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Emissions of ammonia following glyphosate application on *Urochloa decumbens*

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ABSTRACT

This work was carried out with the objective of evaluating the ammonium (NH_4^+) levels and emissions of ammonia (NH_3) after glyphosate application on signal grass (*Urochloa decumbens*). Two experiments were carried out and the following treatments were used: (1) Control – mechanic harvest with no herbicide application on signal grass; (2) Glyphosate – signal grass desiccation with the herbicide glyphosate. Ammonium (NH_4^+); total nitrogen (N_{tot}) levels in plant's tissues (experiment 1) and ammonia (NH_3) emission by the plants or the soil (experiment 2) were evaluated over time, under field conditions. Signal grass desiccation with the herbicide glyphosate enhanced NH_4^+ levels from 2–24% of the N_{tot} at 12 days after herbicide application. The cumulative NH_3 emission by leaves were increased from 2.8–5.3 kg/ha 30 days after herbicide application. Glyphosate application increases NH_3 losses by plant, but it does not affect the NH_3 emissions by soil, the dry mass production and N_{tot} in the aboveground portion of signal grass.

Keywords: weed control; fertilizer; macroelement; atmosphere

Glyphosate [(N-(phosphonomethyl)glycine] is a post emergence herbicide used to weed control, cover crop desiccation in conservationist systems and pre-harvesting desiccation. The widespread use of this herbicide has been related to some environmental concerns. In the last years, some studies have shown that glyphosate could affect nitrogen (N) transformations in the soil-plant systems, enhancing nitrogen losses and changing N mineralization rates (Haney et al. 2002, Damin et al. 2008, 2010a, Castoldi et al. 2012).

Damin et al. (2008) demonstrated that signal grass (*Urochloa decumbens*) desiccation with glyphosate enhances N losses from plant-soil system, with more than 16% of the N from fertilizer being lost 15 days after herbicide application. The higher N losses observed after glyphosate application may be related to ammonia (NH_3) emission by plants. The main factors driving NH_3 emission are the NH_4^+ content in leaves, stomata conductance, plant's age and species and NH_3 levels in the atmosphere (Loubet et al. 2002, Mattsson and Schjoerring 2002). Herbicides affect more than one of these factors, so their application may affect NH_3 emission by plants.

NH_3 emission by agricultural areas account for 10% up to 20% of global NH_3 emission (Behera et al. 2013). It is an important process of N output in agriculture lands, reducing N use efficiency; moreover, it is an environmental concern as well (Galloway et al. 2003).

In Brazilian agroecosystems, signal grass is one of the most widespread weeds (Neto et al. 2008). Moreover, it is used as a cover crop in no-till systems, in the area between rows of plant species such as coffee (*Coffea* spp.) and orange (*Citrus* spp.) and for farming-cattle integration systems; all these systems use glyphosate to control weeds and/or kill cover crops. Within this context, the objective of this study was to evaluate NH_4^+ and total nitrogen (N_{tot}) levels in plant's tissues and NH_3 emission by foliage after glyphosate application on signal grass.

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MATERIAL AND METHODS

Experiment 1

Growing conditions and experimental design. The experiment was conducted in a greenhouse located at the Agricultural Center of Nuclear Energy (CENA) – University of São Paulo, Piracicaba – SP, Brazil (22°42'30''S, 47°38'00''W and 546 m a.s.l.). The temperature range was 18–28°C, with a 13 h/11 h day/night cycle, provided from natural sunlight. The climate is a tropical savanna (AW) with a dry winter and rainy summer, according to Köppen classification.

Pots were filled with 4.5 kg of dried soil classified as Typic Quartzipsamment (Soil Taxonomy, 2006). Soil analysis were determined as described by Raij et al. (2001) and showed the following chemical and physical characterisation of the 0–20 cm layer: pH 4.1; organic carbon 2.3 g/kg; P 1.1 mg/kg; cation exchange capacity (CEC) 20 $mmol_+$/kg; base saturation (%) = 21.8%, with 122 g/kg of clay, 8 g/kg of silt and 870 g/kg of sand.

Treatments were arranged in a factorial scheme, 2 (control – mechanic harvest with no herbicide application on *Urochloa decumbens* and glyphosate – *Urochloa decumbens* desiccation with glyphosate) × 5 times (0, 1, 3, 6 and 12 days after herbicide application); in an entirely randomized block design, with four replicates.

