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Research Article

RESEARCH ARTICLE

Do drying and rewetting cycles modulate effects of sulfadiazine spiked manure in soil?

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One sentence summary: Drying–rewetting cycles modulated the effects of sulfadiazine applied with manure on the soil bacterial community structure but had only a negligible effect on the soil resistome.
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ABSTRACT

Naturally occurring drying–rewetting events in soil have been shown to affect the dissipation of veterinary antibiotics entering soil by manure fertilization. However, knowledge of effects on the soil microbial community structure and resistome is scarce. Here, consequences of drying–rewetting cycles on effects of sulfadiazine (SDZ) in soil planted with *Dactylis glomerata* L. were investigated in microcosms. Manure containing SDZ or not was applied to the pregrown grass and incubated for 56 days in a climate chamber. Water was either added daily or reduced during two drying events of 7 days, each followed by a recovery phase. Total community DNA was analyzed to reveal the effects on the bacterial community structure and on the abundance of *sul1*, *sul2*, *intI1*, *intI2*, *qacE* + *qacE*Δ*1*, *traN* and *korB* genes relative to 16S rRNA genes. 16S rRNA gene-based DGGE fingerprints indicated that drying–rewetting cycles modulated the effects of SDZ on the bacterial community structure in the soil. Furthermore, the SDZ treatment increased the relative abundance of sulfonamide resistance and integrase genes compared to the control. However, this increase was not different between moisture regimes, indicating that drying–rewetting had only a negligible effect on the selection of the resistome by SDZ in the manured soil.

Keywords: drying; rewetting; sulfadiazine; rhizosphere; resistome; mobilome

INTRODUCTION

Sulfadiazine (SDZ) is a veterinary antibiotic commonly used in animal husbandry (Sarmah, Meyer and Boxall 2006). Because of its high excretion rate and persistence in manure, it can reach soils by manure fertilization where it was shown to influence the structure and function of soil bacterial communities and to increase the abundance and transfer of antibiotic resistance genes (Jechalke *et al.* 2014a). In soil, the fate and effects of antibiotic compounds can be influenced by several processes such as microbial degradation, sorption or sequestration, which influence their bioaccessibility (Boxall *et al.* 2004; Sarmah, Meyer and Boxall 2006; Du and Liu 2012; Jechalke *et al.* 2014a). Although the extractability and thus putative bioaccessibility of SDZ declines rapidly in soil, sequestration is a reversible process, and it

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was reported that small concentrations of SDZ can be detectable for extended periods of time by mild extraction designated as the 'potentially bioavailable' fraction (Förster *et al.* 2009; Schauss *et al.* 2009; Zarfl, Klasmeier and Matthies 2009; Rosendahl *et al.* 2011). Furthermore, a recent study by Reichel *et al.* (2014) could show that bioavailable SDZ fractions are strongly influenced by the water regimes of the soil. The authors demonstrated for the same experimental setup described in this study that in a soil that undergoes frequent drying–rewetting cycles the bioavailable fraction of SDZ is larger than in control soils which were hold under a constant water regime. It was suggested that the dissipation of SDZ was accelerated in the permanently moist soil due to a higher accessibility of polar sorption sites of soil organic matter. These results were confirmed by the analysis of *Pseudomonas* and *Betaproteobacteria* communities, which were differing as a result of the different amounts of bioavailable SDZ in the investigated treatments (Reichel *et al.* 2014). Furthermore, for the experiment it was shown by Radl *et al.* (2015) that drying–rewetting changed the response pattern of nitrifiers in soil to the application of manure containing SDZ.

Since nutrients and other resources typically are sparsely available in soil, microbial structure and activity is greatly influenced by plant roots which release nutrients by exudation, secretion and leakage to the plant rhizosphere (Doornbos, van Loon and Bakker 2012). Hence, the rhizosphere is considered to be a hotspot of gene transfer which could be confirmed by previous studies showing a higher transfer frequency of sulfonamide resistance genes in rhizosphere compared to bulk soil samples (Kopmann *et al.* 2013; Jechalke *et al.* 2013c).

In this study, the influence of drying–rewetting cycles on the effects of SDZ on bacterial community structure and resistome in soil planted with *Dactylis glomerata* L. was investigated. To this end, manure spiked with SDZ and unspiked manure was applied to pots with pregrown plants, and the two treatments were incubated in climate chambers for 56 days at constant temperature. Water was either added regularly (constant moisture regime, CMR) to maintain moisture conditions of about 30%–40% of the maximum water holding capacity of the soil (WHC_{max}) or reduced in two drying events of 7 days resulting in a reduction of water contents to 10% WHC_{max}. Each drying event was followed by a recovery phase of 21 days at about 40% WHC_{max} (dynamic moisture regime, DMR). Effects on bacterial community structure as well as on the abundance of sulfonamide resistance genes (*sul1*, *sul2*), class 1 and 2 integron integrase genes (*intI1*, *intI2*) and the associated quaternary ammonium compound resistance gene (*qacE* + *qacE*Δ*1*) were assessed. Furthermore, *traN* and *korB* genes specific for LowGC-type and IncP-1 plasmids, respectively, were quantified. These plasmid groups were previously shown to play an important role in the dissemination of sulfonamide resistance in the agricultural environment (Heuer *et al.* 2009, 2012; Jechalke *et al.* 2013c). Based on the observed increased bioavailability of SDZ in the DMR compared to the CMR treatment, we postulated an increase of resistance genes in the DMR treatments.

