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Legacy effects of soil moisture on microbial community structure and N_2O emissions

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ABSTRACT

Soil moisture is a strong determinant of microbial activity exerting dominant control over gaseous and liquid diffusion rates and affecting O_2 and substrate availability. Often, measures of microbial community structure and soil moisture status fail to inform our understanding of soil processes, particularly those that are governed by complex feedbacks between substrate availability and environmental conditions (e.g. nitrogen transformations). Nitrous oxide (N_2O) emissions, although conceptually regulated by soil moisture, are notoriously difficult to predict based on soil water content and nutrient status. Here, we studied agricultural soils under wetting, drying, and static moisture conditions to assess the impact of current and previous moisture on bacterial 16S rRNA composition; transcription of *amoA, hao, norB*, and *nosZ*; and net N_2O production. Microbial community composition was dependent on previous moisture. As soils dried, bacterial rRNA contained fewer and more evenly distributed genera. We hypothesize that this was linked to the evenness of resource distribution as controlled by differences in substrate diffusion in wetting vs. drying conditions. N_2O flux depended on previous, as well as current, soil moisture status and this legacy effect was greatest at 80% water filled pore space. Overall, we found that previous moisture affected microbial activity, transcription, composition and ultimately, N_2O emissions. Our study demonstrates that, for soil microorganisms and processes, it is not only what soil moisture *is*, but also what it *was* that is important.

1. Introduction

Soil moisture content plays an overarching role on microbial activity because of its dominant control on gaseous and liquid diffusion rates of microbial resources within the soil profile. Increasing soil moisture reduces gaseous diffusion rates, which directly affect microbial physiological status and activities by limiting the supply of the dominant electron acceptors such as oxygen and also gases such as methane (Blagodatsky and Smith, 2012). In contrast, increasing soil moisture increases liquid diffusion rates, providing microorganisms with key substrates such as NH_3, NO_3^- and soluble organic carbon (Blagodatsky and Smith, 2012). Wetting and drying of soil alters the stability of soil aggregates, and can induce cell lysis, both processes shifting the quantity of decomposable organic matter in soil (Morillas et al., 2013). Thus, soil moisture content is a key determinant of C and N availability and plays a pivotal role in structuring microbial communities and activities in soil (Gleeson et al., 2010; Banerjee and Siciliano, 2012; Barnard et al., 2013).

Nitrous oxide (N_2O) is a potent greenhouse gas with 265 times more global warming potential than carbon dioxide (IPCC, 2013). Soils are the most significant source of N_2O, contributing up to 90% of the world's total N_2O emissions; 60% (3.5 Tg N yr^{-1}) are from agricultural soils (Kroeze et al., 1999; Goldberg and Gebauer, 2009). Moreover, the total annual N_2O emission in Canada is approximately 108 Gg N and 55% of that is ascribed to

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agricultural soils (Helgason et al., 2005). While, long terms shifts in soil moisture will alter the sources and sinks of greenhouse gases such as N_2O (Borken and Matzner, 2009; Goldberg and Gebauer, 2009), major N_2O emission events are typically linked to rapid shifts in soil moisture regimes (Pennock et al., 2010). The reason for this later observation, i.e. the high temporality of N_2O emissions, has not been clarified. Despite extensive measurements of soil N_2O emissions and the properties thought to control microbial activity, accurately predicting N_2O emissions remains a challenge. In soil, N_2O forms directly or indirectly through microbial enzymatic transformations of N (Knowles, 1982; Kroeze et al., 1999). In nitrification, ammonia monooxygenase (AMO) oxidizes NH_3 to NH_2OH, and hydroxylamine oxidoreductase (HAO) converts this NH_2OH to NO_2^-, releasing NO, which can thereby further react to produce N_2O (Wrage et al., 2001). In denitrification, NO_3^- can be sequentially reduced to NO_2^-, NO, N_2O, and finally dinitrogen (N_2). Nitric oxide reductase (NOR) reduces NO to N_2O, and nitrous oxide reductase (NOS) then reduces N_2O to N_2 (Knowles, 1982).

