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Soil chemical and microbial responses to biogas slurry amendment and its effect on Fusarium wilt suppression

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ABSTRACT

The rapid development of biogas production will result in increased use of biogas slurry (BS) as organic fertilizer. However, side effects such as suppression of soilborne diseases are not yet well investigated and understood. Therefore, the objectives of the study were to evaluate the effects of biogas slurry application on suppression of Fusarium wilt disease of watermelon and its relationship with soil chemical and microbiological properties. Pot and field experiments were conducted to compare effects of biogas slurry application on Fusarium wilt disease suppression of watermelon in soil with a moisture content of 60% water holding capacity (WHC) or flooded continuously. Fusarium wilt was significantly suppressed in soil from biogas slurry amended plots. Biogas slurry flooding enhanced the degree of suppression in the pot experiment. Moreover, the biogas slurry treatment also significantly suppressed Fusarium wilt in the field with a disease index of 33.2% compared with 69.6% in water treatment. Biogas slurry strongly reduced the pathogen population in rhizosphere soil. The populations were decreased by 43.1% and 95.9% in the biogas slurry moist and flooding treatments, respectively. Biolog data indicated that average well color development (AWCD) and Shannon-weaver index were increased significantly in biogas flooding treatment. Principal component analysis showed that Fusarium wilt was negatively correlated with NH_4^+-N, available K (AK), water-soluble carbon (DOC), water soluble nitrogen (DON) and phenolic acid (PA) contents in soil and positively correlated to soil pH and soil redox potential (Eh). Microbial communities, in general, did not significantly correlate with disease suppression.

1. Introduction

Biogas is an emerging renewable energy source which is derived from the conversion of various organic wastes into biofuels through anaerobic fermentation. Over the last decade, the number of biogas plants has significantly increased in China and other parts of the world (Ward et al., 2008). The rapid development of biogas production will result in increased production of biogas residues. In fact, more than 1 billion tons of biogas slurry (BS), the main by-product of biogas, was produced from 3800 large-scale biogas plants in China annually (Jin and Chang, 2011). These residues are rich in plant nutrients, which can be functioned as fertilizers. It has been shown that BS application enhanced crop yield (Ernst et al., 2008), improved N uptake and soil structure and reduced the cost of fertilizers for farmers (Bachmann et al., 2014; Chen et al., 2012).

Consistent with other organic amendments, it has been reported that intensive application of BS could suppress a number of soilborne diseases (Cao et al., 2013; Jothi et al., 2003). Soil suppressiveness induced by organic amendments has been associated with diverse microbial communities that have a greater probability to contain antagonistic, competitive or parasitic species that can contribute to direct inhibition of a pathogen or the activation of induced systemic resistance (Hadar and Papadopoulou, 2012; Hoitink et al., 1997). There are two types of disease suppression: specific and general suppression, the first type is attributed to a particular antagonist or parasite that has a specific interaction with a pathogen often in a monocropped system, while the second type is often associated with more than one organism and may be directed to several pathogens (Senechkin et al., 2014). Disease suppression caused by organic amendments is assumed to be the second general suppression

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type, which is directed to several pathogens or parasites (Hiddink et al., 2005). Both disease suppression types have been associated with biotic and abiotic soil components (Hoper and Alabouvette, 1996; Postma et al., 2008). Fusarium wilt suppression in organically managed soil was related to numerous soil chemical and soil microbial characteristics (Senechkin et al., 2014; van Bruggen et al., 2015). In addition, the suppression of take-all of wheat has been related to various soil measurements, including microbial diversity. Grünwald et al. (2000) showed that disease suppression was temporarily reduced after addition of organic materials to soil.

Fusarium oxysporum f. sp. *niveum* (FON) is the causal agent of Fusarium wilt of watermelon and is considered to be the most important soilborne facultative parasite that causes economically important losses in watermelon production (Ling et al., 2012). Disease suppression of *F. oxysporum* is quite common, and is often associated with non-pathogenic *Fusarium* sp. (Hoper and Alabouvette, 1996), fluorescent pseudomonads (Larkin et al., 1993) and actinomycete populations (Castano et al., 2011), but is also affected by soil pH, organic matter content (Senechkin et al., 2014; van Bruggen et al., 2015) and water content (Stover, 1953) in particular. Excess of water in soils results in gradual oxygen depletion. As a consequence, physico-chemical properties are severely affected, which in turn has important impact on the activity of soil microorganisms (Unger et al., 2009). Microbial communities shift to anaerobes and potentially toxic chemicals such as phenolic and aromatic acids or hydrogen sulfides may accumulate to decrease the viability of pathogens (Messiha et al., 2007; Seo and DeLaune, 2010). Thus, soil flooding is an effective measure to reduce the incidence of soilborne diseases (Hord and Ristaino, 1992; Niem et al., 2013). Biogas slurry differs from water in that more organic materials and mineral nutrients are added to soil, which may have complicated effects on growth of soilborne pathogens as well as soil chemical and microbiological properties. As a result, better understanding of the effect of soil flooding by BS on disease suppression is necessary.