MATERIALS AND METHODS

Experimental setup

The climate chamber experiment was carried out at the Helmholtz Zentrum München, Germany, and was performed as described previously (Reichel *et al.* 2014; Radl *et al.* 2015). In short, 88 pots (9 × 9 × 20 cm^{-3}) were filled with 1.45 kg Luvisol topsoil (dry mass) from an arable field located in Merzenhausen, Germany, and sowed with *D. glomerata* L. After an 11-week incubation in the greenhouse to obtain dense root masses, stock solutions of SDZ were mixed with pig manure from the Agricultural Experimental Station for Livestock Sciences Frankenforst (University of Bonn, Germany), and this mixture or manure without SDZ (control manure) was applied to the soil surface of each pot, avoiding plant contamination. The amount of manure applied corresponded to typical agricultural praxis (30 m^3 ha^{-1}). Manure parameters were described by Reichel *et al.* (2014). SDZ was spiked to manure to reach final concentrations of 0 (SDZ 0) or 4 mg kg^{-1} soil dry mass (SDZ 4). The pots (four replicates per treatment and sampling) were incubated in climate chambers with 70% humidity, 16 h daylight and a constant temperature of 20 ± 1°C. For the reproducibility of the experiment, the radiation was measured by a double monochromator system TDM300 (Bentham, Reading, UK), and the results were described in detail by Reichel *et al.* (2014).

For the CMR, water was added daily to half of the pots (SDZ 0 and SDZ 4) to maintain stable soil moisture conditions (average 38% WHC_{max}). However, due to the growth of the plants, moisture variations could not be avoided but never dried below 27% WHC_{max} (Reichel *et al.* 2014). For the DMR, the remaining pots were subjected to two 7-day periods (days 0–7 and 28–35) without watering to achieve soil moisture of approximately 10% WHC_{max} while avoiding the wilting point of grass, followed by a 21-day period of daily watering to 40% WHC_{max}.

Sampling and total community DNA extraction

Soil samples were taken from the upper 5 cm after manure application (day 0), after the drying periods (days 7 and 42) and during/at the end of the rewetting periods (days 14, 28 and 56). Total community DNA was extracted and purified as described in Radl *et al.* (2015) from 0.4 g soil. Total community DNA from manure was extracted as described previously (Jechalke *et al.* 2013c).

Quantification of target genes

The sulfonamide resistance genes *sul1* and *sul2*, the *traN* gene specific for LowGC-type plasmids, *intI1* and *intI2* integrase genes of class 1 and 2 integrons, respectively, *korB* of IncP-1 plasmids, quaternary ammonium compound resistance genes *qacE* and the *qacE*Δ*1* variant as well as bacterial 16S rRNA genes (*rrn*) were quantified by real-time PCR 5′-nuclease assays in a CFX96 real-time PCR detection system (Bio-Rad, Hercules, CA) as described previously (Heuer and Smalla 2007; Heuer *et al.* 2009; Barraud *et al.* 2010; Jechalke *et al.* 2013a; Jechalke *et al.* 2014b). To normalize differences in DNA extraction and amplification efficiencies between samples, gene copies were divided by the *rrn* gene numbers (relative abundance) and the results were log transformed.

PCR and DGGE

Amplification of bacterial 16S rRNA gene fragments from total community DNA by PCR and separation of PCR products by denaturing gradient gel electrophoresis (DGGE) were performed as described previously (Weinert *et al.* 2009). Bacterial primers used (F984-GC, R1378) were described by Heuer *et al.* (1997).

Statistical analysis

After the alignment of the DGGE fingerprints, a Pearson correlation was performed applying the GelCompar II® program (version 6.5, Applied Maths, Austin, TX). The resulting similarity

Table 1. MANOVA of factors time after manure application, SDZ treatment, moisture regime and the respective interactions.

