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Temporal variability of soil microbial communities after application of dicyandiamide-treated swine slurry and mineral fertilizers

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ABSTRACT

In modern agriculture, mineral and organic fertilization account for most of the global anthropogenic N_2O emissions. A strategy to prevent or to reduce emissions of greenhouse gases such as N_2O is the use of nitrification inhibitors, which temporarily inhibit the microbial conversion of soil ammonium to nitrate. However, information about the magnitude and duration of disturbance caused by organic fertilization with nitrification inhibitor on the microbial community is lacking. Here we examined N dynamics and how potentially active soil microbial communities changed through time by the addition of dicyandiamide-treated swine slurry and mineral fertilizers. A field experiment (corn/cereal succession under no-tillage system) was carried out using the following treatments: (I) unfertilized control, (II) surface application of mineral nutrients, (III) surface application of swine slurry, and (IV) surface application of swine slurry with dicyandiamide. Soil samples were collected at 0, 3, 6, 11, 25 and 50 days after start of experiment. Total RNA was extracted, synthesized to cDNA and used as template to amplify and sequence the 16S rRNA. Nitrous oxide emissions were also quantified. The organic fertilizers were the main drivers on changes in microbial community structure. Slurry application decreased microbial diversity and changed the microbial structure temporarily but the metabolically active microbial community was resilient, recovering to the original status 50 days post-fertilization. DCD had no effect on metabolically active microbial community and was pathway-specific, having impact only on nitrifiers during a short-term period, which in turn reduced the N_2O emissions.

1. Introduction

Nutrient enrichment through fertilizers input is a source of small-scale anthropogenic disturbance for soil habitats. Interest in the use of organic fertilizers, such as animal manure, has increased with aims to reduce the use of mineral fertilizers for crop production and to develop sustainable global agriculture (De Vries et al., 2015). Animal manure is cost-effective and provides nutrients to plant growth, primarily nitrogen (Gale et al., 2006).

In many regions of Brazil, like in other countries of the globe, the field application of manure is a common practice. According to Aita et al. (2015), doses greater than 100 m^3 ha^{-1} $year^{-1}$ of animal manure (e.g. swine slurry) are commonly used in Southern Brazil. Long and short-term fertilization practices might result in positive, neutral or negative effects in soil microbial community structure (Biederbeck et al., 1996; Hu et al., 2011; Lazcano et al., 2013; Williams et al., 2013; Pan et al., 2014). Hu et al. (2011) documented that fertilizers affected microbial functional diversity, metabolic activity and metabolic quotient. Long-term swine slurry application increased soil microbial quality indicators of biomass and enzyme activity (Balota et al., 2014) while short-term fertilizer regimes stimulated microbial growth, altered the structure of the soil microbial community and increased enzyme activity (Lazcano et al., 2013). However, there is so far very little evidence of a connection between alterations in activity and effects on microbial community composition over time series post disturbances.

Although organic fertilizers are effective in increasing nutrient availability, improving grain yield and are more environmental

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friendly than mineral fertilizers, the disturbance caused by animal manure application might also impose some risks. An important aspect of organic fertilization is the introduction of high loads of exogenous microbes in soil (Durso et al., 2011) and chemical components that might represent a perturbation for soil microbial communities through stimulating specific populations and suppressing others. On the other hand, the soil microbial communities are highly resilient and tend to exclude exotic populations attempting to occupy niches already occupied by the indigenous population (Levine and D'Antonio, 1999).

Furthermore, organic fertilizer additions result in environmental pollution (e.g. nitrate contamination of ground water, eutrophication of water bodies and increase of ammonia and greenhouse gas emissions) when manure is applied beyond the soil retention capacity or above the plant nutrient requirements (Luo et al., 2010). Agricultural sources, including mineral and organic fertilizers, are estimated to account for 60% of the global anthropogenic N_2O emissions (Aguilera et al., 2013) and animal manure is considered the major source of N_2O global emissions (Davidson, 2009). In the 21st century this greenhouse gas (GHG) has become the greatest threat to the ozone layer (Ravishankara et al., 2009) mainly because it has higher global warming potential compared to CO_2 and CH_4. Strategies to avoid adverse impacts on the environment are under development aiming to prevent and/or reduce emissions of N_2O.

The use of nitrification inhibitors such as dicyandiamide (DCD, $C_2H_4N_4$) is a potential alternative to reduce N_2O emissions caused by agricultural use of N sources such as urine and animal manure. DCD temporarily inhibits the microbial conversion of soil ammonium to nitrate by binding to the active site of the enzyme ammonia monooxygenase (AMO), that is responsible for the first step of the nitrification pathway (Amberger, 1989). Dicyandiamide is a white crystalline powder, highly soluble in water, low volatility, biodegradable and totally decomposed in soil to ammonium (NH_4^+) and CO_2 (Amberger, 1989). DCD biodegradation rate is a function of temperature. The half-life of DCD in soils at 25 °C is around 6–20 days depending of DCD rates and experiment conditions (e.g. laboratory or field conditions) (Sing et al., 2008; Kelliher et al., 2008, 2014). On the other hand, high rates of DCD (20 mg kg^{-1} or higher) might cause phytotoxic effects including leaf tip and margin chlorosis and necrosis, and reduction in the biomass yield (Prasad and Power, 1995; Macdam et al., 2003). DCD applied to the soil mixed with manure and urine, presents variable effectiveness on mitigating of N_2O emissions ranging from 11 to 97% depending on the system (Kumar et al., 2000; Luo et al., 2013; Aita et al., 2014; Huang et al., 2014). The effects of DCD on nitrifier and denitrifier communities are relatively well known (O'Callaghan et al., 2010; Wakelin et al., 2013; Di et al., 2014; Morales et al., 2015), but there are no studies on the general effect of DCD-treated swine slurry on the active soil microbial community.

