

## Article

# The Influence of Nitrogen and Phosphorus Addition on Growth of the Invasive C<sub>4</sub> Grass *Saccharum spontaneum*

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**Abstract:** Invasive C<sub>4</sub> grasses can inhibit the natural regeneration of secondary forest in tropical landscapes after the cessation of intensive use for grazing and agriculture. In Panama, invasive *Saccharum spontaneum* forms dense stands that require active management to re-establish forest successional processes. In this region, restoration strategies typically involve clearing grass cover manually and applying fertilizer prior to planting tree seedlings. However, if fertilizers alleviate nutrient limitation and enhance grass competition with tree seedlings, these practices may exacerbate the costs of *Saccharum* control and hamper restoration goals. Here, we evaluated how *S. spontaneum* responds to nitrogen and phosphorus addition in the field to determine whether *S. spontaneum* is nutrient limited in this system. *S. spontaneum* was limited by both nitrogen and phosphorus, as revealed through increased foliar nutrient concentrations. *S. spontaneum* biomass was significantly greater in both nitrogen and phosphorus addition plots after both the first growth period (early rainy season) and second growth period (late rainy season), with stronger effects of nutrient limitation during the second growth period for both N limitation and N and P co-limitation. Nutrient limitation in *S. spontaneum* highlights a potential risk of fertilizer applications during restoration, agriculture, and agroforestry activities in which invasion of this aggressive weed is a challenge to land management.

**Keywords:** biological invasions; restoration ecology; nutrient limitation; *Saccharum spontaneum*; Panama Canal; invasive grasses; tropical ecology; nitrogen limitation; phosphorus limitation; nutrient co-limitation



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## 1. Introduction

Intensive agriculture and grazing in tropical systems can lead to soil degradation and reduced productivity. When such lands are abandoned, succession is expected to lead to habitat recovery. However, low propagule availability, seed and seedling predation, seasonal drought, fire, competition with non-native weeds, and reduced nutrient availability are barriers that can slow or deter the rate of succession [1–5]. Although tropical soils are diverse, they are generally less fertile than temperate soils and more likely to be limited by phosphorus than nitrogen [6–8]. Tropical nutrient availability is strongly regulated by nutrient cycling through decomposition [9]. Forest clearing and removal of above-ground biomass in agriculture reduce the amount of nutrients that cycle back into the system through decomposition [10], leading to a rapid loss of nutrients after as few as three cycles of shifting agriculture [11]. As productivity decreases, increasing amounts of exposed soils can cause further losses of nitrogen (N), phosphorus (P), potassium (K), and other nutrients through erosion and/or run-off, leading to nutrient limitation and delayed succession [6,9,12,13]. Reduced nutrient availability can impede the establishment of native species [9,14].

Numerous species of non-native C<sub>4</sub> grasses were introduced to the American tropics as pasture grasses and many became weeds [1,3,5,15,16]. Invasive C<sub>4</sub> grasses can inhibit forest

succession through accelerated fire regimes [3–5,17–19]. Additionally, in comparison to most native C<sub>3</sub> woody plants, C<sub>4</sub> grasses have high photosynthetic nitrogen use efficiency, allowing them to maintain high photosynthetic rates under the hot, sunny, low-fertility conditions of abandoned agricultural lands. High nitrogen use efficiency gives these grasses an advantage in competition for nutrients and other resources, and it complicates the maintenance or establishment of preferred vegetation [20,21]. Soil degradation via intensive agriculture and grazing has the potential to favor colonization by weedy non-native C<sub>4</sub> grasses [2,3,15,22].

Understanding how soil fertility influences the growth of invasive plants is critical to develop management strategies to restore invaded areas to agricultural use or forest cover. Because fertilizers that are commonly applied as part of reforestation activities to help reestablish trees could preferentially benefit undesired plants, it is important to understand how invasive species will respond to nutrient addition. The same is true for other tropical land use practices that involve grass control, including agriculture.

