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Elevated CO_2 Concentration Modifies the Effects of Organic Fertilizer Substitution on Rice Yield and Soil ARGs

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ABSTRACT

Antibiotic resistance and rising CO_2 levels are considered amongst the most significant challenges we will face in terms of global development over the following decades. However, the impact of elevated CO_2 on soil antibiotic resistance has rarely been investigated. We used a free-air CO_2 enrichment system to investigate the potential risks posed by antibiotics in organic fertilizers applied soil at current CO_2 concentration (370 ppm) and future elevated CO_2 (eCO_2, 570 ppm predicted for 2100). Organic fertilizer substitution (substituting the mineral fertilizer by 50% N) alone increased the plant uptake and soil residue of sulfamethazine, and enriched sulfonamide resistance genes (*sul1*, *sul2*), tetracycline resistance genes (*tetG*, *tetM*) and class 1 integron (*intI1*). But it decreased the rice grain yield (by 7.6%). Comparatively, eCO_2 decreased the *sul2*, *tetG* and *intI1* gene abundances by organic fertilizer substitution, and meanwhile increased grain yield (by 8.4%). Proteobacteria and Nitrospirae were found potential hosts of antibiotic resistance genes (ARGs). Horizontal gene transfer via *intI1* may play an important role in ARGs spread under eCO_2. Results indicated that future elevated CO_2 concentration could offset the adverse effects of organic fertilizer substitution on rice yield and antibiotic risk, with unknown implications for future medicine and human health.

Keywords: Elevated CO_2; Organic fertilizer substitution; Rice; Antibiotic resistance genes.

1. Introduction

The global population has been estimated to reach 10.9×10^9 in 2100, meaning crop and animal production will need to increase from current levels (United Nations, Department of Economic and Social Affairs, Population Division, 2019). Increasing global demand for food will increase agricultural use of fertilizers and antibiotics (Rohrl et al., 2019). However, excessive nitrogen fertilizer application causes low nutrient-use efficiency and serious environmental problems such as eutrophication, global warming, and soil degradation (Gu et al., 2015; Sutton et al., 2013). The key regions suffering from excess nutrients include North America, Europe, and parts of South and South East Asia and Latin America (Sutton et al., 2013). China, the main producer of rice, established a “Zero Increase Action Plan” for fertilizers, to be implemented by 2020, which requires fertilizer use to be reduced while maintaining crop yields. This will be achieved substituting some mineral N fertilizer use with the application of livestock manure to fields and recycling N in organic waste (Liu et al., 2016; Xia et al., 2017). However, given the widespread presence of contaminants of concern in these matrices, this will increase the environmental burden of pharmaceuticals such as antibiotics (Guo et al., 2018; Hu et al., 2010). On a global scale, organic fertilizer is currently being applied to farmland, with the aim to alleviate environmental problems caused by the use of excessive mineral fertilizer whilst also offering a solution to the declining reserves of mineral fertilizer (Sutton et al., 2013).

The application of organic fertilizers, however, result in increases in the abundances of antibiotic-resistant bacteria and antibiotic-resistance genes (ARGs) in the environment (Fang et al., 2015; Tang et al., 2015; Zhu et al., 2013). ARGs can migrate into and be transformed in airborne bacteria, pathogens, soil fauna, and other environmental media (Ding et al., 2019; Fang et al., 2015; Xie et al., 2019; Zhang et al., 2019), where humans can be exposed to biota containing ARGs, thereby contributing to the global AMR (antimicrobial resistance) health crisis (Ben et al., 2019; Forsberg et al., 2012; Zhu et al., 2019).

Current CO_2 concentration in atmosphere is approximately 400 ppm, but the Intergovernmental Panel on Climate Change has predicted that the concentration will increase to 430–1000 ppm by 2100 (IPCC, 2014). Elevated CO_2 concentration (eCO_2) could affect plant performance, change the rhizosphere conditions of plants, affect soil microbial communities, and alter pollutant behavior (Duval et al., 2011; Grover et al., 2015; Sanchez-Carrillo et al., 2018; Xu et al., 2019). We have previously found that eCO_2 could change the soil microbial community composition, inhibit bacteria that degrade polycyclic aromatic hydrocarbons in soil, and may therefore increase polycyclic aromatic hydrocarbon concentrations in soil (Ai et al., 2018). Elevated CO_2 could also increase the solubilities and bio-availabilities of metals in soil and cause more nanoparticle aggregation to occur, both of which will affect soil microbial communities and plant responses (Du et al., 2017; Guo et al., 2011).

Antibiotic resistance is widely acknowledged as the most severe public health threat in the 21century (WHO, 2014). Elevated CO_2 could affect microbial activities such as cell membrane permeability, intracellular substances leakage, carbon transfer efficiency, and biofilm formation, which may influence the transformation of ARGs (Liao et al., 2019a; Yu and Chen, 2019), and cause a modification on the abundance and diversity of ARGs. Liao et al.(2019b) found that eCO_2 promoted the conjugative transfer of ARGs carried on plasmid RP4 within and across genera. These few studies focused on the effects of eCO_2 on transformation of ARGs at the biochemical and cellular levels (Liao et al. , 2019a; Liao et al., 2019b). Research concerning the effects of global climate change on the behavior of antibiotics and ARGs in soil has yet to be investigated.

In this study, we used a free-air CO_2 enrichment (FACE) system to investigate the potential risks posed by applying mineral and organic fertilizers to paddy soil under eCO_2. The aim was to assess the effects of eCO_2 on rice growth, the soil microbial community, antibiotic concentrations in soil, antibiotic uptake by rice, and ARG abundance in response to substituting mineral fertilizers with organic fertilizers. To the best of our knowledge, this is the first study to investigate the responses of a paddy soil–rice system to eCO_2 and organic fertilizer application. The results will provide novel insights into the effects of climate change on the development of antibiotic resistance.

2. Materials and methods

2.1 FACE System and Organic Fertilizer

The FACE system was in Xiaoji Town, Yangzhou City, Jiangsu Province, China (119°42'E, 32°35'N). The system was described in detail by Guo et al (Guo et al. , 2011; Zhu et al., 2016). Briefly, the mean CO_2 concentration was ~370 ppm for the ambient plots (aCO_2, reflecting the current local concentration) and ~570 ppm for the FACE plots (eCO_2, reflecting concentration predicted for 2100). Each test was performed in triplicate. Each eCO_2 was encircled with an octagonal ring (14 m in diameter) with emission tubes that injected pure CO_2 at 30 cm above the plant canopy throughout the growth of rice. Ambient control plots did not receive any supplemental CO_2. The center of eCO_2 was 90 m away from aCO_2. The target CO_2 concentration within eCO_2 was controlled by a computer program with an algorithm based on wind speed and direction.

