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Effect of glyphosate on soil microbial activity and biomass

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Herbicides applied to soils potentially affect soil microbial activity. Quantity and frequency of glyphosate application have escalated with the advent of glyphosate-tolerant crops. The objective of this study was to determine the effect of increasing glyphosate application rate on soil microbial biomass and activity. The soil used was Weswood silt loam. The isopropylamine salt of glyphosate was added at rates of 47, 94, 140, and 234 μg ai g^{-1} soil based on an assumed 2-mm glyphosate–soil interaction depth. Glyphosate significantly stimulated soil microbial activity as measured by C and N mineralization but did not affect soil microbial biomass. Cumulative C mineralization, as well as mineralization rate, increased with increasing glyphosate rate. Strong linear relationships between mineralized C and N and the amount of C and N added as glyphosate (r^2 = 0.995, 0.996) and slopes approximating one indicated that glyphosate was the direct cause of the enhanced microbial activity. An increase in C mineralization rate occurred the first day following glyphosate addition and continued for 14 d. Glyphosate appeared to be directly and rapidly degraded by microbes, even at high application rates, without adversely affecting microbial activity.

Nomenclature: Glyphosate; *Sorghum bicolor* (L.) Moench., sorghum.

Key words: C and N mineralization, degradation, soil microbial biomass, soil microbial activity.

The increasing use of glyphosate-tolerant crops has increased concerns regarding the potential environmental effect of glyphosate. Glyphosate is a nonselective, foliar-applied herbicide used to control weeds preplant or postemergence in tolerant crops or using shielded sprayers. Glyphosate's mode of action is inhibition of 5-enolpyruvylshikimate-3-phosphate synthase, resulting in the depletion of essential aromatic amino acids needed for plant survival (Ahrens 1994). Glyphosate has been shown to be readily adsorbed by clay minerals and hydrous oxides (Glass 1987; McConnell and Hossner 1985). K_d values range from 33 to 660 mL g^{-1} (Glass 1987; USDA 1990). Glyphosate adsorption correlates with the amount of vacant phosphate sorption sites and may occur through binding of the phosphonic acid moiety (Ahrens 1994); yet, glyphosate is degraded microbially in soil and water (Ahrens 1994). It has a reported field half-life of 47 d and a laboratory half-life of < 25 d (Ahrens 1994). However, it is not known what effect glyphosate has on the microbial population.

Although glyphosate is not intentionally soil applied, a significant concentration of material may reach the soil surface during broadcast preplant or early-season applications. The amount of herbicide available to soil microorganisms depends on various edaphic factors, including nutrient and pH status, temperature, and moisture, though they differ in importance depending on the pesticide involved (Weber et al. 1993). Soil moisture and temperature directly affect many biological processes, including plant metabolism and microbial degradation, and thereby influence bioactivity and persistence of the chemicals (Weber et al. 1993).

Inconclusive evidence has been presented to determine the role of sorption on biodegradation and bioavailability to microbes. The mechanism of sorption, the duration of soil contaminant contact, the physicochemical properties of both the sorbate and the sorbent, and the specific characteristics of the degrading organisms are important determinants of sorbed compound bioavailability (Guerin and Boyd 1993).

Average concentrations of surface-applied herbicides to soil may be as low as 1 to 4 mg kg^{-1} (Moorman 1989). These concentrations, however, are usually calculated based on a 15-cm furrow slice. Concentrations based on 15-cm soil depth may be misleading because soil penetration of glyphosate may be only a few millimeters because of its high adsorptivity (Sprankle et al. 1975). Calculations based on a 15-cm soil depth may substantially underestimate the glyphosate concentration that soil microbes are exposed to in the shallow zone of herbicide penetration.

