

Warm-climate, legume-grass forage mixtures versus grass-only swards: An ecosystem services comparison

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ABSTRACT - Integrating warm-climate forage legumes into grass monocultures has received significant research attention during the past 70 years, but widespread adoption by end users has been elusive. The objectives of this review are to provide historical context regarding legume use in warm-climate grasslands; synthesize the current literature addressing contributions to grassland ecosystem services of warm-climate, grass-only vs. legume-grass mixed swards; and consider how to achieve more widespread adoption of legume technology in warm climates. For this review, warm-climate grasslands are considered those in areas between latitudes 30° N and 30° S, where C4 perennial grasses dominate. The literature suggests measurable advantages in animal performance, soil carbon accumulation, and nutrient cycling of legume-grass mixtures are most likely when grass-only swards receive little or no N fertilizer. Advantages are less or may disappear when mixtures are compared with grasses receiving high N fertilizer rates. In contrast, amelioration of greenhouse gas emissions is often most pronounced for mixtures when compared with grass-only swards receiving high rates of N, because of the magnitude of N fertilizer effects on emissions. Going forward, there is need to focus on selecting for, and thoroughly documenting legume persistence, achieving low-risk and affordable legume establishment methods, assessing compatibility of legumes in mixture with grasses prior to legume cultivar release, emphasizing adoption of both legume species and optimal management practices, and recognizing scientists must assume a larger and more intentional role in encouraging adoption by end-users of research innovations, in addition to our traditional role in technology and product development.

Keywords: climate, forage, grass, grazing system, mixed pasture, pasture

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Received: October 27, 2021

Accepted: June 28, 2022

How to cite: Sollenberger, L. E. and Dubeux Junior, J. C. B. 2022. Warm-climate, legume-grass forage mixtures versus grass-only swards: An ecosystem services comparison. *Revista Brasileira de Zootecnia* 51:e20210198. <https://doi.org/10.37496/rbz5120210198>

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1. Introduction

Perhaps the word most commonly associated with warm-climate forage legumes is “potential”. As an example, Schultze-Kraft et al. (2018) stated “legumes have potential to contribute significantly to sustainable intensification of livestock production in the tropics, along with the provision of ecosystem services”. The potential of legume addition to tropical and subtropical forage systems is indeed great, particularly considering the current reliance of such systems on low nutritive value C4 grasses, the widespread nitrogen limitation to productivity of warm-climate grasslands, and the economic and environmental constraints of addressing nitrogen scarcity in these systems by using inorganic fertilizers.

Our fascination with the potential of legumes owes to research documenting their ability to increase litter quality, nutrient release (Kohmann et al., 2018, 2019), and rate of soil organic carbon (SOC) accumulation (Liu et al., 2017) compared with grass monocultures; increase N cycling in grassland soils and reduce grassland degradation (Boddey et al., 2004); increase biodiversity and system resilience (Tracy et al., 2018); increase diet digestibility and livestock performance (Jaramillo et al., 2021b); extend grazing longer into the dry season (Böhnert et al., 1985); decrease N₂O emissions compared with N-fertilized grass swards (Jensen et al., 2012); and decrease enteric CH₄ emissions per unit of ruminant animal product compared with grasses (Hammond et al., 2009, 2013).

The objective of this review is to provide historical context regarding use of legumes in warm-climate grasslands; synthesize the current literature addressing contributions to grassland ecosystem services of warm-climate, grass-only vs. legume-grass mixed swards; and consider how we might move beyond the word “potential” when discussing legume technology and achieve more widespread adoption. For the purposes of this paper, we will consider warm-climate grasslands to be those where C4 perennial grasses dominate and which occur primarily between latitudes 30° N and 30° S. To the extent possible, we will discuss ecosystem services based on literature published in the last ten years, although we will extend that time window as needed to provide appropriate breadth and depth. Lastly, comparisons of ecosystem services from grass-only vs. legume-grass systems will consider the level of N fertilizer input to the grass-only system as an important factor affecting outcomes.

2. Historical context

Approximately 40% of Earth’s ice-free terrestrial surface is covered by grassland (Hewins et al., 2018), and the largest single land-use type is grassland for livestock grazing, occupying 40 million km² (Lambin and Meyfroidt, 2011). Within warm climates, there are huge expanses of C4 grasslands, including approximately 90 million ha of planted forage grass species in the *genus Brachiaria* (syn. *Urochloa* spp.) in Brazil alone (Boddey et al., 2020). Significant proportions of warm-climate grasslands are degraded due to poor grazing management and inadequate soil nutrient replenishment, especially N. In other circumstances, high N fertilizer rates may be applied, but if not used in conjunction with optimal grazing management practice, they can pose an economic burden and environmental risk.

It is within these divergent scenarios that greater use of forage legumes has been proposed. Descriptions of early efforts with warm-climate legumes are found in the literature. Shelton et al. (2005) indicated “The search for adapted tropical forage legumes commenced in earnest after 1950, and by 1990, >17,000 accessions of >20 genera had been introduced into Australia, largely from Central and South America but also from Asia and East Africa”. These authors indicated further “This early Australian enthusiasm for tropical legumes was not shared internationally”. Coleman and Leslie (1966) noted “an anti-legume complex”, which they said was due to the failure of legumes to provide a stable pasture under grazing either in association with grasses or in pure stands. Relative to forage legumes for warm climates, Boddey et al. (2020) surmised that “The problem is the perception, frequently warranted, among pasture specialists that in the tropics, forage legumes do not persist in the sward, and many studies (most unpublished) have shown this generally to be true”. These shortcomings of early legume introductions in Latin America and the Caribbean were emphasized by Peters and Lascano (2003), who indicated “between 1980 and 2000, of 14 legume cultivars that were released none was well adopted”, and Pengelly et al. (2003), who lamented that even after 50 years of investment in tropical forage research, forage legume adoption was relatively poor.

