

## Effect of glyphosate on soil nitrogen mineralization

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### Abstract

*In this study, glyphosate was used to determine the effect of increasing rate of application on soil nitrogen mineralization. The experiment comprised three glyphosate treatments (i.e. 0.3546, 3.456, and 35.46 µg/g soil) with a control (without glyphosate). A Completely Randomized Design (CRD) was used with four replicates. Determination of NH<sub>4</sub><sup>+</sup>-N and NO<sub>3</sub><sup>-</sup>-N were carried out at 1, 3, 5, 7, 10, 14, 21, 35 and 56 days after herbicide application.*

*Result showed that NH<sub>4</sub><sup>+</sup>-N content was not significantly ( $P \leq 0.05$ ) reduced at the recommended field rate after one-day incubation. After 7 days and thereafter, no significant ( $P \leq 0.05$ ) changes were observed among treatments. Results also revealed that NO<sub>3</sub><sup>-</sup>-N content of the treatment 35.46 µg/g soil was significantly ( $P < 0.05$ ) increased from the control after one and three days of incubation period. Thereafter, significant ( $P < 0.05$ ) changes were not observed for any treatment throughout the incubation period. Glyphosate had a positive effect on ammonification at all application rates, in spite of slight inhibition at the beginning of the experiment. In contrast, glyphosate showed no significant ( $P \leq 0.05$ ) stimulatory or inhibitory effects on nitrification. It could be concluded that nitrification was less affected by glyphosate even at higher rates (35.46 µg/g soil) and thus, application of glyphosate in red yellow podzolic soils would not be harmful to of N mineralization.*

**Key words:** Ammonification, nitrification, red yellow podzolic soils

### Introduction

The intensive use of synthetic herbicides has raised increasing concerns today mainly due to their massive pollution of the environment, including the soil systems. Glyphosate {N-(Phosphomethyl)glycine - C<sub>3</sub>H<sub>8</sub>NO<sub>5</sub>P} is a broad-spectrum, nonselective systemic herbicide used for controlling annual and perennial weeds including grasses, sedges, broad-leaved weeds, and woody plants. Assessment of C and N mineralization immediately after application may provide a more realistic understanding of the true effect of this herbicide on soil microbial activity. However studies on N mineralization from glyphosate applied soils have not been reported. Therefore the objective of the present study was to determine the effect of different rates of glyphosate on soil N mineralization.

### Methodology

#### Soil sampling and Sample preparation

The soil belonged to red yellow podzolic great soil group and classified as Hapludults according to the USDA soil taxonomy (Mapa *et al.*, 1999). Soil samples were drawn randomly from selected locations at the research farm of the Faculty of Agriculture, University of Ruhuna, Mapalana, Kamburupitiya. Samples were taken from 0 – 15 cm depth using a soil auger, after removing the surface litter of the soil, and then mixed well to make a composite sample, to be used for the incubation

study. Soils were air-dried and crushed with a rubber tipped pestle and sieved through 2 mm mesh, placed in glass bottles, and pre - incubated for a week at 60 % water holding capacity prior to the application of herbicides.

### Glyphosate treatment

Glyphosate (phosphomethyl glycine/roundup – 360g active ingredient/L) solution was added to the soil at the rate of 35.46, 354.6 and 3546µg/ml to obtain concentrations 0.3546, 3.546 and 35.46 µg/g soil, corresponding to field rate, 10 times, and 100 times of field rate. The conversion of the field rate application to µg of glyphosate per g soil was calculated, assuming an even distribution of glyphosate in the 2 mm soil layer and a bulk density of 1.5 g/cm<sup>3</sup>. Control and herbicide treated soil samples were then placed in the dark at room temperature (25 °C) for incubation. Constant moisture content of the soil was maintained by daily monitoring and adding distilled water when necessary. After the incubation period of 1, 3, 5, 7, 10, 14, 21, 35 and 56 days, four glass bottles including a control for each treatment were removed and analyzed for nitrogen mineralization.

