Nitrogen Fertilization and Glyphosate as a Growth Regulator: Effects on the Nutritional Efficiency and Nutrient Balance in Emerald Grass

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Abstract: Nitrogen (N) is one of the promising nutrients for lawn growth and is required for the lawn’s proper growth and development, but it also increases mowing frequency. Glyphosate herbicide application in sub-doses, as a growth regulator, can reduce the maintenance costs without any adverse reduction in the density and nutritional status of grasses. The objective of this study was to evaluate the influences of nitrogen and glyphosate doses on the growth, aesthetic quality and nutritional status of emerald grass (Zoysia japonica Steud.). The experiment was conducted at the Research and Extension Education Farm of São Paulo State University (UNESP), Ilha Solteira, SP, Brazil, in an Ultisol. The experiment was designed as a randomized block with 12 treatments arranged in a 3 × 4 factorial scheme with 4 replications, comprised of a control (without N), 15 and 30 g N m⁻² of urea, applied in five splits annually, and glyphosate doses (0, 200, 400 and 600 g ha⁻¹) of the active ingredient, a.i.). The split N fertilization at the rate of 15 g m⁻² and glyphosate at the dose of 400 g ha⁻¹ maintained nutritional status of emerald grass. Nitrogen at the rate 15 g N m⁻² (in five splits per year) was observed to produce lower growth traits, an adequate aesthetic quality and longer stability of the nutrients in emerald grasses through lower exportation, with removal of “clipping” after mowing. In addition, glyphosate, at the dose of 400 g a.i. ha⁻¹, was efficient in reducing the leaf area, plant height, shoot dry matter and total dry matter by 18.3, 14.7, 6.8 and 8.1%, respectively, as compared to the control. However, this dose did not impair the coloration and resulted in a lower exportation of nutrients by reducing the need to replenish by fertilization. Therefore, fertilization with 15 g N m⁻², associated with application of 400 g a.i. ha⁻¹ of glyphosate, is recommended for emerald grass in the tropical savannah of Brazil.

Keywords: Zoysia japonica Steud.; lawn; herbicide; nitrogen; plant nutrition

1. Introduction

Emerald Grass (Zoysia japonica Steud.) is one the most commercialized and predominant garden grasses in Brazil [1]. It has an emerald green color and is resistant to trampling and can adapt to high temperatures [2]. The Ministry of Agriculture, Livestock and Supply (MAPA) in Brazil commercialized the production of emerald grasses by legalizing producers [1]. In addition, lawns are highly important for the environment, as they can cover soil (restraining erosion), remove and contain air impurities, provide continuous oxygen and fresh air and ensure thermal comfort in relation to asphalt [3]. The proper growth, development, nutrition and aesthetic quality of these tuff grasses are directly related to adequate fertilization, especially nitrogen fertilization [4,5].

Nitrogen is one of the most important nutrients for the proper growth and development of grasses [6]. It engages in critical interactions with other soil nutrients, improving
the nutrient use efficiency, root architecture, chlorophyll color chart and nutrient homeostasis [7,8]. Low N doses negatively affect the grass sod by limiting the shoot growth and causing a loss of the intense green color (generalized yellowing) [9,10]. In addition, excessive doses of N strengthen the shoot growth and affect the carpet handling after cutting, the “liftability” [9,11], increasing the nitrate leaching to deeper soil layers and number of cuts of the aerial part and promoting nutrient export and dry matter production [12,13]. The higher N doses can enhance the green color of grasses; however, it increases the cost of maintenance [2,4], not only through mowing but also through a higher demand for N fertilization. Therefore, alternative management strategies, such as plant growth regulator utilization, are adapted to maintain the cost of production and quality of the lawn [5].

The use of herbicides as growth regulators is an alternative strategy for the management of turf grasses, employed due to operational difficulties and high maintenance costs [14]. The high availability of active ingredients in glyphosate contribute to the inhibition of the pathway of the 5-enolpyruvylshikimate-3-phosphate synthase enzyme, promoting growth and development [15]. However, its hormetic application to lawns is still restricted due to limited safety measures, while higher doses can damage and even kill the grass [16]. Grasses respond differently to the toxicity of glyphosate, with a wide range of tolerance mechanisms [17].

Glyphosate, as a growth regulator, influences several biochemical and physiological features of grasses, such as the photosynthetic efficiency by inhibiting chlorophyll pigment and carotene production [18], as well as expedite auxin oxidation [19], decreasing the stem elongation and dry matter of *Paspalum notatum* and *Eremochloa ophiuroides* grasses [20,21]. Glyphosate application, at a dose of 200 g a.i. ha$^{-1}$, inhibits the growth of emerald grass without damaging its aesthetic quality (green coloration) [2]. However, this herbicide may alter the availability, absorption and assimilation of several macronutrients and micronutrients in different crops [22].

Glyphosate alters the plant microbial interactions [23] and N metabolism [24]. There are a lack of official or safe dose recommendations regarding herbicides as growth regulators used for managing turf grasses in Brazil due to low commercial interest in the pesticide/herbicide industries [25]. The idea was to control the growth without harming the nutritional status and quality of emerald grass. There exists a research gap in regard to the recommendation of glyphosate doses for the better performance of emerald grasses. Therefore, the purpose of the current study was to determine whether N application and hormetic doses of glyphosate would stimulate growth and nutrient efficiency in emerald grasses. In this context, our aim was to evaluate the influences of N fertilization and hormetic doses of glyphosate on the growth and aesthetic quality of, and nutrient balance in, emerald grass.

2. Materials and Methods

2.1. Location and Management History

The experiment was conducted from October 2015 to July 2016 at the Research and Extension Farm of the Faculty of Engineering, Sao Paulo State University (UNESP), Campus Ilha Solteira (20°22′23.5″ S a 51°22′12.6″ W; 330 m above sea level), in Ultisol soil [26]. The climatic data are summarized in Figure 1.