Reasons for failures have been attributed to several causes. Shelton et al. (2005) suggested that “In many instances, lack of adoption could be related to failure of the technology for technical or socio-economic reasons, i.e., the technology did not live up to expectations or was not targeted at the appropriate production system”. Muir et al. (2011) expressed a somewhat different perspective, noting “The simplicity of managing grass monocultures, pasture production responses to nitrogen fertilizer, and profitability of grass-only systems have limited interest in legume-based approaches”.

With such discouraging assessments of the potential of warm-climate legumes, one might conclude that any embers of hope for legumes that formerly existed have long since been extinguished in the forage-livestock research community. This is not the case, and more recent assessments were considerably more upbeat. Muir et al. (2014) suggested “Forage legumes can be integral components of warm-season, subtropical and tropical systems to minimize inputs ... while still maintaining or improving animal production...”, while Dubeux et al. (2017c) proposed that “Greater use of forage legumes appears to be an opportunity for sustainable livestock intensification in extensive C4 grass-based pastureland commonly found in vast areas of Latin America”. They elaborated further indicating “Tree legumes are a key component for the sustainable intensification of livestock systems in warm-climate regions”. A similar sentiment was echoed in a recent review by Schultze-Kraft et al. (2018) who argued “...legumes have potential to contribute significantly to sustainable intensification of livestock production in the tropics, along with provision of ecosystem services”, and by Dubeux and Sollenberger (2022), who suggested “forage legumes can assist by producing more output per unit of resource input (provisional ecosystem services) while maintaining the delivery of other ecosystem services”. Likewise, it has been proposed that “use of legumes in mixed pastures for tropical regions has emerged as a feasible strategy to keep meat and milk production at levels acceptable to farmers with reduced greenhouse gas emission rates” (Casagrande et al., 2022).

Thus, the enthusiasm for legumes has not disappeared from the forage research community, and studies continue to assess and document the value of legumes. In the following sections, research comparing warm-climate, legume-grass mixtures with grass-only swards is described within an ecosystem services framework. The focus of this review is limited to cases in which grass-only and legume-grass swards were compared in the same experiment. Those who seek more detailed descriptions of the concept of ecosystem services and the categories of such services are referred to D’Ottavio et al. (2018) and Sollenberger et al. (2019).

3. Contributions of legumes within an ecosystems services framework

3.1. Provisioning ecosystem services

Provisioning services will be described for seven cases from the literature. In each case, the N fertilizer regimen of the grass monoculture treatment is noted and its impact discussed. Two cases compared a legume-grass mixture with a grass monoculture receiving moderate to high rates of N, three cases compared a mixture with a grass monoculture receiving no N, and two cases compared the mixture and the grass monoculture, in which the monoculture received N at two levels, either zero or a moderate to high rate.

3.1.1. Mixture vs. monoculture with moderate/high N rate

Overseeding the annual legume *Aeschynomene americana* L.) into limpograss [*Hemarthria altissima* (Poir.) Stapf & Hubb.] during three years increased individual animal gain of yearling steers from 0.39 kg day⁻¹ (limpograss) to 0.70 kg day⁻¹ (mixture) when the limpograss monoculture received an average of 180 kg N ha⁻¹ year⁻¹ in Florida, USA (29°43' N latitude; 1270 mm annual rainfall; daily average temperature of 28 °C during the grazing period; rotational stocking with 7 d of grazing and 35 d of regrowth; pastures grazed to 15-20 cm stubble height; Rusland et al., 1988). Herbage crude protein (CP) concentration of N-fertilized limpograss herbage (hand-plucked to represent the diet) averaged 90 g kg⁻¹. *Aeschynomene* contributed ~8 (early season) to 35% (mid- and late season) of herbage mass, and hand-plucked legume herbage averaged > 200 g CP kg⁻¹ and 700 g *in vitro* digestible organic matter kg⁻¹. Across the three years, limpograss monoculture supported a 30% greater stocking rate (SR) than the mixture, but gain ha⁻¹ was 43% greater for the mixture (377 vs 263 kg year⁻¹) than the monoculture over an average of 112 d of grazing year⁻¹. Thus, in this case, a large annual input of N fertilizer to the monoculture resulted in greater average SR,

but its effect on forage quality could not match that of adding the legume. As a result, both individual animal and per hectare gains were superior for the mixture than the monoculture.

In Bahia, Brazil (16°39' S latitude; mean annual rainfall of 1,311 mm; mean temperature of 25 °C), a mixed Marandu palisadegrass [*Urochloa brizantha* (Hochst. ex A. Rich.) R. Webster] - Belomonte pinto peanut (*Arachis pinto* Krapovickas & W.C. Gregory) pasture was compared with a Marandu monoculture receiving 120 kg N ha⁻¹ year⁻¹ during five periods of more than one year each (rotational stocking; grazing period of 3-7 d; regrowth period of 30-36 d; Pereira et al., 2020). The mixture was planted two years prior to the start of the first period of evaluation. Legume proportion averaged 24 and 21% in the warm and cool seasons, respectively, and forage mass in the mixed pastures was 17% greater than in the monoculture during the warm season but not different in the cool season. Mixtures resulted in 17% greater average daily gain and 29% greater gain ha⁻¹ across seasons (no treatment × season interaction); so, in this case, like that described by Rusland et al. (1988), the addition of more than 100 kg N ha⁻¹ annually to the grass monoculture did not match the benefit of integrating legumes into the mixture.