Soil corrections and sowing. Soil acidity and fertility were corrected. The amount of lime applied was 0.5 g/kg dolomitic lime (TNP = 100%). Single superphosphate was added at a rate of 100 mg of P per kg of dry soil and then a solution containing 8.0 g/L of K, 1.2 mg/L of Zn, 0.6 mg/L of B and 0.8 mg/L of Cu was applied at a rate of 22.5 mL per kg of soil, with 110 mL distilled water per kg of soil (70% of maximum water retention capacity). Pots were incubated for 15 days before sowing.

A hundred *Urochloa decumbens* seeds were sown per pot. The nitrogen fertilizer (ammonium sulfate) was applied after seedling germination at a rate of 225 mg N/kg dry soil. Irrigations were performed maintaining the moisture level at 60% of the maximum water retention capacity.

Herbicide application. The herbicide was applied 63 days after sowing, during the pre-anthesis stage (less than 5% of panicle emission). The commercial product used was Roundup Original at a dosage of 4 L/ha (180 g/L of the acid equivalent – a.e.). The product was diluted to 200 L/ha with water, and herbicide application was performed with a backpack sprayer pressurized with CO_2 and equipped with a 2 m-wide boom with four flat-fan nozzles.

Harvest and dry mass determination. At 0, 1, 3, 6 and 12 days after herbicide application, four pots from each treatment (control and glyphosate) were taken, and all plants from these pots were separated into aboveground portion and roots. Then, the plant's tissues were dried in a forced air circulation oven at 50°C up to a constant weight, to determine dry mass.

Experiment 2

Growing conditions and experimental design. A field experiment was conducted at the Center-South Regional Station of the São Paulo Agency to Agrobusiness Development (APTA), Piracicaba – SP, Brasil (22°42'30''S, 47°38'00''W and 546 m a.s.l.). According to the Köppen classification, the regional climate is Aw. The humidity, precipitation and average daily temperature during the experiment are presented in the Figure 1. The soil was classified as Rhodic Hapludox (Soil Taxonomy, 2006), and the chemical attributes (0–20 cm) were determined as described by Raij et al. (2001). Soil analysis showed the following chemical and physical characterization: pH 5.8; organic carbon 11.8 g/kg; P 2.1 mg/kg; CEC 61.7 $mmol_+$/kg; base saturation (%) = 65%, with 680 g/kg of clay, 70 g/kg of silt and 250 g/kg of sand.

The experimental design was a randomized block, with a split-plot time scheme and 10 replicates of each treatment. The main plots with an area of 10.5 m^2 each were separated by 1.5-m plastic barriers in all sides. The following treatments were allocated to the main plots: A – control, no herbicide application; B – glyphosate, cover crop desiccation with glyphosate; periodic measurements of NH_3 emission by the plants and/or the soil at 48-h intervals were considered secondary plots, and they were performed from 0 to 30 days after herbicide application (DAA).

Urochloa decumbens was sown by broadcasting 22 kg/ha of seeds. On the same day, 87.3 kg/ha of P was applied using a single superphosphate fertilizer and the nitrogen fertilizer, ammonium sulfate, was applied at a dosage of 40 kg N/ha fifteen days after a cut for standardization (crop

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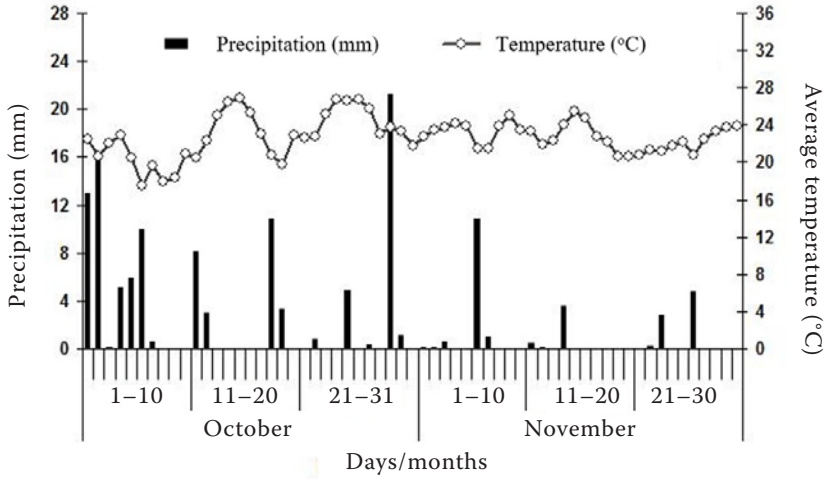

Figure 1. Precipitation and daily average temperatures recorded during the development period of the field experiment; HA – application of herbicides

residues were removed manually from the plots during this procedure).