	MANOVA F values[a]					
Factor	*sul1*	*sul2*	*intI1*	*intI2*	*qacE* + *qacEΔ1*	Total
SDZ treatment	4.72*	63.68***	4.51*	4.10*	3.89	28.87***
Time	11.83***	28.50***	13.33***	24.53***	15.04***	5.23***
Moisture regime	0.13	4.43*	0.11	6.25*	0.64	5.37**
Treatment × time	2.27	6.06**	1.91	1.76	2.01	1.72*
Treatment × regime	0.19	0.04	0.01	0.09	0	0.96
Time × regime	0.83	2.29	0.96	3.25*	1.61	3.05***
Treatment × time × regime	2.4	3.32*	2.65*	1.28	2.71*	1.21

[a]F values are shown and significance is indicated by asterisks, with $P < 0.05$, $P < 0.005$ and $P < 0.0001$ indicated by *, ** and ***, respectively.

matrix was used for permutation tests calculating the d-value from the average overall correlation coefficients within the groups minus the average overall correlation coefficients between samples from treatments to test for significant differences in community composition (Kropf *et al.* 2004). Ordination by non-metric multidimensional scaling (NMDS) was performed using R (version 3.2.1) and the package 'vegan' (version 2.3-0).

The Pearson product-moment correlations between the relative abundances of target genes (*sul1*, *sul2*, *intI1*, *intI2* and *qacE* + *qacEΔ1*) and time (days) were tested by means of the CORR procedure of the SAS 9.4 statistical package (SAS Institute Inc., Cary, NC).

To evaluate the influence of the factors SDZ, time (as a categorical variable) and moisture regime on the relative abundance of the dependent parameters *sul1*, *sul2*, *intI1*, *intI2* and *qacE* + *qacEΔ1*, a multivariate analysis of variance (MANOVA) was calculated applying the procedure GLM of SAS 9.4 (SAS Institute Inc.).

RESULTS

Relative abundance of target genes

Except *traN* and *korB*, which were predominantly below the theoretical limit of quantification (about 10^3 copies g^{-1} soil), all tested genes were detected in TC-DNA from soil samples. On average, the relative abundance of genes was higher in soil treated with manure containing SDZ than in soil treated with control manure (0.1 log units for *sul1*, *intI1* and *qacE* + *qacE1;* 0.2 log units for *intI2;* 0.6 log units for *sul2*) resulting in a significant overall SDZ effect (Table 1, $P < 0.0001$). In particular, significant SDZ effects were observed for the sulfonamide resistance genes *sul1* and *sul2* and for the integrase genes *intI1* and *intI2* (Table 1, see also Table S1, Supporting Information, for the parameter estimates). The factor time had a strong effect on the relative abundance of all target genes (Table 1) which significantly decreased over the course of the experiment (Fig. 1, Table 2). Besides an overall effect of the moisture regime on the relative abundance of target genes, drying–rewetting cycles had a significant effect on *sul2* and *intI2* genes specifically (Table 1) which on average were 0.2 log units more abundant in the CMR soils than in the DMR soils.

Except for *sul1* and *intI2*, significant three-way interactions were found between the factors SDZ, time and moisture regime (Table 1). Significant two-way interactions were found between the factors SDZ and time for *sul2* and between time and moisture regime for *intI2*. For *sul2* relative abundance, significant differences between treatments with and without SDZ were only observed on days 14, 28 and 42 (Bonferroni adjusted *t*-test, $P < 0.01$) with higher mean values in the SDZ manure-treated soil than in the control manure soil. Significant differences between moisture regimes for *intI2* relative abundance were only observed on day 7 (Bonferroni adjusted *t*-test, $P < 0.01$), where the mean value was higher for the DMR. Overall interactions were observed between the factors SDZ and time as well as time and moisture regime. No significant interaction was observed between moisture regime and SDZ treatment.

Considering the data of all treatments and timepoints, strong positive correlations were found between the relative abundance of *sul1*, *intI1* and *qacE* + *qacEΔ1* (correlation coefficient ≥0.96; Table 2). The correlation between relative gene abundance and time was negative and only significant for *sul1*, *intI1* and *qacE* + *qacE1* ($P < 0.003$, Bonferroni corrected).

DGGE analysis of bacterial community structure

The NMDS analysis gave a reasonable representation of the data (stress of 0.121; Bray–Curtis distance index). Day zero soil fingerprints clustered close to the manure fingerprints (Figs 2 and S1, Supporting Information) and using the permutation test based on similarity measures, no significant difference between soil bacterial communities was observed (Table 3). Following the course of the experiment, the NMDS distance to the manure samples increased (Fig. 2). On day 7, the differences of the soil bacterial communities between the treatments with and without SDZ were higher in the CMR than in the DMR according to the calculated d-values (Table 3), while there was a clear separation between the treatments with and without SDZ in the DMR soils on the first two dimensions of the NMDS (Fig. 2). However, the *P*-values were slightly higher than the Bonferroni adjusted alpha level of 0.025. Furthermore, *P*-values ranging from 0.05 to 0.025 were calculated for differences between bacterial communities of the CMR and DMR soils for SDZ and control treatments. On day 56, the differences in bacterial community structure between SDZ and control manure treated soils tended to decrease but were still higher for the CMR. The same trend was observed for the differences between the moisture regimes.

