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Nitrogen removal and nitrifying and denitrifying bacteria quantification in a stormwater bioretention system

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ABSTRACT

In this study, we examine the biological processes involved in ammonia and nitrate removal in a bioretention system characterized by low infiltration rates and long drainage times. The system removed 33% of influent nitrate and 56% of influent total nitrogen. While influent ammonia concentrations were low (<0.3 mg/L), the bioretention cell also removed ammonia produced within the treatment system. Soil cores collected from the bioretention cell were analyzed for total 16S rDNA and both nitrification and denitrification genes (*amoA*, *nirS*, *nirK*, *norB*, and *nosZ*) using quantitative PCR. Total bacterial 16S rDNA levels in the surface layer were similar to those in very sandy soils. Gene counts for both nitrification and denitrification genes decreased as a function of depth in the media, and corresponded to similar changes in total 16S rDNA. The abundance of denitrification genes was also positively correlated with the average inundation time at each sampling location, as determined by modeling of stormwater data from a three-year period. These results suggest that both nitrification and denitrification can occur in bioretention media. Time of saturation, filter medium, and organic carbon content can all affect the extent of denitrification in bioretention systems.

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1. Introduction

Urban runoff is an increasingly important source of excess nitrogen to local receiving waters (United States Environmental Protection Agency, 2002). Unlike many other stormwater pollutants, however, influent nitrogen consists primarily of dissolved compounds that cannot be removed by filtration or settling, making it a particular challenge to remove (Taylor et al., 2005). One promising best management practice (BMP) for stormwater treatment is bioretention. A bioretention cell consists of a vegetated soil filter, with a planting layer overlying a porous medium and, often, an underdrain for effluent collection. Bioretention systems are effective at removing a range

of pollutants, including suspended solids and trace metals (Davis et al., 2009). Nitrogen removal performance, however, has been highly variable, with reported results ranging from as high as 60% removal to net nitrogen export (Davis et al., 2006; Dietz and Clausen, 2006; Hatt et al., 2009; Hunt et al., 2006). Ammonia removal is generally positive due to cation exchange with the filter medium, although this sorbed ammonia can undergo biological nitrification as the filter dries out, resulting in nitrate export in subsequent storm events (Cho et al., 2009; Hsieh et al., 2007). Nitrate and nitrite anions, however, adsorb poorly to most soil or filter media, and removal of these compounds in fast-draining bioretention systems is often quite poor (Davis et al., 2006).

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Several strategies have been employed to improve bioretention performance for nitrate. Lucas and Greenway (2008) observed increased reductions in effluent nitrogen oxides in vegetated mesocosms when retention times were increased to 12–18 h, producing anoxic soil conditions that led to denitrification. Kim et al. (2003) suggested that a permanently saturated zone at the bottom of the bioretention media could be used to increase microbial denitrification. In the field, this approach has led to mixed results. Hsieh and Davis (2005) observed improved nitrate removal with a saturated zone, while Dietz and Clausen (2006) measured a decrease in effluent total nitrogen but no effect on nitrate. Hunt et al. (2006), by contrast, did not see a significant improvement in nitrogen removal due to a saturation zone in a paired-site study. There may also be a significant seasonal component to saturated zone effectiveness, with denitrification occurring primarily in spring and summer (Passeport et al., 2009).

Despite the interest in denitrification in bioretention systems, there has been little direct examination of biological nitrogen transformation processes occurring in the soil media. Denitrification consists of four reaction steps facilitated by four groups of enzymes: nitrate reductases, nitrite reductases, nitric oxide reductases, and nitrous oxide reductases. Because denitrification is accomplished by a diverse range of organisms (Zumft, 1997), most genomic methods target the functional genes rather than the 16S rDNA gene. Real-time PCR assays have been developed for nitrite reductase (both *nirS* and *nirK* enzymes), nitric oxide reductase (*norB*), and nitrous oxide reductase (*nosZ*). Biological nitrification, which can act as an internal source for nitrate in the bioretention media, can be similarly targeted. The most common PCR assay for nitrification is for ammonia monooxygenase (*amoA*), which catalyzes ammonia oxidation to nitrite (Rotthauwe et al., 1997). The quantitative detection of these functional genes has been applied to environmental soils and sediments (Braker and Tiedje, 2003; Henry et al., 2004; Rosch et al., 2002), wastewater (Geets et al., 2007), and urban stormwater catchments (Knapp et al., 2009; Perryman et al., 2011). In this study, we examined nitrogen removal from influent stormwater in a bioretention system characterized by low infiltration rates and long drainage times. We also used quantitative PCR to quantify nitrification and denitrification genes present in the bioretention media and examined the hydrological and soil parameters related to the spatial distribution of these genes throughout the bioretention cell.