### Nitrogen Mineralization

N mineralization was determined using the inorganic N (NH<sub>4</sub><sup>+</sup>-N and NO<sub>3</sub><sup>-</sup>-N) concentration of soil extracted 1, 3, 5, 7, 10, 14, 21, 35 and 56 days after glyphosate treatments. Soil samples containing 10 g of soil, were extracted using 30ml of 2 M KCl and extracts was used to determine NH<sub>4</sub><sup>+</sup>-N and NO<sub>3</sub><sup>-</sup>-N in soil, using a UV/visible spectrophotometer.

### Results and Discussion

#### NH<sub>4</sub><sup>+</sup> - N

Results revealed that average NH<sub>4</sub><sup>+</sup> - N contents of the soil were 13.42, 14.44, 16.4 and 17.44 for treatments C0, C1, C2 and C4, respectively, showing significant (P > 0.05) differences as compared with the control, except C1 (field rate) after one day incubation (Table 1). Soil NH<sub>4</sub><sup>+</sup> - N contents were reduced on the 3<sup>rd</sup> day of incubation period showing no significant (P ≤ 0.05) differences, compared to control except C3. Since the incubation period of 7 days, no significant changes could be observed among treatments until the end of the experiment (56 days) (Fig. 1).

**Table 1. Effect of different rates of glyphosate on NH<sub>4</sub><sup>+</sup> - N contents (mg/kg soil) of soil**

Incuba.period (days)	C3	C2	C1	Co	LSD (Pd <sup>0.05</sup> ) <sup>x</sup>
1	17.43a(2.52) <sup>y</sup>	16.39 ab (0.63)	14.43 bc (0.84)	13.41c (1.26)	2.3
3	11.89 a (0.69)	7.91b (1.09)	9.56b (1.85)	7.54 b (1.57)	2.1
5	13.34 a (0.22)	11.26b (0.57)	10.19b (1.14)	10.50b (0.95)	1.2
7	13.10 a (1.85)	12.13 a (2.75)	11.03 a (1.31)	10.66 a (0.68)	2.8
10	13.68 a (1.66)	12.61a (0.93)	11.49 a (2.25)	11.58 a (1.94)	2.7
14	14.91a (0.61)	13.44 a (1.75)	11.99 a (3.16)	11.87 a (1.22)	3.0
21	15.42 a (3.41)	13.85 a (1.88)	12.52 a (1.68)	12.02 a (3.06)	3.9
35	16.59 a (2.25)	14.48 a (2.01)	13.77 a (1.93)	13.44 a (0.92)	3.4
56	16.13 a (0.58)	15.17 a (1.48)	14.29 a (1.11)	14.24 a (1.66)	1.9

<sup>x</sup>Means followed by the same letter (in each row) are not significantly different at P ≤ 0.05.

<sup>y</sup> Value in parenthesis is the standard deviation of means.

Co – Control (Without herbicide)

C1 – 0.3546 µg/g soil

C2 – 3.546 µg/g soil

C3 –35.46 µg/g soil

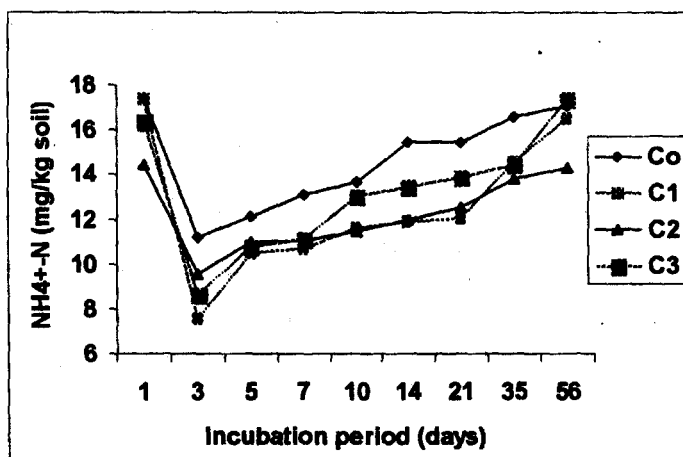


Fig. 1: Effect of glyphosate on NH<sub>4</sub><sup>+</sup> - N content of the soil

Higher extractable NH<sub>4</sub><sup>+</sup> - N levels in soils treated with high propanil concentration in this study could result from stimulation of organic N ammonification, or from depression of ammonium oxidation activity. It could also be due to the release of NH<sub>4</sub><sup>+</sup> - N from lysed microbial cells killed due to herbicide toxicity. Previous investigators (Koike 1961; Chandra and Bollen 1961; Macalady et al., 1998) have also found that higher extractable ammonium levels in soil treated with high concentrations of metam sodium fungicides would be due to organic N ammonification or from depression of ammonium oxidation activity.

