

# ECOSYSTEM SERVICES PROVIDED BY OVERSEEDING AESCHYNOMENE INTO BAHIAGRASS PASTURES IN SOUTH FLORIDA

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**Keywords:** Aeschynomene; Bahiagrass; Ecosystem services.

## Abstract

*Aeschynomene* (*Aeschynomene americana* L.) is a warm-season annual legume commonly overseeded into warm-season perennial grass pastures in tropical and subtropical regions. Although *aeschynomene* usually increases forage production and nutritive value, there is limited information about the ecosystem services provided by this legume. The objective of these studies was to evaluate the effects of overseeding *aeschynomene* into bahiagrass (*Paspalum notatum* Flügge) pastures on nutrient dynamics and microbial N-cycling gene abundances. The studies were conducted in Ona, FL, from April to March 2019-2020 and 2020-2021. Treatments were the split-plot arrangement of two forage systems treatments (overseeding *aeschynomene* into bahiagrass or bahiagrass monoculture; main plots) and two N fertilization levels [0 (control) and 60 kg N ha<sup>-1</sup>; sub-plot], distributed in a randomized complete block design with four replicates. Forage characteristics were evaluated 8-wk after seeding and every 35 d thereafter. The static chamber technique was used to estimate nitrous oxide (N<sub>2</sub>O) emissions. The litter bag technique was used to estimate organic matter (OM) and N decomposition. Nitrogen-cycling gene abundances were measured by qPCR. Bahiagrass-*aeschynomene* had greater crude protein concentration than bahiagrass monoculture but there was no difference in forage accumulation. Nitrogen fertilization increased forage accumulation and daily N<sub>2</sub>O emissions. Litter from bahiagrass-*aeschynomene* had greater OM and N decomposition rates than bahiagrass only, and N fertilization did not affect litter decomposition. There were no differences in N-cycling microbial gene abundances among treatments. Overseeding *aeschynomene* into bahiagrass may provide additional ecosystem services, but the magnitude is conditional to the establishment and proportion of *aeschynomene* in the pasture botanical composition.

## Introduction

Grasslands cover approximately 3.9 million ha in Florida, where 85% of this area is grazed by beef cattle (USDA and NASS, 2019). These are dominated by tropical forages (C4) and usually are allocated to areas with low concentrations of OM, N, K, and other nutrients (Holdridge, 1967). These constraints limit stocking rates, especially when inputs of industrial inorganic fertilizers are minimal (Vendramini et al., 2013). Additionally, there is an increasing concern about the contribution of agriculture to greenhouse gas (GHG) emissions in the United States. Nitrous oxide is a potent GHG (298 times greater warming potential than CO<sub>2</sub> in a 100-yr period) with a long life in the atmosphere (121-yr; IPCC, 2013). Jensen et al. (2012) concluded that N<sub>2</sub>O emissions from legumes tend to be less than N-fertilized pastures or non-legume forage crops. However, there is still limited information regarding the effect of overseeding tropical legumes into warm-season perennial pastures on N<sub>2</sub>O emissions.

Bahiagrass is a warm-season perennial grass, mainly cultivated due its resilience, persistency to grazing, and adaptability to adverse conditions in Florida. *Aeschynomene* is an annual warm-season legume native to the United States, that tolerates wet or temporarily waterlogged soils, and it is commonly overseeded into bahiagrass. There have been previous reports of *aeschynomene* and bahiagrass forage characteristics in the literature (Kalmbacher & Martin, 1983). However, there has not been a previous

evaluation of *aeschynomene* overseeding effect on litter organic matter and N decomposition in pastures. Besides, it is not known the effect of overseeding *aeschynomene* on the abundances of microbial genes associated with N fixation, nitrification, and denitrification, nor on N<sub>2</sub>O emissions in bahiagrass pastures.

## Methods

The experiment was conducted at the University of Florida Range Cattle Research and Education Center, Ona FL, United States (27°23'18"N; 81°56'38"W) from June to March 2019 and 2020. The soil in the study area was Pomona fine sand soil (sandy, siliceous, hyperthermic, Ultic Alaquods. In 2019–2020, soil pH (in water) was 5.0, and Mehlich-3 P, K, Mg, and Ca concentrations in the surface horizon (0–15 cm depth) were 19, 53, 287, and 1811 mg kg<sup>-1</sup>, respectively. In 2020–2021, soil pH was 5.4, and Mehlich-3 P, K, Mg, and Ca concentrations were 26, 71, 317, and 1566 mg kg<sup>-1</sup>, respectively.

Treatments were the split-plot arrangement of two forage systems (overseeding *aeschynomene* into bahiagrass or bahiagrass monoculture; main plots) and two N fertilization levels [0 (control) and 60 kg N ha<sup>-1</sup>; sub-plot], distributed in a randomized complete block design with four replicates.

The study was conducted on established (~ 15-yr old) 'Pensacola' bahiagrass pastures. All plots were mowed at 5-cm stubble height and overseeded with 10 kg ha<sup>-1</sup> of inoculated *aeschynomene* seeds in April 2019 and 2020, using a no-till drill. Plots were fertilized 3-wk after seeding with 13.2 and 25 kg ha<sup>-1</sup> of P and K, respectively.

