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Effect of model root exudate on denitrifier community dynamics and activity at different water-filled pore space levels in a fertilised soil

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ABSTRACT

Although a "rhizosphere effect" on denitrification rates has been established, a clear understanding of the effects of exudate addition on denitrifier community dynamics remains elusive. A microcosm experiment was designed to explore the interaction between exudate addition and soil moisture on community dynamics and denitrification rates. Artificial root exudate at 5 different carbon concentrations was added daily to soil microcosms at contrasting target WFPS (50, 70 and 90%). After a 7-day period, total denitrification and N_2O emission rates were measured and community dynamics assessed using molecular methods. The response of denitrifier genes to exudate addition was different, with *nirS* and *nosZ*-I showing a stronger effect than *nirK* and *nosZ*-II. Distinct community structures were observed for *nirS* and *nosZ*-I at 90% target WFPS when compared to 50% and 70%. *Nir*S denitrifier population size showed a ca. 5-Fold increase in gene copy number at 90% WFPS when exudate was added at the highest C input. Significant total denitrification and N_2O emission rates were observed only at 90% WFPS, which increased with C input. Our study improves the understanding of the complex interaction between microbial communities, the abiotic environment and process rates which can inform management practices aimed at increasing complete denitrification and controlling greenhouse gas production from agriculture.

1. Introduction

Denitrification is a respiratory microbial process in which nitrate (NO_3^-) or nitrite (NO_2^-) are reduced to nitrogen gases (NO, N_2O and N_2) under oxygen limiting conditions (Tiedje, 1988; Philippot et al., 2007). It allows the maintenance of respiration through the use of nitrogen oxides as alternative electron acceptors (Zumft, 1997). This process is of great importance as, in addition to leading to significant N losses in agricultural systems (Rheinbaben, 1990; Shcherbak et al., 2014), it has negative environmental effects due to nitrous oxide (N_2O) production (Mosier et al., 1998; Bouwman et al., 2013). N_2O is an important greenhouse gas with a 100-year warming potential ≈300 greater than that of carbon dioxide (Forster et al., 2007) and has also been shown to contribute to ozone layer depletion (Cicerone, 1987). Atmospheric N_2O levels have increased by 19% since pre-industrial times with current estimates suggesting that agricultural soils contribute as much as 50–60% of N_2O global emissions (IPCC, 2007; Galloway et al., 2008; Bouwman et al., 2013).

The denitrification process occurs through a series of enzymatic steps, each performed by a specific reductase encoded by *narG/napA*, *nirK/nirS*, *norB* and *nosZ* genes (Zumft, 1997). Key process enzymes are nitrite reductase (encoded by *nirK* or *nirS*) catalysing the reduction of NO_2- to NO and N_2O reductase (encoded by *nosZ*) converting N_2O into inert N_2 (Zumft, 1997; Shapleigh, 2013). Graf et al. (2014) confirmed that denitrification is a modular pathway, with some bacteria possessing the full complement and others lacking genes thus performing a subset of the pathway being exclusively N_2O producers (K and S-denitrifier types), N_2O consumers (Z-types) or capable of both processes (KZ and SZ-types) (Jones et al., 2013; Graf et al., 2014). In general, denitrification is promoted under anaerobic conditions, high levels of NO_3^- and the presence of readily available carbon sources (Philippot et al., 2007) but overall denitrification rates, and the proportions of N_2 and N_2O produced, in soil are affected by the interaction of many environmental factors, such as pH, water holding capacity, pore structure, carbon and NO_3^- availability and O_2 levels (Morley and Baggs, 2010; Attard et al., 2011; Butterbach-Bahl et al., 2013; Hu et al., 2015).

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Water-filled pore space (WFPS) has been widely used in denitrification studies as it integrates information about the water content and total porosity of a soil system and has been shown to be closely related to microbial activity and denitrification-derived N_2O emissions, which increase at levels above 60–70% WFPS (Clayton et al., 1997; Bateman and Baggs, 2005).

Plants alter denitrification rates in soil via the growth and activity of the root system (Woldendorp, 1962; Philippot et al., 2013) with increased denitrification in rhizospheric soil compared to bulk soil (Klemedtsson et al., 1987; Bakken, 1988; Mahmood et al., 1997). Rhizodeposition has been suggested as the main factor behind this "rhizosphere effect" (Philippot et al., 2007). It is estimated to account for approximately 11% of the net carbon fixed by photosynthesis or 27% of the allocated C to the roots (Jones et al., 2009) with exuded carbon stimulating the denitrifier community by acting as an electron donor. Additionally, root respiration and exudation decreases O_2 availability, favouring denitrification (Bateman and Baggs, 2005; Hu et al., 2015). However, the high spatial and temporal variability of the denitrification process and the many factors affecting plant-microbe interactions in the rhizospheric soil (Groffman et al., 2009; Giles et al., 2012), have limited the understanding of the dynamics of this and other soil processes. To date, a handful of studies have attempted to elucidate the effect of carbon addition on the denitrifier community (e.g. Murray et al. (2004); Henry et al. (2008); Morley et al. (2014); Giles et al. (2017)). However, no conclusive links between C input quantity or quality, community dynamics and denitrification fluxes were demonstrated. Here, we carried out a controlled 7-day soil microcosm incubation with contrasting target WFPS levels (50, 70, 90%) in which the effect of daily additions of artificial root exudate (ARE) at variable carbon concentration (0–3 mg C day^{-1}), equivalent to a range between 0× and 2× the normal exudation rate of *L. perenne* (Paterson and Sim, 1999) on the denitrifier community size and structure, total denitrification (N_2O + N_2) and N_2O emission rates in soils was studied in an effort to reveal linkage between the amount of C potentially exuded by plants, denitrifier community dynamics and activity.