Increasing soil moisture content raises liquid diffusion rates, providing microorganisms with C and N substrates (Blagodatsky and Smith, 2012) that are key factors structuring microbial communities and activities (Gleeson et al., 2010; Banerjee and Siciliano, 2012; Barnard et al., 2013). Because denitrification is a phylogenetically broad process dominated by heterotrophs, this soil moisture control on resource distribution and availability is expected to directly affect key N transformations. Further, expression of AMO or NOS is influenced by several factors, including temperature, pH, soil moisture content and C, N, and O_2 availability (Giles et al., 2012). Increasing soil moisture content reduces gaseous diffusion rates, limiting oxygen (Blagodatsky and Smith, 2012). Since AMO requires oxygen and NOS operates under anaerobic conditions, i.e. reduced oxygen concentration, soil moisture content is a key factor controlling AMO and NOS activities (Knowles, 1982; Gleeson et al., 2010). Although soil moisture is dynamic, investigators typically fix soil moisture content to a specific level (e.g. Carson et al., 2010; Gleeson et al., 2010) or re-wet soils (e.g. Fierer et al., 2003; Barnard et al., 2013; Placella and Firestone, 2013) to evaluate how a soil moisture content change alters microbial communities. For example, Barnard et al. (2013) found soil microbial communities to be remarkably resilient to soil drying-wetting across multiple sites, returning to pre-drying composition within hours of wetting. To understand the relationship between microbial communities and soil moisture content, we need to consider how changing soil moisture content influences resource availability, then gene transcription, and finally microbial community characteristics. For example, it may be that soils at 60% water filled pore space (WFPS) will behave differently depending on whether they were previously at 80% or 40% WFPS. The reason for this difference may be that if soil moisture content is changing, the conditions under which a newly produced enzyme functions may be different than those that signaled its induction. Here, we evaluated how changing soil moisture affects bacterial community composition, gene transcription, nutrient concentrations, and N_2O emissions. We used quantitative PCR to quantify cDNA of transcripts of *amoA*, *hao*, *norB*, and *nosZ*, and 454-pyrosequencing to survey cDNA reverse transcribed from bacterial 16S rRNA. Specifically, we tested the following hypotheses: 1) the direction of soil moisture change is associated with bacterial richness and community composition; 2) the rate of N_2O emissions varies with the direction of moisture change and the proportion of water filled pore space in soil; 3) the rate of N_2O emission is correlated with substrate availability and transcript abundance across soil moisture regimes.

2. Materials and methods

2.1. Soil collection

Soil samples were obtained from an experimental field near Swift Current, Saskatchewan, in Western Canada (50°12′N; 107°24′W). The area has warm summers, cold winters, and mean annual precipitation of 360 mm. The soils are loam textured Haplic Kastanozems with a surface pH of 6.5 and are under a continuous wheat rotation. Triplicate soil samples were collected at 0–15 cm depth and immediately transferred to the laboratory at the University of Saskatchewan, where they were stored at 4 °C until combined, processed and sieved to <2 mm.

2.2. Soil incubation

Triplicate microcosms were prepared each containing 25 g homogeneous field-moist soil in a 150-mL glass bottle and adjusted to 1.2 g cm^{-3} bulk density. Soil samples were moistened to 40%, 60%, 80%, and 100% water-filled pore space (WFPS) by uniformly pipetting sterile deionized water and a nutrient solution onto the surface. Soil moisture level was obtained by adjusting gravimetric water content. The nutrient solution contained NH_4^+ and NO_3^- and were added at concentrations of 50 mg $N-NH_4^+$ kg^{-1} soil and 50 mg $N-NO_3^-$ kg^{-1} soil. Thus, all the different WFPS contained the same nitrogen concentrations. All microcosms were arranged randomly and incubated at room temperature (~20 °C). Typically, northern agricultural soils reach 20 °C–25 °C in daytime during summer and thus the incubation temperature was selected to resemble the upper *in situ* temperature. Each day, N_2O emissions were measured as follows: a 15 cc disposable syringe equipped with a 25-gauge needle was inserted into the bottle and pumped up and down three times to obtain an evenly mixed sample. Next, 10 mL of gas sample was collected and injected into a pre-evacuated Exetainer® vial (Labco, High Wycombe, UK). After soil and gas sampling at each stage, the bottles were kept opened for 30 min to mix with ambient air and sealed again. Concentrations of N_2O were estimated by a gas chromatograph equipped with an electron capture detector (Yates et al., 2007). The injector temperature = 100 °C, column temperature = 35 °C, detector temperature = 370 °C; separations were performed using Poraplot Q columns (12.5 m by 0.32 mm i.d. fused silica capillary column, DF (film thickness) = 8 μm; includes a 2.5-m particle trap) with ultra high purity He (14.4 mL min^{-1}) as the carrier gas and P5 (95:5 v/v Ar/CH_4 mix) as the make-up gas (12.0 mL min^{-1}). The system was calibrated using standard gases (N_2O in N_2) obtained from PraxAir (Mississauga, ON). Internal calibration curves were calculated by applying linear, least squares regression to the gas concentration (ppbV N_2O) vs. peak area data.

Experimental units were destructively sampled by collecting 5 g of soil and freezing at −80 °C (Fig. 1). The remaining soil samples were frozen at −20 °C for soil characterization. The remaining experimental units had water added (wetting treatment), were maintained at the specified moisture level (static treatment), or had suction applied (drying treatment) to adjust the moisture content. For wetting samples, water was added by weighing soils as water was added. Drying samples were prepared by applying a gentle suction, over a 5–10 min period, to the soil placed on a surface sterilized (95% ethanol) ceramic funnel with periodic weighing to assess moisture loss. For example, the 60% WFPS treatment, initially would have had 3 replicates per treatment (wetting, drying, static) = 9 replicates, in addition to the 3 replicates for the initial static period for a total of 12 replicates. Initially, all 12 replicates were at 60% WFPS. Then after 6 days, 3 of these replicates were chosen randomly and destructively sampled. The remaining 9