Three 0.25-m<sup>2</sup> samples were clipped per plot with a sickle knife at 15-cm stubble height and used to calculate forage accumulation, botanical composition and crude protein (CP) concentration. Harvests occurred each 35 d regrowth interval. Bahiagrass and *aeschynomene* components were hand separated after harvest, dried at 55 °C for 72 h and weighed for forage accumulation determination. A subsample per plot was grounded to pass a 1-mm screen, to analyze total N concentration by dry combustion. Crude protein concentration was calculated by multiplying N concentration by 6.25.

Litter decomposition was determined using the nylon bag technique. Forage biomass were collected in September 2019 and 2020. Bahiagrass and *aeschynomene* components were hand-separated, dried in a forced-air oven at 55 °C for 72 h, and weighed. Plant material was placed in 15 × 30-cm nylon (75-μm pores) bags (12 g bag<sup>-1</sup>), reconstituting the bahiagrass and *aeschynomene* proportions in the bags per plot. Bags were placed on each plot in October 2019 and 2020, and incubated for 0, 10, 20, 40, 50, 100, and 184 d. After each incubation time, the bags retrieved were placed in a forced-air oven set at 55 °C for 72 h, cleaned with a hand brush, weighed, and grounded to 1-mm particle size. An inverse double exponential model was used to estimate the proportion of OM and N that disappeared during incubation.

Nitrous oxide emissions were determined by the closed static chamber technique, using one chamber per plot. Gas samples taken at 0, 10, 20 and 30 min, and analyzed later by gas chromatography. The N<sub>2</sub>O fluxes were calculated by linear interpolation thereafter.

The N-cycling microbial gene abundances in the soil were determined by DNA soil extraction and qPCR. Five soil cores were collected before the N fertilization, 2-wk after the fertilization, during the last plant harvest (September), and in December in 2019 and 2020. Total genomic DNA was extracted using a prefabricated kit and the copy number of *amoA*, *nirK*, *nirS*, and *nifH* functional genes were estimated by qPCR. All standard curves showed PCR efficiencies ranging from 90–99%.

The data were analyzed by mixed-effects models and ANOVA. Fixed effects were forage system and N fertilization level. Years, blocks, and their interactions were considered random effects. Months and days were analyzed as a repeated measure. Treatments were considered different when  $p \leq 0.05$ .

## Results and Discussion

There was a N fertilization effect on forage accumulation. Forage accumulation increased from 1190 to 1410 kg dry matter (DM) ha<sup>-1</sup> in plots receiving N relative to the no N treatment. Greater bahiagrass forage accumulation due to N fertilization has been reported in numerous previous studies under similar environmental conditions (Vendramini et al., 2013) and occurs because N fertilization increases the concentration of enzymes responsible for photosynthesis and increases tiller number and elongation (Beatty et al., 1960). Compared with bahiagrass monoculture, there was no effect of overseeding

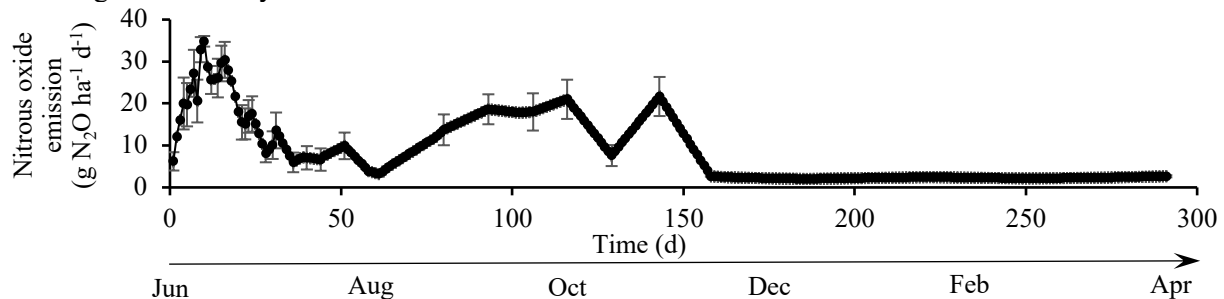
aeschynomene on plot forage accumulation (Table 1). This could be due to relatively poor establishment that limited legume forage accumulation (7 vs. 93% proportion of aeschynomene and bahiagrass in the plots respectively).

**Table 1.** Forage system effect on forage characteristics and litter decomposition. Data were averaged by N fertilization, year, sampling time and replicates.

Response	Forage system		SE	<i>p</i> value
	Bahiagrass monoculture	Bahiagrass-aeschynomene		
Forage accumulation	1249	1356	113	0.2
Crude protein	98	107	3.4	0.02
OM disappearance	330	356	16.2	0.02
N disappearance	152	206	14.7	0.03

There was a forage system effect on crude protein concentration (Table 1). This effect occurred by the greater legume CP concentration (210 vs. 100 g kg<sup>-1</sup>); even with a limited contribution in the canopy, the mixture had greater CP than the monoculture. Kalmbacher & Martin (1983) reported an increase in CP from 70 to 96 g kg<sup>-1</sup> comparing bahiagrass monoculture and mixture with aeschynomene.