The grass was planted using sod carpets (0.63 × 0.40 m) on 3 August 2012 and irrigated by a sprinkling irrigation system according to the methodology of the Penman–Monteith method [27]. The grass species studied was *Zoysia japonica* Steud. It is one of the best-selling commercial grass species in Brazil, planted mostly in residential and playgrounds. It is a perennial grass with small- to medium-sized leaves, depending on the variety. It usually grows as a stoloniferous rhizome in abundant sunlight and has an emerald green coloration. Emerald grass is highly resistant to treading, and its ideal mowing height is 1.25–3.0 cm [4].
A soil chemical analysis were performed according to the methodology of Raij et al. [28]. The soil was collected at a depth ranging from 0.00 to 0.20 m and analyzed for the following characteristics: pH (CaCl₂) = 6.5; organic matter (OM) = 14.8 g dm⁻³; P (resin) = 32 mg dm⁻³; S-SO₄²⁻ = 1.7 mg dm⁻³; K⁺, Ca²⁺, Mg²⁺ and H + Al contents = 1.4; 31.4; 19.7; and 10.0 mmolc dm⁻³, respectively, with a base saturation (V) of 84%. The soil was observed to have low sulfur (S) contents (0–4 mg dm⁻³) [29]; thus, gypsum (500 kg ha⁻¹ with 18% Ca and 15% S) was applied manually on the surface of the lawn on 16 October 2015 (Figure S1).

2.2. Experimental Design and Treatment Application

The treatments used in the present experiment were applied in October 2015. The experiment was designed as a randomized block with 12 treatments arranged in a 3 × 4 factorial scheme and 4 replications. Each plot had a total area of 10 m². The treatments consisted of three nitrogen doses (control, without N; 15 and 30 g m⁻² of N, applied with urea (45% N); and four doses of glyphosate (0, 200, 400 and 600 g ha⁻¹ of active ingredient— a.i.). Nitrogen was applied in five split applications annually, being manually applied to the soil on 16 October and 7 December 2015 and on 29 January, 18 February and 23 March 2016, respectively. Each time, the applied N dose for 15 g m⁻² was 3 g m⁻², and for 30 g m⁻² it was 6 g m⁻² of N. Glyphosate was applied over 15 days after N fertilization during the spring/summer months and 30 days during the autumn/winter months, specifically on 30 October and 21 December 2015 and 12 February, 18 April and 23 May 2016. The different intervals between glyphosate applications were employed due to the Brazilian climatic conditions, whereby grasses do not grow intensively in the autumn/winter as compared to spring/summer [5,30].

Glyphosate was sprayed in the morning in mild temperature conditions using a CO₂-pressurized backpack sprayer equipped with a 2 L-capacity tank (disposable bottles of polyethylene terephthalate, PET) and a 0.50 m-spacing and 4-point anti-drip spigot bar, model 80.02, with the consumption of liquid equivalent to 200 L ha⁻¹ and an operating pressure of 3 psi. The plots were laterally protected with plastic tarpaulin during glyphosate application to avoid spray drift to the adjacent plots.

2.3. Evaluations

The evaluations were performed 30 days after the glyphosate application. After the collection of the plant material, the entire lawn was mowed with a gasoline-powered brush cutter, using a crop collector, at a height of approximately 3 cm from the ground, and the mowing was level so as to standardize the size of the emerald grass in the treatments. The

Figure 1. Monthly averages of pluvial precipitation and minimum, maximum and average temperatures, as well as evaluation timings during the experimental period.
evaluations were made on 30 November 2015 (1st) and 21 January (2nd), 12 March (3rd), 18 May (4th) and 23 July 2016 (5th).

Each of the five evaluations determined the following parameters: First, (a) the plant height was measured with a prism (portable device made of steel and glass (mirror) that reflects light at 90 degrees and contains a scale graded in cm), which was placed on the lawn surface at three points to obtain an average value. Then, (b) the leaf area was determined by collecting 20 leaves per plot, placed in properly identified plastic bags and stored in a polystyrene box containing ice to prevent the leaves from coiling and curling. The leaves were scanned using an HP Deskjet F4480 scanner with a minimum resolution of 75 ppi, and the images were saved in Jpeg format. Then, the leaf area was measured with the ImageJ 1.45 software (National Institutes of Health, Bethesda, Maryland, USA) [31]. Next, (c) the leaf chlorophyll index (LCI) was determined by collecting and examining 15 leaves (middle of the leaf blade) per plot using a portable hand chlorophyll meter (Falker), model CFL 1030.

The last/fifth evaluation performed involved the following steps: (d) the leaf dry matter was determined by collecting the emerald grass plants, cut near the soil surface with manual scissors. The green leaves of the emerald plants were collected from an area of one m$^2$ using 1 m-long rural shears in three replicates per plot to create composite samples, which were packed into labeled paper bags and placed in the oven at 65 °C for 72 h. Subsequently, the samples were then weighed using an analytical balance and the values were presented in g m$^{-2}$. Next, (e) the macronutrient concentrations of the leaves were analyzed according to the methodology adapted from Malavolta et al. [32]. (f) The soil chemical attributes (phosphorus (P), potassium (K$^+$), calcium (Ca$^{2+}$), magnesium (Mg$^{2+}$) and sulphate (S-SO$_4^{2-}$)) were determined according to the methodology of Raij et al. [28]. Six random soil samples were collected per plot at a depth of 0.00–0.20 m using a screw auger and determined for the abovementioned chemical characterization. (g) The root and rhizome dry matter were determined by collecting three sample per plot at depth of 0.00–0.20 m (the most root-dense zone of the grass) using a stainless steel tube of 50.0 cm in length and 8.0 cm in diameter, tapered at the end with a 6.8 cm diameter. Each sample was packed into a plastic bag and kept in a polystyrene box until it was taken to the laboratory, washed with deionized water to remove any soil adhered to the plant material and placed in the shade to dry. Later, the rootstocks and rhizomes were separated with manual scissors and placed in labeled paper bags, and then dried in an oven for 72 h at 65 °C. Next, the samples were weighed and the values were presented in g m$^{-2}$. (h) The macronutrient concentrations of the roots and rhizomes were evaluated according to a methodology adapted from Malavolta et al. [32]. (i) The coefficient of the biological utilization (CUB) of the nutrients was analyzed according to the methodology of Siddiqui and Glass [33]. (j) The nutrient balance of macronutrients was analyzed by considering the application of nutrients such as N (according to the treatments), Ca and S (application of gypsum) minus the total amount of macronutrients exported by the leaves (“clippings”) for all of the five cuttings.