*Saccharum spontaneum* L. subsp. *spontaneum* is a large, exotic C<sub>4</sub> grass species, native to Asia and Northern Africa, that invades abandoned agricultural fields in many tropical countries. It is adapted to drought, burns frequently, and is difficult to eradicate, which is why it is considered one of the most serious weeds where it occurs [18,23]. *S. spontaneum* was most likely introduced to the Republic of Panamá in 1939 with a United States Department of Agriculture (USDA) germplasm collection for sugarcane [24] and by the 1960s, had spread widely through disturbed areas [25]. Control of *S. spontaneum* is actively pursued throughout the country and is especially important in the watershed of the Panama Canal, where *S. spontaneum* is widespread and persistent [24,26]. Fires promoted by abundant *S. spontaneum* reduce the germination and species richness of native trees and encourage additional growth and reproduction of *S. spontaneum* [17,18,27]. In Panama, *S. spontaneum* inhibits agriculture, forest restoration, and succession, generating huge costs associated with weed management [23]. The use of fertilizers to support agricultural production or forest restoration may facilitate *S. spontaneum* growth and persistence, thereby reducing seedling establishment and increasing management costs.

The objective of this experiment was to test whether *S. spontaneum* in this system is nutrient-limited, and specifically evaluate the relative importance of nitrogen and phosphorus limitation. We expected that, as for many other tropical systems, *S. spontaneum* growth would be more P-limited than N-limited.

## 2. Materials and Methods

### 2.1. Site Description

This project was established in Parque Nacional Soberanía in the Panama Canal Watershed, Republic of Panamá (N 9°6'5.5434"; W 79°36'42.8934"), in abandoned agricultural lands now dominated by *S. spontaneum*. This site experiences a mean rainfall of 2226 mm and about 4 dry months annually from December to early April (defined as months with <100 mm rainfall), with the wettest part of the year occurring in October and November [28]. Soils at this site are characterized as ultisols, predominantly clay and silty clay loams [29]. Moist tropical forest was cleared from this site during the 1960s, and the area was then used for grazing and small-scale agriculture, until it was abandoned around 1993.

### 2.2. Experimental Design

A full factorial block design was used to test nutrient limitation of growth of *S. spontaneum*. In June of 2011, a homogeneous area of *S. spontaneum* was cleared of above-ground biomass by machete and gas-powered brush cutter from all blocks before applying fertilizers. This experiment consisted of twelve blocks, each with four nutrient treatments: no nutrient addition (control; C), nitrogen addition (N), phosphorus addition (P), and nitrogen + phosphorus combination (N + P). Plots were 5 × 5 m<sup>2</sup>, with a 2 m buffer between plots within a block and a 3 m buffer between blocks. Buffer zones were maintained free of

*S. spontaneum* to inhibit rhizomatous spread between the plots by *S. spontaneum* and reduce the probability of horizontal movement of nutrients between plots.

Dry fertilizer was added by hand. Nitrogen was added as urea ((NH<sub>2</sub>)<sub>2</sub>CO<sub>2</sub>), and phosphorus was added as triple super phosphate (Ca(H<sub>2</sub>PO<sub>4</sub>)<sub>2</sub>•H<sub>2</sub>O). Nutrient application corresponded to 125 kg ha<sup>-1</sup> N and 50 kg ha<sup>-1</sup> P, as recommended for this region [30]. Based on these values, we calculated a nutrient application of 135.63 g triple super phosphate and 339.06 g urea per plot for each application. Nutrients were applied in July after clearing the site and again in October after the first biomass harvest. Soil cores were taken from each plot at the end of the study (December) to compare final soil nutrient availability across treatments.

