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Soil microbial community responses to sulfadiazine-contaminated manure in different soil microhabitats

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ABSTRACT

Veterinary antibiotics such as sulfadiazine (SDZ) are applied with manure to agricultural soil. Antimicrobial effects of SDZ on soil microbial community structures and functions were reported for homogenized bulk soils. In contrast, field soil is structured. The resulting microhabitats are often hot spots that account for most of the microbial activity and contain strains of different antibiotic sensitivity or resilience. We therefore hypothesize that effects of SDZ are different in diverse soil microhabitats. We combined the results of laboratory and field experiments that evaluated the fate of SDZ and the response of the microbial community in rhizosphere, earthworm burrow, and soil macroaggregate microhabitats. Microbial communities were characterized by phenotypic phospholipid fatty acid (PLFA) and genotypic 16S rRNA gene patterns (DGGE) and other methods. Data was evaluated by principle component analyses followed by two-way ANOVA with post-hoc tests. Extractable SDZ concentrations in rhizosphere soil were not clearly different and varied by a factor 0.7–1.2 from those in bulk soil. In contrast to bulk soil, the extractable SDZ content was two-fold larger in earthworm burrows, which are characterized by a more hydrophobic organic matter along the burrow surface. Also, extractable SDZ was larger by up to factor 2.6 in the macroaggregate surface soil. The rhizosphere effect clearly increased the microbial biomass. Nonetheless, in the 10 mg SDZ kg^{-1} treatment, the biomass deceased by about 20% to the level of uncontaminated bulk soil. SDZ contamination lowered the total PLFA concentrations by 14% in the rhizosphere and 3% in bulk soil of the field experiment. Structural shifts represented by *Pseudomonas* DGGE data were larger in SDZ-contaminated earthworm burrows compared to bulk soils. In the laboratory experiment, a functional shift was indicated by a four-fold reduced acid phosphatase activity in SDZ-contaminated burrows compared to bulk soil. Structural and functional shifts after SDZ contamination were larger by a factor of 2.5 in the soil macroaggregate surface versus interior, but this relation reversed over the long-term under field conditions. Overall, the combined effects of soil microhabitat, microbial community composition, and exposure to SDZ influenced the microbial susceptibility towards antibiotics under laboratory and field conditions.

1. Introduction

Bactericidal or bacteriostatic antibiotics are widely used in agricultural practice to protect or cure livestock from infectious diseases by disrupting the propagation of undesirable bacteria. Global agricultural antibiotic use is estimated to be 50,000–200,000 tons/year (Ok et al., 2011; Du and Liu, 2012; Kumar et al., 2012). Antibiotics of the sulfonamide class are among the most prescribed veterinary antibiotics in North America and Europe (Sarmah et al., 2006). Large antibiotic amounts are excreted unchanged or as metabolites and released into the soil environment by farmyard manure (Thiele-Bruhn, 2003; Sarmah et al., 2006). Sulfadiazine (SDZ) is an often used sulfonamide that inhibits the enzymatic conversion of *p*-aminobenzoic acid during folic acid metabolism (Brown, 1962). SDZ, as a bacteriostatic broad-band antibiotic, inhibits the growth of both $Gram^+$ and $Gram^-$ bacteria (Brown, 1962). Antibiotic effects on soil microbial community activities have been clearly documented for bulk soil samples. They include soil respiration and Fe(III) reduction (Thiele-Bruhn and Beck, 2005), functional aspects (e.g., microbial-derived exo-exoenzymes, nitrification; Gutiérrez et al., 2010; Kotzerke et al.,

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2008), and structure (16S rRNA gene, phospholipid fatty acid patterns; Hammesfahr et al., 2008; Reichel et al., 2013). Dose-response relationships of the microbial community to SDZ were documented in bulk soil (Thiele-Bruhn, 2003; Liu et al., 2009). Despite the rapid dissipation of the operationally defined bio-accessible SDZ fraction (Rosendahl et al., 2011), long-term effects of SDZ in soil have been determined (Hammesfahr et al., 2008; Reichel et al., 2013). This might be explained by a remobilization of small quantities of SDZ over the long-term from the more strongly bound and persistent SDZ residues (Zarfl et al., 2009; Rosendahl et al., 2011). Soil, however, is typically not a homogeneous bulk soil but separated into microhabitats that might be hot spots of microbial activity.

Beare et al. (1995) named five hot spots that account for most of the microbial activity in soil: porosphere, detritusphere, aggregatosphere, drilosphere of earthworms, and rhizosphere. The latter three microhabitats were selected in this study to evaluate antimicrobial effects.

The rhizosphere habitat sums up many different influences of soil type, pH changes, water and nutrient status, organic amendments, plant species and development stage (Hawkes et al., 2007; Buée et al., 2009). The release of low- and high-molecular rhizodeposits is particularly relevant for the growing microbial community (Bertin et al., 2003; Shi et al., 2013). Rhizosphere soil is inhabited by various saprophytic and mycorrhizal fungi along with beneficial bacteria or antagonistic pathogens (Buée et al., 2009). Dominant bacteria are Proteobacteria such as *Pseudomonas* (Hawkes et al., 2007) that often occur in higher abundance in the rhizosphere, comprising also some members that produce antimicrobials (Bergsma-Vlami et al., 2005; Costa et al., 2006). Accordingly, Mavrodi et al. (2012) determined an accumulation of the natural antibiotic *phenazine-1-carboxylic acid* in the rhizosphere of dryland cereals of up to 1.6 $\mu g\,g^{-1}$ fresh roots. Importantly, contamination by synthetic antibiotics applied with manure often ranges at a higher level of $\mu g\,kg^{-1}$ to $mg\,kg^{-1}$ dry soil (Thiele-Bruhn, 2003). Only few reports are available on the effects of anthropogenic antibiotics on rhizosphere microbial communities. SDZ-contaminated manure changed the gene patterns of N-cycling microbes in rhizospheres of clover and maize (Ollivier et al., 2010), and affected the microbial community structure in rhizosphere soil (Reichel et al., 2013). Bio-accessible SDZ dissipated faster near roots than in bulk soil (Rosendahl et al., 2011), but also altered root architecture and function in highly contaminated soil (Michelini et al., 2012).