The pre-anthesis stage was detected at 100 days after plants were cut to standardization; at this time, herbicide application was performed as described to the experiment 1.

Ammonia emission measurements. The ammonia emissions of the plants were measured using semi-open static chambers (Nömmik 1973) with adaptations as described by Castoldi et al. (2014), and the ammonia emitted by the soil was measured using a sampler similar to those described by Araújo et al. (2009). Measurements of the standard ammonia concentration in the air were taken in a fallow area, i.e., without plants. The amount of NH_4^+ was measured by flux injection analyses (FIA), as described by Reis et al. (1997).

The emitted NH_3 was calculated by the equation:

$$NH_3(mg\ per\ sampler) = \left[\frac{NH_4^+}{100} \times V\right] - control$$

Where: NH_3 – NH_3 emitted (mg per sampler); NH_4^+ – ammonium content (mg /L); V – sample volume (mL); control – NH_3 in the air measured in a plant-free area (mg per sample).

The data were presented in kg/ha using the following equation:

$$NH_3(kg/ha) = \left[\frac{NH_3(mg/sampler) \times 1000}{sampler\ area}\right]/100000$$

Harvest and dry mass determination. The dry mass was measured at the same time as herbicide application and after the death of the plants. The harvest was performed using a metallic square with an area of 0.49 m^2. Each plot was sampled twice and sub-sampled to determine the dry mass (65°C, 48 h).

N analyses. For both experiments, plants tissues were ground in a Wiley mill, and the amounts of NH_4^+ in the plant tissues were determined as described by Malavolta et al. (1997). N_{tot} was determined in a mass spectrometer (Barrie and Prosser 1996).

Statistical analyses. To determine differences among treatments, a two-way factorial analysis (ANOVA) was performed, including as factor: herbicide application and period after herbicide application. In the first experiment, the two-way analysis was procedure as a factorial scheme; while in the second one a split-plot was used in time scheme. When the *F*-test was significant, a *T*-test (P = 0.05; 0.01 or 0.001) was applied to compare the herbicide effect. The periodic measurements of behaviour were described using a non-linear regression procedure. The parameters used to choose the model were the *F*-test significance, predicted and adjusted R^2, the residual plots independence (including Durbin-Watson test to verify correlation between adjacent residuals). The statistical analyses were performed using the *R* (R development Core Team, 2009) and the SigmaPlot 10.0 (Systat Software Inc., San Jose, USA).

RESULTS

Experiment 1

The NH_4^+ concentration in the aboveground portion and roots of signal grass treated with glyphosate differed from the control after 6 DAA (Figure 2).

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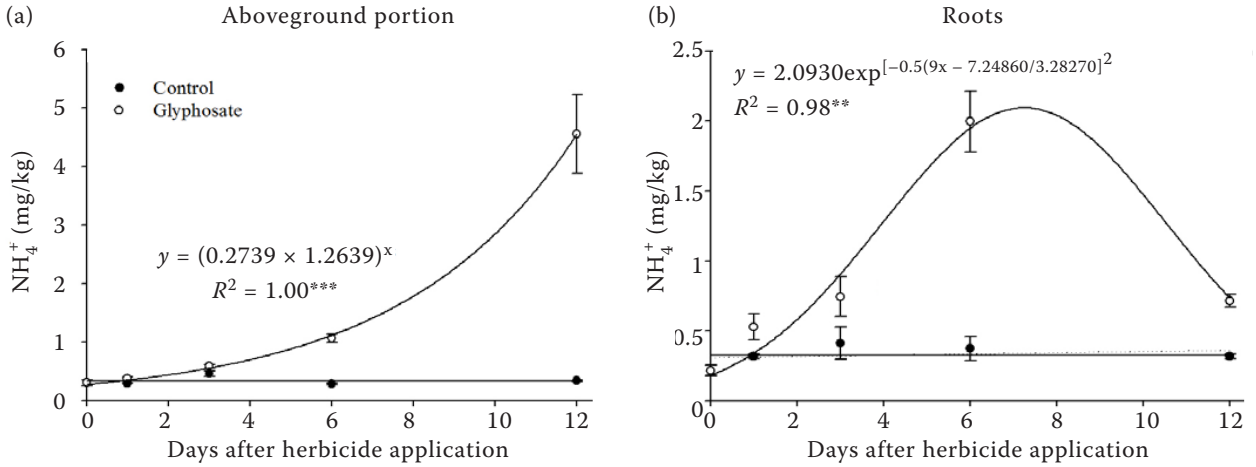