2.4. Statistical Analysis

All data were initially tested using Levene’s homoscedasticity test ($p \leq 0.05$). Then, they were tested for normality using Shapiro–Wilk test, which showed the data to be normally distributed ($W \geq 0.90$). The data were analyzed using analysis of variance (F test) and Tukey’s test at 5% probability to compare the averages of the N applications, which were adjusted according to the polynomial regression for the glyphosate doses using the SAS system (SAS Institute Inc., Cary, NC, USA). Pearson correlations were calculated using the Excel program.

3. Results

3.1. Leaf Chlorophyll Index and Morphological Traits (Plant Height, Leaf Area and Dry Matter)

Nitrogen (N) fertilization, in general, has positive influences on the chlorophyll index and all the determined morphological traits of emerald grasses (Table 1). The highest leaf chlorophyll index (LCI) was observed with a fertilization of 3 g N m$^{-2}$, which was
statistically in accordance with application of 6 g N m\(^{-2}\). The interactions between N and the glyphosate doses were not significant for the leaf chlorophyll index and morphological traits of emerald grasses. Nitrogen application at the rate of 6 g N m\(^{-2}\) increased the plant height, leaf area, shoot and root and rhizome dry matter, as well as the total dry matter of emerald grass, in comparison to the control.

**Table 1.** Leaf chlorophyll index (LCI), plant height (H), leaf area (LA), shoot dry matter (SDM), dry matter of roots and rhizomes (DMR) and total dry matter (T) of emerald grass as a function of nitrogen fertilization.

<table>
<thead>
<tr>
<th>N Rates (g m(^{-2}))</th>
<th>LCI</th>
<th>H</th>
<th>LA</th>
<th>SDM</th>
<th>DMR</th>
<th>T</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>cm</td>
<td>mm(^2)</td>
<td>g m(^{-2})</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>17.1 b</td>
<td>3.7 b</td>
<td>163.5 b</td>
<td>88.5 c</td>
<td>158.9 b</td>
<td>247.3 b</td>
</tr>
<tr>
<td>15</td>
<td>21.0 a</td>
<td>4.7 a</td>
<td>210.4 a</td>
<td>102.1 b</td>
<td>206.4 a</td>
<td>308.5 a</td>
</tr>
<tr>
<td>30</td>
<td>20.6 a</td>
<td>4.9 a</td>
<td>213.9 a</td>
<td>111.6 a</td>
<td>206.4 a</td>
<td>318.0 a</td>
</tr>
<tr>
<td>L.S.D. (5%)</td>
<td>1.0</td>
<td>0.2</td>
<td>14.1</td>
<td>7.8</td>
<td>10.2</td>
<td>14.6</td>
</tr>
<tr>
<td>C.V. (%)</td>
<td>4.92</td>
<td>5.45</td>
<td>7.00</td>
<td>7.56</td>
<td>5.21</td>
<td>4.87</td>
</tr>
<tr>
<td>F-value (\text{nitrogen} \times \text{glyphosate})</td>
<td>1.45 \text{ns}</td>
<td>0.61 \text{ns}</td>
<td>1.81 \text{ns}</td>
<td>1.78 \text{ns}</td>
<td>1.21 \text{ns}</td>
<td>1.07 \text{ns}</td>
</tr>
</tbody>
</table>

Means followed by the same letters in the column do not differ according to Tukey’s test at the 0.05 probability level. \text{ns}: not significant according to the test F.

The higher doses of glyphosate produced a trend of reduction in the chlorophyll index and all the determined morphological traits of emerald grasses (Figure 2). The LCI of the emerald grasses was reduced by 5.1% with a glyphosate application at the dose of 600 in comparison to grasses without glyphosate application (Figure 2D). The morphological traits, such as the plant height, leaf area, dry matter of the shoots, roots and rhizomes, and the total dry matter were reduced by 18.3, 14.7, 6.8, 8.8 and 8.1%, respectively, under higher doses of glyphosate as compared to grasses without herbicide application (Figure 2A–C). Glyphosate application at the dose of 600 g a.i ha\(^{-1}\) reduced the plant height and dry matter of emerald grass (Figure 2A,C), leading to leaf chlorosis, and reduced the green coloration and compromised the aesthetic appearance of the lawn.
A. Height (cm)

\[ Y = 4.91 - 0.0015^\ast X \ (R^2 = 0.94) \]

B. Leaf area (mm^2)

\[ Y = 216.9 - 0.0530^\ast X \ (R^2 = 0.85) \]

C. Dry matter (g m^-2)

\[ \text{SDM } Y = 106.21 - 0.0120^\ast X \ (R^2 = 0.78) \]
\[ \text{DMR } Y = 205.85 - 0.0302^\ast X \ (R^2 = 0.96) \]
\[ \text{T } Y = 312.06 - 0.0422^\ast X \ (R^2 = 0.93) \]

D. Root LCI

\[ Y = 20.1 - 0.0017^\ast X \ (R^2 = 0.60) \]

**Figure 2.** Height, leaf area, shoot dry matter (SDM), dry matter of roots and rhizomes (DMR) and total dry matter (T), along with the leaf chlorophyll index (LCI) of emerald grass as a function of doses of glyphosate (A–D, respectively). The letters correspond to a significant difference at the 5% probability level (\( p \leq 0.05 \)). ** and *: significant at \( p < 0.01 \) and \( 0.01 < p < 0.05 \), respectively. Error bars indicate the standard deviation of the mean \( (n = 4) \).