3.1.2. Mixture vs. monoculture with no N

In Costa Rica (10°13' S latitude; mean annual rainfall of 4535 mm; mean temperature of 25 °C), animal performance on an unfertilized palisadegrass monoculture was compared with a pinto peanut-palisadegrass mixture at SR equivalent to ~ 600 and 1200 kg live weight ha⁻¹ during three periods of more than 300 d each (rotational stocking; 7 d grazing, 21 d regrowth; Hernandez et al., 1995). Cattle diet composition was 10 and 50% pinto peanut at low and high SR, respectively. Average daily gain was greater for the mixture than the monoculture during two of the three periods (average across periods of 0.428 vs. 0.337 kg, respectively) at high SR but only one period at low SR (0.506 vs. 0.452 kg, respectively). Across the three periods (975 d), live weight gain ha⁻¹ was 30% greater for the mixture than the monoculture at the greater SR and 12% greater at the lesser SR. In this example, no N was applied to the grass monoculture and SR was the primary determinant of legume contribution. Advantages of the mixture in animal responses occurred primarily at the greater SR, because it reduced grass competition to pinto peanut, increasing legume forage accumulation (Ibrahim and t'Mannetje, 1998) and percentage in the diet.

In Pernambuco, Brazil (7°23' S; annual rainfall of 1,200 mm; and annual average temperature of 25 °C), a signalgrass [*Urochloa decumbens* (Stapf.) R. Webster] monoculture was compared with mixtures of signalgrass with leguminous trees, either mimosa (*Mimosa caesalpiniiifolia* Benth) or gliricidia [*Gliricidia sepium* (Jacq.) Kunth ex Walp] (continuous stocking with variable stocking rate; Silva et al., 2021a). All pastures received P and K at time of planting the trees into the existing signalgrass, but no additional fertilizer was applied during the two-year study (seven months of grazing per year). Average daily gain was greatest with inclusion of gliricidia followed by the grass monoculture and finally by inclusion of mimosa (0.77, 0.56, and 0.23 kg d⁻¹, respectively). Gain per area during a seven-month period followed the same pattern (423, 347, and 50 kg live weight ha⁻¹). Thus, silvopasture systems using gliricidia in signalgrass enhanced livestock gains compared with the monoculture. Mimosa was less preferred by cattle and outcompeted the grass resulting in poor animal performance; however, timber sales from mimosa could offset some of the economic losses in livestock production. These results illustrate the importance of choice of tree species in silvopasture legume-grass mixtures.

A third case was described for the association of desmodium [*Desmodium ovalifolium* (Prain) Wall.] with koroniviagrass [*Urochloa humidicola* (Rendle) Schweick.] in Bahia, Brazil (16°39' S lat; mean annual rainfall of 1,311 mm; mean annual temperature of 25 °C; continuous stocking; Pereira et al., 2009). During six grazing periods of 9 to 14 months each, mixture and grass monoculture pastures were continuously stocked at three SR (2, 3, and 4 head ha⁻¹) and no N fertilizer was applied. There was no difference in daily live weight gain between the mixture (0.371 kg) and the monoculture (0.369 kg) averaged across SR and periods. Daily gain decreased and gain ha⁻¹ increased for both the mixture and the monoculture as SR rate increased.

3.1.3. Mixture vs. monoculture with both no N and moderate/high N rates

In Minas Gerais, Brazil (21°14' S; annual rainfall of 950 mm during study; 22 °C), a 2.5-year study compared Marandu palisadegrass grown in monoculture with either zero or 150 kg N ha⁻¹ year⁻¹, or grown in mixture with pintoi peanut (Homem et al., 2021). Pastures were continuously stocked using a variable SR to maintain canopy height of 20 to 25 cm. Pintoi peanut proportion in herbage mass ranged from 21 to 34% throughout the study, and mixture CP concentration was 11.5% compared with 9.1 and 13.8 for the monoculture with zero and 150 kg N ha⁻¹. Treatment effects for average daily gain approached significance ($P = 0.081$), and means were 0.64, 0.61, and 0.54 kg for the N-fertilized monoculture, the mixture, and the unfertilized monoculture, respectively. Stocking rate ($P < 0.01$) for the same three treatments was 3.8, 2.8, and 2.3 animal units ha⁻¹, and gain ha⁻¹ ($P < 0.01$) was 219, 143, and 106 kg.

In Florida, USA (30°52' N; average annual rainfall of 1363 mm; average annual temperature of 20 °C), three year-round systems were compared during four years under continuous stocking with a variable stocking rate adjusted every 14 d based on forage allowance (Jaramillo et al., 2021b). During the warm season, treatments were bahiagrass with no N fertilizer in summer (cool-season, grass-clover [*Trifolium* spp.] mixture in winter), bahiagrass with 112 kg N ha⁻¹ during summer (overseeded with a cool-season grass mixture and fertilized with 112 kg N ha⁻¹ during winter), and bahiagrass-rhizoma peanut (*Arachis glabrata* Benth.) mixture during summer (overseeded with a cool-season, grass-clover mixture during winter). During the summer, yearling steers grazing the mixture had 44% rhizoma peanut in their diet and gained 0.61 kg d⁻¹ compared with 0.36 and 0.33 kg for the grass monoculture with and without N fertilizer, respectively. Gain ha⁻¹ was 397, 335, and 278 kg during summer for the same treatments, respectively.

3.2. Provisioning ecosystem services synthesis

In aggregate, these studies demonstrate that daily individual animal performance is generally, but not always, greater for legume-C4 grass mixtures compared with C4 grass monocultures across a wide range of N fertilizer inputs. Only in the case of mimosa silvopasture was daily individual animal performance less for the mixture. Gain ha⁻¹ is a function of both daily gain and SR, the latter dictated in variable SR studies by forage accumulation rate. This can provide advantages in gain ha⁻¹ for monocultures that receive greater amounts of N (e.g., Homem et al., 2021). Thus, unless they are not consumed readily by livestock or compete significantly with the grass component (e.g., mimosa in Silva et al., 2021a), the greater nutritive value of most legumes will support greater daily gain per animal regardless of N rate, but comparisons of gain ha⁻¹ are more likely to be dependent upon amount of N applied to the grass monoculture and its effect on SR.