Heterotrophic soil microorganisms acquire C and N for maintenance and growth by decomposing plant residues and other organic materials added to soils. Herbicides with low C:N ratios (< 15) may potentially be readily mineralized, with N that is in excess of microbial demand being released in the inorganic form (Alexander 1977). Glyphosate has a C:N ratio of 3:1 and may have an immediate effect on soil microbial activity. Several previous studies reported that glyphosate applications had no significant effect on soil microbial activity as measured by C mineralization or only had transitory effects when applied at high rates (Hart and Brookes 1996; Olson and Lindwall 1991; Wardle and Parkinson 1990, 1992). Soils in some of these studies, however, were sampled from 1 to 4 wk after glyphosate application and may have missed the primary effect on microbial activity (Hart and Brookes 1996; Olson and Lindwall 1991). Glyphosate persistence in soils has been reported to range from less than 1 mo (Olson and Lindwall 1991) to years (Torstensson 1985). Assessment of C and N mineralization immediately after application may provide a more realistic evaluation of the true effect of this herbicide on soil microbial activity. Nitrogen mineralization from glyphosate has not been reported.

The objective of this study was to determine the effect of glyphosate application rate on soil microbial biomass and activity.

Materials and Methods

Soil Preparation

The soil used was a Weswood silt loam (fine, mixed, thermic Fluventic Ustochrept) with pH 8.3 (1:2 soil/water), soil organic matter content of 10.6 g kg^{-1} soil, 115 g sand kg^{-1}, 452 g silt kg^{-1}, 310 g clay kg^{-1}, and 123 g $CaCO_3$ kg^{-1}. Extractable soil P was in the very high category as determined by the Texas A&M University Agricultural Extension Service Soil Testing Lab. Soil (surface 5 cm) was collected from unfertilized *S. bicolor* plots at the Texas A&M University Agricultural Research Farm near College Station, TX. The plots sampled had received the same cultural practices for 16 yr prior to sampling. Soils were passed through a 2-mm sieve with obvious roots and plant residue being discarded.

The isopropylamine salt of glyphosate as RoundUp Ultra™ (480 g ai L^{-1}) was added to soil at rates of 1× (47 $\mu g\ g^{-1}$ in soil), 2× (94 $\mu g\ g^{-1}$), 3× (140 $\mu g\ g^{-1}$), and 5× (234 $\mu g\ g^{-1}$). Rate calculations were based on the 1× rate of glyphosate being 0.84 kg ha^{-1} and a shallow 2-mm soil interaction depth due to glyphosate's high adsorptivity and low leachability (McConnell and Hossner 1985; Sprankle et al. 1975). A control treatment with no glyphosate was included to measure background microbial activity.

Carbon Mineralization

Soil samples were dried at 40 C to ensure homogeneity of soil moisture content. Samples (100 g) were subsequently rewetted to approximately 15% moisture to stimulate microbial activity and were incubated at 25 C for 5 d prior to glyphosate addition. The 5-d incubation period prior to glyphosate addition allowed microbial respiration to reach a baseline level after the initial flush of activity from soil drying and rewetting. Franzluebbers et al. (1996) showed that dried and rewetted soils exhibited similar microbial biomass and activities as did continuously moist samples after an incubation period of 5 to 10 d. Glyphosate was added in 5 ml of distilled water to soil samples, increasing the final moisture content to 20% (~ 60% water-filled pore space). Soils were placed in gas-tight 1-L glass containers with a vial containing 10 ml of 1 M KOH to trap evolved CO_2 and a vial of water to maintain humidity. Soils were incubated at 25 C with KOH traps that were replaced 1, 2, 3, 7, 14, 21, 28, 38, and 56 d after the experiment began. Unreacted alkali in the KOH traps was titrated with 1 N HCl to determine CO_2–C (Anderson 1982).

Nitrogen Mineralization

Nitrogen mineralization was determined by subtracting the initial inorganic N concentration of nonincubated soil samples from soil N extracted after 56 d of incubation. Inorganic N was extracted from 7-g soil subsamples using 28 ml of 2 M KCl. Samples were shaken for 30 min on a reciprocal shaker and filtered, and the extracts were analyzed for NH_4–N and NO_2– plus NO_3–N using an autoanalyzer (Technicon 1977a, 1977b). The sum of the above N forms was designated inorganic N.

