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# Sequestration of Manure-Applied Sulfadiazine Residues in Soils

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It is not the total but the (bio)accessible concentration of
veterinary medicines that determines their toxicity in the
environment. We elucidate the changes in (bio)accessibility of
manure-applied sulfadiazine (SDZ) with increasing contact
time in soil. Fattening pigs were medicated with $^{14}$C-labeled SDZ,
and the contaminated manure (fresh and aged) was amended
to 2 soil types (Cambisol, Luvisol) and incubated for 218
days at 10 °C in the dark. Antibiotic residues of different
bioaccessibility were approached by sequential extractions
with 0.01 M CaCl$_2$ (CaCl$_2$ fraction), methanol (MeOH fraction),
and finally acetonitrile/water (residual fraction, microwave
extraction at 150 °C). In each fraction, total radioactivity, SDZ,
and its major metabolites were quantified. The results
showed that both SDZ and, to a lesser extent, 4-hydroxysulfadiazine
(4-OH-SDZ) were rapidly reformed from N-acetylsulfadiazine (N-
ac-SDZ) during the first 2–4 weeks after fresh manure
application, i.e., the N-acetylated metabolite does not sequester
in soil to a significant extent. Yet, the water and methanol
extractable SDZ and 4-OH-SDZ also dissipated rapidly (DT$_{50}$ =
6.0–32 days) for the fresh manure treatment with similar
rate constants for both soil types. In the residual fractions,
however, the concentrations of both compounds increased with
time. We conclude that the residual fraction comprises the
sequestered pool of SDZ and its hydroxylated metabolite. There
they are entrapped and may persist in soil for several years.
Including the residual fraction into fate studies thus yields
dissipation half-lives of SDZ which exceed those previously
reported for sulfonamides by a factor of about 100.

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## Introduction

Veterinary antibiotics are broadly used in animal husbandry and reach the soil via manure application or grazing livestock. The concentrations detected in soils have been frequently on the order of several $\mu$g kg$^{-1}$ (e.g., refs *1–3*); in hot spots concentrations even reached 1.44 mg kg$^{-1}$ soil after application of slurry (*4*). Such high concentrations exceed by far the recommended trigger value of 100 $\mu$g kg$^{-1}$ soil (*5*). Hence, unwanted impacts on microbial community functions (e.g., nitrification rates) and resistant gene formation are likely (*6–8*), especially for compounds that are known to persist in soils, such as tetracyclines (*4*), fluoroquinolones (*9*), and sulfonamides (*2, 10*). Nevertheless, such effects require that the substance is bioaccessible, i.e., that it is freely dissolved and can be desorbed from the solid phase and that microorganisms can have access to its particular location in soil (*11, 12*). In this context, the term bioaccessibility is operationally defined and can be approached by different extraction techniques (*12*).

In general, the bioaccessibility of organic compounds in soil changes with time (*13*). For a range of hydrophobic contaminants (polycyclic aromatic hydrocarbons, polychlorinated biphenyls) and some pesticides (DDT, dieldrin, triazines) it has been shown that the fraction available for degradation, transport, or toxic effects decreases with an increasing residence time of the compounds in soils (*14–16*). However, this decrease in availability could not be attributed to chemical modification or degradation since the substances were still extractable with exhaustive methods. Thus, after "primary sorption" these compounds are subject to "aging" in soils. "Aging" in this context refers to the technical definition of decreasing extractability, and the underlying processes are termed "sequestration" (*17*). While sequestration of hydrophobic compounds is rather well understood (e.g., refs *18–20*), there is a lack of knowledge on the sequestration dynamics of polar compounds such as antibiotics. Here, we focus on the sequestration of sulfadiazine (SDZ), an important sulfonamide antibiotic, which is commonly used therapeutically and prophylactically during, e.g., pig fattening.

Kreuzig and Höltge (*21*) found SDZ concentrations of 20–98 $\mu$g kg$^{-1}$ only directly after manure application. These SDZ concentration levels dropped below the quantification limit 38 days after application in a test plot study. The authors attributed this to rapid formation of bound residues (BR). Later work indicated that covalent bonds to humic materials may in principle account for this (*22–24*). However, the used extraction method of Kreuzig and Höltge (*21*) (acetate buffer [pH 5.2]-ethyl acetate extraction) was not exhaustive. In contrast, Stoob et al. (*25*) and Förster et al. (*26*) were able to liberate substantial amounts of applied SDZ from soils several months after application using high-temperature extractions. This indicates that a portion of sulfonamides formerly assigned to the BR pool is in reality only sequestered and might potentially become released again over long periods into soil solution. Yet, SDZ residues of different degrees of bioaccessibility have not been differentiated in any study. This goal, however, can be achieved using sequential extraction protocols, as, e.g., shown for phenanthrene and various pesticides (*27, 28*) but hardly tested for antibiotics.

