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Fertilization and irrigation practice as source of microorganisms and the impact on nematodes as their potential vectors

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ABSTRACT

The agricultural application of organic fertilizer as well as irrigation with partially treated wastewater is proposed to increase crop yield, but also the incidence of food borne pathogens. Studies have concentrated on the impact of such management practice on soil health, and on spread of pathogens during harvest, transport, and marketing, whereas the role of soil organisms in these processes was largely neglected. Therefore, the objective of the present work was to study microorganisms and nematodes as potential agents in suppression or dissemination of human pathogens. Two experimental field sites were established in semi-arid soils in the West Bank in Palestine. Treatments comprised application of fresh water, wastewater, sewage sludge and chicken manure in three successive crop cycles, and the effects on the microbial and nematode communities were assessed. Microbial biomass was generally fostered by all three organic amendments but the increase was dependent on arable site as well as the type of urban waste. The introduced microorganisms were able to establish in the field environment, indicating that the autochthonous microbial community was not effective in suppressing allochthonous enteropathogens. Nematodes, as potential bacterial vectors, displayed a population increase with organic input across crops and sites. Effects were most distinct by amendment with chicken manure, which additionally resulted in a decrease in diversity, with a distinct increase in bacterial-feeding *r*-strategists and strongly enriched food web conditions. In contrast, application of treated wastewater and sewage sludge fostered *K*-strategist, predominantly omnivores. This enhanced biomass at higher trophic levels indicates ample energy flux along the trophic cascade as well as a stable and structured food web, despite possible contaminants introduced via urban residues. The results highlight the potential role that soil biota play in governing soil responses to the application of biosolids and wastes in agricultural management practice.

1. Introduction

Foodborne diseases are among the most serious health problems affecting public health and development worldwide as changes in agronomic, harvesting, and processing practices increased number of infections associated with consuming fresh produce (Beuchat, 2002; Brandl, 2006; Heaton and Jones, 2008). Foodborne pathogens predominantly originate from sewage sludge or animal manure applied to soil as organic amendment that foster the entry of pathogenic enterobacteria such as *Escherichia coli* and *Salmonella enterica* into soil (Santamaria and Toranzos, 2003; Weinberg et al., 2004; Nicholson et al., 2005). Spreading animal manure on arable lands is a widely accepted practice and may constitute a source of pathogens even when used within current guidelines (Crowther et al., 2002). Besides, recycled semi-purified sewage water in crop irrigation enhances the risk in dispersal of human pathogens but is increasingly applied in countries with water shortage (Altizer et al., 2013; Heinze et al., 2014). The coliform level in treated sewage water can reach up to 10^5 CFU per 100 mL (Israeli Ministry of Health in Weinberg et al., 2004) and countries with widespread use of wastewater irrigation should consider this input in their agricultural management practice.

When released into the environment, enteric bacteria can persist outside their mammalian host from months up to years (Santamaría and Toranzos, 2003; Guan and Holley, 2003; Brennan

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et al., 2013). Survival is affected by soil type and temperature as well as the presence of an intact autochthonous microbial community (Moynihan et al., 2013). Declining reactivation ability was reported for *E. coli* O157 with incubation at soil temperatures below 15 °C for 120 days (Williams et al., 2013). Distribution of these pathogens takes place via active movement as microorganisms are able to migrate significant distances through the soil in vertical as well as horizontal direction (Abu-Ashour et al., 1994). Moreover, bacterial dispersal can be enhanced by irrigation practice in arable land as long-term application of treated wastewater alters soil sorption capabilities (Drori et al., 2006), which in turn affects microbial absorption and transport.

Besides physical transport processes, soil fauna can act as vectors for bacterial pathogens. Nematodes are the most frequent and diverse metazoa in soil with densities of up to 3 million per square meter and more than 200 species at some sites (Yeates, 2003). Many nematodes, at one time during their life history, share their environment with bacteria, leading to multiple bacterial–nematode interactions. Apart from the use as food source, nematodes may harbour, protect, and disperse bacteria, including those ingested and passed in viable form in faeces (Anderson et al., 2003). Bacterial feeders engulf bacteria through their unarmed stoma and, as residence time in the intestine is only 2–10 min, can defecate 30–60% of ingested bacteria in viable conditions (Freckman and Caswell, 1985; Ghafouri and McGhee, 2007). Thus transport between bacterial hot spots, i.e. from manure in bulk soil to plant roots, may reshape the rhizosphere microbiome and thereby impact plant health and disease (Mendes et al., 2013). Nematodes are well known vectors for soil-borne plant diseases such as phytotoxin producing bacteria (e.g. *Pseudomonas*,*Pantoea*, *Enterobacter*; Sutherland and Webster, 1993) and their role in dissemination of human pathogens has already been reviewed by Wasilewska and Webster (1975).