### 3.2. Macronutrients Concentration in the Leaves, Roots and Rhizomes

The higher doses of N fertilization increased the macronutrient concentrations of the leaves of emerald grasses (Table 2). The concentration of all the macronutrients (N, P, K, Ca, Mg and S) in the emerald grass leaves followed an increasing trend with N fertilization at the rate of 6 g N m^-2, which was statistically in accordance with the N rate of 3 g N m^-2. However, the leaf N concentration of the emerald grass was significantly increased by N application at the rate of 6 g N m^-2. The concentrations of Mg and S in the leaves of the emerald grass were increased by the application of 6 g N m^-2, which was statistically similar to that of 3 g N m^-2. The leaf Ca concentration of the emerald grasses was increased by N fertilization at the rate of 6 g N m^-2, as compared to the control (0 g N m^-2).
Table 2. The macronutrient concentrations of the leaves, roots and rhizomes of the emerald grass as a function of nitrogen fertilization in the 10th evaluation.

<table>
<thead>
<tr>
<th>N Rates (g m(^{-2}))</th>
<th>N</th>
<th>P</th>
<th>K</th>
<th>Ca</th>
<th>Mg</th>
<th>S</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>g kg(^{-1})</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Leaves</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>13.1 c</td>
<td>4.6 a</td>
<td>10.4 a</td>
<td>3.4 b</td>
<td>1.6 b</td>
<td>3.1 b</td>
</tr>
<tr>
<td>15</td>
<td>17.9 b</td>
<td>4.2 a</td>
<td>11.9 a</td>
<td>3.7 ab</td>
<td>1.8 a</td>
<td>3.7 a</td>
</tr>
<tr>
<td>30</td>
<td>20.4 a</td>
<td>4.6 a</td>
<td>12.1 a</td>
<td>3.8 a</td>
<td>2.0 a</td>
<td>3.8 a</td>
</tr>
<tr>
<td><strong>L.S.D. (5%)</strong></td>
<td>1.9</td>
<td>0.9</td>
<td>1.9</td>
<td>0.3</td>
<td>0.2</td>
<td>0.4</td>
</tr>
<tr>
<td><strong>C.V. (%)</strong></td>
<td>10.77</td>
<td>19.82</td>
<td>16.00</td>
<td>8.70</td>
<td>9.40</td>
<td>11.20</td>
</tr>
<tr>
<td><strong>Roots and rhizomes</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>5.1 c</td>
<td>1.0 a</td>
<td>2.8 a</td>
<td>2.9 a</td>
<td>0.6 a</td>
<td>2.6 a</td>
</tr>
<tr>
<td>15</td>
<td>7.5 b</td>
<td>1.0 a</td>
<td>2.8 a</td>
<td>2.8 a</td>
<td>0.6 a</td>
<td>2.4 a</td>
</tr>
<tr>
<td>30</td>
<td>9.8 a</td>
<td>1.0 a</td>
<td>2.7 a</td>
<td>2.6 a</td>
<td>0.5 a</td>
<td>2.4 a</td>
</tr>
<tr>
<td><strong>L.S.D. (5%)</strong></td>
<td>1.0</td>
<td>0.1</td>
<td>0.3</td>
<td>0.5</td>
<td>0.2</td>
<td>0.7</td>
</tr>
<tr>
<td><strong>C.V. (%)</strong></td>
<td>13.16</td>
<td>13.02</td>
<td>11.27</td>
<td>16.50</td>
<td>27.82</td>
<td>27.72</td>
</tr>
<tr>
<td><strong>F-value</strong></td>
<td>0.89 (^\text{ns})</td>
<td>1.05 (^\text{ns})</td>
<td>2.01 (^\text{ns})</td>
<td>1.84 (^\text{ns})</td>
<td>0.49 (^\text{ns})</td>
<td>1.76 (^\text{ns})</td>
</tr>
</tbody>
</table>

Means followed by the same letters in the column do not differ according to Tukey’s test at the 0.05 probability level. \(^\text{ns}\) not significant by the test F.

The nutrient concentrations of the roots and rhizomes of the emerald grass were higher in the case of the low N dose (3 g m\(^{-2}\)) and the control (Table 2). The concentration of N (9.8 g kg\(^{-1}\)) was only significantly increased with application of N at the rate of 6 g m\(^{-2}\).

The leaf concentrations of N, K, Ca, Mg and S of the emerald grass were linearly increased with increasing doses of glyphosate (Figure 3A,C). Glyphosate, at high doses (600 g ha\(^{-1}\) of a.i.), decreased the nutrient concentrations of the dry matter. However, the leaf nutrient concentrations were increased compared to the control (Figure 2C).

Interestingly, the application of glyphosate reduced the root growth of the emerald grasses (Figure 2C), whereas it did not influence the nutrient uptake, producing no reduction in the root and rhizome nutrient concentrations (Figure 3B,D).
Figure 3. N, P and K concentrations of the leaves (A), roots and rhizomes (B), and the Ca, Mg and S in the leaves (C), roots and rhizomes (D) of the emerald grass as a function of doses of glyphosate. The letters correspond to a significant difference at the 5% probability level ($p < 0.05$). #, ** and *: not significant, significant at $p < 0.01$ and $0.01 < p < 0.05$, respectively. Error bars indicate the standard deviation of the mean ($n = 4$).

3.3. Coefficient of the Biological Utilization of the Nutrients

The application of the N fertilizer resulted in a higher coefficient of the biological utilization (CBU) of P, K, Ca, Mg and S in the emerald grass. However, the high N dose (6 g m$^{-2}$) decreased the CBU of N in comparison to 0 and 3 g N m$^{-2}$ (Table 3). The impacts of N fertilization at the rates of 3 and 6 g N m$^{-2}$ were statistically similar in terms of the total dry matter production of the emerald grass (Table 1), indicating that higher N doses did not produce a greater total dry matter. The highest CBU of the P, K, Ca, Mg and S of the emerald grass is due to the greater production of shoots, roots and rhizomes, and the total dry matter as a result of N fertilization (Table 1).
Table 3. Coefficient of the biological utilization of macronutrients by emerald grass as a function of nitrogen fertilization.

