

## Environmental Fate of Two Sulfonamide Antimicrobial Agents in Soil

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Veterinary antimicrobial agents have been detected in a number of environmental samples, including agricultural soils. In this study, we investigated the persistence and sorption of the sulfonamides sulfamethazine (SMZ) and sulfachloropyridine (SCP) in soil and their potential effects on soil microorganisms. The sulfonamides dissipated more rapidly from the silt loam soil as compared to the sandy soil. Average half-lives of SMZ and SPC among the two soils were 18.6 and 21.3 days, respectively. The presence of liquid swine slurry (5% v/w) decreased sulfonamide persistence in the silt loam soil. The lower persistence of the antimicrobials in liquid swine slurry-amended soil was likely due to higher microbial activity, as compared to unamended soil, and/or to the greater bioavailability of the sulfonamides to degrading microorganisms, as estimated by sorption isotherms. Concentrations of SMZ and SPC up to 100  $\mu\text{g g}^{-1}$  had no effect on antimicrobial degradation rates and soil microorganisms. These studies suggest that higher sulfonamide concentrations would be necessary to affect the main processes controlling their environmental fates in soil, but at the concentrations normally found in the environment, there would be little or no effects.

**KEYWORDS:** Antimicrobials; veterinary pharmaceuticals; sulfonamides; sorption; soil degradation; soil microbial community structure

### INTRODUCTION

Antimicrobial agents are useful tools to prevent and treat human and animal diseases. Since the early 1950s, the use of antimicrobial agents has been a standard practice to increase food conversion efficiency in livestock production systems (1). Following administration, a significant fraction of the antimicrobial agents is excreted unaltered into feces and urine and may enter the soil through land application of animal manure or slurry. The primary concern with introducing antimicrobials into soil is the spread of antimicrobial resistance (2). While the development of antimicrobial resistance in bacteria, and its transfer to other members of the soil microbial community, is a well-known phenomenon, the importance of soil factors and environmental pollution pathways to the proliferation of resistant bacteria has only recently received attention (1). Although contamination of agricultural soils and water reservoirs by veterinary antimicrobials has been widely documented, only a few studies have been undertaken to estimate the persistence and bioavailability of these compounds in the soil ecosystem (3).

The most widely used veterinary antimicrobials in industrialized countries include tetracyclines, macrolides, penicillins, aminoglycosides, and sulfonamides (4, 5). Sulfonamides (sulfa drugs) are synthetic antimicrobial agents with a wide spectrum and inhibit the growth of a large number of Gram-positive and Gram-negative bacteria. These antimicrobials are competitive antagonists of *para*-aminobenzoic acid, interrupting bacterial utilization of this compound in the synthesis of folic acid. Sulfonamides are both fairly water-soluble and polar compounds, which ionize depending on the pH of the medium (6). Similar to other organic contaminants (i.e., soil-applied pesticides), the environmental fate of antimicrobials in soil is governed by transformation, retention, and transport processes. Research indicates that sulfonamides are sorbed on soil particles with distribution constants ( $K_d$ ) ranging from 0.9 to 3.5 (6–8). Thiele-Bruhn et al. (9) showed that soil pH and properties of soil organic colloids are important factors in determining the strength of sulfonamides sorption on soil. High mobility of sulfonamides through the soil profile has been observed under field conditions (6).

Concentrations of sulfonamides in soil after application of manure or slurry to agricultural fields have not been extensively measured. In a monitoring study done in Southern Germany, Christain et al. (10) found detectable sulfamethazine (SMZ) concentrations in soil 7 months after application of manure to

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**Table 1.** Properties of WA and PR Soils

soil textural class		particle size (%)				pH <sup>a</sup>	organic C	soil bacterial numbers <sup>b</sup> (Log CFUs g <sup>-1</sup> soil)
		sand	silt	clay				
WA	silt loam	19.9	56.5	23.6	7.5	1.8	9.85 ± 0.55	
PR	sand	93.5	2.7	3.8	7.2	0.94	8.17 ± 0.39	

<sup>a</sup> Soil pH measured in 1:2.5 (w/w) soil/deionized water mixture. <sup>b</sup> Colony forming units. The colony count was done by spread-plating soil dilutions onto 0.1× tryptone soy agar.

