RAPID REPORT

Loss of soil microbial diversity exacerbates spread of antibiotic resistance

Qing-Lin Chen¹, Xin-Li An¹, Bang-Xiao Zheng^{1,2}, Michael Gillings³, Josep Peñuelas^{4,5}, Li Cui¹, Jian-Qiang Su¹, Yong-Guan Zhu^{1,6,*}

¹ Key Laboratory of Urban Environment and Health, Institute of Urban Environment, Chinese Academy of Sciences, Xiamen 361021, China

² University of Chinese Academy of Sciences, 19A Yuquan Road, Beijing 100049, China

³ Department of Biological Sciences, Macquarie University, Sydney, NSW, 2109, Australia

⁴ Consejo Superior de Investigaciones Científicas (CSIC), Global Ecology Unit, Centre for Ecological Research and Forestry Applications (CREAF)–CSIC– Universitat Autònoma de Barcelona (UAB), Bellaterra, 08193 Barcelona, Catalonia, Spain

⁵ CREA, Cerdanyola del Vallès, 08193 Barcelona, Catalonia, Spain

⁶ State Key Laboratory of Urban and Regional Ecology, Research Center for Eco-Environmental Sciences, Chinese Academy of Sciences, Beijing 100085, China

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ABSTRACT

Loss of biodiversity is a major threat to the ecosystem processes upon which society depends. Natural ecosystems differ in their resistance to invasion by alien species, and this resistance can depend on the diversity in the system. Little is known, however, about the barriers that microbial diversity provides against microbial invasion. The increasing prevalence of antibiotic-resistant bacteria is a serious threat to public health in the 21st century. We explored the consequences of the reduction in soil microbial diversity for the dissemination of antibiotic resistance. The relationship between this diversity and the invasion of antibiotic resistance was investigated using a dilution-to-extinction approach coupled with high-capacity quantitative PCR. Microbial diversity was negatively correlated with the abundance of antibiotic-resistance genes, and this correlation was maintained after accounting for other potential drivers such as incubation time and microbial abundance. Our results demonstrate that high microbial diversity can act as a biological barrier resist the spread of antibiotic resistance. These results fill a critical gap in our understanding of the role of soil microbial diversity in the health of ecosystems.

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1 Introduction

Biodiversity is fundamental to the maintenance of ecosystem functioning. Diverse species pools sustain healthy ecosystems by supporting multiple ecosystem functions and ser-

vices, including biogeochemical cycling, primary production, litter decomposition and climatic regulation (Wardle et al., 2004; van der Heijden et al., 2008; Bardgett and van der Putten, 2014; Wagg et al., 2014). Genomic techniques have identified immense microbial diversity in both soil and oceans (Torsvik et al., 1998; Torsvik and Ovreas, 2002; DeLong et al., 2006; Sogin et al., 2006), but the role of microbial diversity in controlling ecosystem function remains unclear (Hillebrand and Matthiessen, 2009; Peter et al., 2011; Miki et al., 2013).

* Corresponding author

E-mail address: ygzhu@iue.ac.cn (Y.G. Zhu)

Anthropogenic activities such as agricultural intensification, altered land use and nitrogen enrichment might reduce microbial diversity and potentially affect ecosystem functions (Mäder et al., 2002; Gans et al., 2005; Maestre et al., 2015).

Debate continues about the relationship between microbial diversity and ecosystem functioning (Bell et al., 2005; Allison and Martiny, 2008). Soil microorganisms are key drivers of biogeochemical flows, but the functional redundancy in soil microorganisms suggests that some loss of microbial diversity might not substantially affect ecosystem functions (Bell et al., 2005; Wertz et al., 2006; Wertz et al., 2007), especially if the relationship between microbial diversity and ecosystem functioning is saturated (Cardinale et al., 2011; Reich et al., 2012). For example, communities of microbial decomposers are often highly redundant for a single function, such as microbial respiration or biomass production (Reinthal et al., 2005; Reich et al., 2012). The relationship between biodiversity and function, however, might depend on the presence of key species, particularly if the function of concern is more specific and limited to a few taxa (such as pesticide degradation) (Gamfeldt et al., 2008; Obernosterer et al., 2010; Singh et al., 2014). The buffering capacity of ecosystems might be limited for specialized functions so that a moderate loss of diversity could substantially impair key processes (Singh et al., 2014). Relationships between microbial diversity and ecological functions may nevertheless involve trade-offs and may not necessarily be generalized as positive, neutral or negative (Jung et al., 2016). Investigating the consequences of loss in biodiversity for the process and functioning of ecosystems is thus of considerable interest (Swift et al., 1998; Chapin et al., 2000; Hunt and Wall, 2002; Loreau, 2004; Wardle et al., 2011; Perveen et al., 2014).