### 2.3. Nutrient Concentration Analysis

We compared soil and leaf nutrient concentration data across treatments. In December, we randomly collected and homogenized 10 soil cores at 10 cm depth for each plot; a 20 g subsample was used to extract soil nitrogen and another for phosphorus. We followed the potassium chloride (KCl) [31] and Mehlich [32] standardized protocols to extract available nitrate (NO<sub>3</sub><sup>-</sup>), ammonium (NH<sub>4</sub><sup>+</sup>), and phosphate (PO<sub>4</sub><sup>-2</sup>) [33]. Soil samples were placed directly into solution in the field and processed in the lab within 24 h of being collected. For plant tissue nutrient analysis, we collected the third mature leaf from the base of 15 randomly selected individuals in each plot. Leaves were dried for 3 days at 60 °C, and samples were processed at the University of California Santa Cruz. Five leaves were selected from each plot, and leaf N and P were extracted following the Kjeldahl acid digestion protocol [34] using a Lachat BD 46 block digester (Lachat Instruments, Milwaukee, WI, USA).

We assessed *S. spontaneum* performance, in terms of density and above-ground biomass, in September and December 2011. Measurements of both biomass and density are useful because they each affect the establishment and persistence of native species in this system; biomass is an indicator of the overall dominance of *S. spontaneum* in the field; beyond biomass, the density of individual stems influences physical availability of space for other plants to grow. The ability of *S. spontaneum* to spread through underground rhizomes makes it very difficult to differentiate between genetic individuals of *S. spontaneum* in the field. For this reason, we estimated density as the number of stalks; a tiller visibly emerging from a stalk was considered a part of that stalk. In September, we measured density by counting the number of stalks of *S. spontaneum* in 3 randomly placed 1 m<sup>2</sup> quadrats in each plot. *S. spontaneum* height varied across treatments but reached as high as 2 m (personal observation). We randomly collected 10 stalks of *S. spontaneum* from half of the 1 m<sup>2</sup> area used for quantifying *S. spontaneum* density to calculate above-ground biomass. *S. spontaneum* biomass was dried at 60 °C for 3 days and then weighed. We estimated *S. spontaneum* biomass as the product of the number of stalks and mean biomass per stalk. At the end of September, we cleared the plots of *S. spontaneum* to simulate common management practices in areas under restoration. In October, we re-applied nutrients and allowed *S. spontaneum* to grow back until the onset of the dry season in December. Individuals of *S. spontaneum* appeared much smaller during the December harvest, with few individuals reaching heights greater than 2 m and many individuals less than 1 m, suggesting a reduction in above-ground growth after clearing. Data collection in December followed the methods used in September.

### 2.4. Data Analysis

We used two-way factorial ANOVA to compare the response of soil nutrients, leaf nutrients, and *S. spontaneum* growth to application of nitrogen, phosphorus, or their combination. Blocks were included in the model as a random effect. Soil nutrient response variables included nitrate, ammonium, total N (nitrate + ammonium), phosphate, and N:P ratio (total inorganic nitrogen/phosphate). Plant nutrient variables were leaf N and P concentrations (mg/g), as well as leaf N:P ratios. Plant growth response variables included

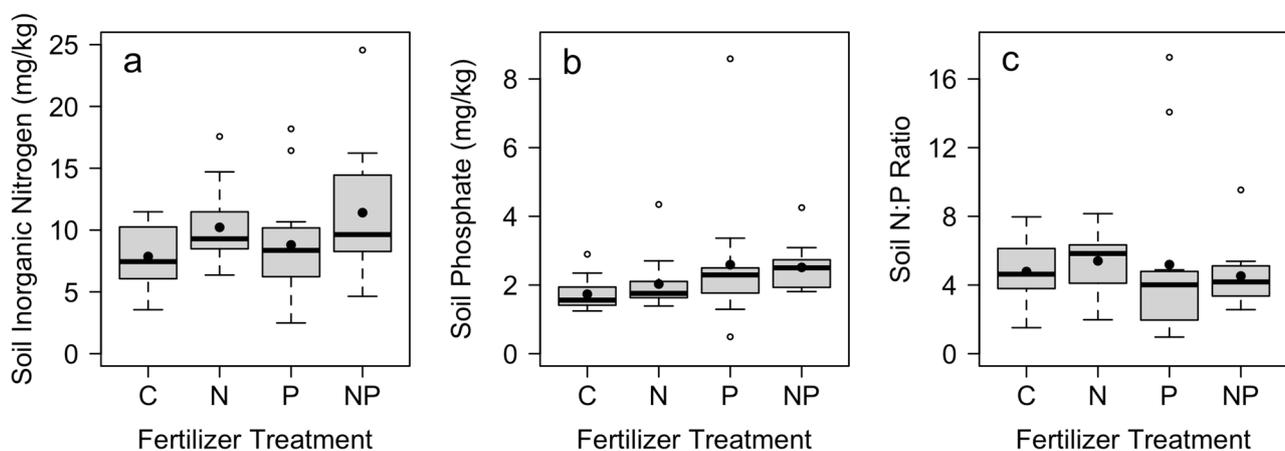
*S. spontaneum* density and biomass measured in both September (July–September growth) and December (October–December growth).

