in Concepción, Panama. Samples were dried at 60°C for 48 hours to weight constancy before determination of dry biomass weight.

L. leucocephala and *T. diversifolia* Shrubs

We coppiced all *L. leucocephala* shrubs at 150 cm in height once during the dry season (March) and once during the wet season (September) following recommendations for optimal biomass yield given by Duguma et al. (1988). *T. diversifolia* shrubs were coppiced to 50 cm in height once in September of 2015 and once in September of 2016, following the recommendations of Partey (2011). Biomass (leaves + small green branches) of five target shrubs was weighed in a burlap sack, quartered to extract a 400 g subsample, and sent to the Cooleche Laboratory in Concepción, Panama. Samples were dried at 60°C for 48 hours to weight constancy before determination of dry biomass weight. All remaining biomass clippings from shrubs were spread throughout their respective plot and where possible, mixed into the soil sub-surface.

Growth and survival of *A. saman* saplings

The nine central timber seedlings per subplot were measured with a metric tape and a Vernier caliper to an accuracy of 1 cm and 0.1 mm, respectively, two weeks after planting and thereafter twice every six months: twice in the rainy season (August and November 2015), twice in the first dry season (January and April 2016), and twice in the middle of the second rainy season (August and November 2016). Height (cm) and root collar diameter at ground level (from here also rereferred to as “diameter”; cm) were

selected as the main indicators for tree growth. Dead seedlings were replaced with seedlings of approximately the same size before September 2015.

Data Analysis

We analyzed planting regime effects on response variables including soil chemical properties, seedling height and diameter growth rate, shrub and grass dry matter yield, and PMS, between treatments and sampling dates using a generalized linear mixed model analysis of variance (ANOVA) using the MIXED procedure in SAS (SAS® 2013). We considered the planting regime treatments as main plots and sampling dates as repeated measures (Steel and Torrie 1980). Treatment, sampling date, and their interactions were considered fixed effects while the replicate block, treatment nested under replicate block, and sampling date nested under treatment and replicate block were considered random effects. We examined height and diameter growth increment by calculating an absolute growth rate (AGR; cm month^{-1}) following Kikvidze and Armas (2010) by using the following equation: $\text{AGR} = (W_2 - W_1) / (t_2 - t_1)$, where W represents either height or root collar diameter at t_1 at the beginning of the study and t_2 at the end of the study (in months). All increment parameters were subsequently transformed using the natural logarithm to be used for analysis.

Soil bulk density and VWC were analyzed with a two-way analysis of variance (ANOVA) using a mixed model with a split plot in space and time (Steel and Torrie 1980). The split plot design considered planting regime treatments as main plots, sampling depth as sub-plots, and sampling dates as sub-sub-plots (Steel and Torrie 1980). Sampling dates were analyzed as repeated measures. The advantage of using a mixed

model approach to analyze these data is the inclusion of a random intercept for each plot that serves to account for the clustered nature of tree plantings (Piepho et al. 2003).

For the parameters sapling height/diameter and shrub fodder dry matter yield (kg ha^{-1}), we used a commonly applied statistical methodology known as the “top height tree collective” (Pretzsch 2009). This approach considers only the five most vital seedlings in each plot as targets for analysis; thus, the 15 tallest trees per treatment were selected as the sample population. These trees are most representative of those individuals that could potentially develop a competitive advantage over others. Additionally, the use of only five target trees avoids differences in means that could be caused by high differences in survival between treatments. Treatments with higher survival rates are likely to have wider ranges of heights and diameters than treatments with lower survival rates (Paul and Weber 2016).

To analyze differences in seedling survival, we used a logistic mixed effects analysis of variance (ANOVA) model that provided an odds ratio, or probability of death, for each treatment. To better understand the structure of errors due to treatment, replicate, and tree number, we ran three null models without testing for fixed effects of treatment and month and recorded the -2 Residual Log Pseudo-Likelihood and General Chi-Square / DF fit statistics available using the Glimmix procedure in SAS (SAS® 2013). The second model using the compound symmetry (CS) covariance matrix structure gave the smallest fit statistics, so we used the CS structure for errors.

All dependent variables were tested for normality and \log_{10} transformed when necessary to meet the assumption of homoscedasticity. A simple variance component matrix was used to include the random plot effect and the residual maximum likelihood

estimation was used to estimate covariance parameters. When significant effects were detected, pair-wise comparisons were performed using the Tukey-adjusted least squares method. Main effects and all interactions were considered statistically significant at $\alpha = 0.05$.

Results

Soil Physical and Chemical Properties

Analysis of physical and chemical soil properties revealed a wide array of soil fertility among sites. Soils in Pedasi were relatively low in fertility, with a mean pH of 6.34, a mean base saturation (BS) of 91.96%, a sandy loam texture (Table 1) and a relatively high bulk density ranging between 1.37 and 1.62 g cm⁻³ (Table 2). Soils in Los Asientos were higher in fertility, with a mean pH of 6.73, a mean BS of 98.95%, a loam to clay-loam texture and a lower bulk density ranging between 0.875 and 1.02 g cm⁻³. Soil fertility was highest in Calabacito, with a mean pH of 6.71, a mean BS of 99.63%, a loam texture and a bulk density between .704 and 1.02 g cm⁻³.

There were significant treatment ($P = 0.0427$), depth ($P = 0.0001$), and year ($P = 0.0227$) effects for bulk density. Soil mean bulk density in C was significantly greater than all other treatments, while all other treatments did not statistically differ from one another. Bulk density increased with sampling depth and differed significantly between all three depth profiles. Soil mean bulk density in 2017 was significantly less than that in 2015, decreasing from 0.9238 to 0.9169 g cm⁻³ overall. There were no interaction effects.

Table 1 Soil texture determined at three sampling depths (0-5, 5-10, and 10-15 cm) from ten locations within each of the three study sites, Pedasi (P), Los Asientos (L), and Calabacito I.

Soil depth	Soil Texture (%)			Texture class
	Sand	Silt	Clay	
Profile 1 (P)				
0-5	58	23.5	18.5	Sandy Loam
5-10	55	27.5	17.5	Sandy Loam
10-15	54	24	22	Sandy Loam
Profile 2 (L)				
0-5	45	34.5	20.5	Loam
5-10	42.5	32.5	25	Loam
10-15	40	34	26	Clay Loam
Profile 3 I				
0-5	51	30.5	18.5	Loam
5-10	50	30	20	Loam
10-15	48	30	22	Loam

Overall, CEC increased significantly from 13.80 to 15.63 cmol kg⁻¹ between pre-treatment and post-treatment sampling dates, with the highest value of 16.01 in T and the lowest value of 13.59 in M. All treatments increased significantly between sampling dates with the exception of treatments M ($P = 0.6971$) and C ($P = 0.7648$). Significant differences did not exist between treatments but there were significant treatment*date interactions, most notably in 2017 between treatments L-T and M ($P = 0.0312$) and between treatments M and T ($P = 0.0304$) (Table 3).

There was no overall difference among treatments for base saturation ($P = 0.6024$), which ranged from 96.20% in M to 97.70% in L-T. Much like CEC, BS significantly increased from 96.27% in 2015 to 97.43% in 2017 ($P = 0.0058$). There were no treatment*date interactions. Planting regime did not affect soil pH ($P = 0.6613$), nor were there changes in soil pH over time ($P = 0.4501$).

The major cations Ca and K both showed marked increases in association with plots that included shrubs. Ca concentrations ranged from 8.39 cmol kg⁻¹ in M to 11.46 cmol kg⁻¹ in T. Although not statistically significant ($P = 0.3130$), the two treatments without shrubs, C and M, contained the lowest Ca concentrations. Sampling date significantly affected Ca concentration, with an overall concentration of 9.58 cmol kg⁻¹ in 2015 and 10.390 in 2017 ($P = 0.0001$). Similarly, K concentrations were lowest in M and C and higher in all plots with shrubs, but were affected significantly only by sampling date ($P = 0.0001$), increasing from .074 cmol kg⁻¹ in 2015 to 0.121 cmol kg⁻¹ in 2017. Mg concentrations followed a different pattern, with the highest concentration of 4.88 cmol kg⁻¹ found in T-M and the lowest concentration of 3.58 cmol kg⁻¹ found in L-T. Mg was not affected by treatment ($P = 0.2793$), but concentrations differed in respect to sampling date ($P = 0.0001$), increasing from 3.80 cmol kg⁻¹ in 2015 to 4.78 cmol kg⁻¹ in 2017. There was a significant interaction between treatment and date ($P = 0.0327$), with Mg concentrations in treatment M decreasing between sampling dates.

Table 2 Bulk Density recorded pre-trial (2015) and post-trial (2017) at three depths for each of the eight planting regimes at the three study sites in Los Santos, Panama.

Treatments	Bulk Density (g cm ⁻³)											
	Calabacito				Los Asientos				Pedasi			
	0-5 cm	5-10 cm	10-15 cm	Mean	0-5	5-10	10-15	Mean	0-5	5-10	10-15	Mean
<u>2015</u>												
C	0.912	0.9843	0.9922	0.9628	0.8	0.914	1.014	0.9093	1.575	1.577	1.579	1.577
L	0.744	0.977	1.148	0.9563	0.843	0.974	1.102	0.973	1.333	1.511	1.624	1.4893
L-M	0.839	1.032	1.077	0.9827	0.897	1.151	0.921	0.9897	1.481	1.714	1.675	1.6233
L-T	0.733	0.827	0.959	0.8397	0.746	0.876	1.002	0.8747	1.481	1.495	1.556	1.5107
L-T-M	0.824	0.858	1.077	0.9197	0.704	0.849	1.082	0.8783	1.489	1.511	1.517	1.5057
M	0.854	0.917	0.942	0.9043	0.794	0.853	1.287	0.978	1.314	1.398	1.412	1.3747
T	0.957	1.019	1.09	1.022	0.974	1.044	1.045	1.021	1.33	1.484	1.346	1.3867
T-M	0.86	0.893	0.935	0.896	0.75	1.101	1.119	0.99	1.489	1.517	1.463	1.4897
<u>2017</u>												
C	1.054	1.098	1.138	1.0967	0.898	0.912	1.012	0.9407	1.591	1.611	1.643	1.615
L	0.7312	0.932	1.128	0.9304	0.812	0.913	1.102	0.9423	1.301	1.497	1.413	1.4037
L-M	0.754	0.957	1.021	0.9107	0.785	1.012	0.991	0.9293	1.324	1.712	1.689	1.575
L-T	0.704	0.7981	0.917	0.8064	0.7112	0.7987	0.943	0.8176	1.298	1.431	1.499	1.4093
L-T-M	0.798	0.811	0.987	0.8653	0.616	0.779	1.001	0.7987	1.218	1.499	1.502	1.4063
M	0.7988	0.884	0.913	0.8653	0.698	0.881	1.201	0.9267	1.212	1.298	1.361	1.2903
T	0.931	1.005	1.167	1.0343	0.897	1.002	1.023	0.974	1.214	1.398	1.412	1.3413
T-M	0.778	0.8512	0.991	0.8734	0.712	0.913	1.102	0.909	1.213	1.491	1.513	1.4057

Soil organic matter (SOM) increased significantly overall from 2.74% in 2015 to 3.32% in 2017 ($P = 0.0001$). All treatments showed numerical increases in SOM between sampling dates, with the exception of C, which declined by 0.286% (Fig. 6). L-T-M revealed the greatest change in SOM between sampling dates, increasing by 1.17%. When compared to all other treatments, L-T-M had significantly greater mean SOM than treatments M and C, while all treatments contained more SOM than C ($P = 0.0039$). There was a significant interaction between treatment and sampling date ($P = 0.0001$), with all treatments increasing SOM values in time with the exception of treatment C (Table 4).

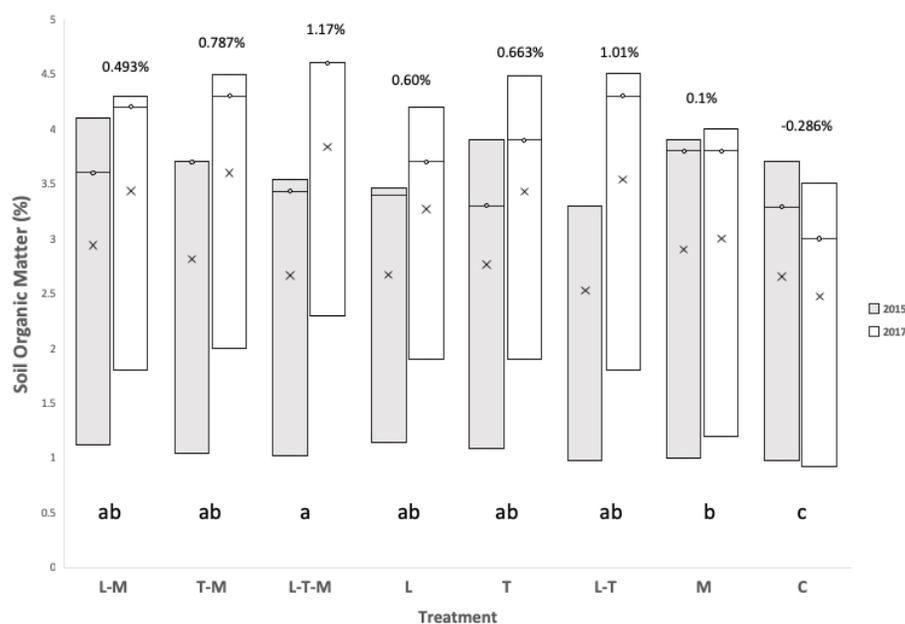


Fig. 6 Changes in mean soil organic matter between 2015 and 2017 for eight planting regime treatments at all three sites combined. Means sharing the same letter are not significantly different from one another at $P < 0.05$.

Table 3 Pre-trial (2015) and post-trial (2017) measurements of effective cation exchange capacity (ECEC), base saturation (BS), and several important macronutrients for soils collected within the eight planting regime treatments.

Site	Treatment	ECEC (cmol kg ⁻¹)		BS (%)		Ca		K		Mg	
		2015	2017	2015	2017	2015	2017	2015	2017	2015	2017
Calabacito	C	27.16	27.36	99.60	99.77	19.12	18.91	0.04	0.09	7.63	8.01
	L	22.92	24.38	99.54	99.77	17.19	17.78	0.07	0.16	5.28	6.07
	L-M	23.49	24.62	99.43	99.77	16.47	17.38	0.09	0.12	6.51	6.69
	L-T	21.16	26.02	99.50	99.78	17.17	17.74	0.06	0.14	3.53	7.76
	L-T-M	20.66	21.20	99.24	99.76	15.18	14.18	0.06	0.13	5.01	6.48
	M	23.97	23.52	99.55	99.73	15.29	15.03	0.10	0.11	8.22	8.04
	T	25.27	26.38	99.60	99.81	18.87	19.21	0.08	0.13	5.96	6.69
	T-M	20.13	23.42	99.37	99.83	13.35	15.02	0.08	0.13	6.31	7.86
	Mean	23.09	24.61	99.48	99.78	16.58	16.91	0.07	0.13	6.06	7.20
Los Asientos	C	14.49	15.02	98.71	99.36	8.40	8.46	0.13	0.15	5.55	6.01
	L	17.72	18.60	98.88	99.34	12.11	12.01	0.13	0.16	5.12	6.11
	L-M	16.26	18.95	98.11	99.06	9.38	11.27	0.10	0.20	6.28	7.02
	L-T	14.62	19.78	98.87	99.60	11.49	13.38	0.15	0.21	2.70	5.93
	L-T-M	14.15	19.56	98.07	99.12	10.93	12.97	0.08	0.17	2.74	6.02
	M	14.35	14.35	98.65	99.31	8.12	8.22	0.12	0.13	5.68	5.59
	T	17.45	20.49	98.78	99.47	12.21	13.92	0.08	0.18	4.73	6.02
	T-M	17.50	20.78	98.62	99.32	10.79	12.22	0.11	0.19	6.20	8.03
	Mean	15.82	18.45	98.58	99.32	10.43	11.56	0.11	0.19	4.87	6.34
Pedasi	C	2.07	1.97	90.72	90.32	1.43	1.33	0.06	0.05	0.34	0.33
	L	2.56	4.39	88.40	92.05	1.53	2.89	0.04	0.09	0.63	1.07
	L-M	2.73	3.86	91.94	94.42	1.93	2.64	0.01	0.03	0.54	0.97
	L-T	2.74	5.06	92.38	96.08	1.94	3.66	0.05	0.11	0.48	1.06
	L-T-M	2.92	4.85	92.85	95.87	2.13	3.48	0.03	0.07	0.51	1.12
	M	2.87	2.49	91.15	88.80	1.99	1.68	0.05	0.04	0.52	0.47
	T	2.33	4.14	89.66	93.46	1.58	2.98	0.04	0.09	0.42	0.79
	T-M	1.78	3.86	88.79	94.54	1.25	3.01	0.01	0.03	0.28	0.63
	Mean	2.50	3.83	90.74	93.19	1.72	2.71	0.04	0.06	0.47	0.80

Total C ranged from 14132 mg kg⁻¹ in C to 15483 mg kg⁻¹ in L-T-M and was strongly positively correlated with SOM ($r = 0.95288$, $P = 0.0001$) (Fig. 7). Total C was significantly affected by planting regime ($P = .0115$), with all six treatments including shrubs producing significantly more total C than those without (M and C). Over the course of the study, overall total C increased significantly by 9.56% from 14795 mg kg⁻¹ in 2015 to 15194 mg kg⁻¹ in 2017 ($P = 0.0002$). There was a borderline treatment * date effect, with treatments T, M, L, and C showing no significant change over the two years, while treatments T-M, L-T-M, L-T, and L-M did.

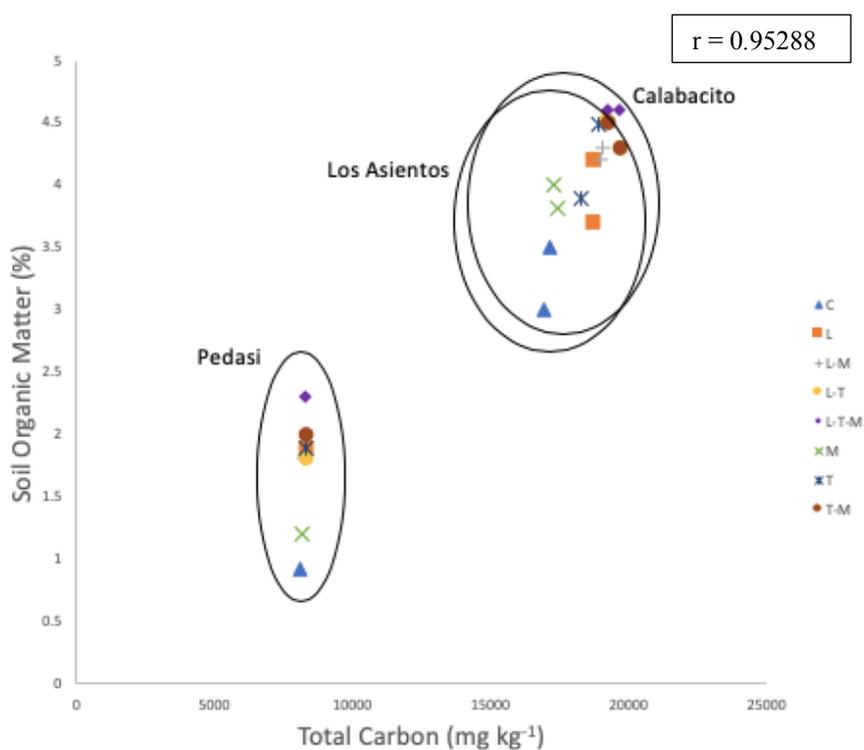


Fig. 7 Relationship between mean soil organic matter (SOM) and mean total carbon (TC) sampled in 2017 for three sites and eight treatments.

Table 4 Pre-trial (2015) and post-trial (2017) soil organic matter (OM), total carbon (TC), total nitrogen (TN), carbon to nitrogen ratio (C:N), nitrate, ammonium, and Mehlich-3 P for all eight planting regime treatments at three sites in Los Santos, Panama.

