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A comparison of different approaches for measuring denitrification rates in a nitrate removing bioreactor

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ARTICLE INFO

Article history:
Received 15 January 2011
Received in revised form
15 May 2011
Accepted 22 May 2011
Available online 31 May 2011

Keywords:
Denitrification
^{15}N
Push–pull
Stable isotopes
Acetylene block
Natural abundance

ABSTRACT

Denitrifying woodchip bioreactors (denitrification beds) are increasingly used to remove excess nitrate (NO_3^-) from point-sources such as wastewater effluent or subsurface drains from agricultural fields. NO_3^- removal in these beds is assumed to be due to microbial denitrification but direct measurements of denitrification are lacking. Our objective was to test four different approaches for measuring denitrification rates in a denitrification bed that treated effluent discharged from a glasshouse. We compared these denitrification rates with the rate of NO_3^- removal along the length of the bed. The NO_3^- removal rate was 8.73 ± 1.45 g m^{-3} d^{-1}. *In vitro* acetylene inhibition assays resulted in highly variable denitrification rates (DR_{AI}) along the length of the bed and generally 5 times greater than the measured (NO_3^-—N removal rate. An *in situ* push–pull test, where enriched ^{15}N—NO_3^- was injected into 2 locations along the bed, resulted in rates of 23.2 ± 1.43 g N m^{-3} d^{-1} and 8.06 ± 1.64 g N m^{-3} d^{-1}. The denitrification rate calculated from the increase in dissolved N_2 and N_2O concentrations (DR_{N2}) along the length of the denitrification bed was 6.7 ± 1.61 g N m^{-3} d^{-1}. Lastly, denitrification rates calculated from changes in natural abundance measurements of $\delta^{15}N$–N_2 and $\delta^{15}N$—NO_3^- along the length of the bed yielded a denitrification rate (DR_{NA}) of 6.39 ± 2.07 g m^{-3} d^{-1}. Based on our experience, DR_{N_2} measurements were the easiest and most efficient approach for determining the denitrification rate and N_2O production of a denitrification bed. However, the other approaches were useful for testing other hypotheses such as factors limiting denitrification or may be applied to determine denitrification rates in environmental systems different to our study site. DR_{N_2} does require very careful sampling to avoid atmospheric N_2 contamination but could be used to rapidly determine denitrification rates in a variety of aquatic systems with high N_2 production and even water flows. These measurements demonstrated that the majority of NO_3^- removal was due to heterotrophic denitrification.

1. Introduction

The global production of anthropogenic nitrogen (N) is increasing due to food and energy production (Vitousek et al., 1997; Canfield et al., 2010). This N also has lasting adverse effects on the environment, including increased greenhouse gas emissions, stratospheric ozone depletion, pollution of drinking water, and eutrophication of streams, lakes and coastal waters (Galloway et al., 2004, 2008; Canfield et al., 2010). There are a range of strategies to reduce the N load to

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0043-1354/$ – see front matter © 2011 Elsevier Ltd. All rights reserved.
doi:10.1016/j.watres.2011.05.027

aquatic ecosystems from agricultural practices, such as the construction or preservation of wetlands and riparian buffers, and installation of denitrification beds or walls (Dinnes et al., 2002; Vymazal et al., 2006; Schipper et al., 2010). Denitrification beds are large containers filled with wood by-products that act as a carbon source to support heterotrophic denitrification, which converts nitrate (NO_3^-) to nitrous oxide (N_2O) and N_2 gases (Seitzinger et al., 2006). These beds are increasingly being installed to remove NO_3^- from point-source discharges such as effluent streams and drainage systems (Schipper et al., 2010).

It is generally presumed that microbial denitrification is predominantly responsible for the NO_3^-—N removal in these beds (Schipper et al., 2010) and that other NO_3^- removal processes such as dissimilatory NO_3^- reduction to ammonium (DNRA), anammox and microbial/plant uptake are relatively low. For example, Greenan et al. (2006, 2009) showed that less than 4% of NO_3^-—N removal in woodchip columns was due to DNRA and that microbial uptake only accounted for 2–3.5% of NO_3^-—N removed. Isotopic enrichment of natural abundance of ^{15}N in NO_3^- was measured in the outflow of a denitrification bed and in a column study while NH_4^+ concentrations were low, was also suggestive of microbial denitrification (Robertson et al., 2000; Robertson, 2010). However, there are various processes beside heterotrophic denitrification that can account for ^{15}N—NO_3^- increase in natural systems (Bedard-Haughn et al., 2001). Therefore, measurement of the products of denitrification (N_2, N_2O), is critical to establish that denitrification is responsible for NO_3^- removal. Our previous work suggested that denitrification is the primary pathway for NO_3^- removal in denitrification beds because we measured very high potential rates of denitrification using the acetylene inhibition method. Anammox and DNRA were likely negligible due to low NH_4^+ concentrations and the lack of plant/algae growth on the denitrification bed ruled out biotic uptake of NO_3^- (Warneke et al., 2011). However, there are no direct measurements of denitrification rates in operating denitrification beds to demonstrate that denitrification dominates other NO_3^- removal processes. Developing a method to directly measure denitrification rates would also allow reliable determination of NO_3^- removal rates in denitrification beds and potentially in other similar aquatic systems because determining NO_3^- removal via measurement of inflow and outflow NO_3^- concentrations is difficult in many of these systems due to high temporal variability in NO_3^- concentrations and flow rates at inflow and outflow (Schipper et al., 2010).