### 3. Results

Soil nutrient concentrations responded significantly to nutrient additions. Although there was no difference in soil nitrate across treatments, soil ammonium and total N were significantly higher in plots where N was added (Table 1). Nutrient concentrations varied across blocks for all nitrogen measures (Table 1). Soil phosphate was greatest in plots where P was added (Table 1; Figure 1). There was no difference in soil N:P ratios across nutrient treatments (Table 1; Figure 1).

**Table 1.** Two-way factorial analysis of final soil nutrient availability across treatments, showing the degrees of freedom (df), F ratios (F), and probability (p). Nutrients were added at the beginning of the study in July and again after the first harvest in October, and nutrient availability was measured in December. Nutrient additions are treated as fixed effects with blocks as random effects. Bold values represent significant treatment effects.

Treatment	df	Ammonium (mg/kg)		Nitrate (mg/kg)		Total Inorganic N (mg/kg)		Phosphate (mg/kg)		N:P	
		F	p	F	p	F	p	F	p	F	p
N addition	1.33	9.20	<b>0.005</b>	0.10	0.10	9.73	<b>0.004</b>	0.11	0.75	0.001	0.98
P addition	1.33	1.77	0.19	0.59	0.59	1.78	0.19	4.14	<b>0.05</b>	0.08	0.78
N × P interaction	1.33	0.16	0.70	1.49	0.23	0.03	0.87	0.32	0.35	0.57	0.46
Block	11.33	5.91	<b>&lt;0.001</b>	2.51	<b>0.02</b>	5.95	<b>&lt;0.001</b>	1.17	0.58	1.12	0.38

