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Soil & Water Management



Effect of Roundup Ultra on Microbial Activity and Biomass from Selected Soils

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By R. L. Haney, S. A. Senseman,
and F. M. Hons

The escalating increasing use of Roundup-tolerant crops has increased concerns regarding the potential environmental impact of glyphosate. Roundup Ultra (RU) is a nonselective, foliar-applied herbicide used to control weeds preplant or postemergence in tolerant crops or by using shielded sprayers in nontolerant crops. The active ingredient of RU is glyphosate, or more accurately the isopropylamine salt of glyphosate, which inhibits production of 5-enolpyruvylshikimate-3-phosphate synthase, resulting in death of susceptible plants by depleting essential aromatic amino acids needed for plant survival.

Glyphosate is readily adsorbed by clay minerals and hydrous oxides. The K_d values have been reported to range from 33 to 660 L kg⁻¹. Glyphosate adsorption correlates with the amount of vacant phosphate sorption sites and may occur through binding of the phosphonic acid moiety. Glyphosate is microbially degrad-

ed in soil and water and has a reported field half-life of 47 days and a laboratory half-life of <25 days. However, it is not known what effect the product-grade RU (which includes surfactant and other inert products) has on indigenous microbial populations and activities across a range of soils varying in fertility.

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

In our previous work, we found that increasing rates of RU resulted in linear responses of C and N mineralization while no response was observed in soil microbial biomass (SMB) C and N when incubated at 25°C. Soil microbial biomass C and N were determined 14 days after addition of RU instead of 3 days, allowing more time for RU-derived C and N to be incorporated into microbial cells.

Since 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 to N ratios (<15) may potentially be readily mineralized, with N that is in excess of microbial demand being released in the inorganic form. A study in 1999 showed evidence of unculturable microorganisms when

exposed to glyphosate. We chose C and N mineralization and soil microbial biomass C and N as indicators of microbial activity since these methods are usually sensitive to low C to N ratio substrates and allow for a more realistic study of the effect of RU on field soils since most laboratory studies use analytical glyphosate, which farmers do not use.

The objective of this study was to determine whether RU application that reaches the soil has a detrimental or favorable effect on the microbial biomass and activities as measured by C and N mineralization or SMB from soils covering a wide array of soil characteristics.

Nine soils from Georgia and Texas were used in this study. The soils varied in soil pH (4.7 to 8.2), soil organic C (4.1 to 52.3 g C kg⁻¹ soil) and clay content (6 percent to 45 percent) (Table 1). Land management of these soils ranged from row crop production to permanent pasture. The isopropylamine salt of Roundup as RU (480 g active ingredient L⁻¹) was added to soil at a rate of 234 mg kg⁻¹. This amount was based on the recommended rate of RU being 0.84 kg ha⁻¹ and a shallow 2-mm soil interaction depth due to glyphosate's high adsorptivity and low leachability. A control treatment with no RU was included for each soil; therefore, comparison with RU additions to each soil would measure the positive or negative influences on microbial biomass and activity for each soil.

Carbon Mineralization

Soil samples were initially dried at 40°C for 24 hours to ensure homogeneity of soil moisture content. Samples were subsequently wetted and preincubated for 7 days and incubated at 30°C prior to RU



addition. Wetting amounts are calculated using the 90% of clay content technique. For example, if a soil contains 10% clay then $10\% \times 0.9 = 9$ mL water per 100 g soil; if only 50 g are used, then 4.5 mL water is added to the soil sample. The 7-day incubation period before RU addition allowed microbial respiration to reach a baseline level after the initial flush of activity from soil drying and rewetting.

A study in 1996 showed that dried and rewetted soils exhibited similar microbial biomass and activities as continuously moist samples after an incubation period of 5 to 10 days. RU was added in 5 mL of distilled water to soil samples, increasing the final moisture content to 20% w/w (approximately 60% water filled pore space). Soils were placed in gas-tight 1-L glass containers along 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 30°C with KOH traps replaced daily until 7 days and then at days 14, 24, 28 and 50. Unreacted alkali in the KOH traps was titrated with 1 M HCl to determine $\text{CO}_2\text{-C}$.

Nitrogen Mineralization

Nitrogen mineralization was determined by subtracting the initial inorganic N con-

centration of nonincubated soil samples from soil N extracted after 50 days of incubation. Inorganic N was extracted from 7-g soil subsamples using 28 mL of 2 M KCl. Samples were shaken for 30 minutes on a reciprocating shaker and filtered, and the extracts were analyzed for $\text{NH}_4\text{-N}$ and $\text{NO}_2\text{-}$ plus $\text{NO}_3\text{-N}$ using an autoanalyzer. The sum of the above N forms was designated inorganic N.

