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Q3 Effect of *Trichoderma viride* biofertilizer on ammonia volatilization from an alkaline soil in Northern China

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A B S T R A C T

Ammonia (NH_3) volatilization is one of the primary pathways of nitrogen (N) loss from soils after chemical fertilizer is applied, especially from the alkaline soils in Northern China, which results in lower efficiency for chemical fertilizers. Therefore, we conducted an incubation experiment using an alkaline soil from Tianjin (pH 8.37–8.43) to evaluate the suppression effect of *Trichoderma viride* (*T. viride*) biofertilizer on NH_3 volatilization, and compared the differences in microbial community structure among all samples. The results showed that viable *T. viride* biofertilizer (T) decreased NH_3 volatilization by 42.21% compared with Q7 conventional fertilizer ((CK), urea), while nonviable *T. viride* biofertilizer (TS) decreased NH_3 Q8 volatilization by 32.42%. NH_3 volatilization was significantly higher in CK and sweet potato starch wastewater (SPSW) treatments during the peak period. *T. viride* biofertilizer also improved the transfer of ammonium from soil to sweet sorghum. Plant dry weights increased 91.23% and 61.08% for T and TS, respectively, compared to CK. Moreover, *T. viride* biofertilizer enhanced nitrification by increasing the abundance of ammonium-oxidizing archaea (AOA) and ammonium-oxidizing bacteria (AOB). The results of high-throughput sequencing indicated that the microbial community structure and composition were significantly changed by the application of *T. viride* biofertilizer. This study demonstrated the immense potential of *T. viride* biofertilizer in reducing NH_3 volatilization from alkaline soil and simultaneously improving the utilization of fertilizer N by sweet sorghum.

Introduction

NH_3 volatilization is one of the main pathways for gaseous nitrogen release from the plant–soil system (Xia et al., 2016). In 2010, the total amount of NH_3 volatilization from agricultural fertilizer was 10.7 Tg NH_3/year, 41.9% of which came from synthetic fertilizer, and this NH_3 emission took place primarily in the northern farmland of China (Xu et al., 2015). China, as one of the world's largest agricultural countries, is also the world's largest manufacturer and consumer of fertilizer. In 2010, China produced 37.1 Tg N (nitrogen), of which agricultural consumption amounted to 28.1 Tg N (Zhang et al., 2013). However, the assimilation of fertilizer N by crops was inefficient (Zhu et al., 2005), due to substantial N losses through various pathways, including leaching, surface runoff, gaseous emissions of nitrous oxide (N_2O), and ammonia (NH_3) volatilization (Alva et al., 2006;

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Duretz et al., 2011). Urea is one of the common fertilizers used as a nitrogen source because of its high nitrogen content, low cost and convenient usage. Urea undergoes a series of reactions that will finally lead to N losses through NH_3 volatilization. Such processes may take hours to weeks depending on soil characteristics, environmental conditions and fertilizer practices. (Bolan et al., 2004; Ferguson et al., 1984).

Besides lowered fertilizer utilization efficiency, NH_3 volatilization also has many negative impacts on the natural environment (Bolan et al., 2004), such as air pollution in the atmosphere when transformed to N_2O (Sutton et al., 2008), eutrophication in various aquatic environments (Hellsten et al., 2008), and acidification in soils (Van der Eerden et al., 1998). Excessive NH_3 deposition into various water bodies and soils will not only affect the ecological balance in water and soil, but also influence the aboriginal biodiversity (Aneja et al., 2003). Therefore, ammonia volatilization from fertilization in agricultural systems is one of the universal concerns in many studies.

There are many factors affecting ammonia volatilization, such as soil pH, soil NH_4^+ concentration and fertilization practices (Ferguson et al., 1984; Soares et al., 2012). Nitrification is a very important part of the N cycle, which is related to the supply of soil elements, greenhouse gas emissions and nitrate leaching (Purkhold et al., 2000). The first step of nitrification is driven by ammonium-oxidizing archaea (AOA) and ammonium-oxidizing bacteria (AOB), which are mainly responsible for the oxidization of NH_4^+ to NO_2^-. Therefore, the abundance of AOA and AOB is closely related to the soil concentration of NH_4^+. Studies have shown that AOB play a predominant role in alkaline soil nitrification, while AOA carry out more important functions in acid soil (Hu et al., 2014).