**Table 2.** Selected Properties of the Filtered Swine Slurries

pH	7.8
total organic C (%)	20.2
NO <sub>3</sub> -N (mg L <sup>-1</sup> )	<limit of detection (0.2)
NO <sub>2</sub> -N (mg L <sup>-1</sup> )	<limit of detection (1.0)
NH <sub>4</sub> -N (mg L <sup>-1</sup> )	3,008
total P (mg L <sup>-1</sup> )	83.6
aerobic bacteria (Log CFUs mL <sup>-1</sup> )	7.9 (±0.88)
BOD (mg L <sup>-1</sup> )	10365
COD (mg L <sup>-1</sup> )	17400

agricultural fields. These findings suggest that under field conditions, sulfonamides may be more persistent than would be predicted from information obtained in laboratory studies (2). Although veterinary antimicrobials are expected to have the potential to exert biological effects against soil microorganisms, this aspect has not been well-elucidated. On the basis of the available information, the effects of sulfonamides on soil microorganisms would likely depend on antimicrobial concentration and on the activity level of the whole microbial community (11, 12).

The primary objective of this study was to investigate the degradation and sorption of SMZ and sulfachloropyridazine (SCP) in two agricultural soils. In addition, potential effects of SMZ and SCP on the soil microbial community were also determined.

## MATERIALS AND METHODS

**Soils and Liquid Swine Slurry (LSS).** Two agricultural soils from Minnesota were selected for this study. Surface soil samples (0–20 cm) of a Waukegan (WA) silt loam (Typic Hapludoll, mixed, mesic) and a Princeton (PR) sandy soil (Argic Udipsamments, mixed, frigid) were air-dried and passed through a 2 mm sieve. Physicochemical properties and counts of culturable bacteria of the WA and PR soils are given in **Table 1**. LSS was collected from a lagoon located in Northern Italy (Imola, Bologna). Prior to use, the LSS was filtered through Whatman #42 filter paper. The composition of the filtered LSS is reported in **Table 2**.

**Sulfonamides Degradation.** Sulfonamide degradation experiments were performed in WA and PR soil samples treated with increasing concentrations of SMZ and SCP (1, 10, and 100 μg g<sup>-1</sup> soil). The effect of LSS on sulfonamide degradation was studied in the WA soil treated with 10 μg g<sup>-1</sup> soil of SMZ and amended with filtered LSS (5% v/w).

Sulfonamides were applied as concentrated methanol solutions to 5 g portions of soil. After the solvent was allowed to evaporate for 30 min, a sufficient mass of untreated soil was thoroughly mixed with the antimicrobial-treated soil to attain the desired concentration. Soil samples (15 g) were weighed into 50 mL screw-top tubes, the soil moisture was adjusted to the gravimetric content at –33 kPa using distilled water, and samples were incubated in the dark at 25 °C. In the case of LSS-amended samples, a fixed aliquot of filtered LSS (equivalent to 5% v/w of soil) was dissolved in the water that was used to bring the soil to the field capacity (–33 kPa). At 3 day intervals during the 40 day incubation period, soil moisture was determined by weighing samples, and water was added as needed. Sulfonamide

degradation was monitored by removing tubes for analysis at 0, 5, 10, 15, 30, and 40 days after treatment. Collected samples were stored at –20 °C until analyzed. The experiment was conducted in triplicate.

Sulfonamides were extracted from soil samples by adding 15 mL of methanol to each tube and shaking vigorously for 12 h on a horizontal shaker. Supernatants were collected by centrifugation at 5000g for 20 min, and a 10 mL aliquot of the supernatant was filtered through a 0.2 μm filter. The filtrate was passed through a solid-phase extraction cartridge using a SPE Strata-X 200 mg/6 mL cartridge (Phenomenex, Aschaffenburg, Germany). Preliminary investigations showed that the average recovery efficiency of SMZ and SCP among the two soils was >93 and 91%, respectively.