2. Materials and methods

2.1. Field soil

A brown forest soil of the Carpow series (Laing, 1976) with a sandy loam texture and a pH (measured in 0.01 M $CaCl_2$) of 5.95 was collected on April 2013 from an arable field at The James Hutton Institute (56°27′20″N, 3°04′35″W). Soil characteristics were 60% sand, 25% silt, 11% clay, 3.68% C and 0.12% N. Soil dissolved organic carbon (DOC) was 14.38 ± 2.65 mg kg^{-1} dry soil. Soil was sieved to 4 mm mesh size and stored at 4 °C until microcosm preparation the following week. Moisture content was determined by drying at 105 °C.

2.2. Experimental setup

A fully factorial soil microcosm experiment consisting of three target WFPS levels (50, 70 and 90%) and five root exudate C inputs (0, 0.375, 0.75, 1.5 and 3 mg C day^{-1}) was established. C input levels selected were equivalent to 0× (water-only control), 0.25×, 0.5×, 1× and 2× the normal exudation rate of the model grass species *Lolium perenne*, as previously estimated by Paterson and Sim (1999). Prior to microcosm set-up, potassium nitrate (KNO_3) was added to the soil as a solution and mixed thoroughly to obtain a rate of 100 mg N kg^{-1} dry soil and water content adjusted to 50% WFPS. The amount of N added is representative of the recommended fertiliser N addition rates under current UK agricultural practices (Defra, 2010) and is similar to that used in other developed countries (Potter et al., 2010). Additionally, it also falls within the range of N rates previously shown to stimulate denitrification and lead to the production of N_2O and N_2 (Giles et al., 2012; Morley et al., 2014). To establish the microcosms, 120 g dry soil equivalent was added to 150 identical plastic pots (6.6 cm height x 5 cm diameter) with 10 replicates and packed to a bulk density of 1.2 g cm^{-3}. Subsequently, water was added as required to establish the 70 and 90% WFPS treatments. A completely randomized block design was established in a controlled temperature room (darkness, 21 °C) and pre-incubated for seven days prior to ARE addition. Additional microcosms were established for each moisture level and sampled upon the start of the experiment (day 0) to allow the evaluation of changes in community dynamics and soil parameters between the start and the end of the experiment in water-only (0 C) controls.

2.3. Artificial root exudate addition

A single artificial root exudate mix containing five sugars (glucose, sucrose, fructose, ribose and arabinose), five amino acids (glycine, valine, glutamine, serine and alanine) and five organic acids (malic, citric, malonic, oxalic and fumaric acid) at equimolar C concentrations was used for the experiment (Paterson et al., 2007). This ARE mix was previously designed to represent a model mixture of the major chemical classes of carbon compounds (sugars, amino acids and organic acids) found in plant root exudates (Uren, 2001; Paterson et al., 2007; Dennis et al., 2010). Although amino acids also provided an additional N input to the system, amino-N accounted for less than 25% of the total N added as KNO_3 in the highest C input treatment. The KNO_3 added is known to be sufficient to support unrestricted denitrification over the time frame of the experiment (see Giles et al., 2012), and the amino-N supplied was not expected to affect or alter this process.

Distinct ARE solutions at the different final C input levels previously mentioned (0–3 mg C ml^{-1}) were prepared daily. After degassing, 1 ml of the corresponding ARE solution for each treatment was applied to the centre of the soil microcosms using a syringe at a depth of 2 cm depth (simulating root exudation and generating a model "rhizospheric" zone in the centre of the microcosm, Supplementary Fig. S1). Microcosms were then immediately adjusted to the target WFPS by further addition of water to the surface based on the overnight weight loss from each microcosm. This rapid water adjustment ensured WFPS changes around the injection zone due to ARE addition were kept to a minimum. ARE solution application and moisture adjustment were repeated daily for seven days, thus allowing sufficient time for the bacterial and denitrifier community to be affected by their respective C inputs. After this period, gas sampling and soil sampling were carried out to evaluate the effect of the 7-day ARE application regime on gas emissions and community dynamics.

2.4. Gas sampling and analysis

After ARE addition and WFPS adjustment on day 7, microcosms were placed in 500 ml Kilner jars and sealed. In half the microcosms for each treatment, 10% v/v of the headspace air was replaced with acetylene (C_2H_2) to measure total denitrification rates (N_2O + N_2) by inhibiting N_2O reductase activity. In the remaining half of the microcosms, headspace air was not modified to allow the measurement of actual N_2O emission rates. All microcosms were then incubated at 21 °C for a 4-h period after which a 5.9 ml gas sample was taken using a syringe and placed in a sealed Exetainer® vacuum vial (Labco Limited, Lampeter, UK). Preliminary tests were run on the same soil and with extremes of treatments to determine the headspace incubation time for the entire experiment taking samples every 30 min for 8 h. N_2O was found to accumulate linearly over 8 h in this system and a 4-h incubation reliably provided measurable N_2O concentrations (data not shown). Although ideally several time points should be taken our approach was used to allow the high number of samples required in this experiment. Samples were analysed for N_2O concentrations using a TRACE™ Gas Chromatograph (GC) fitted with an electron capture detector (Thermo Scientific, Hemel Hempstead, UK) maintained at 300 °C. GC response was calibrated using certified standard N_2O gas mixtures