Site	Treatment	OM		TC		TN		C:N		Nitrate		Ammonium		Mehlich-3 P	
		2015	2017	2015	2017	2015	2017	2015	2017	2015	2017	2015	2017	2015	2017
<u>C</u>	C	3.60	3.50	17544	17110	1569	1594	11.19	10.76	2.24	2.50	9.22	9.22	1.41	1.31
	L	4.20	4.20	18143	18700	1599	1713	11.35	8.60	2.01	2.68	9.51	9.92	1.90	1.79
	L-M	3.60	4.20	18054	18940	1647	1771	10.96	10.70	2.48	2.78	8.27	10.27	1.49	1.40
	L-T	3.30	4.30	18741	19697	1644	1806	11.40	10.96	1.99	2.83	9.88	10.44	1.75	1.95
	L-T-M	3.43	4.60	18847	19920	1656	1811	11.38	11.01	2.45	2.84	9.13	10.50	2.30	2.50
	M	3.80	4.00	17400	17320	1562	1514	11.14	11.47	2.15	2.37	8.12	8.76	1.68	1.48
	T	3.30	3.90	18131	18240	1718	1796	10.55	10.47	2.11	2.81	8.78	10.38	2.02	2.32
	T-M	3.70	4.30	18960	19680	1603	1695	11.83	11.64	2.12	2.65	8.43	9.80	1.55	1.75
	Mean	3.62	4.13	18228	18701	1625	1713	11.23	10.70	2.19	2.68	8.92	9.91	1.76	1.81
<u>L</u>	C	3.10	3.40	17008	16980	1506	1538	11.30	11.10	2.31	2.40	8.55	8.87	1.40	1.80
	L	3.40	3.70	18522	18710	1588	1685	11.67	11.38	2.08	2.64	8.89	9.74	2.63	2.43
	L-M	4.10	4.30	18248	19020	1505	1613	12.12	11.81	2.04	2.53	8.99	9.34	1.43	1.73
	L-T	3.30	4.20	17993	19200	1593	1668	11.29	11.39	2.29	2.61	9.12	9.63	2.44	3.10
	L-T-M	3.54	4.60	18092	19420	1600	1799	11.30	11.03	2.41	2.76	9.01	10.21	1.70	2.07
	M	3.90	3.80	17621	17430	1490	1491	11.82	11.70	2.27	2.34	8.88	8.64	2.05	1.99
	T	2.90	3.90	18021	18920	1600	1714	11.26	10.71	2.32	2.68	8.01	9.92	2.66	2.66
	T-M	3.70	4.50	18476	19230	1606	1726	11.50	11.18	2.43	2.70	8.13	9.98	1.51	1.91
	Mean	3.49	4.05	17998	18614	1561	1654	11.53	11.29	2.27	2.58	8.70	9.54	1.98	2.21
<u>P</u>	C	0.97	1.10	8040	8109	682	714	11.80	11.86	1.02	1.11	4.25	4.12	2.87	2.87
	L	1.14	1.90	8241	8310	878	940	9.38	8.84	1.05	1.48	4.92	5.45	4.18	4.08
	L-M	1.12	1.80	8054	8210	816	855	9.87	9.66	1.08	1.33	3.54	4.93	3.20	3.50
	L-T	0.98	1.80	8096	8308	748	845	10.82	9.65	0.97	1.32	4.72	4.87	3.27	4.14
	L-T-M	1.02	2.30	8208	8410	814	912	10.09	9.24	1.11	1.43	4.41	5.28	3.02	3.52
	M	1.00	1.20	8182	8140	664	668	12.33	12.33	0.95	1.04	3.89	3.83	3.84	3.54
	T	1.19	1.90	8208	8320	854	897	9.61	9.35	1.09	1.40	4.90	5.16	4.91	5.17
	T-M	1.04	2.00	8257	8330	708	794	11.66	10.54	0.98	1.24	3.76	4.58	3.03	3.23
	Mean	1.06	1.75	8161	8267	770	828	10.69	10.19	1.03	1.29	4.30	4.78	3.54	3.76

Total N was also affected significantly by treatment ($P = 0.0001$) and date ($P = 0.0001$). Total N ranged from $1267.02 \text{ mg kg}^{-1}$ in C to 1430 mg kg^{-1} in T. Much like for TC, all treatments including shrubs had significantly greater total N than those without shrubs (M and C), however, T-M had significantly lower TN than the other treatments with shrubs. TN increased significantly by 5.9% from $1318.72 \text{ mg kg}^{-1}$ in 2015 to $1397.17 \text{ mg kg}^{-1}$ in 2017. There was a significant interaction between treatment and date ($P = 0.0009$), with TN increasing in all treatments except for M, where it decreased.

Plant available N made up 0.687% of total N and was highest in the form of ammonium. Ammonium ranged between 7.021 mg kg^{-1} in treatment M and 8.11 mg kg^{-1} in treatment L-T. Both planting regime ($P = 0.0007$) and date ($P = 0.0001$) had significant effects on soil ammonium concentrations (Table 5). Treatments L-T, L-T-M, and L had significantly higher mean ammonium concentrations than the remaining treatments. Overall ammonium concentrations increased by 10.56% from $7.3052 \text{ mg kg}^{-1}$ in 2015 to $8.0765 \text{ mg kg}^{-1}$ in 2017. Nitrate levels differed significantly between treatments ($P = 0.0295$) and date ($P = 0.0001$). L-T-M had the highest nitrate concentration of 2.167 mg kg^{-1} , which was significantly greater than treatments L-T, L, C, and M. M had the lowest nitrate concentration at 1.8527 . Overall nitrate concentration increased from 1.83 mg kg^{-1} in 2015 to 2.185 mg kg^{-1} in 2017 (Table 5).

The overall C:N ratio significantly decreased over the course of the study, from 11.151 in 2015 to 10.724 in 2017 ($P = 0.1223$) (Table 4). However, there were no treatment ($P = 0.1223$) or treatment*date effects ($P = 0.3572$).

Significant differences existed for Mehlich-3 P between treatments ($P = 0.0219$), dates ($P = 0.0008$), and for the treatment*date interaction ($P = 0.0044$). Post-hoc tests

revealed significantly greater P concentrations in treatment T than for all other treatments with the exception of L-T ($P = 0.1455$) and L ($P = 0.1947$) (Fig. 9). However, treatments differed significantly in 2015 at the start of the experiment, making absolute value comparisons impossible. Assessing the differences in relative changes between years among treatments is a more appropriate analysis in this case. Two treatments, L and M, showed non-significant decreases in plant available P between 2015 and 2017. Conversely, the treatments T-M ($P = 0.0325$), L-T-M ($P = 0.0064$), and L-T ($P = 0.0001$), showed significant increases in plant available P over the study (Fig. 8).

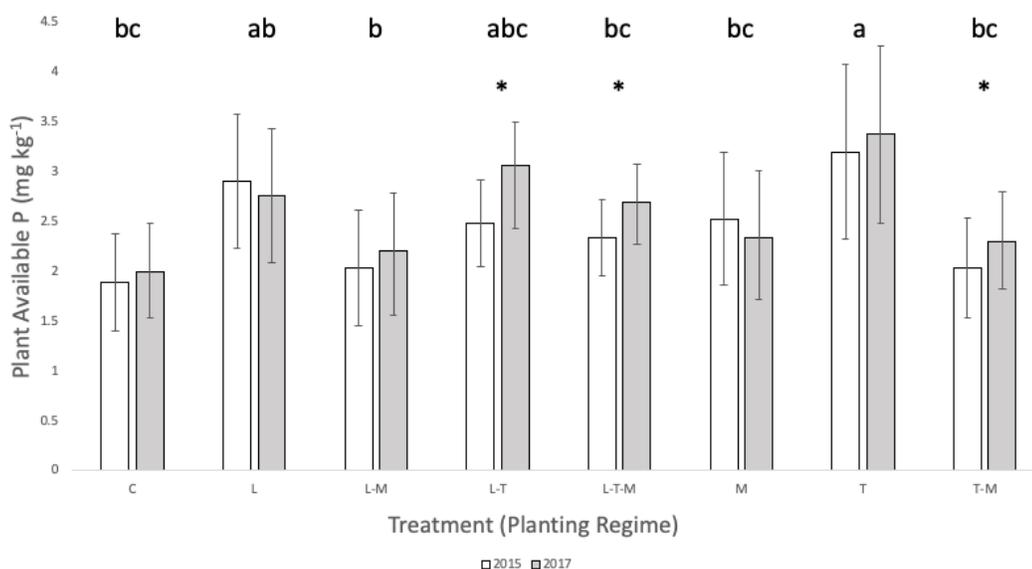


Fig. 8 Least squares means (LSMs) with standard errors for plant available P estimated with the Mehlich-3 P extraction. Means sharing the same letter are not significantly different from one another at $P < 0.05$. Significant differences in LSMs between years are indicated with an *.

Table 5 Pre-trial (2015) and post-trial (2017) measurements taken for plant available ammonium (NH_4^+) and nitrate (NO_3^-). Overall, ammonium and nitrate increased over time, with lowest concentrations in M and highest concentrations in L-T-M for both variables.

Site	Variable (year)	C	L	L-M	L-T	L-T-M	M	T	T-M
Calabacito	NH_4^+ (2015)	9.22	9.51	8.27	9.88	9.13	8.12	8.78	8.43
Los Asientos		8.55	8.89	8.99	9.12	9.01	8.88	8.01	8.13
Pedasi		4.25	4.92	3.54	4.72	4.41	3.89	4.90	3.76
Calabacito	NH_4^+ (2017)	9.22	9.92	10.27	10.44	10.50	8.76	10.38	9.80
Los Asientos		8.87	9.74	9.34	9.63	10.21	8.64	9.92	9.98
Pedasi		4.12	5.45	4.93	4.87	5.28	3.83	5.16	4.58
Calabacito	NO_3^- (2015)	2.24	2.01	2.48	1.99	2.45	2.15	2.11	2.12
Los Asientos		2.31	2.08	2.04	2.29	2.41	2.27	2.32	2.43
Pedasi		1.02	1.05	1.08	0.97	1.11	0.95	1.09	0.98
Calabacito	NO_3^- (2017)	2.50	2.68	2.78	2.83	2.84	2.37	2.81	2.65
Los Asientos		2.40	2.64	2.53	2.61	2.76	2.34	2.68	2.70
Pedasi		1.11	1.48	1.33	1.32	1.43	1.04	1.40	1.24

Soil Volumetric Water Content (VWC)

There were no significant treatment effects for soil VWC ($P = 0.8417$). Variability was high for all treatments and all sampling dates, making it impossible to discern treatment differences (Fig. 9). There were strong month ($P = 0.0001$) and sampling depth differences ($P = 0.0001$). Post-hoc tests showed that mean VWC differed significantly between all months, with the exception of July and September ($P = 0.1463$) and July and November ($P = 0.0823$). As expected, months falling within the dry season (January, March, and May) had significantly lower mean VWC (26.11, 9.58, and 30.39%, respectively) than those falling within the wet season (July: 46.24%; September: 49.65%; and November: 42.51%). VWC increased consistently with sampling depth, with a least squares mean of 26.25% at 3.8 cm, 33.20% at 7.5 cm, 35.79% at 12 cm, and 41.09% at 20 cm. There were also treatment*depth ($P = 0.0001$) and month*depth ($P = 0.0001$) interactions. The pattern of increasing VWC with depth was not observed in M and L-M during July and M and T-M during November, two of the three rainiest months of the year. Rather, highest VWC was measured at 12 cm and 7.5 cm, for July and November, respectively.

Plant Moisture Stress

There were no treatment effects on sapling mean leaf water potential ($P = 0.6285$). Mean leaf water potential varied across sites, with saplings in Pedasi exhibiting the lowest value at -3.48 Mpa in treatment C during the month of March (Fig. 10). Treatment least squares means ranged between -1.75 Mpa in M to -2.03 in T-M. Significant differences existed between all months ($P = 0.0001$) with the exception of

January and May, two months coinciding with the beginning of the dry season and the beginning of the rainy season ($P = 0.2955$). There were no interaction effects.

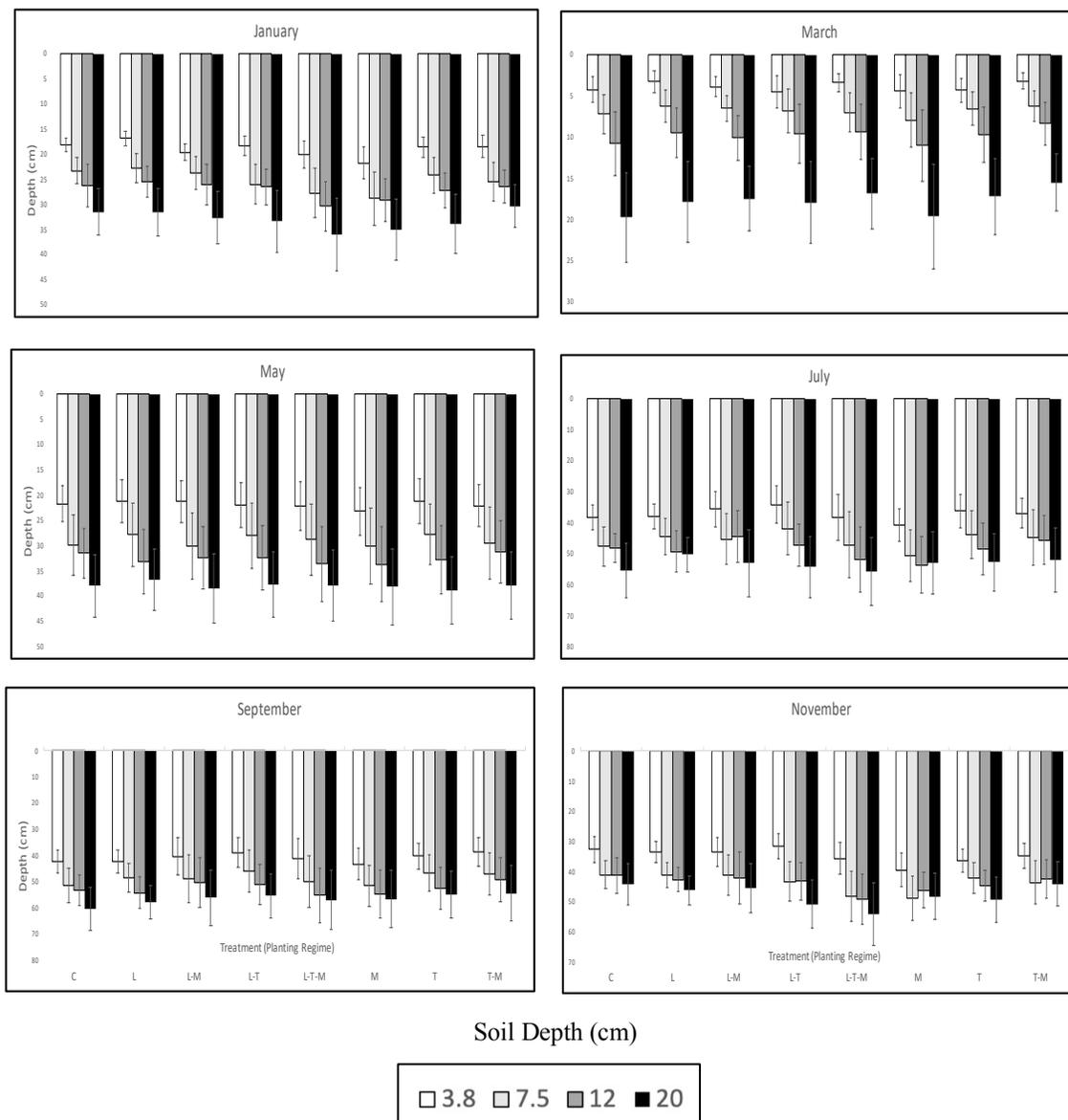


Fig. 9 Soil volumetric water content (VWC) at four sampling depths (3.8, 7.5, 12, and 20 cm) for the eight planting regimes sampled over 6 months throughout 2016. Least squares means with standard error are presented.

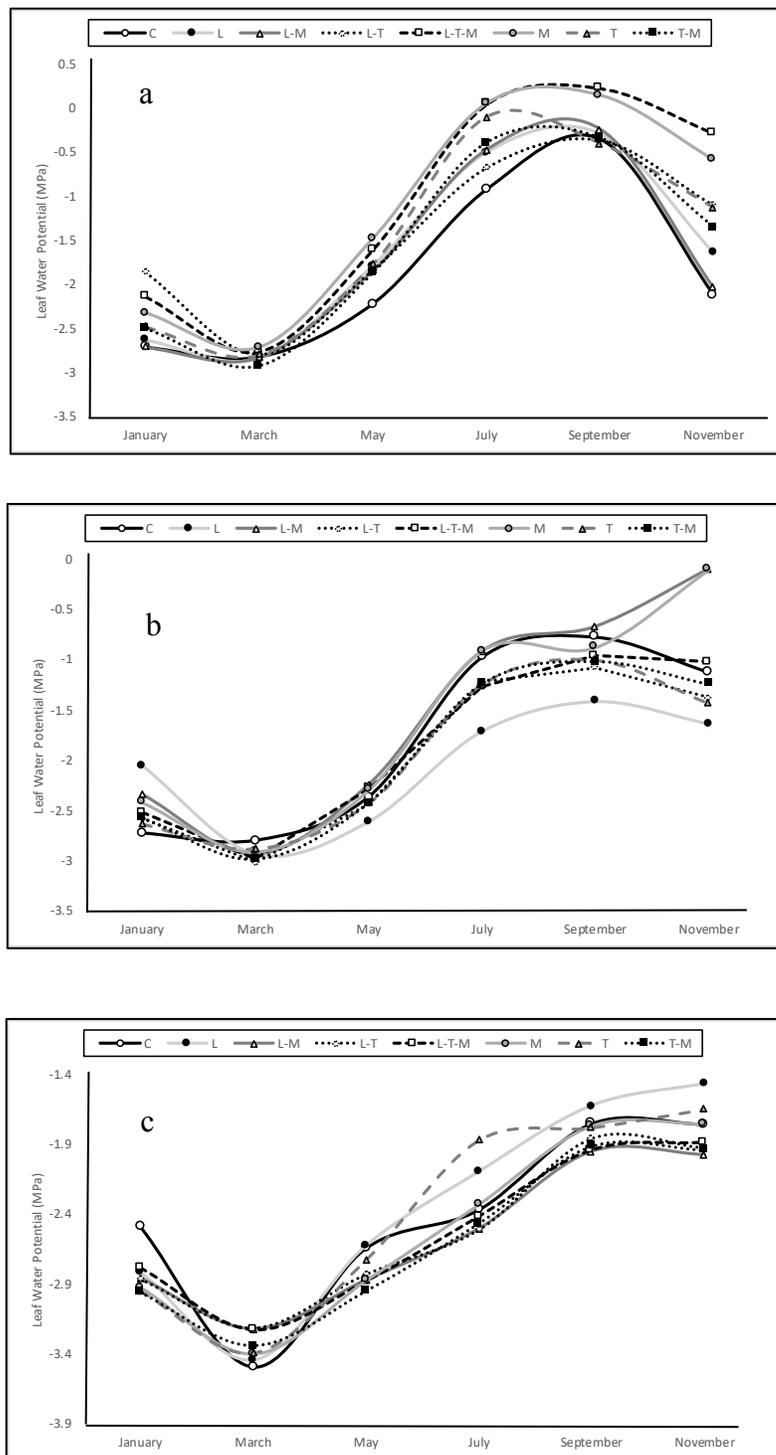


Fig. 10 Mean leaf water potential (Mpa) for *A. saman* saplings growing in association with eight different planting regimes in 2016 at the three sites a) Calabacito, b) Los Asientos, and c) Pedasi.

Massai Grass Dry Matter Production

Cumulatively, \log_{10} mean grass dry matter (DM) production was greatest in L-M for all sites (Fig. 11) but was only marginally significantly different from the other treatments ($P = 0.0552$). Mean annual DM production ha^{-1} totaled $11,761.87 \pm 168.42$, $7,098.73 \pm 287.57$, $8,258.13 \pm 547.12$, and $8,980.4 \pm 101.33$ for L-M, M, L-T-M, and T-M, respectively. As expected, there were significant differences between sampling periods ($P = 0.0001$), with greater quantities of dry matter produced in the months of September and November than in the months of February and April. There were no treatment*month interaction effects ($P = 0.9137$).