4. Supporting ecosystem services

Supporting services are diverse and include forage production, nutrient cycling in plant litter and excreta, N fixation, and crop pollination. This section of the review will focus briefly on each of these categories of ecosystem services for experiments comparing grass-only and legume-grass swards.

4.1. Forage production

Results are not consistent for experiments comparing forage production of grass-legume mixtures vs. grass monocultures. The outcome of the comparison is affected by the N rate applied to the grass, compatibility of the legume and grass species in the mixture, the grazing management imposed, and other factors that are not always well defined.

The importance of species compatibility was illustrated in Costa Rica, where mixtures of several tropical legumes with palisadegrass and signalgrass were compared with grass monocultures at two SR (Hernandez et al., 1995; Ibrahim and t'Mannetje, 1998). Pintoi peanut-palisadegrass mixtures had

similar pre-grazing forage mass as palisadegrass receiving no N fertilizer when SR was low, but when SR was doubled, the mixture had greater forage mass than the monoculture because of increased contribution from the legume (Hernandez et al., 1995). Greater grazing pressure resulted in superior performance of the relatively low-growing legume in association with a taller-growing grass. At the same location, pinto peanut performed better at both SR when planted in lower-growing signalgrass compared with palisadegrass (Ibrahim and t'Mannetje, 1998). In contrast, stylo [*Stylosanthes guianensis* (Aublet) Sw.], with a more upright growth habit than pinto peanut and lacking stolons, was negatively affected by greater SR and made important contributions to forage mass only when grown with shorter-growing signalgrass and grazed at a low SR. These data illustrate the importance of growth habit of the species in the mixture and grazing management in determining legume productivity and the relative performance of mixtures vs. grass monocultures.

The amount of N fertilizer applied to the grass monoculture is also an important determinant of their productivity relative to grass-legume mixtures. For example, in Florida USA, yearling steer SR was 30% greater for limpograss monoculture receiving an average of 180 kg N ha⁻¹ year⁻¹ vs. aescynomene-limpograss mixtures during three years of grazing. A variable SR was used to maintain similar forage allowance, so the SR was a useful proxy for forage accumulation. Also in Florida, rhizoma peanut was grown in strips in association with bahiagrass during four years and compared with the bahiagrass monoculture receiving either 0 or 120 kg N ha⁻¹ during summer (Jaramillo et al., 2021b). A variable SR methodology was used to maintain constant forage allowance, so observed SR is a response to forage accumulation. Stocking rate was ranked N-fertilized bahiagrass > grass with no N > grass-rhizoma peanut mixture (6.3, 5.6, and 4.3 AU ha⁻¹, respectively, when 1 AU = 350 kg live weight). In a separate study at the same location, forage accumulation of rhizoma peanut-Tifton 85 bermudagrass mixtures was similar to that of Tifton 85 monocultures receiving no N fertilizer during a three-year study (Jaramillo et al., 2018).

One challenge for sustaining forage accumulation of legume tree-grass mixtures is the potential for shading of the grass understory that decreases grass forage accumulation. In Pernambuco state, Brazil, signalgrass was subjected to shading from the tree legumes gliricidia and mimosa planted in rows 10-m apart (Lima et al., 2020). Greater forage accumulation occurred for signalgrass in full sun than under shade (54 vs. 27 kg ha⁻¹ d⁻¹), indicating that the trees would need to provide significant additional forage accumulation to compensate for the losses in grass forage accumulation associated with shade.

4.2. Nutrient cycling

Nutrient cycling is key to sustaining pasture productivity over time. Reducing nutrient losses and enhancing the delivery of products and services per unit of cycled nutrient is the ultimate goal to enhance efficiency of grassland ecosystems (Dubeux et al., 2017c). Nitrogen is perhaps the most dynamic nutrient in grassland ecosystems, and it is easily lost via decomposition processes. This portion of the review will focus on N dynamics for C4 grass swards in monoculture or in mixture with legumes.

Major N inputs in grassland ecosystems include biological N fixation (symbiotic and non-symbiotic) and N fertilizer application, with limited contributions from atmospheric deposition and livestock supplementation (Dubeux et al., 2007; Vendramini et al., 2007). Warm-climate C4 grasses have the ability to swap N sources when grown in association with legumes. Villegas et al. (2020) assessed paired sites comparing C4 grass monocultures or in mixture with legumes. They monitored grass $\delta^{15}\text{N}$ and observed changes indicating that grasses were able to obtain N from legumes when in mixtures and likely from non-symbiotic N fixation when growing in monocultures. Nitrogen is lost mainly via denitrification, ammonia volatilization, and nitrate leaching (Longhini et al., 2020). Organic N might also undergo decay and transformation, forming recalcitrant compounds protected in the soil organic matter (Cotrufo et al., 2013).

Plant litter and livestock excreta are the major pathways of nutrient cycling in grasslands (Dubeux et al., 2007). Therefore, improving the quality of both excreta and litter (i.e., greater N concentration, lesser

recalcitrant compounds) will enhance rate and extent of decomposition. Integrating forage legumes typically reduces litter C:N ratio and enhances litter decomposition (Thomas and Asakawa, 1993; Silva et al., 2012). Nitrogen-fertilized C4 grasses typically have greater litter deposition rates and litter mass than grass-legume mixtures with limited or nil N input (Jaramillo et al., 2021a; Silva et al., 2021b); however, because of greater litter N concentration, grass-legume mixtures may result in similar N return from litter compared with N-fertilized grass (Jaramillo et al., 2021a). There are exceptions to this outcome. For example, when N rate applied to a year-round, grass-only system was quite high (290 kg N ha⁻¹ year⁻¹), N concentrations of the grass litter were at least as great as those for mixture litter, leading to greater litter N return for the grass-only system than the mixture (Silva et al., 2021b).