DISCUSSION

The MANOVA method revealed that SDZ applied with manure to soil planted with *D. glomerata* L. increased the relative abundance of *sul1* and *sul2* genes but also the relative abundance of class 1 and 2 integron integrases compared to the control manure treatment (Table 1, Fig. 1). This is in line with previous studies reporting an increase in the relative abundance of *sul* genes in soil and rhizosphere following the application of manure containing SDZ (Jechalke *et al.* 2014a). The relative abundances of *sul1*, *intI1* and the *qacE* gene with its *qacEΔ1* variant were highly

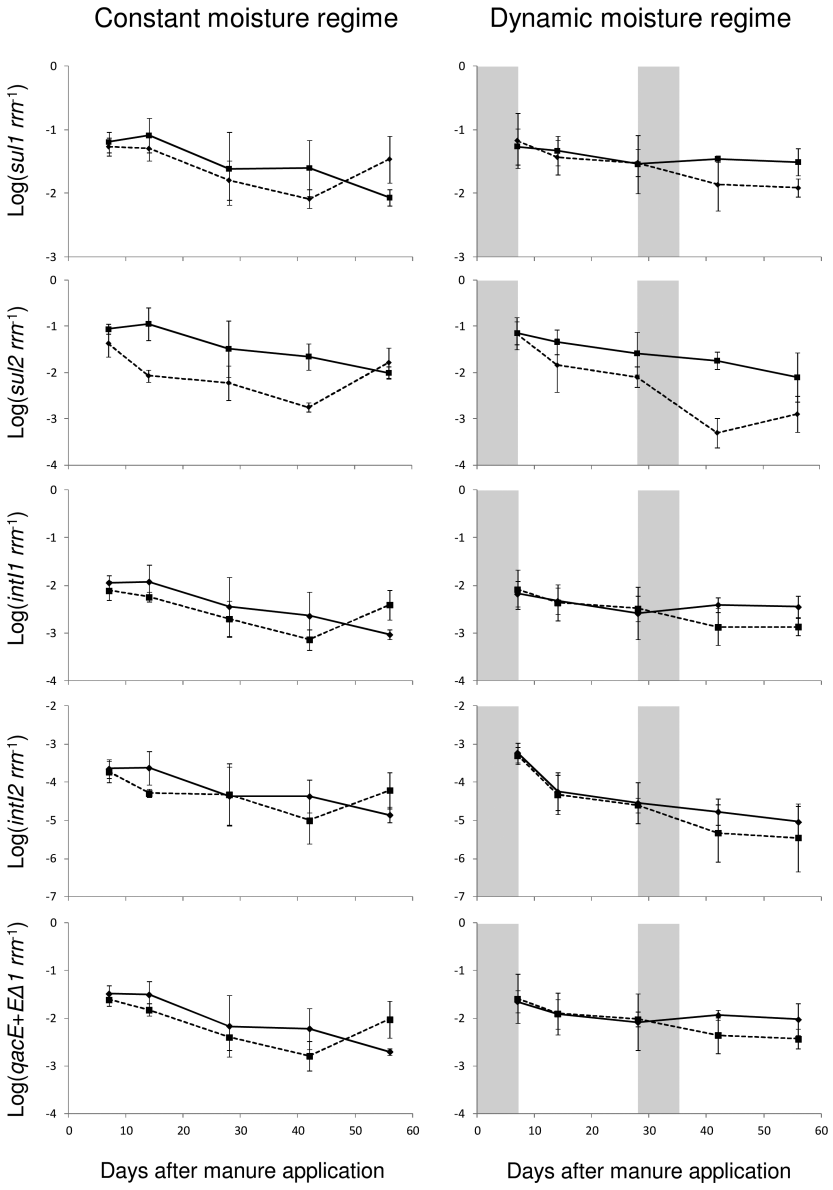

Figure 1. Time course of the relative abundance of SDZ resistance genes *sul1* and *sul2*, *intI1* and *intI2* integrase genes of class 1 and 2 integrons, respectively, as well as *qacE* + *qacEΔ1* resistance genes in soil planted with *D. glomerata* L. Soils treated with manure with SDZ added or not are indicated by boxes connected by solid lines and diamonds connected by dashed lines, respectively. Data points are connected by mere convenience and drying events are highlighted by gray background. Error bars indicate standard deviations of three to four replicates.

correlated (Table 2), which suggests that *sul1* was mainly located on class 1 integrons within a 3′ conserved segment consisting of the *qacEΔ1* variant and *sul1* that is characteristic for many clinical class 1 integrons (Gillings 2014). However, compared to the *sul2* relative abundance, MANOVA showed higher *P*-values for the influence of the factor SDZ treatment on *sul1*, *intI1* and *intI2* ($P < 0.05$ compared to $P < 0.0001$ for *sul2*, Table 1), and differences between SDZ treatment and control tended to be bigger for the *sul2* relative abundance (Fig. 1). This might indicate that bacterial populations carrying *sul2* resistance genes were more severely affected by SDZ.