Although organic fertilization is a practice disseminated around the world, information about the N dynamics, magnitude and duration of disturbance caused by organic fertilization with nitrification inhibitor as well as the resistance and resilience of microbial communities in a time series still need to be better understood. Microbial communities can change abruptly in response to perturbations and recover quickly to its original state. A time series study allows us to analyze the stability and dynamics of microbial communities while single sampling points might only capture a specific status of soil microbes that might not represent the true microbial response to perturbations. The aim of this study was to examine N dynamics and how the potentially active soil microbial communities changed through time by the addition of DCD-treated swine slurry and mineral fertilizers using 'post-light'-based sequencing technology. Special attention was devoted to nitrous oxide emissions because of the link to the nitrogen cycle and the importance in global warming potential.

2. Material and methods

2.1. Experimental field description and soil sampling

The experiment was carried out in the Federal University of Santa Maria (UFSM), Rio Grande do Sul State, Brazil (29°43′ S, 53°43' W, altitude 105 m). The soil in the experimental area was characterized as Typic Paleudult (USDA classification). Soil samples were collected from the 0.00–0.10 m soil layer and contained: 19.2% of clay and 44.3% of sand (pipette method, Embrapa, 1997); pH 5.9 pH (H_2O) 5.9, determined in a soil:water suspension (1:1, m/v). The exchangeable concentrations of Ca, Mg and Al were 9.8, 3.1 and 0.0 cmolc kg^{-1}, respectively (extractor KCl 1 mol L^{-1}) (Tedesco et al., 1995). The determination of the concentrations of Ca and Mg in the extracts was performed in an atomic absorption spectrophotometer (AAS) and Al by titration (Tedesco et al., 1995). The concentration of available P was 6.7 mg kg^{-1} and exchangeable K was 39.0 mg kg^{-1} (extractor Mehlich 1 solution – HCl 0.05 mol L^{-1} + H2SO4 0.0125 mol L^{-1}) (Tedesco et al., 1995). The P was measured using a spectrophotometer and the K was measured using a flame photometer. Cation exchange capacity (CEC) was 12.9 cmolc kg^{-1}. The CEC was calculated following Summer and Miller (1996). Total C (20.5 g kg^{-1}) and N (1.6 g kg^{-1}) contents were analyzed by dry combustion with a graphite furnace (FlashEA 1112, Thermo Finnigan, Milan, Italy). For more details about the soil and experimental area see Aita et al. (2014).

The experimental field was divided into plots of 5.25 m × 6.00 m and each treatment was replicated three times in a complete randomized block design. Four treatments were applied: (I) control (unfertilized), (II) surface application of urea (NPK), (III) surface application of swine slurry (slurry) at 50 $\text{m}^3 \text{ ha}^{-1}$, and (IV) surface application of swine slurry with dicyandiamide (slurry with DCD) at 50 $\text{m}^3 \text{ ha}^{-1}$. Rates of swine slurry were determined to provide a target total N supply of 130–140 kg total N ha^{-1}, equivalent to the application of urea in the treatment II.

The field was cultivated during 2 years before soil sampling (2011 and 2012) with a corn/cereal succession (corn/oat/corn/wheat) under a no-tillage system. The treatments were applied twice a year during two years (before the summer - corn; and the winter crops - oat or wheat) on soil surface with residues of the preceding crop (a few days before sowing the next crop). The average daily temperature was obtained from the University meteorological station, located approximately 500 m from the experiment. The amount of rainfall and irrigation water at each site was measured using rain gauges. The average annual rainfall was 1700 mm, and the mean annual temperature was 19.9 °C (Supplementary Figure 1).

The swine slurry was obtained from fattening pigs and stocked in an anaerobic tank at the experimental field. The main characteristics of the swine slurry at each growing no-tillage succession season are presented in the Supplementary Table 1. The swine slurry applied in the experiment presented pH 7.6, 23 kg m^{-3} of dry matter, 8.2 kg m^{-3} of total carbon, 3.29 kg m^{-3} of total N and 2.6 kg m^{-3} of total ammoniacal N (NH_3 + NH_4^+). In treatment IV, DCD was applied at a rate of 10 kg ha^{-1}, and mixed with the slurry swine immediately prior to soil application. The surface application was performed using watering cans. During application, samples of slurry and slurry with DCD were collected for microbial molecular analysis. Urea fertilizer was applied on the soil surface of treatment II at a target rate of 130 kg N ha^{-1}, one third pre-plant and two thirds at the maize sixth-leaf stage (34 days after sown). The treatment with urea was also fertilized with P and K in a rate of