Highly biodiverse ecosystems are less vulnerable to disturbances (Elton, 1958; Levine, 2000; Ricciardi and MacIsaac, 2008) and can be resistant to invasion by alien species (Vitousek et al., 1996; Kennedy et al., 2002). Whether this principle can be applied to microbial ecology remains largely unresolved. The relationship between invasibility and microbial diversity has been investigated by manipulating microbial communities (van Elsas et al., 2007; Wertz et al., 2007; van Elsas et al., 2012; Philippot et al., 2013; Sun et al., 2015). Survival of inoculant *Escherichia coli* O157:H7 has been positively correlated with the depth of soil fumigation using a dilution–reinoculation approach, where soil microbial diversity was negatively correlated with survival of the invader (van Elsas et al., 2007; van Elsas et al., 2012; Vivant et al., 2013). Survival rate has been negatively correlated with the degree of microbial diversity for inoculation with *Pseudomonas aeruginosa*, *Ralstonia solanacearum* and *Listeria monocytogenes* (Matos et al., 2005; Irikiin et al., 2006; Vivant et al., 2013). Such data shed light on the relationship between complexity and invasiveness within microbial communities and are valuable but cannot be regarded as universal due to the limited number of species investigated.

Only a fraction of bacteria in natural environments can be cultivated, so we focused on the relationship between

invasibility and microbial diversity at the gene level using the antibiotic resistome as a proxy for invasion (Wright, 2007). The emergence and spread of antibiotic resistance has become a global health threat (Bush et al., 2011; Pruden et al., 2013; Zhu et al., 2013; Su et al., 2017; Zhu et al., 2018), but the factors regulating the environmental dynamics of antibiotic resistance, especially biotic factors, have not been directly addressed. This neglect has hampered our ability to assess the risks of antibiotic resistance due to anthropogenic activities and global environmental change.

The main objective of this study was to determine if soil microbial diversity could act as a biological barrier to prevent the invasion of antibiotic resistance by addressing the consequences of a loss of soil microbial diversity on the fate of antibiotic-resistance genes (ARGs). Microbial diversity was manipulated using a dilution–reinoculation approach, and pig manure was used as the source of determinants of antibiotic resistance. We characterized bacterial communities using Illumina Hiseq profiles of 16S rRNA genes and the resistome was determined using high-capacity quantitative PCR that targeted major classes of ARGs. Our study provides empirical evidence that high microbial diversity lowers the survival and dissemination of ARGs in soil ecosystems, suggesting that the loss of microbial diversity could exacerbate the spread of resistance.

2 Materials and methods

2.1 Microcosms and experimental setup

Surface soil (0–20 cm) was collected from a rice cropland in Jiaying, Zhejiang, southern China (30°50'7.7"N, 120°43'5.7"E) in August 2016. Pig manure was obtained from a local commercial pig farm. The properties of the soil and manure are described in Table S1.

The soil was sieved to <2 mm, and some of the soil was sterilized by γ -irradiation (35 kGy) (Philippot et al., 2013). The remaining soil was used as an inoculum in a dilution-to-extinction experiment. The sterility of the irradiated soil was tested by spreading 0.5 g onto plates containing tryptic soy broth (TSB) and potato-dextrose agar (PDA) (Yan et al., 2017). No bacterial or fungal growth was observed after six days.