Grazing management may play an important role in altering proportion of legume in the botanical composition and in livestock diets. Depablos et al. (2021) indicated that less frequent defoliation of Marandu palisadegrass and calopo (*Calopogonium mucunoides* Desv.) mixed pastures, with regrowth periods ending when 100% light interception (LI) was achieved, resulted in lesser proportion of legume and lower N intake and biological N fixation compared with 90 or 95% LI treatments. They recommended 95% LI as the upper limit to start grazing these mixtures to enhance nutrient cycling. In another study with Marandu palisadegrass and pinto peanut, legume integration replaced N fertilizer application and resulted in similar conservation of soil N reserves compared with the C4 grass monoculture without legume or N (Homem et al., 2021). Kohmann et al. (2018, 2019) found similar results on bahiagrass in monoculture or mixed with rhizoma peanut, indicating that grass-legume mixtures are an alternative to N fertilizer for enhancing litter N cycling in grasslands when contrasted with bahiagrass in monoculture.

Belowground litter might be a significant source of nutrients for annual and perennial species. Santos et al. (2022) assessed rhizoma peanut root-rhizome decomposition under grazing or clipping in a two-year study. Defoliation management did not affect decomposition, and root-rhizome turnover was more responsive to season than management. In the same experimental area, Santos et al. (2019a) assessed rhizoma peanut root-rhizome N pool, turnover, and half-life. Assuming a 30% participation of rhizoma peanut in the pasture botanical composition, the annual N contribution by peanut root-rhizome turnover would be 22 kg N ha⁻¹. The combination of above- and belowground litter adds to the N pool and contributes to plant N uptake.

Grass-legume mixtures affect output and chemical composition of livestock excreta. Garcia et al. (2021) investigated nutrient excretion from cattle grazing N-fertilized grass or grass-legume pastures in three year-round production systems. In the warm-season, Argentine bahiagrass was the perennial C4 grass species under one of the following managements: no N fertilizer, fertilizer application of 112 kg N ha⁻¹, or mixed with strip-planted Ecoturf rhizoma peanut. In the cool season, these systems were respectively overseeded with oat (*Avena sativa* L.) and rye (*Secale cereale* L.) for treatment 1 and the same grass mixture plus a blend of clovers (*Trifolium* sp.) for treatments 2 and 3 during the cool season. Urinary volume was greater in the warm season (122 to 182 L ha⁻¹ d⁻¹) compared with the cool season (56 to 70 L ha⁻¹ d⁻¹). During the warm season, urinary N concentration was greater in the grass-legume mixture (4.4 g kg⁻¹) compared with N-fertilized grass (3.1 g kg⁻¹), and fecal output was greater for N-fertilized grass. One of the major take-home messages from this study is that grass-legume systems receiving only 34 kg N ha⁻¹ year⁻¹ recycled 80% the amount of N recycled in the grass system receiving 224 kg N ha⁻¹ year⁻¹, indicating the role of forage legumes to increase N cycling efficiency in grasslands. In fact, these grass-legume systems were able to sustain similar productivity compared with the high-N input system, despite using only 15% of the N fertilizer input (Jaramillo et al., 2021b). Homem et al. (2021) also investigated excreta return in N-fertilized Marandu palisadegrass (150 kg N ha⁻¹ year⁻¹) or palisadegrass mixed with pinto peanut without N fertilizer application, or the grass monoculture without N fertilizer or legume. They observed greater fecal and urinary N output per area in the N-fertilized grass. Both N-fertilized grass and grass-legume mixtures, however, had a positive N balance, whereas the grass monoculture without N fertilizer had a negative N balance.

To summarize the findings for nutrient cycling, when warm-climate legume-grass mixtures are compared with grass-only systems receiving no N or low rates of N, the mixture often has lesser

litter mass deposition rates, but litter quality may be superior to the grass alone, leading to equal or greater N cycling for the mixture. However, at moderate to high N rates applied to the grass, litter N concentrations may be similar to that of the mixture, and in conjunction with often greater litter deposition rates of the grass-only system may result in greater N cycling for these grass-only swards. A similar relationship can be observed for N return in cattle excreta. Cattle grazing grasses receiving zero or low amounts of N fertilizer will likely have lesser excreta N concentration than for the mixture, which can result in greater N cycling through excreta for mixtures than grass-only systems. This may not be the case if more N fertilizer is applied to the grass. In any case, integrating legumes with grasses provides an alternative to N fertilizer for enhancing litter N cycling in grasslands.

4.3. Biological N fixation

Symbiotic biological N fixation (BNF) is perhaps one of the most important reasons to integrate forage legumes into livestock systems. The amount of BNF varies with species, proportion in the botanical composition, management, soil N, and other environmental conditions that affect the growth potential of the target legume species. Although variable, typical BNF in warm-climate, grass-legume mixtures ranges from 50 to 150 kg N ha⁻¹ year⁻¹ (Homem et al., 2021; Jaramillo et al., 2021a). If legumes are grown in monoculture, BNF can exceed these values. An average BNF of 200 kg N ha⁻¹ year⁻¹ was reported for rhizoma peanut in monoculture and harvested every 56 d (Dubeux et al., 2017a). Considering a target legume proportion of 30% by weight in the mixture, these numbers would decrease to 60 kg N ha⁻¹ year⁻¹ assuming no interaction with the grass species. Some research, however, indicates that the presence of a grass with the legume benefits BNF because N uptake by the grass diminishes soil N and provides a feedback loop to the companion legume that stimulates BNF (Ledgard and Steele, 1992).