In the past, 0.01 M CaCl$_2$ was recommended for extraction of plant available nutrients because it mimics the soil solution regarding pH, concentration, and composition (e.g., ref *29*). Also batch sorption experiments usually rely on 0.01 M CaCl$_2$ (*30*). It is thus a reasonable extract also to predict the readily

bioaccessible amount of SDZ residues in soils. Methanol has been promising for the subsequent extraction step to describe field aging of pesticides (28, 31) or in combination with 0.01 M $CaCl_2$ to predict bioavailability of xenobiotics (32). During aging, however, xenobiotics become resistant to mild extraction procedures (e.g., refs 14 and 27), i.e., harsh conditions are required for releasing sequestered SDZ from soil, e.g., using hot microwave extractions with acetonitrile/water (26).

In addition to aging, metabolite formation may also form a sink of SDZ in soils. Some metabolites are already formed during the passage through the animal. To consider this process, our research unit treated fattening pigs with $^{14}$C-labeled SDZ. Lamshöft et al. (33) investigated the pharmacokinetics and subsequent transformation of SDZ during manure storage and observed that 96% of the administered radioactivity (RA) was excreted by the animals within 10 days. Although a large part of excreted RA was SDZ (44% of RA), 47% of RA were quantified as the two main metabolites N-acetylsulfadiazine (N-ac-SDZ) and 4-hydroxysulfadiazine (4-OH-SDZ). There was no significant BR formation in the manure.

The objective of this study was to elucidate the sequestration and transformation of manure-applied SDZ and its major metabolites in soils. For this purpose, we used the manure from the pig-medication experiment containing the excreted $^{14}$C-SDZ and its animal-derived metabolites and applied it to two common agricultural soils (Cambisol, Luvisol). During an incubation at controlled conditions we monitored the dynamics and distribution pattern of the different target compounds in fractions of different bioaccessibility using a sequential extraction method. The nonextractable RA allowed for estimating the BR pool, which likely comprised covalent bondings to soil organic matter (24).

## Material and Methods

**Studied Compounds and Chemicals.** Detailed information on the studied compounds are given in Förster et al. (26) and in Table S4, Supporting Information. The purity of the reagents used for the study was at least pro-analysis grade for salts, Millipore grade for water, and HPLC grade for organic solvents.

**Soils.** The soils used in the study were topsoil horizons of a Dystric Luvisol (pH, 6.3; $C_{org}$, 12.2 g kg$^{-1}$; sand, 60 g kg$^{-1}$) and an Endogleyic Cambisol (pH, 6.0; $C_{org}$, 9.9 g kg$^{-1}$; sand, 750 g kg$^{-1}$; see Supporting Information for further details).

**Sequential Extraction of Soils.** To obtain residue fractions of different degrees of bioaccessibility, soil samples (equivalent to 10 g dry weight, weighed into glass centrifuge tubes with Teflon-lined screw caps) were subjected to a sequential extraction procedure. Initially the soil was extracted with 25 mL of 0.01 M $CaCl_2$ on an end-over-end shaker (approximately 30 rpm, ambient temperature) for 24 h. Afterwards the samples were centrifuged at 900g for 30 min (Allegra 6KR Centrifuge; Beckman-Coulter, Krefeld, Germany), and after decanting and weighing the total extracts, weighed aliquots were taken for determination of $^{14}$C-SDZ-equivalents ($^{14}$C-SDZ$_{eq}$) by liquid scintillation counting (LSC) and liquid chromatography tandem mass spectrometry (LC-MS/MS) determinations. This extract represented the "$CaCl_2$ fraction". The soil was subsequently extracted with 25 mL of methanol for 4 h (end-over-end shaker, ambient temperature) and centrifuged again under the above-mentioned conditions. The extracts, representing the "MeOH fraction", were decanted and weighed. The weighed aliquots were taken for subsequent LSC measurements and LC-MS/MS determinations. Afterwards, the soil was extracted with acetonitrile: water 1:4 (v/v) according to Förster et al. (ref 26, termed "residual fraction") using microwave energy (MLS-Ethos 1600; MLS GmbH, Leutkirch, Germany). The extracts were

weighed, and an aliquot of 10 mL was transferred into glass centrifuge tubes and centrifuged for 30 min at 40 000g (Model J2-21 High Speed Centrifuge; Beckman, Krefeld, Germany) at ambient temperature. Afterwards, weighed aliquots of these extracts were taken for LSC measurements and LC-MS/MS determinations. The extracted soil plus remaining extraction solvent was quantitatively transferred into glass centrifuge tubes with Teflon-lined screw caps and centrifuged at 900g for 10 min at ambient temperature. Subsequently, the supernatant was discarded and the extracted soils were dried at 60 °C overnight, ground, and stored in glass vials at −20 °C until bound residues were determined via combustion.

