EFFECT OF THE HERBICIDE GLYPHOSATE ON NITRIFICATION, DENITRIFICATION, AND ACETYLENE REDUCTION IN SOIL

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Abstract. The effect of glyphosate on N_2 fixation, denitrification, and nitrification in an agricultural soil was investigated. Effects of the pure herbicide and commercial formulation, Roundup® (Monsanto Company), were compared in soil under aerobic and anaerobic conditions. Anaerobic C_2H_2 reduction was inhibited by high herbicide levels. Denitrification in non-amended soil was either unaffected (N_2O reduction) or stimulated (NO_3^- reduction); in glucose-amended soil, N_2O reduction was inhibited and NO_3-reduction unaffected by both glyphosate and Roundup. Roundup caused greater stimulation of N_2O reduction than pure glyphosate; no other significant formulation effects were observed. Nitrification was inhibited by the two formulations. Ammonium oxidation were both influenced. Pure glyphosate was more inhibitory than Roundup. No toxicity to any of these activities should be seen at recommended field application rates of the herbicide.

1. Introduction

Increasing herbicide usage has led to concern about the effects of these chemicals on non-target soil microorganisms. Microorganisms involved in N cycling are among the groups for which toxicity testing has been recommended under the Toxic Substances Control Act (Stern, 1980). Various microbial activities have been studied in order to assess herbicide toxicity to these organisms: soil respiration (Anderson *et al.*, 1981); estimation of N_2 fixation by the acetylene (C_2H_2) reduction assay (Tam and Trevors, 1981; Tu, 1978a); nitrification (Tu, 1978b); P solubilization and S oxidation (Lewis *et al.*, 1978).

Glyphosate (*N*-phosphonomethyl glycine) is a relatively new herbicide that is already widely used. It is rapidly inactivated in most soils, being bound to clays through the phosphonate group (Sprankle *et al.*, 1975), and is degraded at rates that range from 50% in 28 days (Rueppel *et al.*, 1977) to as low as 0.8% in 60 days (Nomura and Hilton, 1977), depending on soil type. Roslycky (1982) found that soil respiration was either stimulated or inhibited by glyphosate, depending on the dose. High levels of glyphosate caused an initial increase in bacterial and actinomycete numbers, but the herbicide had little effect on the final populations of bacteria, actinomycetes, or fungi after a 214 day period. Marsh *et al.* (1977) studied the effect of the herbicide on N_2 transformation and respiration in soil; the effect on cellulose degradation has also been investigated (Grossbard and Wingfield, 1978). Glyphosate can be used in a P source by some

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Pseudomonas species (Moore *et al.*, 1983; Shinabarger and Braymer, 1985; Shinabarger *et al.*, 1984; Talbot *et al.*, 1984) and by *Alcaligenes* (Talbot *et al.*, 1984). Other studies have examined adsorption and degradation of glyphosate in soils (Nomura and Hilton, 1977; Sprankle *et al.*, 1975); persistence in soil (Eberbach and Douglas, 1983); detoxification in soil (Rueppel *et al.*, 1977; Torstensson and Aamisepp, 1977; Moshier and Penner, 1978); the effect on Ca^{+2} uptake and translocation in soybean seedlings (Duke *et al.*, 1983) and on selected plant processes (Cole *et al.*, 1983); the mode of action of glyphosate (Bode *et al.*, 1984; Steinrücken and Amrhein, 1980); microbial resistance to the herbicide (Comai *et al.*, 1983; Schulz *et al.*, 1984). Few of these studies are concerned with the effects of the commercial formulation, Roundup® (Monsanto Company) on microbial activities (Marsh *et al.*, 1977; Torstensson and Aamisepp, 1977), and there are no reported comparisons of the effects of pure glyphosate and the commercial formulation.

In a previous study, we compared the effects of pure glyphosate and Roundup on soil respiration and H_2 oxidation in an agricultural soil, under both aerobic and anaerobic conditions (Carlisle and Trevors, 1985). Now we report the effects of the two herbicide formulations on N cycling in the same soil: aerobic and anaerobic C_2H_2 reduction (an estimate of N_2 fixation), denitrification, and nitrification.

2. Materials and methods

2.1. Soil samples

Sandy loam soil was collected from the top 10 cm of an agricultural field at Floradale, Ontario, Canada. The soil was sieved, and the 2 to 4 mm soil aggregates were stored at 4 °C. Various characteristics of the soil (pH 6.5; combustible matter, 10.2% (dry weight); sand, 56%; silt, 34%; clay, 10%; carbohydrate, 54.4 mg^{-1}; NH_4^+–N; 2.5 $\mu g\, g^{-1}$) have been described by Tam and Trevors (1981).

All soil treatments were performed in triplicate. All results are averages, expressed on a g dry weight soil basis.

Pure glyphosate or the commercial formulation of the isopropylamine salt, Roundup® (Monsanto Chemical Co., Mississauga, Ontario), were dissolved or diluted in distilled water to appropriate concentrations. All herbicide concentrations are given as concentrations of active ingredient (AI).

2.2. Nitrogen fixation

The C_2H_2 reduction assay (Hardy *et al.*, 1973) was used to estimate N_2 fixation rates in soil under both aerobic and anaerobic conditions.