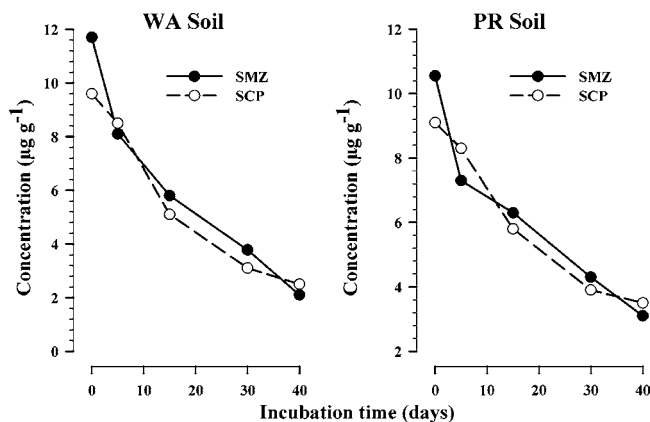
Sulfonamide concentrations in samples were determined by using a System Gold 126 liquid chromatograph (Beckman Inc., Palo Alto, CA) equipped with a diode array detector and a Spherisorb C18, 5 μm, reverse phase column (ODS II, 250 mm × 4.6 mm; Supelco Inc. Bellefonte, PA). Isocratic elution was carried out at room temperature using a methanol/phosphate buffer (10 mM, pH 3.1) solution (30/70) with an eluent flow rate of 1 mL min<sup>-1</sup>. Sulfonamides were detected at 260 nm.

Concentration data were conventionally analyzed assuming the following first-order kinetics:  $\ln C = \ln C_0 - Kt$ , where  $t$  is days after treatment,  $C_0$  (100%) is the initial amount of pesticide,  $C$  is the percentage of the initial amount remaining at time  $t$ , and  $K$  is a rate constant in this equation. Parameters were obtained using linear regression. Formal  $F$  tests for a best-fit line were performed by the analysis of variance of regression results. The half-life ( $t_{1/2}$ ) was calculated by the following equation:  $t_{1/2} = (\ln 2)/K$ .

**Sulfonamides Sorption Isotherms.** Isotherms for sorption of SMZ and SCP on WA and PR soils were determined using the batch equilibrium method. Briefly, 5 g of soil was placed in 25 mL centrifuge tubes, and a 10 mL aliquot of sulfonamide solution prepared in 0.01 M CaCl<sub>2</sub> was added. For each soil type and antimicrobial chemical, sorption isotherms were determined using triplicate samples at five initial concentrations, ranging from 1 to 100 μg mL<sup>-1</sup>. Tubes were sealed with Teflon-lined caps, mechanically shaken at 20 °C for 14 h, and centrifuged at 5000g for 20 min. Supernatant aliquots (5 mL) were removed, filtered through a 0.2 μm filter and analyzed by high-performance liquid chromatography as described above. The same procedure was adopted to estimate sorption isotherms of SMZ and SPC in WA and PR soils containing 5% LSS.

The amount of antimicrobial sorbed was calculated from the concentration differences between the supernatant of the equilibrated solutions and those of the corresponding initial solutions. Sorption data were fitted to the log form of the Freundlich equation:  $\log C_s = \log K_f + (1/n) \log C_e$ , where  $C_s$  is concentration of antimicrobial sorbed (μg g<sup>-1</sup> soil),  $C_e$  is the equilibrium concentration (μg mL<sup>-1</sup> solution), and  $K_f$  and  $1/n$  are the empirical Freundlich constants. Values of  $K_f$  and  $1/n$  were estimated by linear regression after log–log transformation.