Significant overall SDZ effects on the *sul2* relative abundance were observed on days 14, 28 and 42 in both moisture regimes, while on day 56 in the control of the CMR soil the relative abundance of *sul2* increased. This increase might have been triggered by other, so far unknown factors in the CMR soil without SDZ.

Remarkably, no significant interactions were observed between the factors SDZ and moisture regime for the tested genes (Table 1), indicating that drying–rewetting had a negligible effect on the soil bacterial population's response to the presence of SDZ. This was surprising since the extractable SDZ fractions were repeatedly larger in DMR relative to CMR soils (Reichel *et al.* 2014), indicating a faster dissipation and a lower bioavailability of SDZ in the permanently moist soil. Furthermore, it was shown that drying and rewetting cycles can enhance microbial biomass and activity, for example in surface and subsurface California grassland soils (Xiang *et al.* 2008), and that the application of nutrients to the oligotrophic soil environment by manure or root exudates is further supposed to enhance the effect of bacteriostatic antibiotics such as SDZ on horizontal gene transfer and bacterial growth (Brandt *et al.* 2009). It was reported that only if the water potential of the soils was previously reduced at least

Table 2. Pearson correlation coefficients between abundance of target genes (relative to 16S rRNA genes) and time after manure application (days).

	Pearson correlation coefficient[a]					
	sul1	*sul2*	*intI1*	*intI2*	*qacE* + *qacE*Δ*1*	Time
sul1	1					
sul2	0.81***	1				
intI1	0.97***	0.80***	1			
intI2	0.74***	0.85***	0.76***	1		
qacE + *qacE*Δ*1*	0.96***	0.77***	0.96***	0.73***	1	
Time	−0.56***	−0.59*	−0.57***	−0.67**	−0.59***	1

[a]Significance is indicated by asterisks, with $P < 0.05$, $P < 0.01$, $P < 0.0001$ indicated by *, ** and ***, respectively.

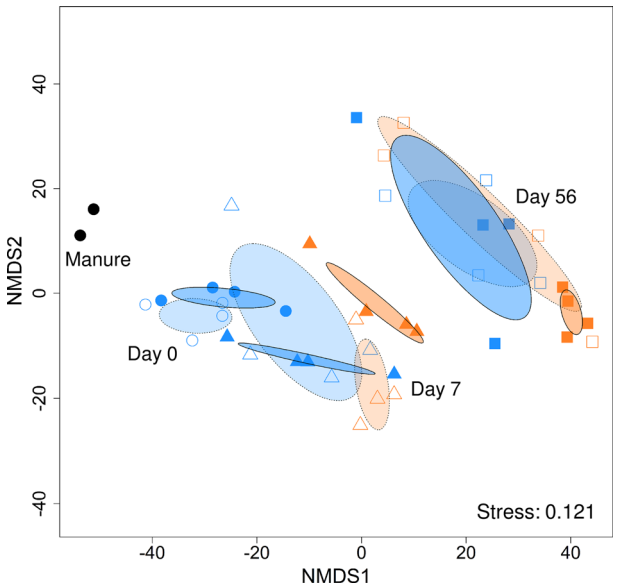

Figure 2. Results of NMDS analysis based on Pearson correlations of background-subtracted densitometric curves from DGGE analysis. Differences in bacterial community structure are shown. CMR samples from days 0 (filled circle), 7 (filled triangle) and 56 (filled squares) are indicated in blue, DMR samples in orange, while control treatments without SDZ application are indicated by open symbols. Ellipses show the 95% confidence interval (four replicates) of control (dashed line) and SDZ treatments (solid line). Two technical replicates for the manure bacterial community are indicated by black circles.

Table 3. Percent difference (d-values) and respective *P*-values (Bonferroni adjusted alpha level 0.025) of soil bacterial community structure based on Pearson correlations of background-subtracted densitometric curves from DGGE analysis (days 0, 7 and 56). Differences are shown between the treatments manure (control) and manure spiked with SDZ (SDZ) for constant and dynamic moisture regimes (CMR and DMR, respectively) and between the moisture regimes for each treatment. For day 0, the difference between manured soils with and without SDZ is shown.