Experiments were performed using 10 g fresh sieved soil (or sterile soil for the sterile controls) in 50 mL Erlenmeyer flasks sealed with serum stoppers (Suba-Seal, Barnsley, England). Aerobic soil treatments were brought to 65% soil water holding capacity by adding 0.7 mL liquid (distilled water, glucose solution, herbicide solution); the flasks were left air-filled, and sealed with serum stoppers. Anaerobic soil treatments were

saturated by adding 2.0 mL liquid; the flasks were sealed with serum stoppers, and evacuated and backfilled with He five times. Acetylene was added by removing 5 mL of the gas phase from each flask and adding 5 mL pure C_2H_2 gas. Flasks were incubated in the dark at 20 °C; gas samples were taken at 24 or 48 hr intervals during a 7-day (anaerobic) or 9-day (aerobic) incubation for ethylene (C_2H_4) and (aerobic flasks only) O_2 analysis.

For each experiment, four treatments of the two herbicides were used: no herbicide; a low level of herbicide, 12.7 μg active ingredient (AI) g^{-1} dry soil; a medium level, 127 μg AI g^{-1}; a high level, 635 μg AI g^{-1}. Preliminary experiments showed that the C_2H_2 reduction rates in non-amended soils were negligible; therefore, soils were amended with 0.9 mg glucose g^{-1} dry soil. All solutions were distributed evenly over the soil surface using a 1 mL syringe.

Ethylene levels were measured using an Antek 300 gas chromatograph with a stainless steel, 189 cm × 3.2 mm Porapak N 80/100 mesh column and a flame ionization detector. Detector and injector temperatures were 150 °C; the column temperature was 100 °C. The carrier gas, N_2, was maintained at a pressure of 144 kPa. The flow rate of air was 150 mL min^{-1}; of H_2, 15 mL min^{-1}. Oxygen was measured using a Gow-Mac 69–150 gas chromatograph equipped with a thermal conductivity detector and a stainless steel, 92 cm × 6 mm Molecular Sieve 5A column. The bridge current was maintained at 150 mA, the column temperature at 50 °C, and the He carrier gas at a flow rate of 50 mL min^{-1}. All gas concentrations were determined by comparison to pure gas standard curves.

2.3. Denitrification

Two assays were used to determine denitrification rates in soil; the depletion of nitrous oxide (N_2O) by conversion to nitrogen gas (N_2) (Evans *et al.*, 1985); the conversion of nitrate (NO_3^-) to N_2O in the presence of C_2H_2, which inhibits further denitrification (Yoshinari *et al.*, 1977). Both assays were performed under anaerobic conditions, with and without glucose amendment.

Erlenmeyer flasks containing 10 g fresh soil were treated with the four herbicide levels (0 μg AI g^{-1}, 12.7 μg g^{-1}, 127 μg g^{-1}, 635 μg g^{-1}) as described above. Glucose was added to one set of treatments to a concentration of 0.9 mg g^{-1} dry soil. For the second assay (NO_3^- reduction), 1.0 mL of 1 mg mL^{-1} KNO_3 was added to each flask. A total of 2.0 mL liquid was added to each flask, distributed evenly over the soil surface using a 1 mL syringe. Flasks were sealed with serum stoppers, evacuated and backfilled with He 5 times.

For the N_2O reduction assay, 1 mL of the gas phase was removed from each flask and replaced with 1 mL pure N_2O. Control flasks were also prepared, lacking added N_2O, to monitor production of N_2O from endogenous NO_3^-. Control values were used to correct the experimental values for N_2O production during the experiment. Flasks were incubated at 20 °C, in the dark. Samples were taken at 24 or 48 hr intervals over a 6-day period, using a Hamilton gas-tight syringe, and the N_2O concentration determined.

For the NO_3^- reduction assay, 5 mL of the gas phase were removed and replaced with 5 mL pure C_2H_2. The rate of N_2O generation was monitored by taking samples over a 4-day period and measuring the amount of N_2O present.

Nitrous oxide was measured with a Gow-Mac 69–150 gas chromatograph equipped with a thermal conductivity detector and a stainless steel, 152 cm × 6 mm Porapak Q column. Operating conditions were as previously described for O_2 analysis.

2.4. Nitrification

Rates of conversion of ammonium (NH_4^+) to nitrite (NO_2^-) and nitrate (NO_3^-) were measured in soil perfusion columns (Lees and Quastel, 1946; Quastel and Scholefield, 1957). Each soil column contained 50 g fresh soil, and was perfused by 200 mL of liquid (perfusate) at a flow rate of 2 mL min^{-1}. Five herbicide levels were tested for each formulation: 0 μg AI g^{-1} dry soil; 76.7 μg g^{-1}; 230 μg g^{-1}; 767 μg g^{-1}. Ammonium sulphate was added to the perfusate to a concentration of 50 μg NH_4^+–N mL (157 μg NH_4^+–N g^{-1} dry soil). Samples were taken periodically over a 10-day period, after which the control NO_2^- and NO_3^- levels had stabilized. Levels of NO_2^- were measured using the modified Griess–Ilosvay method; NO_3^- concentrations were determined by the phenoldisulphonic acid method (Bremner, 1965).

2.5. Sterile controls

Sterile controls were run for the N_2 fixation and denitrification experiments. Sterile soil was obtained by autoclaving sieved soil for 1 hr on three consecutive days. All solutions added to sterile controls were either autoclaved (water) or filter-sterilized (NO_3^-, glucose, herbicides). Sterile controls were run for the herbicide-free and high-level herbicide treatments.