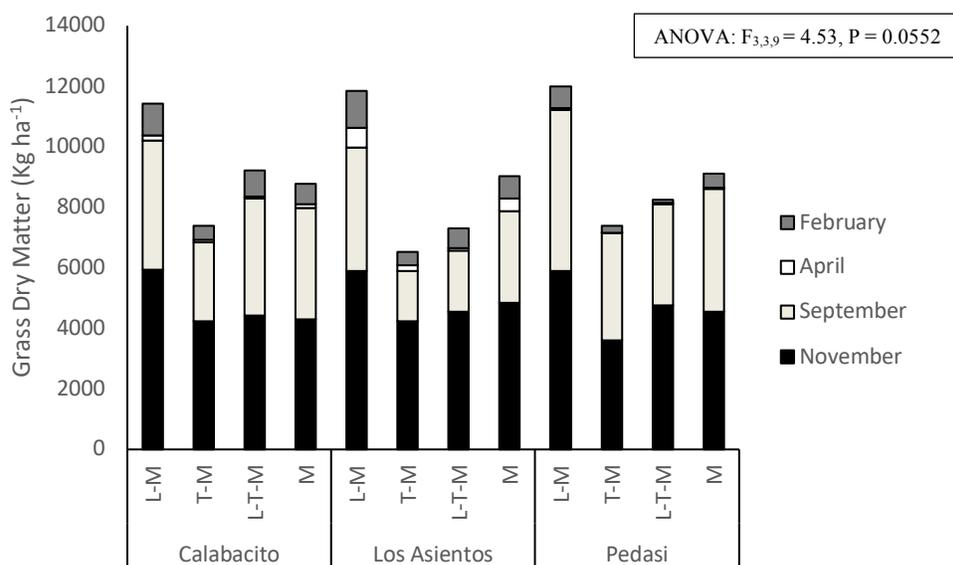


Fig. 11 Massai dry matter production sampled twice in the dry season and twice in the wet season of 2016 in the four planting regimes where grass was included at Calabacito, Los Asientos, and Pedasi in Los Santos, Panama. Values were \log_{10} transformed for the analysis, but are depicted here as back-transformed values for ease of interpretation.

Shrub Dry Matter Production

There were significant differences in \log_{10} dry weight of forage between treatments ($P = 0.0208$). Treatment T-M produced significantly more DM than all other treatments. *L. leucocephala* produced significantly less fodder than *T. diversifolia* and Massai ($P = 0.0477$). When DM values were back-transformed, least squares means ranged between 203.95 kg ha⁻¹ in L-L to 3120.59 kg ha⁻¹ in T-M. When seasons were summed, cumulative annual forage production was as much as 18,723 and 16,353.56 kg ha⁻¹ in T-M and L-T-M, respectively. During the dry season, M produced 4.33, 10.95, and 13.7% less combined forage than L-M, L-T-M, and T-M, respectively (Table 6). There was a significant difference between seasons, with overall lower DM production during the dry season ($P = 0.0001$) (Table 7). In the dry season, shrubs made up 81.64% of the available forage in plots including both grasses and shrubs. In the rainy season, only 35.45% of available forage came from shrubs; the remainder was provided by grass (Fig. 12).

Growth rate of *A. saman* saplings

A. saman saplings exhibited a non-linear growth pattern, with variation among sites (Fig. 13). Final sapling height and diameter differed between sites, with saplings in Calabacito attaining noticeably smaller heights and diameters than those in Los Asientos and Pedasi (Table 7). At Calabacito, saplings growing in association with Massai attained the greatest heights and diameters. At Los Asientos, saplings in C performed consistently well, along with those in L and L-M, which initially grew the quickest, followed by a

Table 6 Species contributions to total mean DM yield and total mean annual DM yield for each treatment over the three study sites.

Treatment	Species	Dry	Wet	Total Species Contribution	Annual Total (kg ha ⁻¹)
C	Leucaena	--	--	--	--
	Massai	--	--	--	--
	Tithonia	--	--	--	--
L-L	Leucaena	555 ± 147	669 ± 188	1224	1224
	Massai	--	--	--	
	Tithonia	--	--	--	
L-M	Leucaena	1236 ± 133	2179 ± 218	3415	14320
	Massai	629 ± 252	10276 ± 480	10905	
	Tithonia	--	--	--	
L-T	Leucaena	1646 ± 169	2113 ± 103	3758	8618
	Massai	--	--	--	
	Tithonia	2290 ± 104	2570 ± 84	4860	
L-T-M	Leucaena	1550 ± 112	2249 ± 78	3799	16354
	Massai	751 ± 163	6698 ± 311	7449	
	Tithonia	2419 ± 189	2687 ± 111	5105	
M	Leucaena	--	--	--	7851
	Massai	431 ± 37	7420 ± 704	7851	
	Tithonia	--	--	--	
T-M	Leucaena	--	--	--	18724
	Massai	617 ± 218	6870 ± 512	7487	
	Tithonia	5301 ± 74	5936 ± 241	11236	
T-T	Leucaena	--	--	--	11411
	Massai	--	--	--	
	Tithonia	5100 ± 57	6312 ± 80	11411	

Table 7 Least squares means of total dry matter production (kg ha⁻¹) in each of the eight planting regimes, from each of three forage sources, and from dry (February and April) and wet (November and December) seasons.

	Treatment								ANOVA	
Treatment	C	L	M	L-T	T	L-M	L-T-M	T-M	F	P
	0 (396)	204a (396)	1309b (396)	1436bc (396)	1902c (396)	2387cd (396)	2726d (396)	3121e (396)	7.66	0.0208
Fodder	<i>L. leucocephala</i>		<i>T. diversifolia</i>			Massai				
	762 (243)a		2038(243)b			2106 (243)b			5.26	0.0477
Season	Dry				Wet					
	938 (256)a				2332 (256)b				38.28	< .001

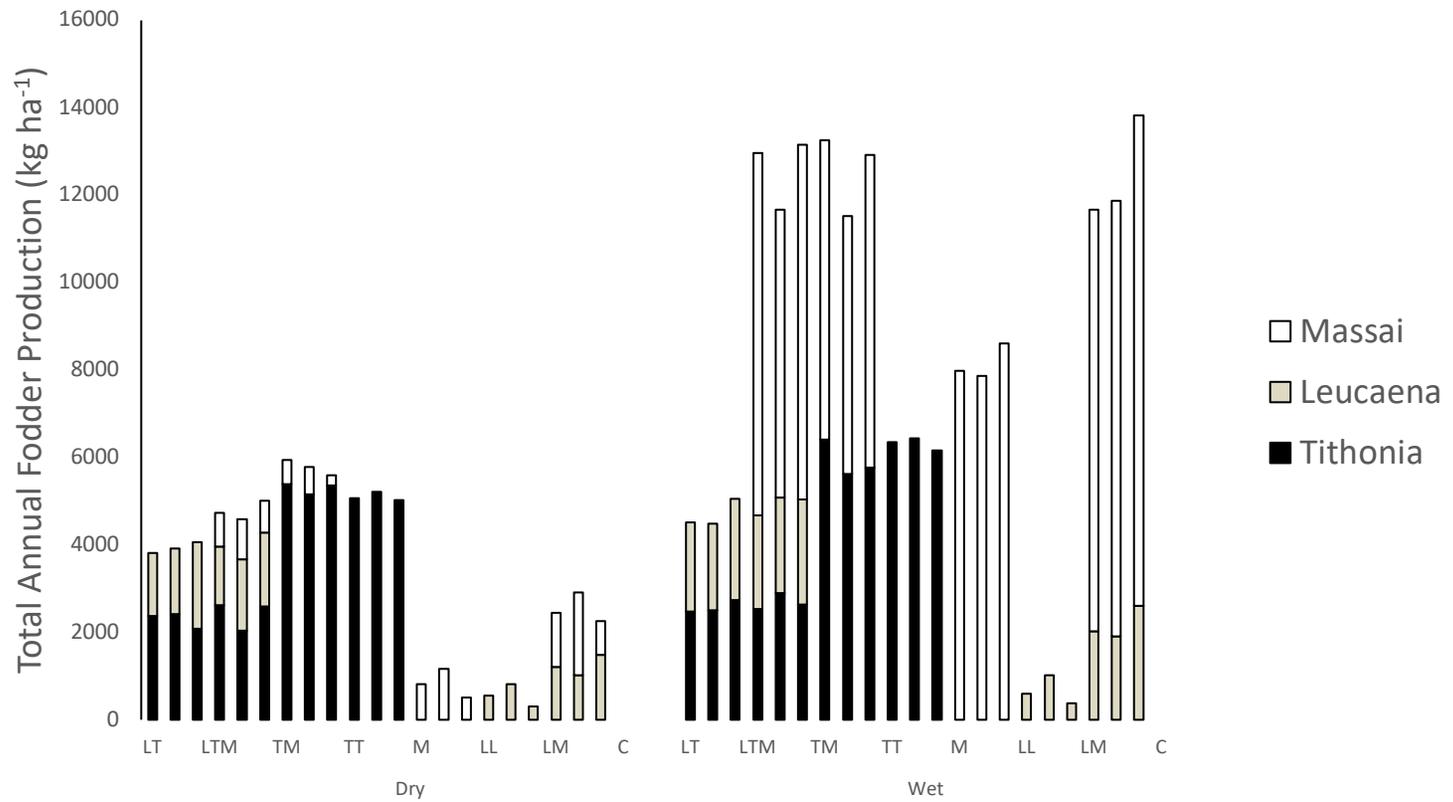


Fig. 12 Total annual DM forage production among the various planting regimes, including contributions from the grass Massai and the shrubs *T. diversifolia* and *L. leucocephala* on eight plots at the three sites Calabacito, Los Asientos, and Pedasi.

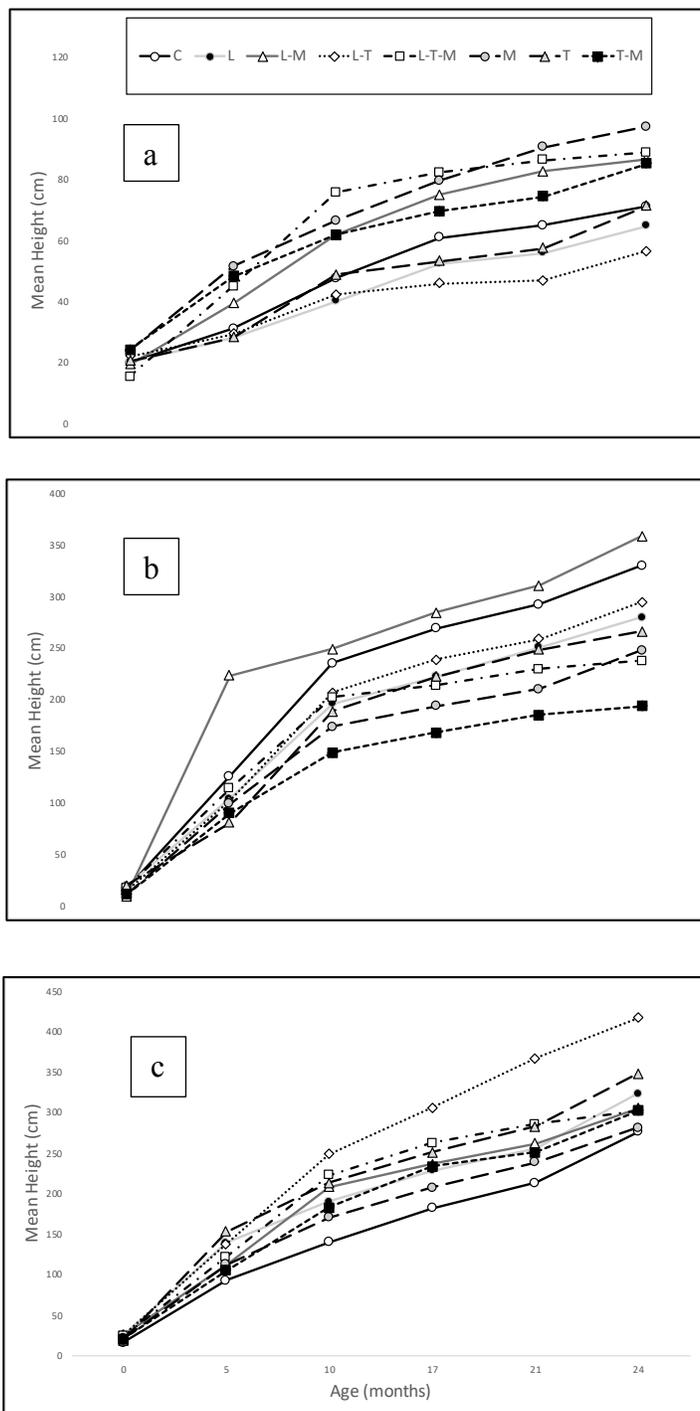


Fig. 13 Mean height of the five *A. saman* target saplings in association with the different planting regimes over the two-year period at the three sites a) Calabacito, b) Los Asientos, and c) Pedasi.

reduction in growth rate. At Pedasi, saplings in L-T, T, and L attained greatest heights and diameters (Table 8). Despite this site having the lowest overall soil fertility, *A. saman* sapling growth in Pedasi outperformed all the others (Fig. 15). Consequently, there were no statistically significant differences observed between treatments for height ($P = 0.7802$) nor diameter ($P = 0.5798$) absolute growth rate (AGR) (Table 9).

Table 8 *A. saman* sapling final height (cm) and diameter (cm) growing in association with eight different planting regimes on farms at Calabacito, Los Asientos, and Pedasi.

Treatment	Calabacito		Los Asientos		Pedasi	
	Height	Diameter	Height	Diameter	Height	Diameter
C	71.28 ± 2.71	1.524 ± 1.36	329.94 ± 23.45	7.126 ± 4.30	276.62 ± 12.83	5.118 ± 5.02
L	64.82 ± 10.67	1.417 ± 1.71	280.26 ± 18.78	5.74 ± 4.92	323.66 ± 16.74	5.3 ± 3.94
L-M	86.86 ± 8.89	1.96 ± 1.25	358.66 ± 17.67	6.394 ± 5.47	305.16 ± 25.78	5.358 ± 3.19
L-T	56.46 ± 5.28	1.404 ± 1.41	294.68 ± 13.67	5.054 ± 3.64	416.94 ± 25.44	6.644 ± 8.05
L-T-M	88.9 ± 8.62	1.668 ± 1.49	237.86 ± 28.61	5.06 ± 6.78	301.82 ± 22.47	5.078 ± 3.07
M	97.34 ± 7.02	1.898 ± 1.35	248.06 ± 24.29	4.656 ± 4.96	281.76 ± 25.85	5.23 ± 3.75
T	71.34 ± 6.12	1.522 ± 1.20	266.6 ± 21.57	4.88 ± 3.81	348.1 ± 35.50	6.536 ± 6.75
T-M	85.34 ± 6.87	1.734 ± 2.01	193.98 ± 14.80	3.448 ± 2.25	302.52 ± 25.36	5.622 ± 4.87

Although not significant, saplings in M and L-M had the greatest mean monthly height and diameter increment at Calabacito, particularly in the last two sampling periods. At Los Asientos, saplings in C, L, and L-M grew relatively quickly during the

first two sampling periods and then later slowed their growth rate. Another non-significant, but important result to note is that L-T consistently had the highest mean monthly height and diameter increment in Pedasi, with the exception of the first 5 months, where T was greater (Figs. 15 and 16). Seasonal differences in growth were distinct, with significant differences occurring between all sampling periods with the exception of the first and second rainy seasons ($P = 0.870$). There were no treatment*sampling period interactions for height nor diameter AGR (Table 9).

Table 9 ANOVA table of generalized linear mixed effects model results for the response parameters height and diameter, expressed as absolute growth rate (AGR, cm month⁻¹). Data was collected from July 2015 to August 2017 and only the top five “target” saplings were included in the analysis. The natural log of both parameters was used in the analysis.

Height (AGR)	Num DF	Den DF	F-value	P-value
Treatment (Planting Regime)	7	21	0.56	0.7802
Sampling Period	4	96	40.42	< .0001
Treatment*Sampling Period	28	96	1	0.4767
<hr/>				
Diameter (AGR)	Num DF	Den DF	F-value	P-value
Treatment (Planting Regime)	7	21	0.8	0.5798
Sampling Period	4	96	78.62	< .0001
Treatment*Sampling Period	28	96	0.57	0.9529



Fig. 14 An example of one of the several *A. saman* seedlings growing over 3 m tall after two years of growth in the T-M treatment at Pedasi, Los Santos, Panama.

Survival of *A. saman* seedlings

Seedling survival was assessed in July 2016, one year after planting, and then again in August of 2017. In 2016, 546 of 600 seedlings remained, amounting to an overall survival rate of 95%, 91.5%, and 87.5% of seedlings survived at Pedasi, Los Asientos and Calabacito, respectively (Table 10). In August of 2017, 506 out of 600 seedlings remained, with an overall survival rate of 84.33%.

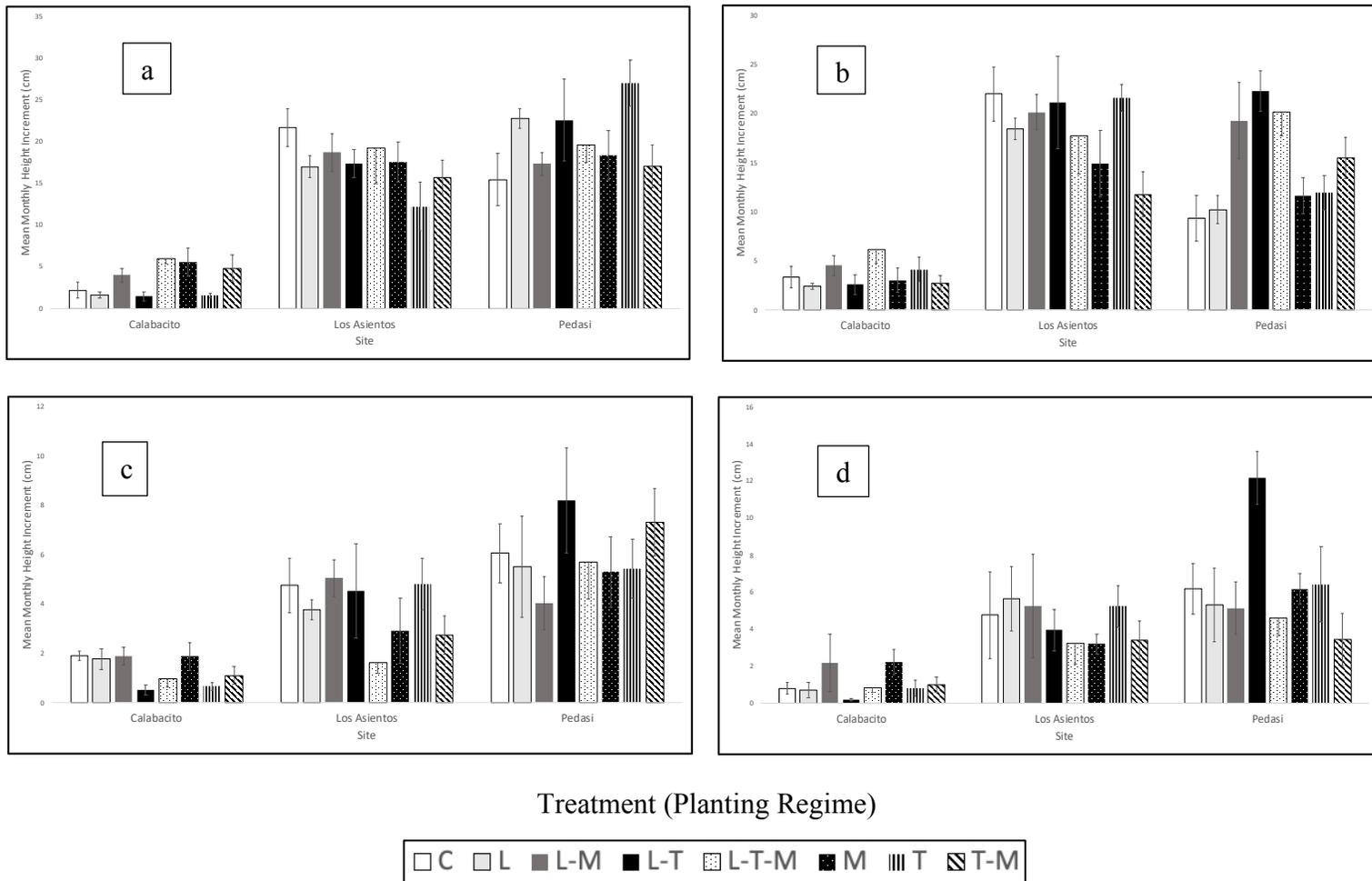


Fig. 15 Mean monthly height increment (cm) of the target saplings during the periods from a) 0-5 months (wet), b) 5-10 months (dry), c) 10-17 months (wet), and d) 17-21 months (dry) after tree planting.