Day	Treatment	Comparison	d-Value (%)	*P*-Value
0		SDZ/control	−0.9	0.68
7	CMR	SDZ/control	13.9	0.03
7	DMR	SDZ/control	3.8	0.14
56	CMR	SDZ/control	8.5	0.14
56	DMR	SDZ/control	1.5	0.32
7	SDZ	DMR/CMR	15.0	0.03
7	Control	DMR/CMR	10.0	0.03
56	SDZ	DMR/CMR	13.1	0.03
56	Control	DMR/CMR	6.1	0.11

3-fold compared to the constantly moist soil, a flush of respiration was induced (Chowdhury *et al.* 2011). This was the case in this study, where the soil moisture ranged from approximately 10% WHC_{max} in the dry phase to 40% WHC_{max} during the recovery phase, compared to an average of 38% WHC_{max} during the CMR (Reichel *et al.* 2014). Hence, due to the potentially higher bioavailability of SDZ connected with an anticipated higher bacterial activity in the DMR soil during the recovery phase, bigger effects of the bacteriostatic SDZ were to be expected in this moisture regime. Since this was not the case, probably the observed differences in extractable SDZ between the moisture regimes were not sufficient to obtain differences in relative gene abundance that were big enough to be detectable by the qPCR technique. Furthermore, it cannot be excluded that the drying periods selected to avoid wilting of the grass were too short to produce detectable effects.

Another explanation for the missing interaction between the factors moisture regime and SDZ might be that the drying periods induced plant stress that led to a reduction in root exudation and hence a successive reduction of bacterial activity. For example, it was shown in a recent study that drying and rewetting reduced the rhizodeposition and total input of plant C in microbial biomass and in the soil mineral phase, which was mainly due to a reduction in plant biomass (Canarini and Dijkstra 2015). Therefore, this potential reduction in nutrient availability in the DMR soil might have counteracted the presumed higher bioavailability of the bacteriostatic SDZ by reducing the bacterial activity. Thus, results may differ if samples from bulk soil are investigated.

The results of the DGGE analysis showed a higher effect of the treatment on the bacterial community structure for the CMR than for the DMR soil as judged from the d-values (Table 3), while a clear separation was apparent for the DMR soils between the SDZ-treated and untreated samples in the first two dimensions of the NMDS analysis (Fig. 2). Based on these results, it cannot be clearly decided whether a reduced bacterial activity during the time of drying counteracted the theoretically higher bioavailability of SDZ. In tendency, this difference was less pronounced on day 56 which might indicate that during the rewetting phase the bacterial community recovered from the effects of the second drying phase. Additionally, the moisture regime influenced the bacterial community structure and the difference between the regimes apparently was bigger in the SDZ than in the control treatment (Table 3). Accordingly, a previous study by Reichel *et al.* (2014) using the same samples observed significant interactions between SDZ treatment and moisture regime for *Pseudomonas*

but no interaction was found for *Betaproteobacteria*. Copiotrophic species like those of the genus *Pseudomonas* are frequently reported members of the rhizosphere bacterial community and hence might have been affected by a reduction of nutrient availability (Van Overbeek and Van Elsas 1997). Furthermore, the effect of soil rewetting on bacterial communities was investigated in great detail by Aanderud and Lennon (2011) using barcoded pyrosequencing of 16S rRNA genes. They observed that rewetting led to an increased relative recovery of *Alpha-*, *Beta-* and *Gammaproteobacteria* but to a decreased recovery of *Chloroflexi* and *Deltaproteobacteria*, which was speculated to be due to copiotrophic and oligotrophic behavior. However, it has to be considered that in the study of Reichel *et al.* (2014) shifts in the DGGE fingerprints were analyzed based on band appearance or loss, while in this study also the relative intensity of bands is considered which might have influenced the sensitivity in the detection of community changes. The impact of bacteria applied with manure to soil on the bacterial community structure decreased over the course of the experiment (Fig. 2), which is in line with previous studies reporting that manure bacteria are typically not well adapted to soil environmental conditions causing only transient effects on the soil bacterial community structure (Sengelov *et al.* 2003; Heuer *et al.* 2008; Jechalke *et al.* 2013b; Ding *et al.* 2014; Riber *et al.* 2014).

Manure bacteria are reported to be a reservoir of resistance genes, frequently located on mobile genetic elements (Smalla *et al.* 2000; Binh *et al.* 2008; Heuer, Schmitt and Smalla 2011; Heuer *et al.* 2012; Zhu *et al.* 2013). Although bacteria applied with manure are considered not to be persistent in soil, horizontal gene transfer which is enhanced by manure application likely relocated these resistance genes to the indigenous soil bacterial community (Heuer and Smalla 2012; Musovic *et al.* 2014). Remarkably, the abundance of the *traN* gene specific for LowGC-type plasmids and *korB* of IncP-1 plasmids was predominantly below the limit of quantification. These plasmid groups were frequently captured by exogenous plasmid isolation from manure and manured soil and hence were proposed to play an important role in the spread of antibiotic resistance in the agricultural environment (Binh *et al.* 2008; Heuer *et al.* 2009, 2012; Jechalke *et al.* 2014a). However, although these plasmids were readily transmissible to the bacterial recipients used in these studies, indicating their importance for the spread of antibiotic resistance, they are often scarce in soil (Heuer and Smalla 2012; Kopmann *et al.* 2013). Furthermore, it cannot be excluded that other plasmid groups that were not tested in this study might have played an important role in the spread of antibiotic resistance within the soil bacterial community. For example, it was reported that the *sul2* genes can be associated with IncQ and IncN plasmids and were found to be located outside of class 1 integrons (Smalla *et al.* 2000; Binh *et al.* 2008; Vinue *et al.* 2010).

