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Changes in microbial properties and community composition in acid soils receiving wastewater from concentrated animal farming operations

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ABSTRACT

The increasingly intensified animal industry in recent decades has resulted in the discharge of a large amount of wastewater with high concentrations of organic matter and nutrients into the ambient environment, which influences soil properties. In this study, we applied a multi-parameter approach to investigate changes in soil microbial properties and community compositions from three acid soil sites that differed in land-use patterns and histories of receiving wastewater. Wastewater had been applied to the sites for 2–20 years. Compared to controls, soil pH, EC and total nutrients were significantly higher in soils receiving wastewater, as well as average increases of 149 mg kg^{-1} for microbial biomass carbon and 0.19 mg CO_2–C kg^{-1} h^{-1} for basal respiration; whereas the metabolic quotient and the ratio of saturated to monounsaturated phospholipid fatty acids decreased by 13% to 31%, and 32% to 61%, respectively. Soil microbial communities of all sites changed with the impact of wastewater application and showed significant increases in bacteria, especially Gram-negative bacteria. The differences in microbial metabolic profiles from all sites were reduced by wastewater application. Soil pH and EC were the two most important factors controlling microbial community composition under wastewater application. These results suggested that wastewater application could reduce stress on acid soil microorganisms by providing more organic carbon and nutrients, and through neutralization of soil acidity.

1. Introduction

The recent intensification of the swine industry has resulted in overproduction of swine manure and wastewater in confined areas (Bernet and Béline, 2009; Deng et al., 2007; Fernandes et al., 2012; Girard et al., 2009), thus increasing the risk of excessive land application of the wastes, which can affect soil quality (Petersen et al., 2007). The impacts of swine manure on soil microorganisms, since they play pivotal roles in soil ecosystem functioning and can reveal changes in soil status (Bending et al., 2000), has received considerable attention (e.g. Ros et al., 2006). Most of such studies have focused on manure types such as solid manure or its compost, which can improve microbial activity (Fließbach et al., 2007) and biomass carbon (Guerrero et al., 2007) and alter community profiles (Ros et al., 2006). The effects of slurry manure have also received much attention and are believed to increase microbial biomass and enzyme activity (Plaza et al., 2004; Rochette et al., 2000), and may stimulate bacterial rather than fungal populations (Bittman et al., 2005; Walsh et al., 2012). However, less is known about the responses of the soil microbial community in soils receiving wastewater. The wastewater of concentrated animal farming operations is richer in small molecular weight organic matters than solid manure due to the hydrolysis and fermentation of complex polymers in the digestion process (Gómez-Brandón et al., 2013). Consequently, the application of wastewater may exert different effects on soil properties compared to other types of animal manure.

Previous study found no pronounced stimulation of the soil microbial community with wastewater application, but only a slight enrichment of the fungal community (Iyyemperumal and Shi, 2007). Wastewater application also inhibited microbial communities as reflected by stress indicators based on phospholipid fatty acid (PLFA) biomarkers (Iyyemperumal and Shi, 2007). However, Iyyemperumal and Shi (2007) focused on the lasting effects of wastewater application on soil microbial characteristics 3 years after wastewater was applied. Therefore, the resilience of

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microbial community (Saison et al., 2006) might mask the actual microbial status in soil in the process of receiving wastewater. With the increasingly intensified swine industry, adjacent land is forced to digest not only more concentrated wastewater than previously, but also to receive wastewater more frequently, which might result in stronger responses of soil microorganisms subject to high inputs of nutrients (Saison et al., 2006). Consequently, the microbial characteristics in soil that continuously receives high rates of wastewater need to be addressed in order to evaluate the influence of the intensive swine industry on soil quality. Furthermore, the effects of wastewater application on soil microorganisms might depend on factors such as soil and cover types (Grayston et al., 1998; Larkin et al., 2006), the measured biological indicators (Knapp et al., 2010) and application history (Fauci and Dick, 1994). Hence, more comprehensive investigations examining multi-factors are greatly needed.

We investigated microbial biomass, activity and community compositions of acid soils receiving wastewater consecutively for 2–20 years from different land-use patterns. Both PLFA and community level physiological profiles (CLPP), the latter of which is also considered to be a sensitive indicator for detecting soil management changes (Gomez et al., 2006), were applied in this study. We hypothesized that the application of wastewater in a frequently high rate could significantly affect microorganisms in acid soils, and bacteria may be more sensitive.

2. Materials and methods

2.1. Site description and soil sampling

Three soil sets representing typical land-use patterns and wastewater receiving histories were collected in Yujiang, Jiangxi, China during the drought season in June. The county is characterized by a subtropical monsoon climate with a mean annual temperature of 17.6 °C, annual rainfall of 1789 mm and a frost-free period of 258 d. Over 400 swine farms with a total annual waste output of approximately 2.24×10^6 t (Zhou et al., 2013) are distributed across the area of 936 km^2. Site 1 is a grassland (28°17′42″N, 116°56′44″E) and had been receiving wastewater frequently for 2 years. Site 2 is a 5-year old vineyard (28°10′57″N, 116°57′26″E) located next to a swine farm, and has had wastewater applied in the drought season annually. Site 3 is a paddy field (28°15′38″N, 116°55′38″E) adjacent to a swine farm, plots with double-crop rice were irrigated with wastewater in the growing seasons for almost 20 years. Soils from site 1–3 were classified respectively as Stagnic Anthrosols, Rhodic Ferralsols and Ferric-Stagnic Anthrosols in the FAO classification system.