**Metabolic Potential.** The influence of SMZ and SCP on the metabolic potential of the soil microbial community was assessed by measuring mineralization of glucose and glyphosate, in treated and untreated soils. Soils received increasing sulfonamides concentrations (1, 10, and 100 μg g<sup>-1</sup>) were prepared as described above for the sulfonamide degradation experiment. Untreated soils and soils treated with the antibiotic nalidixic acid (100 μg g<sup>-1</sup> soil) were included for comparison. The experiments were conducted in triplicate. Aqueous solutions of glucose and glyphosate (N-phosphonomethyl-2-14C-glycine) were individually added to soils, as a mixture of unlabeled and <sup>14</sup>C-labeled compounds, to obtain a final concentration of 1 μg g<sup>-1</sup> of each substrate. Unlabeled glucose (chemical purity >99%), uniformly labeled <sup>14</sup>C-glucose (radiopurity >98%; specific activity 245 mCi mmol<sup>-1</sup>), unlabeled glyphosate (purity >99%), and <sup>14</sup>C-glyphosate (radiopurity >99%, specific activity 5.4 mCi mmol<sup>-1</sup>) were purchased from Sigma (St. Louis, MO). After solution addition, the soil moisture was adjusted to a gravimetric content of –33 kPa, and samples were incubated for 28 days in the dark at 25 °C. The moisture content of incubated samples was checked at 3 day intervals and adjusted to the initial –33 kPa as needed. Glucose and glyphosate mineralization were monitored by trapping the evolved <sup>14</sup>CO<sub>2</sub> in vials containing 5 mL of



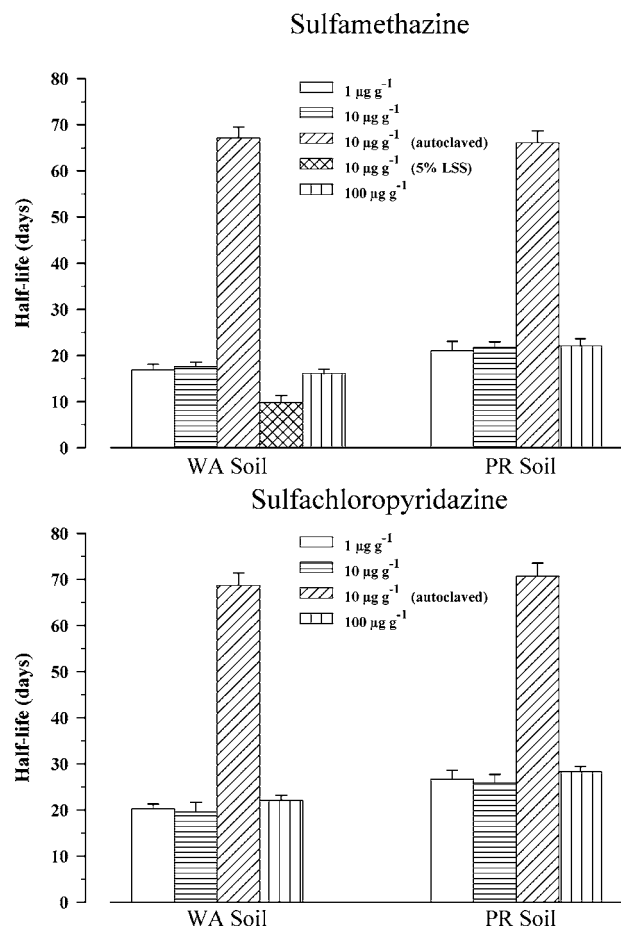
**Figure 1.** Concentration values of SMZ and SCP in the WA and PR soils during the 40 day incubation period. Bars represent standard errors of the mean.

1 M NaOH, and the solution was replaced at each sampling, facilitating flask aeration. Trapped  $^{14}\text{C}$  was determined by mixing a 1 mL aliquot of NaOH solution with 5 mL of EcoLite scintillation cocktail (ICN Pharmaceuticals Inc., Costa Mesa, CA), and the amount of radioactivity was determined by liquid scintillation counting for 10 min using a 1500 Tri-Carb Packard (Meriden, CT) liquid scintillation analyzer. Samples were kept in the dark for 12 h prior to analysis, and no chemiluminescence was observed.