Soil Microbial Biomass C and N

Soil microbial biomass C was determined by fumigation–incubation (Jenkinson and Powlson 1976) by exposing 40-g soil samples that had been rewetted and incubated for 5 d to alcohol-free $CHCl_3$ vapor for 24 h. Following evacuation and removal of vapors, soil was incubated in 1-L gas-tight glass containers for 10 d at 25 C. Carbon dioxide evolved during the 10-d incubation period following fumigation was trapped in 1 M KOH and determined as described previously. The quantity of evolved CO_2–C was divided by an efficiency factor of 0.41 to calculate microbial biomass C (Voroney and Paul 1984).

Soil microbial biomass N was determined by analyzing soil NH_4–N concentrations of fumigated samples following the 10-d incubation period minus initial NH_4–N prior to fumigation, divided by an efficiency factor of 0.41 (Carter and Rennie 1982). Extraction and analysis of NH_4–N was accomplished as previously described.

Statistical Analysis

All treatments were replicated three times. Analysis of variance was used for generation of means and for determination of standard error terms. Linear regression was used to assess relationships among variables. Model adequacy was based on residual plot analysis. Treatment means within each incubation interval were separated using Tukey's HSD test at the 5% level of significance (SPSS 1997).

Results and Discussion

Carbon and Nitrogen Mineralization

Cumulative soil C mineralization increased with increasing glyphosate rate (Figure 1a) and was significantly greater for all applications compared to controls by day 3, with the trend continuing for 38 d. Only the 3 and 5× rates were different than controls by 56 d.

The first day after glyphosate addition, all treatments for C mineralization were significantly different than the control except the 5× rate. The influence of the surfactant may be partially responsible for the lag of the 5× rate, possibly from surfactant lysis of microbial cells or from a surfactant–glyphosate interaction. This result was repeated in a separate experiment with product-grade glyphosate and analytical-grade glyphosate, where it was apparent that the surfactant suppressed the 5× rate initially in the product-grade compared with the 5× analytical-grade material that contained no surfactant (data not shown).

All glyphosate rates were significantly different from the control by day 28 for N mineralization; however, the 1, 2, and 3× rates were not different from each other. The 5× rate was different from the 1 and 2× rates but not the 3× rate (Figure 1b). By day 56, N mineralization data showed that all treatments were significantly different than the control and different from each other.

The largest difference in C mineralization rate between different glyphosate additions occurred 2 d after application when all glyphosate treatments were significantly different

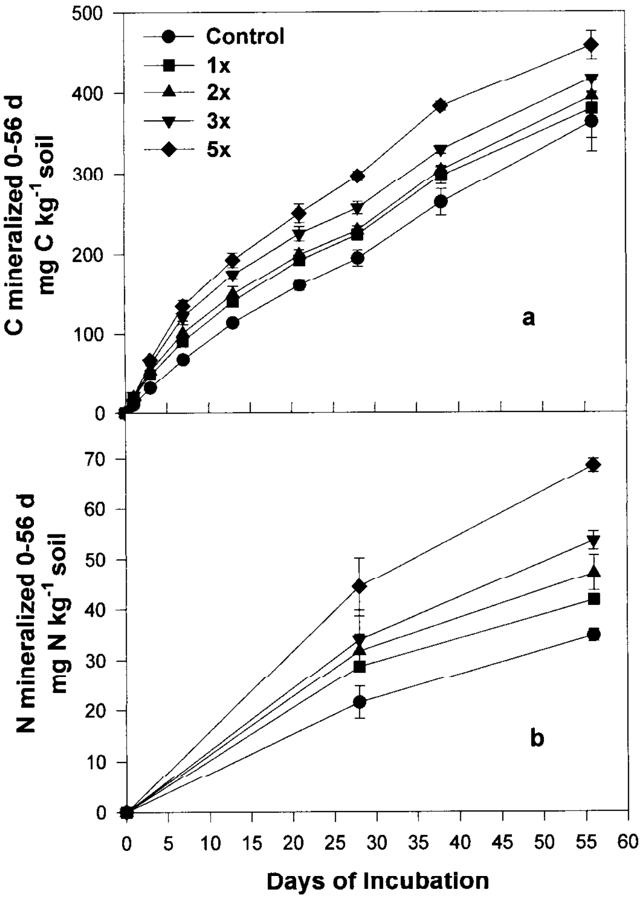

FIGURE 1. Effect of glyphosate rate on (a) carbon mineralization and (b) nitrogen mineralization from soil after 56 d of incubation. The 1, 2, 3, and 5× represent glyphosate addition rates of 47, 94, 140, and 234 μg ai g^{-1} soil, respectively. Error bars indicate one standard deviation.