The soil was classified as a Shajiang Aquic Cambosol containing 57.8% sand, 28.5% silt, and 13.7% clay, with pH at 7.2, total N content 1.45 g kg^{-1}, and organic matter content 18.4 g/kg (Xu et al., 2019). Organic fertilizer was obtained from a factory that produces composted pig manure and had a mean N content of 1.66%, P_2O_5 content 2.66%, and K_2O content 1.35%. Antibiotics including ciprofloxacin, enrofloxacin, ofloxacin, sulfadiazine, sulfamerazine, sulfamethazine (SMZ), sulfamethoxazole, and sulfamethoxypyridazine were detected in the organic fertilizer. The SMZ content was 17.8 mg kg^{-1}, with the other antibiotic concentrations <0.67 μg kg^{-1}.

2.2 Crop Cultivation

Five kilograms of soil was placed in a series of plastic pots (20 cm in diameter and 35 cm high), then water was applied to the soil until field water capacity was reached. Rice (*Oryza sativa* L. cv. Wuyunjing 23) was germinated for 28 days, transplanted into the different pots in early June 2016, and irrigated to keep submersed in water until 7 days before harvest in the end of October 2016 (Figure S1). Two fertilizer treatments were used, mineral fertilizer (MF; 225 kg N ha^{-1}, 225 kg P_2O_5 ha^{-}1, and 225 kg K_2O ha^{-1}), and MF with 50% of the N content replaced with organic fertilizer (MF+OF; 6777 kg ha^{-1} of organic fertilizer and mineral fertilizer providing 112.5 kg N ha^{-1}, 112.5 kg P_2O_5 ha^{-1}, and 112.5 kg K_2O ha^{-1}). The MF+OF treatment therefore had the same N content as the MF treatment. For the MF treatment, the MF was applied to each pot in three applications, 50% as a basal application applied in mid-June 2016, 25% in mid-July, and 25% in late August. For the MF+OF treatment, the organic fertilizer was applied as a basal application, and the MF was added 50% applied in mid-July, 50% in late August. These fertilizer treatments were selected based on previous studies that substituting mineral fertilizer with organic fertilizer by half maintained or even increased the crops yields (Bi et al., 2009; Singh et al., 2016). In all, four conditions were tested: MF.A, mineral fertilizer with ambient CO_2; MF.F, mineral fertilizer with elevated CO_2; MF+OF.A, organic fertilizer substitution with ambient CO_2; MF+OF.F, organic fertilizer substitution with elevated CO_2. Each treatment consisted of three replicates. After fertilization, the SMZ content in soil of the MF+OF treatment was 102 ± 16.0 μg kg^{-1}, with no significant difference between aCO_2 and eCO_2. The SMZ content in soil of the MF treatment was below the routine limit of quantification (0.08 μg kg^{-1}).

2.3 Rice Harvesting and Sampling

The rice was harvested 130 days after transplanting and biomass produced by each pot was determined. The plants were separated into roots, stems, leaves, and grains, freeze-dried, weighed, and ground to powder. Then stored them at −20 °C until analysis. Soil samples in each pot were collected for soil heavy metal content (Cu, Zn, Pb, Ni, etc.) analysis according to the Technical Specification for Soil Environmental Monitoring of China (HJ/T 166-2004) using atomic absorption spectrometry (Thermo M6, USA) (Zhang et al., 2020). Fresh soil samples were respectively stored at −80 °C for DNA analysis and stored at −20 °C for antibiotic analysis.

2.4 Antibiotic Content Analysis

SMZ in the soil and organic fertilizer samples was determined using a method published by Dalkmann et al. (2012). Each plant sample was extracted using a procedure described by Dolliver et al.(2007) and Ahmed et al.(2015) with some modifications. The method is described in detail in Text S1 in Supplementary Information (SI). The SMZ concentrations in the extracts were determined by an Agilent 1260 infinity high performance liquid chromatography coupled with an API 4000 triple quadrupole mass spectrometry (AB Sciex, Concord, ON, Canada). The instrument conditions are described in Text S1. The properties and conditions used to determine SMZ are given in Table S1 in SI. The SMZ concentration in a sample was determined as a ratio between the target peak area and the internal standard (SMZ-D4). The SMZ recoveries were 65.7%–88.9% (see Table S2 in SI). The routine limit of quantification (defined as the lowest standard concentration that was used) (Dalkmann et al., 2012) for organic fertilizer, soil, and plant matter were 0.67, 0.08, and 0.1 μg kg^{-1}, respectively.

2.5 DNA Extraction and ARG Quantification

DNA was extracted using a D5625 soil DNA kit (Omega Bio-Tek, Norcross, GA, USA) following the instructions provided by the manufacturer. The absolute numbers of the target genes were determined using a Light-Cycler 480 (Roche, Penzberg, Germany). The target genes were two *sul* genes (*sul1* and *sul2*), four *tet* genes (*tetG*, *tetM*, *tetW* and *tetX*), class 1 integron (*intI1*), and 16S rRNA. Each PCR reaction was performed in triplicate. Melting curve analysis was performed for each PCR run to verify that nonspecific amplification had not occurred. The relative abundance of the ARGs was defined as the absolute target gene number to 16S rRNA ratio. The method is described in detail in Text S2 and Tables S3 and S4 in SI.

2.6 High-throughput Sequencing of the Soil Bacterial Community

Once the DNA qualities and quantities had been determined, the 16S rRNA genes in the V3–V4 region of the bacteria were amplified using the primers 338F (5'-ACTCCTACGGGAGGCAGCA-3') and 806R (5'-GGACTACHVGGGTWTCTA AT-3). The PCR reactions were amplified by thermo cycling. The program was 5 min at 94 °C, 31 cycles of 94 °C for 30 s, 52 °C annealing for 30 s, and extension for 45 s at 72 °C, then 10 min final elongation at 72 °C. The lengths and concentrations of the PCR products were determined by gel electrophoresis using 1% agarose. The PCR products were purified, quantified, and then sequenced using an Illumina Miseq PE300 platform. The raw data were processed using pipeline coupling mothur (Schloss et al., 2009) and QIIME (Caporaso et al., 2010) software. Quality sequences were clustered into operational taxonomic units at the 97% similarity level (Edgar, 2010), and the Greengenes operational taxonomic units database (gg_13_8_otus) was used to provide reference sequences. The α-diversity indices (Chao1, Shannon, and Simpson indices) were then determined. The raw sequencing data were submitted to the NCBI Sequence Read Archive database (accession no. SRP224943).

2.7 Statistical Analyses

Each result is expressed as the mean ± standard deviation (n=3). One-way analysis of variance (Fisher's least significant differences tests, significance level $p<0.05$) and spearman 's correlation analysis were performed using SPSS 22.0 software (IBM, Armonk, NY, USA) to identify statistically significant differences ($p<0.05$) and correlations. Apart from SMZ content and CO_2 levels, pH values and contents of Cu and Zn were significant changed (Figure S2) and chosen for environmental variables. Principal component analysis, based on the Bray–Curtis distance matrix, was performed using Canoco 5.0 software. Heatmaps were generated using R 3.5.2. Then redundancy analysis (RDA) and variation partitioning analysis were generated using R 3.5.2 (Huerta et al., 2013). In addition, for the co-occurrence patterns between ARGs and the bacterial genera, a correlation matrix was calculated using spearman's correlations between ARGs that occurred in all the samples and top 50 genera with an average abundance (Li et al., 2015; Li et al., 2020). A correlation was considered statistically robust between two items with Spearman's correlation coefficient (ρ) > 0.8 and the *P*-value < 0.01 (Björn H. Junker, 2008; Li et al., 2015). False-positive correlations were avoided by adjusting the *P*-values with Benjamini-Hochberg method (Benjamini and Hochberg, 1995). The network analysis was conducted using R 3.5.2 with psych package and visualized in Gephi 0.9.2 based on the Fruchterman-Reingold algorithm (Bastian, 2009; Li et al., 2015).