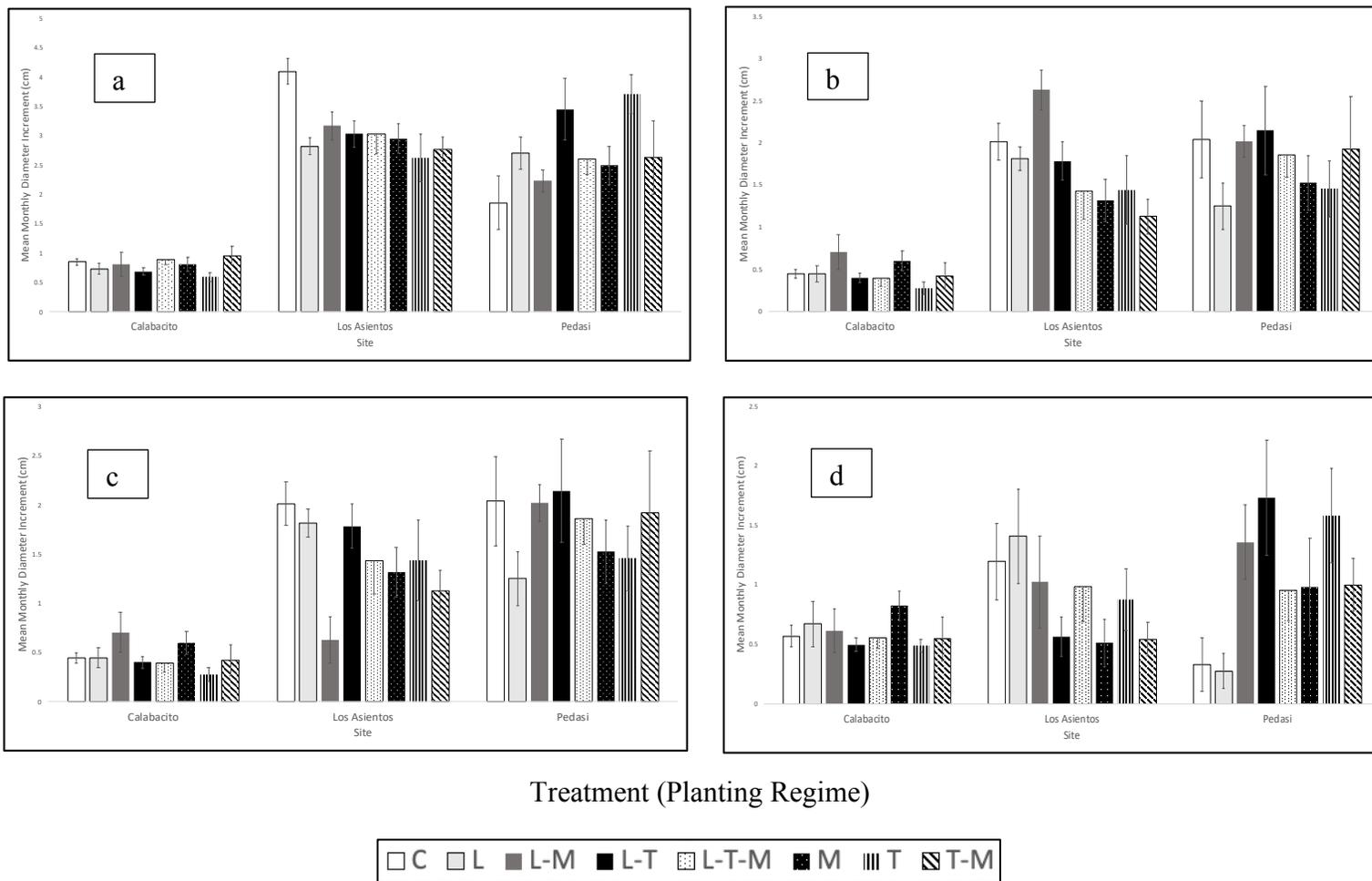


Fig. 16 Mean monthly root collar diameter increment (cm) of the target trees during the periods from a) 0-5 months (wet), b) 5-10 months (dry), c) 10-17 months (wet), and d) 17-21 months (dry) after tree planting

Table 10 Percentage of *A. saman* seedlings surviving in association with eight planting regime treatments at farms in Pedasi, Los Asientos, and Calabacito.

Treatment	Pedasi		Los Asientos		Calabacito	
	2016	2017	2016	2017	2016	2017
C	80	68	100	96	84	68
L	88	84	96	96	88	80
L-M	100	100	40	40	84	76
L-T	92	92	100	100	92	80
L-T-M	100	96	100	100	92	84
M	100	96	96	96	88	88
T	100	100	100	100	84	68
T-M	100	92	100	100	88	84
Year Average	95	91	91.5	91	87.5	78.5
Site Average	93		91.25		83	

There was no treatment effect on seedling survival ($P = 0.8442$). Interestingly, lowest survival in Calabacito and Pedasi occurred in C, the only treatment where seedlings were planted in isolation of neighboring plants. In Los Asientos, lowest survival was documented in L-M (Table 10). Month affected seedling survival, with significantly higher mortality during the dry season than during the rainy season ($P = 0.0432$). There were no significant interaction effects.

Discussion

Soil chemical and Physical Properties

Our hypothesis predicting increased soil fertility in plots that included shrubs was supported. The data clearly suggest that the inclusion of perennial shrubs, and in some cases, perennial grasses, significantly increased soil fertility parameters over time. The macronutrients N, P, K, and Ca all showed significant increases when associated with the shrubs *L. leucocephala* and *T. diversifolia*, however, we were unable to discern the relevant nutrient contributions provided by each shrub.

Significantly higher concentrations of ammonium in L-T, L-T-M, and L suggest that *L. leucocephala* may be provisioning plant available N to the surrounding soil. This result is similar to that presented by Mafongoya and Nair (1997), who showed that the incorporation of *Cajanus cajans* and *L. leucocephala* prunings into the soil released relatively large amounts of N that became available to surrounding *Zea mays*. Palm and colleagues (1997) claimed that tree prunings applied at 5 tons ha⁻¹ can provide between 60 and 150 kg ha⁻¹ of N, supporting *Z. mays* yield of up to 4 tons ha⁻¹ without any added synthetic fertilizer.

The exact mechanism responsible for this increase in ammonium remains unknown, largely because this study involved periodic coppicing and the subsequent addition of organic leaf matter to the soil, two management techniques that act together to confound inquiries into mechanism. Coppicing has been shown to induce root die-back, followed by the subsequent release of N-rich exudates to the soil, while application of organic matter to the soil surface results in N transfer through decomposition and mineralization (Munroe and Isaac 2014). Further, some authors have speculated that the

addition of organic matter or inorganic fertilizers to the soil can impede nodulation and nitrogen fixation in certain species (Kadiata 1997). Thus, it is impossible to conclude exactly what proportion of the recorded ammonium was contributed by leaf litter decomposition and mineralization.

Interestingly, the lowest recorded ammonium and nitrate concentrations were from treatment M, where no shrubs were present. Over the course of the study, the greatest increases in ammonium and nitrate were in L-T (1.01%) and L-T-M (1.17%). Some authors have speculated that there are likely synergistic effects when nitrogen-fixing rhizobium bacteria (from *L. leucocephala*) and vesicular arbuscular mycorrhizal fungi (from *Tithonia diversifolia*) are combined. In a phosphate-deficient soil, De la Cruz and authors (1988) obtained little increase in the N per plant of *A. auriculiformis*, *A. mangium* and *A. falcata* from the inoculation with rhizobium alone, but found 8- to 25-fold increases when inoculated both with rhizobium and selected vesicular arbuscular mycorrhizal (VAM) fungi.

Plant available P increased significantly in T-M, L-T-M, and L-T, indicating that *T. diversifolia* may be playing an important role in P provisioning. In a review of several agroforestry systems in Colombia, León and Osorio (2014) claimed that soil chemical changes from litterfall and turnover are typically not observed until between 6 and 13 years after tree establishment. However, this review did not consider biomass transfer systems, where leaf litter prunings are applied directly to the soil surface and sub-surface. Not only does *T. diversifolia* rapidly produce green leaf biomass high in foliar nutrients; it decomposes quickly after incorporation into the soil (Jama et al. 2000). This rate of

litter turnover greatly enhances biogeochemical cycling, particularly during the wet season, and could be at least partially responsible for the observed increases in P.

Gachengo et al. (1998) applied *T. diversifolia* green biomass at 5 tons ha⁻¹ to maize fields in Kenya and showed that half the leaves decomposed in 1.1 weeks and that N and P were released immediately. In under one year, the amount of N and P supplied to surrounding maize was equivalent to the amount of commercial NPK fertilizer recommended, doubling maize yields in comparison to the unfertilized control. In a similar study, Mutuo (2000) failed to detect increased levels of microbial P in maize fields following the application of *T. diversifolia* green biomass at 1.8 tons ha⁻¹. Mean *T. diversifolia* shrub biomass presented in our study measured 2.038 tons ha⁻¹, all of which was returned to the soil. Differences in P response could be due to differences in the timing of litter application with the nutrient demands of the crop. Effectively synchronizing plant litter nutrient release with crop demands is extremely important for the successful application of biomass transfer systems.

Alternatively, elevated levels of soil plant available P could be attributed to belowground processes not explored here. Belowground associations with vesicular arbuscular mycorrhizal (VAM) fungi and plant-moderated alterations of the rhizosphere via root exudation of organic anions are other plausible explanations for elevated levels of plant available P (George et al. 2002). The increase in root surface area under intercropping systems allows for greater soil exploration, the modification of root distribution in response to nutrient availability (Cahill et al. 2010), and the ultimate enhanced uptake of P and other nutrients.

Soil organic matter and total C increased moderately over time in all treatments except for C, where the ground remained bare. This is not surprising, as bare ground is vulnerable to severe erosion and runoff, violating one of the central tenets of conservation agriculture. In 2017, treatments M and C differed significantly from L-T-M, suggesting that the contribution of both shrubs had an additive effect on SOM. This is not the first study concluding that the intercropping of fertilizer shrubs can have significant positive effects on SOM. In a study examining the intercropping of *Gliricidia sepium* with *Zea mays* in Southern Africa, Beedy et al. (2010) reported that 14 years after establishment, SOM was 12% higher in the *Gliricidia* intercrop compared to the *Zea mays* monoculture. Intercropping has also been shown to increase SOM decomposition, presumably through reduced SOM recalcitrance resulting from lower C:N ratio, higher litter input and better N retention (Cong et al. 2015).

Our hypothesis predicting significantly lower bulk density in treatments with shrubs was only partially supported, because treatment M also reduced bulk density. These data indicate that the establishment of perennial vegetation, herbaceous or woody, is important in the reduction of bulk density. Other studies have reported significantly greater bulk density of soil planted in pasture grasses versus that of woody perennials (Belsky 1994; Staley et al. 2008; Orefice et al. 2017). It is possible that not enough time has passed since tree and shrub planting to observe noticeable bulk density declines in treatments including shrubs. Bulk density also has important implications for soil moisture, in that it directly influences the rate and conversion of precipitation into plant available soil water and other physical properties such as porosity and hydraulic conductivity (Li and Shao 2006).

Soil Volumetric Water Content

Our hypotheses predicting greater VWC at deeper soil depths and during the wet season were supported. However, we did not find any treatment differences for VWC during either season. In March, the driest month, least square mean VWC from 20 cm depth was marginally greater in C and M, but only for that depth. It could be possible that rooting depths of the trees and shrubs are greater than that of grasses, giving them access to moisture found at greater soil depths, depleting more of the moisture from that depth. When woody perennials are planted closely together in arid environments, their root systems tend to be highly plastic, favoring the diminishment of lateral spread, sending their roots deeper into the soil (Lehmann et al. 1998). During March and April, all *Massai* grasses had already lignified and yellowed, while foliage on the trees and shrubs remained green (Fig. 17).



Fig. 17 The green leaves of *T. diversifolia* (foreground) and *A. saman* (background) contrasted with the yellowing tillers of surrounding *Massai* grass in March 2016 at the plantation in Pedasi.

Plant Moisture Stress

Our hypothesis predicting seedling PMS to be affected by month was supported but we did not observe the treatment differences that we expected. The presence of multiple competing species in treatments including shrubs did not affect plant water availability. There was wide variation in treatment response to PMS across sites, which indicates that differences in physical soil structure and topography played an important role in water availability. Several studies have suggested that mixed-species plantings can actually reduce water competition and benefit plants in drought-impacted areas. One study showed that when the commercial timber tree Amarillo (*Terminalia amazonia*) was planted in monocultures, it was less tolerant of drought stress than when it was planted in mixed plots with the nitrogen fixing pioneer Cocobolo (*Dalbergia retusa*) (Craven et al. 2011). Another study in Panama showed that solitary tree saplings exhibited significantly higher $\delta^{13}\text{C}$ values (measure of plant stress) than those surrounded by nitrogen-fixing and non-nitrogen-fixing companion plants. This may indicate increased water stress due to a more intense exposure to sunlight and higher temperatures in solitary individuals (Plath et al. 2010). Although we did not determine this to be true in our study, we did not find any evidence of drought stress in seedlings surrounded by neighboring shrubs and grasses.

Massai DM Production

Our hypothesis predicting significantly more grass DM production in all treatments with shrubs was not supported. Massai grasses growing in treatment L-M outperformed all other treatments, producing 39.3% more biomass than M and more than twice as much biomass than L-T-M and T-M. Reported DM yields for *M. maximus* and

its cultivars vary widely, with open pasture wet season estimates ranging from 2,400 kg ha⁻¹ (Brâncio et al. 2003) to as much as 20,900 kg ha⁻¹ for open pastures fertilized with 150 to 200 kg ha⁻¹ of nitrogen (Fernandes et al. 2014). These estimates depend greatly on site-specific climatic and edaphic conditions, management, and sampling protocol; thus, comparisons should be made cautiously. In a study examining the effects of the fertilizer shrub *Erythrina poeppigiana* on the commonly planted African grass *Brachiaria brizantha*, Bustamante and colleagues (1998) found that production of *B. brizantha* was 41% higher in silvopastoral systems in association with *E. poeppigiana*, which was pruned every 6 months, compared to production measured in *B. brizantha* monocultures. Several other studies looking at the effects of *L. leucocephala* on surrounding Massai production have either found no difference (Wilson 1998) or a reduction in grass dry matter production when compared with grass monocultures (Kumar et al. 2001). Both studies documented a significant reduction of grass DM production after three years, when the tree canopy began to close, shading out the grasses. It is important to reiterate that all eight of our plots contained *A. saman* seedlings, including the control, so Massai grasses were not free of competition like they were in the studies mentioned above. Investigating DM production of Massai monocultures adjacent to our test plots would enhance this study. Nevertheless, these data provide strong evidence that the facilitative effects of *L. leucocephala* are responsible for increased Massai production observed on these plots.

Massai DM production was lowest for plots including *T. diversifolia*. This result was surprising, considering that soils in these plots contained significantly greater concentrations of plant-available P. It is possible that competitive forces may have

superseded more facilitative ones, resulting in grass productivity decline. *T. diversifolia* is known in many regions of the world as a weedy species, that has the potential to grow and spread rapidly. Even with periodic coppicing, foliage returned quickly, which occupied space and created shade (Fig. 18). Furthermore, several authors have reported that *T. diversifolia* may contain allelopathic chemicals that can impede the growth of other plants. Tongma and colleagues (2001) claimed that plants whose seeds were planted in soil where *T. diversifolia* had been growing for five years had significantly less germination, shoot growth, and root growth than those that were planted in a similar soil without the presence of *T. diversifolia*. They also concluded that *T. diversifolia* shrubs growing in water-limited environments contained greater amounts of allelopathic substances per dry weight than in the absence of water stress. Additionally, Otusanya and others (2007) found a significant reduction in the germination, growth, and dry matter production of *Amaranthus cruentus* by four different aqueous extracts containing *T. diversifolia* plant parts. Additional research is needed to determine which of these competitive forces is responsible for the observed reduction in grass biomass on these plots.



Fig. 18 *Tithonia diversifolia* shrubs (background) growing in the T-M plot at Los Asientos. Notice the severely reduced grass biomass in comparison to the M plot in the foreground.

When fertilizer shrub DM production was considered a supplemental addition to Massai forage, cumulative annual DM yielded more than 18 and 16 tons of fodder ha⁻¹ in T-M and L-T-M, respectively. Despite these numbers, it is important to note how much of the biomass produced will actually be consumed by livestock (Devendra and Ibrahim 1999). Anecdotal reports from the Los Santos region suggest that cattle actively browse *T. diversifolia* only sparingly at the end of the dry season. This observation, coupled with the findings presented here showing a moderate reduction in Massai productivity in association with *T. diversifolia*, suggest that this species may be relatively unfit for three-strata silvopastoral systems.

Sapling Growth

Growth results presented here (with the exception of Calabacito) compare similarly to those presented in the literature from the same region. Wishnie et al. (2007) reported a 2-year mean height of 364 cm and mean basal diameter of 7.55 cm for *A. saman* saplings planted in mixed species plantations in Los Santos, Panama. Another study from the Los Santos region reported a mean annual increment height and basal diameter of 143 and 3.84 cm yr⁻¹, respectively (Hall et al. 2011a). Our study recorded the following 2-year site averages for *A. saman* height and diameter: 77.79 cm / 1.64 cm (Calabacito); 276.25 cm / 5.294 cm (Los Asientos); 319.57 / 5.610 cm (Pedasi).

Our hypothesis predicting *A. saman* saplings to benefit in terms of growth from the presence of neighboring fertilizer shrubs was not supported. Treatments had no effect on sapling total height and diameter nor mean monthly height and diameter increment. This result is not dissimilar from that presented by Plath et al. (2010), who found that supplemental planting with companion species had neither a competitive nor facilitative effect on survival and growth performance of timber species. Conversely, Paul and Weber (2016) showed that when planted in association with *Zea mays* and *C. cajan*, the height increment of the timber species *Astronium graveolens*, *Cedrela odorata*, and *Terminalia amazonia* quadrupled that found in pure timber plantations. More time may be needed to form a clearer picture of how these planting regimes ultimately affect the growth of *A. saman*. It is likely that a facilitation effect could develop over a longer period of time.

Sapling Survival

Our hypothesis predicting *A. saman* saplings to benefit from the presence of neighboring fertilizer shrubs in terms of survival was not supported. No treatment differences existed for survival, but several interesting patterns emerged. Sapling survival in Calabacito and Pedasi was lowest in treatment C, where saplings were planted in the absence of neighboring plants. Fertilizer shrubs may be protecting tree saplings from the intensity of sunlight and wind, reducing levels of evapotranspiration, and thus maintaining a more humid microclimate, creating a “nurse effect” (Vandermeer 1989) for tree saplings. In Calabacito, tree saplings were particularly vulnerable to deer herbivory during the dry season both years, resulting in relatively inhibited survival and growth of saplings. All four treatments containing grasses had the highest rates of survival at this site. This may be an indication that established grasses provided a visual barrier that deterred browsing by deer. At Los Asientos, sapling survival was lowest in L-M, the treatment with the highest production of *Massai*. Management intervention was taken to prevent competition-induced mortality with the regular application of *rodajeo* (spot-ringed weeding), so it is unlikely that grass competition was the sole reason for mortality on this plot.

Conclusion

In conclusion, this experiment has demonstrated the successful integration of the native tree species *A. saman* with the non-native shrubs *L. leucocephala* and *T. diversifolia*. We have shown that with active management, *A. saman* tree saplings can be grown simultaneously with shrubs and grasses without negatively affecting growth and

survival of all system components. Further, we have shown that the overall productivity of Massai pastures associated with *L. leucocephala* shrubs was enhanced, suggesting a potential facilitative interaction in these plots.

Two years after planting, the simulated silvopastures showed significant changes in several soil properties. Bulk density was significantly reduced in all plots except for C, where the soil was left bare. There were significant increases in Ca, K, ECEC and soil organic matter in plots that included shrubs throughout the course of the study. Additionally, plant available N and P increased in plots containing *Leucaena* and *Tithonia* shrubs, respectively.

There were no planting regime effects on soil water availability and *A. saman* sapling moisture stress. Additionally, *A. saman* growth and survival was not affected by planting regime, indicating that there were neither competitive nor facilitative effects strong enough to influence any of these factors, at least at this early stage in growth. Massai grass DM production showed a marginally significant positive response to the association with *L. leucocephala*, and although not significant, was reduced in the presence of *T. diversifolia*. Further research is needed regarding if and to what degree Massai grasses are affected by exposure to allelopathic chemicals released by this species. Although the findings presented here indicate facilitation by *A. saman* and *L. leucocephala* on surrounding pasture growth, plantations are still in their early stages, and results should be heeded with caution.

Management Recommendations

It is expected that continued *A. saman* growth and canopy expansion will begin to limit pasture productivity, including that of the shade intolerant shrubs. Thus, periodic thinning of *A. saman* to a desired density is recommended and regular coppicing of shrubs will be needed to maintain nutrient levels and reduce spatial competition. Once introduced to the plots, cattle will help to control rampant growth of grasses and shrubs as well as recycle nutrients back to the soil via urine and manure. However, this is a very different kind of biomass transfer than the green mulching activities used in this study, and different results could be expected in the future.

