New insights for benefit of legume inclusion in grazing systems

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Abstract
The benefits and challenges of legume inclusion in grazing systems have been well documented over time and across different regions. Recent investigations have provided novel insights into the benefits of legume inclusion in grazing systems. Our objective is not to provide a wide overview of the benefits of legume inclusion but to explore novel insights of recent advancements made from studies evaluating legume inclusion in grazing systems. Efficiency of resource use through legume inclusion in grazing systems can reduce the water footprint associated with beef production through improvements in forage nutritive value and animal performance. These efficiencies also translate into improvements in nutrient cycling and nutrient transfer, which are critical for sustaining productivity of grazing systems. Moreover, evidence exists highlighting the importance of root contact between grasses and legumes for sharing N. Provisioning of floral resources from legumes has also been shown to be important for providing habitat for pollinator species. Lastly, soil microbial abundance of microorganisms associated with N_2 fixation can be altered according to species present within a pasture, especially when legumes are present. Insights derived from such recent studies continue to provide evidence for the need to continue to develop legume-based grazing agroecosystems.

Introduction
The benefits of legume inclusion in pasture agroecosystems have been well documented across different regions. These benefits have been largely shown through increases in pasture productivity, improvements in nutritive value, or improvements in animal performance. Challenges associated with legume adoption at the producer level have also been well documented, oftentimes citing poor persistence and issues related to establishment as reasons for not adopting legume use. Recent investigations have provided novel insights into other aspects and benefits of legume inclusion into grazing systems. The objective is not to provide a wide-ranging overview of the benefits of legume inclusion in grazing systems, but rather explore recent insights and advancements made in the literature regarding the use of forage legumes in grazing agroecosystems.

Water Footprint
Water footprint is the volume of water used to produce a given mass or volume or product (Hoekstra et al., 2011). Water use assessments have mostly focused on withdrawals from aquifers or water bodies for irrigation, with limited information available regarding water footprint in grazing systems (Ran et al., 2016). Such assessments are critical to evaluate the environmental footprint of livestock production systems (Ran et al., 2016), and the majority of reports have been conducted through large-scale modeling efforts, such as cradle-to-farm models (Rotz et al., 2015), with limited studies reporting water footprints using direct measurements.

Legume inclusion into pasture systems is hypothesized to decrease the water footprint, per unit of defined product, by increasing efficiency of production through improvements in the forage nutritive value of the pasture systems, resulting in greater gains, compared to grazing systems with no legume components. In the southeastern U.S., the water footprint in beef grazing systems was reduced by introducing the legume rhizoma peanut (*Arachis glabrata* Benth) into bahiagrass (*Paspalum notatum* Flügge), as compared to bahiagrass monocultures receiving no N-fertilizer (Jaramillo et al., 2021). Considering rainfall and drinking water as the inputs into the grazing systems, the water footprint in bahiagrass receiving no N fertilizer was 25 m^3 water kg^{-1} bodyweight gain, compared to 18 m^3 water kg^{-1} bodyweight gain from bahiagrass-rhizoma peanut systems. Moreover, when considering only the drinking water as the only exogenous water input into the non-irrigated grazing systems, the water footprint was reduced by 45% in bahiagrass-rhizoma peanut system, compared to both grazing systems consisting of N-fertilized bahiagrass and bahiagrass receiving no N fertilizer, where the water footprint was 0.06 and 0.11 m^3 water kg^{-1} bodyweight gain for bahiagrass-rhizoma peanut and remaining systems, respectively (Jaramillo et al., 2021). These data provide evidence that increasing system productivity through legume inclusion is a key management practice for improving the efficiency of water-resource use, which will become increasingly important in regions where water resource may become scarce.

Nutrient Cycling
Nutrient cycling is vital for sustaining productivity of pasture agroecosystems. Both plant litter and excreta produced by grazing livestock are valuable resources that enhance nutrient cycling. Nutrient returns through plant litter or excreta can differ according to environmental conditions, management, and other factors (Dubeux et al., 2007). Introducing legumes into pasture systems has the potential to increase the efficiency of N cycling.

Accounting for stocking rate, Garcia et al. (2021) reported that pasture systems containing legumes during both the cool-and-warm season periods, exhibited greater total N excretion when compared to grazing systems without legumes and relying on N-fertilizer application. In their study, the grazing systems consisted of warm-season perennial pastures, overseeded with cool-season grasses or legumes during the cool-season months. Overall, Garcia et al. (2021) showed that grazing systems consisting of grass-legume mixtures throughout the year were capable of re-cycling 80% of the total N recycled in N-fertilized systems, with no legume additions. Moreover, mineral nutrient excretion (e.g. P, K, Ca, and Mg) and total urinary and fecal N were greater during the cool-season, likely due to improved forage nutritive value of temperate species, allowing for greater intake (Garcia et al., 2021).

Soil Microbial Diversity

Rhizosphere microorganisms are critical members of the plant microbiome. Plant species present within pastures have a direct effect on soil microbial communities, mainly through providing nutrients through root exudates (Berg and Smalla, 2009). The amount of soil available N also has a direct impact on soil bacteria composition (Fierer et al., 2012), but increasing pasture species diversity does not always result in increased microbial species diversity. Diazotrophic microorganisms, which include rhizobia that form symbioses with legume species, represent a minor fraction of the total soil microbial community, but they are highly beneficial to grasslands for their ability of converting atmospheric N_2 into plant-usable N via biological N_2-fixation (BNF), contributing to improvements in nutrient cycling (Guerra et al., 2022).