3. Results and discussion

3.1 Rice Growth.

The 1000-grain weight and grain biomass of rice from the MF+OF treatment were 7.6% and 10.4% lower than those from the MF treatment. Elevated CO_2 alone or combined with MF+OF did not significantly affect the rice biomass

(Table 1). However, MF+OF treatment with eCO_2 significantly increased grain biomass (by 8.4%) relative to those from MF+OF with aCO_2. This indicated that partial substitution of MF with organic fertilizer could decrease crop growth, but this change is offset by eCO_2 concentrations. Decrease in grain yield in MF+OF treatment might due to lower available nutrients content of organic fertilizer than mineral fertilizer (Pan et al., 2009; Zhang et al., 2018). Although equal N among treatments, the basal fertilizer for MF+OF treatment was organic, and its nutrients were released slowly in rice early stages, not meeting the nutrient requirement for crop, and subsequently affecting crop growth. While, elevated CO_2 has been shown to affect root production, physiological activity and morphology, thus increased plant nutrient uptake (Kim et al., 2001). Hence, eCO_2 could alleviate the shortage of available nutrients caused by MF+OF treatment (Table 1).

3.2 Concentration of SMZ in Soil and Rice

In MF+OF treatment, SMZ was detected at 27.5–36.3 μg kg^{-1} and 6.42–7.83 μg kg^{-1} in soil and rice root, respectively, with no significant difference between the aCO_2 and eCO_2 treatments (Table 1). This indicated that organic fertilizer substitution indeed increased risk of antibiotics entering soil and plants at both current and future CO_2 conditions. Although elevated CO_2 has the potential to affect plant performance and modify the soil microbial community, thereby indirectly affecting the behaviors of pollutants such as antibiotics (Duval et al., 2011), results indicated that organic fertilizer substitution might play a more important role than eCO_2 in terms of the soil microbial community which is confirmed as followings.

3.3 Soil ARGs Abundances

ARGs and *intI1* were detected in soils from all treatments, including the organic fertilizer, MF and MF+OF (Figure 1). Amongst, *tetX* had the highest average relative abundance (1.38×10^{-4} copies/16S rRNA gene copies), followed by *tetW*, *sul1*, *intI1*, *tetG*, and *tetM*, with lowest values found for *sul2* (Figure 1a, 1b). Interestingly, when compared with MF, MF+OF showed 3.28 times, 2.90 times, 5.39 times, 6.17 times higher in *sul1*, *sul*2, *tetG*, and *intI1,* respectively (Figure 1a, 1b), but no difference in *tetX* or *tetW*. Organic fertilizer was a main source of environmental ARGs (Zhu et al., 2013) and *sul* genes were more recalcitrant and easier to increase than other ARGs (Wang et al., 2020). Hence, ARG changes in MF+OF may be explained by the relative abundances order of *sul1*>*sul2*>*intI1*>*tetX*>*tetG*>*tetW*>*tetM* in organic fertilizer (Fig 1c). Elevated CO_2 alone did not significantly affect the relative abundances of ARGs (Figure 1a, 1b), but the relative abundances of *sul2*, *tetG* and *intI1* were significantly lower (51.6%, 66.9% and 74.5%, respectively) for its combination with MF+OF than those of MF+OF alone (Figure 1a, 1b). Considering *intI1* could enhance the transmission and accumulation of ARGs through horizontal gene transfer (HGT) , HGT might be an alternative reason for ARG changes in MF+OF treatments (Han et al., 2018). This was further supported by the results that *sul1*, *sul*2, and *tetG* significantly positively correlated with *intI1* at $p<0.05$ ($r=0.66$), $p<0.05$ ($r=0.64$), and $p<0.01$($r=0.72$) levels, respectively (Figure 2). These indicated that MF+OF increase the relative abundance of ARGs in soil potentially through horizontal gene transfer by a selective environmental pressure, or a supply of related genes in the organic fertilizer (Zhu et al., 2013). Meanwhile, eCO_2 inhibited the transmission of detected ARGs in organic fertilizer substitution soil by decreased rates of HGT (Figure 1a,1b).

ARGs are widely acknowledged as emerging contaminants of concern, and *IntI1* has been suggested to be used as an indicator of the prevalence of ARGs in the environment (Ma et al., 2017). The co-enrichment of detected *sul*2, *tetG* and *intI1* genes exacerbate the transfer risks of ARGs from livestock animals to human-associated pathogens, and finally to humans via increasing environmental exposure to antibiotic resistance genes selected for in the human gastrointestinal tract (Forsberg et al., 2012; Perry and Wright, 2013; Zhao et al., 2018). Exposure to resistance genes in the environment pose a significant threat to the public health and ecosystem safety. However, our results suggest that this threat may be mitigated with an elevated CO_2 concentration predicted in 2100.

3.4 Soil Microbial Community

No statistically significant difference in the bacterial α-diversity indices (Chao1, Shannon, and Simpson indices) was observed for MF or MF+OF treatments with aCO_2 or eCO_2 (Table S5). Principal component analysis was performed to identify differences between the soil bacterial community structures of all treatments and shown in Figure S3a. The MF+OF treatment was clearly separated from the MF treatment on axis 1, explaining 54.8% of the total variance in soil bacterial community structures in all samples (Figure S3a). The MF treatments with aCO_2 and eCO_2 were separated along axis 2, which explained only 7.4% of the total variance in soil bacterial structures in all samples (Figure 3a). The MF+OF treatment with eCO_2 clustered with the MF+OF treatment with aCO_2. These results suggest that organic fertilize substituting played a more important role than eCO_2 in terms of altering the soil bacterial structure as mentioned in 3.2. The variances of bacterial phyla and top 50 genera in soil are described in Figure 3a and 3b. Proteobacteria, Firmicutes, Chloroflexi and Acidobacteria were the dominant phyla in soil (Figure 3a). The relative abundance of Gemmatimonadetes and Nitrospirae were lower under MF with eCO_2 as compared with MF with aCO_2 (Figure S3b). While the relative abundances of Gemmatimonadetes and Actinobacteria were higher in MF+OF with eCO_2 when compared with that of MF+OF with aCO_2 (Figure S3b). At the top 50 genera, eCO_2 alone significantly decreased the relative abundances of *c_Gemm-5*, *f_Chitinophagaceae*, *f_Gaiellaceae*, *g_Phycicoccus*, *o_Acidimicrobiales*, *f_Koribacteraceae*, *g_Candidatus Solibacter* and *o_Solibacterales* (Figure 3b). In contrast, MF+OF with eCO_2 enriched *c_Gemm-1*, *f_Micrococcaceae*, *f_Rhodospirillaceae*, *f_Syntrophobacteraceae* and *o_NB1-j*, compared to MF+OF with aCO_2 (Figure