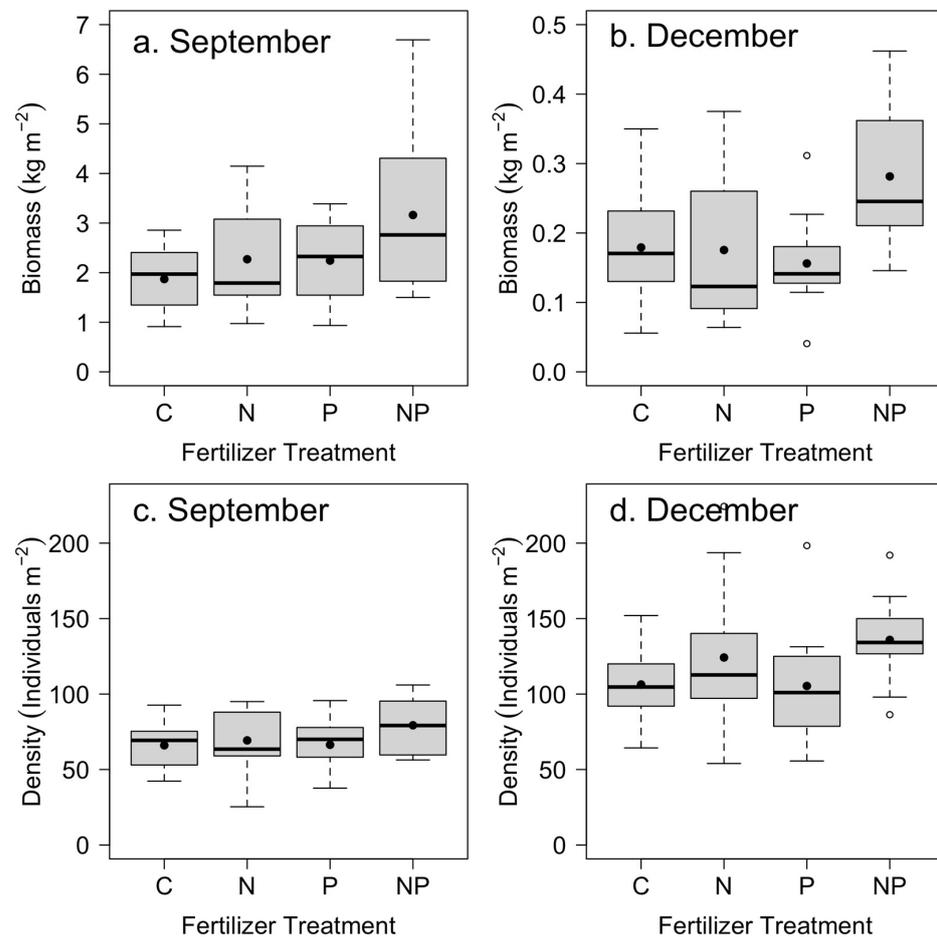


**Figure 1.** Mean total soil (a) inorganic nitrogen and (b) phosphate nutrient concentrations, and (c) soil N:P across control (C), nitrogen (N), phosphorus (P), and nitrogen and phosphorus (NP) fertilizer treatments ( $n = 12$ ). Mean soil nutrient concentrations were measured in December at the end of the study, with fertilizer applications in July and October. Box plots indicate median (thick line), 1st and 3rd quartiles (grey box), and range whiskers, with extreme values as open circles. Filled circles indicate the mean. See Table 1 for associated statistics.

The above-ground biomass of *Saccharum spontaneum* showed rapid growth from July to September (Figure 2), averaging approximately 2–3 kg dry biomass per  $m^2$  across the four treatments. The growth from October through to December was nearly an order of magnitude slower than the growth from July to September. However, stem density was higher in the later period (Figure 2).

We found a significant positive effect of nitrogen and phosphorus addition on mean *S. spontaneum* biomass from July to September (Table 2; Figure 2a). From October to December, we found a significant positive effect of nitrogen addition and a significant

positive interaction between nitrogen and phosphorus on *S. spontaneum* above-ground biomass (Table 2; Figure 2b). Stem density in September increased by an average of 14% with the addition of N ( $p = 0.10$ ), whereas in December, it increased by 25% ( $p = 0.02$ ) (Table 2; Figure 2c,d). We found no significant effect of P on density in September or December (Table 2; Figure 2c,d).



**Figure 2.** Mean *Saccharum spontaneum* above-ground biomass in (a) September and (b) December (note different scales) across control (C), nitrogen (N), phosphorus (P), and nitrogen and phosphorus (NP) nutrient treatments ( $n = 12$ ). Mean stem density in (c) September and (d) December. Box plots indicate median (thick line), 1st and 3rd quartiles (grey box), and range whiskers, with extreme values as open circles. Filled circles indicate the mean. See Table 2 for associated statistics.