Determination of $^{14}$C-SDZ$_{eq}$ was at least done in triplicate. In most instances LC-MS/MS measurements for each compound were also performed in triplicate ($n = 153$). In some cases, determination of SDZ and its metabolites was carried out in duplicate ($n = 17$) or in singular determination ($n = 8$), mostly due to analyte concentrations outside the valid range of calibration. This occurred predominantly for samples that had been diluted 1:9 (v/v) with acetonitrile: water 1:4 (v/v) after addition of internal standard to reduce quantification interferences due to coextracted matrix.

**Analysis of RA and Test Compounds: Data Analysis.** Detailed descriptions of the analysis of RA and the test compounds as well as of the equations of the fitted regression curves are given in the Supporting Information. The balances of RA were within the range of 95−101% for all samples, reflecting quantitative and reliable analyses of all fractions.

## Results and Discussion

**RA in Soils and Soil Extracts.** The level of total RA in soil (determined before extraction) did not change during incubation (218 days) for any of the three treatments (Figure 1), indicating that no significant mineralization of SDZ residues into $^{14}$CO$_2$ occurred during the experiment. This finding corroborated results by Schmidt et al. (24), who directly quantified evolving $^{14}$CO$_2$ from the manure-amended soils and reported mineralization rates of less than 2% within the incubation. A comparable low mineralization of SDZ was also reported by Kreuzig and Höltge (21) during laboratory experiments and test plot studies. Compared to the mineralization of other antibiotics ($^{14}$C-sarafloxacin hydrochloride: 0.58% (34)) or pesticides (e.g., nicosulfuron: <1.3% (35)) mineralization of SDZ residues in our experiment was in a similar range.

The extracted RA in the $CaCl_2$ and MeOH fractions decreased exponentially for Luvisol and Cambisol with fresh manure, resulting in dissipation half-life times (DT$_{50}$) of 16−11 days (Figure S1, Supporting Information). In parallel to this rapid decline of $^{14}$C-SDZ$_{eq}$ concentrations in these two fractions, an initial rise of extracted RA in the residual fraction occurred from 2490 to 3400 $\mu$g $^{14}$C-SDZ$_{eq}$ kg$^{-1}$ in the Luvisol and from 2060 to 3580 $\mu$g $^{14}$C-SDZ$_{eq}$ kg$^{-1}$ in the Cambisols in the first 14−29 days after application. After these temporary peak concentrations the amount of $^{14}$C-SDZ$_{eq}$ decreased slowly in this fraction until the end of the experiment (DT$_{50}$ of 900 days for the Luvisol and 1120 and 2410 days for Cambisol with fresh and aged manure, respectively; $p < 0.2$, $R^2 > 0.66$), leading to $^{14}$C-SDZ$_{eq}$ concentration levels comparable to those measured at the beginning of the aging experiment.

