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Nitrate removal, communities of denitrifiers and adverse effects in different carbon substrates for use in denitrification beds

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ABSTRACT

Denitrification beds are containers filled with wood by-products that serve as a carbon and energy source to denitrifiers, which reduce nitrate (NO_3^-) from point source discharges into non-reactive dinitrogen (N_2) gas. This study investigates a range of alternative carbon sources and determines rates, mechanisms and factors controlling NO_3^- removal, denitrifying bacterial community, and the adverse effects of these substrates. Experimental barrels (0.2 m^3) filled with either maize cobs, wheat straw, green waste, sawdust, pine woodchips or eucalyptus woodchips were incubated at 16.8 °C or 27.1 °C (outlet temperature), and received NO_3^- enriched water (14.38 mg N L^{-1} and 17.15 mg N L^{-1}). After 2.5 years of incubation measurements were made of NO_3^-–N removal rates, *in vitro* denitrification rates (DR), factors limiting denitrification (carbon and nitrate availability, dissolved oxygen, temperature, pH, and concentrations of NO_3^-, nitrite and ammonia), copy number of nitrite reductase (*nirS* and *nirK*) and nitrous oxide reductase (*nosZ*) genes, and greenhouse gas production (dissolved nitrous oxide (N_2O) and methane), and carbon (TOC) loss. Microbial denitrification was the main mechanism for NO_3^-–N removal. Nitrate–N removal rates ranged from 1.3 (pine woodchips) to 6.2 g N m^{-3} d^{-1} (maize cobs), and were predominantly limited by C availability and temperature (Q_{10} = 1.2) when NO_3^-–N outlet concentrations remained above 1 mg L^{-1}. The NO_3^-–N removal rate did not depend directly on substrate type, but on the quantity of microbially available carbon, which differed between carbon sources. The abundance of denitrifying genes (*nirS*, *nirK* and *nosZ*) was similar in replicate barrels under cold incubation, but varied substantially under warm incubation, and between substrates. Warm incubation enhanced growth of *nirS* containing bacteria and bacteria that lacked the *nosZ* gene, potentially explaining the greater N_2O emission in warmer environments. Maize cob substrate had the highest NO_3^-–N removal rate, but adverse effects include TOC release, dissolved N_2O release and substantial carbon consumption by non-denitrifiers. Woodchips removed less than half of NO_3^- removed by maize cobs, but provided ideal conditions for denitrifying bacteria, and adverse effects were not observed. Therefore we

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recommend the combination of maize cobs and woodchips to enhance NO_3^- removal while minimizing adverse effects in denitrification beds.

1. Introduction

Anthropogenic production of reactive nitrogen (N), through the Haber Bosch process, cultivation of N-fixing crops, and combustion of fossil fuels, contributes 45% of global N fixation (Canfield et al., 2010). This human impact on the nitrogen cycle leads to N enrichment of surface waters, with consequences including eutrophication, hypoxia, harmful algae blooms and habitat degradation in lakes, rivers and coastal zones, and an increase in N_2O emissions (Howarth et al., 2002; Rabalais, 2002; Phoenix et al., 2006). Denitrification beds are a promising approach to reduce reactive N release from point source discharges into waterways. These denitrifying bioreactors are containers filled with wood by-products, where the wood acts as carbon and energy source for denitrifying microorganisms (Schipper et al., 2010), which convert NO_3^- to unreactive N gas *via* microbial denitrification (Warneke et al., 2011b).