**Bacterial Community Structure.** At the end of the incubation period, bacterial community structures in selected soil samples from the metabolic potential experiment were analyzed using the polymerase chain reaction–denaturing gradient gel electrophoresis (PCR-DGGE) technique as previously described (13). Total bacterial genomic DNA was extracted from soil samples using the QBioGENE FastDNA spin kit for soil (QBioGENE, Carlsbad, CA). PCR amplification of the variable V3 region of 16S rDNA was performed using universal bacterial primers in a PTC100 Thermal Controller (MJ Research, Inc., Watertown, MA). Primers were PRBA338F (5'-ACTCCTACGGGAG-GCAGCAG) with a GC clamp needed to stabilize products in the DGGE and PRUN518R (5'-ATTACCGCGGCTGCTGG). The PCR cycle consisted of a 92 °C enzyme activation step for 5 min, followed by 30 cycles of 30 s each at 92, 55, and 72 °C, and ended with a 15 min incubation at 72 °C. PCR product quality and quantity were confirmed by running 5  $\mu\text{L}$  of product on a 1% agarose gel. Community structure was analyzed using the DCode Universal Mutation Detection system for DGGE gels (Bio-Rad Laboratories, Hercules, CA) as previously described (13). DNA fragments were separated on 8% (w/v) polyacrylamide gels (37.5:1 acrylamide:bisacrylamide) with a urea–formamide denaturing gradient of 35–60% (where 100% denaturant contains 7 M urea and 40% v/v formamide). Equal amounts of PCR product were loaded in each well of the preheated gels. Gels were run for 5.5 h at 60 °C, with a constant voltage of 200 mV in  $1 \times$  TAE buffer that was constantly stirred. Gels were stained with 1:10000 dilution of SYBR Green I (Molecular Probes, Eugene, OR), and bands were visualized on a UV transilluminator and photographed with a digital CCD camera (BioChem System; UVP, Upland, CA).

## RESULTS AND DISCUSSION

**Sulfonamides Degradation and Sorption.** The quantity of SMZ and SPC recovered from the silt loam (WA) and sandy (PR) soils over the 40 day incubation period is shown in Figure 1. The decline of extractable residues was found to follow first-order kinetics in both soils (coefficients of determination  $\geq 0.87$ ), suggesting that dissipation of both SMZ and SCP was mainly due to microbiological processes. The silt loam soil had a greater capacity for degradation of both antimicrobial compounds than did the sandy soil. Average half-life values of SMZ and SCP among the two soil types were of 18.6 and 21.3 days, respectively. Half-lives for SMZ and SCP were significantly



**Figure 2.** Half-life values of SMZ and SCP in the WA and PR soils as a function of initial concentrations, the presence of LSS (5%), and soil autoclaving.

longer in autoclaved soils than in nonautoclaved soils (Figure 2). Because autoclaving does not completely stop microbial activity (14), these results suggest that microbial degradation was the predominant mechanism in the dissipation of the two sulfonamides in soil. The difference in the degradation rates between the two soils is consistent with the greater abundance of culturable bacteria in the silt loam than the sandy soil (Table 1).

Our results were consistent with other reported research, which indicated that half-lives of these two chemicals ranged from 10 to 30 days in soil (15–17). In contrast to this current study, Ingerslev and Halling-Sørensen (18) found that the degradation of 12 different sulfa drugs in sewage sludge was preceded by a lag phase of approximately 10 days. Similar findings were reported by Pérez et al. (19), who concluded that degradation of structurally related sulfonamides occurred with comparable half-lives after development of microbial adaptation to a single sulfonamides. Although no lag phase was observed in the experiment described here and considering that the two soils have never been exposed to sulfa drugs, persistence of the two sulfonamides confirmed this tendency.