The amount of RA determined in the BR fraction increased throughout the whole experiment, rising from initially 1030 to 1770 $\mu$g $^{14}$C-SDZ$_{eq}$ kg$^{-1}$ at 1 day after application to 2810−3370 $\mu$g $^{14}$C-SDZ$_{eq}$ kg$^{-1}$ at the end of the incubation period (Figure 1). About 8 weeks after the start of the experiment the distribution of RA across all fractions remained fairly stable. In the experiment with aged manure the concentration of BR was even lower than for freshly applied manure and remained below the concentration of
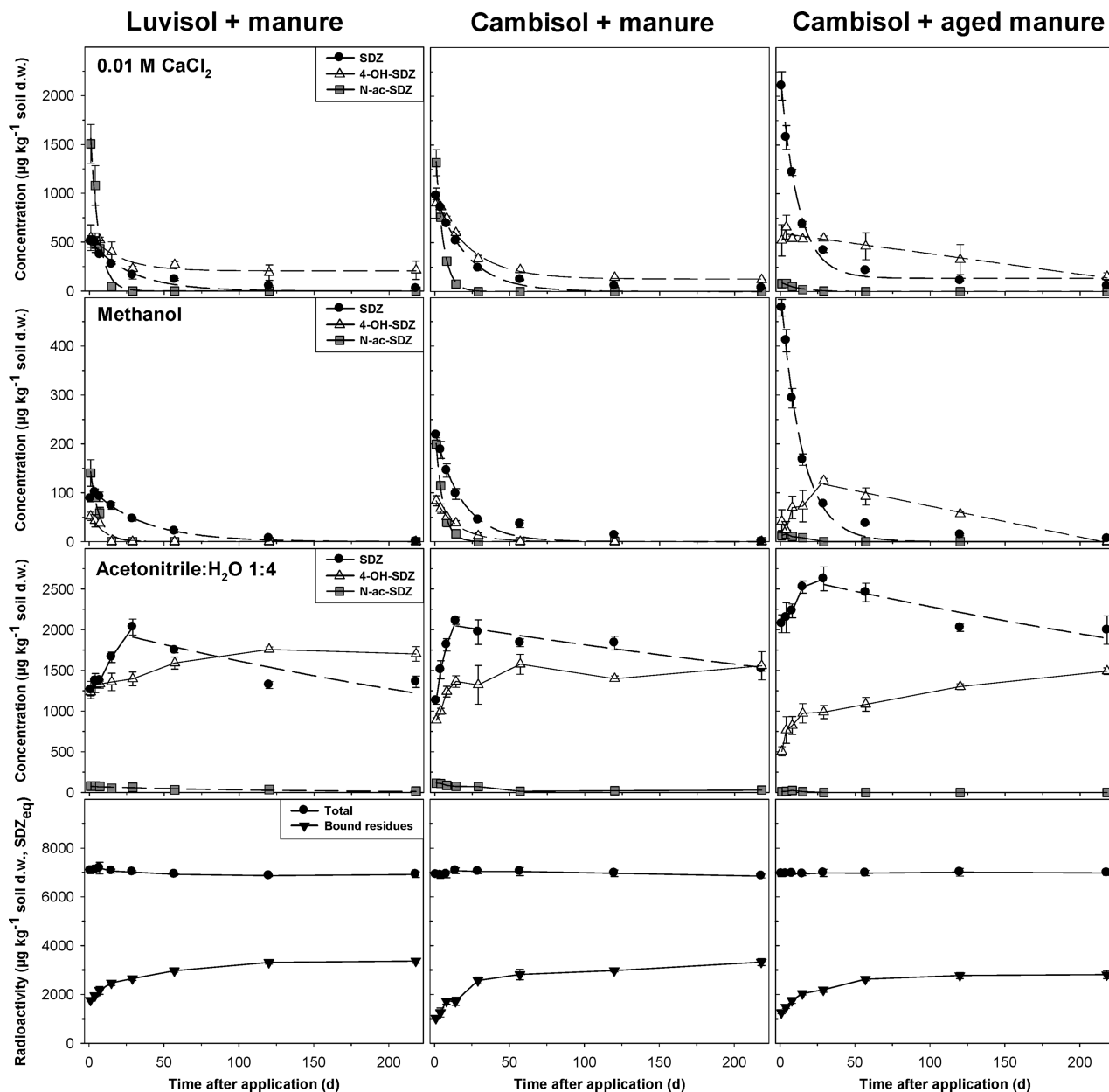
**FIGURE 1.** Concentrations (± standard deviation) of sulfadiazine (SDZ), and 4-hydroxysulfadiazine (4-OH-SDZ), *N*-acetylsulfadiazine (*N*-ac-SDZ) and total radioactivity and bound residue (sulfadiazine equivalents, $SDZ_{eq}$) in different soil extracts of the sequential extraction (model fits, dashed lines). 4-OH-SDZ concentrations were corrected to comply with the value of measured total RA in extracts (LSC measurements) for the sum of extracted compounds (SDZ, *N*-ac-SDZ, 4-OH-SDZ).

SDZ residues in the residual fraction throughout the incubation period. According to Barriuso et al. (*36*), who reviewed the BR formation for >90 pesticides, 50% of the these compounds showed a BR formation of <30%, whereas 12% of the pesticides exceeded 70% of initially applied RA in the BR fraction. Compared with our data (40−49% BR), BR formation of pesticides containing a pyrimidine moiety was in the same range. For sulfonamides, Bialk and Pedersen (*37*) reported formation of covalent bonds to quinone moieties via the aniline group of these compounds. It may therefore be speculated that SDZ and 4-OH-SDZ may bind to soil organic matter in a similar way, but a detailed characterization of BR (e.g., by NMR (*37*)) was beyond the scope of our study. We conclude that the main redistribution of SDZ residues among the fractions occurs within the first 2 months after application of manure. The overall influence of soil type and manure age on the dynamics of the fractions was low, suggesting that the given differences in soil texture were obviously not large enough to significantly affect SDZ

dynamics. Besides, also the aging of SDZ residues in manure before application (approximately 6 months) did not substantially change the time course of the extractability and binding of RA in the Cambisol.

The fate of manure was not explicitly monitored in this study as this is only possible using additional stable isotope labeling approaches (e.g., ref *38*) However, as indicated in this study, most of the added manure dissipates with the first 10 weeks.