2. Materials and methods

2.1. Field site

The bioretention cell, constructed in 2004 to treat stormwater runoff from a four-lane arterial road in Lenexa, Kansas, has a surface area of 0.02 ha (0.06 acres). It receives runoff from the two northbound lanes of the road, a portion of the roadway embankment, and a paved footpath. This watershed is 0.25 ha (0.63 acres) and contains approximately 40% impervious surface. The cell consists of a 7.6 cm (3 inch) wood chip mulch layer over a soil mixture consisting of 50% sand, 20% shredded hard wood mulch, and 30% sandy loam planting soil. This soil

mixture extends to an average depth of 71 cm, where a clay layer serves as a barrier to further runoff infiltration. In 2009, the site had approximately 85% surface vegetative cover, consisting primarily of prairie cord grass (*Spartina pectinata*) and sumpweed (*Iva annua*).

Stormwater runoff from the road is collected in curbside sewers and drained to the bioretention cell through a single concrete pipe. A catch basin insert (Flogard + Plus, Hydro International) installed in the curbside sewer removes large particulates and trash from the runoff before it enters the bioretention cell. Treated effluent from the bioretention cell is collected in a perforated pipe underdrain and then discharged to an adjacent lake through a subsurface culvert. An overflow sewer with an opening approximately 23 cm (9 inches) above the soil surface is located in the southern portion of the bioretention cell, allowing some influent stormwater to bypass the bioretention cell during high rainfall events.

2.2. Water sampling and analysis

Runoff sampling for nitrogen analysis was conducted from March to August, 2009. Samples were collected at the entrance to the curbside storm sewer, before the catch basin insert, at the storm sewer discharge to the bioretention cell, and at the discharge point for the bioretention cell underdrain. At the storm sewer, stormwater runoff was captured by a plastic tube on the road surface and funneled into a 1 L plastic bottle. At the cell inlet, runoff was collected by a programmable auto-sampler (ISCO 6700, Teledyne ISCO, Inc.) triggered by an attached flow meter. During each rainfall event, samples were collected every 15 min in 250 mL glass bottles throughout the storm event or until all sample bottles were filled. The maximum total time for sample collection was four and one-half hours (18 samples). An automated sampler (WS 750, Global Water Instrumentation) attached to a flow meter located in the discharge pipe was used to collect a flow-weighted composite sample of the cell effluent from each storm event from March through June. An ISCO 6700 auto-sampler was used to collect time sequence samples at this location for five storms during July and August using the same approach described above. Due to difficulties with performance of the autosamplers, grab samples were also collected by hand in 1 L plastic bottles within 24 h of storm events at this location.

All samples were taken back to the laboratory within 24 h of collection. For the autosampler time sequence samples, every four samples were combined in a 1 L bottle and homogenized to produce a single sample for analysis. These samples were then averaged to provide a single time-weighted concentration value for each storm event.

Ammonia nitrogen analysis was performed following Standard Method 4500-NH₃ (American Public Health Association et al., 2005). Nitrate and nitrite concentrations were determined by ion chromatography using a Dionex ICS-2000 (Dionex Corp.) with 30 mM KOH as the eluent. Total nitrogen samples were digested according to Standard Method 4500-N C, followed by spectrophotometric detection for nitrate using Standard Method 4500-NO₃-B. Quantitative detection limits were 50 µg/L as N for ammonia (NH₃-N) and total nitrogen and 100 µg/L as N for nitrate (NO₃-N) and nitrite (NO₂-N).

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