One of the biggest drawbacks of this system is the labor required to periodically coppice shrubs and return green biomass to the soil. Moreover, the opportunity cost that is required to establish these systems can be prohibitive. During the early stages of silvopasture establishment, tree seedlings are particularly vulnerable to browsing and trampling by cattle. This makes the inclusion of livestock imprudent until tree saplings have grown above the browse line. A more practical alternative for producers would be to plant trees and shrubs in rows protected by polywire electric fencing with grasses planted on both sides. Shrubs should be coppiced several times and applied as green mulch to nearby grasses. Once the saplings reach an appropriate height, fencing should be removed to permit livestock to browse the shrubs, naturally coppicing them and returning nutrients to the soil.

Combining grasses with shrubs and trees creates three distinct foraging layers that not only increases system productivity but more importantly, mitigates forage losses during severe droughts and provides nutritional diversity to livestock. The increased

adoption of similar agro-ecological systems will be critical for sustainable and environmentally sound livestock production in a future compromised by climate change.

Chapter 4

Initial Performance of Red Mulberry (*Morus rubra* L.) Under a Light Gradient: An Overlooked Alternative Livestock Forage?

Abstract

Climate change creates much uncertainty for the future of agriculture, particularly due to an increase in summer droughts that result in the loss of range productivity. The integration of perennial shrubs into pasture is one way to diversify forage resources and compensate for losses caused by droughts during the summer forage gap. One species that has shown promise for use as woody fodder is white mulberry (*Morus alba* L.). While the yield and nutritional quality of *M. alba* has been widely studied, there remains a paucity of information for its native congener red mulberry (*M. rubra*). We report on the initial survival, growth, yield, and nutritive value of *M. rubra* seedlings planted under a cherrybark oak (*Quercus pagoda*) canopy at four overstory densities: 3x3m (D), 6x6m (S-D), 9x9m (S-O), and 12x12m (O), representing a light gradient. Global Site Factor (GSF), the proportion of global radiation under a plant canopy relative to that in an open area, ranged from 23.91 to 92.29%. Despite summer drought conditions, 81.25% of seedlings survived, ranging from 70% in D to 90% in O. The odds of seedlings surviving in D were significantly lower than those of surviving in S-O and O. Seedlings expressed morphological plasticity with increased diameter growth, decreased specific leaf area, and increased DM yield as canopy openness increased. Seedlings appeared to reach a light saturation point somewhere between 66.21 and 92.03% Global Site Factor (GSF) in terms of DM yield, obtaining highest leafiness at 66.21% GSF. Nutritive value assessed during the summer forage slump surpassed the quality of many common pasture forages. Nutritive value did not differ significantly above 66.21% GSF in terms of CP and fiber fractions and did not differ significantly for any of the treatments in respect to digestibility (TDN and RFV). Our results indicate that *M. rubra* seedling survival, growth, productivity, and nutritive value can be optimized on this site at 66.21% GSF, an irradiance level that corresponds to an overstory planting density of 9 x 9m (~500 trees ha⁻¹). Due to unseasonably high protein and mineral content with relatively low fiber fractions, *M. rubra* could serve as a highly nutritional supplemental forage to livestock in multi-strata silvopastures during drought conditions, but more research is needed to determine seedling response to repeated defoliation events.

Key words: Tree-shrub interactions, multi-strata agroforestry, forage production, silvopasture

Introduction

The climate of the central United States has changed measurably over the last half century and significant changes in temperature and precipitation are predicted for the future. Extreme heat waves associated with less rainfall will become more frequent, resulting in an increase in the number of short-term summer droughts and precipitation is likely to arrive in the form of heavy rains (Dai 2013; Trenberth et al. 2014). Climate change creates uncertainty for the future of agriculture in the Midwest and will likely have far-reaching consequences for animal production through its effects on forage and range productivity (FAO 2009). Higher temperatures tend to lower feed intake and feed conversion rates (Rowlinson 2008) and increased drought frequency may decrease range production, forage longevity, and the availability of feedstock for the winter.

In Missouri, climate change has already had demonstrable effects on livestock production. In August of 2012, 93% of the state experienced extreme drought (D3) conditions, creating pasture deficits and dwindling hay supplies that forced many cattle ranchers to sell their herds (Gustin 2012). Estimated financial losses to livestock and poultry operations were more than \$547 million (Nixon 2013). In the summer of 2018, an extreme drought (D3) plagued 19% of Missouri, resulting in 72% of cattle pastures rated as poor or very poor. Additionally, haymaking was cut short and hay prices skyrocketed to \$165 Mg⁻¹ (Dailey 2018). To make matters worse, most of Missouri's most widely used forage crop tall fescue (*Festuca arundinacea*) has become infected with the fungal endophyte *Neotyphodium coenophialum*, elevating body temperatures and respiratory rates in cattle, making animals more vulnerable to heat-related stress (Roberts 2010).

One way to mitigate the impacts of climate change is by intentionally integrating trees, forages, and livestock into one cohesive management unit. This agroforestry practice, known as silvopasture, has been shown to increase overall production, animal welfare, and environmental benefits (Broom et al. 2013; Jose et al. 2019). Silvopasture can reduce heat stress in livestock, increase weight gain, reduce calving difficulty, provide high quality forage, extend the grazing season, and increase the value of timber (McIlvain and Shoop 1971; McArthur 1991; Garrett et al. 2004; Kallenbach et al. 2006; Kallenbach 2009).

Trees and shrubs can also provide alternative and highly nutritious forage sources during critical periods. In other parts of the world, forage shrubs (also known as fodder shrubs) can be a strategic resource for farmers during the worst drought periods that often occur during the summer slump or forage gap. Some forage shrubs retain green foliage amidst even the harshest droughts due to their deep root systems that have specialized access to the water table. As the summer progresses, forage shrubs have been shown to lose nutritive value, digestibility and palatability at a slower rate than herbaceous forages (Talamuci and Pardini 1999), providing relatively high quality supplemental forage during these times. In Bali, Indonesia, the production of a shrub layer creates a three strata forage system that has resulted in an increase in stocking rates by one animal ha⁻¹ and an increase in live weight gain by 153 kg ha⁻¹ yr⁻¹ (Devendra 2012). In Colombia, palatable nitrogen-fixing shrubs are planted at high densities amidst grasses and scattered trees and have increased milk yields by as much as 130% (Murgueitio et al. 2011). Woody fodder has been reported to improve digestion, reduce parasitic infestation, and decrease methane emissions, but anti-nutritional components and toxins can limit their

use (Vandermeulen et al. 2018a). In the temperate Mediterranean region of Italy, Papanastasis and colleagues (2008) concluded that natural woody shrub communities can be browsed directly by livestock and used as supplemental feed reserves during critical forage shortages.

The use of woody trees and shrubs for livestock fodder in the United States has been limited primarily due to a relatively limited plant selection and existing cultural and behavioral norms. Temperate regions lack the diversity of nutritious, nitrogen fixing woody plants that are used readily for fodder in the tropics. The few trees that produce palatable fodder only do so during the growing season when highly preferred herbaceous forage is available, unless compromised by extreme weather. In the winter, dried grasses and legumes in the form of hay are used as a primary feed source.

Orefice and colleagues (2016) surveyed landowners practicing hardwood silvopasture in the Northeastern U.S. and concluded that farmers valued trees for multipurpose uses. When asked for their opinion on important areas for future silvopasture research, a popular response was to further investigate the quality, selection, and management of plants serving as potential forage alternatives. Three out of 20 studied farms were managing woody browse as a component of forage in their silvopastures, but no specifics were provided in terms of species preferences.

In spite of these barriers, several perennial species have shown potential to provide woody fodder in temperate silvopastoral systems, including willow (*Salix spp.*) (Moore et al. 2003; Pitta et al. 2005), osage orange (Forwood and Owensby 1985), bristly locust (Burner and Burke 2012), and mulberry (*Morus spp.*). White mulberry (*Morus alba*), widely known for its use as a fodder for silk worm cultivation in Asia, can

produce higher quantities of digestible nutrients than most traditional forages (Sánchez 2000). Its leaves are highly palatable and digestible (70-90%) with a protein content varying from 15-28% and relatively low fiber and tannin contents, establishing its potential as a strategic forage source in temperate as well as tropical regions of the world (Sharma and Zote 2010). The optimum temperature for white mulberry biomass yield is reported to be 32 °C (Fukui 2000), within a range of temperatures that corresponds with the summer forage slump in Missouri. Both ruminants and monogastrics are known to consume the leaves of *M. alba* by either directly browsing the shrub or more commonly by ingesting clippings that are cut and carried to the location of the animal. Mulberry leaves can be used in poultry production and provide a valuable source of vitamin A due to their high carotene content. Moreover, pigs relish the maturing fruits which are relatively high in protein, readily devouring them throughout the summer (Shepard 2013).

M. alba was introduced to North America from East Asia in the 1600s and has since become naturalized in eastern North America. Although genetically differentiated from its native, shade tolerant congener red mulberry (*Morus rubra*), *M. alba* poses a growing threat to *M. rubra* trees throughout the eastern and central United States through competition and hybridization (Burgess and Husband 2006). This is concerning, considering this species' vital importance to native wildlife. One study in Arkansas reported 32 species of native songbirds foraging on red mulberry fruits over a 67-hour period (Kannan 2018). The planting of *M. rubra* in temperate silvopastures could be an effective means of restoring this native species while provisioning fruit and fodder to livestock during the summer forage gap. While the yield and nutritional quality of *M.*

alba has been widely studied (Sánchez 2000), there remains a paucity of information in this capacity for *M. rubra*.

A number of studies have been published on the growth and nutritional response of herbaceous forages to different shade intensities with implications for silvopasture (Lin et al. 2001, Belesky 2005, Buerger et al. 2006, Kallenbach et al. 2006). However, there is limited data available on how shade affects the survival, growth, yield, and nutritive content of naturally occurring understory woody shrubs, largely because they have not been viewed as a forage resource for livestock in the United States.

Objectives

The objectives of this study were to: 1) Establish plantings of *M. rubra* seedlings under four different overstory densities: 3x3m (D), 6x6m (S-D), 9x9m (S-O), and 12x12m (O) of cherrybark oak (*Quercus pagoda*), 2) quantify understory light levels with the use of hemispherical photography, 3) compare *M. rubra* survival among treatments, 4) evaluate differences in *M. rubra* growth rate by measuring height (cm), root collar diameter (mm), crown width (cm), and specific leaf area (SLA; $\text{cm}^2 \text{g}^{-1}$), 5) estimate differences in *M. rubra* stem and leaf dry matter yield, and 6) evaluate differences in *M. rubra* leaf nutritive value.

We hypothesized that *M. rubra* survival would be greatest in open areas. Although *M. rubra* is a shade tolerant species, it should reach its full photosynthetic potential in open sunlight and achieve greatest growth rates and dry matter yield in treatment O. We hypothesized that SLA would be greatest in treatment D due to morphological differences stimulated by lower light levels. We expected *M. rubra* leaf

nutritive value to increase as understory light levels decreased, predicting highest values for total digestible nutrients (TDN) and crude protein (CP) content and lowest values for the fiber fractions acid detergent fiber (ADF) and neutral detergent fiber (NDF) in D.

Materials and Methods

Study Site

This study was conducted at the Horticulture and Agroforestry Research Center (HARC) in New Franklin, Missouri (longitude 92° 44' W; latitude 39° 01' N). In November of 1997, an area encompassing 96,000 ft² (~2.2 acres) on the southern border of the farm was planted in cherrybark oak (*Quercus pagoda*) seedlings at four different densities (Fig. 1). The soil type at this location is a Menfro silt loam (Fine-silty, mixed, superactive, mesic Typic Hapludalfs) and is well-drained. The soil is relatively fertile (Table 1) with textures ranging from loam to silt loam and an average soil pH of 5.01 (\pm 0.16). Historic mean annual precipitation and temperature was 94.31 cm and 12.3 °C, respectively, compared to 85.8 and 13.37 °C for the overall mean of data collected in 2016, 2017, and 2018 (Table 2). Mean monthly values are displayed for each year (Fig. 2).

Experimental Design

A randomized complete block design was used with overstory tree density as the treatment with two replications. Overstory *Q. pagoda* were planted at three different densities: 3x3m, 6x6m, 9x9m, and 12x12m spacing between trees. Hereafter,

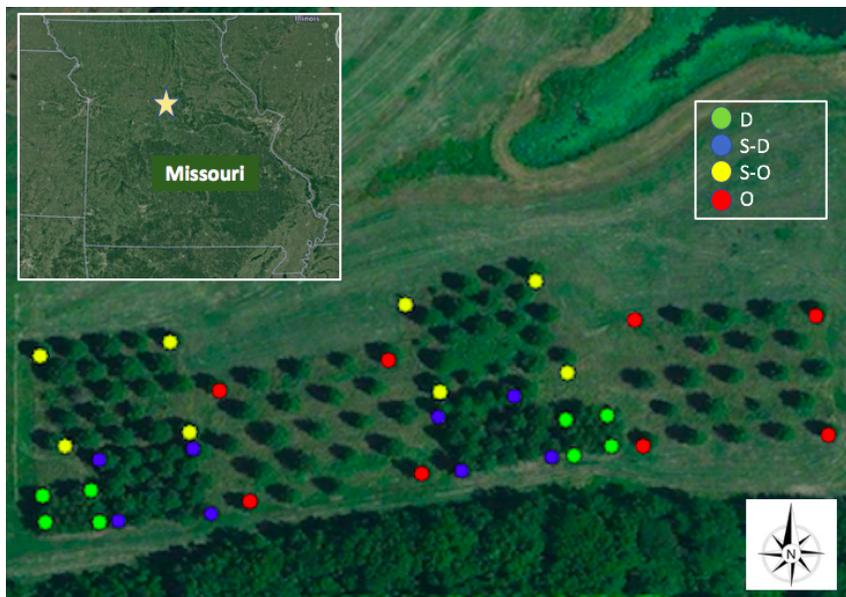


Fig. 1 Randomized complete block design with four cherrybark oak (*Quercus pagoda*) overstory tree spacings: D (3x3m), S-D (6x6m), S-O (9x9m), and O (12x12m) at the Horticulture and Agroforestry Research Center (HARC) in New Franklin, Missouri. Twenty red mulberry (*Morus rubra*) seedlings were planted on 3x3m spacings in the understory of each plot.

treatments will be referred to as dense (D), semi-dense (S-D), semi-open (S-O), and open (O) for convenience. Diameter at breast height (DBH; cm) was measured for all overstory *Q. pagoda* trees to calculate stand basal area and quadratic mean diameter (Table 3). Twenty *M. rubra* seedlings were planted on a 3x3m spacing in the understory of each plot, which differed in size according to overstory tree spacing (Fig. 3). Seedlings were protected with plastic tubing and measured periodically throughout the study (described below).

Table 1 Chemical soil properties for composite samples collected in May of 2018.

Treatment	pH	N.A.	OM	Bray I P	Ca	Mg	K	CEC
Overstory Tree Spacing (m)		Meq 100g ⁻¹	%	Lb Ac ⁻¹	Lb Ac ⁻¹	Lb Ac ⁻¹	Lb Ac ⁻¹	Meq 100g ⁻¹
3 x 3	4.95 ± 0.15	4.75 ± 0.25	3.15 ± 0.15	95 ± 20	2895 ± 95	549.5 ± 107.5	528 ± 154	14.95 ± 0.75
6 x 6	4.9 ± 0.30	4.75 ± 1.25	3.75 ± 0.25	74 ± 30	3068.5 ± 247.5	542.5 ± 128.5	325 ± 21	15.1 ± 2.4
9 x 9	4.95 ± 0.15	5 ± 0.50	4.35 ± 0.15	92.5 ± 26.5	3363.5 ± 121.5	684 ± 11	460 ± 19	16.85 ± 0.25
12 x 12	5.25 ± 0.05	4 ± 0.00	4.3 ± 0.10	56.5 ± 7.5	2756.5 ± 16.5	479 ± 12	431.5 ± 4.5	13.45 ± 0.05

Table 2 Precipitation (cm) and mean monthly temperature (°C) at the Horticulture and Agroforestry Research Center (HARC) in New Franklin, Missouri. Source: NOAA Midwestern Regional Climate Center.

Year	Precipitation (cm)	Temperature (°C)
2016	93.71	13.51
2017	85.62	13.26
2018	78.08	13.32
3-year Mean	85.80	13.36

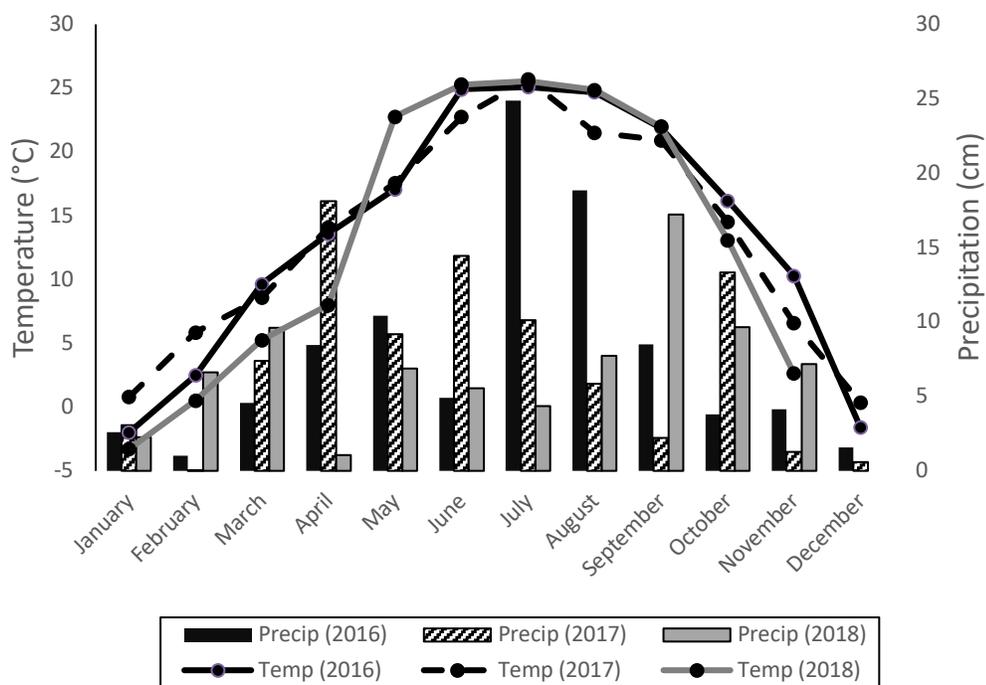


Fig. 2 Mean monthly precipitation (cm) and temperature (°C) for three years (2016-2018) at the Horticulture and Agroforestry Research Center (HARC) in New Franklin, Missouri. Source: NOAA Midwestern Regional Climate Center.



Fig. 3 Planting arrangement of the twenty red mulberry seedlings planted per treatment. Seedlings were planted on a 3x3m spacing under four different densities of cherrybark oak trees.

Table 3 Overstory composition with four planting densities in a 21-year old cherrybark oak (*Quercus pagoda*) plantation at the Horticulture and Agroforestry Research Center (HARC) in New Franklin, Missouri.

Treatment	D	S-D	S-O	O
Trees ha ⁻¹	551 ± 10	269 ± 10.64	148 ± 3.55	91 ± 3.32
DBH (cm)	28.47 ± 1.50	39.53 ± 1.86	40.72 ± 2.71	44.45 ± 1.44
Basal Area (m ² ha ⁻¹)	36.69 ± 3.04	33.39 ± 1.33	20.67 ± 2.71	14.34 ± 0.66
Quadratic Mean Diameter (cm)	29.11 ± 0.41	39.75 ± 0.03	42.07 ± 0.39	44.66 ± 0.31
Global Site Factor (%)	23.91 ± 3.51	46.28 ± 4.11	71.38 ± 6.08	92.29 ± 2.81

Means and standard error are reported. Global Site Factor is the percentage of total incident solar radiation reaching the understory canopy.

Understory Light Environment

Light quantity was determined by measuring solar transmission radiation with hemispherical photography. Upward hemispherical images were photographed in the late summer of 2017 and the winter of 2018 using a Cannon EOS Rebel with an Opteka Vortex Fisheye Lens. Images were taken 1 m above the ground on a tripod equipped with a Delta-T devices mount used for horizontal leveling and north-south orientation of the camera. Measurements were taken at dawn to maximize light evenness. Three photographs were taken at the middle and corners of each plot with automatic exposure compensation and bracketing at -2.0, -1.0, and 0. This was done to mitigate the effects of over-exposure that can result from using the automatic exposure in dense canopies. Images were analyzed with Hemiview software (Delta-T Devices Ltd., Cambridge, U.K.), which classifies pixels into two classes: open sky and obstructed sky. The software calculates the global site factor (GSF), which is the sum of direct and diffuse radiation (excluding reflected radiation) entering through canopy openings as a proportion of the amount of radiation that would fall on the same point given no overhead obstructions over the course of a year (Griscom et al. 2009). Values range from 0 to 1, which 0 being no radiation (complete sky obstruction) and 1 being the radiation for an open location, where the sky is completely visible (Rich et al. 1999).