**Concentrations of SDZ, 4-OH-SDZ, and *N*-ac-SDZ in the Sum of Extracts.** The results showed that the concentrations of the animal-derived conjugate *N*-ac-SDZ rapidly and exponentially decreased in the sum of extracts for the soils incubated with fresh manure ($DT_{50} = 3.8$ to 4.1 days; Figure S1, Table S2, Supporting Information). In parallel to the almost complete disappearance of *N*-ac-SDZ from levels of 1630−1720 $\mu g\ kg^{-1}$ within the first weeks after application the concentrations of SDZ and its main metabolite 4-OH-SDZ increased in the sum of extracts to maximum values of

2240 and 1940 $\mu$g kg$^{-1}$ 29 and 120 days after application for the Luvisol and to concentrations of 2760 and 2130 $\mu$g kg$^{-1}$ 8 days after application for the Cambisol, respectively. Hence, deacetylation of the N-ac-SDZ metabolite to SDZ and its subsequent transformation to 4-OH-SDZ is not only a relevant mechanism in manure ([33]) but also in soils. After 8–29 days the concentrations of SDZ showed a relatively steady but slow decline for the soils with fresh manure (DT$_{50}$ = 250 days for the Luvisol), which still left substantial residues at the end of the experimental period (>1400 $\mu$g kg$^{-1}$, Figure S1, Supporting Information). For the Cambisol with fresh manure a decline of the 4-OH-SDZ concentrations was observed 8 days after manure application until the end of the experiment, resulting in a DT$_{25}$ value of 16.8 days and a soil concentration of 1670 $\mu$g kg$^{-1}$ after 218 days. Both soils with fresh manure showed high residue concentrations at the end of the incubation experiment (>3220 $\mu$g kg$^{-1}$ for the sum of SDZ and 4-OH-SDZ). As for total RA, the metabolism pattern was similar for both soil types. Our dissipation rates therefore contradict the ones of Kreuzig and Höltge ([21]), who reported half-life times of SDZ in the range of ≤3 days in spiked soils with and without the presence of manure using, however, a relatively mild extraction procedure to recover SDZ from soil. Similarly, Blackwell et al. ([39]) reported a DT$_{50}$ of 3.5 days for the dissipation of sulfachloropyridazine in a sandy loam field soil also using a mild extraction protocol for analysis of antibiotics in soil rather than an exhaustive extraction. Slightly higher DT$_{50}$ of 18.6 and 21.3 days were reported for dissipation of sulfamethazine and sulfachloropyridazine in two different soils by Accinelli et al. ([40]). Studying the degradation of manure-derived sulfadimethoxine in soil, Wang et al. ([41]) reported DT$_{50}$ values of 3.0 to 11 days depending on initial substance concentration, manure application rate, and soil moisture. These data, however, are also based on mild extraction techniques for analyses of sulfonamides in soils. We conclude that a major portion of SDZ assigned to "bound residues" in former studies was actually reversibly sequestered in a residual fraction, which remained extractable though at low degree of bioaccessibility (requiring hot extraction in the microwave ([26])).

In contrast to soils applied with fresh manure, the amount of extractable N-ac-SDZ remained low throughout the experiment with aged manure (<110 $\mu$g kg$^{-1}$), indicating that N-ac-SDZ was already transformed to SDZ during the aging of manure (see also ref [33]). Consequently, the concentration of SDZ remained at a higher level in soil of this treatment as those in the soils applied with fresh manure (Table S2, Supporting Information). We conclude that SDZ and 4-OH-SDZ are of long-term relevance in the residual fraction of soils that receive manure from SDZ-treated pigs, and thus, they may impact the soil ecosystem due to slow release processes or accumulation. The soil type did not exert a substantial influence on total substance pattern and kinetics of SDZ residues in our experiments.

**Concentrations of SDZ, 4-OH-SDZ, and N-ac-SDZ in the Extracts.** As only a minor portion of total antibiotic concentrations is presumably available for transformation reactions and uptake by microorganisms, we aimed at capturing this fraction using "mild" extraction procedures ([27, 28]). Using aqueous CaCl$_2$ as extraction solvent, we observed an exponential decrease of substance concentrations over time for all monitored compounds with a DT$_{50}$ value of 3.4–3.9 days for N-ac-SDZ, 15–19 days for SDZ, and 21–32 days for 4-OH-SDZ in both soils that received fresh manure (Figure 1, Table 1). In comparison, SDZ dissipation was slightly enhanced and N-ac-SDZ and 4-OH-SDZ disappearance slower in soils applied with aged manure (Table 1).