3b). Results indicated that the response of soil microbial species to eCO_2 is related to the fertilizer type. Moreover, the decrease of chemoautotrophic bacterium Nitrospirae in MF and increase of photosynthetic bacterium *f_Rhodospirillaceae* in MF+OF by eCO_2, respectively, may lead to modification in process or efficiency of soil microbial CO_2 fixation (Liu et al., 2018; Shi et al., 2020). Redundancy analysis was performed to investigate the influence of environmental variables (SMZ, pH, Cu, Zn, and CO_2) on soil bacterial community, and found that these environmental variables explain 45.3% of the variance in microbial communities (Figure 3c). Among these environmental factors, SMZ positively correlated with Proteobacteria (Figure 3c).

In previous studies, the genera *Acinetobacter, Methylobacterium*, and *Pseudomonas* have been described as sulfonamide antibiotic degrading bacteria (Deng et al., 2018; Mulla et al., 2016; Zhang et al., 2012a; Zhang et al., 2012b). In this study, combinated MF+OF with eCO_2 enriched *Methylobacterium*, but the relative abundance of *Methylobacterium* was < 0.03%. While, *Acinetobacter* and *Pseudomonas* were not significantly changed among all treatments (Figure S3c). However, *Thauera,* which has been found to be related to sulfonamide antibiotic degradation (Yang et al., 2018), was only detected in MF+OF treatments, both aCO_2 and eCO_2 (Figure S3c). Whilst the underlying mechanism is unclear, this finding verified that *Thauera* genus is involved in SMZ degradation.

3.5 Factors shaping ARGs abundance

The abundance of ARG in organic fertilizer was the higher than soils (Figure. 1), verifying that organic fertilizer was a major source of ARGs in MF+OF soils. After entering soil with organic fertilizer application, the ARG abundance is shaped by several factors, including environmental variables and the microbial community (Forsberg et al., 2014; Wang et al., 2020). Here, factors that could affect ARGs were investigated by performing spearman's correlation analyses on the detected ARGs and environmental variables including SMZ, Cu, Zn contents, pH values, and CO_2 concentrations (Figure 2). Results showed that SMZ, pH, Cu and Zn significantly positively correlated with most ARGs (Figure 2), indicating that soil environmental variables did partly shape ARGs abundances. But a non-significant negative correlation was found between ARGs and eCO_2. These results suggest that environmental variables induced by the addition of organic fertilizer are more important than eCO_2 in shaping ARGs abundance. As one of most important mobile genetic elements for HGT, *intI1* showed no significantly correlation with environmental variables and phylum, but positively correlated with the *sul1*, *sul2* and *tetG*. This suggest that under future eCO_2, the HGT of ARGs will be more important than other factors in shaping ARGs abundance.

Studies have speculated and verified some potential hosts of ARGs through the significantly similar abundance trends between ARGs and co-occurred bacteria (Forsberg et al., 2014; Jia et al., 2020). In this study, spearman analysis (Figure 2) and network analysis (Figure 4) were constructed to search the potential hosts of target ARGs. At phylum level, Proteobacteria, Nitrospirae, and Planctomycetes were significantly positively correlated with most detected ARGs, while Gemmatimonadetes and Chlorobi exhibited a negative correlation (Figure 2). Both positive and negative correlations suggest that these bacteria may play roles stimulating or inhibiting ARG survival or dissemination (Peng et al., 2016). Moreover, the co-occurrence patterns between ARGs and the top 50 genera were investigated based on strongly and significantly correlations ((ρ) > 0.8, *P*-value < 0.01) (Figure 4). The network consists of 47 nodes and 138 edges, with a high modularity index of 0.564, and parsed into five modules (Figure 4a). Some topological properties of the network analysis were summarized in Table S6 in SI. Nodes within the same module were more frequently connected than those cross modules (Han et al., 2017). The *sul1* genes were correlated with *sul2*, *tetM* and *tetG* genes, indicating that they might be located in the same genetic elements or carried by specific bacterial species (Han et al., 2017). Moreover, ARGs (*sul1*, *sul2*, *tetM* and *tetG* genes) and some genera were in the module 2 (Figure 4). Co-occurrence patterns that between *sul1* and *f_Alcaligenaceae*, *f_syntrophobacteraceae*, *f_Rhodospirillaceae*, *g_Nitrospira* and *f_Micrococcaceae* were observed, and also between *sul2* and *f_Alcaligenaceae*, *g_Nitrospira*. The *tetG* gene was significantly correlated with *f_Alcaligenaceae*, as well as *tetM* and *g_Nitrospira*. Previous study reported that *Rhodospirillaceae* acquired cross-resistance in a municipal sewage

(Schreiber and Kistemann, 2013). These results indicated that the above bacteria probably carried the ARGs, and some bacterium could carry multiple ARGs. The ecological risk of bacteria that carrying multiple ARGs need more concern. Since the most of genera involved in these co-occurrence patterns belong to Protebacteria and Nitropirae (Figure 4b), which were suggested to be the potential hosts of soil ARGs (Chen et al., 2019; Liu et al., 2019). In addition, *f_Alcaligenaceae*, *f_syntrophobacteraceae*, *f_Rhodospirillaceae*, *g_Nitrospira* and *f_Micrococcaceae* were enriched 2.22-fold, 3.26-fold, 1.59-fold, 1.96-fold, and 5.15-fold in MF+OF.A, respectively, compared to MF.A (Figure 3b). Therefore, the relative abundance of ARGs (*sul1*, *sul2*, *tetG* and *tetM*) carried by these bacteria increased in MF+OF.A. *Alcaligenaceae* was described as NO_3^- reducing microbes in soil (Qin et al., 2019). *Syntrophobacteraceae* plays an important role in propionate-dependent sulfate reduction in anoxic microcosms of paddy soil (Liu and Conrad, 2017). And *Nitrospira* is the chemolithoautotrophic bacterium that can utilize inorganic carbon (such as HCO_3^- and CO_2). This indicated that the potential hosts of soil ARGs may also played important roles in C, N transformations.

Variation partitioning analysis was also performed to explore the contribution of environmental variables and the potential hosts on the variation of ARGs (Figure S4). Results showed that a total of 69.4% of the ARGs variation could be explained by these factors. The potential hosts (including the relative abundances of Proteobacteria and Nitropirae) contributed 32.4% to the variations, whereas environmental variables (pH and SMZ) contributed 29.8%. Their joint effect defined 7.2 % of the variations. These results suggested the importance of potential hosts in structuring soil antibiotic resistome under organic fertilizer substitution. However, the effects of elevated CO_2 concentration on ARGs abundance still need further in-depth studies.