Soil Microbial Biomass Carbon and Nitrogen

Soil microbial biomass C (SMBC) and N (SMBN) were determined 14 days after RU addition for each soil. SMB C was determined by fumigation-incubation by exposing 40 g of soil to alcohol-free CHCl_3 vapor for 24 hours. Following evacuation and vapor removal, soil was incubated in 1-L gas-tight glass containers for 10 days at 30°C. Carbon dioxide evolved during the 10-day incubation period following fumigation was trapped in 1 M KOH and determined as described previously. The quantity of evolved $\text{CO}_2\text{-C}$ was divided by an efficiency factor of 0.41 to estimate microbial biomass C.

SMB N was determined by analyzing soil $\text{NH}_4\text{-N}$ concentrations of fumigated samples following the 10-day incubation

period minus initial $\text{NH}_4\text{-N}$ prior to fumigation, divided by an efficiency factor of 0.41.

Clay Content, Soil Organic Carbon, and pH

Soil pH was estimated with 2:1 water to soil. Clay content was determined by the hydrometer method and soil organic C from the modified Mebius method.

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 test at the 5 percent level of significance.

Results and Discussion

The first day after RU addition, C mineralization for all soils was significantly different from the control. The 30°C incubation temperature appeared to enhance microbial activity and resulted in no apparent lag phase after RU addition when compared with a similar study using Weswood soil, and conducted at 25°C.

TABLE 1. CHARACTERISTICS OF SOILS.

Location	Series	Texture†	USDA soil classification	Soil pH (water)	Clay content %	Organic C gkg^{-1}	Depth of sampling cm	Land management‡
Waynesboro, GA	Lakeland	S	thermic, coated Typic Quartzipsamment	4.7	10.1	5.9	30-60	cropped (cotton, soybean)
Watkinsville, GA	Cecil	SL	fine, kaolinitic, thermic Typic Kanhapludult	4.8	12.8	15.0	0-15	pasture (bermudagrass)
Amarillo, TX	Pullman	SCL	fine, mixed, superactive, thermic Torrertic Paleustoll	5.7	28.7	11.6	0-7.5	cropped (sorghum, wheat)
Overton, TX	Bowie	fSL	fine-loamy, siliceous, semiactive, thermic Plinthic Paleudult	6.3	6.0	4.1	0-7.5	pasture (bermudagrass)
Stephenville, TX	Windthorst	fSL	fine, mixed, thermic Udic Paleustalf	6.4	13.0	18.3	0-7.5	pasture (bermudagrass)
Watkinsville, GA	Pacolet	SCL	fine, kaolinitic, thermic Typic Kanhapludult	6.6	26.9	52.3	0-5	pasture (tall fescue)
Malone, TX	Houston Black	C	very-fine, smectitic, thermic Oxyaquic Hapludert	7.8	45.0	13.7	0-10	cropped (sorghum, wheat)
College Station, TX	Weswood	SiCL	fine-silty, mixed, superactive, thermic Udifluventic Ustochrept	8.0	28.4	23.7	0-7.5	pasture (bermudagrass)
Weslaco, TX	Hidalgo	SCL	fine-loamy, mixed, hyperthermic Typic Calcicustoll	8.2	22.3	9.8	0-7.5	cropped (cotton, corn)

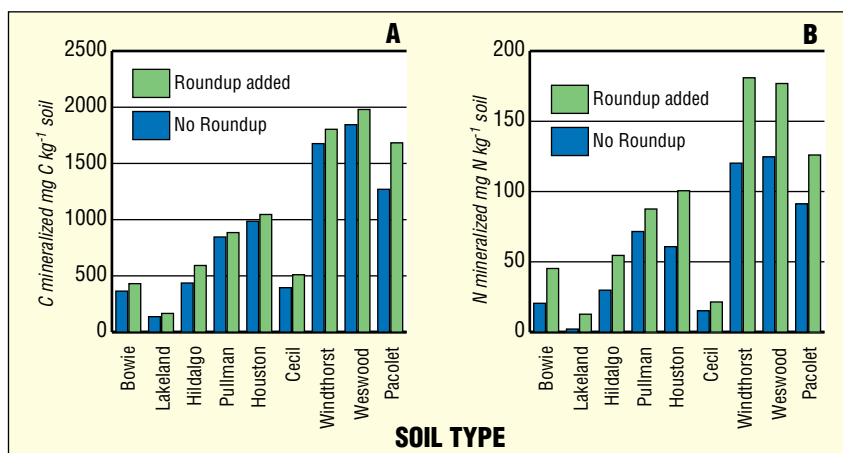
† S, sand; SL, sandy loam; SCL, sandy clay loam; fSL, fine sandy loam; C, clay; SiCL, silty clay loam.