An initial soil suspension was prepared by mixing 30 g of unsterilized soil with 50 mL of sterile demineralized water using an ultraviolet-sterilized blender at maximum speed for 5 min (Philippot et al., 2013). Four concentrations of the soil suspension were used as inocula to create a diversity gradient of soil microorganisms ranging from undiluted (D0) to 10^{-2} (D1), 10^{-4} (D2) and 10^{-8} (D3) dilutions, and 5 mL of each suspension were subsequently inoculated into the 200-mL flasks containing 30 g of sterilized soil, equivalent to 10^{-1} , 10^{-3} , 10^{-5} , 10^{-9} g of inoculum per gram of sterile soil. Positive and negative controls using non-sterile soil (S) and sterile soil (SS), to inoculate the same volume of sterile demineralized water, were also prepared. Each treatment had four repli-

cates. Each flask received 1.2 g of pig manure as the source of ARGs (Fig. 1). The flasks were then closed with sterile lids, and the inoculated microcosms were incubated at 20°C and at 65% water-holding capacity by adding sterile demineralized water (van Elsas et al., 2012). Four replicates of each treatment were collected on days 0, 10, 30, 60 and 90 for the analysis of bacterial abundance and diversity, and the profile of antibiotic resistance.

2.2 Assessing microbial diversity

Total DNA was extracted from 0.5 g of soil for each replicate microcosm from each dilution using a FastDNA® Spin Kit for soil following the supplier's manual (MP Biomedical, Santa Ana, USA). The quality of the DNA was assessed using an ND-1000 spectrophotometer (NanoDrop Technology, Wilmington, USA). The concentration of DNA was determined using a Qubit™ dsDNA HS Assay kit with a Qubit™ 3.0 fluorometer (Thermo Fisher Scientific Inc, Waltham, USA). Four replicate 50-μL PCR reactions were prepared using 1 μL each of a forward (515F) and reverse (907R) bar-coded primer (F515: GTGCCAGCMGCCGCGG, R907: CCGTCA ATTCMTTTRAGTTT) (Zhou et al., 2011), 25 μL of 2 × ExTaq polymerase (Takara Bio Inc, Otsu, Japan), 1 μL of sample DNA as the template and 22 μL of nuclease-free PCR-grade water. The PCR program was: 95°C for 5 min, followed by 30 cycles of 95°C for 30 s, 58°C for 30 s, 72°C for 30 s and a final extension at 72°C for 10 min. Negative controls (template DNA replaced with water) were included for detecting any contamination during PCR preparation. PCR products for each sample and replicate were purified using the Wizard SV Gel and PCR Clean-Up System (TIANGEN Biotech, Beijing, China). The purified PCR products were quantified and

pooled at the same concentration and then sequenced using an Illumina HiSeq2500 platform (Novogene, Beijing, China).

The quality of downstream analyses was guaranteed by filtering raw pair-end reads to discard raw reads containing three or more ambiguous nucleotides, reads with a low (<20) average quality score, short reads (<100 nt) and barcodes to generate clean joined reads spanning the complete V4-V5 region of the 16S rRNA gene. High-quality sequences were processed and analyzed using the QIIME pipeline (Caporaso et al., 2010). Operational taxonomic units (OTUs) were identified using the UCLUST algorithm with a phylotype defined at 97% sequence similarity (Edgar, 2010). Chimeric sequences, chloroplast and mitochondrial OTUs (around 1%) and singleton OTUs were discarded from the final OTU data set. The database from the Ribosomal Database Project was used for taxonomic classification (Wang et al., 2007; Cole et al., 2009). Raw sequences were deposited in the National Center for Biotechnology Information (NCBI) Sequence Read Archive (SRA) under accession number SRP108158.

2.3 Assessing ARGs

A total of 296 primer sets (Zhu et al., 2017) were used to analyze the soil DNA (Table S2). The primers targeted resistance genes for all major classes of antibiotics (285 primer sets), transposase genes (eight primer sets), the universal class 1 integron-integrase gene (*intl1*), the clinical class 1 integron-integrase gene (*cint1*) and the 16S rRNA gene. All soil DNA was diluted to 50 ng·μL⁻¹ using sterile water and amplified in triplicate for each primer set with a Wafergen SmartChip Real-time PCR system in 100-nL reaction volumes. Negative controls without template were