4 Conclusions

Sustainable agricultural practices offer a means of increasing our crop productivity to meet the food demands of a rapidly growing global population. However, we need to account for the environmental transfer of ARGs, following approaches such as the application of organic fertilizers, to ensure we are not contributing to the global spread antibiotic resistance. This study demonstrated that elevated CO_2 concentration in future could mitigate the adverse effects of organic fertilizer substitution, decreasing *sul2, tetG* and *intI1* gene abundances in paddy soil and increasing rice yield. Network analysis and variation partitioning analysis suggested that the potential hosts of soil ARGs (Proteobacteria and Nitropirae) play important roles in structuring soil antibiotic resistome, and under future elevated CO_2, the HGT of ARGs is more important than other factors in shaping ARGs abundance in this study. Currently, it is imperative that alternative strategies are put in place to alleviate pressure from increased ARG abundance such as reducing the use of antibiotics in animal husbandry and establishing dispersal barriers between animals, human and the external environment. Future studies involving the co-occurrences of different types of ARGs and mobile genetic elements are needed to provide comprehensive insight into the overall ARG profile response following organic manure substitution under elevated CO_2 concentrations.

Declaration of competing interest

The authors declare no competing financial interest.

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References

Ahmed, M.B.M.; Rajapaksha, A.U.; Lim, J.E.; Vu, N.T.; Kim, I.S.; King, H.M.; Lee, S.S.; Ok, Y.S., 2015. Distribution and accumulative pattern of tetracyclines and sulfonamides in edible vegetables of cucumber, tomato, and lettuce. J Agr Food Chem. 63, 398-405. https://doi.org/10.1021/jf5034637.

Ai, F.X.; Eisenhauer, N.; Jousset, A.; Butenschoen, O.; Ji, R.; Guo, H.Y., 2018. Elevated tropospheric CO_2 and O_3 concentrations impair organic pollutant removal from grassland soil. Sci Rep-Uk. 8, 5519. https://doi.org/10.1038/s41598-018-23522-z.

Bastian, M., Heymann, S., Jacomy, M., . 2009. Gephi: an open source software for exploring and manipulating networks. ICWSM. 8, 361–362.

Ben, Y.J.; Fu, C.X.; Hu, M.; Liu, L.; Wong, M.H.; Zheng, C.M., 2019. Human health risk assessment of antibiotic resistance associated with antibiotic residues in the environment: A review. Environ Res. 169, 483-493. https://doi.org/10.1016/j.envres.2018.11.040.

Benjamini, Y.; Hochberg, Y., 1995. Controlling the False Discovery Rate - a Practical and Powerful Approach to Multiple Testing. J R Stat Soc B. 57, 289-300.

Bi, L.; Zhang, B.; Liu, G.; Li, Z.; Liu, Y.; Ye, C.; Yu, X.; Lai, T.; Zhang, J.; Yin, J.; Liang, Y., 2009. Long-term effects of organic amendments on the rice yields for double rice cropping systems in subtropical China. Agric Ecosyst Environ. 129, 534-541. https://doi.org/10.1016/j.agee.2008.11.007.

Björn H. Junker, F.S., 2008. Correlation Networks. Analysis of Biological Networks: Wiley-Interscience.

Caporaso, J.G.; Kuczynski, J.; Stombaugh, J.; Bittinger, K.; Bushman, F.D.;

Costello, E.K.; Fierer, N.; Pena, A.G.; Goodrich, J.K.; Gordon, J.I.; Huttley, G.A.; Kelley, S.T.; Knights, D.; Koenig, J.E.; Ley, R.E.; Lozupone, C.A.; McDonald, D.; Muegge, B.D.; Pirrung, M.; Reeder, J.; Sevinsky, J.R.; Tumbaugh, P.J.; Walters, W.A.; Widmann, J.; Yatsunenko, T.; Zaneveld, J.; Knight, R., 2010. QIIME allows analysis of high-throughput community sequencing data. Nat Methods. 7, 335-336. https://doi.org/10.1038/nmeth.f.303.

Chen, Z.; Zhang, W.; Yang, L.; Stedtfeld, R.D.; Peng, A.; Gu, C.; Boyd, S.A.; Li, H., 2019. Antibiotic resistance genes and bacterial communities in cornfield and pasture soils receiving swine and dairy manures. Environ Pollut. 248, 947-957. https://doi.org/10.1016/j.envpol.2019.02.093.

Dalkmann, P.; Broszat, M.; Siebe, C.; Willaschek, E.; Sakinc, T.; Huebner, J.; Amelung, W.; Grohmann, E.; Siemens, J., 2012. Accumulation of Pharmaceuticals, Enterococcus, and Resistance Genes in Soils Irrigated with Wastewater for Zero to 100 Years in Central Mexico. Plos One. 7, e45397. https://doi.org/10.1371/journal.pone.0045397.

Deng, Y.; Li, B.; Zhang, T., 2018. Bacteria that make a meal of sulfonamide antibiotics: Blind spots and emerging opportunities. Environ Sci Technol. 52, 3854-3868. https://doi.org/10.1021/acs.est.7b06026.

Ding, J.; Zhu, D.; Hong, B.; Wang, H.T.; Li, G.; Ma, Y.B.; Tang, Y.T.; Chen, Q.L., 2019. Long-term application of organic fertilization causes the accumulation of antibiotic resistome in earthworm gut microbiota. Environ

Int. 124, 145-152. https://doi.org/10.1016/j.envint.2019.01.017.

Dolliver, H.; Kumar, K.; Gupta, S., 2007. Sulfamethazine uptake by plants from manure-amended soil. J Environ Qual. 36, 1224-1230. https://doi.org/10.2134/jeq2006.0266.

Du, W.; Gardea-Torresdey, J.L.; Xie, Y.; Yin, Y.; Zhu, J.; Zhang, X.; Ji, R.; Gu, K.; Peralta-Videa, J.R.; Guo, H., 2017. Elevated CO_2 levels modify TiO_2 nanoparticle effects on rice and soil microbial communities. Sci Total Environ. 578, 408. https://doi.org/10.1016/j.scitotenv.2016.10.197.

Duval, B.D.; Dijkstra, P.; Natali, S.M.; Megonigal, J.P.; Ketterer, M.E.; Drake, B.G.; Lerdau, M.T.; Gordon, G.; Anbar, A.D.; Hungate, B.A., 2011. Plant-soil distribution of potentially toxic elements in response to elevated atmospheric CO_2. Environ Sci Technol. 45, 2570-2574. https://doi.org/10.1021/es102250u.

Edgar, R.C., 2010. Search and clustering orders of magnitude faster than BLAST. Bioinformatics. 26, 2460-2461. https://doi.org/10.1093/bioinformatics/btq461.

Fang, H.; Wang, H.F.; Cai, L.; Yu, Y.L., 2015. Prevalence of antibiotic resistance genes and bacterial pathogens in long-term manured greenhouse soils as revealed by metagenomic survey. Environ Sci Technol. 49, 1095-1104. https://doi.org/10.1021/es504157v.