In summary, the results indicated that drying–rewetting cycles can modulate the effects of SDZ on the bacterial community structure in soil planted with *D. glomerata* L. However, while the SDZ treatment increased the relative abundance of sulfonamide resistance and integrase genes compared to the control, no interaction was observed between the factors SDZ and moisture regime, indicating that drying–rewetting cycles had a negligible effect on the selection of tested genes by the presence of SDZ.

SUPPLEMENTARY DATA

Supplementary data are available at FEMSEC online.

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REFERENCES

Aanderud ZT, Lennon JT. Validation of heavy-water stable isotope probing for the characterization of rapidly responding soil bacteria. *Appl Environ Microb* 2011;**77**:4589–96.

Barraud O, Baclet MC, Denis F *et al.* Quantitative multiplex real-time PCR for detecting class 1, 2 and 3 integrons. *J Antimicrob Chemoth* 2010;**65**:1642–5.

Binh CTT, Heuer H, Kaupenjohann M *et al.* Piggery manure used for soil fertilization is a reservoir for transferable antibiotic resistance plasmids. *FEMS Microbiol Ecol* 2008;**66**:25–37.

Boxall AB, Fogg LA, Blackwell PA *et al.* Veterinary medicines in the environment. *Rev Environ Contam T* 2004;**180**:1–91.

Brandt KK, Sjøholm OR, Krogh KA *et al.* Increased pollution-induced bacterial community tolerance to sulfadiazine in soil hotspots amended with artificial root exudates. *Environ Sci Technol* 2009;**43**:2963–8.

Canarini A, Dijkstra FA. Dry-rewetting cycles regulate wheat carbon rhizodeposition, stabilization and nitrogen cycling. *Soil Biol Biochem* 2015;**81**:195–203.

Chowdhury N, Yan N, Islam MN *et al.* The extent of drying influences the flush of respiration after rewetting in non-saline and saline soils. *Soil Biol Biochem* 2011;**43**:2265–72.

Ding GC, Radl V, Hai B *et al.* Dynamics of soil bacterial communities in response to repeated application of manure containing sulfadiazine. *PLoS One* 2014;**9**:e92958.

Doornbos RF, van Loon LC, Bakker P. Impact of root exudates and plant defense signaling on bacterial communities in the rhizosphere. A review. *Agron Sustain Dev* 2012;**32**:227–43.

Du LF, Liu WK. Occurrence, fate, and ecotoxicity of antibiotics in agro-ecosystems. A review. *Agron Sustain Dev* 2012;**32**:309–27.

Förster M, Laabs V, Lamshöft M *et al.* Sequestration of manure-applied sulfadiazine residues in soils. *Environ Sci Technol* 2009;**43**:1824–30.

Gillings MR. Integrons: past, present, and future. *Microbiol Mol Biol R* 2014;**78**:257–77.

Heuer H, Binh CTT, Jechalke S *et al.* IncP-1e plasmids are important vectors of antibiotic resistance genes in agricultural systems: diversification driven by class 1 integron gene cassettes. *Front Microbiol* 2012;**3**:2.

Heuer H, Focks A, Lamshöft M *et al.* Fate of sulfadiazine administered to pigs and its quantitative effect on the dynamics of bacterial resistance genes in manure and manured soil. *Soil Biol Biochem* 2008;**40**:1892–900.

Heuer H, Kopmann C, Binh CTT *et al.* Spreading antibiotic resistance through spread manure: characteristics of a novel plasmid type with low %G plus C content. *Environ Microbiol* 2009;**11**:937–49.

Heuer H, Krsek M, Baker P *et al.* Analysis of actinomycete communities by specific amplification of genes encoding 16S rRNA and gel-electrophoretic separation in denaturing gradients. *Appl Environ Microb* 1997;**63**:3233–41.

Heuer H, Schmitt H, Smalla K. Antibiotic resistance gene spread due to manure application on agricultural fields. *Curr Opin Microbiol* 2011;**14**:236–43.

Heuer H, Smalla K. Manure and sulfadiazine synergistically increased bacterial antibiotic resistance in soil over at least two months. *Environ Microbiol* 2007;**9**:657–66.

Heuer H, Smalla K. Plasmids foster diversification and adaptation of bacterial populations in soil. *FEMS Microbiol Rev* 2012;**36**:1083–104.

Jechalke S, Dealtry S, Smalla K *et al.* Quantification of IncP-1 plasmid prevalence in environmental samples. *Appl Environ Microb* 2013a;**79**:1410–3.