Interestingly, the dissipation dynamics of SDZ and its metabolites were similar for both the CaCl$_2$ fraction and the fraction extracted with methanol, which is known to increase

**TABLE 1. Concentrations ($\pm$ standard deviation, $n = 3$) of Sulfadiazine (SDZ), 4-Hydroxysulfadiazine (4-OH-SDZ), and N-Acetylsulfadiazine (N-ac-SDZ) in Soil Extracts and Calculated Dissipation Times (4-OH-SDZ concentrations were corrected to comply with the value of measured total RA in extracts (LSC measurements) for the sum of extracted compounds (SDZ, N-ac-SDZ, 4-OH-SDZ))**

| analyte | soil type | manure type | CaCl$_2$ fraction concentration ($\mu$g kg$^{-1}$) max | min | DT$_{50}$[a] (days) | R$^2$[b] | MeOH fraction concentration ($\mu$g kg$^{-1}$) max | min | DT$_{50}$ (days) | R$^2$ | residual fraction (acetonitrile:water) concentration ($\mu$g kg$^{-1}$) max | min | DT$_{50}$ (days) | R$^2$ |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| SDZ | Luvisol | not aged | 506 ± 59 | 24.2 ± 4.9 | 19[c] | 0.963 | 100 ± 7 | 6.64[n] | 24[c,h] | 0.998 | 2030 ± 100 | 1260 ± 110 | 290[c,i] | 0.754[f] |
|  | Cambisol | not aged | 978 ± 38 | 34.5 ± 1.0 | 15[c] | 0.993 | 218 ± 5 | 12.8 ± 3.5 | 13[c] | 0.981 | 2110 ± 40 | 1130 ± 40 | 490[c,j] | 0.904 |
|  | Cambisol | aged | 2100 ± 150 | 53.5 ± 4.2 | 10[d] | 0.993 | 478 ± 17 | 5.65 ± 0.53 | 10[c] | 0.994 | 2630 ± 150 | 2000 ± 170 | 440[c,k] | 0.829 |
| 4-OH-SDZ | Luvisol | not aged | 545 ± 132 | 188 ± 80 | 32[d] | 0.935 | 51.6 ± 8.4 | 37.1[n] | 6.0[c] | 0.935 | 1760 ± 30 | 1230 ± 50 |  |  |
|  | Cambisol | not aged | 905 ± 155 | 119 ± 5 | 21[d] | 0.995 | 83.5 ± 10.4 | 11.8 ± 5.9 | 11[c] | 0.998 | 1580 ± 120 | 889 ± 30 |  |  |
|  | Cambisol | aged | 659 ± 121 | 151 ± 40 | 140[e,l] | 0.952 | 125 ± 4 | 23.9 ± 17.3 | 110[e,m] | 0.985 | 1490 ± 40 | 505 ± 56 |  |  |
| N-ac-SDZ | Luvisol | not aged | 1510 ± 200 | 44.8 ± 7.6 | 3.9[c] | 0.981 | 140 ± 27 | 58.6 ± 11.4 | 4.2[c] | 0.988 | 78.1 ± 13.8 | 15.4 ± 6.5 | 81[c] | 0.837 |
|  | Cambisol | not aged | 1320 ± 130 | 76.1 ± 7.1 | 3.4[c] | 0.999 | 199 ± 9 | 16.2 ± 4.0[o] | 3.3[c] | 0.997 | 112 ± 13 | 13.0 ± 10.1[o] | n.d.[g] | n.d. |
|  | Cambisol | aged | 81.7 ± 18.2 | 4.64 ± 1.22 | 7.9[c] | 0.964 | 15.4 ± 5.9 | 7.83 ± 3.62[o] | n.d. | n.d. | 25.1 ± 32.0 | 9.48 ± 1.26[o] | n.d. | n.d. |

[a] Time period in which 50% of initial concentration (start of dissipation) vanishes. [b] Coefficient of determination. [c] Exponential dissipation model. [d] Exponential dissipation model with constant concentration fraction (iterative determination). [e] Linear dissipation model. [f] Parameter estimation not significant at the $p < 0.1$ probability level. [g] Not determined due to overall low substance concentrations. [h] Time period in which 50% of initial concentration (day 1) disappears: 36 days. [i] Time period in which 50% of initial concentration (day 1) disappears: 530 days. [j] Time period in which 50% of initial concentration (day 1) disappears: 940 days. [k] Time period in which 50% of initial concentration (day 1) disappears: 630 days. [l] Time period in which 50% of initial concentration (day 1) disappears: 160 days. [m] Time period in which 50% of initial concentration (day 1) disappears: 210 days. [n] $n = 1$. [o] $n = 2$.