2.6. Statistical analysis

All statistical testing was performed on an Apple II + microcomputer using an Ed–Sci Statistics program (Ed–Sci Developments, Modesto, CA).

Means and standard deviations were determined for each data point ($n = 3$). Effect of herbicide dose was determined by analyzing selected data points by analysis of variance and the Student–Newman–Keuls multiple range test. Formulation effects were tested by comparing the data points using a two-tailed, unpaired Student's t-test. Final and initial gas concentrations in individual flasks were compared using a two-tailed, paired Student's t-test. All tests were performed at the 95% level of significance.

3. Results

3.1. Nitrogen fixation

Rates of N_2 fixation were statistically analyzed at the end of the experiments, on day 9 for aerobic flasks and day 7 for anaerobic flasks.

Aerobic C_2H_2 reduction rates were very low, even in glucose-amended soil (mean

C_2H_4 increase, controls: 0.64 nmol g^{-1} dry soil over 9 days). In half of the treatments (12.7 μg glyphosate g^{-1}; 635 μg glyphosate g^{-1}; Roundup control; 635 μg Roundup g^{-1}) there was no increase in C_2H_4 levels over the 9 day incubation period (data not shown). There was no significant difference between final C_2H_4 concentrations in any of the glyphosate or Roundup treatments. No difference in the effects of the two formulations was observed (Table I).

TABLE I

Acetylene reduction in glucose-amended soil. Comparison of effect of herbicide formulation on final C_2H_2 concentrations. A two-tailed unpaired Student's *t*-test was used, at the 95% confidence level

Conditions	Herbicide concentration (μg AI g^{-1} dry soil)	C_2H_4 concentration[a] (μmol g^{-1} dry soil)		Test result: significantly different
		G	R	
Aerobic	12.7	0.53 ± 0.36	0.75 ± 0.16	No
	127	0.47 ± 0.07	0.74 ± 0.20	No
	635	0.52 ± 0.44	0.13 ± 0.32	No
Anaerobic	12.7	601 ± 87.5	466 ± 36.6	No
	127	184 ± 113	176 ± 193	No
	635	1.98 ± 0.88	3.43 ± 3.37	No

[a] Mean (n = 3) ± 1 × Standard Deviation.
G: Glyphosate; R: Roundup.

Soils amended with glucose and incubated anaerobically showed a two-day lag in C_2H_4 production, after which C_2H_4 levels rose sharply in most treatments (Figure 1). Inhibition of C_2H_2 reduction by both medium and high levels of the two herbicide formulations was observed. Low herbicide levels caused no significant inhibition. No formulation effect was observed (Table I). Medium glyphosate treatments showed an average of 70.1% inhibition of C_2H_4 production compared to the controls, medium Roundup treatments showed, on average, 64.2% inhibition; however, the response of the soil N_2 fixing microorganisms was not significantly different in these treatments from the response in either the low or high dose treatments. Inhibition by 635 μg g^{-1} soil was very high. Glyphosate at this dose caused 99.3% inhibition; Roundup, 98.8%.

3.2. Denitrification: N_2O reduction

In non-amended soils, no initial lag was observed in reduction of N_2O, but rates were low. No inhibition or stimulation of this activity was seen in any of the herbicide treated soil over a 6-day period. The only significant variation in the data was that N_2O reduction was lower in the low Roundup treatment (12.7 μg g^{-1}) than in the equivalent glyphosate treatment (Table II).

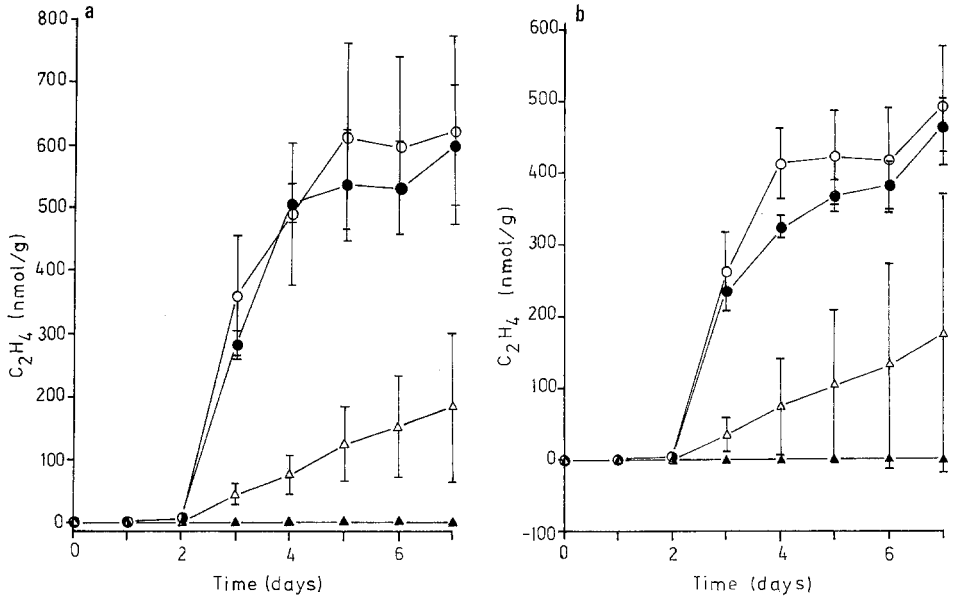

Fig. 1. Effect of glyphosate on anaerobic C_2H_2 reduction in glucose-amended soil: C_2H_2 production. (a) pure glyphosate; (b) Roundup. (○) Control, 0 μg active ingredient (AI) g^{-1} dry soil; (●) 12.7 μg AI g^{-1}; (△) 127 μg AI g^{-1}; (▲) 635 μg AI g^{-1}. All values were determined on a g dry soil basis. All points are the means of three determinations; error bars represent one standard deviation from the mean.

