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DECOMPOSITION AND NUTRIENT RELEASE FROM *CROTALARIA SPECTABILIS* WITH GLYPHOSATE APPLICATION

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ABSTRACT

Legumes used as green manure provide essential chemical elements to the soil through their biomass decomposition. However, nutrient availability may be significantly affected by herbicide application such as glyphosate. Thus, we evaluated the decomposition and nutrients release from the shoot dry phytomass and the root system of *Crotalaria spectabilis* with and without glyphosate application. After 90 days of *C. spectabilis* cultivation, the shoot and the root system were collected to determine the remaining phytomass. The analysis of variance for the remaining dry mass indicated the two-way interaction between herbicide and age was significant ($P < 0.0001$). For most elements being released, the two-way interaction was significant. The remaining dry mass that received herbicide presented greater mineralization and faster reduction for most of the elements, resulting in the considerable nitrogen and phosphorus loss compared to the material without the herbicide, concluding that their decomposition was affected by the glyphosate application.

Key words: legume; green manure; herbicide; mineralization.

DESCOMPOSICIÓN Y LIBERACIÓN DE NUTRIENTES DE *CROTALARIA SPECTABILIS* CON APLICACIÓN DE GLIFOSATO

RESUMEN

Las legumbres utilizadas como abono verde proporcionan elementos químicos esenciales al suelo a través de la descomposición de su biomasa. Sin embargo, la disponibilidad de nutrientes puede verse significativamente afectada por la aplicación de herbicidas, como el glifosato. En este trabajo se evaluó la descomposición y liberación de nutrientes de la fitomasa seca de parte aérea y sistema radicular de *Crotalaria spectabilis* con y sin aplicación de glifosato. *C. spectabilis* fue cultivada por 90 dias, luego se colectó y separó el material en parte aérea y sistema radicular para determinar la fitomasa remanente. El análisis de varianza de la masa seca remanente indicó la doble interacción entre herbicida y edad que fue significativa ($P < 0.0001$). Para la mayoría de los elementos liberados, la interacción doble fue significativa. La masa seca remanente que recibió herbicida presentó una mayor mineralización y una reducción más rápida para la mayoría de los elementos, dando como resultado una pérdida considerable de nitrógeno y fósforo en comparación con el material sin herbicida, permitiendo concluir que su descomposición se vio afectada por la aplicación de glifosato.

Palabras clave: leguminosa; abono verde; herbicida; mineralización.

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INTRODUCTION

Green manuring improves the physical, chemical and biological soil characteristics through intercropping crops of agronomic interest, incorporating the residues in the soil or keeping them on the surface. Legumes used as green manure have been an alternative for agricultural regions by providing soil nutrients from the decomposition and release of nutrients from their biomass (Pereira *et al.*, 2016). Among these legumes, stands out *Crotalaria spectabilis*, a species with fast growth, high biomass production and nematode control (Inomoto *et al.*, 2008).

The symbiotic association between legumes and diazotrophic bacteria results in expressive amounts of nitrogen (N) in the soil-plant system, in addition, the low C:N ratio of these plants and the presence of soluble compounds favor the decomposition and release of other nutrients to the soil (Redin *et al.*, 2014). The nutrients availability to the soil depends on the amount of N accumulated in the dry matter, litter C:N ratio and, in great importance, the use of broad spectrum herbicides such as glyphosate (Cavalieri *et al.*, 2012).

Glyphosate is a non-selective herbicide used in weed control, which inhibits the enzyme 5-enolpiruvilshiquimato-3-fosfato-sintase (EPSPS) responsible for the synthesis of the aromatic amino acids phenylalanine, tyrosine and tryptophan, which are precursors of lignin, alkaloids, flavonoids, benzoic acids and vitamin K. Thus, plants treated with glyphosate tend to die slowly, causing a delay in the process of decomposition of residues, reducing the supply of nutrients by the crop (Timossi *et al.*, 2006).

It is already known that foliar contents of N, calcium (Ca), magnesium (Mg), iron (Fe) and copper (Cu) can also be reduced with high doses of glyphosate (Santos *et al.*, 2007). Although glyphosate has been used as a sugarcane maturing (Dalley & Richard Junior, 2010), soybean production (Gonçalves *et al.*, 2014) and as a plant size controlling agent, it has not been reported the effect of this herbicide on the nutrient release of *C. spectabilis*. Thus, we evaluated the decomposition and release of nutrients rates from the shoot and root system phytomass in *C. spectabilis* assuming that the application of glyphosate exerts influence mainly on N and P release.

