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Reducing water use by alternate-furrow irrigation with livestock wastewater reduces antibiotic resistance gene abundance in the rhizosphere but not in the non-rhizosphere

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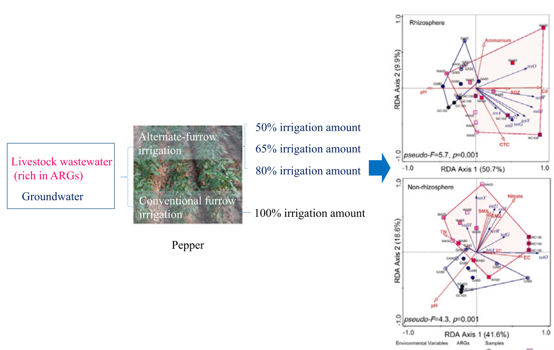
HIGHLIGHTS

- First study of alternate-furrow irrigation effects on ARGs in soil
- Rhizosphere was more sensitive to water source than non-rhizosphere soil.
- Cd had greater influence on ARGs distribution than antibiotics.
- Sulfonomides had a greater influence on ARGs distribution than tetracyclines.
- Reducing irrigation amount with AFI reduced ARGs dispersion only in rhizosphere

GRAPHICAL ABSTRACT



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ABSTRACT

Livestock wastewater is rich in nutrients but may contain antibiotics and antibiotic resistance genes (ARGs). Their discharge to watercourses or soil may result in proliferation of ARGs. Irrigation with wastewater appears to be the most feasible option of disposing of it. One efficient irrigation technology used in arid regions is alternate-furrow irrigation (AFI) by alternately drying part of the plant roots for a prolonged period to physiologically reduce transpiration without compromising yield. However, the extent to which AFI with wastewater influences the concentration of antibiotics and spread of ARGs in soil is poorly understood. The purpose of this paper is to investigate how AFI using swine wastewater alters antibiotic kinetics and ARGs abundance under different irrigation rates, using pepper as the model plant. We examined three AFI treatments using 50%, 65% and 80% of the amount of water employed in sufficient conventional furrow irrigation. Each treatment had a groundwater irrigation control. The results showed that antibiotic concentrations and relative ARGs abundance in the top 20 cm of soil did not increase with the irrigation amount, although they were higher than those in the groundwater-irrigated soils. The relative ARGs abundance in the soil was modulated by irrigation amount and reducing the irrigation amount in AFI reduced ARGs dispersion only in rhizosphere. When the soil moisture was close to field capacity, ARGs were more abundant in rhizosphere than in non-rhizosphere, possibly because the rhizosphere is rich in microbes and increasing antibiotic concentrations due to an increase in irrigation rate favors antibiotic-resistant microbiome in competing for substrates. These, however, were not mirrored in the relative

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ARGs abundance in the roots. These results have important implications as it revealed that reducing the input of antibiotics and ARGs into soil with AFI does not necessarily reduce ARGs proliferation.

1. Introduction

Water used in agricultural production accounts for 50–80% of freshwater consumed globally (Palese et al., 2009). The combined pressures from agricultural production, increasing demand for water from population growth and global climate change have necessitated the use of recycled wastewater for agricultural irrigation to relieve water scarcity (Stroosnijder et al., 2012). Concurrently, livestock production is shifting towards large and more specialized farms, producing greater and more centralized quantities of wastes. For example, in China, >3 × 10^9 tons of manures are produced each year (Xie et al., 2018). In Jiangsu Province of China alone, the overall output of livestock was 4.2 × 10^6 tons in 2013, and the output of pork was 2.3 × 10^6 tons and 2.3 × 10^6 tons in 2013 and 2014, respectively (Wang et al., 2016). In United States, the total production of recoverable beef, dairy and swine manures was 37.7 × 10^6 tons (dry weight) in 2016 (Milbrandt et al., 2018). There are potential benefits of using wastewater from livestock production for irrigation due to its richness of nutrients and it is also an effective way to reduce pollution resulting from arbitrary discharge of such wastes to the environment.

However, livestock wastewaters are reservoirs of both antibiotics and microbial antibiotic resistance genes (ARGs) (Qiao et al., 2018). For example, wastes from swine and chicken farms are shown to be associated with high concentrations of antibiotics and abundant antibiotic resistance genes (Lu et al., 2010; Sayah et al., 2005; Wei et al., 2011). Over the past few decades, development of large-scale, concentrated animal feeding operations has increased the extensive use of veterinary antibiotics for infection treatment, disease prevention and growth promotion. Daily global consumption of antibiotics had increased from 2000 to 2015 (Klein et al., 2018). In China alone, it is estimated that 53,800 tons of antibiotics entered the environment in 2013 even after waste treatment (Zhang et al., 2015). Residual antibiotics could exert selection pressure on environmental microorganisms, contributing to the spread of ARGs and antibiotic resistant microorganisms (Pruden et al., 2006). This pressure-driven spread of antibiotic resistance compromises the efficacy of antibiotics in animal and human medicine and is a global public health threat. The United Nations recently warned that antibiotic resistance is a crisis that cannot be ignored and has called for responsible use of antibiotics at the World Antibiotic Awareness Week held in 2017.