Forsberg, K.J.; Patel, S.; Gibson, M.K.; Lauber, C.L.; Knight, R.; Fierer, N.; Dantas, G., 2014. Bacterial phylogeny structures soil resistomes across habitats. Nature. 509, 612-616. https://doi.org/10.1038/nature13377.

Forsberg, K.J.; Reyes, A.; Bin, W.; Selleck, E.M.; Sommer, M.O.A.; Dantas, G., 2012. The Shared Antibiotic Resistome of Soil Bacteria and Human Pathogens. Science. 337, 1107-1111. https://doi.org/10.1126/science.1220761.

Grover, M.; Maheswari, M.; Desai, S.; Gopinath, K.A.; Venkateswarlu, B., 2015. Elevated CO_2: Plant associated microorganisms and carbon sequestration. Applied Soil Ecology. 95, 73-85. https://doi.org/10.1016/j.apsoil.2015.05.006.

Gu, B.J.; Ju, X.T.; Chang, J.; Ge, Y.; Vitousek, P.M., 2015. Integrated reactive nitrogen budgets and future trends in China. P Natl Acad Sci USA. 112, 8792-8797. https://doi.org/10.1073/pnas.1510211112.

Guo, H.Y.; Zhu, J.G.; Zhou, H.; Sun, Y.Y.; Yin, Y.; Pei, D.P.; Ji, R.; Wu, J.C.; Wang, X.R., 2011. Elevated CO_2 levels affects the concentrations of copper and cadmium in crops grown in soil contaminated with heavy metals under fully open-air field conditions. Environ Sci Technol. 45, 6997-7003. https://doi.org/10.1021/es2001584.

Guo, T.; Lou, C.L.; Zhai, W.W.; Tang, X.J.; Hashmi, M.Z.; Murtaza, R.; Li, Y.; Liu, X.M.; Xu, J.M., 2018. Increased occurrence of heavy metals, antibiotics and resistance genes in surface soil after long-term application of manure. Sci Total Environ. 635, 995-1003. https://doi.org/10.1016/j.scitotenv.2018.04.194.

Han, X.M.; Hu, H.W.; Chen, Q.L.; Yang, L.Y.; Li, H.L.; Zhu, Y.G.; Li, X.Z.; Ma, Y.B., 2018. Antibiotic resistance genes and associated bacterial communities in agricultural soils amended with different sources of animal manures. Soil Biol Biochem. 126, 91-102. https://doi.org/10.1016/j.soilbio.2018.08.018.

Han, Y.; Wang, J.; Zhao, Z.L.; Chen, J.W.; Lu, H.; Liu, G.F., 2017. Fishmeal application induces antibiotic resistance gene propagation in mariculture sediment. Environ Sci Technol. 51, 10850-10860. https://doi.org/10.1021/acs.est.7b02875.

Hu, X.G.; Zhou, Q.X.; Luo, Y., 2010. Occurrence and source analysis of typical veterinary antibiotics in manure, soil, vegetables and groundwater from organic vegetable bases, northern China. Environ Pollut. 158, 2992-2998. https://doi.org/10.1016/j.envpol.2010.05.023.

Huerta, B.; Marti, E.; Gros, M.; Lopez, P.; Pompeo, M.; Armengol, J.; Barcelo, D.; Luis Balcazar, J.; Rodriguez-Mozaz, S.; Marce, R., 2013. Exploring the links between antibiotic occurrence, antibiotic resistance, and bacterial communities in water supply reservoirs. Sci Total Environ. 456, 161-170. https://doi.org/10.1016/j.scitotenv.2013.03.071.

IPCC, 2014. Climate Change 2014: Synthesis Report. Contribution of Working Groups I, II and III to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change [Core Writing Team, R.K. Pachauri and L.A. Meyer (eds.)]. Geneva, Switzerland.

https://www.ipcc.ch/site/assets/uploads/2018/02/SYR_AR5_FINAL_full.pdf

Kim, H.Y.; Lieffering, M.; Miura, S.; Kobayashi, K.; Okada, M., 2001. Growth and nitrogen uptake of CO_2-enriched rice under field conditions. New Phytol. 150, 223-229. https://doi.org/10.1046/j.1469-8137.2001.00111.x.

Li, B.; Yang, Y.; Ma, L.; Ju, F.; Guo, F.; Tiedje, J.M.; Zhang, T., 2015. Metagenomic and network analysis reveal wide distribution and co-occurrence of environmental antibiotic resistance genes. Isme J. 9, 2490-2502. https://doi.org/10.1038/ismej.2015.59.

Li, Y.; Wang, X.J.; Wang, Y.; Wang, F.; Xia, S.Q.; Zhao, J.F., 2020. Struvite-supported biochar composite effectively lowers Cu bio-availability and the abundance of antibiotic -resistance genes in soil. Sci Total Environ. 724, 138294. https://doi.org/10.1016/j.scitotenv.2020.138294

Liao, J.Q.; Chen, Y.G.; Huang, H.N., 2019a. Effects of CO_2 on the transformation of antibiotic resistance genes via increasing cell membrane channels. Environ Pollut. 254, 113045. https://doi.org/10.1016/j.envpol.2019.113045.

Liao, J.Q.; Huang, H.N.; Chen, Y.G., 2019b. CO_2 promotes the conjugative transfer of multiresistance genes by facilitating cellular contact and plasmid transfer. Environ Int. 129, 333-342. https://doi.org/10.1016/j.envint.2019.05.060.

Liu, H.; Sun, H.; Zhang, M.; Liu, Y., 2019. Dynamics of microbial community and tetracycline resistance genes in biological nutrient removal process. J Environ Manage. 238, 84-91. https://doi.org/10.1016/j.jenvman.2019.02.123.

Liu, P.F.; Conrad, R., 2017. Syntrophobacteraceae-affiliated species are major propionate-degrading sulfate reducers in paddy soil. Environ. Microbiol. 19, 1669-1686. https://doi.org/10.1111/1462-2920.13698.

Liu, X.J.; Vitousek, P.; Chang, Y.H.; Zhang, W.F.; Matson, P.; Zhang, F.S., 2016. Evidence for a Historic Change Occurring in China. Environ Sci Technol. 50, 505-506. 10.1021/acs.est.5b05972.

Liu, Z.; Sun, Y.F.; Zhang, Y.Q.; Feng, W.; Lai, Z.R.; Fa, K.Y.; Qin, S.G., 2018. Metagenomic and C-13 tracing evidence for autotrophic atmospheric carbon absorption in a semiarid desert. Soil Biol Biochem. 125, 156-166. https://doi.org/10.1016/j.soilbio.2018.07.012.

Ma, L.P.; Li, A.D.; Yin, X.L.; Zhang, T., 2017. The Prevalence of Integrons as the Carrier of Antibiotic Resistance Genes in Natural and Man-Made Environments. Environ Sci Technol. 51, 5721-5728. https://doi.org/10.1021/acs.est.6b05887.