‡ Cotton, *Gossypium hirsutum* L.; soybean, *Glycine max* (L.) Merr.; bermudagrass, *Cynodon dactylon* (L.) Pers.; sorghum, *Sorghum bicolor* (L.) Moench; wheat, *Triticum aestivum* L.; tall fescue, *Festuca arundinacea* Schreb.; corn, *Zea mays* L.

The stimulation of soil microbial activity appeared to be unique to each soil, as soils lower in clay content and soil organic C tended to reach their peak mineralization rate a few days later than the soils with higher clay and organic matter content. Carbon mineralization rates for the higher organic C soils tended to peak earlier and then dropped significantly in contrast to the lower organic C soils that may have first had to increase their microbial biomass in response to an easily mineralizable substrate. The flush of C mineralization was greater in the higher organic C soils possibly due to a greater population of microbial biomass, which mineralized the added substrate more quickly. Of the nine soils tested, two reached their peak on Day 2, five by Day 3, one on Day 4, and one on Day 5. By Day 7, only the Lakeland soil had yet to release more C as CO₂ than was added as RU. By the 14th day of incubation, however, all soils had evolved more C as CO₂ than was added as RU.

Soils differed greatly in organic C content (Table 1). Some soils responded similarly to RU addition at the end of 7 days while others did not. These data showed that generally as soil organic carbon (SOC) increased, C mineralization did also; however, when SOC, soil pH, and clay content are regressed with the flush in CO₂ (the difference between RU and no RU after 14 days) the relationships were poor. Therefore, SOC, soil pH, and clay content do not necessarily indicate how rapidly RU will be mineralized in a given soil.

Fourteen days after RU addition, all soils returned to background CO₂ levels. SMB C and SMBN were determined at this time, as the added RU had apparently been completely mineralized. Five of the nine soils showed significant increases in SMBC with RU addition. However, all nine soils exhibited significantly increased SMBN with RU addition. RU addition to soil appeared to affect the microbial N content to a greater extent than microbial C, thereby effectively lowering the microbial biomass C to N ratio and releasing N to the soil. The average increase in SMBC due to the addition of RU was 17% compared with 76% for SMBN. This result may indicate an enhanced ability to min-



eralize N that is in excess of microbial demand from RU degradation.

After 50 days of incubation following RU addition, five of the nine soils tested had significantly greater C mineralization and eight showed significantly greater N mineralization (charts A and B). The average increase for all soils for C mineralization due to RU addition was 18% compared with 108% for N mineralization. After 50 days of incubation all soils had released more C above the control than was added by RU. RU appeared to be readily mineralized regardless of soil type, clay content, pH or soil organic C content.

The literature suggests that the methods for SMBC and SMBN usually do not separate differences between herbicide treatments. In our study, SMBC was highly correlated to SMBN with and without RU addition. Carbon and N mineralized were also highly correlated. The slopes of the regression were reduced by roughly three with the addition of RU. Since the C to N ratio of the isopropylamine of glyphosate is 3:1, these data support the hypothesis that the addition of isopropylamine of glyphosate in RU was directly responsible for the increase in SMBC, SMBN, and C and N mineralization. These results support earlier work done on Weswood soil.

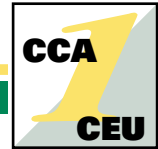
SMBC was estimated on the 14th day after addition of RU and was highly related to the amount of C mineralized at both 7 and 14 days. The relationships were not as strong on Day 7 as on Day 14, which may indicate that soil microbial respiration is coupled to the size of the population when substrate is not limiting. In both

instances, RU addition increased C mineralized per unit of microbial biomass.