the swelling of soil organic matter (*18*), though the latter fraction exhibited substantially lower initial concentrations in comparison with the $CaCl_2$ extract (soils with fresh manure, 51.6–218 vs 506–1510 $\mu$g kg$^{-1}$; soils with aged manure, 13.0–478 vs 79.8–2100 $\mu$g kg$^{-1}$). Also, the computed $DT_{50}$ values for all compounds were of similar magnitude for both extracts (3.3–7.9 days for *N*-ac-SDZ, 10–24 days for SDZ, and 6.0–140 days for 4-OH-SDZ, Table 1). We conclude that the methanol-extractable antibiotics are likely bound in a similar manner to soil as the ones in the $CaCl_2$ extract, presumably representing the fraction in sorption equilibrium with the portion extracted by $CaCl_2$. Hence, in contrast to pesticides with similar water solubility and sorption coefficients as SDZ (e.g., atrazine, metolachlor, alachlor *28, 31, 32*), methanol extraction did not capture a SDZ residue pool of lower bioaccessibility in the studied soils. To which extent the coapplication of antibiotics with manure contributed to this contrasting behavior of SDZ residues in soils cannot be answered in our study. We conclude that for modeling of sequestration dynamics and related antimicrobial effects the $CaCl_2$ and MeOH fractions, as operationally defined in our study, may be considered as one fraction for the soils studied.

In contrast to the two more readily available fractions, different residue dynamics are observed in the residual fractions for both soils: While SDZ concentrations increased until 14–29 days after application and then began to decline, 4-OH-SDZ increased until the end of the incubation experiment (Figure 1). Hence, identifying the residual fraction by our optimized extraction procedure (*26*) allowed for differentiating between real dissipation and mere sequestration dynamics of antibiotic residues in soils.

The increase of SDZ and 4-OH-SDZ concentrations in the residual fraction at the beginning of the experiment coincided with the steep decline of *N*-ac-SDZ concentrations in the easily extractable fractions. Obviously, the transformation products of *N*-ac-SDZ in the available fractions do not increase the available pools of SDZ and 4-OH-SDZ; the latter are quickly sequestered into the residual fraction during the first 2 months of incubation. In contrast, the residual fraction contained *N*-ac-SDZ at low initial concentrations only (<120 $\mu$g kg$^{-1}$), which also dissipated with a more or less steady decline to concentrations below 30 $\mu$g kg$^{-1}$ until 218 days after application. Similar dynamics are seen for the soil applied with aged manure, though at slightly higher or lower absolute concentrations levels for SDZ and 4-OH-SDZ, respectively. The $DT_{50}$s of SDZ in the residual fraction for both soils and manure treatments were outside the experimental observation period (290–490 days), indicating a high persistence of these substances in this fraction (Table 1). In comparison to $DT_{50}$s reported by Kreuzig and Höltge (*21*) for SDZ and the $DT_{50}$ values reported by Blackwell et al. (*42*) for sulfachloropyridazine in soils we observed ca. 100 times higher half-life times for sequestered SDZ.

Besides SDZ, also 4-OH-SDZ therefore is a long-term soil contaminant, occurring at similarly high concentration levels as the parent (ca. 1500–2000 $\mu$g kg$^{-1}$) in all treatments of our study at 218 days after application. In contrast, *N*-ac-SDZ is not sequestered into the residual fraction at relevant amounts, which may be due to its fast transformation into SDZ and/or the blocking of the free amino group, being crucial for a first attachment and later covalent bond formation of the sulfonamide aniline group with quinone moieties in organic matter (e.g., refs *37* and *43*). In summary, our results thus support the assumption that the residual fraction is a fraction of low accessibility of its residues for (bio)degradation processes.

**Development of Soil Sorption Coefficients (apparent $K(CaCl_2)$) with Time.** Sorption experiments with sulfonamides and pure minerals revealed that diffusion of sulfonamide residues into micropores of ferrihydrite may contribute
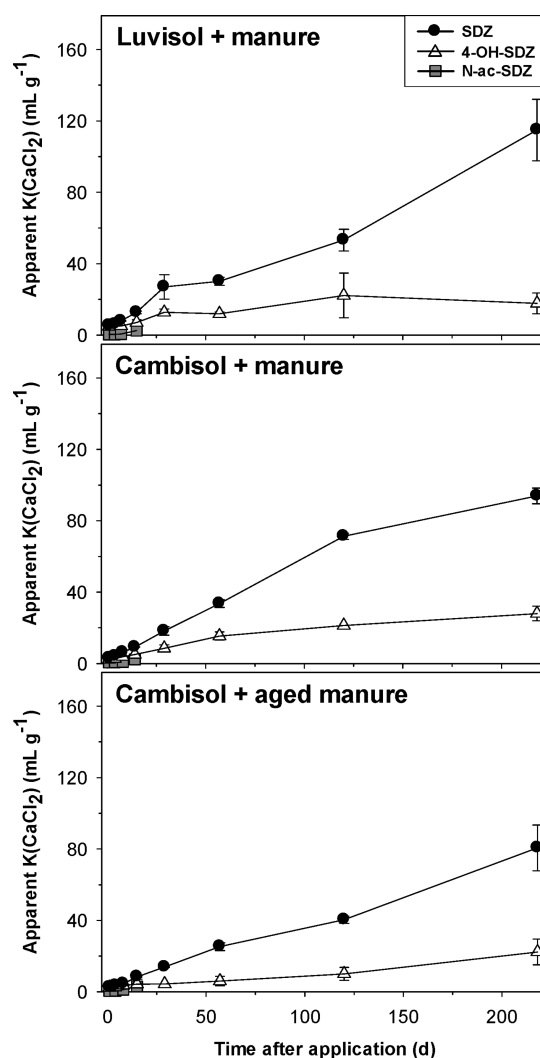


