Pedosphere **24**(4): 516–528, 2014
ISSN 1002-0160/CN 32-1315/P

Published by Elsevier B.V. and Science Press

PEDOSPHERE

www.elsevier.com/locate/pedosphere

Denitrification Rates and Their Controlling Factors in Streams of the Han River Basin with Different Land-Use Patterns[*1]

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(Received October 29, 2013; revised May 28, 2014)

ABSTRACT

Land-use patterns can affect various nutrient cycles in stream ecosystems, but little information is available about the effects of urban development on denitrification processes at the watershed scale. In the presented study, we investigated the controlling factors of denitrification rates within the streams of the Han River Basin, Korea, with different land-use patterns, in order to enhance the effectiveness of water resource management strategies. Ten watersheds were classified into three land-use patterns (forest, agriculture and urban) using satellite images and geographic information system techniques, and *in-situ* denitrification rates were determined using an acetylene blocking method. Additionally, sediment samples were collected from each stream to analyze denitrifier communities and abundance using molecular approaches. *In-situ* denitrification rates were found to be in the order of agricultural streams (289.6 mg N_2O-N m^{-2} d^{-1}) > urban streams (157.0 mg N_2O-N m^{-2} d^{-1}) > forested streams (41.9 mg N_2O-N m^{-2} d^{-1}). In contrast, the average quantity of denitrifying genes was the lowest in the urban streams. Genetic diversity of denitrifying genes was not affected by watershed land-use pattern, but exhibited stream-dependent pattern. More significance factors were involved in denitrification in the sites with higher denitrification rates. Multiple linear regression analysis revealed that clay, dissolved organic carbon and water contents were the main factors controlling denitrification rate in the agricultural streams, while dissolved organic carbon was the main controlling factor in the urban streams. In contrast, temperature appeared to be the main controlling factor in the forested streams.

Key Words: denitrifier communities, denitrifying genes, nutrient cycle, stream ecosystem

Citation: Jung, S. P., Kim, Y. J. and Kang, H. 2014. Denitrification rates and their controlling factors in streams of the Han River Basin with different land-use patterns. *Pedosphere*. **24**(4): 516–528.

For effective water quality management in streams, a comprehensive understanding on the entire stream ecosystem is necessary, due to the complex interaction of water bodies, soil properties, geomorphology, vegetation and climate. The amount of nitrogen (N) is one of most important indexes within water management, as N is typically a limiting factor in streams and downstream estuaries, often being solely associated with eutrophication, and also because N is the most widespread drinking water pollutant (Hill *et al.*, 2004). In particular, human activities have doubled global nitrate supply over the last century (Galloway *et al.*, 2004; Allan and Castillo, 2007) and it has substantially altered the ecosystems of streams. Because the widespread prevention of N inflow into streams from non-point sources is highly difficult to implement, enhanced N removal through optimization of denitrification in streams has drawn much attention as an effective approach towards N control (Allan and Castillo, 2007). Denitrification is the microbiological transformation of nitrate to dinitrogen or nitrous oxide gases, which can permanently remove nitrate from a water body (Martin *et al.*, 1999). It has been reported that up to 75% of total N can be removed by denitrification in aquatic ecosystems (Howarth *et al.*, 1996).

Due to its importance, many research efforts have been made towards understanding denitrification controlling factors within aquatic ecosystems. Denitrification rates in aquatic ecosystems are known to be influenced by various factors, such as soil grain size, organic matter content, temperature and nitrate concentration. Stream subsurfaces consisting of fine sands exhibit higher denitrification rates than those consisting of large pebbles, due largely to the increased specific surface area, and the provision of a greater volume of matrix for the propagation of microorganisms (Inwood *et al.*, 2007). Denitrification rates have been found to be positively related to the volume of benthic organic matter in those streams with high nitrate concentrations above the half-saturation constant (Arango *et al.*,

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2007). And nitrate concentration and temperature are positive controlling factors for denitrification in various aquatic ecosystems (Inwood *et al.*, 2007; Herrman *et al.*, 2008; Song *et al.*, 2012).