from controls (Figure 2). However, only the 5× rate was significantly different from the other glyphosate treatments at this time. The third day after application, all glyphosate rates were significantly different from each other, and all additions were significantly different from the control. By 14 d after glyphosate addition, however, the rate of C mineralization had essentially returned to background levels for all glyphosate treatments, and rate differences were no longer observed (Figure 2). The C flush 2 d after application suggested that glyphosate was either readily and directly utilized by soil microbes or made other resources available (Forlani et al. 1999).

Highly significant linear relationships were determined between C and N mineralized vs. C and N added as glyphosate (Figures 3 and 4), indicating that glyphosate was directly mineralized or made other resources proportionally available for mineralization. The slope of the regression line for N mineralized vs. glyphosate N added was 1.16, whereas that for C mineralized vs. glyphosate C added was 1.31. These slopes indicated that for each unit of glyphosate C and N added, there was approximately 1 unit of C or N mineralized. The implication is that the mineralized C and N came from the applied glyphosate. The slope of 1.31 for C mineralization suggests that the stimulation of some microorganisms resulted in indigenous soil C also being mineralized or the decomposition of microbes susceptible to the herbicide (Wardle and Parkinson 1990). Forlani et al. (1999) determined degradation kinetics of glyphosate in soil and concluded that it was involved in non-growth-linked microbial metabolism.

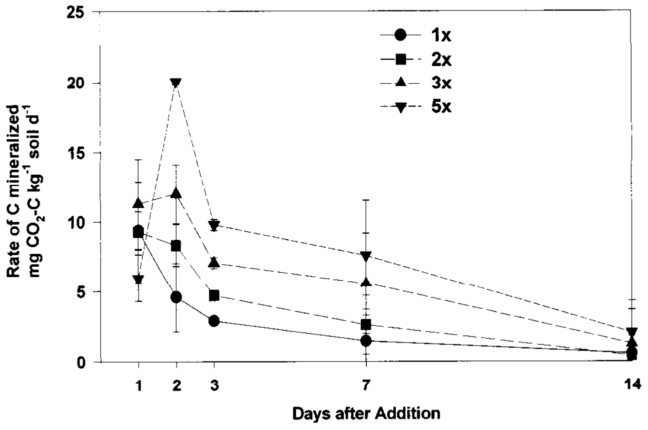

FIGURE 2. Effect of glyphosate addition rate on soil carbon mineralization. Carbon mineralized from basal microbial respiration in control samples has been subtracted. The 1, 2, 3, and 5× represent glyphosate addition rates of 47, 94, 140, and 234 μg ai g^{-1} soil, respectively. Error bars indicate one standard deviation.

The quantities of C and N mineralized after glyphosate addition resulted in a C:N mineralization ratio of 2.89 (Figure 5). Because the C:N ratio of the isopropylamine salt of glyphosate is three, mineralized C and N most likely resulted from the addition of glyphosate. N mineralization increased linearly with glyphosate concentration (Figure 6). The magnitude of N mineralized between 28 and 56 d increased with increasing rates of glyphosate. These data support the C mineralization data and indicate that the increase in N mineralization came solely from the addition of glyphosate.