Summary

RU was readily mineralized by indigenous soil microbes and increased their population and activity. Soils with higher organic matter tended to mineralize RU more quickly initially than soils with lower organic C, possibly due to a greater microbial biomass. Soils with less organic C tended to mineralize RU at a slower rate, while their microbial biomass increased in response to the added substrate. Soil organic C, soil pH and clay content do not necessarily indicate a soil's ability to mineralize RU. Available N may have been more limiting than substrate C in the studied soils as RU application significantly increased SMBN in all soils, but increased SMBC in only five soils. The amount of C mineralized was more highly correlated to SMBC at 14 days after addition of RU than at 7 days. Since the method for SMBC was estimated at 14 days, it appeared that activity was coupled to microbial population when substrate was not limiting. These data suggest that RU actually enhances microbial activity and biomass and does not adversely affect the soil microbial community.

Editor's note: Content was adapted from the paper "Effect of Roundup Ultra on Microbial Activity and Biomass from Selected Soils," which was published in J. Environ. Qual. 2002 31, and is courtesy of the authors R. L. Haney, S. A. Senseman, and F. M. Hons.



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DIRECTIONS

1. Read the self-study article on pages 44-46 carefully.
2. Answer the questions by clearly marking an "X" in the box next to the best answer for each question.
3. Complete the self-study exam registration form on the back of this page.
4. Clip out this self-study examination page, fold and place in envelope.
5. Enclose a check for \$10.00 made payable to the American Society of Agronomy, for processing fees. Payment in U.S. funds only.
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Effect of Roundup Ultra on Microbial Activity and Biomass from Selected Soils

September Self-Study Examination

1. Roundup Ultra is a herbicide that is:
 - a. selective and subsoil-applied.
 - b. nonselective and subsoil-applied.
 - c. selective and foliar-applied.
 - d. nonselective and applied postemergence.
2. Glyphosphate has a reported field half-life of:
 - a. 27 days.
 - b. 37 days.
 - c. 47 days.
 - d. 57 days.
3. Many biological processes are directly affected by water and:
 - a. soil pH.
 - b. nutrients.
 - c. temperature.
 - d. all of the above.
4. As indicators of microbial activity, this study chose to use:
 - a. C and N mineralization and soil microbial biomass (SMB).
 - b. leaching rates.
 - c. nitrification rates and soil microbial biomass (SMB).
 - d. SMB accumulation.
5. Study soils were dried at 40° C for 24 hours to ensure:
 - a. homogeneous microbial activity.
 - b. homogeneity of soil moisture content.
 - c. even texture.
 - d. homogeneous structure.
6. Final moisture content of the study soils was:
 - a. 5% w/w.
 - b. 10% w/w.
 - c. 15% w/w.
 - d. 20% w/w.
7. SMB nitrogen was determined by:
 - a. $(\text{NH}_4\text{-N}_{\text{after 10 days}} - \text{NH}_4\text{-N}_{\text{initial}}) / 0.41$.
 - b. $(\text{NH}_4\text{-N}_{\text{after 10 days}} - \text{NH}_4\text{-N}_{\text{initial}}) \times 0.41$.
 - c. $(\text{NH}_4\text{-N}_{\text{after 10 days}} - \text{NH}_4\text{-N}_{\text{initial}}) - 0.41$.
 - d. $(\text{NH}_4\text{-N}_{\text{after 10 days}} - \text{NH}_4\text{-N}_{\text{initial}}) + 0.41$.
8. All treatments were replicated:
 - a. two times.
 - b. three times.
 - c. four times.
 - d. five times.

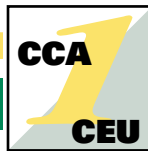
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Continuing Education Self-Study Test

Soil and Water Management Test (continued)



9. Soil organic C, soil pH and clay content:
- a. indicate a soil's ability to mineralize Roundup Ultra.
 - b. do not necessarily indicate a soil's ability to mineralize Roundup Ultra.
 - c. define a microbial environment.
 - d. were homogeneous in the nine soils studied.
10. These data suggest that Roundup Ultra:
- a. does not adversely affect soil microbial communities.
 - b. adversely affects soil microbial communities.
 - c. slows microbial activity and biomass accumulation.
 - d. kills indigenous soil microbes.



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This exam issued September 2003 expires September 2006.

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Rating Scale: 1=Poor 5=Excellent

Information presented will be useful in my daily crop advising activities: 1 2 3 4 5

Information was organized and logical: 1 2 3 4 5

Graphics/tables were appropriate and enhanced my learning: 1 2 3 4 5

I was stimulated to think how to use and apply the information presented: 1 2 3 4 5

This article addressed the stated competency area and performance objective(s): 1 2 3 4 5

Briefly explain any "1" ratings: _____

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