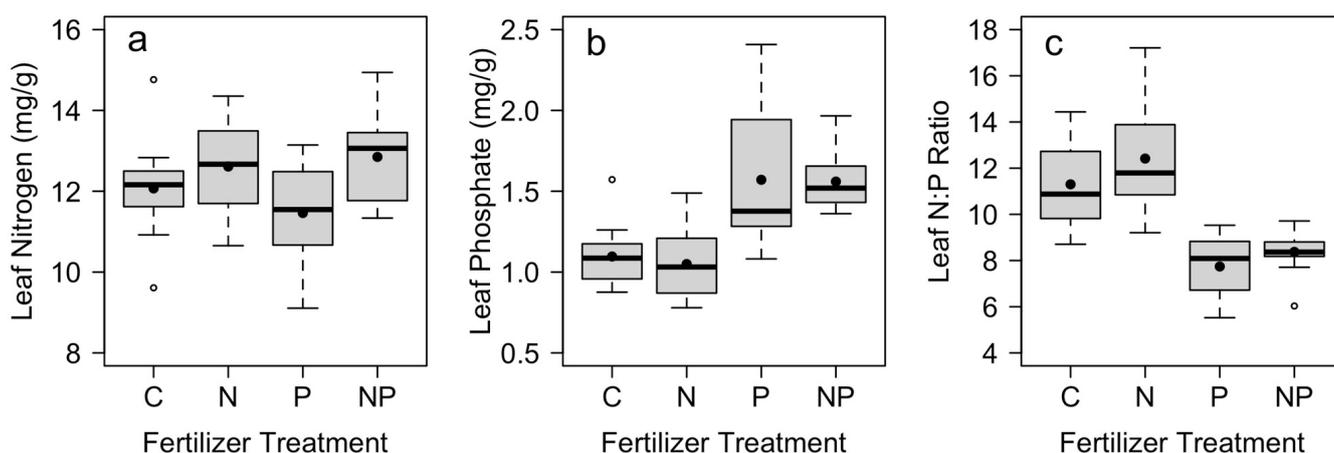
**Table 2.** Two-way factorial analysis of *S. spontaneum* density and biomass in response to nutrient additions, after growth from July to September (“September”) and from October to December (“December”). Nutrient additions are treated as fixed effects with blocks as random effects. Columns show the degrees of freedom (df), F ratios (F), and probability (p) for each treatment. Bold values represent significant treatment effects.

Treatment	df	Density				Biomass			
		September		December		September		December	
		F	p	F	p	F	p	F	p
N addition	1.33	2.87	0.10	5.84	<b>0.02</b>	6.78	<b>0.01</b>	6.88	<b>0.01</b>
P addition	1.33	1.17	0.29	0.29	0.59	6.23	<b>0.02</b>	3.20	0.08
N × P interaction	1.33	1.02	0.17	0.39	0.31	0.81	0.31	7.74	<b>0.008</b>
Block	11.33	1.53	0.32	1.22	0.53	3.36	<b>0.003</b>	2.20	<b>0.04</b>

*S. spontaneum* responded to nutrient additions by increasing internal nutrient concentrations. In plots where N was added, we found significantly higher leaf N and no significant effect on leaf P (Table 3; Figure 3). In plots where P was added, we found significantly higher leaf P and no significant increase in leaf N (Table 3; Figure 3). Both leaf N and leaf P also varied by block (Table 3). In addition, there was a significant effect of both N and P fertilization on the leaf N:P ratio, where the leaf N:P was significantly lower in plots where P was added (Table 3; Figure 3). There was no significant interaction between N and P treatments for leaf nutrient concentrations ( $p > 0.15$ , Table 3).

**Table 3.** Two-way factorial analysis of *S. spontaneum* leaf nutrient concentrations across treatments, showing the degrees of freedom (df), F ratios (F), and probability (p). Leaves were collected during the December *S. spontaneum* harvest, following July and October fertilization treatments. Nutrient additions are treated as fixed effects with blocks as random effects. Bold values represent significant treatment effects.