Schematic of the experimental design

Fig. 1 Schematic of the experimental design. Soil was sterilized by γ -irradiation, microbial diversity was manipulated using a dilution–reinoculation approach and pig manure was used as the source of ARGs.

included. A more detailed description of the experimental procedure can be found in a previous study (Chen et al., 2017b), except we used a threshold cycle (C_T) of 29 as the detection limit. The HT-qPCR data were analyzed using the SmartChip qPCR software (V 2.7.0.1). Reactions with poor melting curves and reactions with <90% or >110% amplification efficiencies were discarded. Retained reactions had both a threshold cycle <29 and three positive replicate samples. Relative copy number was calculated as: relative gene copy number = $10^{(29 - C_T)/(10/3)}$, where C_T refers to the results of the quantitative PCR.

Absolute 16S rRNA gene copy numbers were quantified separately using standard curves with a Roche 480 system (Roche Molecular Systems Inc., Branchburg, USA) for determining bacterial abundance. Each 20- μ L qPCR mixture consisted of 10 μ L of $2 \times$ LightCycler 480 SYBR Green I Master, 0.5 μ g $\cdot$ μ L⁻¹ bovine serum albumin, 1 μ M each primer, 5 ng $\cdot$ μ L⁻¹ template DNA and 6 μ L of nuclease-free PCR-grade water. The thermal cycling was set the same as for the HT-qPCR. A plasmid control containing a cloned and sequenced 16S rRNA gene fragment (1.18×10^{10} copies $\cdot$ μ L⁻¹) was used to generate eight-point calibration curves from 10-fold dilutions for standard calculation. All qPCRs were performed in technical triplicates with template-free negative controls.

2.4 Statistical analysis

We used ordinary least squares (OLS) regression models to determine the relationships between the pattern of antibiotic resistance and microbial diversity estimated with OTU richness, diversity and evenness. OLS regression was conducted with R (R Core Team, 2016) and visualized with the “ggplot 2” package (Wickham, 2009). We used structural equation models (SEMs) to evaluate the direct and indirect relationships between dilution, incubation time, bacterial diversity, bacterial abundance, mobile genetic elements (MGEs) abundance and pattern of antibiotic resistance. SEM is an *a priori* approach able to identify casual relationships between variables by fitting data to the models representing causal hypotheses (Eisenhauer et al., 2015). The first step in SEM analysis thus requires establishing an *a priori* model based on the known effects and relationships among the drivers affecting antibiotic resistance. The theoretical model assumptions were: (i) dilution and incubation time may directly influence MGE and ARG patterns, (ii) dilution and incubation time may indirectly influence MGE and ARG patterns by affecting bacterial abundance and diversity and (iii) bacterial abundance and diversity may directly influence ARG patterns and may indirectly influence the patterns by affecting MGE abundance and number detected. We determined the distributions of all our variables and tested their normality before modeling. All data were standardized using Z-scores in the downstream analysis. Bivariate correlation determined the pairwise correlations among these variables using SPSS 20 (IBM, Armonk, USA), and the covariance

matrix was imported into AMOS 21 (SPSS Inc., Chicago, USA) for SEM construction using maximum-likelihood estimation.

We parameterized our model using our data set and tested its overall goodness of fit. The overall goodness of fit for SEMs does not have a universally accepted test, so we used multiple goodness-of-fit criteria: the chi-square (χ^2) test (the model has a good fit when χ^2 is low and P is high, traditionally $P > 0.05$), the root mean square error of approximation (RMSEA; the model has a good fit when RMSEA is near 0), a high goodness-of-fit index (GFI, traditionally GFI > 0.90) and a low Akaike information criterion (from the default model lower than that from saturated and independence models). A reasonable model fit allowed us to interpret the path coefficients of the model and their associated P -values. A path coefficient is analogous to a partial correlation coefficient and describes the strength and sign of the relationship between two variables. SEMs can also partition direct and indirect effects that one variable may have on another and estimate the strengths of these multiple effects. We calculated the standardized direct, indirect and total effects of dilution, incubation time, bacterial abundance, bacterial diversity and MGE abundance to aid final interpretation. The net effect of one factor on another was calculated by summing all direct and indirect pathways between the two factors. If the model fitted the data well, the total effect should approximate the bivariate correlation coefficient for that pair of factors.