The persistence of SMZ and SCP was not affected by initial chemical concentration in both soil types (Figure 2). Considering that the highest tested concentration was 100  $\mu\text{g g}^{-1}$  and that the antimicrobials tested are specifically designed to have an adverse effect on bacteria, these findings suggest that a much higher concentration would be necessary to affect microbial processes involved in sulfonamide degradation in the soil. Moreover, because the toxicity of antimicrobials is convention-

**Table 3.** Sorption Coefficients of SMZ and SCP Determined in the WA and PR Soils

	WA soil			PR soil		
	$K_f^a$ ( $\mu\text{g}^{1-1/n}$ $\text{g}^{-1} \text{mL}^{1/n}$ )	$1/n^b$	$r^{2c}$	$K_f$ ( $\mu\text{g}^{1-1/n}$ $\text{g}^{-1} \text{mL}^{1/n}$ )	$1/n$	$r^2$
SMZ	6.75 (6.63–6.81)	0.79	0.93	4.21 (4.01–4.44)	0.75	0.91
SMZ/LSS <sup>a</sup>	5.04 (4.79–5.23)	0.82	0.90	3.52 (2.99–3.97)	0.73	0.89
SCP	6.11 (6.01–6.41)	0.88	0.88	3.97 (3.11–4.57)	0.78	0.87

<sup>a</sup> Soil amended with LSS (5% w/v). <sup>b</sup> Numbers in parentheses are 95% confidence intervals. <sup>c</sup> Correlation coefficients of linear regression of linearized Freundlich isotherms.

ally tested against a single bacterial strain, it is likely that this parameter would not be correctly transferable to the soil ecosystem. Moreover, under some circumstances, a decrease of sulfonamide degradation rate with increasing initial concentration in soil has been reported (17).

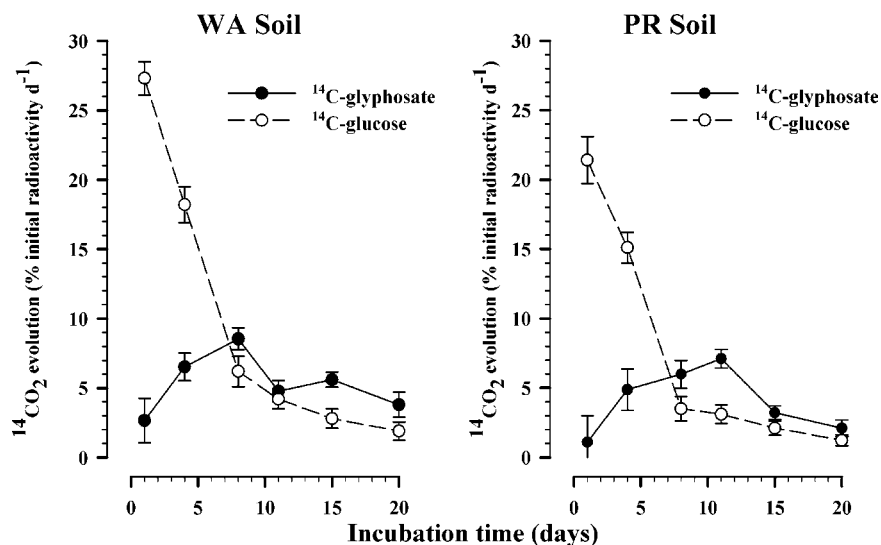
As expected, the addition of LSS addition to soil led to a significant increase in the degradation rates of SMZ, presumably the result of the addition of a large microbial population present in the LSS (Table 2). The stimulatory effect of animal manure slurries on degradation of antimicrobial chemicals has been reported previously (17). Aside from the stimulation of microbial activity due to a greater availability of LSS-derived nutrients (i.e., nitrogen), the observed increase in the rate of sulfonamides degradation in LSS-amended soil samples may be more likely due to the greater size of the microbial population with respect to unamended soil. These results reinforced the importance of microbial processes in the overall degradation of sulfonamides in soil.