**FIGURE 2. Apparent $K(CaCl_2)$ values ($\pm$ standard deviation) of sulfadiazine (SDZ), 4-hydroxysulfadiazine (4-OH-SDZ), and *N*-acetylsulfadiazine (*N*-ac-SDZ) in different soils. For calculations, 4-OH-SDZ concentrations were corrected to comply with the value of measured total RA in extracts (LSC measurements) for the sum of extracted compounds (SDZ, *N*-ac-SDZ, 4-OH-SDZ).**

to sorption and aging processes (*44*). For natural soils it was suggested that the cationic species of sulfonamides interacts with organic matter (*45*) and the anionic ones predominantly with positively charged soil oxides (*43*). Little is known on the long-term sorption interactions. In general, the soil sorption coefficient is regarded as an equilibrium constant, which is then also used for the long-term modeling of compound fate in soils. However, increasing evidence has been collected during the last two decades that sorption coefficients of organic pollutants in soils tend to increase with time (e.g., refs *28, 46,* and *47*), presumably due to pore diffusion in the fixed intraparticle pore system or slow intraorganic matter diffusion in "condensed" organic matter phases in which diffusing molecules are retained by adsorption to high affinity microvoids or pores in the organic matter matrix (*18, 19*). In our experiment we calculated "apparent" $K(CaCl_2)$ values by dividing the soil concentration of SDZ compounds (equivalent to substance mass extracted within the residual fraction and MeOH fraction) by the concentrations measured in the $CaCl_2$ extracts. Consequently, we were able to plot the "short-term" distribution coefficients (equilibration time, 24 h; defined as apparent $K(CaCl_2)$) of the target compounds vs incubation time (Figure 2). The initial apparent

K(CaCl$_2$) values for SDZ measured in our study are slightly larger than the one (2 mL g$^{-1}$) reported by Thiele-Bruhn et al. (43) for SDZ sorption to a silt loam Chernozem (1.6% organic carbon, pH 7.0). Possibly, the lower pH in our soils led to increased adsorption (48) in spite of lower organic C contents than in the Chernozem. The SDZ showed an increase of the apparent K(CaCl$_2$) by a factor of 22 and 32 during the 218 day incubation for Luvisol and Cambisol, respectively. A similar increase (31-fold) was observed for the Cambisol when aged SDZ residues were applied with manure. A slightly less extent of sorption increase with time was observed for 4-OH-SDZ, for which apparent K(CaCl$_2$) values increased by a factor of 5 (Luvisol) and 10 and 12 (Cambisol treated with aged and fresh manure, respectively). The apparent K(CaCl$_2$) increase of $N$-ac-SDZ (maximum factor of 9) could only be followed until 16 days after application at high replicate variability due to rapidly decreasing concentrations of this compound in the water extract. Concerning SDZ, Stoob et al. (49) calculated distribution ratios between soil matrix and pore water, which, similar to our apparent K(CaCl$_2$) values, increased with time.

The time-dependent evolution of apparent K(CaCl$_2$) values may be well fitted using the Richter model, which is based on a three-compartment model assuming two binding sites: a reversible one in equilibrium with a solution phase and an irreversible one ($R^2 > 0.88$ for all compounds (50)). This model assumes, however, that there is a difference between the decay rates in this irreversible fraction and the aqueous phase, which according to our fitting was sometimes positive and sometimes negative. This made no sense. Hence, we conclude that although the data may be fitted using standard models from, e.g., pesticide fate analyses, the processes behind are more complex than those considered in these models.

## Acknowledgments

## Supporting Information Available

Description of the analysis of RA and test compounds; information on the physicochemical properties of the test compounds, data analysis, detailed soil properties, min/max concentrations, and DT$_{50}$ values of the compounds in the sum of extracts; min/max values of apparent K(CaCl$_2$); set of figures with concentration trends of SDZ$_{eq}$ for the extracts and of the compounds in the sum of extracts. This material is available free of charge via the Internet at http://pubs.acs.org.

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ES8026538