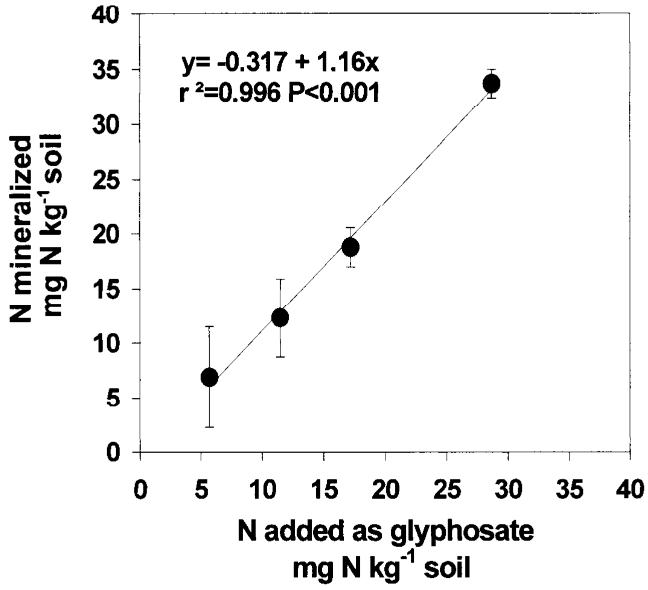

FIGURE 3. Relationship of nitrogen added from glyphosate and nitrogen mineralized from soil in 56 d following glyphosate addition. Controls have been subtracted. Error bars indicate one standard deviation.

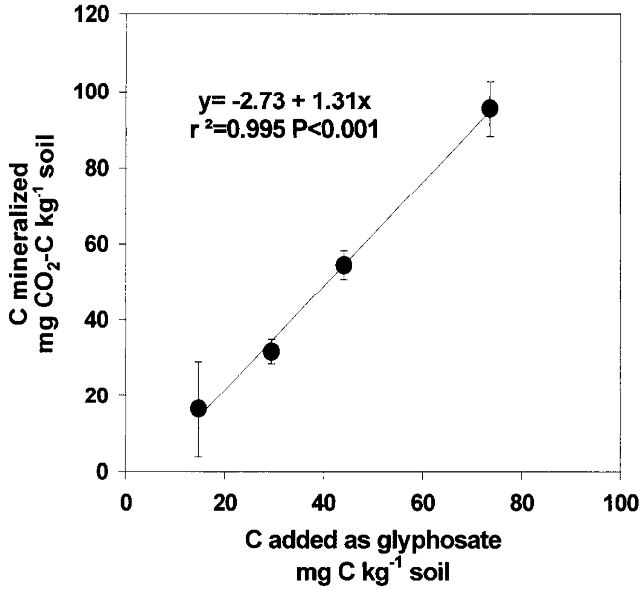

FIGURE 4. Relationship of carbon added from glyphosate and carbon mineralized from soil in 56 d following glyphosate addition. Controls have been subtracted. Error bars indicate one standard deviation.

Soil Microbial Biomass C and N

Neither soil microbial biomass C (SMBC) nor N (SMBN) were significantly affected by glyphosate addition (Figure 7). Carlisle and Trevors (1986) showed that glyphosate can either stimulate or inhibit soil organisms depending on the soil and herbicide concentration used. Wardle and Parkinson (1990) and Hart and Brookes (1996) observed no glyphosate effects on soil microbial biomass or activity. Glyphosate rates used in their studies were probably too low for detecting effects by the methods employed. However, Wardle and Parkinson (1990) reported that glyphosate at 200 μg ai g^{-1} soil increased microbial biomass C 3 d after application, but that the effect was highly transitory." Wardle and Parkinson used substrate-induced respiration (SIR) for their soil microbial biomass C test. This was accomplished by the addition of 6,000 μg g^{-1} glucose to the soil 3 d after the addition of 200 μg g^{-1} glyphosate. SIR is one of many methods for SMBC and the methodology does not always yield the same estimate. In their paper, after addition of glucose, samples for SMBC were incubated 4 h while trapping CO_2. In the fumigation–incubation method we used, soil is exposed to chloroform in a vacuum desiccator for 24 h then incubated for an additional 10 d. Wardle and Parkinson found a significant difference between 14.8 μg g^{-1} C for the 20 μg g^{-1} glyphosate addition and 17.6 μg g^{-1} C h^{-1} from the 200 μg g^{-1} glyphosate treatment. Although statistically significant, a difference of only 2.8 μg g^{-1} C h^{-1} in 4 h was determined. The application rates of glyphosate in our study probably were not high enough to produce detectable increases in SMBC or SMBN above background levels. Based on this information, it is our belief that microbial activity estimated through C and N mineralization is a more sensitive and useful tool than estimating soil microbial biomass.