Estimated sorption coefficients for SMZ and SCP are summarized in Table 3. The low  $K_f$  coefficients indicated that these compounds most likely are highly mobile in soil. Sorption of SMZ and SCP was greater in the silt loam than in the sandy soil, consistent with the tendency for sulfonamides to be preferentially retained by fine-textured particles (9). Less antimicrobials were sorbed on the LSS-amended soils than onto control soils (Table 3). This was likely due to the high affinity of sulfonamides for organic matrices, as reported by others (6, 9). Sulfonamides are fairly water soluble and polar compounds, and thus, soil sorption is a pH-dependent process. Boxall et al. (6) reported that less sulfachloropyridazine was sorbed on soil amended with swine slurries than onto unamended soil. These

findings were thought to be due, in part, to the increase of solution pH caused by ammonia released from swine slurry. In our studies, the decrease in sorption due to LSS addition was correlated with increased biodegradation of the sulfonamides. This most likely is directly related to the increased bioavailability of the two antimicrobial compounds. Because the main route by which veterinary antimicrobial chemicals enter the environment is by the spreading of contaminated manure or slurries onto soil, our findings suggest that the interaction of animal wastes and antimicrobials with soils should be a large consideration in predicting the environmental fate of these chemicals in ecosystems.

#### Metabolic Potential and Bacterial Community Structure.

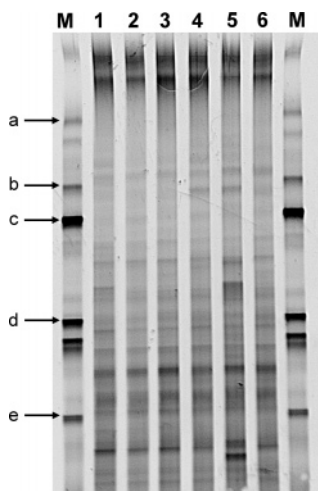
In the present study, glucose and glyphosate were chosen as models for highly and moderately biodegradable compounds, respectively (12, 13). Studies have shown that mineralization of these two compounds reflects the metabolic potential of soil (13, 20). Representative patterns of radiolabeled glucose and glyphosate mineralization, expressed as  $^{14}\text{CO}_2$  evolution, are shown in Figure 3. As expected, mineralization of glucose proceeded at an initially higher rate than did glyphosate. Moreover, the silt loam WA soil exhibited greater potential for mineralization of both compounds than did the sandy soil. Several studies have confirmed that glucose and glyphosate mineralization occurs chiefly via microbial processes. Consequently, greater glucose and glyphosate mineralization in the WA soil, as compared to that found in the PR soil, is consistent with the larger size of the culturable microbial population in the WA soil (Table 1). The high increasing concentrations of either SMZ and SPC had no significant impact on glucose and glyphosate mineralization in the WA and PR soils (Table 4). Previous studies that examined the effects of sulfonamides on soil microorganisms have presented limited, and often contradictory, results. Thiele-Bruhn and Beck (11) demonstrated that concentrations of sulfonamide sulfapyridine up to 1000  $\mu\text{g g}^{-1}$  soil did not affect microbial respiration. Similar findings were observed with other sulfonamides (21, 22). In contrast, detrimental effects of increasing sulfonamide concentration on soil microorganisms and sulfonamide degradation in glucose-amended soils have also been reported (11, 21). These authors suggested that the stimulatory effect of glucose on soil microorganisms increases their vulnerability to this class of antimicrobials, due to growth stimulation. Because the primary action of sulfonamides on bacteria is believed to be bacterio-

**Figure 3.** Representative  $^{14}\text{C}$ -glyphosate and  $^{14}\text{C}$ -glucose mineralization in the WA and PR soils treated with SMZ and SCP ( $10 \mu\text{g g}^{-1}$ ).

**Table 4.** Mineralization of  $^{14}\text{C}$ -Glyphosate and  $^{14}\text{C}$ -Glucose, Expressed as Cumulative  $^{14}\text{CO}_2$  Evolution, during the 20 Day Incubation Period from WA and PR Soils Treated with the Highest Concentration of the Following Compounds: SMZ, SCP, and Nalidixic Acid

	$^{14}\text{CO}_2$ (% initial radioactivity)			
	WA soil		PR soil	
	glyphosate	glucose	glyphosate	glucose
– (control)	31.9 ± 2.3	60.6 ± 4.1	24.4 ± 1.9	46.4 ± 2.7
SMZ (100 $\mu\text{g g}^{-1}$ )	34.1 ± 1.5	58.9 ± 5.6	26.9 ± 4.3	43.9 ± 1.9
SCP (100 $\mu\text{g g}^{-1}$ )	35.9 ± 4.4	63.1 ± 2.7	28.5 ± 5.1	45.7 ± 3.3
nalidixic acid (100 $\mu\text{g g}^{-1}$ )	30.8 ± 3.5	61.9 ± 4.1	23.7 ± 2.1	47.1 ± 3.2

<sup>a</sup> Values are means of three replicates ± standard deviations.