The application of novel molecular technologies has resulted in increased abilities of studying soil microbial communities. In recent investigations, Guerra et al. (2022) utilized 16S rRNA genes to investigate how soil bacterial communities differed across pastures systems with contrasting N management regimes. The three bahiagrass-based systems consisted of 1) bahiagrass fertilized with mineral N (224 kg N ha^{-1} yr^{-1}), 2) bahiagrass pastures with combination of mineral N (34 kg N ha^{-1} yr^{-1}) and legume N via overseeded clovers during the cool season, or 3) bahiagrass with combination of overseeded clovers during the cool season, and strips of Ecoturf rhizoma peanut during the warm season. The soil bacteria alpha diversity was greater in pastures receiving 34 kg N ha^{-1} yr^{-1} compared to pasture systems with bahiagrass-rhizoma peanut during the warm season (Guerra et al., 2022). This indicates that increasing the species diversity within a pasture system did not result in increased diversity of the soil microorganisms. However, further characterization of the soil microorganisms indicates that relative abundance of *Bradyrhizobium* spp., a genus important for N_2-fixing abilities, was 44% greater in bahiagrass systems with legume components, whereas bahiagrass pastures managed with mineral N fertilizer showed greater abundance of *Bryobacter* spp. and *Pseudolabrys* spp., which are associated with C cycling (Guerra et al., 2022). This study shows that soil bacterial communities are impacted by the pasture N management practices, especially through inclusion of forage legumes. Increasing abundance of soil microorganisms that enhance N cycling are important for improving the productivity of grasslands and helping to build soil OM through improvements in nutrient cycling processes.

Pollinator Habitat

Pollinators are a diverse group of animals, dominated by insects, and are largely responsible for pollinating large proportions of global crops. Bees are the most-widely recognized pollinators species, however land-use changes, agricultural intensification, pesticide use, and environmental pollution have all contributed to declines in native bee populations. Legumes can be important contributors to providing habitat for pollinator species through provisioning of floral resources and increasing diversity of floral structures in grasslands (Cole et al., 2022). Indeed, Garcia (2019) showed grazing systems with year-round inclusion of legumes had greater abundance of flowers, compared to grass monocultures, which also resulted in greater abundance of pollinator diversity in systems with legume inclusion. Pasture systems that combine productive grass-legume species, with focus on utilization of legumes with distinct flowering structure and phenology will be capable of supporting greater abundance of pollinator species, providing valuable habitat to species in decline (Cole et al., 2022).

Nitrogen Transfer and Biological N_2-Fixation

Nitrogen transfer from legume to grasses can be a bi-directional process, occurring mainly from donor plants possessing abundance of N, to plants with greater demand for N. This transfer can occur both above and belowground. Aboveground N transfer from legume to grass can occur to a high extent in grazing systems, mainly through plant litter decomposition and excreta (Dubeux et al., 2007). Belowground N transfer, however, remains challenging to quantify, compared to other aboveground processes, but occurs mainly through decomposition of legume tissue, root exudation, and via associations with mycorrhizae fungi (Queiroz, 2021). Stable isotope techniques, using enriched $^{15}N_2$ gas have been employed in controlled environments to track N transfer from legumes to grasses. Queiroz (2021) utilized H-format pots, in which two separate pots are interconnected with a middle portion, allowing roots to grow into opposite sides. Injecting $^{15}N_2$ gas directly into the root zone in these H-format pots showed that direct root contact, as well as association with mycorrhizal fungi, were main pathways for belowground N transfer, when annual ryegrass (*Lollium multiflorum* L.) and crimson clover (*Trifolium incarnatum* L.) were planted in opposite sides in H-format pots (Queiroz, 2021). Moreover, when membrane was included in the H-pots, direct root contact and mycorrhizae growth was prevented and thus N transfer did not occur between both species (Queiroz, 2021). These data provide evidence of novel techniques for measuring N-transfer, while also serving to indicate the importance of direct root contact for N transfer in grass-legume mixtures.

Biological N_2-fixation (BNF) is an important ecosystem service obtained through inclusion of legume species (Sollenberger et al., 2019). Total BNF in a system is contingent upon several factors, including pasture botanical composition, herbage accumulation of the legume, legume N concentration, and the proportion of N derived from the atmosphere. In pure stands, clovers (*Trifolium* spp.) can fix up to 155 kg N ha^{-1} yr^{-1} (Brink, 1990), however, Jaramillo et al. (2021) showed continuously stocked pastures consisting of mixtures between cool-season grasses and clovers fixed up to 44 kg N ha^{-1} yr^{-1}, across a 4-yr study. In subtropical regions, warm-season legumes also provide options for legume inclusion, however the options are limited in relation to temperature regions. Rhizoma peanut (*Arachis glabrata* Benth.) is an option and has been utilized in the U.S. Gulf Coast Region. In mixture with bahiagrass, Jaramillo et al. (2021) reported rhizoma peanut with BNF ability of 16 kg N ha^{-1} yr^{-1} managed under continuous stocking for 4-yr. While these values were less than what is reported in other tropical legumes, the study by Jaramillo et al. (2021) utilized strip-planting method (Castillo et al., 2013) which enabled selection of the rhizoma peanut over bahiagrass, resulting in suppressed herbage accumulation rates and lesser overall contribution to the pasture botanical composition.

Implications

Forage legume inclusion into grazing systems has its benefits and challenges. Recent investigations have provided insight into various aspects and ecosystem services provided by legume inclusion into grazing systems, but the aspects discussed are not an exhaustive list. These benefits go beyond the traditional improvements in forage production and nutritive value. The use of forage legumes in grazing systems has the potential to improve resource-use efficiency related to water and nutrients, while also improving nutrient cycling. The challenges pertaining to poor adoption among livestock producers must still be addressed in order to increase the use of forage legumes across all regions.

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