MATERIAL AND METHODS

The experiment was carried out in fields of Coruripe plant, at an altitude of 75 m, at 10°08'31"S and 36°18'16"W, in Coruripe municipality (Alagoas state, Brazil). The average annual rainfall is 1500 mm and the average temperature is 27°C and maximum and minimum relative humidity of 95% and 65%, respectively.

The soil is a Distrocoesian Argisol, medium texture, very clayey. Samples were taken in the 0-10, 10-20 cm layers to determine the chemical attributes, according to EMBRAPA methodology (1997) (**Table 1**).

The *C. spectabilis* was cultivated during 90 days and was collected immediately the first part of the material without the glyphosate application. The shoot was collected and conditioned in identified paper bags, heated to 65ºC to obtain the dry mass. Then, the glyphosate was applied with an herbicide applicator Case model Patriot 350 at the dose of 3 L ha^{-1}.

Three days after the application, the material that received herbicide was collected to monitor the decomposition and release of nutrients from the shoot of *C. spectabilis*, with and without application of glyphosate, basing on the bags of residues decomposition technique, described by Rezende *et al.* (1999). Polyamide bags of 30 cm x 30 cm were prepared to keep 30 g of shoot and root sys-

Table 1. Mean values of soil chemical analysis at two depths (0-10 and 10-20 cm)

Tabla 1. Valores medios del análisis químico del suelo a dos profundidades (0-10 y 10-20 cm)

Depths	pH	Ca^{2+}	Mg^{2+}	Zn	P	Mn	Cu	Fe	B	K	SB	OM	CTC
0-10	6.1	1.5	0.7	3.9	25	6.3	0.8	49	0.4	78	2.5	1.3	5.1
10-20	5.5	1.1	0.5	3.5	27	5.3	0.6	132	0.4	50	1.8	0.8	5

SB: Sum of Exchangeable Bases, CTC: Cation Exchange Capacity; OM: Organic Matter. Zn, P, Mn, Cu, Fe, B, K are in $mg.dm^{-3}$. Ca^{2+} and Mg^{2+} are in $cmolc.dm^{-3}$.

tem phytomass of plants that received or not glyphosate. The bags were distributed in the experimental area at 15 cm depth, in 160 units.

The treatments were formed by a factorial arrangement between: eight incubation dates (0, 2, 8, 16, 32, 64, 128, 230 days of incubation), with or without glyphosate application, with five replicates. After the incubation period, the bags were collected from the experimental area. Then, the remaining biomass was determined, as well as chemical analyzes were carried out to determine the remaining nutrient contents in the Laboratório de Análise de Solos (Viçosa municipality, Minas Gerais state, Brazil).

The total N contents were determined by the micro Kjeldahl method, according to Malavolta *et al.* (1997). In the extract obtained by nitroperchloric digestion, the levels of P were determined by colorimetry; the contents of Ca, Mg, Cu, Fe, Mn was determined by atomic absorption spectrophotometry; K by flame photometry and S by turbidimetry (Malavolta *et al.*, 1997). B was extracted by incineration and determined by curmumin colometry, described by Malavolta *et al.* (1997).

The estimation of decomposition and nutrient release rates of shoots with and without herbicide application was obtained, according to Rezende *et al.* (1999). The decomposition and daily release of nutrients were calculated based on the information collected at the specific dates of incubation, according to Dubeux *et al.* (2006).

A completely randomized design with five replications has been used. Regressions was made for the samples, which had significant time effects. The analysis of variance was performed using SAS-ANOVA (SAS, 2004) and the regressions using Sigma Plot 10.0.

RESULTS AND DISCUSSION

The analysis of variance for the remaining dry mass (**Table 2**) indicated that the double interaction between herbicide and age was significant (P <0.0001). For the N, P, Ca, Mg, B, Fe and Mn contents remaining, the double interaction was significant, but the same did not occur for K, S and Cu, whose relationship between herbicide and time worked independently (P < 0.0001).

The glyphosate has affected the dry mass decomposition rate (**Figure 1**) and the highest decomposition was observed in the first 30 days of incubation. The plants that received herbicide showed less decomposition in this period until the following 90 days, whose shoot dry matter values were equivalent for the two treatments.

After 90 days, there was greater decomposition of the shoot with glyphosate until the evaluation realized at 230 days, where we obtained a total decomposition. Zobiole *et al.* (2011) reported a similar situation in a study with transgenic soybeans, although at different dosages of glyphosate used.