The outcome of such nematode–bacteria interactions is strongly affected by farming practice as this impacts the biomass and structure of both the microbial and faunal populations in soil. Much work has been carried out on agricultural application of sewage sludge and animal manure with the focus on altered nutrient supply, potential contaminants introduced or specific aspects such as pathogen survival (e.g.Santamaria and Toranzos, 2003; Toyota and Kuninaga, 2006; Briar et al., 2011). The role of soil autochthonous microbial communities and their faunal grazers has been little studied, although they likely govern the establishment or fate of introduced microorganisms. Only recently, Harris et al. (2011) proposed a conceptual model on the effects of input history on soil microbial community configuration and Moynihan et al. (2013) showed a suppressing effect of the inhabiting microflora on enteropathogens.

The present study investigates agricultural sites in the West Bank in Palestine, where both modern as well as traditional management practices frequently use treated wastewater in surface irrigation and animal manure as amendment. Two experimental field sites at Sir and Ramallah were established, on loamy clay and loamy sand, receiving the treatments fresh water, wastewater, sewage sludge and chicken manure in three successive crop cycles. The same chicken manure was used at both sites, whereas sewage sludge and effluent water were derived from local treatment plants nearby. Microbial biomass and community structure were determined using phospholipid fatty acids (PLFAs) and population density, structure and key food-web indices were assessed for the nematode fauna. The effects of agricultural management are reported under two crops, green sweet pepper and winter lettuce. The following questions were addressed: (i) how does organic and organismic allochthonous input affect the autochthonous soil microbial and nematode assemblages? (ii) do differences in structure of residing organismic communities or crops produce different outcome with the same organic amendment (i.e. manure)? (iii) do comparable urban waste types differing in origin result in similar soil biological community configuration? The overall goal was to assign changes in biological soil conditions as prerequisite for survival and transport of human pathogens.

2. Materials and methods

2.1. Field sites

The experiments were carried out at two field site in the West Bank in Palestine. Both sites were established on long-term arable land, without any recorded previous amendment with urban wastes. One site at 320 m a.s.l. was located close to the village Sir in the north-west, and the second at 814 m a.s.l. near the city Ramallah in the mid West Bank. In Sir the mean day temperature is 29.5 and 13.3 °C and in Ramallah 27.4 and 9.4 °C in July and January, respectively. The mean annual rainfall is 635 mm in Sir and 586 mm in Ramallah.

The field sites were established on a terra rossa chromic luvisol, which typically contains pedogenic clay. The soil type in Sir was brown loamy clay, whereas in Ramallah it was light red loamy sand. Three crops cycles were maintained at the experimental arable fields, first tomato (May–July 2011), then pepper (September–December 2011) and finally lettuce (February–March 2012). Of these three crops, the green sweet pepper (*Capsicum annuum*) and the local short season winter lettuce (*Lactuca sativa*), were investigated in the present work.

2.2. Experimental design and treatments

The same experiment setup was applied at both sites on plots with areas around 250 m^2 each. The experiment had a completely randomized strip plot design with three replications each that had the following treatments: Control plots were irrigated with fresh water (FW) without any sort of additional amendment, whereas wastewater (WW) plots used treated wastewater from nearby domestic sewage treatment plants. Fresh water was also used for irrigation of plots amended with sewage sludge (FS) and chicken manure (FM). Each strip had a length of 10 m and a width of 3 m, i.e. comprised a 1 m area for sampling in the middle and a 1 m buffer zone at each site. The soil was ploughed to a depth of 30 cm to prepare seedling beds and crop seedlings were planted in rows at 50 cm spacing. This strip plot design was established at the sites in both Sir and Ramallah.

At both sites, the fresh sewage sludge used was partially stabilized with a solid content of about 12–16%. The application rate was 6 kg fresh sludge per stripe, accounting for 1.2 kg m^{-2} solids. The sewage sludge was applied to the soil surface and then mixed well with the upper soil layer to a depth of 20 cm. Please note that there is no established Palestinian standard for reusing the sewage sludge in agriculture and this research was performed after receiving a special permission from the ministry of agriculture since application is officially prohibited. For both field sites chicken manure from a layers farm near Ramallah was used. The manure was almost dry with less than 5% moisture and mixed with some secondary waste material such as feathers. At each stripe 6 kg of manure was applied in a width of 0.5 m, which represents a focused application rate in planting rows of 12,000 kg ha^{-1}. The application method for chicken manure was similar to sewage sludge.

All treatments and replicates received surface drip irrigation twice a week via permanent dripper lines installed at both sites. This method is required when using recycled domestic wastewater, as regulations typically do not permit spraying water that has not been fully treated to potable water standards. Fresh water was used