Figure 2. NH_4^+ concentration in plant's tissues after glyphosate application on signal grass: (a) Aboveground portion (Fherbicide = **; FDAA = **; Fherbicide × DAA = **); and (b) roots (Fherbicide = **; FDAA = **; Fherbicide × DAA = **). DAA – days after herbicide application. $^{**}P < 0.01$

In aboveground portion, it was described for an exponential growth model, with a 15 times increase of the NH_4^+ concentration at 12 DAA. The root's NH_4^+ concentration was described by a peak model, in which glyphosate increased ten times the NH_4^+ concentration at 7.2 DAA, following by a decrease on it after 7,2 DAA, being only 2 times higher than control at 12 DAA (Figure 3b).

The N_{tot} concentration in the aboveground portion did not differ between treatments (Figure 3a). In roots, herbicide application decreased N_{tot} only at 12 DAA (Figure 4b).

In entire plants, glyphosate application increased the amount of NH_4^+ after 6 DAA (Figure 4a) and it decreased N_{tot} at 12 DAA (Figure 5b, with NH_4^+ representing 5% and 20% of the N_{tot} 6 and 12 DAA (Figure 5), respectively.

Experiment 2

The NH_4^+ concentration in the aboveground portion were enhanced after glyphosate application; however, dry matter and N_{tot} in the aboveground portion of signal grass did not differ between treatments (Table 1). The NH_3 emission by signal grass was increased after 16 DAA (Figure 6a), with cumulative NH_3 emissions ranging to 2.8 kg/ha in

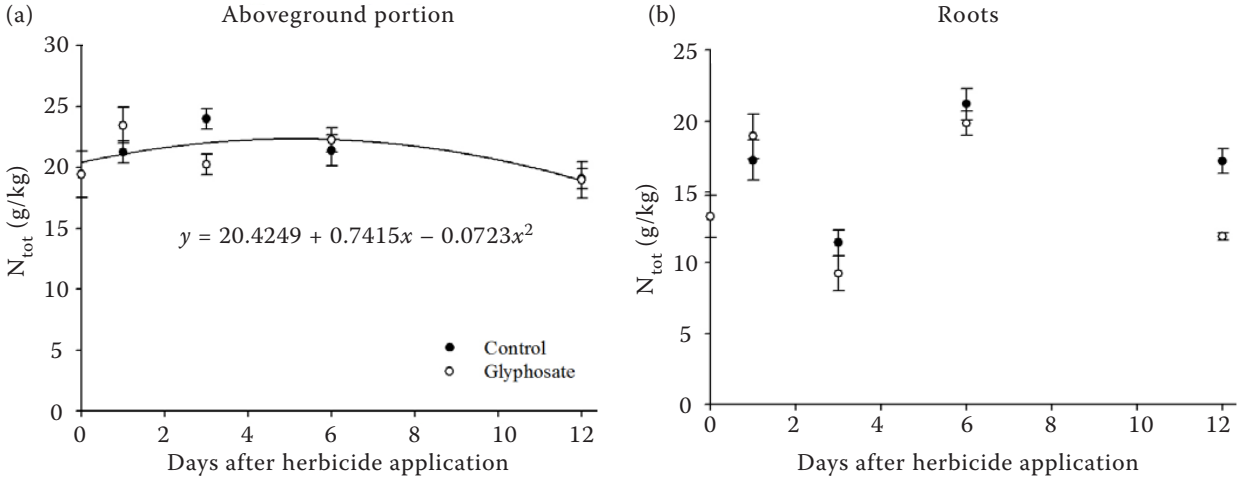

Figure 3. Total nitrogen in plant's tissues after glyphosate application on signal grass: (a) Aboveground portion (Fherbicide = ns; FDAA = *; Fherbicide × DAA = ns); and (b) roots (Fherbicide = *; FDAA = **; Fherbicide × DAA = *). DAA – days after herbicide application. $^{*}P < 0.05$; $^{**}P < 0.01$; ns – not significant