<table>
<thead>
<tr>
<th>N Rates (g m⁻²)</th>
<th>N</th>
<th>P</th>
<th>K</th>
<th>Ca</th>
<th>Mg</th>
<th>S</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>31.7 a</td>
<td>107.0 b</td>
<td>44.9 b</td>
<td>80.5 b</td>
<td>259.1 b</td>
<td>90.4 b</td>
</tr>
<tr>
<td>15</td>
<td>28.4 a</td>
<td>154.8 a</td>
<td>54.3 a</td>
<td>101.7 a</td>
<td>316.8 a</td>
<td>113.0 a</td>
</tr>
<tr>
<td>30</td>
<td>23.8 b</td>
<td>142.9 a</td>
<td>54.0 a</td>
<td>107.2 a</td>
<td>314.7 a</td>
<td>112.4 a</td>
</tr>
<tr>
<td>L.S.D. (5%)</td>
<td>3.4</td>
<td>22.2</td>
<td>7.1</td>
<td>13.9</td>
<td>51.0</td>
<td>16.5</td>
</tr>
<tr>
<td>C.V. (%)</td>
<td>11.68</td>
<td>16.04</td>
<td>13.62</td>
<td>14.01</td>
<td>16.76</td>
<td>15.28</td>
</tr>
<tr>
<td>F-value (N × glyphosate)</td>
<td>1.73 ns</td>
<td>1.57 ns</td>
<td>1.34 ns</td>
<td>1.47 ns</td>
<td>0.86 ns</td>
<td>0.72 ns</td>
</tr>
</tbody>
</table>

Means followed by the same letters in the column do not differ according to Tukey’s test at the 0.05 probability level. ns: not significant according to the F test.

Glyphosate reduced the CBU of N, K, Ca and Mg (Figure 4A,B). The highest dose of glyphosate (600 g a.i. ha⁻¹) was observed with the lowest CBU of the emerald grass. The lowest CBU was due to the lower production of total dry matter (Figure 2C) and higher leaf nutrient concentration of the grass (Figure 3A,C) with glyphosate application at the rate of 600 g ha⁻¹. In this sense, there was a lower dry matter production coupled with a higher concentration of nutrients, which indicated their low utilization by the lawn.

**Figure 4.** Coefficient of the biological utilization (CBU) of N, P and K (A), Ca, Mg and S (B) of emerald grass as a function of doses of glyphosate. The letters correspond to a significant difference at the 5% probability level (p < 0.05). ns and **: not significant and significant at p < 0.01, respectively. Error bars indicate the standard deviation of the mean (n = 4).

3.4. Nutrient Balance of the Macronutrients

The nutritional balance of the macronutrients of the emerald grass was assessed to verify the quantities of those nutrients that remained in the area after the removal of “clippings” by mowing. The data obtained from the five evaluations were considered for the five applications of N at doses of 15 and 30 g N m⁻², corresponding to 3 and 6 g N m⁻² for each application, respectively (Table 4).
Table 4. The macronutrient balance obtained with the removal of leaves (“clippings”) and the application of N, Ca and S to the emerald grass, according to its treatments with nitrogen fertilization and doses of glyphosate.

<table>
<thead>
<tr>
<th>Glyphosate doses (g ha(^{-1}))</th>
<th>N Rates (#) g m(^{-2})</th>
<th>N</th>
<th>P</th>
<th>K g m(^{-2})</th>
<th>Ca</th>
<th>Mg</th>
<th>S</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0</td>
<td>−7.4</td>
<td>−3.0 a</td>
<td>−5.2</td>
<td>7.8 a</td>
<td>−0.8 a</td>
<td>6.2 a</td>
</tr>
<tr>
<td>15</td>
<td>15</td>
<td>4.0</td>
<td>−3.6 b</td>
<td>−6.7</td>
<td>7.5 b</td>
<td>−1.0 b</td>
<td>5.9 b</td>
</tr>
<tr>
<td>30</td>
<td>30</td>
<td>15.7</td>
<td>−4.2 c</td>
<td>−7.8</td>
<td>7.3 c</td>
<td>−1.2 c</td>
<td>5.6 c</td>
</tr>
<tr>
<td>L.S.D. (5%)</td>
<td>1.1</td>
<td>0.5</td>
<td>0.5</td>
<td>0.1</td>
<td>0.1</td>
<td>ns</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Glyphosate doses (g ha(^{-1}))</th>
<th>N Rates (#) g m(^{-2})</th>
<th>N</th>
<th>P</th>
<th>K g m(^{-2})</th>
<th>Ca</th>
<th>Mg</th>
<th>S</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0</td>
<td>2.6</td>
<td>−4.1 (1)</td>
<td>−7.4</td>
<td>7.5 ns</td>
<td>−1.0 ns</td>
<td>5.7 (2)</td>
</tr>
<tr>
<td>200</td>
<td>200</td>
<td>4.3</td>
<td>−3.7</td>
<td>−6.6</td>
<td>7.5</td>
<td>−1.0</td>
<td>5.9</td>
</tr>
<tr>
<td>400</td>
<td>400</td>
<td>4.0</td>
<td>−3.5</td>
<td>−6.7</td>
<td>7.5</td>
<td>−1.0</td>
<td>5.9</td>
</tr>
<tr>
<td>600</td>
<td>600</td>
<td>5.5</td>
<td>−3.1</td>
<td>−5.8</td>
<td>7.6</td>
<td>−0.9</td>
<td>6.0</td>
</tr>
<tr>
<td>C.V. (%)</td>
<td>26.21</td>
<td>12.91</td>
<td>7.87</td>
<td>2.01</td>
<td>9.87</td>
<td>2.48</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>F-value (\text{N x glyphosate})</th>
<th>Equations</th>
<th>(R^2)</th>
</tr>
</thead>
<tbody>
<tr>
<td>3.41 *</td>
<td>(\hat{Y} = -4.08 + 0.0016 \times X)</td>
<td>0.98</td>
</tr>
<tr>
<td>0.39 ns</td>
<td>(\hat{Y} = 5.74 + 0.0005 \times X)</td>
<td>0.85</td>
</tr>
</tbody>
</table>

Means followed by the same letters in the column do not differ according to Tukey’s test at the 0.05 probability level. ns, ** and *: not significant, significant at \(p < 0.01\) and \(0.01 < p < 0.05\), respectively. \(\#\): total of five applications (sixth, seventh, eighth, ninth and tenth evaluations).