The contact surface has favored the microbiological action, accelerating the decomposition because the addition of glyphosate stimulated the microorganisms activity that degrade the recalcitrant organic matter present in the soil The chemical compounds introduction by decomposition served as a source of nutrients for soil, mainly carbon, nitrogen and phosphorus (Imparato *et al.*, 2016). Matos *et al.* (2011) also attributed the accelerated decomposition of green manure to the

Table 2. Variance analysis of the remaining dry mass and element content.

Tabla 2. Análisis de varianza de la masa seca restante y el contenido del elemento.

	DM	N	P	K	Ca	Mg	S	B	Cu	Fe	Mn
G	8.10^{-21}	3.10^{-23}	0.05	0.8	0.017	0.7	9.10^{-10}	1.10^{-17}	0.05	3.10^{-5}	2.10^{-15}
A	1.10^{-48}	5.10^{-33}	2.10^{-25}	1.10^{-34}	3.10^{-34}	6.10^{-32}	1.10^{-27}	5.10^{-38}	5.10^{-27}	2.10^{-31}	8.10^{-33}
GxA	3.10^{-10}	6.10^{-10}	0.4	0.003	1.10^{-6}	0.007	0.04	0.009	0.003	0.003	1.10^{-8}
R^2	0.99	0.99	0.94	0.96	0.99	0.97	0.97	0.98	0.98	0.96	0.99
CV	9.3	10.6	11.4	17	18	5	9.5	2.1	5.8	3.1	3.7

G: With Glyphosate; A: Age; Dm: Dry mass (g); CV: Coefficient of Variation

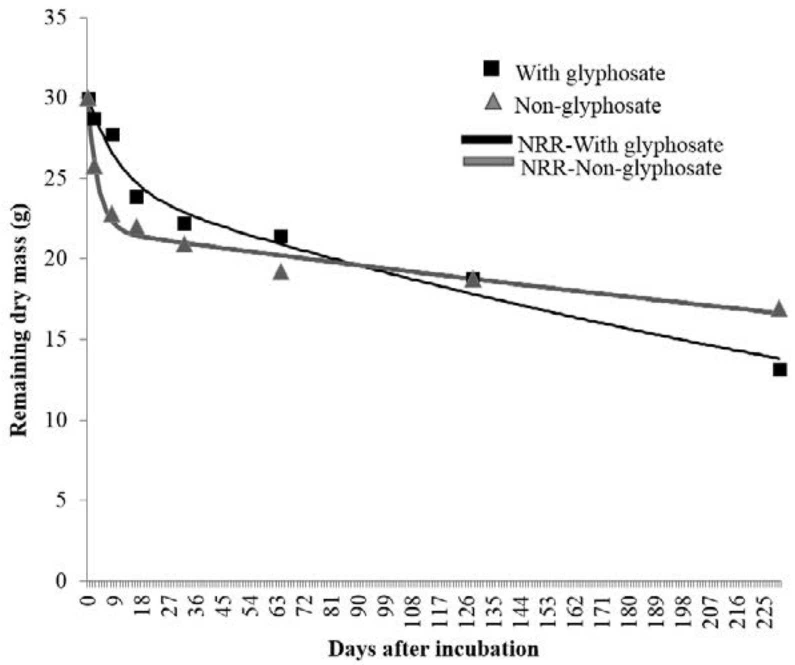

Figure 1. Shoot dry mass remaining from *Crotalaria spectabilis*. Nutrients release rate (NRR): estimation of decomposition rates and nutrient release from the shoot with and without glyphosate.

Figura 1. Masa seca de la parte aérea restante de *Crotalaria spectabili*s. Tasa de liberación de nutrientes (NRR): estimación de las tasas de descomposición y liberación de nutrientes de la parte aérea con y sin glifosato.

incorporation of their residues compared to their maintenance on the soil surface.

The mineralized nutrient contents of the remaining shoot dry mass from *C. spectabilis* with and without the glyphosate application are represented in **Figure 2**.

The residues decomposition rate and the release of nutrients can be influenced mainly by N, C:N ratio, lignin and polyphenols ratio (Bono & Alvarez, 2012). In the present work, there was a rapid reduction of the N content, which can lead to losses of this element to the environment, in case there is no synchronism between this release and the necessity of the successor culture. This release occurred more markedly for the shoot with glyphosate, and at 35 days more than half of the N content had already been mineralized, whereas for the shoot without herbicide, slower.