### NO<sub>3</sub><sup>-</sup> - N

Results revealed that there was little or no effect by the applied herbicide on NO<sub>3</sub><sup>-</sup> - N content. However, treatment C3 was significantly (*P* < 0.05) different from the control after one and three days of incubation. Since then, significant differences were no longer observed for any treatment until the end of the incubation period. However, the average NO<sub>3</sub><sup>-</sup> - N contents of the soil increased with the increasing incubation period (Fig. 2). After one day of incubation, the average NO<sub>3</sub><sup>-</sup> - N contents of the soil were 4.83, 4.52, 4.37 and 6.17 for treatments C0, C1, C2 and C3 respectively and at the end of the experiment (56 days) NO<sub>3</sub><sup>-</sup> - N contents have increased to 24.99, 24.62, 24.82 and 24.79 for the respective treatments (Table 2).

Table 2. Effect of different rates of glyphosate on NO<sub>3</sub><sup>-</sup> - N (mg/kg soil) content of soil

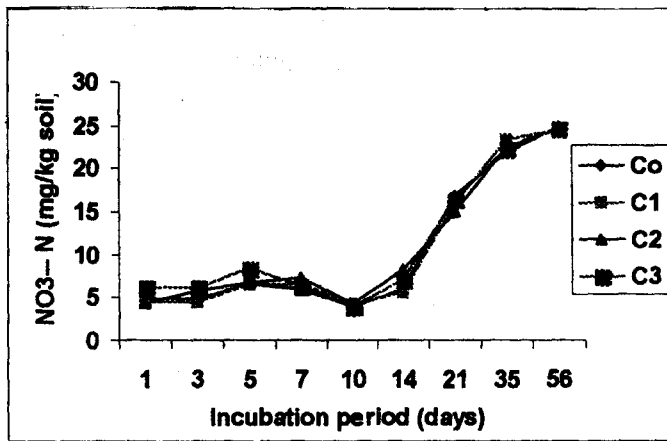
Incuba.period (days)	C3	C2	C1	Co	LSD (Pd <sup>0.05</sup> ) <sup>x</sup>
1	6.16 <sup>a</sup> (0.74) <sup>y</sup>	4.36 <sup>b</sup> (0.34)	4.52 <sup>b</sup> (0.69)	4.83 <sup>b</sup> (0.93)	1.1
3	6.24 <sup>a</sup> (0.59)	5.72 <sup>ab</sup> (0.76)	4.41 <sup>b</sup> (0.68)	4.90 <sup>b</sup> (1.28)	1.3
5	8.51 <sup>a</sup> (2.48)	6.65 <sup>a</sup> (0.32)	6.60 <sup>a</sup> (0.46)	6.53 <sup>a</sup> (0.86)	2.0
7	6.39 <sup>a</sup> (0.97)	7.32 <sup>a</sup> (1.13)	6.49 <sup>a</sup> (0.27)	6.02 <sup>a</sup> (1.22)	1.5
10	3.90 <sup>a</sup> (0.99)	4.24 <sup>a</sup> (1.82)	4.38 <sup>a</sup> (1.51)	4.01 <sup>a</sup> (1.32)	2.2
14	7.14 <sup>a</sup> (0.95)	8.27 <sup>a</sup> (3.22)	5.67 <sup>a</sup> (0.53)	5.98 <sup>a</sup> (0.14)	2.6
21	16.42 <sup>a</sup> (1.16)	14.93 <sup>a</sup> (2.12)	15.90 <sup>a</sup> (1.63)	16.80 <sup>a</sup> (0.80)	2.3
35	22.36 <sup>a</sup> (1.17)	22.43 <sup>a</sup> (1.47)	23.49 <sup>a</sup> (1.48)	21.91 <sup>a</sup> (0.50)	1.9
56	24.78 <sup>a</sup> (1.20)	24.81 <sup>a</sup> (1.74)	24.61 <sup>a</sup> (1.75)	24.99 <sup>a</sup> (2.65)	2.9

<sup>x</sup>Means followed by the same letter (in each row) are not significantly different at *P* ≤ 0.05.