Mulla, S.I.; Sun, Q.A.; Hu, A.Y.; Wang, Y.W.; Ashfaq, M.; Eqani, S.A.M.A.S.; Yu, C.P., 2016. Evaluation of sulfadiazine degradation in three newly isolated pure bacterial cultures. Plos One. 11, 1-14. https://doi.org/10.1371/journal.pone.0165013.

Pan, G.X.; Zhou, P.; Li, Z.P.; Smith, P.; Li, L.Q.; Qiu, D.S.; Zhang, X.H.; Xu, X.B.;

Shen, S.Y.; Chen, X.M., 2009. Combined inorganic/organic fertilization enhances N efficiency and increases rice productivity through organic carbon accumulation in a rice paddy from the Tai Lake region, China. Agric Ecosyst Environ. 131, 274-280. https://doi.org/10.1016/j.agee.2009.01.020.

Peng, S.; Zhou, B.B.; Wang, Y.M.; Lin, X.G.; Wang, H.M.; Qiu, C.P., 2016. Bacteria play a more important role than nutrients in the accumulation of tetracycline resistance in manure-treated soil. Biol Fert Soils. 52, 655-663. https://doi.org/10.1007/s00374-016-1105-9.

Perry, J.A.; Wright, G.D., 2013. The antibiotic resistance "mobilome": searching for the link between environment and clinic. Front Microbiol. 4, 138. https://doi.org/10.3389/fmicb.2013.00138.

Qin, S.P.; Yu, L.P.; Yang, Z.J.; Li, M.Y.; Clough, T.; Wrage-Monnig, N.; Hu, C.S.; Liu, B.B.; Chen, S.M.; Zhou, S.G., 2019. Electrodes Donate Electrons for Nitrate Reduction in a Soil Matrix via DNRA and Denitrification. Environ Sci Technol. 53, 2002-2012. https://doi.org/10.1021/acs.est.8b03606.

Rohrl, J.R.; Barrett, C.B.; Civitello, D.J.; Craft, M.E.; Delius, B.; DeLeo, G.A.; Hudson, P.J.; Jouanard, N.; Nguyen, K.H.; Ostfeld, R.S.; Remais, J.V.; Riveau, G.; Sokolow, S.H.; Tilman, D., 2019. Emerging human infectious diseases and the links to global food production. Nat Sustain. 2, 445-456. https://doi.org/10.1038/s41893-019-0293-3.

Sanchez-Carrillo, S.; Alvarez-Cobelas, M.; Angeler, D.G.; Serrano-Grijalva, L.;

Sanchez-Andres, R.; Cirujano, S.; Schmid, T., 2018. Elevated Atmospheric CO_2 Increases Root Exudation of Carbon in Wetlands: Results from the First Free-Air CO_2 Enrichment Facility (FACE) in a Marshland. Ecosystems. 21, 852-867. https://doi.org/10.1007/s10021-017-0189-x.

Schloss, P.D.; Westcott, S.L.; Ryabin, T.; Hall, J.R.; Hartmann, M.; Hollister, E.B.; Lesniewski, R.A.; Oakley, B.B.; Parks, D.H.; Robinson, C.J.; Sahl, J.W.; Stres, B.; Thallinger, G.G.; Van Horn, D.J.; Weber, C.F., 2009. Introducing mothur: open-source, platform-independent, community-supported software for describing and comparing microbial communities. Appl Environ Microb. 75, 7537-7541. https://doi.org/10.1128/AEM.01541-09.

Schreiber, C.; Kistemann, T., 2013. Antibiotic resistance among autochthonous aquatic environmental bacteria. Water Sci Technol. 67, 117-123. https://doi.org/10.2166/wst.2012.539.

Shi, M.; Li, J.Y.; Zhou, Q.; Wang, G.B.; Zhang, W.G.; Zhang, Z.H.; Gao, Y.; Yan, S.H., 2020. Interactions between elevated CO_2 levels and floating aquatic plants on the alteration of bacterial function in carbon assimilation and decomposition in eutrophic waters. Water Research. 171, https://doi.org/10.1016/j.watres.2019.115398.

Shuyu Jia, K.B., Peng Shi, Lin Ye, Chang-Hong Liu, 2020. Metagenomic profiling of antibiotic resistance genes and their associations with bacterial community during multiple disinfection regimes in a full-scale drinking water treatment plant. Water Res. 176. https://doi.org/10.1016/j.watres.2020.115721

Singh, R.J.; Ghosh, B.N.; Sharma, N.K.; Patra, S.; Dadhwal, K.S.; Mishra, P.K., 2016. Energy budgeting and emergy synthesis of rainfed maize-wheat rotation system with different soil amendment applications. Ecological Indicators. 61, 753-765. https://doi.org/10.1016/j.ecolind.2015.10.026.

Sutton, M.A.B., A.; Howard, C.M.; Bekunda, M.; Grizzetti, B.; de Vries, W.; van Grinsven, H.J.M.; Abrol, Y.P.; Adhya, T.K.; Billen, G.; Davidson, E.A.; Datta, A.; Diaz, R.; Erisman, J.W.; Liu, X.J.; Oenema, O.; Palm, C.; Raghuram, N.; Reis, S.; Scholz, R.W.; Sims, T.; Westhoek, H.; Zhang, F.S.. , 2013. Our nutrient world: the challenge to produce more food and energy with less polution. Edinburgh: Centre for Ecology and Hydrology.https://edepot.wur.nl/249094.

Tang, X.J.; Lou, C.L.; Wang, S.X.; Lu, Y.H.; Liu, M.; Hashmi, M.Z.; Liang, X.Q.; Li, Z.P.; Liao, Y.L.; Qin, W.J.; Fan, F.; Xu, J.M.; Brookes, P.C., 2015. Effects of long-term manure applications on the occurrence of antibiotics and antibiotic resistance genes (ARGs) in paddy soils: Evidence from four field experiments in south of China. Soil Biol Biochem. 90, 179-187. https://doi.org/10.1016/j.soilbio.2015.07.027.

United Nations, Department of Economic and Social Affairs, Population Division, 2019. World Population Prospects 2019: Highlights

(ST/ESA/SER.A/423). https://population.un.org/wpp/Publications/Files/WPP2019_Highlights.pdf.

WHO, 2014. Antimicrobial resistance: global report on surveillance.Geneva, World Health Organization. https://www.who.int/antimicrobial-resistance/publications/surveillancereport/en/.

Xia, L.L.; Lam, S.K.; Yan, X.Y.; Chen, D.L., 2017. How Does Recycling of Livestock Manure in Agroecosystems Affect Crop Productivity, Reactive Nitrogen Losses, and Soil Carbon Balance? Environ Sci Technol. 51, 7450-7457. https://doi.org/10.1021/acs.est.6b06470.

Xie, J.W.; Jin, L.; He, T.T.; Chen, B.W.; Luo, X.S.; Feng, B.H.; Huang, W.; Li, J.; Fu, P.Q.; Li, X.D., 2019. Bacteria and antibiotic resistance genes (ARGs) in $PM_{2.5}$ from China: implications for human exposure. Environ Sci Technol. 53, 963-972. https://doi.org/10.1021/acs.est.8b04630.