In glucose-amended soil, rates of N_2O reduction were low for the first 24 h, but then increased rapidly. Nitrous oxide was totally depleted in the controls near the end of the second day of incubation (data not shown).

Low and medium concentrations of glyphosate had no effect on the rate of N_2O reduction. High glyphosate (635 μg g^{-1}) inhibited reduction 37% compared to the control and the low glyphosate treatment, but the amount of N_2 reduced was not significantly different from that observed in the medium dose treatment. Similar effects were seen with Roundup; both medium and high levels of Roundup inhibited N_2O reduction by 21.4 and 37.3%, respectively. Nitrous oxide reduction in the low treatment was not significantly different from the control, but was different from both the higher treatments. The high level of Roundup was significantly more inhibitory than the medium dose. No difference between the effects of Roundup and glyphosate were observed (Table II).

3.3. Denitrification: NO_3^- reduction

Rates of N_2O production in non-amended soils were very low (Figure 2). Strong dose-dependent stimulation was observed in all but the lowest glyphosate treatment, which showed no significant difference from the control. The high glyphosate treatment stimulated N_2O production 3.3-fold, significantly more than the medium dose (2.5-fold). Similarly, high Roundup caused greater stimulation (4.1-fold) than the medium dose

TABLE II

Denitrification in glucose-amended and non-amended anaerobic soil. Comparison of effect of herbicide formulation on NO_3^- and N_2O reduction. A two-tailed unpaired Student's *t*-test was used, at the 95% confidence level

Activity measured	Herbicide concentration (μg AI g^{-1} dry soil)	Change in N_2O concentration[a] (μmol g^{-1} dry soil)		Test result: significantly different
		G	R	
		N_2O consumption		
N_2O reduction	12.7	1.15 ± 0.22	0.71 ± 0.10	Yes
(no glucose)	127	1.29 ± 0.22	0.88 ± 0.17	No
(day 6)	635	1.06 ± 0.28	1.02 ± 0.28	No
N_2O reduction	12.7	3.61 ± 0.27	4.29 ± 0.49	No
(glucose-amended)	127	3.00 ± 0.43	3.14 ± 0.09	No
(G: 48 hr; R: 41 hr)	635	2.19 ± 0.72	2.75 ± 0.43	No
		N_2O production		
NO_3^- reduction	12.7	0.36 ± 0.06	0.50 ± 0.06	Yes
(no glucose)	127	0.79 ± 0.07	0.88 ± 0.09	No
(day 4)	635	1.02 ± 0.07	1.28 ± 0.11	Yes
NO_3^- reduction	12.7	3.11 ± 0.08	2.96 ± 0.11	No
(glucose-amended)	127	2.82 ± 0.11	2.78 ± 0.09	No
(day 2)	635	2.95 ± 0.12	2.77 ± 0.21	No

[a] Mean ($n = 3$) ± 1 × Standard Deviation.
G: Glyphosate; R: Roundup.

(2.8-fold); both caused more stimulation than the low dose (1.6-fold). Some formulation effects were observed: low and high levels of Roundup stimulated NO_3^- reduction to a greater extent than equivalent glyphosate treatments. The effects of the medium doses of the two formulations were not significantly different (Table II).

In glucose-amended soil, NO_3^- reduction to N_2O showed a 1-day lag followed by a rapid increase over the next 24 hr, after which levels plateaued (Figure 3). At the end of the sampling period, some inhibition was caused by medium and high levels of glyphosate (9.62 and 8.65%, respectively). The low glyphosate treatment, and all Roundup treatments, had no significant effect on NO_3^- reduction. No difference between the effects of the two formulations was detected (Table II).

The effects of herbicide treatments were compared at day 2, also. High levels of glyphosate inhibited NO_3^- reduction by 20.2% compared to the control; lower doses of glyphosate, and all Roundup treatments, had no effect.

3.4. Nitrification

Nitrite and NO_3^- levels were compared at three points during the experiments: day 3, day 6, and the final day (glyphosate, day 9; Roundup, day 10). Levels of NO_2^-