**Figure 4.** PCR-DGGE fingerprints of the V3 region of 16S rDNA isolated from incubated samples of the WA soil. Lanes 1–3 glucose amendments: control of only glucose (1  $\mu\text{g g}^{-1}$ ) (lane 1), glucose plus nalidixic acid (100  $\mu\text{g g}^{-1}$ ) (lane 2), and glucose plus SMZ (100  $\mu\text{g g}^{-1}$ ) (lane 3); lanes 4–6 glyphosate amendments: control of only glyphosate (1  $\mu\text{g g}^{-1}$ ) (lane 4), glyphosate plus nalidixic acid (100  $\mu\text{g g}^{-1}$ ) (lane 5), and glyphosate plus SMZ (100  $\mu\text{g g}^{-1}$ ) (lane 6). Outside lanes flanking the numbered lanes contain reference fingerprints that were as used to confirm a consistent gradient of denaturants across the gel. Letters in the margin refer to DNA fragments of the V3 region of 16S rDNA amplified from *Pseudomonas putida* (a), *Acinetobacter ADP1* (b), *Escherichia coli* (c), *Comamonas testosterone* (d), and environmental isolate BW7 (e).

static, rather than bacteriocidal, sulfonamides would affect only growing bacteria rather than dormant cells, which represent the predominant fraction of the whole soil microbial community (23).

Similar to results of the metabolic potential experiments, PCR-DGGE analyses showed no major differences between the samples receiving sulfonamide treatments and the controls (Figure 4). Although slight differences within each gel lane were observed, the small variation in banding patterns was thought not to be significant, in part due to gel errors. This was reflected in statistical analyses. Dice similarity coefficients were all above 0.9 (data not shown). In addition, while several band classes were evident in all of the treatments, no unique bands were observed in any of the treatments. This further indicated that the communities shared a high degree of similarity.

Vaclavik et al. (21) and Thiele-Bruhn and Beck (11) reported that stimulation of soil microorganisms would result in a greater sensitivity to sulfonamides. Results from the bacterial community analysis of WA soil receiving only glucose or glucose

plus SMZ or nalidixic acid did not confirm this predicted result. Because SMZ and SCP up to 100  $\mu\text{g g}^{-1}$  did not appreciably affect soil microorganisms in our analyses, we investigated whether these findings would be restricted to these chemicals or can be extended to other antimicrobial compounds. Unexpectedly, samples of the WA soil treated with nalidixic acid (100  $\mu\text{g g}^{-1}$ ) did not affect metabolic potential and bacterial structure community as estimated by glucose and glyphosate mineralization (Table 4) and PCR-DGGE analysis, respectively (Figure 4). Because nalidixic acid has different physicochemical properties and a different bacterial target site with respect to sulfonamides, our findings further suggest that higher concentrations of these antimicrobial compounds would be necessary to grossly affect the soil microbial community structure and function.

In conclusion, results from these laboratory investigations indicated that the two sulfonamides SMZ and SPC did not persist in the silt loam and sandy soils tested. The persistence of the tested sulfonamides in soil was further reduced in samples amended with LSS. A major role for microbial processes in sulfonamide dissipation in soil was observed. We have also estimated the effects of increasing concentrations of SMZ and SCP on their degradation rates and on the soil microbial community. The results obtained indicated that concentrations of SMZ and SCP up to 100  $\mu\text{g g}^{-1}$  had no effect on degradation rates and on the soil microbial community, thus indicating that higher concentrations than those commonly encountered would be required to eventually influence the dissipation of sulfonamides in soil.

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