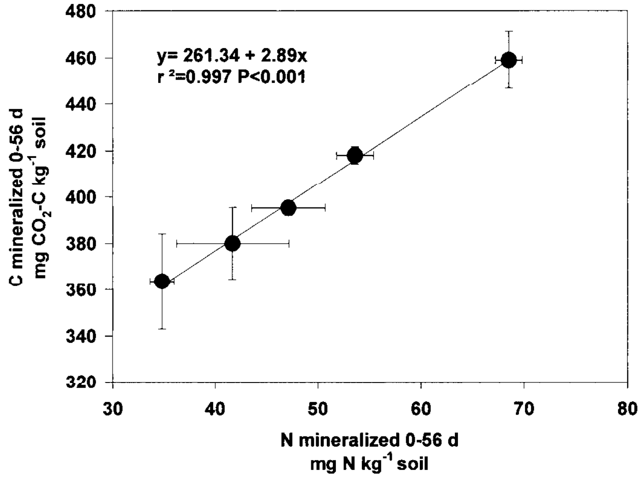

FIGURE 5. Relationship of carbon and nitrogen mineralized from soil in 56 d following glyphosate addition. Error bars indicate one standard deviation.

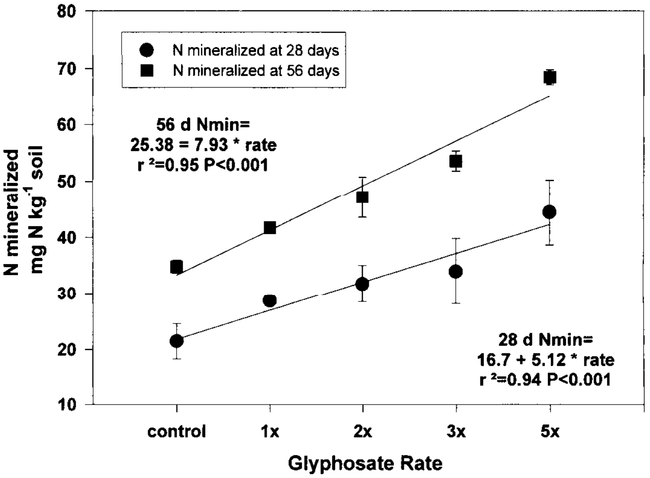

FIGURE 6. The effect of glyphosate rate on N mineralization at 28 and 56 d of incubation.

Glyphosate application rapidly stimulated soil microbial activity as measured by C and N mineralization. The highly significant relationships observed for amounts of glyphosate C and N added vs. C and N mineralized, as well as the ratio of mineralized C vs. N, suggested that glyphosate was the direct source of increased microbial activity. Soil C and

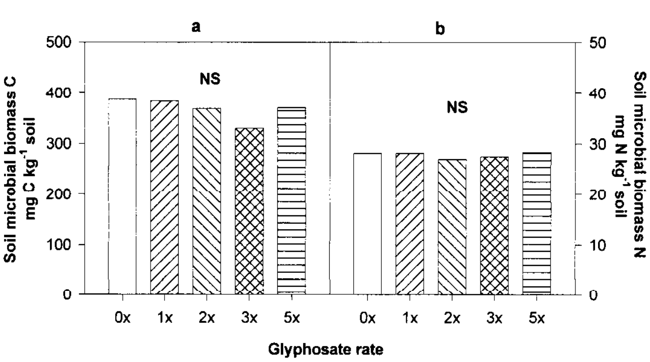

FIGURE 7. Effect of glyphosate addition rate on (a) soil microbial biomass C and (b) soil microbial biomass N 3 d after addition of glyphosate. NS indicates nonsignificance at $P < 0.05$.

N mineralization were much more sensitive to glyphosate addition than SMBC and SMBN, probably because insufficient substrate was added to alter microbial biomass. Glyphosate that reaches the soil surface should be quickly degraded by soil microorganisms without adversely affecting them, even at excessive application rates.

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