Earthworm activity often stimulates the biological activity in soil due to beneficial influences on soil porosity, aggregation, nutrient and substrate availability, and bioturbation (Edwards, 2004; Blouin et al., 2013). The earthworm drilosphere is characterized by structures such as casts and burrows (Brown, 1995). Burrow walls can be lined with a mixture of soil, mucus and organic matter, temporarily stimulating the microbial biomass and activity up to ten or more times (Lavelle et al., 1995; Brown and Doube, 2004). Anecic earthworms such as *Lumbricus terrestris* (L.) create vertical burrows that facilitate the infiltration of water from the soil surface (Ernst et al., 2009). These macropores can also promote the transport of dispersed or dissolved manure compounds, microorganisms (Joergensen et al., 1998; Chadwick and Chen, 2002), as well as veterinary antibiotics into the soil profile (Kay et al., 2004). Xenobiotics bind onto the burrow walls and can accumulate (Edwards et al., 1992). Such transport and accumulation of antibiotics in earthworm burrows will have further consequences for dose-related adverse effects on microorganisms. Kotzerke et al. (2010) found that denitrificants (gene copies) were reduced tenfold by the exposure to SDZ in earthworm guts. Forty percent of the radioactivity of ^{14}C-ciprofloxacin was transported down the soil profile by earthworm activity (Mougin et al., 2013).

Soil aggregates are part of the soil structure and are operationally defined units of soil that are revealed after mechanical disruption along zones of weakness or pores (Young and Ritz, 2005). Different aggregate types are defined according to size as well as physical or chemical characteristics: small (2–20 μm) and large microaggregates (20–250 μm) are very stable and formed by e.g., bacterial polysaccharides, clays, and highly aromatic organic matter; small (250–2000 μm) and larger macroaggregates (>2 mm up to several centimeters) are less stable and built-up from preceding aggregates that are linked by bacteria, fungi, roots, and organic matter (Gobat et al., 2004). Microbial communities of different aggregate fractions (outer surface and interior) differ in their composition, functions, and activity (Mummey and Stahl, 2004; Mummey et al., 2006) due to small-scale gradients of pH, water, gases such as O_2, and organic matter as well as predation (Standing and Killham, 2007; Davinic et al., 2012; Mueller et al., 2012). Microbial biomass and activity are higher at pore system-connected aggregate surfaces with a better accessibility to growth substrates compared to the interior of soil macroaggregates (Jasinska et al., 2006). SDZ concentrations also showed small-scale gradients and gradually declined towards the interiors (Rosendahl et al., 2011). Time-dependent diffusion into the interior of aggregates was previously documented for pesticides by Van Beinum et al. (2005). We hypothesized that antibiotic fate and effects are different and more pronounced in soil microbial hot spots such as soil macroaggregate surfaces, earthworm burrows, and rhizosphere microhabitats of structured soil. This was evaluated by investigating SDZ fate and effects in different soil microhabitat samples from laboratory and field experiments on the same Luvisol topsoil.

2. Material and methods

2.1. Experimental

Soil material from the Ap horizon of a silt loam Luvisol was sampled from an arable field at Jülich-Merzenhausen, Germany (50°55′48,77″N, 6°17′20,02″E). The same soil was used for the laboratory and field experiments. The soil had the following properties: pH ($CaCl_2$) 6.3; clay 16%; silt 78%; sand 6%; C_{org} 1.2%; maximum water holding capacity (WHC_{max}) 45.8 $g\,g^{-1}$ (Förster et al., 2009). The pig manures were cumulatively collected from a group of pigs at the Agricultural Experimental Station for Livestock Science of the University of Bonn (Germany). The animals were fed with the same food mixture over the whole sampling period. For the field experiment, the same group of pigs was medicated intramuscularly with 30 $mg\,SDZ\,kg^{-1}$ bodyweight on four consecutive days, following the recommended dosage for the SDZ injectable solution (200 $mg\,ml^{-1}$), supplied by Vetoquinol Biowet (Gorzow Wielkopolski, Poland). Pig manures of the field experiment were stored in dark at 15 °C. Aliquots of the uncontaminated manure were stored at −20 °C, acclimatized over one week, and sieved before conducting the laboratory experiments with and without spiking in artificial sulfadiazine sodium salt (99.0% minimum, CAS: 547-32-0, Sigma–Aldrich, Germany). The processed manure was characterized by pH 6.0; C:N 7.0, and 1.2% dry matter (dm). Generally, the laboratory experiments were conducted at a manure-to-soil ratio of 1:25 (w/dm). The supplementary information (SI) of this manuscript provides more detailed method descriptions and result data.

2.1.1. Experimental design and sampling of laboratory experiments

2.1.1.1. Rhizosphere soil. Effects of SDZ-spiked manure in rhizosphere soil were investigated using inert polypropylene Kick-Brauckmann pots (25.5 cm height, 28.5 cm external diameter),