The lowest amounts of P, Ca, Mg and S in the leaves of the emerald grass were obtained through the application of 30 g N m\(^{-2}\) in comparison to the control and 15 g N m\(^{-2}\) (Table 4). The higher amount of N resulted in greater nutrient exportation, with the removal of “clippings”, due to a higher dry matter production and/or nutrient concentrations of −1.2, 0.5, −0.4 and 0.6 g m\(^{-2}\) of P, Ca, Mg, and S, respectively.

A linear increase in the leaf concentrations of P and S of the emerald grass as a function of glyphosate application was observed (Table 4). The higher glyphosate dose (600 g a.i. ha\(^{-1}\)) resulted in 1.0 and 0.3 g m\(^{-2}\) of P and S, respectively, without exportation upon the removal of “clippings”. The higher dose of the herbicide was observed to produce a lower dry matter production (Figure 2C). In this sense, there is a lesser necessity to replenish these nutrients through fertilization.

There was an interaction between the nitrogen fertilization and glyphosate doses regarding the N and K balances (Table 4; Figure 5A,B) in the leaves of the emerald grass. Regardless of the glyphosate dose, the lowest leaf N content of the emerald grasses was obtained through the control treatments (Figure 5A), with a deficit of 7.4 g N m\(^{-2}\). The lowest K concentration was observed with the highest N dose (Figure 5B). Therefore, the greater growth of the fertilized emerald grass (Table 1) was characterized by a greater exportation of K upon the removal of “clippings”.

The glyphosate doses influenced the N and K balance, and the highest residues of these nutrients were obtained with the highest glyphosate dose (Figure 5A,B). The fertilization of 15 g N m\(^{-2}\) was observed to produce 2.6 and 2.0 g m\(^{-2}\) of N and K in the emerald grass, respectively, whereas the application of 30 g N m\(^{-2}\) resulted in 4.1 and 1.9 g m\(^{-2}\) of N and K residues, respectively.

The lower preservation of macronutrients in the leaves of the emerald grass required a greater attention in order to replace them through fertilization, since the leaves were removed in the mowing operations. Thus, the highest N dose was not appropriate. In addition, the largest amounts of nutrient residue in the lawn area were generally obtained with application of 400 and 600 g a.i. ha\(^{-1}\) of glyphosate (Table 4; Figure 5). This is interesting, because it would entail a lower cost for the mowing as well as a reduced need to replenish the exported nutrients through fertilization.
Figure 5. Nutritional balance of N and K ((A,B), respectively) obtained upon the removal of leaves (“clippings”) and application of N to emerald grass as a function of doses of glyphosate. The letters correspond to a significant difference at the 5% probability level (p ≤ 0.05). ns and **: not significant and significant at p < 0.01, respectively. Error bars indicate the standard deviation of the mean (n = 4).

3.5. Soil Chemical Attributes

Nitrogen fertilization did not influence the P, K⁺, Ca²⁺, Mg²⁺ and S-SO₄²⁻ contents in the soil (Table 5), yielding medium to high P contents (13–30 and 31–60 mg dm⁻³), respectively, high Ca²⁺ and Mg²⁺ (> 8 and 7 mmol c dm⁻³), respectively, low to medium S-SO₄²⁻ (0–4 and 5–10 mg dm⁻³), respectively, and low K⁺ (0.8–1.5 mmol c dm⁻³) [22].

Table 5. Macronutrient contents in soil as a function of treatments with nitrogen fertilization and doses of glyphosate in emerald grass in the 10th evaluation.

<table>
<thead>
<tr>
<th>N Rates (g m⁻²)</th>
<th>P_resin mg dm⁻³</th>
<th>S-SO₄²⁻</th>
<th>K⁺ mmol dm⁻³</th>
<th>Ca²⁺ mmol dm⁻³</th>
<th>Mg²⁺</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>31.9 a</td>
<td>4.5 a</td>
<td>1.3 a</td>
<td>39.4 a</td>
<td>18.7 a</td>
</tr>
<tr>
<td>15</td>
<td>32.2 a</td>
<td>3.5 a</td>
<td>1.3 a</td>
<td>35.5 a</td>
<td>20.2 a</td>
</tr>
<tr>
<td>30</td>
<td>29.5 a</td>
<td>3.8 a</td>
<td>1.2 a</td>
<td>33.6 a</td>
<td>17.4 a</td>
</tr>
<tr>
<td>L.S.D. (5%)</td>
<td>5.4</td>
<td>1.0</td>
<td>0.2</td>
<td>7.0</td>
<td>5.6</td>
</tr>
</tbody>
</table>

Glyphosate doses (g ha⁻¹)

| 0               | 34.3 ns        | 3.8 ns  | 1.2 (1)      | 31.6 ns        | 16.8 ns|
| 200             | 31.0           | 4.2     | 1.2          | 37.8           | 21.4   |
| 400             | 26.8           | 3.9     | 1.3          | 36.7           | 18.7   |
| 600             | 32.8           | 3.9     | 1.4          | 38.7           | 18.2   |

C.V. (%) 17.00 22.25 15.88 18.94 29.16

F-value (nitrogen x glyphosate) 0.38 ns 1.83 ns 0.98 ns 1.09 ns 0.36 ns

Equation

(1) \( \hat{Y} = 1.17 + 0.0004 \times X \)

\( R^2 \)

Means followed by the same letters in the column do not differ according to Tukey’s test at the 0.05 probability level. ns: not significant according to the F test. *: significant at 0.01 < p < 0.05.