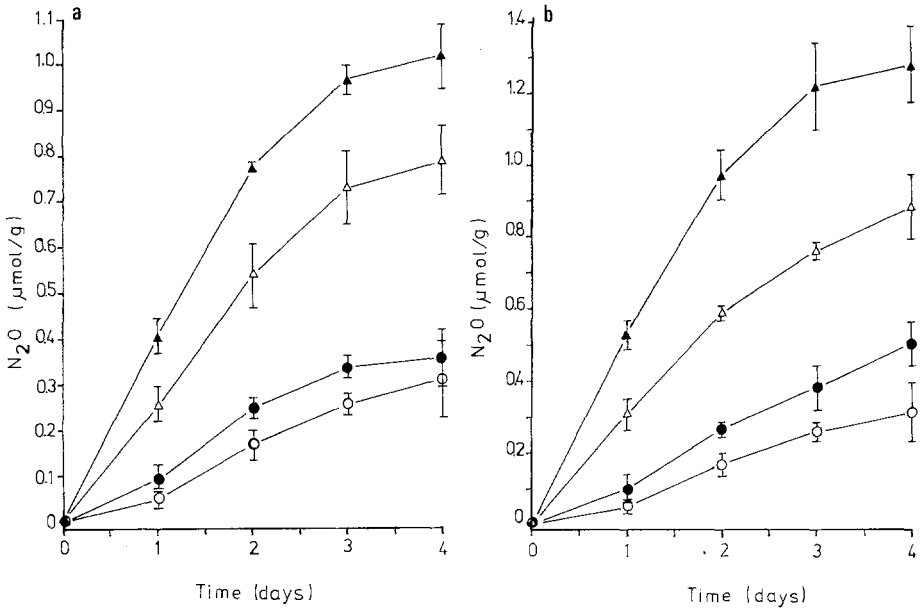

Fig. 2. Effect of glyphosate on N_2O reduction in non-amended soil: N_2O produced. (a) Pure glyphosate; (b) Roundup. Symbols are as described in Figure 1.

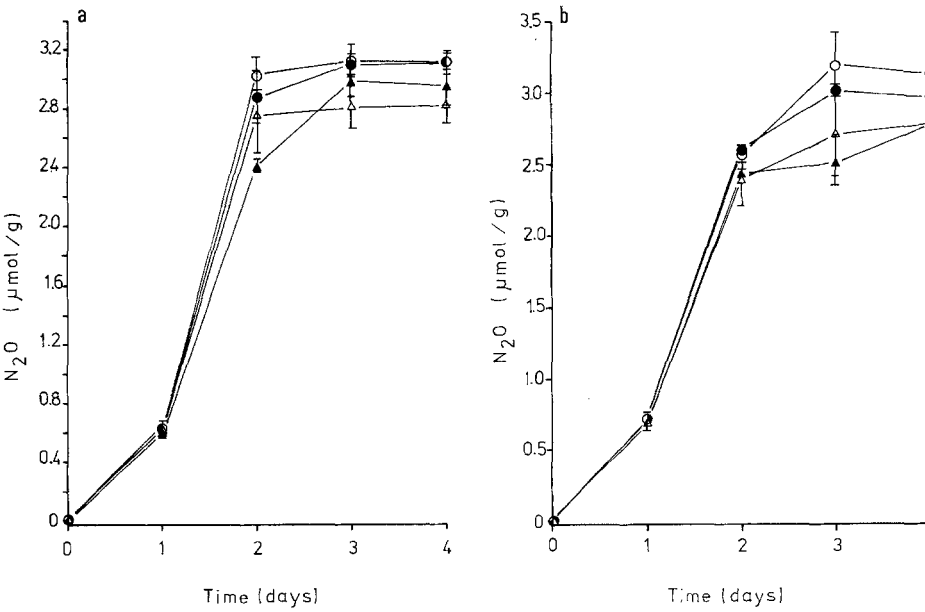

Fig. 3. Effect of glyphosate on N_2O reduction in glucose-amended soil: N_2O produced. (a) Pure glyphosate; (b) Roundup. Symbols are as described in Figure 1.

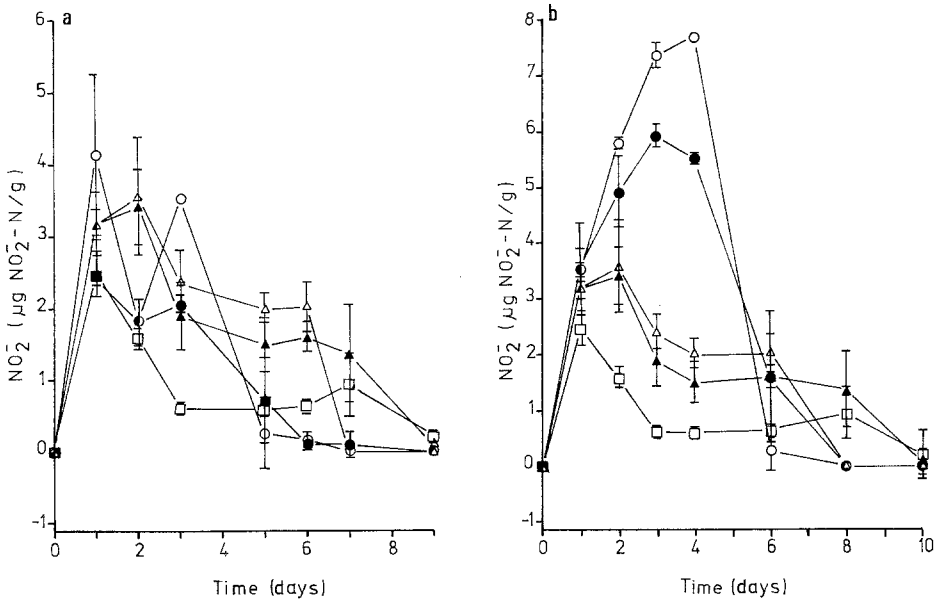

Fig. 4. Effect of glyphosate on NO_2-production in $(NH_4)_2SO_4$-amended soil: (a) pure glyphosate; (b) Roundup. (○) Control, 0 μg AI g^{-1} dry soil; (●) 76.7 μg AI g^{-1}; (△) 230 μg AI g^{-1}; (▲) 460 μg AI g^{-1}; (□) 767 μg AI g^{-1}. All values were determined on a g dry soil basis. All points are the means of three determinations; error bars represent one standard deviation from the mean.

generally increased initially, then dropped (Figure 4). Nitrate levels increased to a maximum, and then remained constant (Figure 5).