Soil Properties

Soil samples were collected preliminarily to confirm that all plots were relatively homogeneous. Twenty randomly distributed soil samples (200g) were collected on a zig-

zag path at 0 -15 cm depth with an augur from each plot and analyzed for chemical and physical properties at the University of Missouri's soil testing laboratory.

Growth, Specific Leaf Area, and Dry Matter Yield

A total of 160 comparably sized (~30 cm) *M. rubra* bare root seedlings were purchased from Forrest Keeling Nursery in October, 2015, refrigerated throughout the winter and planted in early May, 2016. Twenty seedlings were planted in four rows (five per row) on a 3m x 3m spacing in the understory of each treatment (Fig. 3). All plots were fertilized after planting using a compound fertilizer (12:24:12 N/P/K) at a rate of 180 kg ha⁻¹. In May of 2018, two years after seedling establishment, we broadcast seeded Orchardgrass (*Dactylis glomerata*) and Panicked Tick-trefoil (*Desmodium paniculatum*) to establish forages at the ground level. Due to a particularly dry spring and lack of irrigation, the forage stand failed to establish. Instead, a variety of broadleaf weeds emerged, including pokeweed (*Phytolacca americana*), ragweed (*Ambrosia spp.*), smartweed (*Polygonum*), beggar-ticks (*Bidens pilosa*), ironweed (*Vernonia fasciculata*), bush honey suckle (*Lonicera maackii*), poison ivy (*Toxicodendron radicans*) and some white clover (*Trifolium repens*) and tall fescue (*Festuca arundinacea*) that remained in the seedbed. It is important to note that the establishment of a ground forage component is necessary and critical to the success of any silvopasture operation. Time constraints prohibited re-seeding of ground forages and the assessment of interactions between woody and herbaceous components.

For the growth parameters height (cm), diameter (mm), crown width (cm), specific leaf area (cm² g⁻¹), and dry matter yield (kg ha⁻¹), we used a commonly applied

methodology known as the “top height tree collective” (Pretzsch 2009). This approach considers only the five most vital seedlings in each plot as targets for analysis; thus, the 10 tallest trees per treatment were selected as the sample population. These trees are most representative of those individuals that could potentially develop a competitive advantage over others. Additionally, the use of only five target trees avoids differences in means that could be caused by high differences in survival between treatments. Treatments with higher survival rates are likely to have wider ranges of heights and diameters than treatments with lower survival rates (Paul and Weber 2016).

M. rubra seedlings were measured with a metric tape and a vernier caliper to an accuracy of 1 cm and 0.1 mm, respectively, twice in the summer of 2017 and twice in the summer of 2018 (Fig. 4). Height (cm), root collar diameter at ground level (from here also referred to as “diameter”; mm), and mean crown diameter (N-S, E-W; cm) were selected as the main indicators for tree growth. Parameters from the top five most vital seedlings were used for analysis.

Survival was recorded as *either* dead or *alive* and at the end of the first year, all dead seedlings were replaced with similar-sized bare root seedlings.

In mid-August of 2018, eight healthy leaves were collected from current year shoot growth on the five target trees. Specific leaf area (SLA; $\text{cm}^2 \text{g}^{-1}$) was measured on a total of 40 leaves per plot using the Leafscan © iOS app. Leafscan is a new technology, but accuracy is reported to be good. Errors of between two and five percent have been recorded when correlated with the Licor LI-3100C leaf area meter (Leafscan 2019). All measurements were made in the field when leaves were still fresh.



Fig. 4 Height and root collar diameter measurements were taken on all *M. rubra* seedlings.

To estimate dry matter yield, destructive sampling was used in late August of 2018. Seedlings were bottom pruned and fresh weight and the number of stems were recorded for the five most vital seedlings in each plot. Whole plant dry matter (DM) was recorded and leaf to stem (L:S) ratio was determined by separating leaves with petiole from the stems. Sub-samples of plant fractions (whole plant, leaf, and stem) were dried in an oven at 60 °C for 48 h for DM determination. Fresh yield was converted to DM yield with the following equations:

$$\text{DM yield plot}^{-1} = \text{weight of fresh material} \times \text{DM}\%$$

$$\text{DM yield ha}^{-1} = \text{DM yield plot}^{-1} \times \text{number of plots ha}^{-1}$$

Leaf Nutritive Value

Nutritive value was assessed in late August of 2018. Ten leaves on each of the five target shrubs were collected and combined for each treatment. Composite samples of *M. rubra* leaves were dried to weight constancy, milled to a homogenized powder, and analyzed using wet chemistry procedures for dry matter crude protein (CP, %), acid detergent fiber (ADF, %), and neutral detergent fiber (NDF, %), total digestible nutrients (TDN), relative feed value (RFV), and mineral content at the Dairy One Forage Lab in Ithaca, NY.

Data Analysis

To analyze differences in seedling survival, we used a logistic mixed effects analysis of variance (ANOVA) model that provided an odds ratio, or probability of death, for each treatment. To better understand the structure of errors due to treatment, replicate, and tree number, we ran three null models without testing for fixed effects of treatment and month and recorded the -2 Residual Log Pseudo-Likelihood and General Chi-Square / DF fit statistics available using the Glimmix procedure in SAS (SAS® 2013). The second model using the compound symmetry (CS) covariance matrix structure gave the smallest fit statistics, so we used the CS structure for errors.

Tree density effects on all response variables were analyzed with a linear mixed model analysis of variance (ANOVA) using the MIXED procedure in SAS (SAS® 2013). We considered the overstory tree density treatments as main plots and sampling dates as repeated measures (Steel and Torrie 1980). Treatment, sampling date, and their interactions were considered fixed effects while the replicate block, treatment nested

under replicate block, and sampling date nested under treatment and replicate block were considered random effects. The advantage of using a mixed model approach to analyze these data is the inclusion of a random intercept for each plot that serves to account for the clustered nature of tree plantings (Piepho et al. 2003). Additionally, we examined height and diameter growth increment by calculating an absolute growth rate (AGR; cm month⁻¹) following Kikvidze and Armas (2010) by using the following equation: $AGR = (W_2 - W_1) / (t_2 - t_1)$, where W represents either height or root collar diameter at t_1 at the beginning of the study and t_2 at the end of the study (in months). All increment parameters were subsequently transformed using the natural logarithm to be used for analysis.

All dependent variables were tested for normality and \log_{10} transformed when necessary to meet the assumption of homoscedasticity. A simple variance component matrix was used to include the random plot effect and the residual maximum likelihood estimation was used to estimate covariance parameters. When significant effects were detected, pair-wise comparisons were performed using the Tukey-adjusted least squares method. Main effects and all interactions were considered statistically significant when $P = < 0.05$.

Results

Understory Light Environment

All four treatments differed significantly with respect to understory light levels in the summer and winter ($P = < 0.0001$). There was a significant difference found between seasons ($P = 0.0012$), but no interaction was found between treatment and season. Mean

Global Site Factor ranged from summer values of 21.86% in D to winter values of 93.21% in O (Table 4).

Survival

In May of 2017, one year after the planting, 92 out of 160 seedlings remained, amounting to an overall survival rate of 57.5% (Table 5). In May of 2018, 131 out of 160 seedlings remained, with an overall survival rate of 81.88%.

Table 4 Global Site Factor (GSF) values representing the sum of direct and diffuse radiation (excluding reflected radiation) entering through canopy openings as a proportion of the amount of radiation that would fall on the same point given no overhead obstructions over the course of a year. Estimates given for dense (D), semi-dense (S-D), semi-open (S-O), and open (O) treatments.

Global Site Factor (%)		
<u>Treatment</u>	<u>Summer</u>	<u>Winter</u>
D	21.86 ± 1.74	27.41 ± 1.56
S-D	43.80 ± 1.98	49.99 ± 0.68
S-O	66.21 ± 2.11	76.47 ± 1.42
O	92.03 ± 1.92	97.44 ± 1.62
<u>Factor</u>	<u>F-value</u>	<u>P-value</u>
Treatment	1298.68	<0.0001
Season	66.43	0.0012
Interaction	1.86	0.2766

Means and standard errors are reported. F-values and significance values for the linear mixed model analysis are given.

Overstory tree density significantly affected seedling survival ($P = 0.0442$). The odds of seedlings surviving in D were significantly lower than those surviving in S-O and O ($P = 0.0472$ and $P = 0.0130$, respectively), but not for S-D. No significant differences in odds of mortality existed between S-D, S-O, and O for any of the measurement periods (Table 5). Sampling period also affected seedling survival ($P = 0.0132$), but only for O between month 16 and months 26 and 28 ($P = 0.0039$ and $P = 0.0039$, respectively). There were no significant interaction effects.

Table 5 Percentage of *M. rubra* seedlings ($N = 20$) surviving during each measurement period in dense (D), semi-dense (S-D), semi-open (S-O) and open (O) treatments. Replanting activities occurred at the dashed line.

Age (months)	Tree Density				Overall
	D	S-D	S-O	O	
0	100 ^{aA}	100 ^{aA}	100 ^{aA}	100 ^{aA}	100
12	57.5 ^{aB}	60 ^{aB}	57.5 ^{aB}	55 ^{aB}	57.5
16	70 ^{aC}	77.5 ^{abC}	87.5 ^{bC}	95 ^{bA}	82.5
24	70 ^{aC}	77.5 ^{abC}	87.5 ^{bC}	92.5 ^{bA}	81.88
26	70 ^{aC}	77.5 ^{abC}	87.5 ^{bC}	90 ^{bC}	81.25
28	70 ^{aC}	77.5 ^{abC}	87.5 ^{bC}	90 ^{bC}	81.25

Numbers that share a letter are not statistically different from one another at $\alpha = .05$ (lower case indicates treatment differences; upper case indicates differences in measurement period).

Growth

Seedling growth was less vigorous than expected, with a mean seedling height of 83.77 cm, mean diameter of 1.28 mm, and mean crown width of 59.33 cm after 28

months of growth. There was no overall treatment effect on seedling height ($P = 0.0637$), although least squares mean estimates increased numerically under increasing canopy openness (Table 6). As expected, significant differences existed between sampling dates ($P = 0.0001$), with the exception of growth between months 14 and 16 ($P = 0.3016$) and months 26 and 28 ($P = 0.5940$) (Table 6). A statistically significant interaction was found between overstory treatment and sampling date ($P = 0.0005$). Although the treatment groups never became significantly different at one sampling point, the strength of the treatment effect increased over time (Fig. 5).

The analysis of mean AGR for height similarly revealed no treatment differences, but showed differences for year and treatment*year. Height mean AGR was significantly greater in 2017 than it was for 2018 ($P = 0.0196$). The treatment*year effect showed that mean AGR in treatment D was significantly less than mean AGR in S-D, S-O, and O in 2017 but not for 2018 ($P = 0.0129$). Interestingly, in 2018, when all AGRs were lower, treatment D had an AGR of 22.43 cm yr⁻¹ compared with 19.70, 18.93, and 14.88 cm yr⁻¹ for O, S-O, and S-D, respectively (Fig. 6).

Generally, seedling diameter increased as tree density decreased (Table 6). Significant differences in diameter existed between all sampling dates ($P = 0.0001$), with the exception of months 0 and 14 ($P = 0.1381$), 0 and 16 ($P = 0.0639$), 14 and 16 ($P = 0.9924$), and 16 and 26 ($P = 0.0536$). A statistically significant interaction was found between overstory treatment and sampling date ($P = 0.0405$). Similar to results presented for height, the strength of the treatment effect increased over time (Fig. 7). In fact, in the last measurement period, significant differences existed between D and O ($P = 0.0028$) and between S-D and O ($P = 0.0016$).

The analysis of diameter AGR showed significant treatment ($P = 0.0255$) and year effects ($P = 0.0102$), but no interaction effect ($P = 0.2826$). Post-hoc Tukey tests showed a significant difference between treatments S-D and O ($P = 0.0463$), with O displaying significantly greater mean diameter AGR than S-D (Fig. 8). Diameter AGR was significantly less in 2017 than it was for 2018 ($P = 0.0102$).

Crown width (cm) was not affected by treatment ($P = 0.2289$) but sampling dates differed significantly ($P = 0.0001$). There was a statistically significant interaction between overstory treatment and sampling date ($P = 0.0003$) (Fig. 9). The treatment effect varied for each sampling date, with some treatments affecting crown widths significantly during particular sampling dates and not during others (Table 6).

Dry Matter Production

Overstory tree density significantly affected *M. rubra* dry matter yield ($P = 0.0008$). Post-hoc tests showed that seedlings in D and S-D produced significantly less dry matter than those in O ($P = 0.0352$ and $P = 0.0026$, respectively) and S-O ($P = 0.0012$ and $P = 0.0019$, respectively) (Fig. 10). Seedlings in S-O produced the greatest amount of biomass with a total of $169.15 \text{ kg ha}^{-1}$, followed closely by O which produced $164.37 \text{ kg ha}^{-1}$. Leaf weight differed significantly between treatments ($P = 0.0023$), increasing with overstory openness and differing significantly between all groups with the exception of S-O and O and D and S-D. Stem weight also differed significantly between treatments ($P = 0.0143$). S-O had the greatest mean stem weight (77.76 kg ha^{-1}), which differed significantly from D ($P = 0.0259$) and S-D ($P = 0.0242$), but not for O ($P = 0.8840$). There were no treatment effects on stem count ($P = 0.2878$). Lastly, the leaf to stem ratio differed significantly between treatments ($P = 0.0452$), ranging from 0.8390 in D to

1.1890 in S-O (Table 7). Post-hoc tests showed that the leaf to stem ratio in D was significantly lower than S-O ($P = 0.0084$) and O ($P = 0.031$), but did not differ from S-D ($P = 0.2015$).

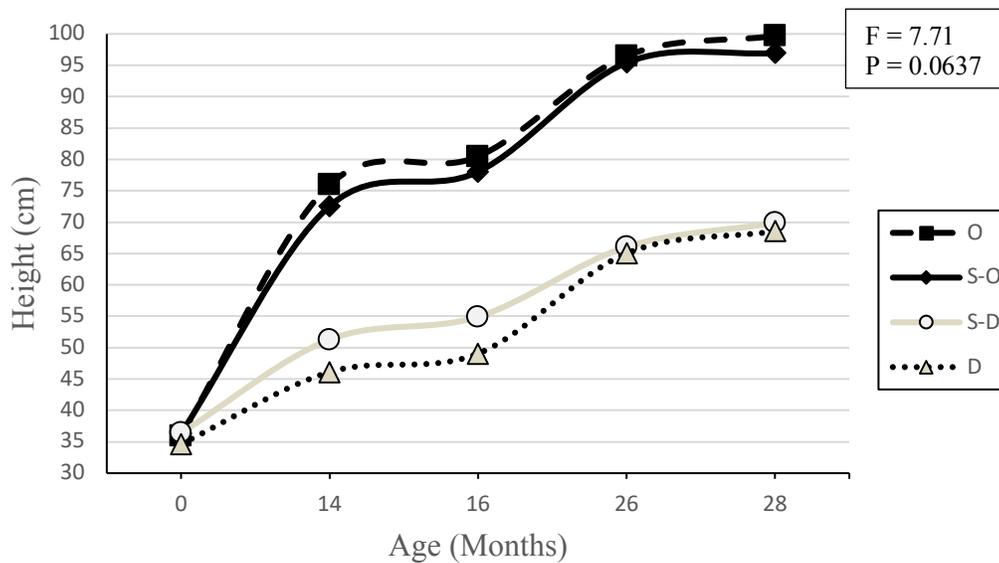


Fig. 5 Mean height (cm) of the target trees growing under four different tree densities throughout the 28-month sampling period.

Table 6 Least squares means estimates obtained for the growth parameters height (ht; cm), root collar diameter (rcd; mm), and crown width (cw; cm) in *M. rubra* seedlings planted under four tree densities at HARC in New Franklin, MO.

Treatment	Time Period (months after planting)											
	0-14			14-16			16-26			26-28		
	ht	rcd	cw	ht	rcd	cw	ht	rcd	cw	ht	rcd	cw
D	46.12aA	0.61aA	42.37aA	49.04aA	0.67aA	46.38aA	65.03aB	1.09aB	52.11aC	68.55aB	1.19aB	53.93aD
S-D	51.29abA	0.68abA	39.18aA	54.92aA	0.74abA	48.95aB	66.06aB	1.09aA	55.58aC	69.86aB	1.09abA	55.58aC
S-O	72.56abA	0.96abA	39.94aA	78.03abA	1.04abA	49.17aB	95.42bB	1.67abB	57.37aC	96.97bB	2.44bC	62.48bC
O	75.95bA	1.06bA	43.36aA	80.46bA	1.18bA	51.54aB	96.51bB	1.83bB	55.44aB	99.69bB	3.17abC	65.33C

Means sharing the same letter in a row or column are not significantly different from one another at $P < 0.05$. Lower case letters represent treatment differences (columns); upper case letters represent differences between sampling dates (rows).

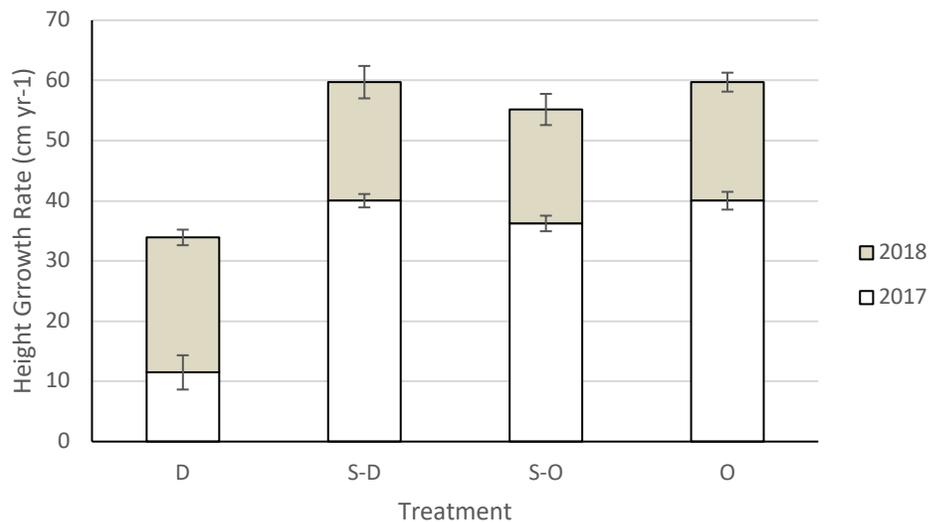


Fig. 6 Mean absolute growth rate for height (cm yr⁻¹) in 2017 and 2018. Significant differences existed between treatment D and all other treatments for 2017 but not for 2018.

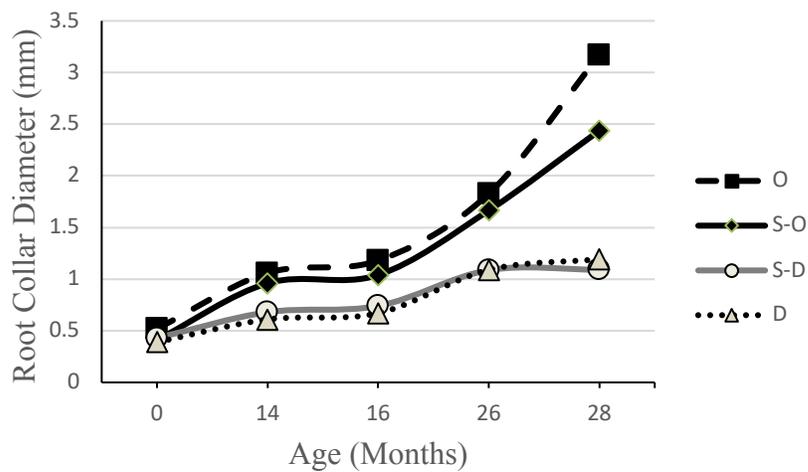


Fig. 7 Mean root collar diameter (mm) of the target trees growing under four different tree densities throughout the 28-month sampling period.