<sup>y</sup> Value in parenthesis is the standard deviation of means.

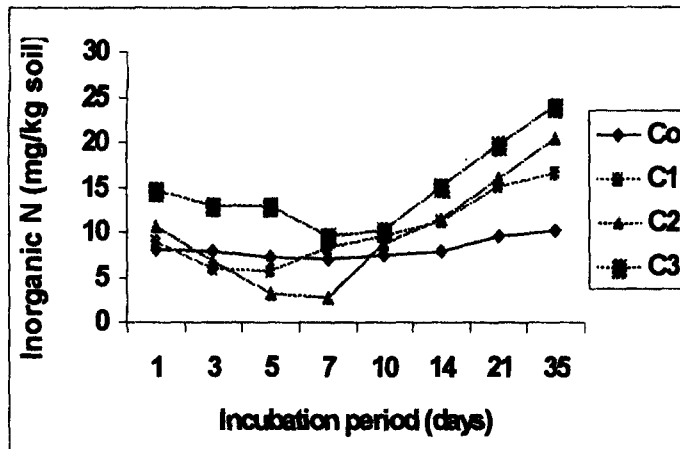
Co – Control (Without herbicide)      C1 – 0.3546 µg/g soil

C2 – 3.546 µg/g soil                              C3 – 35.46 µg/g soil



**Fig. 2: Effect of glyphosate on NO<sub>3</sub><sup>-</sup> - N content of the soil**

Fig. 3 shows the response of total inorganic nitrogen content to the applied glyphosate treatments. Accordingly more mineral N was observed in soil containing higher concentration of glyphosate, which may have been due to enhanced mineralization of organic N.



**Fig. 3: Effect of different rates of glyphosate on nitrogen mineralization**

Co – Control (Without herbicide)

C1 – 0.3546 µg/g soil

C2 – 3.546 µg/g soil

C3 – 35.46 µg/g soil

Stratton and Stewart (1991), tested the effects of glyphosate on ammonification, nitrification and denitrification using forest litter and forest soil. According to them, there was a stimulation of activity in ammonification both in the forest litter and forest soil after exposure to glyphosate. Nitrification rates in forest litter and forest soil were very low and glyphosate had no appreciable stimulatory or inhibitory effect on nitrification. They found that, glyphosate stimulated ammonification by 50% at concentrations ranging from 140-550. µg g<sup>-1</sup>. With regard to nitrification, it was estimated that glyphosate concentrations greater than 1000-2000. µg g<sup>-1</sup> would be required to cause a 50% inhibition of activity.

Carlisle and Trevors (1986) also found that ammonium oxidation rate was reduced by the applied glyphosate and thus no influence on NO<sub>3</sub><sup>-</sup> - N content of the soil.

Heterotrophic soil microorganisms acquire C and N for maintenance and growth by decomposing plant residues and other organic materials in soils. Herbicides with low C: N ratios may potentially be readily mineralized with N that is in excess of microbial demand being released in the inorganic form (Alexander, 1977). When compared with propanil C: N ratio was low in glyphosate (propanil 9:1 and glyphosate 3:1) and thus low C: N ratio may have an immediate effect on soil microbial activity. Haney *et al.*, (2002) also found that addition of low C: N ratio herbicides, stimulates microbial activity and enhances the eventual mineralization of the compounds.

## Conclusions

- ❖ Glyphosate had a positive effect on ammonification at all application rates, in spite of slight inhibition at the beginning of the experiment.
- ❖ Glyphosate showed no significant ( $P > 0.05$ ) stimulatory or inhibitory effects on nitrification. Therefore, it could be concluded that nitrification was less affected by glyphosate even at higher rates (35.46  $\mu\text{g/g}$  soil).

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