During irrigation with livestock wastewater, ARGs spread through soil, plants and surface runoff (Ghosh and LaPara, 2007; Joy et al., 2013). For example, compared to a wastewater-irrigated soil in summer, the total ARGs in soil fell 1.66 log-fold in winter when no irrigation was applied (Sui et al., 2016). Bastida et al. (2017) reported that both water quality and irrigation amount affected soil microbial communities in a semi-arid citrus orchard, and Mavrodi et al. (2018) found that irrigation influenced the overall diversity of a wheat rhizosphere microbiome and the relative abundance of specific operational taxonomic units (OTUs) in a three-year field irrigation experiment by altering soil water potential and pH. Ma et al. (2018) revealed that irrigation water sources affected accumulation and transport of pharmaceutical and personal care products (PPCPs) in vadose zone soils, but they did not consider ARGs.

Antibiotic resistance in soil spreads preferentially along water flow paths (Lüneberg et al., 2018) and ARGs dissemination depends on the mobility of individual antibiotic in soil. Santiago et al. (2016) found that high soil moisture resulted in high concentrations of PPCPs, including ofloxacin - a quinolone antibiotic - in irrigation with recycled wastewater, suggesting that the mobility of PPCPs in soil increased with soil moisture. Increasing irrigation frequency with reclaimed water was found to increase the level of ARGs in soil (Fahrenfeld et al., 2013). In addition to irrigation timing and amount, irrigation methods may also affect the spread of ARGs due to their potential influence on soil microorganisms, antibiotic distribution and other factors such as soil moisture, pH, organic matter and nutrients. However, to what extent these factors combine to modulate antibiotics and ARGs in soil-plant system is poorly understood.

Many irrigation methods have been developed to increase water use efficiency (WUE) in arid or semi-arid regions. Conventional furrow irrigation (CFI) is arguably the most traditional method despite its low WUE. Alternate-furrow irrigation (AFI) is a more efficient and easily implemented method by alternately irrigating two adjacent furrows to promote abscisic acid (ABA) synthesis by roots in the dry side in attempts to reduce stomatal conductance and thus transpiration (Graterol et al., 1993; Kang et al., 2000a; Kang et al., 2000b). AFI has been replacing CFI in most semiarid regions as the dominant irrigation method. The purpose of this study is to investigate the effects of AFI on the spread of antibiotics and ARGs in a pepper-cultivation field experiment using swine wastewater, with groundwater irrigation as control. We hypothesized that irrigation method, water quality and irrigation rates all influence the abundance of ARGs in soil. Our objective was to identify the associations between environmental factors and ARGs distribution in soil and plant tissues under AFI with livestock wastewater to improve our understanding of the environmental risks of irrigation with livestock wastewater and provide some reference information for safe use of livestock wastes in agricultural production.

2. Materials and methods

2.1. Soil

The experiment was carried out in a vinyl tunnel at the Agriculture Water and Soil Environmental Field Science Research Station, Chinese Academy of Agricultural Science at Xinxiang (Henan Province, 35°15′44″N, 113°55′6″E). The vinyl tunnel acted only to intercept rainwater and had no temperature, light, CO_2 or moisture control. The field soil was a sandy loam (fluvo-aquic soil according to Chinese classification, Fluvic Cambisol according to the World Reference Base). The chemical properties of the top soil (0–20 cm) were: pH 8.5, electrical conductivity (EC) 87.7 mS m^{-1}, organic matter (OM) 9.0 g kg^{-1}, total N 0.7 g kg^{-1}, nitrate nitrogen (NO_3^--N) 136 mg kg^{-1}, ammonium nitrogen (NH_4^+-N) 7.9 mg kg^{-1}, available potassium (K) 252 mg kg^{-1}, available phosphorus (P) 33.2 mg kg^{-1}, total copper (Cu) 25.7 mg kg^{-1}, total zinc (Zn) 72.4 mg kg^{-1}, total lead (Pb) 22.0 mg kg^{-1}, total cadmium (Cd) 0.60 mg kg^{-1}, available Cu 1.5 mg kg^{-1}, available Zn 1.8 mg kg^{-1}, available Pb 1.9 mg kg^{-1}, available Cd 0.20 mg kg^{-1}.

2.2. Water

Groundwater and swine wastewater were used in our study. The groundwater was pumped to the field through plastic pipes with a flow meter from a depth of 4.5 m below the ground level at the experimental site. Swine wastewater was taken from a fermentation tank in a pig farm near the research station. The farm has an annual stock of about 3000 pigs, producing approximately 40,000 tons of wastewater annually. Water properties are presented in Table 1.