For each site, the wastewater-treated field was located along the downstream of the draining passage, and the control field that never receives wastewater was located horizontally adjacent to the wastewater treated field. It should be noticed that the control and the wastewater treated fields had the same land-use pattern before and throughout wastewater application, the parent material and soil type were identical, and the management practices were similar throughout time. Therefore, the potential influences of previous soil management practices were minimized, and we considered the only factor that brought changes was the receiving of wastewater. For each site, soils receiving wastewater (W1–W3 for sites 1–3, respectively) were sampled from the top 20 cm in triplicate, and corresponding controls (C1–C3) were collected in triplicate. Pigs of all farms are fed with mixes of corn, wheat bran and soybean. The chemical properties of wastewater in each site are listed in Table 1. Particle size distribution of sand, clay and silt of soils from the three sites were 22.1%, 57.4% and 20.6%; 11.2%, 33.7% and 55.2%; and 18.2%, 48.5% and 33.4%, respectively. Soils were sieved (2 mm) after removing roots and litter, and then divided into three subsamples: one was air-dried for chemical analysis; one was freeze-dried and kept at −20 °C for PLFA extraction; and one preserved at 4 °C for analyses of soil microbial properties and CLPP.

2.2. Soil chemical properties

Soil pH and EC were measured in 1:2.5 and 1:5 soil-to-water solution (w/v), respectively. Total organic carbon (TOC), total nitrogen (TN), total phosphorus (TP) and total potassium (TK) were measured using $K_2Cr_2O_7$–H_2SO_4 oxidation, Kjeldahl digestion, H_2SO_4–$HClO_4$ digestion (colorimetric detection) and HF–$HClO_4$ digestion (flame photometry detection), respectively, according to methods proposed by Soil Science Society of China (Lu, 1999). The ratio of carbon-to-nitrogen (C/N) was then calculated.

2.3. Microbial biomass and basal respiration

Microbial biomass carbon (MBC) was extracted using the chloroform-fumigation extraction method (Vance et al., 1987) and measured by Jena 3100c TOC analyzer (Analytik Jena AG, Jena, Germany). Basal respiration was measured using 50 g of soil adjusted to 60% water holding capacity in a 500-ml jar after preincubation for 7 d at 25 °C; the evolved CO_2 was trapped with NaOH solution over a period of 24 h and back-titrated with standard HCl (Cheng et al., 2013). Metabolic quotient (qCO_2) was calculated as the amount of CO_2–C produced per unit of MBC.

2.4. CLPP

Soil samples of 5 g were added to 50 ml of sterile 0.85% NaCl solution and shaken for 30 min, then let stand for 10 min. A tenfold dilution of the supernatant was made and 150 μl of each sample was inoculated into the wells of Biolog EcoPlate™ (Biolog Inc., Hayward, CA, USA). The plates were incubated at 25 °C and read every 24 h for 7 d using a plate reader at 590 nm. Average well color development (AWCD), substrate richness (S), evenness (E) and the Shannon–Weaver index (H') of the readings at 72 h were calculated after subtracting the absorbance of the control well according to Garland (1996) and Kong et al. (2006).

2.5. PLFA

PLFA was extracted as described by Buyer et al. (2010). Briefly, 4 g of freeze-dried soil was used for lipid extraction by phosphate buffer (pH 7.4), chloroform and methanol in 0.8:1:2 volume ratio. The lipid classes were separated by solid phase extraction cartridge to collect phospholipids. Transesterification was performed by alkaline method and 19:0 methyl ester was added as internal standard. The derived methyl esters were subjected to Agilent 7890 chromatography (Agilent Technologies, Santa Clara, CA, USA) and identified by MIDI Sherlock system (MIDI, Inc., Newark, DE, USA).

The fatty acid 18:2 ω6c was used as the biomarker for fungi (Frostegård and Bååth, 1996). Actinomycetes was identified by fatty acids with a methyl branch on the 10th C (16:0 10-methyl, 17:0 10-methyl and 18:0 10-methyl) (Zelles, 1999). The branched, saturated fatty acids (13:0 iso, 14:0 iso, 15:0 iso, 15:0 anteiso, 16:0 iso, 16:0 anteiso, 17:0 iso, 17:0 anteiso, 18:0 iso, 19:0 iso and 20:0 iso) were chosen to represent gram-positive bacteria and the monounsaturated fatty acid as well as OH-substituted fatty acids (15:1 ω6c, 16:1 ω6c, 16:1 ω7c, 16:1 ω9c, 17:1 ω8c, 18:1 ω5c, 18:1 ω7c, 20:1 ω9c, 10:0 3OH, 16:0 2OH) to represent gram-negative bacteria (Zelles, 1999). The ratio of fungal to bacterial biomass (F/B) was calculated as the ratio of 18:2 ω6c to bacterial fatty acids. The ratio of total saturated to total monounsaturated fatty acids (12:0 + 13:0 + 14:0 + 16:0 + 17:0 + 18:0 + 20:0/