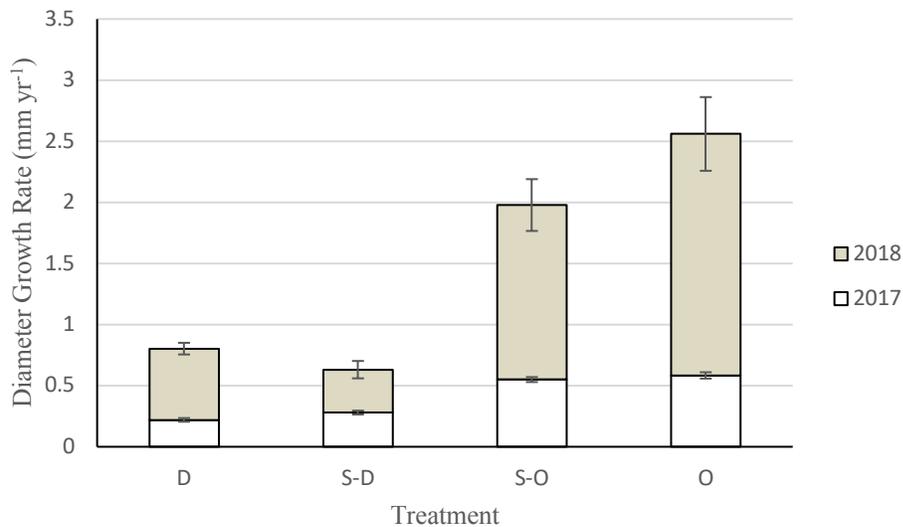


Fig. 8 Mean absolute growth rate for diameter (mm) in 2017 and 2018. A significant difference existed between treatments S-D and O.

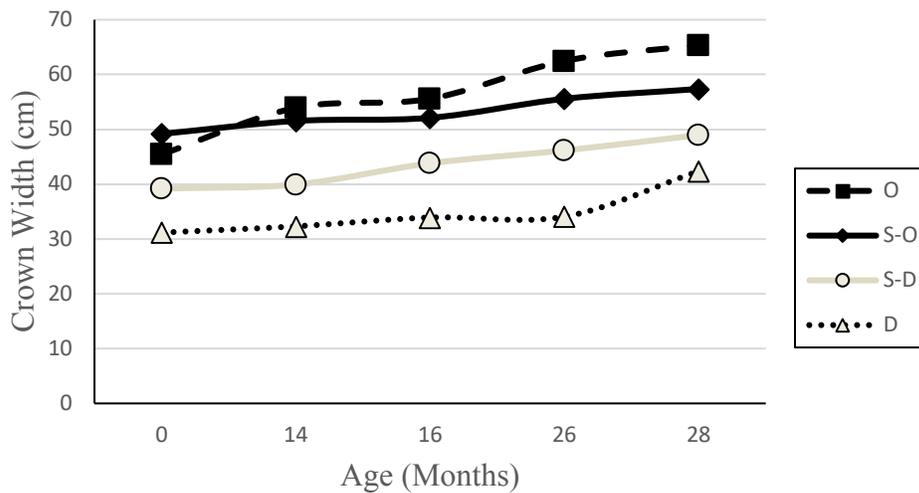


Fig. 9 Mean crown width (cm) of the target trees growing under four different tree densities throughout the 28-month sampling period.

Table 7 Least squares means estimates obtained for mean dry matter weight of individual plant (kg), number of stems, leaf weight (kg ha⁻¹), stem weight (kg ha⁻¹), leaf to stem ratio (L:S), and total dry matter yield (kg ha⁻¹) for five target *M. rubra* shrubs planted in dense (D), semi-dense (S-D), semi-open (S-O) and open (O) treatments at HARC in New Franklin, MO.

Treatment	Number of Stems	Leaf Weight (g)	Stem Weight (g)	L:S	DM (kg plant ⁻¹)	DM (kg ha ⁻¹)
D	5 ± 0.6 a	46 ± 2 a	54.9 ± 6 a	0.84 ± 0 a	101 ± 12 a	112 ± 10 a
S-D	4 ± 0.4 a	53.9 ± 5 a	54.5 ± 7 a	1.00 ± 0.1 a	108 ± 12 a	120 ± 13 a
S-O	5 ± 0.8 a	82.3 ± 6 b	69.9 ± 7 b	1.20 ± 0.1 b	152 ± 17 b	169 ± 16 b
O	5 ± 0.6 a	79.9 ± 6 b	68 ± 8 b	1.20 ± 0.1 b	148 ± 16 b	164 ± 12 b

Means sharing the same letter in a column are not significantly different from one another at P < 0.05.

Specific Leaf Area

The specific leaf area of *M. rubra* differed significantly between treatments ($P = 0.0062$). Shrubs in the understory of D and S-D had the greatest specific leaf areas, with 114.85 and 77.29 $\text{cm}^2 \text{g}^{-1}$, respectively, compared with specific leaf areas of 49.86 and 33.25 $\text{cm}^2 \text{g}^{-1}$ in S-O and O, respectively. Mean specific leaf area in D was significantly greater than that in O and S-O ($P = 0.0058$ and $P = 0.0113$, respectively) while mean specific leaf area in S-D was significantly greater than that in O ($P = 0.0336$) (Fig. 11).

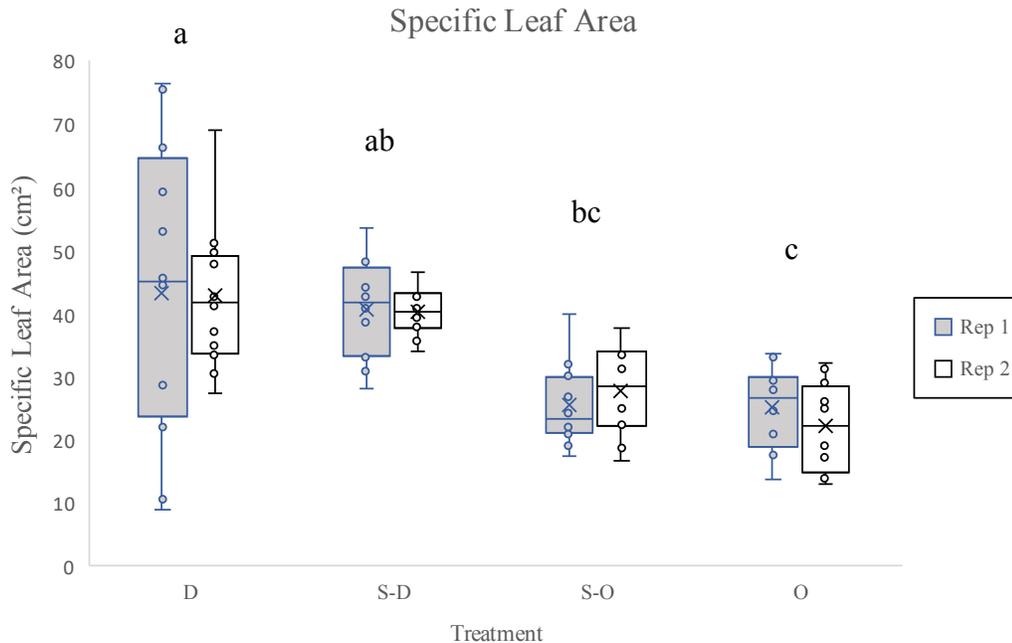


Fig. 10 Mean specific leaf area (SLA) values measured from 40 leaves per plot growing on *M. rubra* seedlings planted in dense (D), semi-dense (S-D), semi-open (S-O) and open (O) treatments at HARC in New Franklin, MO.

Nutritive Value

There were significant differences between treatment groups in regards to several important nutritive value parameters (Table 8). *M. rubra* leaf crude protein (CP) content differed significantly between treatments ($P = 0.0075$). CP content reached its highest value of 24.10 % in S-D and its lowest value of 15.25% in S-O. Post-hoc Tukey tests revealed that significant differences in CP only existed between D and S-O ($P = 0.0184$), D and O ($P = 0.0223$), S-D and S-O ($P = 0.0040$), and S-D and O ($P = 0.0047$). The fiber components of *M. rubra* leaves differed between treatments for ADF ($P = 0.0120$), but not for NDF ($P = 0.1663$). ADF increased directly proportionally to tree density, with a range of 12.35% to 17.7%. Significant differences existed for ADF between D and S-O ($P = 0.0263$), D and O ($P = 0.0116$), and S-D and O ($P = 0.038$). NDF followed a similar pattern as ADF, with values generally increasing with overstory tree density. However, ADF content of leaves from S-D was marginally greater than D.

Total digestible nutrients (TDN) and relative feed value (RFV) did not differ among treatments (TDN: $P = 0.2846$; RFV: $P = 0.1170$). Both measurements decreased numerically with increasing tree cover, but these differences were not statistically significant. Most major mineral concentrations did not differ significantly in *M. rubra* leaves between treatments, but an overall pattern of declining mineral concentration at lower tree densities emerged (Table 9). K, Mg, Ca, Fe, Zn, Cu, and Mb all showed numerical declines as tree density decreased. There was a significant treatment effect in leaf concentrations of Mg ($P = 0.0372$). Mg concentrations in *M. rubra* leaves growing under D were significantly greater than those in O ($P = 0.0321$).

Table 8 Least squares mean estimates obtained for the major nutritive value parameters acid detergent fiber (ADF, %), neutral detergent fiber (NDF, %), crude protein (CP, %), total digestible nutrients (TDN, %), and relative feed value (RFV) in *M. rubra* leaves collected in dense (D), semi-dense (S-D), semi-open (S-O), and open (O) treatments at HARC in New Franklin, MO.

Treatment	ADF (%)	NDF (%)	CP (%)	TDN (%)	RFV
D	17.7 ± 0.9 a	31.8 ± 1.35 a	22.9 ± 0.65 a	64.5 ± 0.5 a	221 ± 11.5 a
S-D	15.9 ± 0.05 ab	33.3 ± 1.35 a	24.1 ± 1.4 a	64.5 ± 0.5 a	215 ± 8.5 a
S-O	13.7 ± 0.1 bc	29.0 ± 2.95 a	15.3 ± 0.35 b	65.0 ± 1.0 a	254 ± 26 a
O	12.4 ± 0.35 c	25.3 ± 1.3 a	15.8 ± 1.15 b	66.5 ± 0.5 a	292 ± 14 a

Means ± SE sharing the same letter in a column are not significantly different from one another at P < 0.05.

Table 9 Least squares mean estimates obtained for the minerals Potassium (K, %), Magnesium (Mg, %), Phosphorus (P, %), Calcium (Ca, %), Sodium (Na, %), Iron (Fe, ppm), Zinc (Zn, ppm), Copper (Cu, ppm), Manganese (Mn, ppm), and Molybdenum (Mb, ppm) in *M. rubra* leaves collected in dense (D), semi-dense (S-D), semi-open (S-O) and open (O) treatments at HARC in New Franklin, MO.

Treatment	K (%)	Mg (%)	P (%)	Ca (%)	Na (%)	Fe (ppm)	Zn (ppm)	Cu (ppm)	Mn (ppm)	Mb (ppm)
D	2.665 ± 0.145 ^a	0.45 ± 0.02 ^a	0.42 ± 0.07 ^a	2.62 ± 0.6 ^a	0.0175 ± 0.0005 ^a	251 ± 32 ^a	52 ± 1.0 ^a	10 ± 2.0 ^a	141 ± 33.0 ^a	0.95 ± 0.05 ^a
S-D	2.5 ± 0.44 ^a	0.325 ± 0.045 ^{ab}	0.415 ± 0.005 ^a	2.315 ± 0.295 ^a	0.01 ± 0.004 ^a	200 ± 76 ^a	37.5 ± 9.5 ^a	7 ± 1.0 ^a	88.5 ± 24.5 ^a	0.95 ± 0.05 ^a
S-O	2.205 ± 0.165 ^a	0.365 ± 0.005 ^{ab}	0.5 ± 0.0 ^a	2.375 ± 0.055 ^a	0.004 ± 0.002 ^a	187 ± 16 ^a	36.5 ± 5.5 ^a	7 ± 1.0 ^a	108.5 ± 3.5 ^a	0.6 ± 0.2 ^a
O	2.025 ± 0.035 ^a	0.275 ± 0.005 ^b	0.4 ± 0.0 ^a	2.13 ± 0.15 ^a	0.006 ± 0.002 ^a	120 ± 3.0 ^a	27.5 ± 0.5 ^a	6 ± 0.0 ^a	65.5 ± 2.5 ^a	0.35 ± 0.15 ^a

Means ± SE sharing the same letter in a column are not significantly different from one another at P < 0.05

Discussion

Survival

Our hypothesis predicting highest survival in O was only partially supported. We reported an overall seedling survival rate of 57.55% in 2017 and 81.88% in 2018, with lowest survival in D, where overstory tree density was highest. In 2018, the majority of seedling mortality occurred in D within the first four months after replanting during a period coinciding with an extreme drought (D3) causing very poor pasture conditions (Dailey 2018). However, there were no differences in survival between treatments S-D, S-O, and O, suggesting that *M. rubra* seedlings are capable of equal survival under moderate shade of at least up to 43.80% GSF on this site.

Shade tolerance is well known for this species, as it grows naturally in shaded understory environments throughout eastern North America. In fact, some authors have even reported that *M. rubra* seedling emergence is negatively associated with irradiance and positively associated with groundcover (Burton and Bazzaz 1991). Shade tolerance, along with a productive lifespan of up to 125 years is why this species is recommended as one of many multifunctional perennial cropping (MPC) species (Lovell and Wilson 2017). Surprisingly, Burgess and Husband (2006) showed that *M. rubra* seedlings growing in Ontario, Canada had consistently lower survival in both shaded and unshaded habitats when compared with *M. alba* and *M. alba x rubra* hybrids. Only 18.4% of *M. rubra* survived in their study, compared to 81.3% of *M. alba x rubra* hybrids and 91.2% of *M. alba*. The observed inferior fitness of *M. rubra* could be due to differences in species distributions, as Ontario is at the northern limit of its geographical range while that of *M. alba* extends further north. However, improved fitness of white-red crosses

could be due to hybrid vigor, which could jeopardize future survival of wild populations of *M. rubra* (Burgess and Husband 2006).

Will et al. (2013) examined seedling response to drought on a forest-grassland ecotone in Oklahoma and found that field-planted *M. rubra* seedlings died only five days after watering was discontinued. This was faster than all nine other species tested, suggesting that *M. rubra* is relatively susceptible to drought-induced mortality. There is some indication that *M. alba* is slightly more drought tolerant than *M. rubra* (California Rare Fruit Growers 1997). Studying the growth and survival of *M. alba* in China, Huang et al. (2013) showed that seedlings that were hardened to drought-like conditions performed significantly better in the field than those that were not. They concluded that *M. alba* trees showed strong resistance to drought that could be improved with drought-hardening. Further study is needed to compare the drought tolerance and survival of these two species to help determine how climate change may affect their respective populations and appropriateness of use in silvopastoral systems in the future.

Growth

Growth was limited across all four treatments when compared with reports of heights between 1.5 and 1.75 m within six months (Sharma and Zote 2010). This could be due to either this site's pH of 5.01, which is well outside the optimal range of 6.5 – 6.8 reported for this species (Sharma and Zote 2010), or to limited precipitation in both years of the study (Fig. 2). Our hypothesis predicting *M. rubra* growth to be significantly enhanced under increasing canopy openness was not supported for height and crown width but was supported for diameter. Height was not significantly affected by treatment,

however there were numerical increases in height as canopy openness increased. Height and diameter responses were similar in treatments S-O and O and similar in S-D and D. Crown width followed a similar trend, increasing numerically with canopy openness. Further study is needed to observe how these parameters change through time, as the treatment effect was strongest for both variables at the final measurement period.

By month 28, diameter in O had increased to a point at which it was significantly different from that of the diameter of seedlings in D and S-D. These trends indicate that *M. rubra* grows most rapidly in open environments, a finding consistent with other reports (McClendon and McMillen 1982). Reporting on the growth and productivity of *M. alba*, Fukui (2000) claimed that the growth of one-year-old mulberry in the field is less uniform and inferior to that of mulberry grown for more than two years. Our finding that the seedlings put a disproportionate amount of energy into height increment the first year and diameter increment the second year supports this claim. Fukui (2000) suggested that these inconsistencies may be due to limitations of the root system during early growth. Continual study of growth parameters into the future will help elucidate how this species is affected by changes in canopy cover.

Dry Matter Yield

M. rubra seedlings produced significantly more DM in S-O and O than they did in S-D and D, partially supporting our hypothesis. Rather than demonstrating a step-wise positive response to natural light availability like we expected, DM production displayed a nearly asymptotic reaction, leveling off and even decreasing slightly moving from

treatment S-O to O. This indicates that *M. rubra* seedlings on this site may be reaching a light saturation point somewhere between 66.21 and 92.03% GSF.

Leaf production was also greatest in S-O, corresponding with the highest leaf to stem ratio. Seedlings generally produced fewer stems as canopy openness increased, diverting more photosynthetic energy for the production of leaves. Leaves contain more protein and less fiber than stems, thus, an increased leaf to stem ratio has a positive effect on the quality of the forage (Lin et al. 2001). In a study conducted at HARC, Lin and colleagues (2001) showed that the leaf to stem ratio of alfalfa (*Medicago sativa* L.) was reduced by 20 and 30% in 50 and 80% shade, respectively. This is similar to our finding that leaf to stem ratio in *M. rubra* seedlings was reduced by 19 and 41.7% in 56.2% and 78.14% shade, respectively. Conversely, Annighöfer (2018) showed that woody fractions of shade tolerant beech seedlings increased in response to greater light availability. Morphological adaptation to altered light availability in terms of leaf to stem ratio appears to depend on many factors, as other authors have reported that shade has little to no effect on the leaf to stem ratio of forage plants (Samarakoon 1990; Kephart and Buxton 1993)

Our data confirming greater specific leaf area (SLA) for shaded plants was not surprising, as this morphological adaptation to low irradiance levels is commonly reported in the literature (Loach 1970; Kephart and Buxton 1993). Plants exposed to reduced light often, but not always, compensate for reduced photosynthesis by producing leaves that are larger in area than related sun leaves (Hanson 1917). The large area of these leaves is correlated with reduced specific leaf dry weight, leaf thickness, and density (McClendon and McMillen 1982; Allard et al. 1991). The *M. rubra* seedlings in

treatments D and S-D produced fewer, larger, presumably thinner leaves with reduced density than those in S-O and O. This likely contributed to overall lower DM production in D and S-D.

In terms of usable forage for livestock, leaf DM productivity is paramount. A literature review of DM productivity resulted in data reported for *M. alba* only, much of which dwarfed numbers reported here. Direct comparisons should not be made between studies due to many differing variables, including cultivar, latitude, climate, site fertility, fertilizer application, harvesting techniques, and plant density. However, we provide several examples of *M. alba* DM yield for reference. Sanchez (2000) claimed that white mulberry produces more DM than most traditional forages, with production of up to 40 tons ha⁻¹ yr⁻¹ in intensively managed operations in the tropics. When planted with subterranean clover (*Trifolium subterraneum*) in a silvopasture in central Italy, *M. alba* produced between 4.2 and 5.3 tons DM ha⁻¹ (Talamuci and Pardini 1999). Armand and Meuret (1993) demonstrated that the Japanese cultivar Kokuso 21 produced up to 2.2 tons ha⁻¹ on good sites in France, but in poorer sites production was much lower at 444 kg ha⁻¹.

There are many important differences in our study compared with those reported above, but several should be noted here. The first is that we report on DM yield produced from the initial pruning event. Once a shrub is bottom pruned, it will begin to sprout vigorously, producing greater quantities of total DM whereupon the tree can be shoot harvested, typically 12 weeks later (Sharma and Zote 2010). Although no direct evidence was found in the literature, there is some speculation that *M. rubra* does not pollard well, and this may be reduced even more in the shade. Due to time limitations, we were unable

to determine whether or not this is true. The second difference to note is that DM yield estimates in the literature are reported for a variety of planting densities. It is well known that as plant spacing is reduced, yield plant⁻¹ decreases owing to competition, but total forage yield ha⁻¹ increases, as does the leaf to stem ratio (Gong et al. 1995). Periodic pollarding along with increasing the planting density could be two effective means of increasing overall DM production.

When *M. rubra* seedling DM production was compared with DM yield of sun-grown non-pollarded *M. alba*, *M. rubra* produced more. After 32 months of growth, Fukui (2000) reported a stem and leaf DM mean of 63.4 g (70.44 kg ha⁻¹) and 55.4 g (61.55 kg ha⁻¹) for 1995 and 1996, respectively. S-O and O grown *M. rubra* seedlings produced more than double that (169.15 and 164.37 kg ha⁻¹). Certainly, environmental and site differences prevent direct comparisons, but the example is used to demonstrate that when managed in a similar way, *M. alba* may not always be more productive than *M. rubra*.