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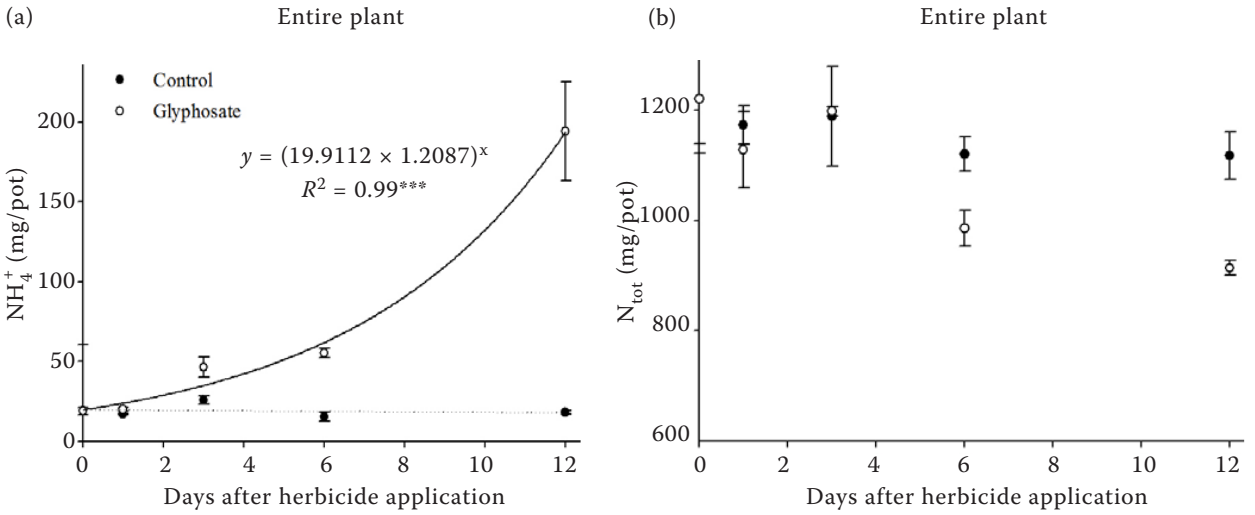

Figure 4. (a) Ammonium (NH_4^+) amount in entire plant after glyphosate application to signal grass (Fherbicide = **; FDAA = **; Fherbicide × DAA = **); and (b) total nitrogen (N_{tot}) amount in entire plant after glyphosate application to signal grass (Fherbicide = *; FDAA = *; Fherbicide × DAA = *). DAA – days after herbicide application. *P < 0.05; **P < 0.01; ***P < 0.0001

the control up to 5.4 kg ha in the glyphosate treatment. Herbicide application did not affect NH_3 emission by soil, with an average of cumulative NH_3 emissions being 5 kg/ha (Figure 6b).

DISCUSSION

Some studies have shown a positive correlation between NH_4^+ levels in the aboveground portion and NH_3 emission by plants (Husted et al. 2000). NH_4^+ in the aboveground portion and entire plants enhanced exponentially from the day that herbicide was applied up to the complete senescence, suggesting that NH_3 emission potential is higher near to the death of the plant. Increasing NH_4^+ after glyphosate application is a secondary effect of this molecule in plant's metabolism (Duke and Hoagland 1985). This herbicide inhibits phenylalanine, tyrosine and tryptophan synthesis; however, as a feedback effect, it increases the phenylalanine ammonia-lyase activity, with degradation of phenylalanine and formation of phenolic acids and NH_4^+ (Duke and Hoagland 1985).

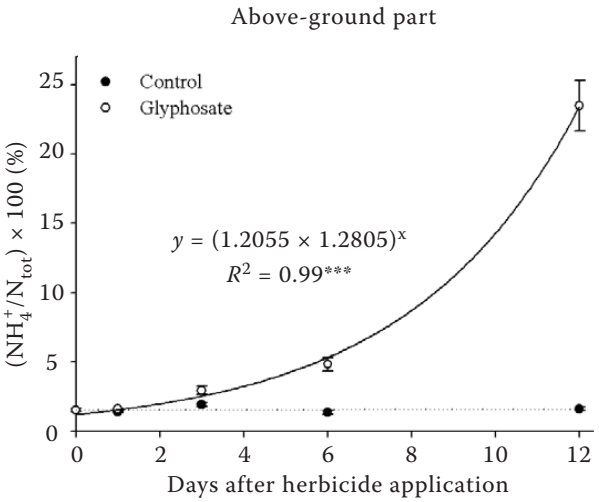

Figure 5. Ammonium (NH_4^+) percentage in the aboveground portion after glyphosate application on signal grass (Fherbicide = **; FDAA = **; Fherbicide × DAA = **). DAA – days after herbicide application. ***P < 0.001