The soil K⁺ content increased linearly with increasing glyphosate doses. The high dose of glyphosate (600 g a.i. ha⁻¹) increased the K⁺ content by 20.5% in comparison to the treatments without glyphosate application (Table 5). This increase in the K⁺ content with the herbicide doses may be explained by the longer preservation of K in the lawn due to its less removal through “clippings” (Figure 5B), thus becoming more widely available in the soil.
4. Discussion

4.1. Leaf Chlorophyll Index and Productive Components

Nitrogen (N) fertilization was observed as having positive effects on the growth and development of grasses in the current and in some other studies. Dinalli et al. [2] observed an increased leaf chlorophyll index (LCI), shoot growth and leaf dry matter of emerald grass fertilized with 20 g m\(^{-2}\) N, applied five times per year. The application of N at the dose of 2.5 g N m\(^{-2}\) increased the leaf area index of emerald grass by 57% in comparison to the control [34].

Our evaluations found a positive correlation between the values of the leaf area and shoot dry matter \((r = 0.94 \text{ **})\) of emerald grass. The higher leaf area was noted as a result of the high level of interception of sunlight incident that ultimately increased the plant growth [35]. However, the increase in growth provided by higher doses of N is not economically interesting due to the higher number of mowing operations, nutrient removal and maintenance costs of the lawn [4,36].

The results obtained for LCI of the emerald grass demonstrated that a high dose of N increased the green coloration, which is aesthetically desirable. Gazola et al. [5] also verified that high doses of N fertilization increased the dark green color index. The application of N, regardless of the N source, increased the green color of Zoysia grass (Zoysia matrella (L.) Merr.) [37]. Under the effects of physiological responses, plants with more intense green coloration have a greater photosynthetic capacity due to their higher chlorophyll concentration, with a greater number of molecules that are responsible for capturing the light energy of solar radiation [4].

The effects of glyphosate, as a growth regulator, on grasses have already been demonstrated. Dinalli et al. [2] observed the least growth and minimum accumulation of leaf dry matter of emerald grass with the application of 200 g a.i. ha\(^{-1}\) of glyphosate. Fry [21] observed a decreased plant height of centipede grass with the application of 600 g a.i. ha\(^{-1}\), and Barbosa et al. [21] observed the existence of dwarf plants and a low dry matter production in Batatais grass. This implies a lower export of the nutrients upon the removal of dry matter from the leaves in the mowing operation.

This reduction in growth can be explained by the mechanism of action of glyphosate. It acts on the shikimic acid pathway by inhibiting the enzyme 5-enolpyruvylshikimate-3-phosphate (EPSP) synthase and, therefore, affects the synthesis of alkaloids, auxins and ethylene [38], as well as phenolic compounds [39].

In relation to plant hormones, the inhibition of the EPSP enzyme compromises the formation of aromatic amino acids, such as tryptophan, which is the precursor of indole-3-acetic acid (IAA biosynthesis) [40], as well as the synthesis of gibberellin, which is promoted by IAA [41]. The increased synthesis of ethylene inhibits the plant growth [38,41,42]. In this sense, blocking the shikimic acid route using glyphosate leads to a reduction in the plant growth. The blockage of this route also affects photosynthesis [41] through the accumulation of shikimic acid and carbon drainage in the Calvin–Benson cycle [43] through the diversion of erythrose-4-phosphate that regenerates ribulose-1,5-bisphosphate, a CO\(_2\) acceptor in the carboxylation stage of the cycle [44], therefore resulting in a reduction in the CO\(_2\) fixation [45] and, consequently, the plant growth.

The high doses of glyphosate reduced the LCI, plant height and leaf dry matter (Figure 2A,C,D), which lead to leaf chlorosis and compensate for the aesthetic aspect of the grass. Such a reduction in the intensity of the green coloration may be associated with the degeneration of chloroplasts [46], as well as the inhibition of the synthesis of chlorophyll molecules [41,47], which are the pigment responsible for the green coloration of leaves. The application of glyphosate and appearance of chlorosis in the leaves (yellowing) resulted in the reduction in the green color of the lawn and, consequently, a reduced photosynthetic process, thus leading to a lower production of dry plant matter (Figure 2C). This finding agrees with the statement of Su et al. [17] that chlorosis and necrosis are caused by the application of glyphosate to grasses and associated with the inhibition of biomass production. Dinalli et al. [2] indicated that 200 g a.i. ha\(^{-1}\) of glyphosate resulted in a
lower growth of emerald grass while maintaining its green coloration. Gazola et al. [5] also verified that this herbicide did not reduce the dark green color index of the emerald grass.

4.2. Macronutrient Concentrations of the Leaves, Roots and Rhizomes

The application of N increased the nutrient concentrations and availability in the emerald grasses. The application of N increased its availability and that of other nutrients so as to stimulate plant growth [48,49], confirming our results.

According to Godoy et al. [4], the concentrations of N in grass leaves was increased in the range from 20 to 50 g N kg\(^{-1}\), with a minimal deficiency mean concentration of 14 g N kg\(^{-1}\). Godoy et al. [12] found that the treatments of emerald grass without the application of N fertilizer produced N concentrations ranging from 14 to 16 g kg\(^{-1}\), indicative of severe deficiency. Likewise, our results also indicated that treatments without the application of N resulted in a low concentration of N (13.1 g kg\(^{-1}\)) and less green coloration of the lawn, which, therefore, compromised its growth and appearance.