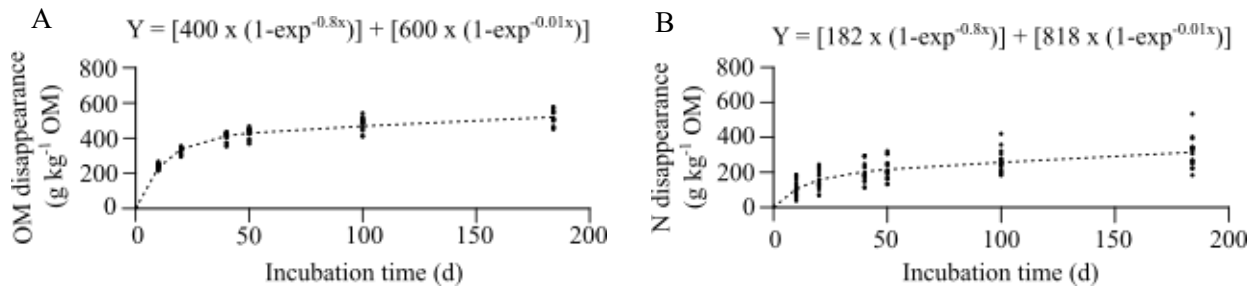
There was a day effect on N<sub>2</sub>O emissions. This occurred due to two main emission peaks during the experimental period (Figure 1). The first peak occurred during the first 50 d after N fertilization. The available N not taken up by the plants or microorganisms may be directed to N leaching or N<sub>2</sub>O emission. Once the N from the fertilizer was consumed, leached, or volatilized, the microbial activity decreased again, reducing the N<sub>2</sub>O fluxes (Dijkstra et al., 2013), as observed 30 d after N fertilization. The second N<sub>2</sub>O peak started in August and likely happened due to greater soil microbial activity caused by greater rainfall, soil moisture and air temperature. Nitrous oxide emissions are expected to increase in soils with greater moisture content (Dijkstra et al., 2013). The decrease in air and soil temperature likely impacted microorganism activity and reduced N<sub>2</sub>O emissions in November.



**Figure 1.** Nitrous oxide emissions in the bahiagrass pastures fertilized with N or overseeded with aeschynomene.

There was a forage system and month effect on OM and N litter disappearance. Bahiagrass-aeschynomene plots had greater OM disappearance than bahiagrass monoculture (Table 1). Legumes stimulate soil microorganism activity and diversity in grasslands due to greater input of soluble N by atmospheric fixation, root exudation, and litter decomposition (Balestrini et al., 2015). Aeschynomene had greater N concentration than bahiagrass aeschynomene and bahiagrass respectively; (Vendramini et al., 2013). Thus, legume overseeding could increase litter N content and promote soil microbial activity and decomposition (Balestrini et al., 2015). The incubation time effect occurred due to rapid OM disappearance in the first 40 d of incubation, where more than 41% of the initial litter OM disappeared

(Figure 2A). Furthermore, there was a faster N disappearance during the first 50 d (45% of the total N disappeared), followed by a lesser decay rate until day 184 (Figure 2B).



**Figure 2.** Plant litter decomposition of bahiagrass monoculture or mixture with aeschynomene with or without N fertilization, separated in A) organic matter and B) nitrogen disappearance.

There were no interaction or treatment effects on the abundances of microbial genes associated with soil N-cycling processes. Thus, there was no effect of overseeding aeschynomene on soil nifH gene abundance, likely because there may already be N-fixing microbial populations in the experimental area before the start of the experimental period and for the low aeschynomene botanical composition in the bahiagrass plots. Dobereiner (1966) isolated a new N-fixing species-specific for bahiagrass: *Azotobacter paspali*, which was predominant in several root samples. Similarly, to other warm-season perennial grass species, bahiagrass is highly responsive to N fertilization (Beaty et al., 1960). Hence, the N input provided by the N fertilization rate and aeschynomene could be quickly used by the bahiagrass, leaving the microorganisms in similar conditions prior the treatment application. Additionally, reduced soil inorganic N concentration ( $0.01\text{--}0.03\text{ g kg}^{-1}$ ), acidic conditions ( $5.0\text{--}5.4\text{ pH}$ ), and sandy texture may have promoted an oligotrophic environment, and fostered greater growth of AOA and nirK over AOB and nirS populations (5070 and 5,829,000 vs. 80 and 24,000 gene copy numbers  $\text{g}^{-1}$  soil respectively).

## Conclusions and Implications

Overseeding aeschynomene provide ecosystem services to bahiagrass pastures, with the increase in CP concentration and the decomposition of OM and N from the litter, which would imply a greater release of nutrients to the soil. These effects then depend on the success establishment of the legume. Additionally, N fertilization increased biomass accumulation, but also daily  $\text{N}_2\text{O}$  emissions. Finally, soil microorganisms were not affected by the treatments, which could be due to oligotrophic soil conditions, high response of bahiagrass to N input, low N fertilization, and low aeschynomene proportion in the pasture.

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