A number of different techniques may be used to measure denitrification rates in terrestrial and aquatic environments (Groffman et al., 2006). The acetylene inhibition method has probably been the most commonly used approach for measuring denitrification (Groffman et al., 2006). Acetylene inhibits the reduction of N_2O to N_2 and accumulated N_2O can be measured using gas chromatography. However, the acetylene block technique can lead to inaccurate measurements of denitrification rates because acetylene has a number of other unwanted effects on microbial populations e.g., acting as an inhibitor of nitrifiers or as a carbon source (Groffman et al., 2006). Denitrification rates measured in soils using acetylene inhibition technique are generally an underestimate of actual rates (Groffman et al., 2006).

Denitrification rates in water-saturated environments (e.g., groundwater or wetlands) can also be estimated using the push–pull method (Addy et al., 2002) where a slug of ^{15}N-labelled NO_3^- is added into the denitrifying environment and the accumulation of ^{15}N–N_2 and ^{15}N–N_2O is measured with time (Hauck and Melsted, 1956; Addy et al., 2002; Baker and Vervier, 2004).

Direct quantification of denitrification by measuring N_2 emissions from soils has also been attempted, although it is technically challenging due to the high atmospheric background concentration of N_2 (Butterbach-Bahl et al., 2002). Similarly, in aquatic environments, increases in dissolved N_2 can be measured but are also confounded by high background levels of dissolved N_2 concentrations derived from the atmosphere (Groffman et al., 2006). However, conditions for measuring denitrification in rivers via dissolved N_2 concentrations described by Laursen and Seitzinger (2005) suggested that it may be possible to directly measure increases in dissolved N_2 concentrations along the length of denitrification beds due to their turbulent-free water flow and their potentially high production of N_2 through denitrification.

A final approach that could be used to demonstrate denitrification as the main mechanism for NO_3^- removal in denitrification beds is the measurement of changes in the $^{15}N/^{14}N$ natural abundance of NO_3^- and nitrogen gases along the length of the bed. If denitrification was the main mechanism of NO_3^- removal then there should be increases in natural abundance $^{15}N/^{14}N$ in NO_3^-, observed as $\delta^{15}N$–NO_3, due to the strong discrimination against ^{15}N during denitrification (Mariotti et al., 1981) and a negative congruent decrease in the $^{15}N/^{14}N$ of N_2 gas produced, reported as $\delta^{15}N$–N_2.

The main objectives of this study were to determine whether denitrification rates were high enough to account for the observed NO_3^- removal in an operational denitrification bed and to compare different methods for measuring denitrification rates in denitrification beds. A range of the techniques were trialled for accuracy, ease, and expense of measurement, including measuring changes in the dissolved nitrogen gases and natural abundance stable isotope (^{15}N–N_2 and ^{15}N—NO_3^-) along the length of the bed, acetylene inhibition assays, and accumulation of ^{15}N-labelled N_2 and N_2O following introduction of an ^{15}N-labelled NO_3^- spike.

2. Materials and methods

2.1. Study site

This study was performed at a large denitrification bed (176 m × 5 m × 1.5 m) constructed in 2006 and filled with a mixture of woodchips and sawdust (Warneke et al., 2011). The bed treated effluent from a glasshouse, which grew hydroponic cucumbers, tomatoes and capsicums at Karaka, New Zealand. The effluent from the glasshouse was pumped into one end of the denitrification bed through a PVC pipe 1 m below the surface of the woodchips and was discharged from the other end of the bed into a drainage ditch. Twelve fully screened PVC wells (2 m long; diameter 0.05 m) were installed along the length of the bed at 16 m intervals for effluent sampling. Mechanical water metres (LXLG-80, Bil, China) at