In subtropical regions, there is an opportunity to overseed perennial warm-season grasses with cool-season forages, including legumes. This enhances the opportunity to add N to the system via BNF. Jaramillo et al. (2021a) measured BNF in the cool and warm seasons in a grazing system in north Florida, USA (30°52' N, 85°11' W, 35 m asl). Rhizoma peanut was the legume component during the warm season, and clovers (*Trifolium* sp.) during the cool season. They observed annual BNF of 54 kg N ha⁻¹, with 70% of BNF occurring during the cool season. In a clipping trial, Santos et al. (2018) assessed rhizoma peanut and bahiagrass entries, and BNF ranged from 11 to 44 kg N ha⁻¹ harvest⁻¹, with three to four harvests per year occurring every 6 wk to a 7.5-cm stubble.

In summary, there is a wide range in BNF of grass-legume mixtures, but the most frequently observed values are in the range of 50 to 150 kg N ha⁻¹ year⁻¹. The great advantage is the more efficient N cycling compared with N input from fertilizer, resulting in a more efficient N use.

4.4. Pollination

Grasslands represent one of the most important terrestrial agroecosystems and play a key role providing habitat for pollinating insects. Increasing floristic diversity in grasslands is a possible way to enhance pollinator habitat. Integrating legumes and forbs enhances resources for pollinators and increases their abundance, which is correlated with legume and forb flower density (Woodcock et al., 2014; Orford et al., 2016). Even a modest increase in conventional grassland plant diversity with legumes and forbs enhances pollinator functional diversity, richness, and abundance, improving pollination services in surrounding crops (Orford et al., 2016). Results indicate that multi-species grasslands can be designed to support high diversity of pollinators without compromising pasture productivity (Cong et al., 2020).

Grass monocultures in Latin America have been identified as one of the reasons for insect pollinator decline in the region (Narjes Sanchez et al., 2021). Integration of herbaceous legumes or tree legumes in silvopasture systems enhances pollinator habitat in the region; however, availability of legume seed at an economic price is still a problem (Narjes Sanchez et al., 2021). In subtropical northern

Florida, USA, Garcia-Jimenez (2019) assessed contrasting grazing systems presenting different floristic composition. Integration of the warm-season perennial legume, rhizoma peanut, and overseeding clovers during the cool season enhanced flower diversity and resulted in greater abundance of bees when compared with grass-only grazing systems. Most of the bee species collected in this study were small bees, indicating that they were foraging and had nests in a short radius. The author concluded that inclusion of legumes in forage-livestock systems enhanced ecosystem services related to pollination. In summary, integration of forage legumes into warm-climate livestock systems has potential to enhance insect pollinator habitat, but in some cases, limited seed availability is a barrier to establishment of legumes in these regions.

5. Regulating ecosystem services

5.1. Ameliorating greenhouse gas emissions

Agriculture is responsible for approximately 9% of total greenhouse gas emissions, and ruminant livestock systems emit CO₂, CH₄, and N₂O (IPCC, 2019). Enteric CH₄ is the largest contributor to agricultural greenhouse gas emissions (Herrero et al., 2016), while approximately 18% of global N₂O emissions are attributed to grasslands, primarily associated with N deposition in excreta and losses from N fertilization (Abalos et al., 2014). Boddey et al. (2020) state “The possibility that the introduction of legumes to the diet may reduce these emissions is a powerful motivation to introduce incentives for the adoption of mixed pastures for beef or dairy production”. Superior nutritive value of legumes than grasses and ability of legumes to replace N fertilizer through BNF contribute to achieving reduced emissions.

Relative to nutritive value effects, CH₄ emissions per unit of animal product were inversely correlated with forage digestibility for several ruminant species (Hristov et al., 2013). A meta-analysis concluded that ruminants fed C4 grasses produced 20% more CH₄ per kg of intake than those fed warm-climate legumes (Archimède et al., 2011). They attributed this response to greater fiber concentration and rumen retention time for the C4 grasses (Archimède et al., 2011). Presence of secondary metabolites in legumes, including condensed tannins, has been associated with reductions in CH₄ emissions (Naumann et al., 2017).

Because most legume-grass mixtures are minimally or not fertilized with N, this eliminates one source of N₂O emissions as well as avoiding CO₂ emissions associated with manufacture and transportation of N fertilizer. Nitrogen fertilization of grasses can result in large peaks in N₂O fluxes immediately following fertilization events (Soussana et al., 2010; Klumpp et al., 2011). Cumulative N₂O emissions from legume swards can be similar to N-fertilized systems, but more commonly, soil N₂O losses from N-fertilized grasslands are greater than those from legumes, with differences up to four-fold reported (Jensen et al., 2012). Although N₂O losses per unit of N applied are quite variable depending upon climate, soil, and management, an overall mean of 0.9% has been proposed (Bouwman et al., 2002). If urea is the N source, ammonia volatilization of 30% of applied N or more can be anticipated (de Morais et al., 2013), leading to N deposition and subsequent emissions of N₂O (IPCC, 2019).

Studies comparing greenhouse gas emissions for mixed legume-grass vs. monoculture grass pastures are rare in warm climates. A mixed pasture of Marandu palisadegrass and BRS Mandobi pintoi peanut reduced enteric CH₄ emissions by grazing heifers by 12% relative to palisadegrass monoculture pastures with or without N fertilizer (148, 170, and 165 g day⁻¹ for the mixture, monoculture with N, and monoculture without N, respectively) (unpublished data of B.G.C. Homem cited by Boddey et al., 2020). These authors reported a reduction in CH₄ emissions per unit of daily weight gain of 9 and 15% for animals in the mixed pastures vs. the monoculture with and without N fertilizer, respectively. They attributed this response in part to greater condensed tannin intake of animals grazing mixtures vs. monocultures (0.12 vs. 0.03 g kg⁻¹ body weight day⁻¹, respectively).