Treatment	df	Leaf N (mg/g)		Leaf P (mg/g)		Leaf N:P	
		F	p	F	p	F	p
N addition	1.33	10.82	<b>0.002</b>	0.22	0.64	6.71	<b>0.01</b>
P addition	1.33	0.40	0.53	65.20	<b>&lt;0.001</b>	126.63	<b>&lt;0.001</b>
N × P interaction	1.33	2.10	0.16	0.08	0.77	0.51	0.48
Block	11.33	2.19	<b>0.04</b>	3.94	<b>0.001</b>	5.14	<b>&lt;0.001</b>



**Figure 3.** Mean leaf concentrations of (a) nitrogen and (b) phosphorus, together with (c) leaf N:P ratio across control (C), nitrogen (N), phosphorus (P), and nitrogen and phosphorus (NP) fertilizer treatments ( $n = 12$ ). Mean leaf nutrient concentrations were measured in December at the end of the study, with fertilizer applications in July and October. Box plots indicate median (thick line), 1st and 3rd quartiles (gray box) and range whiskers, with extreme values as open circles. Filled circles indicate the mean. See Table 2 for associated statistics.

#### 4. Discussion

Biomass growth of *S. spontaneum* showed a positive response to both N and P addition, suggesting that *S. spontaneum* is both nitrogen and phosphorus limited in this system. This positive effect is likely linked to increased photosynthetic capacity under increased nutrient availability. The net CO<sub>2</sub> assimilation rate increases nonlinearly with increasing leaf N for eight species of *Saccharum* [35], likely reflecting an increase in the concentration of Rubisco. Similarly, leaf P and growth in C<sub>4</sub> grasses increased with increasing soil P supply in other studies [36,37]. We also found evidence for N and P co-limitation of biomass in *S. spontaneum*, in particular in the later growing period.

Final soil N and P concentrations were significantly higher in plots where N and P were added. This indicates that treatments were effective in increasing nutrient availability

throughout the study. Leaf N and P were greater in plots where we added N and P, respectively, indicating that *S. spontaneum* had increased nutrient uptake in fertilized plots.

In our system, nitrogen addition produced a slight increase in stem density. Tillering of both C<sub>3</sub> and C<sub>4</sub> grasses in temperate systems increased in response to increased nitrogen [38–40]. In addition, frequent cutting can increase tillering in *Hyparrhenia rufa*, another tropical invasive C<sub>4</sub> grass [41]. Our results suggest that *S. spontaneum* responds similarly to other grasses by increasing tillering in response to both nitrogen addition and cutting.

Growth in above-ground biomass was an order of magnitude lower in December than September. After the first growing period, the energy reserves in the rhizomes may have been severely depleted, meaning there was little stored carbohydrate left to sustain growth during the second growth period. Alternatively, this growth pattern may suggest that resource allocation varies temporally in *S. spontaneum*. In our study, the period from July to September represents the middle of the wet season, with peak flowering in August–September. The October–December periods include the wettest and cloudiest parts of the rainy season, along with the transition into the dry season. Investment in above-ground growth in the early rainy season may allow the plant to shade competitors and attain heights that optimize pollination and seed dispersal, with a shift in allocation to below-ground growth before the onset of the dry season. Other studies of tropical C<sub>4</sub> grasses have also found a high growth rate at the beginning of the rainy season (e.g., [42]).

In conjunction with resource reallocation, multiple rounds of removal of above-ground biomass may have reduced the growth ability of *S. spontaneum*. Repeated clearing can reduce below-ground carbon stores [43]. For a number of temperate grass species, increased annual above-ground biomass clearing reduced carbohydrate reserves [43]. Our results are consistent with studies that show that repeated clearing of *S. spontaneum* can reduce *S. spontaneum* growth [44]. Future studies should evaluate whether growth after clearing varies seasonally.

## 5. Conclusions

In summary, we found that nutrient addition increased the growth of *Saccharum spontaneum*, but these effects varied seasonally and differed between biomass and density responses. Given the substantial impacts of *S. spontaneum* within the Panama Canal Watershed [24] and in many other parts of the world where it is a weedy invader [45], there is a great need to understand the management activities that help suppress, or unintentionally promote, its growth. This study highlights a potential risk of fertilizer applications during restoration, agriculture, agroforestry, or other activities in which *S. spontaneum* invasion is a challenge to land management.

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