Several methods have been reported to reduce NH_3 volatilization by regulating NH_4^+ concentration in the soil, such as using a urease inhibitor to delay urea hydrolysis (Carmona et al., 1990; Watson et al., 1994), using biochar to increase nitrification and N immobilization (Mandal et al., 2016), and regulating urea release and absorption (coating urea with sulfur, resins or polymers) to increase fertilizer utilization efficiency. However, those approaches are fundamentally based on physical and chemical approaches, and all are costlier than using traditional urea. Thus, a new environment-friendly and biology-based alternative is needed.

Plant growth promoting fungi (PGPF) and plant growth promoting rhizobacteria (PGPR) have played an important role in plant growth and health. In recent years, these beneficial microorganisms have been used in agricultural systems as biofertilizers and biocontrol agents. The direct influences of biofertilizer contribute to nitrogen absorption, phosphorus solubilization, and plant hormone production, and thus promote plant growth (Berg, 2009; Kumar et al., 2007). Diverse mechanisms are involved in biological disease control. Using biofertilizer as a biocontrol agent not only avoids environmental pollution, but also cuts down the chemical pesticide input (Saldajeno and Hyakumachi, 2011). In addition, the microbial community can be altered due to the application of biofertilizer. For example, the abundance of beneficial microbial consortia, such as *Firmicutes* and *Bacillus*, significantly increased in response to the application of biofertilizer (Shen et al., 2015b; Zhao et al., 2005), and the soil properties were considerably improved thereafter.

Trichoderma viride (*T. viride*) has been broadly reported as an effective biofertilizer, soil amendment and biocontrol agent for a long time, and has been widely studied and commercially marketed all around the world (Bai et al., 2008; Vessey, 2003; Vinale et al., 2008). In addition, it was reported that *T. viride* biofertilizer could significantly reduce N_2O emissions by 33.3%–71.8% at the usage of 225 kg N/(ha·year) (Xu et al., 2014b). However, few studies on the reduction of NH_3 volatilization through microbiological methods are available. Therefore, we used *T. viride* biofertilizer to explore its effects on ammonia volatilization from an agricultural system. Production of *T. viride* with conventional potato dextrose agar (PDA) culture medium is very costly in industrial-scale mass production. Sweet potato starch wastewater has been reported to be an alternative microbial culture medium, which can substantially support microorganism growth (Xu et al., 2014a).

In this study, *T. viride* was cultivated using sweet potato starch wastewater for biofertilizer production. The effects of the combination of biofertilizer and traditional fertilizer on reducing NH_3 volatilization as well as the potential mechanisms were collectively investigated in this study. More importantly, the effects of *T. viride* biofertilizer on the microbial community in the experimental soils were also studied.

1. Materials and methods

1.1. Site description and soil samples

The experiment was conducted in a greenhouse from June to July 2016. The soil for this study was collected from the Dagang District of Tianjin. The surface soils (0–20 cm) from uncultivated land were collected, air-dried and thoroughly homogenized prior to subsequent experiments.

All air-dried soil samples were passed through a 2-mm sieve for measurement of pH, available potassium and available phosphorus, total N and C, NH_4^+ and NO_3^-. Soil pH (1:2.5 soil-1 mol/L KCl solution) was measured using a pH meter. NH_4^+-N and NO_3^--N concentrations were measured by extracting the soil with 1 mol/L KCl at 200 r/min for 30 min (Zaman et al., 2009). After centrifugation at 5000 r/min for 5 min, the supernatant filtered through 0.45 μm filter was measured for NH_4^+-N and NO_3^--N using a continuous-flow analyzer (BRAN+LUEBBE, AA3, Germany). Total C and N were determined by a Vario Max elemental analyzer (Vario EL cube, Elementar, Germany). Available potassium and available phosphorus were analyzed as described previously (Soares et al., 2012). The physical and chemical properties of soil are presented in Table 1.

1.2. Experimental design

Every pot, which contained 4 kg soil, was pre-cultivated with two sorghum plants for 24 days starting May 28, 2016. Each pot received the same amount of nitrogen (0.25 g/kg soil), potassium (0.15 g/kg soil) and phosphorus (0.1 g/kg soil) fertilizer on 21 June 2016. The nitrogen fertilizers applied to these treatments were urea or urea and biofertilizer (0.89:0.11). Sweet potato starch wastewater, which contained approximately 20 g/L COD and 1.12 g/L total nitrogen, was sterilized at 121°C for 25 min and

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