After 3 days, a dose-dependent inhibition of NO_2^- production was seen with both herbicide formulations in all except the lowest glyphosate treatment, which was not significantly different from the control. The NO_2^- concentrations in the two lowest Roundup treatments (76.7 μg g^{-1}, 230 μg g^{-1}) were not significantly different.

By day 6, a more complex response had developed. The level of NO_2^- in the glyphosate control and the low glyphosate treatment (76.7 μg g^{-1}) had dropped considerably. The two intermediate treatments (230 μg g^{-1}, 460 μg g^{-1}) showed decreased NO_2^- compared to day 3 levels. Nitrite in the high dose treatment (767 μg g^{-1}) had increased slightly. There was no significant difference between NO_2^- levels in the control and low treatments. All other treatments had NO_2^- levels that were significantly higher than the control, and significantly different from each other. The highest NO_2^- treatment; the level of NO_2^- was higher in the 460 μg g^{-1} treatment than in the 767 μg g^{-1} treatment. Due to high variation among the Roundup treatment replicates, no significant difference between treatments was found; however, the means show a similar trend. Levels of NO_2^- in the three higher glyphosate treatments were significantly lower than in the equivalent Roundup treatments; the controls and low herbicide treatments were not significantly different (Table III).

At the end of the experiment, no NO_2^- was detectable in controls, 76.7 μg g^{-1} and

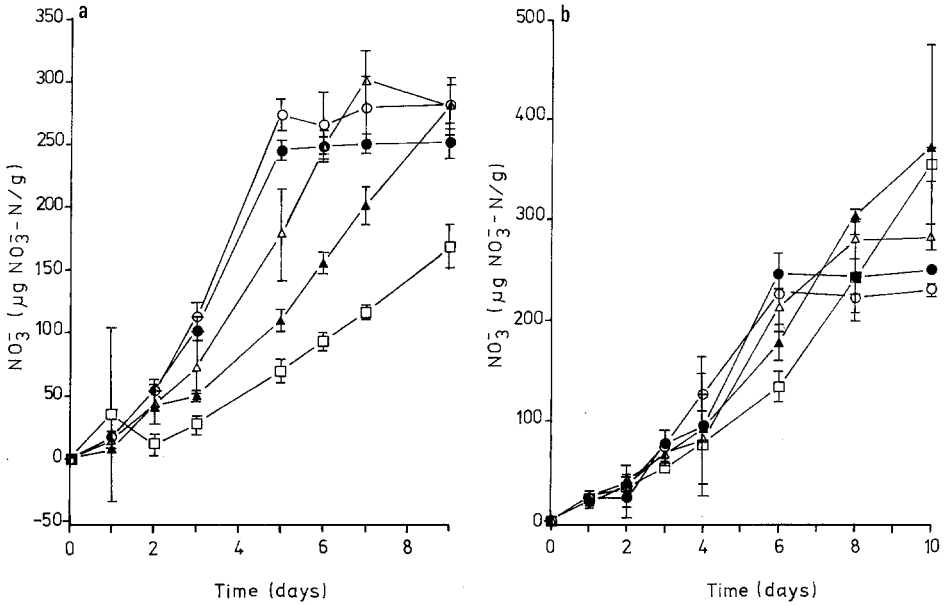

Fig. 5. Effect of glyphosate on NO_3^- production in $(NH_4)_2SO_4$-amended soil: (a) pure glyphosate; (b) Roundup. Symbols are as described in Figure 4.

230 μg g^{-1} treatments for either formulation. Low, but significant, levels were still present in the two highest glyphosate treatments; there was significantly more NO_2^- present in the 430 μg g^{-1} perfusate than in the 767 μg g^{-1} treatment. Although NO_2^- was still detectable in the two highest Roundup treatments, levels were not significantly different from the control. There was more NO_2^- in the highest glyphosate treatment than the equivalent Roundup treatment; otherwise, no formulation effect was found (Table III).

At the end of the experiment, only the highest level of glyphosate resulted in NO_3^- levels that were different from the control, with significantly less NO_3^- present in this perfusate than for any other treatment. In contrast the two highest levels of Roundup (460 μg g^{-1}, 767 μg g^{-1}) caused significantly more NO_3^{-1} to be produced than in any of the other Roundup treatments; the two treatments were not significantly different from each other.

After three days of perfusion, a dose-dependent response to glyphosate was observed. Increasing levels of glyphosate above 76.7 μg g^{-1} caused increased inhibition of NO_3^- production. The effect of Roundup was less obvious. Doses of 76.7 μg g^{-1} and 230 μg g^{-1} had no effect on NO_3^- production. High doses, 460 μg g^{-1} and 767 μg g^{-1}, inhibited NO_3^- production equally. There was no significant difference between NO_3^- levels in these two high treatments and the 230 μg g^{-1} treatment.