Jechalke S, Focks A, Rosendahl I *et al.* Structural and functional response of the soil bacterial community to application of manure from difloxacin-treated pigs. *FEMS Microbiol Ecol* 2013b;**87**:78–88.

Jechalke S, Heuer H, Siemens J *et al.* Fate and effects of veterinary antibiotics in soil. *Trends Microbiol* 2014a;**22**:536–45.

Jechalke S, Kopmann C, Rosendahl I *et al.* Increased abundance and transferability of resistance genes after field application of manure from sulfadiazine-treated pigs. *Appl Environ Microb* 2013c;**79**:1704–11.

Jechalke S, Schreiter S, Wolters B *et al.* Widespread dissemination of class 1 integron components in soils and related ecosystems as revealed by cultivation-independent analysis. *Front Microbiol* 2014b;**4**:420.

Kopmann C, Jechalke S, Rosendahl I *et al.* Abundance and transferability of antibiotic resistance as related to the fate of sulfadiazine in maize rhizosphere and bulk soil. *FEMS Microbiol Ecol* 2013;**83**:125–34.

Kropf S, Heuer H, Grüning M *et al.* Significance test for comparing complex microbial community fingerprints using pairwise similarity measures. *J Microbiol Methods* 2004;**57**:187–95.

Musovic S, Klümper U, Dechesne A *et al.* Long-term manure exposure increases soil bacterial community potential for plasmid uptake. *Environ Microbiol Rep* 2014;**6**:125–30.

Radl V, Kindler R, Welzl G *et al.* Drying and rewetting events change the response pattern of nitrifiers but not of denitrifiers to the application of manure containing antibiotic in soil. *Appl Soil Ecol* 2015;**95**:99–106.

Reichel R, Radl V, Rosendahl I *et al.* Soil microbial community responses to antibiotic-contaminated manure under different soil moisture regimes. *Appl Microbiol Biot* 2014;**98**:6487–95.

Riber L, Poulsen PHB, Al-Soud WA *et al.* Exploring the immediate and long-term impact on bacterial communities in soil amended with animal and urban organic waste fertilizers using pyrosequencing and screening for horizontal transfer of antibiotic resistance. *FEMS Microbiol Ecol* 2014;**90**:206–24.

Rosendahl I, Siemens J, Groeneweg J *et al.* Dissipation and sequestration of the veterinary antibiotic sulfadiazine and its metabolites under field conditions. *Environ Sci Technol* 2011;**45**:5216–22.

Sarmah AK, Meyer MT, Boxall ABA. A global perspective on the use, sales, exposure pathways, occurrence, fate and effects of veterinary antibiotics (VAs) in the environment. *Chemosphere* 2006;**65**:725–59.

Schauss K, Focks A, Heuer H *et al.* Analysis, fate and effects of the antibiotic sulfadiazine in soil ecosystems. *TRAC-Trend Anal Chem* 2009;**28**:612–8.

Sengelov G, Agerso Y, Halling-Sørensen B *et al.* Bacterial antibiotic resistance levels in Danish farmland as a result of treatment with pig manure slurry. *Environ Int* 2003;**28**:587–95.

Smalla K, Heuer H, Götz A *et al.* Exogenous isolation of antibiotic resistance plasmids from piggery manure slurries reveals a high prevalence and diversity of IncQ-like plasmids. *Appl Environ Microb* 2000;**66**:4854–62.

Van Overbeek LS, Van Elsas JD. Adaptation of bacteria to soil conditions: applications of molecular physiology in soil microbiology. In: Van Elsas JD, Wellington EMH, Trevors JT (eds). *Modern Soil Microbiology*. New York: Marcel Dekker, Inc., 1997, 441–7.

Vinue L, Saenz Y, Rojo-Bezares B *et al.* Genetic environment of *sul* genes and characterisation of integrons in *Escherichia coli* isolates of blood origin in a Spanish hospital. *Int J Antimicrob Ag* 2010;**35**:492–6.

Weinert N, Meincke R, Gottwald C *et al.* Rhizosphere communities of genetically modified zeaxanthin-accumulating potato plants and their parent cultivar differ less than those of different potato cultivars. *Appl Environ Microb* 2009;**75**:3859–65.

Xiang SR, Doyle A, Holden PA *et al.* Drying and rewetting effects on C and N mineralization and microbial activity in surface and subsurface California grassland soils. *Soil Biol Biochem* 2008;**40**:2281–9.

Zarfl C, Klasmeier J, Matthies M. A conceptual model describing the fate of sulfadiazine and its metabolites observed in manure-amended soils. *Chemosphere* 2009;**77**:720–6.

Zhu Y-G, Johnson TA, Su J-Q *et al.* Diverse and abundant antibiotic resistance genes in Chinese swine farms. *P Natl Acad Sci USA* 2013;**110**:3435–40.