A wide range of carbon substrates have been trialled in column studies to find appropriate media for bioreactors (Volokita et al., 1996a,b; Soares and Abeliovich, 1998; Della Rocca et al., 2005, 2006; Saliling et al., 2007; Gibert et al., 2008; Cameron and Schipper, 2010). Nitrate removal rates in column studies range from 3 g N m^{-3} d^{-1} (woodchips; Cameron and Schipper, 2010) to 96 g N m^{-3} d^{-1} (rice husk; Shao et al., 2008). The exceptionally high NO_3^- removal rates of many carbon substrates (e.g., rice husks, wheat straw, cotton) were attributed to a large organic carbon release in the start-up phase of the columns, and were not sustainable over a longer time period (Cameron and Schipper, 2010). In a long-term study, barrels filled with maize cobs removed 3–6.5 times more NO_3^-–N than wood substrate, but also had higher carbon leaching in the effluent (Cameron and Schipper, 2010). Greenan et al. (2006) also reported that maize stalks produced greater NO_3^- removal than woodchips. However, little is known about the mechanism responsible for NO_3^- removal, the controlling factors, denitrifying bacterial communities or adverse effects, such as greenhouse gas release, when using different carbon substrates than woodchips. Warneke et al. (2011a, b) demonstrated that the mechanism responsible for NO_3^- removal in a full-scale woodchip bioreactor was microbial denitrification, and the removal process was limited by microbially available carbon and temperature. Smaller-scale studies have also determined that microbial denitrification is the dominant N removal mechanism, rather than dissimilatory NO_3^- reduction to ammonium DNRA or NO_3^- immobilization (Robertson, 2010; Greenan et al., 2006, 2009; Gibert et al., 2008).

Greenhouse gas (GHG) production during denitrification is an important issue to address when studying denitrification beds. An *in field* woodchip bioreactor study by Warneke et al. (2011a) yielded total N_2O release of 4.3% of removed NO_3^-–N, whereas Greenan et al. (2009) reported negligible release of dissolved N_2O in a woodchip column study. However, there have been no studies examining GHG production in denitrification beds containing different carbon sources.

So far, the population of denitrifying bacteria has not been investigated in substrates for use in denitrification beds. The abundance of denitrifying communities can be estimated by quantifying the functional gene copy numbers for nitrite reductase, *nirS* and *nirK*, and nitrous-oxide reductase, *nosZ*. These denitrification genes express reductase enzymes involved in denitrification. *NirS* expresses the cytochrome *cd*1-containing nitrite reductase (which catalyses the reduction of nitrite to nitric-oxide), *nirK* expresses the copper containing nitrite reductase, and *nosZ* expresses nitrous oxide reductase (which catalyses the reduction of N_2O to N_2) (Zumft, 1997; Braker et al., 1998). The two different genes for nitrite reductase, *nirS* and *nirK*, have coevolved to produce two independent pathways and no denitrifier is known to contain both pathways (Philippot, 2002). Interestingly many denitrifying organisms have been shown to reduce NO_3^- only to nitrous oxide (Cheneby et al., 1998, 2004) and some, such as *Agrobacterium tumerfaciens* C58 do not possess nitrous oxide reductase (*nosZ*) (Wood et al., 2001). Many studies have shown that differences in the diversity and abundance of denitrifying bacterial genes were correlated to a variety of physical and chemical conditions; organic carbon in glacier foreland (Kandeler et al., 2006), temperature in constructed wetlands (Chon et al., 2010), water logging in rice paddy soils (Yoshida et al., 2009), organic or conventional fertilizer in agricultural soils (Dambreville et al., 2006; Enwall et al., 2005), native and cultivated soils (Stres et al., 2004), soil pH in grassland soils (Cuhel et al., 2010), nitrous-oxide emissions (Philippot et al., 2009) and NO_3^- concentration in woodlands with different vegetation (Lindsay et al., 2010). However, the diversity and abundance of denitrifying bacteria under consistent environmental conditions (e.g., same temperature, NO_3^- concentration, DO concentration, flow rate), but with different carbon substrates are poorly known.

This study followed a 2.5-year trial by Cameron and Schipper (2010), where different C substrates were compared for their ability to remove NO_3^- from water at two temperatures. The main objectives of the present study were to determine the limiting factors and the microbial mechanisms of the NO_3^- removal for different C substrates such as woodchips (Pine and Eucalyptus), sawdust, green waste, maize cobs and wheat straw in these barrels. The abundance of the denitrification functional *genes nirS, nirK* and *nosZ* were compared across replicate barrels, different temperatures and substrates. The factors affecting denitrifying communities were examined and whether NO_3^- removal could be predicted from the copy number of denitrification genes. Adverse effects, including production of N_2O and methane (CH_4), and total organic carbon (TOC) release, were also determined to evaluate the benefit of the different C