Table 1. Dry mass, total nitrogen (N_{tot}) and NH_4^+ in the aboveground portion of signal grass 30 days after glyphosate application

Treatment	Dry mass	N_{tot}	NH_4^+
	(kg/ha)		
Control	12.3	151.7	2.1[b]
Glyphosate	12.7	176.4	9.5[a]
CV (%)	20.4	25.3	30.9
P	ns	ns	**

Means followed by the same letter in the columns are not different according to the least significant difference – *LSD* of *T*-test (P = 0.05). **P < 0.01; ns – not significant

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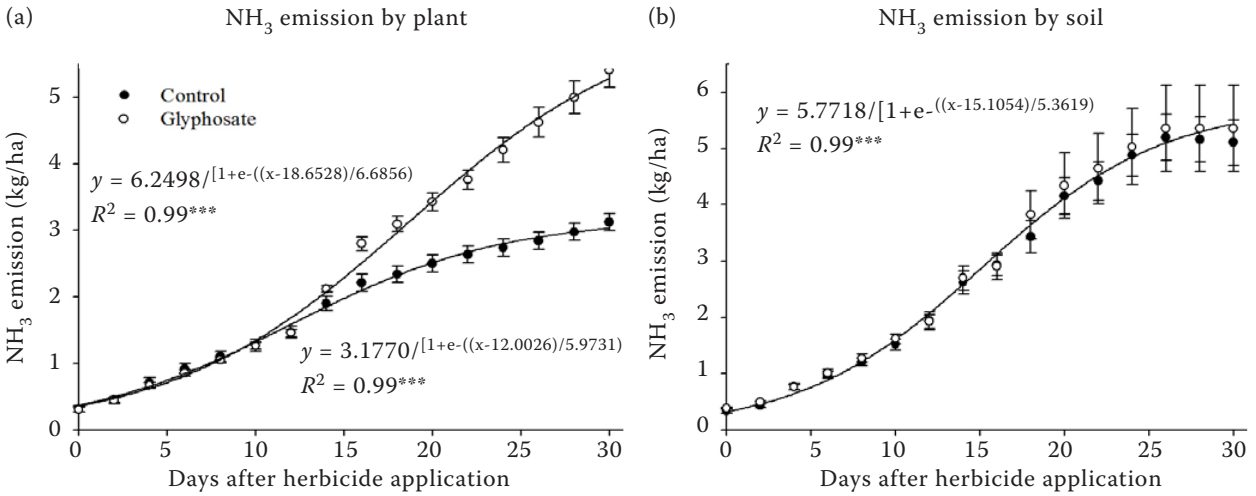

Figure 7. Cumulative NH_3 emission (a) by plants (Fherbicide = *; FDAA = **; Fherbicide × DAA = **); and by the soil (Fherbicide = *; FDAA = **; Fherbicide × DAA = **) after herbicide application to signal grass. DAA – days after herbicide application. *$P < 0.05$; **$P < 0.01$; ***$P < 0.000$

Higher NH_4^+ concentration in the aboveground portion followed by enhanced NH_3 emission by plants were observed at the field experiment, confirming that glyphosate affects this N output pathway, as suggested by Damin et al. (2008). As expected, even using soils with a very low CEC, which guaranteed that NH_4^+ was available for transformation, herbicide did not affect N losses by soil. The soil losses were separated from the losses by plants, since a closed soil chamber was used.

The N losses by foliage magnitude is difficult to predict since foliar ammonia samplers can detect some NH_3 emitted by soil as well (Schjoerring and Mattsson 2001). However, since losses from the soil did not differ between treatments, the difference of 5.3 kg/ha of ammonia emission in plots with glyphosate application to 2.8 kg/ha in the control can be attributed to the herbicide application.

The NH_3 emissions observed in this study are similar to those estimated by Schjoerring and Mattsson (2001), who observed NH_3 emissions by barley, peas and canola ranging from 1–5 kg/ha. Although accumulated NH_3 emissions by signal grass were increased after glyphosate application, the magnitude of these losses may not affect the main crop production, growing under residues from cover crops desiccated with glyphosate. In fact, the total-N and dry mass of signal grass plants were not affected, which is in accordance with previous results obtained by Damin et al. (2008).

Even though the herbicide application may not affect dry mass production and N_{tot}, glyphosate use is certainly an environmental concern in countries like Brazil that have an extensive area under conservationist systems, which use glyphosate application for cover crop management.

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