3 Results

3.1 Microbial community assembly and diversity

A total of 7 251,527 high quality sequences were obtained from all of the samples with sequences per sample ranging from 40 962 to 136,022. The soil microbial community was assembled into 17 phyla (Figure S1A) using the database from the Ribosomal Database Project (Cole et al., 2009). The community was dominated by Actinobacteria, Firmicutes, Proteobacteria, Bacteroidetes, Planctomycetes and Chloroflexi, which are common soil bacterial phyla worldwide (Fierer et al., 2013; Maestre et al., 2015). Firmicutes percentage significantly decreased over time, in contrast to Actinobacteria and Bacteroidetes. It has been reported that the decline of Firmicutes abundance suggested the decrease of manure-derived microbiome over time in the soil environment (Gou et al., 2018). The dominant bacterial phyla varied slightly among the dilution treatments. Proteobacteria were nevertheless significantly ($P < 0.01$) enriched after extensive dilution, especially at day 10 after incubation.

Rarefaction curves were constructed for each sample showing the number of operational taxonomic units (OTUs) (Figure S1B), defined at a sequence-similarity cut-off of 97% in QIIME. Sequencing depths assessed by calculating Good's coverage scores were highly comparable for all samples, ranging from 94.25% to 98.51%, indicating that the sequen-

cing depth was adequate to reliably describe the bacterial microbiome. As expected, species richness (observed OTUs) progressively decreased in the dilution treatments (Fig. 2, Figure S2), indicating that the dilution-to-extinction method could effectively manipulate the microbial biodiversity of complex natural ecosystems such as soil (Sun et al., 2015). Species richness also decreased over time. A principal coordinates analysis (PCoA) based on weighted UniFrac distances between samples further indicated that dilution and incubation time significantly shifted the overall profile of bacterial composition (Figure S3). Microbial communities were clustered by their initial dilution levels along PC2, which explained 10.41% of the total variation, whereas microbial communities were clustered by incubation time along PC1 (explaining 53.55% of the total variation). This result suggested that time played a more important role than dilution in shaping bacterial composition.

Bacterial abundance was also measured using real-time qPCR for the 16S rRNA gene (Figure S4). Bacterial abundance was higher at the beginning (day 0) in treatments S and D0 (because the native microbiota were more abundant), but decreased over time. Bacterial abundance was more stable in D1, D2, D3 and SS (where the native microbiota was less diverse) than S and D0.

3.2 Resistome assembly and diversity

HT-qPCR was used to determine the abundance and diversity of ARGs. A total of 195 genes consisting of 186 unique ARGs, the class 1 integron-integrase gene and eight transposase genes were detected (Figure S5). The numbers of ARGs detected in each sample ranged from 47 to 116 with an

average of 85. These ARGs can confer resistance to most of the major classes of antibiotics that are commonly administered to humans and included resistance determinants for aminoglycosides, beta-lactams, Macrolide-Lincosamide-Streptogramin B (MLSB), multidrug efflux, sulfonamide, tetracycline and vancomycin. The abundance of ARGs ranged from 5.53×10^{10} to 1.65×10^{12} copies·g⁻¹ soil (Fig. 2). ARG abundance was higher in S at the beginning (day 0) due to a higher abundance of native antibiotic-resistant soil microbiota. This observation was consistent with the bacterial abundance (Figure S4). The dilution treatment affected ARG abundance by 10 days after the incubation, with the abundance of ARG increasing as dilution increased (Fig. 2).

3.3 Correlation between the fate of the resistome and microbial diversity

We then used an ordinary least-squares (OLS) regression model to determine the relationship between species diversity and the abundance of ARGs (Fig. 3). Loss of species richness (OTU richness) was linearly associated with an increase in ARG abundance ($P = 0.0016$, $R^2 = 0.0810$). The inverse Simpson diversity of OTUs was highly and negatively correlated with the abundance of ARGs ($P = 0.0003$, $R^2 = 0.1043$). In contrast, evenness was not significantly correlated with ARG abundance ($P = 0.8122$, $R^2 = 0.0005$). Further analyses provided evidence (Figure S6) that OTU richness was correlated positively with OTU diversity ($P < 0.0001$, $R^2 = 0.4264$) and negatively with OTU evenness ($P = 0.0064$, $R^2 = 0.0532$).