At day 6, the two highest doses of glyphosate were inhibitory , but lower doses did not have any significant effect on NO_3^- levels. The highest dose, 767 μg g^{-1}, was

TABLE III

Nitrification in $(NH_4)_2SO_4$-amended soil columns. Comparison of effect of herbicide formulation on NO_2^- and NO_3^- production. A two-tailed unpaired Student's *t*-test was used, at the 95% confidence level

Activity measured	Herbicide concentration (μg AI g^{-1} dry soil)	Change in N concentration[a]		Test result: significantly different
		G	R	
NO_2^- production (μg NO_2^-–N g^{-1} dry soil)				
(day 3)	control	3.53 ± 1.2E-4[b]	7.36 ± 0.22	Yes
(day 6)	control	0.16 ± 0.084	0.28 ± 0.39	No
	76.7	0.11 ± 0.084	2.30 ± 1.62	No
	230	1.09 ± 0.15	2.04 ± 0.34	Yes
	460	0.65 ± 1.5E-5	1.61 ± 0.21	Yes
	767	0.31 ± 0.084	0.65 ± 0.082	Yes
(final day: G, day 9; R, day 10)	control	0.00 ± 0.00	0.00 ± 0.00	No
	76.7	0.00 ± 0.00	0.00 ± 0.00	No
	230	0.00 ± 0.00	0.00 ± 0.00	No
	460	0.76 ± 0.43	0.095 ± 0.16	No
	767	0.29 ± 8.0E-6	0.20 ± 0.041	Yes
NO_3^- production (μg NO_3^-–N g^{-1} dry soil)				
(day 3)	control	113 ± 10.5	75.6 ± 13.6	Yes
(day 6)	control	265 ± 27.0	228 ± 38.4	No
	76.7	249 ± 4.73	247 ± 5.03	No
	230	248 ± 37.5	213 ± 15.9	No
	460	157 ± 8.19	178 ± 18.8	No
	767	93.8 ± 7.10	135 ± 14.4	Yes
(final day: G: day 9; R: day 10)	control	283 ± 20.2	231 ± 4.04	Yes

[a] Mean (n = 3) ± 1 × standard deviation.
[b] 3.53 ± 1.2 × 10^{-4}.
G: Glyphosate; R: Roundup.

significantly more inhibitory than the 460 μg g^{-1} dose; both doses caused significantly more inhibition than lower treatments. A similar effect was seen with Roundup, except that the responses to 460 μg g^{-1} and to 230 μg g^{-1} were not significantly different. No significant difference was found between any of the equivalent treatments except the highest, with 767 μg g^{-1} glyphosate being more inhibitory to NO_3^- production than 767 μg g^{-1} Roundup.

4. Discussion

4.1. Nitrogen fixation

Aerobic C_2H_2 reduction occurred at a very low rate in glucose-amended soil; only 0.64 nmol $C_2H_4\ g^{-1}$ dry soil were evolved in controls after 9 days. This is lower than the rate reported by Tam and Trevors (1981) in glucose-amended soil from the same site (250 nmol g^{-1} over 12 days). Acetylene reduction rates were too low to determine whether glyphosate had any significant effect on aerobic N_2 fixation in soil. Anaerobic C_2H_2 reduction rates in glucose-amended soil were much higher, although control rates (601 and 466 nmol $C_2H_4\ g^{-1}$ dry soil evolved over 7 days) were lower than those measured by Tam and Trevors (1981) (2800 nmol g^{-1}, 7 days). The two formulations caused equal inhibition of C_2H_2 reduction at concentrations of 127 $\mu g\ g^{-1}$ and higher. Similar high variation in the effect of low levels of glyphosate on O_2 uptake in soil was reported by Roslycky (1982). At the high concentration (635 $\mu g\ g^{-1}$) there was little variation in the inhibition of C_2H_2 reduction. At this level, the herbicide almost completely blocks anaerobic C_2H_4 production.

4.2. Denitrification: N_2O reduction

In non-amended soil, glyphosate and Roundup had no effect on N_2O reduction. However, in glucose-amended soil, high levels of glyphosate (635 $\mu g\ g^{-1}$) and Roundup (127 $\mu g\ g^{-1}$ and higher) inhibited this process. Glucose may preferentially stimulate growth of a small group of glyphosate-sensitive denitrifying microorganisms; inhibition of this group would have a large effect on total N_2O reduction in glucose-amended soil, but little effect in non-amended soil where these species are only a small part of the denitrifying population. The herbicide may be utilized as a nutrient source in non-amended soil, stimulating growth of some species while inhibiting others, resulting in no net effect on N_2O reduction.

4.3. Denitrification: NO_3^- reduction

Both glyphosate and Roundup stimulated NO_3^- reduction in non-amended soil, Roundup having a greater effect. In glucose-amended soil, glyphosate was inhibitory, but Roundup had no effect on NO_3^- reduction. In non-amended soil the herbicide may act as a nutrient source for some NO_3^- reducing microorganisms. The greater stimulatory effect of Roundup compared to glyphosate in non-amended soil was also seen in studies of O_2 uptake and CO_2 evolution in this soil (Carlisle and Trevors, 1985). Glucose may stimulate growth of glyphosate-sensitive denitrifiers, as suggested above; any inhibition caused by Roundup may be masked by the strong stimulation of NO_3^- reduction observed in non-amended soil, resulting in no net effect of this formulation being observed in amended soil. Pure glyphosate may not actually be more inhibitory than Roundup; the apparently greater degree of inhibition observed in amended soil may be due to the lower degree of stimulation casued by this formulation. Again, NO_3^-

reducing chemolithoautotrophs may be more sensitive to the effects of the herbicide, whereas other denitrifiers are not adversely affected.