It has been reported that intensive application of BS contributes to soil suppressiveness by enhancing soil microbial biomass and diversity (Abubaker et al., 2013). In contrast, Makadi et al. (2007) showed that BS application has not caused drastic changes in soil microbiological properties. Toxicity caused by some trace contaminants during the anaerobic fermentation, such as phenolic compounds, volatile fatty acids and polycyclic aromatic hydrocarbons (Cao et al., 2013), may have negative effects on some microbial enzyme activities in soils (Elfstrand et al., 2007), but also suppress a large number of plant pathogens, including *Fusarium* spp. (Blok et al., 2008). The limited number of studies does not allow drawing conclusions concerning the underlying mechanism towards suppression of soilborne pathogens after applying BS to soils. For example, it is unknown which biotic or abiotic factors are directly associated with root disease suppression by BS application. It is also not known whether application of BS in flooded condition leads to greater suppression than in soil with lower moisture content. Therefore, a more comprehensive study on the chemical and microbiological characteristics of soil is necessary to elucidate soil suppressiveness induced by BS. The objectives of the study were: (1) to evaluate the effect of BS application on suppression of watermelon Fusarium wilt disease in the greenhouse and in the field; (2) to determine the impacts of BS application on soil chemical properties and ecological characteristics of soil microorganisms through soil dilution and plate counts and community level physiological profiles (Biolog); and (3) to explore the relationship between the incidence of Fusarium wilt of watermelon and an array of microbiological and chemical characteristics after BS application.

2. Materials and methods

2.1. Biogas slurry characteristics

The biogas slurry (mesophilic 25–30 °C, 12-d retention time), produced from pig farm wastes, was collected from the large-scale biogas plant in Luhe Animal Science Base, Jiangsu Academy of Agricultural Sciences, Nanjing, Jiangsu Province, China. About 100 L of the biogas slurry was collected and used immediately in the pot experiment. A representative sample (approximately 500 mL) of the biogas slurry was portioned into 500-mL bottles and stored at −20 °C until chemical analysis. The chemical characteristics of the BS were: dry matter 6.2 g kg^{-1}, chemical oxygen demand 587 mg L^{-1}, total N 661 mg L^{-1}, total P 533 mg L^{-1}, total K 147 mg L^{-1}, NH_4^+-N 378 mg L^{-1}, NO_3^--N 85 mg L^{-1}, and the contents of Zn, Cu, As were 0.5, 0.35 and 0.02 mg L^{-1}, respectively.

2.2. Soil sampling and characteristics

The soil in the experiments was collected from Luhe Animal Test site, located 60 km north-west of Nanjing (32°2′N, 118°8′E), which is a conventional farm with a crop rotation consisting of rice and rapeseed. The soil was a clay Tye-Fel-Stagnic Anthrosols soil (IUSS Working Group WRB, 2007), with the following characteristics at the beginning of the experiment: clay 32.36%, silt 30.58%, sand 37.06%, pH 7.4, organic matter (OM) 14.81 g kg^{-1}, total nitrogen (TN) 1.68 g kg^{-1}, available phosphorous (AP) 77 mg kg^{-1}, available potassium (AK) 131 mg kg^{-1}. About 200 kg wet soil were taken from the 0–20 cm layer, immediately transported to the laboratory and air-dried. During drying, the soil was mixed manually every day to ensure homogeneous moisture distribution. Soil was then sieved through a 4-mm screen and inoculated with conidia suspension of FON and thoroughly mixed to obtain a homogeneous blend before being used in the pot experiment. FON spore concentration in the soil was 5×10^5 cfu g^{-1} dry soil.

2.3. Preparation of pathogen inocula

F. oxysporum f. sp. *niveum* (FON) was isolated from infected watermelon in the field, and was grown in Potato Dextrose Agar (PDA) plates at 28 °C for 10 days till spores were densely produced. A colony showing typical FON morphologies was isolated and confirmed as the responsible pathogen by carrying out Koch's postulates in pot experiments (Fang, 1998). The plates were flooded with sterile distilled water, and the spores were scraped and collected into a tube, and filtered through four layers of sterile gauze to remove solids. The spore number was counted by a hemacytometer. The spore solution was thoroughly mixed and 10 μL of the spore solution was loaded in both chambers of a hemacytometer under a coverslip and examined with a microscope (Nikon Eclipse E200, Japan) at ×400. Spore numbers in 5 squares (each square contained 16 smaller squares) were counted in each chamber, and counts on both sides were averaged (N). The number of spores per mL (4×10^7 cfu/mL) was calculated by the following equation: spore concentration = $N/80 \times 400 \times 10^4$. The inoculum concentration of FON in the pot experiment was 5×10^4 cfu/g.

2.4. Pot experiment design

Watermelon seeds, cultivar Zaojia 84–24, were surface sterilized in 2% NaClO for 3 min, rinsed three times in sterile water, and then germinated in a standard 9-cm Petri dish filled with 7 mL sterile water and then incubated in dark at 30 °C until the seeds germinated. The germinated seeds were then moved into 72-hole seedling trays each containing 500 g peat. After grown for 30 days,