Xu, M.L.; Zhu, Y.G.; Gu, K.H.; Zhu, J.G.; Yin, Y.; Ji, R.; Du, W.C.; Guo, H.Y., 2019. Transcriptome Reveals the Rice Response to Elevated Free Air CO_2 Concentration and TiO_2 Nanoparticles. Environ Sci Technol. 53, 11714-11724. https://doi.org/10.1021/acs.est.9b02182.

Yang, C.W.; Tsai, L.L.; Chang, B.V., 2018. Anaerobic degradation of sulfamethoxazole in mangrove sediments. Sci Total Environ. 643, 1446-1455. https://doi.org/10.1016/j.scitotenv.2018.06.305.

Yu, T.; Chen, Y.G., 2019. Effects of elevated carbon dioxide on environmental microbes and its mechanisms: A review. Sci Total Environ. 655, 865-879. https://doi.org/10.1016/j.scitotenv.2018.11.301.

Zhang, M.; Yao, Y.L.; Tian, Y.H.; Ceng, K.; Zhao, M.; Zhao, M.; Yin, B., 2018. Increasing yield and N use efficiency with organic fertilizer in Chinese intensive rice cropping systems. Field Crop Res. 227, 102-109. https://doi.org/10.1016/j.fcr.2018.08.010.

Zhang, W.W.; Wen, Y.Y.; Niu, Z.L.; Yin, K.; Xu, D.X.; Chen, L.X., 2012a. Isolation and characterization of sulfonamide-degrading bacteria *Escherichia* sp HS21 and *Acinetobacter* sp HS51. World J Microb Biot. 28, 447-452. https://doi.org/10.1007/s11274-011-0834-z.

Zhang, W.W.; Xu, D.X.; Niu, Z.L.; Yin, K.; Liu, P.; Chen, L.X., 2012b. Isolation and characterization of *Pseudomonas* sp DX7 capable of degrading sulfadoxine. Biodegradation. 23, 431-439. https://doi.org/10.1007/s10532-011-9522-9.

Zhang, Y.J.; Hu, H.W.; Chen, Q.L.; Singh, B.K.; Yan, H.; Chen, D.L.; He, J.Z., 2019. Transfer of antibiotic resistance from manure-amended soils to vegetable microbiomes. Environ Int. 130, 104912. https://doi.org/10.1016/j.envint.2019.104912.

Zhang, Y.X.; Chen, H.Y.; Jing, L.J.; Teng, Y.G., 2020. Ecotoxicological risk assessment and source apportionment of antibiotics in the waters and sediments of a peri-urban river. Sci Total Environ. 731, 139128. https://doi.org/10.1016/j.scitotenv.2020.139128.

Zhao, W.X.; Wang, B.; Yu, G., 2018. Antibiotic resistance genes in China: occurrence, risk, and correlation among different parameters. Environ Sci Pollut R. 25, 21467-21482. https://doi.org/10.1007/s11356-018-2507-z.

Zhi Wang, M.H., Enhua Li, Xi Liu, Huimin Wei, Chao Yang, Shaoyong Lu, Kang Ning,, 2020. Distribution of antibiotic resistance genes in an agriculturally disturbed lake in China: Their links with microbial communities, antibiotics, and water quality. J Hazard Mater. 393, 122426. https://doi.org/10.1016/j.jhazmat.2020.122426.

Zhu, C.W.; Xu, X.; Wang, D.; Zhu, J.G.; Liu, G.; Seneweera, S., 2016. Elevated atmospheric [CO_2] stimulates sugar accumulation and cellulose degradation rates of rice straw. Gcb Bioenergy. 8, 579-587. https://doi.org/10.1111/gcbb.12277.

Zhu, Y.G.; Johnson, T.A.; Su, J.Q.; Qiao, M.; Guo, G.X.; Stedtfeld, R.D.; Hashsham, S.A.; Tiedje, J.M., 2013. Diverse and abundant antibiotic resistance genes in Chinese swine farms. P Natl Acad Sci USA. 110, 3435-3440. https://doi.org/10.1073/pnas.1222743110.

Zhu, Y.G.; Zhao, Y.; Zhu, D.; Gillings, M.; Penuelas, J.; Ok, Y.S.; Capon, A.; Banwart, S., 2019. Soil biota, antimicrobial resistance and planetary health. Environ Int. 131, 105059. https://doi.org/10.1016/j.envint.2019.105059.

Figure captions

Figure 1. (a, b) Relative abundances of *sul1*, *sul2*, *intl1*, *tetX*, *tetW*, *tetM*, and *tetG* genes (normalized to the 16S rRNA copies) in soil, (c) Relative abundances of these genes in organic fertilizer, (d) 16S rRNA copies in organic fertilizer and soil samples. Ambient CO_2 concentration=370 ppm, and elevated CO_2 concentration = 570 ppm. MF.A = mineral fertilizer under the ambient CO_2 concentration; MF.F = mineral fertilizer under the elevated CO_2 concentration; MF+OF.A = organic fertilizer substitution under the ambient CO_2 concentration; MF+OF.F = organic fertilizer substitution under the elevated CO_2 concentration. Different letters among bars indicate statistically significant differences at $p \le 0.05$.

Figure 2. Spearman correlation among the relative abundances of ARGs, *intl1* and environmental variables, and microorganism (phylum) data. ** Correlation significant at the 0.01 level ($p<0.01$); * correlation significant at the 0.05 level ($p< 0.05$).

Figure 3. (a) Composition of the soil bacterial community in the different fertilization treatments at the ambient CO_2 concentration (370 ppm) and elevated CO_2 concentration (570 ppm). (b) Heatmap of the top 50 abundant genera in soil in soil from the different fertilization treatments at the ambient CO_2 concentration (370 ppm) and the elevated CO_2 concentration (570 ppm). (c) Redundancy analysis of bacterial community data constrained by environmental variables. The abundance of bacteria phylum and environmental variables were analyzed after “Hellinger” and log transformation,

respectively. MF.A = mineral fertilizer under the ambient CO_2 concentration; MF.F = mineral fertilizer under the elevated CO_2 concentration; MF+OF.A = organic fertilizer substitution under the ambient CO_2 concentration; MF+OF.F = organic fertilizer substitution under the elevated CO_2 concentration. Different letters among bars indicate statistically significant differences at $p \leq 0.05$.

Figure 4. Network analysis of co-occurring ARGs and the top 50 bacterial genera in soil. Each connection represented a strongly (Spearman's coefficient (ρ) > 0.8) and significantly (*P*-value < 0.01) correlation. The size of the nodes is proportional to the number of connections and the weight of edge between two nodes was calculated according to the Spearman's correlation coefficient. Red and green lines represented positive and negative correlations, respectively. (a) Co-occurrence network colored by different modularity; (b) Co-occurrence network colored by ARGs and phylum types.