Glyphosate is probably not acting as a C source in soil. Attempts to isolate microorganisms capable of using glyphosate as sole C source have failed; however, species that can utilize glyphosate as a P source have been identified (Moore *et al.*, 1983; Shinabarger and Braymer, 1985; Shinabarger *et al.*, 1984; Talbot *et al.*, 1984). Microorganisms that can degrade glyphosate to CO_2 have been found in soil (Moshier and Penner, 1978; Nomura and Hilton, 1977; Rueppel *et al.*, 1977; Sprankle *et al.*, 1975); however, this degradation appears to be cometabolic, and did not support growth of the microorganisms. These findings suggest that, if glyphosate and Roundup are acting as a nutrient source, they are probably supplying P, rather than C, to the microbial community. The greater stimulatory effect of Roundup may be due to utilization of the additional isopropylamine group on the herbicide molecule, or of other compounds, such as solubilizing agents, in the commercial formulation.

4.4. Nitrification

Nitrite production was noticeably affected by the herbicide formulations. A decrease in maximum NO_2^- concentration and a slower decrease in NO_2^- disappearance after this maximum was attained were observed in perfusates with high levels of herbicide. The decrease in maximum NO_2^- level caused by glyphosate at 230 $\mu g\ g^{-1}$ or more, and Roundup at 460 $\mu g\ g^{-1}$ or more, suggests that NH_4^+-oxidizing bacteria are inhibited by these herbicide doses. The prolonged high NO_2^- levels in perfusates with 230 $\mu g\ g^{-1}$ pure glyphosate or more indicates inhibition of NO_2^- oxidizing bacteria. In perfusates treated with 460 $\mu g\ g^{-1}$ or more herbicide, NO_2^- was detectable throughout the experiment. Glyphosate had a greater effect than Roundup on both maximum NO_2^- concentration and persistence of NO_2^- in the perfusate.

Nitrate production was also influenced by the herbicide. Glyphosate at 230 $\mu g\ g^{-1}$ or higher, and Roundup at 460 $\mu g\ g^{-1}$ or more, initially caused significant inhibition of NO_3^- production. This reflects both the decreased NO_2^- production in these treatments, shown by the decreased maximum NO_2^- concentration, and the inhibition of NO_2^- oxidation, as demonstrated by the slower decrease in NO_2^- levels in perfusates with high herbicide concentrations. Inhibition appears to reduce the rate of NO_3^- production, but not the final levels of NO_3^-. Although the 767 $\mu g\ g^{-1}$ glyphosate treatment showed a lower final accumulation of NO_3^- than other glyphosate treatments, the NO_3^- level is still increasing. High levels of Roundup (460 $\mu g\ g^{-1}$ or more) actually result in final NO_3^- levels that are greater than those observed in the controls. The increased final NO_3^- concentrations in high Roundup treatments indicate that either Roundup is being nitrified, or in some way facilitates release of NO_3^- into the perfusate.

Both nitrification steps, therefore, appear to be inhibited by the herbicide, pure glyphosate having a stronger inhibitory effect than Roundup.

The effects of glyphosate and Roundup on soil N cycling appear to be minimal, except at very high concentrations. Anaerobic N_2 fixation was the most susceptible activity in

glucose amended soils, with very high inhibition occurring at herbicide concentrations of 635 $\mu g\ g^{-1}$.

Reduction of N_2O in non-amended soil was not affected by the herbicide; however, when glucose was present, inhibition was observed. Nitrate reduction was stimulated by herbicide treatments in non-amended soil, but inhibited by pure glyphosate in glucose-amended soil. This strongly suggests that at least some NO_3^- reducing microorganisms are able to use glyphosate and Roundup as a nutrient source, probably P; it also indicates that the organisms stimulated by the herbicide are not the same organisms as those stimulated by glucose, since soil populations in the glucose-amended soil do not respond in the same manner as those in non-amended soil.

Both NH_2^+ oxidation and NO_2^- oxidation appear to be sensitive to herbicide treatments. *Nitrosomonas* is the genus most commonly found to catalyze NH_4^+ oxidation; *Nitrobacter*, NO_2^- oxidation (Alexander, 1977). Thus, *Nitrosomonas* and *Nitrobacter* appear to be sensitive to this herbicide, especially to the pure formulation. The commercial formulation is less inhibitory to these processes.

In all cases, inhibition and stimulation occurs at herbicide levels far greater than those involved in normal field application of glyphosate. Recommended field rates are up to 4.48 $kg\ ha^{-1}$ (WSSA, 1983), or roughly 2 ppm in the top 13 cm of soil (Brown, 1978); dose rates investigated here were from 12.7 to 767 ppm. In this study, the lowest treatments had no effect at all on soil microbial processes. Thus, at recommended application rates of this herbicide, no effects on soil N cycling activities should be observed.

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