Nutritive Value

Our hypothesis predicting leaf nutritive value to increase as understory light levels decreased was not supported. Treatments D and S-D had the highest CP content but they also had the highest ADF, resulting in negligible treatment differences in TDN and RFV. It is possible that elevated ADF levels in D and S-D were due to an increase in lignification caused by the reduction of soluble sugars and starches in the leaves. Many authors have made similar postulations, claiming that exposure to shade causes an increase in cell wall content and a reduction in forage digestibility (Samarakoon 1990;

Lin et al. 2001). The higher CP content of shade-grown leaves seen in this study may be due to canopy-induced microclimatic differences, such as increased soil moisture and moderate soil temperatures that can speed up the rate of N mineralization and turnover (Wilson 1996). Other authors have speculated that increased CP in shaded plants is due to the reduction in cell size attributed to leaf etiolation. These smaller cells contain similar concentrations of N, resulting in a concentrating effect within each cell (Kephart and Buxton 1993).

Leaf mineral content was relatively high, with denser overstory treatments generally containing leaves with greater mineral content. Leaf phosphorus levels ranged from 0.35% to 0.50%, with no significant change due to treatment. These numbers are greater than P concentrations reported for white mulberry in a review by Sanchez (2000), where concentrations of 0.14 – 0.24% were reported. Calcium concentrations were similar to those reported in the literature for *M. alba* (1.8 – 2.4%). Espinoza et al. (1999) reported similar values for potassium in leaves (1.9 – 2.87%), but higher values for magnesium (.47 – 0.63%).

Overall, the nutritive value of *M. rubra* seedlings assessed during the summer forage slump surpassed the quality of many common pasture forages. Mean TDN for all four treatments was 65.13%, comparable to that of leafy, immature legumes. Mean RFV for all four treatments was 245.25, a number that is exceedingly high and indicative of high dry matter digestibility. Mean CP content for all four treatments was 19.48%. This is comparable to CP levels recorded for alfalfa (*M. sativa* L.) and white clover (*Trifolium repens* L.) in the spring during the vegetative stage on the same site (Lin et al. 2001). We reported an overall mean ADF and NDF of 14.9 and 29.81%, respectively, even lower

than the 26.67 and 33% reported for white clover in the same study (Lin et al. 2001). A study comparing the nutritive value of several woody species in northern Greece showed that *M. alba* had significantly less fiber (ADF 18.3%; NDF 31.3%) and significantly higher digestibility (IVOMD 75.6%) than the other species tested (Ainalis et al. 2006). Kandylis et al. (2009) partially replaced alfalfa (*M. sativa*) hay and concentrates with mulberry leaves and found no significant differences in DM, CP, or crude fiber digestibility of the entire diet when fed to sheep.

Any presentation of woody forage nutritive value should mention the presence of anti-nutritional compounds that have the ability to severely restrict nutrient utilization (Papanastasis et al. 2008). Secondary compounds such as condensed tannins, alkaloids, saponins, and oxalates are known to occur in many woody perennials and can have detrimental effects to the animal if consumed in high quantities. However, diets containing herbaceous forage with a high level of digestible CP have been shown to counteract the negative effects of tannins (Yiakoulaki 1995). Further, tannins in low to moderate concentrations (20-40 g kg⁻¹ DM) can induce beneficial effects, which are associated with suppression of bloat in ruminants (Jones et al. 1973). No anti-nutritional factors or toxic compounds have been identified in *M. alba* (Sanchez 1999). Makkar (1989) identified mulberry as a “low tannin” fodder tree, with feeding experiments in India showing that up to 6 kg of leaves per day can be fed to cattle without adversely affecting the health of the animals or the yield and butter content of milk.

Conclusion

M. rubra seedlings responded positively to increased light availability in terms of survival, growth, and productivity, but were not affected in terms of leaf nutritive value. Overall seedling survival was relatively high despite extreme drought conditions in 2018, and significant seedling mortality did not occur until below 43.8% GSF on this site. Seedlings expressed morphological plasticity with increased diameter growth, decreased specific leaf area, and increased DM yield as canopy openness increased. Seedlings appeared to reach a light saturation point somewhere between 66.21 and 92.03% GSF in terms of DM yield, obtaining highest leafiness at 66.21% GSF. Nutritive value did not differ significantly above 66.21% GSF in terms of CP and fiber fractions and did not differ significantly for any of the treatments in respect to digestibility (TDN and RFV). Our results indicate that *M. rubra* seedling survival, growth, productivity, and nutritive value can be optimized on this site at 66.21% GSF, an irradiance level that corresponds to an overstory planting density of 9 x 9m.

Management Recommendations

Producers should pay careful attention to soil conditions and topographic landform when selecting perennial species for use in silvopastoral systems. *M. rubra* thrives in valleys, floodplains, and on mesic hillsides, with best growth reported for well-drained moist coves and along streams (Martin et al. 1961). Geophysical site conditions are likely to be of supreme importance to biological interactions between plants. Thus, plant selection should depend on the appropriateness of the site.

It is highly recommended that future *M. rubra* management efforts are geared toward dual-purpose production of fodder and fruits to increase food supply and diversify on-farm resources. *M. rubra* has a dioecious reproductive strategy that would allow producers to create silvopastures with separate fodder and berry production. Male plants can be coppiced and browsed up to twice a year while female plants can be allowed to grow taller into fruit producing shrubs (Shepard 2013). However, shrubs are not good sources of energy after fruit development, so it is important to keep fodder shrubs pollarded, preferably above browse level where deer herbivory is prevalent.

Study Limitations and Future Directions

There were several limitations of this study which should not go unmentioned. Seedling growth is influenced by many different factors, including – but not limited to – nutrient, space, and water availability; thus, the seedling responses reported here cannot be attributed solely to differences in light availability. We acknowledge that differences in overstory density will also result in variable microclimatic conditions, creating unforeseen stressors to plants growing in the understory. Additional study on seedling moisture stress, soil volumetric water content, and variable seedling planting distances could help elucidate which of these competitive forces is most responsible for differences in seedling response.

DM production estimates reported here should be interpreted with caution. Our measurements of DM production were assessed in an environment void of regular fertilizer application and nitrogen-fixing legumes. Despite thriving well with minimum fertilization (Sharma and Zote 2010), mulberry is a non-legume and planting this species

in combinations with nitrogen-fixing forages can improve overall yield (Saddul et al. 2004). Moreover, DM yield was assessed at one point in time from the initial bottom pruning event. Both DM yield and nutritive value are known to vary widely throughout various stages of plant maturity and different harvesting cycles can dramatically affect both variables (Saddul et al. 2004; Kabi and Bareeba 2008).

Although we collected data during the most critical time of year, our results are limited in scope. Further research is needed to evaluate seedling response to defoliation and harvesting frequency under varying shade levels. It may be that white mulberry (*M. alba*) is a more appropriate choice for forage production under drought scenarios. Research is needed comparing productivity and growth responses of both species to understory light availability. Furthermore, future research should investigate livestock behavior in multi-strata systems to identify if and under what conditions livestock will consume *M. rubra* (Vandermeulen et al. 2018b) and what this means in terms of animal production during critical forage shortages.

Chapter 5

Conclusion

Results reported in the preceding chapters have indicated that trees have both competitive and facilitative effects on forage production in silvopastoral systems, yet facilitation may be more prevalent during droughts.

In chapter two, we showed that grasses growing under increasing canopy cover demonstrated higher nutritive value and decreased production, with the exception of the cultivar Massai under moderate tree densities in February, which produced significantly more dry matter than dense and open treatments. This observed extension of the growing season under moderate tree densities could result in reduced animal weight loss between the months of January and April for this region.

In chapter three, we showed that there were no additional constraints to water availability and tree and grass growth when intercropped with fertilizer shrubs in the first two years after silvopasture establishment. Conversely, the planting, coppicing, and mulching of *T. diversifolia* and *L. leucocephala* increased several important soil fertility parameters, including plant available N and P. Additionally, the intercropping of the N-fixing *L. leucocephala* marginally increased annual grass dry matter production. However, grass productivity was decreased in the presence of *T. diversifolia*, suggesting there are competitive forces at work that we could not discern in this study. During the dry season, cumulative forage production in plots with shrubs provided on average 9.66% more forage than plots without shrubs, diversifying and supplementing forage reserves.

In chapter four, we presented results showing that *M. rubra* seedling survival, growth, productivity, and nutritive value on this site appeared to reach a light saturation

point between 66.21 and 92.03% GSF in terms of DM yield, obtaining highest leafiness at 66.21% GSF. This value corresponds to an overstory planting density of 9 x 9m (~500 trees ha⁻¹). The nutritive value of *M. rubra* seedlings assessed during the summer forage slump surpassed the quality of many common pasture forages and could serve as a highly nutritional supplemental forage to livestock in multi-strata silvopastures during drought conditions. However, more research is needed to determine how *M. rubra* responds to repeated coppicing events, particularly during severe droughts.

The overall results of this research demonstrate that food security can be enhanced with the appropriate use and management of silvopastoral systems, but may not be ensured used in complete isolation of traditional rotational stocking of open pastures. We recommend the use of integrated systems that utilize silvopasture during critical periods of the year, such as January through April in Panama, and July and August in Missouri. During these times, moderate shade may provide a temporary boost in grass production as well as supplemental forage in the form of fruit from overstory trees and foliage from browsable fertilizer shrubs. Silvopasture should be used as a tool to diversify and increase forage production during these critical periods of the year, ensuring adequate animal nutrition and health throughout the year.

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APPENDICES

Appendix A

Protocol for Hemispherical Canopy Photos with the Canon Eos Rebel T5

Helene Muller-Landau, Version September 21, 2014. Adjusted by Lea Bernath, Version March 17, 2015 and later adjusted by Ryan Dibala, Version June 6, 2018.

Things you need to do once before you start taking pictures

1. **Mode of operation:** The camera should be in Program mode, represented by the letter P on the dial on the top right of the camera.
2. **File type:** The camera should save files in the highest quality formats – both RAW and JPEG. To do this,
 - Turn on the camera
 - Press the MENU button
 - In the first menu (very left), select the top line, “quality” and press SET
 - Use the keys to the right and left of the SET button to move among the options and choose RAW + half-circle L
 - Press SET
3. **Automatic exposure compensation and automatic exposure bracketing:** Automatic exposure bracketing makes it easy to take multiple photos on the same site with systematic variation in exposure. Multiple photos at different exposures provide additional information for processing images. Exposure bracketing commands the camera to adjust the default exposure values to several values from lower to higher exposure compensation (darker to lighter). Specifically, we want to take photos at -2.0, -1.0, and 0 exposure compensation.
 - Turn on the camera
 - Keep the “Av +/-” button pressed and use the dial on the top right of the camera to set the exposure to -1
 - Press the menu button
 - Press to the right of the SET button to move among the menus, to the second menu, where the top line is AEB
 - Select AEB, then press SET
 - Press to the right of the SET button to separate the three lines so they are at -2, -1, and 0
 - Press SET
 - Every time you turn off the camera you need to set the exposure bracketing again
4. **Focus:** The lens should be set the manual focus (M) and the focus ring should be set on infinity.
5. **Self-timer:** It is important that the camera is not moved in any way while the photo is taken and the easiest way to accomplish this is to take the photos using the self-timer so that the operator is not touching the camera at the time the photo is taken.
 - Press to the left of the SET button
 - Select the 2-second self-timer

6. **Metering mode:** Make sure that the metering mode is on “Evaluative Metering.” Press the button on top of the SET button to do this.
7. **Picture Style:** Make sure that the picture style is “Neutral.” Press the button below the SET button to do this.
8. **Check configuration:** When the camera is fully and correctly configured the LCD should look like this:



(Note: the number on the bottom right in brackets will change depending on the amount of space left on the memory card.

Steps to follow when taking hemispherical photographs:

1. **Check configuration:** Proper configuration of the camera, as described above, is necessary to achieve efficiency under field conditions. In general, these settings should be retained by the camera from the last time it is used. However, the automatic exposure bracketing returns to default values (no bracketing) every time you turn off the camera.
2. **Sky conditions:** Take photos only when all the following conditions are met:
 - a. No rain
 - b. No direct sunlight in the forest – Direct sun can damage the camera, and strong reflections make it difficult to analyze the images
 - c. It is earlier than 9 am or later than 3 pm, or the sky is completely overcast
3. **Position:** The camera should be placed on a tripod so that the lens faces up and the top of the lens is 1 m above the ground.
4. **Orientation:** Orient the camera so that the bottom of the lens (and the paper triangle on it) faces magnetic north. To do this, use the compass. Note – the magnetic compass is sensitive to metal bodies and/or batteries in the camera as well as to metal

necklaces and cell phones, so you should be careful to keep the compass away from these objects.

5. Leveling: Level the camera so that the top of the lens is perfectly flat. Do not tilt the camera to match the slope. It is important to have the camera level according to the bubble level. You can use the cubic bubble level that attaches to the camera to do this. Once the camera is leveled, you need to check the north once more to make sure the leveling has not affected the orientation. If reorientation is required, then check the leveling again.

6. Remove the lens cap and ring: Remove both the lens cap and the ring around the fisheye. Make sure the triangular piece of paper is within the field of view of the lens.

7. Check that the lens is clean.

8. Check the focus: The lens should be on manual focus (M) and should be focused on infinity. It is easy for the focus ring to get accidentally moved when the lens cap is removed.

9. Take the photographs: With the camera turned on, press the shutter once to activate the timer and move yourself below the horizon of the fisheye lens. The camera should automatically take 3 photographs in a row, one at each of the exposure compensation settings. If it does not take three photographs, double-check the configuration.

APPENDIX B

Relative Survival of *T. guayacan* seedlings in August 2016

Initially, the fertilizer shrub experiment detailed here in Chapter 3 was set up as a randomized complete block split plot design, with each study site containing two plots, one planted in guachapalí de ganado (*A. saman*) and the other planted in guayacan (*T. guayacan*). Each plot contained eight split-plots, where central timber saplings were surrounded by the different combinations of fertilizer shrubs and grasses. The strong ENSO event during the dry season of 2015-2016 resulted in the loss of 46.83% of the *T. guayacan* seedlings (Fig.1). Due to limited resources, we made the decision to abandon the *T. guayacan* plots and focus our efforts on the *A. saman* plots.

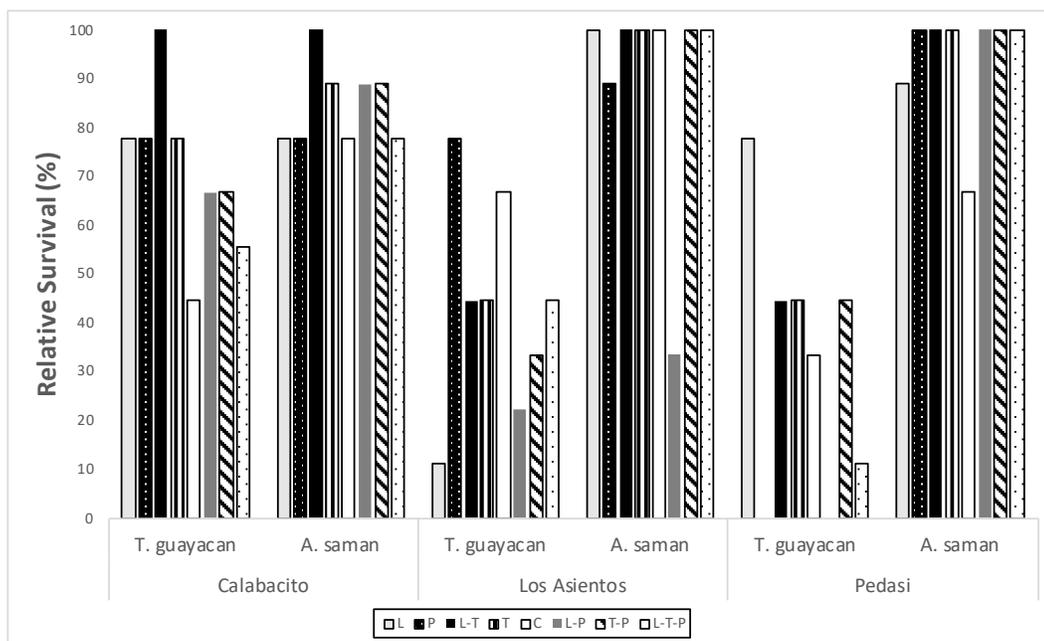


Fig. 1 Relative survival of *T. guayacan* and *A. saman* seedlings planted at Calabacito, Los Asientos, and Pedasi. The percentage of *T. guayacan* seedlings surviving was 53.17% overall in August, one year after planting.

APPENDIX C

Images of Plantation Establishment and Research in Chapter 2



Fig. 1 Three *M. maximus* cultivars growing in the understory of treatment D during the wet season of 2016 at the IDB Forestal Ranch near Playa Venao, Panama.



Fig. 2 The 0.5 x 0.5 m square grid used to sample pasture grasses.

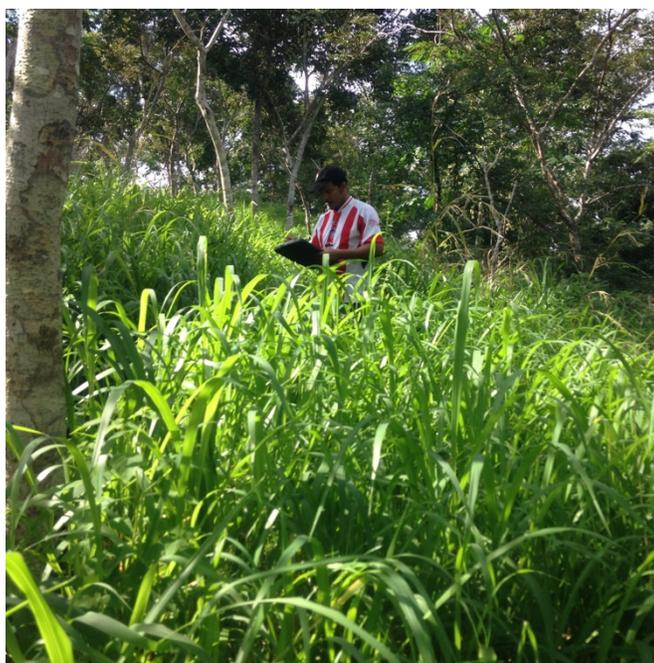


Fig. 3 Field assistant Manuel Facio helps record data during the assessment of grass dry matter production.

APPENDIX D

Images of Plantation Establishment and Research in Chapter 3

Fig. 1 Field assistant Dutch Silfer helps to demarcate treatment plots at Calabacito.



Fig. 2 Hauling seedlings via horseback to the planting site at Calabacito



Fig. 3 Treatment plots during the dry season (March) at Calabacito. Tree seedlings are difficult to perceive in this photo, as growth and survival was inhibited by deer herbivory at this site.



Fig. 4 Treatment plots at Pedasi during the rainy season.



Fig. 5 Treatment plots at Los Asientos during the rainy season.



Fig. 6 Preparing to assess sapling moisture stress with the pressure chamber.



Fig. 7 Measuring soil volumetric water content at Los Asientos during the dry season.



Fig. 8 Central *A. saman* sapling (middle) surrounded by *Leucaena leucocephala* (right) and *Tithonia diversifolia* (left).



Fig. 9 *A. saman* saplings at Los Asientos during the dry season.



Fig. 10 Measuring sapling height at Pedasi during the rainy season.

APPENDIX E

Images of Plantation Establishment and Research in Chapter 4

Fig. 1 *M. rubra* seedlings planted and protected by plastic tubing under a cherrybark oak canopy at HARC, New Franklin, Missouri.



Fig. 2 Measuring the root collar diameter of a *M. rubra* seedling at HARC in Missouri.

VITA

Ryan Haynes Dibala was born in Willimantic, CT, USA. He grew up in rural northeastern Connecticut where he quickly took a fascination in animals, agriculture, and the environment. His favorite subject in high school was Ecology, and remembers actively learning about the natural world on field trips to State parks with his favorite high school teacher Mr. Edmund Smith. Early on, Ryan was most interested in wildlife ecology, particularly forest management effects on wildlife populations. As a Master's student, he studied Cerulean Warbler settlement patterns in a managed forest in Southern Indiana to better understand the effects of silviculture on this threatened migratory songbird. After graduating, he longed to pursue less species-specific research and to develop questions with greater social relevance. Time spent in Latin America as a Peace Corps volunteer had Ryan yearning to conduct research with direct implications for developing socially, ecologically and economically feasible land use options for smallholding farmers in developing countries.

Over the last four years, Ryan has created the opportunity to work directly with ranchers in Panama, reforest badly degraded pastures, and explore a number of highly complex ecological processes during his doctorate work at the University of Missouri-Columbia. In a time when food security and ecosystem integrity is being confronted with a changing global climate, Ryan remains passionate and optimistic about our planet's ecological and agricultural future. In the next few years, Ryan hopes to continue international development work that supports progressive, agroforestry-based alternative agriculture.