The shoot with glyphosate presented greater initial mineralization, equilibrating at the end of the incubation period. This occurred due to the translocation effect of the N between the period of herbicide application and the collect of this material, because the plants without herbicide had higher levels of N on the first day of analysis. Haney *et al.* (2002) reported that N mineralization increased with glyphosate concentration, as observed in our work, and the magnitude of N mineralized between 28 and 56 days increased with increasing doses of this herbicide. Damin *et al.* (2012) reported losses of N in the soil-plant system after application of glyphosate and glufosinate, which may have affected mineralization rates.

Decrease in the essential chemical elements concentration such as N accompany a decrease in the size and formation of plant roots, thus reducing phytomass, as observed by Zablotowicz & Reddy (2007) in a study with transgenic soybean after the application of glyphosate. This damage is caused by the production of aminomethylphosphonic acid (AMPA) obtained by the degradation of the herbicide active principle when it participates in the vegetable metabolic route. High concentrations of AMPA reduce chlorophyll synthesis, photosynthesis and phytomass accumulation (Zobiole *et al.*, 2012).

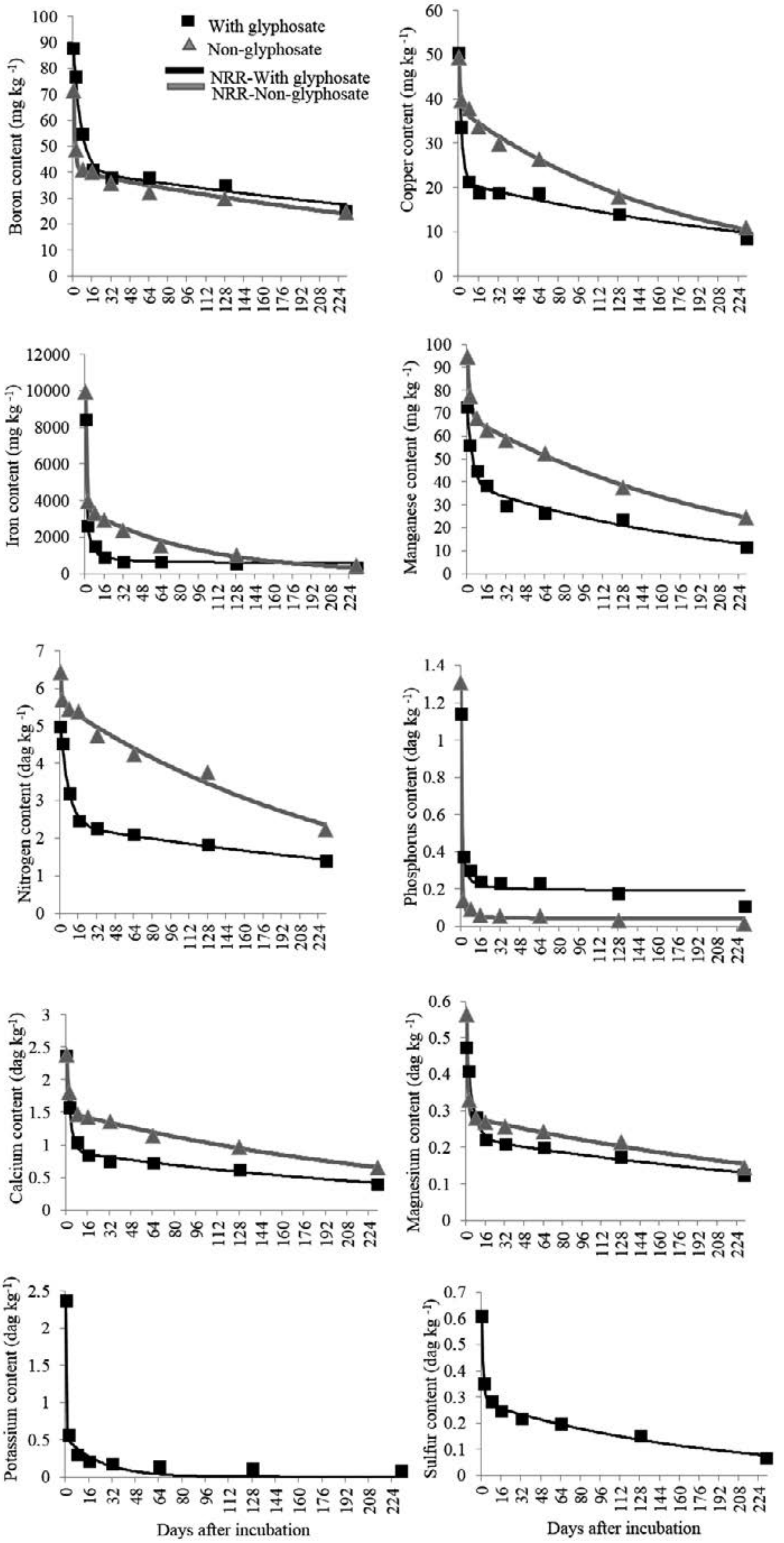

Figure 2. B, Cu, Fe, Mn, S and K (mg.kg^{-1}) content and N, P, Ca and Mg (dag Kg^{-1}) mineralized from the dry mass remaining on *Crotalaria spectabilis* with and without the application of herbicide. NRR: Nutrient release rate.

Figure 2. Contenido de B, Cu, Fe, Mn, S y K (mg. kg-1) y N, P, Ca y Mg (dag Kg-1) mineralizados de la masa seca remanente en *Crotalaria* spectabilis con y sin la aplicación de herbicida. NRR: tasa de liberación de nutrientes.

There was a similarity in the P release curves with and without glyphosate application. Initially there was a rapid release in the first evaluations and at 8 days there had already been a reduction of more than 50% in the P content, tending to a slower release in the subsequent evaluations. This P released from the remaining dry mass may be available for both uptake by the root system of the subsequent crop and for immobilization into mineral compounds. In this situation, the glyphosate adsorption by soil occurred due to the formation of complexes with the metals of the oxides, through the competition between the herbicide and the phosphate ions by specific adsorption sites in the soil (Prata *et al.*, 2003).

Calcium mineralization was more intense around 32 days of analysis with or without glyphosate. In general, there was rapid release of Ca at the beginning of experiment, stabilizing as the dry mass was mineralized. Crusciol *et al.* (2005) observed the same situation when analyzing the release rate of this nutrient in the radish forage. This rapid initial release is due to the very low rate of Ca redistribution due to the low concentration in the phloem and, unlike K, most of the Ca found in the plants is in insoluble forms.