The OLS regression model indicated that ARG abundance

Fig. 2 Abundance of ARGs and number of OTUs associated with the six treatments during the 90-day incubation. OTUs were defined at a 97% sequence similarity cut-off in QIIME. Bars represent averages of four replicates.

Fig. 3 Ordinary least squares (OLS) regression model showing the relationships of ARG abundance with OTU richness, diversity and evenness. The solid lines indicate significant relationships. The shaded areas show the 95% confidence interval of the fit.

decreased over time ($P < 0.0001$, $R^2 = 0.4065$) (Fig. 4). This result indicates that steep increases in ARG abundance following manure application are not maintained in the long-term. We generated SEMs based on known effects and relationships to test whether the relationship between microbial diversity and the fate of ARGs was maintained when accounting for other possible drivers (Fig. 5). The model is an *a priori* approach that can separate multiple pathways of influence and is useful for exploring the complex networks of

relationships in ecosystems (Eisenhauer et al., 2015). Dilution, incubation time and abundance of MGEs and bacteria were included in the model because of their effects on ARG profiles. The model explained 86% of the variance in ARG patterns. Incubation time and dilution could directly affect the patterns of ARGs or could do so indirectly by strongly affecting the diversity and abundance of bacteria and the abundance of MGEs. Bacterial diversity significantly and directly affected ARG abundance ($\rho = -0.09$, $P < 0.05$) and indirectly affected the ARG patterns by strongly affecting MGE abundance ($\rho = -0.07$, $P < 0.05$). Bacterial abundance, though, had nonsignificant direct impacts on ARG abundance ($\rho = 0.02$, $P > 0.05$).

Fig. 4 Relationship between ARG abundance and incubation time. The solid blue line represents the fitted OLS model, and the shaded area shows the 95% confidence interval of the fit. Box plots show the first (25%) and third (75%) quartiles, the median and the maximum and minimum values within each data set.

4 Discussion

4.1 Effects of biological barriers on the resistome

The largest obstacle to understanding the importance of microbial biodiversity for the functioning of ecosystems is the lack of sound experimental methods to induce directed and predictable changes in the diversity of microbial communities in soil (Yan et al., 2015). The present and prior studies (van Elsas et al., 2012; Philippot et al., 2013) have demonstrated that the dilution-to-extinction method could effectively manipulate soil microbial communities to produce a gradient in species richness and diversity (Figs. 2 and S5).

Soil microbial diversity was negatively correlated with ARG abundance, indicating that the loss of microbial diversity could facilitate the proliferation and spread of antibiotic resistance in the soil environment. Our results provide the first empirical genetic evidence that natural ecosystems may be variably resistant to invasion by aliens, and this resistance is associated with the species richness and diversity of the

Fig. 5 Structural equation models showing the direct and indirect effects of dilution, time, bacterial abundance, bacterial diversity and MGEs on the ARG patterns. Black and red arrows indicate positive and negative relationships, respectively. Solid and dashed arrows indicate significant and nonsignificant relationships, respectively. Numbers adjacent to the arrows are path coefficients, and the width of the arrows is proportional to the strength of the path coefficients. R^2 denotes the proportion of variance explained. Significance levels are indicated: * $P < 0.05$, ** $P < 0.01$ and *** $P < 0.001$. Standardized effects (total, direct and indirect effects) are derived from the structural equation models. The hypothetical models fit our data well: $\chi^2 = 0.49$, $P = 0.48$, $df = 1$, GFI = 0.99 and RMSEA = 0.00.

system. Previous studies have suggested that functional redundancy is prevalent in soil microorganisms and may overwhelm any type of relationship between diversity and function, e.g. an initial loss of microbial diversity would not likely substantially affect ecosystem function (Wertz et al., 2007; van Elsas et al., 2012). Functional redundancy, however, may have been overestimated, because the effect of the biological barrier established by microbial diversity was not taken into account.