Glyphosate application reduced the root growth. However, the nutritional status was not affected. The nutritional status of the root system and rhizomes (subterranean stems) is important, because it is reflected in the growth, density and color of lawns. The inadequate concentration and availability of nutrients lead to weak, discolored grasses and their and slow recovery [50]. Glyphosate has an antagonistic relationship with the absorption, transportation and accumulation of Ca and Mg that might be due to the formation of poorly soluble complexes with these cations [51]. However, the scientific literature is limited in terms of its parameters or results related to the concentrations of macronutrients in the roots and rhizomes of implanted grasses, which could be compared with the results obtained in this study in order to determine the adequate nutritional status.

Santos et al. [52] evaluated the nutrient concentrations of eucalyptus under simulated glyphosate drift and observed that plants subjected to treatments of 345.6 and 691.2 g ha\(^{-1}\) of glyphosate resulted in higher concentrations of foliar Ca and Mg, corresponding to a lower accumulation of dry matter. This higher concentration could be interpreted as a an effect of the concentration of these nutrients in the leaves, reducing the growth, as observed in the present study on emerald grass.

4.3. Coefficient of the Biological Utilization of the Nutrients

N fertilization provided a higher coefficient of the biological utilization (CBU) of P, K, Ca, Mg and S by the emerald grass. The higher N supplied by fertilization did not result in a higher total dry matter production. According to Silva et al. [53], the greater absorption of a nutrients by plants is due to their increased concentration in the soil solution. However, if the growth rate of plants is less than that of absorption, then the efficiency of the utilization of the nutrients in question is reduced. This fact explains the lower coefficient obtained with the higher amount of N applied. This was a case of luxury consumption, whereby the plant absorbs more than it needs, and this is not reflected by a higher growth [54].

The highest CBU of P, K, Ca, Mg and S of the emerald grass were due to the higher yield of the dry matter the shoots, roots and rhizomes, and the total matter provided by nitrogen fertilization. This increase in the N availability as a function of its application stimulated the plant growth and, consequently, the absorption of other nutrients [48,49], supporting the present study’s results.

The high dose of glyphosate reduced the CBU of N, K, Ca and Mg. Glyphosate influences protein synthesis [41], whereas N is a constituent of several enzymes [55]. The application of glyphosate modified the N metabolism of the plant and inhibited its transportation and accumulation [24]. Potassium is the activator of more than 50 enzymes, but its availability was reduced with application of glyphosate [55]. Magnesium activates the ribulose-1,5-bisphosphate enzyme in the carboxylation stage of the Calvin cycle [44] and the content of chlorophyll molecules, since the application of glyphosate reduces their synthesis [41,48]. Glyphosate also reduces the Ca content in plants, with a lower growth
and hindered cell formation and structure [54,55]. Therefore, the application of glyphosate changes the coefficient of the biological utilization of the nutrients.

4.4. Nutrient Balance of the Macronutrients

The nutritional balance of the macronutrients of the emerald grass was assessed in order to verify the quantities of the nutrients that remained in the area after the removal of “clippings” by mowing. The higher amount of N resulted in greater nutrient exportation upon the removal of “clippings” due to the higher dry matter production and/or foliar concentration of the nutrient. The K and N had antagonistic effects on the grasses. The higher N fertilizer exported greater amounts of K. According to Godoy et al. [12], K is the second most frequently extracted nutrient by emerald grass, which requires its replacement in the soil through fertilization.

The glyphosate doses influenced the N and K balance, and the highest residues of these nutrients were observed with the highest dose of this herbicide. The greater stability of these nutrients is due to their lower exportation upon the removal of “clippings”. The reduction in the leaf dry matter provided by glyphosate is related to its mechanism of action [40,41]. As these nutrients are intensely exported by emerald grasses, they are replenished through fertilization [12,56]. The higher doses of glyphosate, interestingly, were reported to produce higher amounts of residues of nutrients, which can help to lower the cost of mowing and also reduce the replenishment through fertilization.

4.5. Soil Chemical Attributes

Nitrogen fertilization did not influence the P, K\(^+\), Ca\(^{2+}\), Mg\(^{2+}\) and S-SO\(_4^{2-}\) contents of the soil, whereas the K\(^+\) content in the soil increased linearly with higher doses of glyphosate. This increase in the K\(^+\) content with the glyphosate doses may be explained by the longer preservation of K in the lawn and its reduced removal through “clippings”, thus enabling it to become more available in the soil. In addition to the exportation of K through the grass leaves’ removal by “clipping”, under higher Ca and Mg contents in the soil, the content of replaceable K\(^+\) and its availability may be reduced [57], therefore explaining our results. The replaceable cations are retained by the soil in a sequence called the lyotropic series (Al\(^{3+}\) > Ca\(^{2+}\) > Mg\(^{2+}\) > K\(^+\) > Na\(^+\)) [57]. Thus, in this work, K was more effectively absorbed by the lawn and may also have been leached in the soil profile due to its higher fixation in the solution and lesser retainment of cation exchange capacity points in the replicable form.

5. Conclusions

Fertilization with 15 g N m\(^{-2}\) applied in five splits per year resulted in a lower growth, adequate aesthetic quality and longer stability of nutrients in the lawn through their lower exportation upon the removal of “clippings” after mowing.

The glyphosate dose of 400 g a.i. ha\(^{-1}\) was efficient in reducing the growth of emerald grass and did not impair its coloration, and it resulted in less exportation of the nutrients by reducing the need to replenish them through fertilization.

Therefore, fertilization with 15 g N m\(^{-2}\), associated with application of 400 g a.i. ha\(^{-1}\) of glyphosate, is recommended for emerald grass.

Future researchers are encouraged to assess the interactive hormetic effects of nitrogen fertilization coupled with the application of herbicide doses on lawn grasses, focusing on plant nutrition and the physiological and biochemical responses of key enzymes whose activities are related to N metabolism in different environments. Therefore, more studies are needed in order to understand how lawn grass species respond to these variables. This will allow researchers not only to better understand this biological phenomenon but also to obtain greater confidence in regard to N and herbicide management.

Supplementary Materials: The following are available online at https://www.mdpi.com/article/10.3390/agronomy12102473/s1, Figure S1: Harvest of sod carpets of emerald grass (Zoysia japonica Steud.).


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