Glyphosate interferes in the transport of Ca and Mg in reproductive organs, and can immobilize these elements in the root or in vegetative tissues (Cakmak *et al.*, 2009). In this work, there was a high Mg release up to 30 days by the plants that received the herbicide and in contrast to Mg, there was rapid K release with or without glyphosate. In the first 30 days there was a decrease in the content of this nutrient, probably by leaching, transferring K to the soil, since mineralization is not a prerequisite for their release. At 100 days, all K had already been mineralized from the remaining dry mass, which was also demonstrated by Rosolem *et al.* (2003). In this context, there was also a rapid release of S, associated with the rapid mineralization of this element already in the first evaluations, although it slowed in the last days of analysis, emphasizing however that the glyphosate and the period of evaluation (age) acted independently for the mineralization for this element.

For the element B, a high release occurred in the first evaluations, and for the shoot without herbicide had released 50% of B remaining, however, the material that received herbicide presented greater stability in the process of B mineralization over time. For Cu, the release was initially high, highlighting the shoot with glyphosate and the continuous release of Cu until the last evaluation. These results differ from the Santos *et al.* (2007) works, where the authors observed a reduction of Cu in soybean when treated with glyphosate.

There was a rapid initial release of Fe up to 16 days, decreasing until 170 days, when the shoot mineralization from *C. spectabilis* without glyphosate was equal to the remaining Fe mineralization of shoot dry mass with herbicide. For the Mn, it was more liberated until the 32 days and then less marked and constant release. Thus, as observed by Eker *et al.* (2006), the uptake and translocation to the shoot of Mn and Fe were affected by the application of glyphosate, since the Fe and Mn uptake systems are more susceptible to action of this herbicide.

CONCLUSION

In this work, we have assumed that the application of glyphosate would influence the release of the evaluated nutrients. The remaining dry mass of *Crotalaria spectabilis* that received glyphosate presented greater mineralization and faster reduction for most of the elements, causing considerable loss of N and P mainly in comparison to the material without this herbicide. Thus, we should take into account that the decrease in the concentration of essential chemical elements, such as N, leads to a decrease in the size and formation of the plants roots and its can reducing their phytomass. Finally, we confirm that glyphosate has affected the decomposition e release of nutrients from this legume and its requiring another's cautious studies on the use of this herbicide.

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