Kohmann (2017) compared bahiagrass monocultures receiving 50 kg N ha⁻¹ year⁻¹ with bahiagrass-rhizoma peanut mixtures ranging from 45 to 78% legume. There was no effect of pasture type on N₂O-N

emissions from animal excreta (the sum of dung + urine) due primarily to no differences in urine N concentration. Methane emissions from dung were approximately twice as great for animals grazing the mixture due to greater N concentration in dung derived from mixed pastures. When expressed on a CO₂-equivalence basis, total emissions from animal excreta plus those calculated from N fertilizer application were 2.5 times greater for the N-fertilized grass than the legume-grass system, without accounting for emissions associated with fertilizer production and transport. The author concluded that inclusion of legumes in grass-based systems results in lesser greenhouse gas emissions compared with typical N fertilization regimes in the southeastern USA. In São Paulo state, Brazil, NH₃ emissions were compared for N-fertilized (150 kg N ha⁻¹ year⁻¹ as urea) palisadegrass and palisadegrass-pintoi peanut mixtures (Longhini et al., 2020). The NH₃ emissions from excreta (urine plus dung) of cattle grazing N-fertilized palisadegrass pasture was greater than for excreta from cattle grazing the mixture, and NH₃ emissions from application of urea fertilizer were approximately as great as those from excreta. The authors concluded that N-fertilized grass systems increase N losses as NH₃ over those observed for mixtures, and emissions from urea fertilizer can be significant and must be accounted for.

There are insufficient data to draw firm conclusions regarding the impact of legume-grass mixtures vs. N-fertilized grasses on greenhouse gas emissions in warm climates. Early results from these comparisons on grazed pastures suggest reductions in emissions for mixtures are likely to be associated more with the absence of N fertilizer application than lesser emissions from enteric fermentation or emissions from livestock excreta, although these responses may also occur.

5.2. Soil carbon

In temperate grasslands, presence of legumes or forage species with particular rooting characteristics may positively influence soil C accumulation (De Deyn et al., 2009, 2011; Skinner and Dell, 2016). There have been relatively few quantitative studies of the impact of legume introduction on soil organic carbon accumulation (Jensen et al., 2012), and there is a scarcity of comparisons of grass monocultures with legume-grass mixtures.

Integration of legumes in koroniviagrass swards increased soil C accumulation in two studies. In Colombia, soil C accumulation to a 100-cm depth was 20% greater in pintoi peanut-koroniviagrass [*Urochloa humidicola* (Rendle) Schweick.] mixtures relative to the grass monoculture (Fisher et al., 1994; swards fertilized every two years, but specific nutrients and rates not identified). Introduction of the legume desmodium [*Desmodium heterocarpon* (L.) DC subsp. *ovalifolium* (Prain) H. Ohashi] into a koroniviagrass sward in Bahia, Brazil, increased soil C accumulation (0–100 cm depth) from 0.66 Mg C ha⁻¹ year⁻¹ for the monoculture, which received no N fertilizer, to 1.17 Mg C ha⁻¹ year⁻¹ for the mixture during a nine-year period (Tarré et al., 2001).

Relative performance of mixtures and monocultures was less consistent when the grass monoculture received significant N fertilizer inputs. When a low stocking rate was imposed for 19 years in Texas USA, SOC concentration in the upper 15 cm increased 39% for N-fertilized bermudagrass [*Cynodon dactylon* (L.) Pers.]–annual ryegrass [*Lolium multiflorum* Lam.] pastures (350 kg N ha⁻¹ year⁻¹; annual ryegrass grazed in the cool season) and 67% for a bermudagrass-clover (clover overseeded into the bermudagrass during the cool season) mixture receiving no N fertilizer (Wright et al., 2004). In contrast, at a high stocking rate, bermudagrass-clover pasture SOC concentration did not increase beyond eight years, but in Coastal bermudagrass-ryegrass pastures, it continued to increase for 25 years.

In Florida, USA, bahiagrass monocultures fertilized with 90 kg N ha⁻¹ year⁻¹ were compared with mixtures of rhizoma peanut and bahiagrass in terms of bulk soil C and N accumulation and particulate organic matter accumulation over periods of four and five years (Santos et al., 2019b). Plots were harvested by clipping one to four times year⁻¹ over the five years, with three or four harvests year⁻¹ in years 4 and 5. Bahiagrass-rhizoma peanut mixtures had similar whole-soil C and soil particulate organic matter carbon stocks as N-fertilized bahiagrass. Also in Florida, USA, year-round forage systems of N-fertilized bermudagrass in summer plus cool-season grasses in winter (290 kg N ha⁻¹ year⁻¹) were compared with rhizoma peanut-grass mixtures in summer plus cool-season grass-clover mixtures

in winter (Silva et al., 2022). Annual C accumulation (0-0.1 m depth) during the six-year period was 1.3 and 0.2 Mg C ha⁻¹ for N-fertilized grass and legume-grass systems, respectively. Thus, a year-round forage system based on N-fertilized grasses accumulated more surface-soil C and N than a legume-grass mixture, with the grass system benefitting from presence of a perennial C4 species and application of high N fertilizer rates.