Our results may be correlative in nature, so are not definitive proof of causation, but they are nevertheless consistent with those from theoretical and experimental studies reporting that highly diverse soil microbial communities can act as a biological barrier against invasion (Case, 1990; Hodgson et al., 2002; Kennedy et al., 2002; Vivant et al., 2013). For example, a recent study found that the survival rate of inoculated *Listeria monocytogenes* L9 was negatively correlated with inverse Simpson diversity ($\rho = -0.817$, $P < 0.05$) and suggested that the loss of microbial diversity might enhance the circulation of pathogenic microorganisms in soil (Vivant et al., 2013). In addition, our previous study demonstrated that the manure-borne ARGs proliferated more easily in γ -irradiated soil than natural soil, indicating that indigenous soil microorganisms could prevent the dissemination of ARGs from manure to soil (Chen et al., 2017a). Some manure-borne antibiotic-resistant microbiota may not have been well adapted to the soil environment. The underlying mechanisms may involve resource competition or a negative relationship between native-soil and manure-borne microbiota, which were correlated with the richness and diversity of native soil species. Shifts in conditions from manure to soil

may also impede the proliferation of some manure-borne antibiotic-resistant microbiota. We will discuss these possible mechanisms in the following section.

4.2 Possible mechanisms for the effects of biological barriers on the resistome

Competition for nutrients is an important mechanism that may limit invasions in highly diverse communities (Fargione and Tilman, 2005). Resource-based niche theories suggest that the establishment of invading species is dependent on the amount of (limiting) resources not consumed by native species and on the rate at which native and invader species consume resources (Tilman, 1999). Invader species with higher consumption rates may be more efficient in extracting resources and thus more competitive with indigenous species and vice versa. In addition, some bacterial phyla such as Pseudomonads and Actinobacteria can produce ample secondary metabolites (including antibiotics), which play an important role in determining the fate of invading species (Forsberg et al., 2014). For example, van Elsas et al. (2012) found a highly significant negative correlation between invader survival (*E. coli* strain T) and Actinobacteria richness ($R^2 = 0.893$; $P = 0.0001$), suggesting that Actinobacteria were the major contributor in determining the survival of the invader species in the soil (van Elsas et al., 2012). Furthermore, decreasing the diversity of soil microorganisms would lower the degree of functional overlap and allow invaders to find, conquer and become established in a niche and subsequently persist as part of the community (van Elsas et al., 2007).

Changes in environmental conditions could also affect the

fate of invader species and genes, which is supported by previous studies reporting that some manure-derived bacteria could not thrive in soil and gradually decreased in abundance after manure treatment, at least partly due to the differences in environmental conditions between soil and the animal gut (Hammesfahr et al., 2008; Heuer et al., 2008; Chee-Sanford et al., 2009). Most of the microbiota from the guts of humans and other animals are restricted to growth under anaerobic conditions (Goodman et al., 2011), which are highly different from the aerobic conditions in our microcosms. Changes in oxygen concentration or pH would likely affect the fate of manure-borne microbiota in soil. Other mechanisms, e.g. predation and negative species interactions, might also affect the survival of manure-borne species (van Elsas et al., 2012).

4.3 Implications

The failure of antibiotic treatments is typically attributed to the “weapon-shield” role that ARGs play in clinical settings (Martínez, 2008), and the pace of development of novel antibiotics is now alarmingly slow (Walsh, 2003). The emergence and spread of antibiotic resistance has now become a global threat to human health (Bush et al., 2011; Pruden et al., 2013). Agricultural use of animal manures is believed to contribute to the spread of antibiotic resistance. The survival in soil of invading manure-borne antibiotic-resistant bacteria depends strongly on the diversity of soil microorganisms. This soil-borne legacy, however, has long been overlooked. Human activity and climate change have reduced soil microbial diversity in recent decades, which may promote the circulation of ARGs in the environment. Maintaining soil microbial diversity and identifying the driving forces that shape soil microbial diversity are therefore crucial for maintaining the soil-borne legacy.

Conflict of interest

The authors declare no conflict of interest.

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Electronic supplementary material

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