A question regarding impact on soil C accumulation and addition of legumes is whether greater quality of senescing legume plant biomass and the associated greater decomposition rates (Kohmann et al., 2019) represent losses to the system, while the litter that does not decompose at a measurable rate contributes to stable soil organic matter (Berg and McLaugherty, 2008). Using isotopically enriched litter, several studies suggest that rapidly degraded material actually contributes a large amount of C to soil (Bird et al., 2008; Rubino et al., 2010). This contribution is thought to derive from microbial compounds produced during decomposition (Mambelli et al., 2011) rather than by recalcitrant components present in the litter (Marschner et al., 2008). It has been suggested that labile plant constituents, not recalcitrant ones, are the dominant source of microbial products because they are used more efficiently by microbes (Cotrufo et al., 2013). These findings support a conclusion that greater degradability of legume residues does not diminish the potential for soil C accumulation.

The literature on mixture impacts on soil C accumulation is somewhat variable. Because of the importance of above- and belowground plant biomass in affecting soil C accumulation, it is likely that the N status of system is an important factor determining the response to integration of legumes. Mixtures are expected to demonstrate greater advantage when no N fertilizer is applied to the grass monoculture and when soil N is limiting.

6. Moving beyond potential to adoption

The potential of legume-grass mixtures to provide valued ecosystem services is irrefutable, but as any mentor, parent, or coach knows, potential does not guarantee a desired outcome. How can we move beyond recognized potential of legumes to their successful adoption? A long treatise could be written about this subject, but our comments will be brief and limited to several key points. Others have discussed the issue of legume adoption in much greater breadth and depth, and readers are referred to these papers (Dubeux et al., 2017b, 2017c; Muir et al., 2014; Schultze-Kraft et al., 2018; Shelton et al., 2005).

For legumes to be adopted in on-farm grassland systems, they need to, first of all, be persistent. Lack of reliable stand persistence is the death knell of any perennial forage, and many forage legumes have underperformed in this area. In some instances, it may require consideration of non-traditional species (e.g., tree legumes), and in other cases, it may require additional plant collection, more complete evaluation of existing germplasm, or simply evaluation with greater researcher focus on long-term persistence (Schultze-Kraft et al., 2018; Sollenberger et al., 2015). Clearly, the genus *Arachis* has contributed attractive forage legumes in both tropical and subtropical environments, and both species in use are characterized by persistence under defoliation and competitiveness with C4 grasses. There are pastures of rhizoma peanut in Florida, USA, that were planted in the 1960s and are still in production. The senior author assisted in planting rhizoma peanut pastures in 1983 that will be used in upcoming research, nearly 40 years later.

After persistent types are identified, establishment methods that are low risk and affordable must be developed. In some cases, this is a gradual progression of learning how to manage the planting process, how to more inexpensively produce seed or vegetative propagules, or breeding/selecting new material with a focus on establishment success. All of these elements have been part of the sequential improvement made in rhizoma peanut establishment in the southern USA (Quesenberry et al., 2010; Castillo et al., 2013a, 2013b; Aryal et al., 2020). In Brazil, the Pinto Peanut Breeding Program has as one of its primary objectives the release of high seed-yielding cultivars of pinto peanut, because low seed availability and high costs limit producer adoption in mixed pastures (De Assis et al., 2013).

Often, plant compatibility in mixtures is an afterthought in forage evaluation programs, but it has been argued that the success of legume technology depends on development of compatible grasses

and legumes (Andrade et al., 2015). Typically, compatibility in mixtures is assessed after a cultivar is released, with justification for release based on performance in monoculture. Recent work in Brazil has focused on evaluation of compatibility of pinto peanut genotypes with koroniviagrass, and results suggest legume genotypes can be identified with both high seed yield and compatibility with the grass (De Andrade et al., 2021).

Lastly, there are questions regarding interaction of scientists with end users. Others have addressed “front-end” integration of producers into research prioritization and planning to insure relevance of research (Shelton et al., 2005; Muir et al., 2011). At the other end of the process, there are questions as to whether we are effectively communicating our scientific outputs (discovery, technology, or product) to potential adopters leading to adoption and impact. Fundamentally, do scientists believe that we have a stake in technology or product adoption or is the science leading to development of these products our only responsibility? Is it a legitimate, or perhaps even essential role, for us to be a champion (Shelton et al., 2005), advocating for adoption of our research outputs? Perhaps we need to reassess our role as research scientists and include in our job descriptions a larger and more intentional stake in technology or product adoption, in addition to our traditional role in development. From the Florida, USA, context, it is quite likely that some of our most impactful scientific products of the recent decades would never have been adopted by producers if it were not for the persistent, unflagging effort by champions of those technologies.

7. Conclusions

The history of integrating warm-climate forage legumes into grass swards has not been an overwhelming success, but the literature clearly demonstrates numerous advantages in ecosystem service delivery for legume-grass mixtures compared with grass-only swards. Those advantages in terms of animal performance, soil carbon accumulation, and nutrient cycling are most clearly measurable when mixtures are compared with grass swards receiving little or no N fertilizer. Advantages are often less or may disappear altogether when mixtures are compared with grasses receiving moderate to high N rates. In contrast, amelioration of greenhouse gas emissions is often most obvious when the comparison grass receives relatively high rates of N because of the scale of N fertilizer impacts on greenhouse gas emissions. Looking ahead, there is need to focus on adoption of existing legume technologies in addition to ongoing technology development. To enhance adoption, it is suggested we emphasize selection for legume persistence; identify low risk and affordable legume establishment methods; assess compatibility of legumes in mixture with grasses prior to legume cultivar release; emphasize the need for adoption of both legume species and optimal management practices for their success; and recognize that, in addition to our traditional role in technology or product development, scientists need to assume a larger and more intentional role in encouraging adoption by end-users, perhaps by being champions of our research innovations.

Conflict of interest

The authors declare no conflict of interest.

Author contributions

Conceptualization: L.E. Sollenberger and J.C.B. Dubeux Junior. Writing – original draft: L.E. Sollenberger and J.C.B. Dubeux Junior. Writing – review & editing: L.E. Sollenberger and J.C.B. Dubeux Junior.

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