Denitrification processes are mainly mediated by a group of microorganisms called denitrifiers. They are facultative anaerobes and reduce nitrates or nitrites to dinitrogen gas or denitrification intermediates. They include very diverse groups of microorganisms, such as *Thiobacillus denitrificans*, *Micrococcus denitrificans*, *Paracoccus denitrificans*, and some species of *Serratia* and *Pseudomonas* (Madigan *et al.*, 2002). Previous studies have reported on community structure and abundance of denitrifiers in aquatic ecosystems exposed to various changes in their environment. For examples, environmental perturbations have been found to affect microbial community compositions in aquaecosystems (Findlay, 2010). Bacterial community composition was significantly different in the streams surrounded by urban or rural areas (Lear and Lewis, 2009). In addition, denitrifying communities were shifted in response to elevated atmospheric carbon dioxide (Lee *et al.*, 2012) and presence of vegetation (Song *et al.*, 2010, 2012) in constructed wetlands. However, in other studies, it is still doubtable whether denitrification is influenced by microbial community changes. For example, denitrification rates were not found to be correlated with denitrifying community shifts associated with hydrologic pulsing in constructed wetlands (Song *et al.*, 2010). Degree of watershed urbanization influenced bacterial communities, but correlations between denitrification and bacterial community shifts were not significant (Wang *et al.*, 2011).

Previous studies of urbanization on stream denitrification have been mainly concerned with denitrification rates, soil chemistry and water chemistry, but few of them have concerned with denitrifying community structures (Groffman *et al.*, 2002; Inwood *et al.*, 2007; Lofton *et al.*, 2007; Kaushal *et al.*, 2008; Mulholland *et al.*, 2008; Klocker *et al.*, 2009; Harrison *et al.*, 2011). In order to find a practical strategy for comprehensive N reduction management within streams, we selected 10 study sites in 9 streams of the Han River in Seoul, where surrounding watersheds were dominated by forest, agricultural or urban land uses, to study the factors controlling denitrification rates (*i.e.*, biogeochemistry, water chemistry and denitrifying community structures) within the streams.

MATERIALS AND METHODS

Site selection and sampling

Ten study sites in 9 streams (Chang-Reung, CR; Yong-Am, YA; Wang-Suk, WS; Hong-Reung, HR; Gul-Po, GP; Sung-Nae, SN; An-Yang, AY; Yang-Jae, YJ; and Tan, T) of the Han River, 14-km away from the center of Seoul, Korea, were selected according to the following considerations (Fig. 1). Fifth-order streams in the Strahler stream order were selected (Chorley, 1995) because the size of the fifth-order stream was deemed to be small enough to be highly sensitive to general localized background N inputs. A sampling site with a meandering part in the streams and with an alluvial island was then selected because the hyporheic exchange flow are active in this geographic configuration (Fig. 2a) (Ward *et al.*, 2002). However, an alluvial island was not present in stream GP.

For determination of land-use patterns, geographical range of a 1-km radius was defined in satellite images (NHN Co., Seongnam, Korea) using AutoCAD 2008 (Autodesk Inc., USA) (Fig. 1b) (Barringer *et al.*, 1990). There were no overlapping parts among the geographical ranges for land-use pattern determination. To determine proportional areas of different land uses in the defined sections, national geographical information system (http://ngis.go.kr/) was utilized. Ten sites were classified as forested (YA and CR-up), urban (AY, YJ, SN, and T), and agricultural streams (GP, CR-down, WS, and HR), based on the dominant land-use pattern within each site (Fig. 1c).

Four habitats with different microtopographic characteristics, *i.e.*, riparian, channel, front of alluvial island and back of alluvial island, within each site were designated for sampling and *in-situ* measurements (Fig. 2a). Duplicate soil cores of 5-cm diameter and 10-cm depth were collected from the four habitats. Single water sample was collected in polyethylene bottles from overlaying water in three habitats, where riparian was excluded for water sampling. Soil and water samples were kept in an insulated ice container and transported immediately to the laboratory for the analyses. Soil samples for molecular biological characterization were stored at −20 °C prior to the analyses. All the sampling and *in-situ* measurement were performed from July 27 to August 3 in 2009.

Measurement of denitrification rate

Denitrification rates were measured *in-situ* by an acetylene blocking assay (Tiedje, 1982; Seitzinger *et al.*, 1993) in duplicate at the sampling spots of the four habitats in ten sites (Fig. 2b). For more secure measurement, a polyvinyl chloride chamber (45-cm height and 5.5-cm diameter) was fixed by inserting its 15-cm base through the sediment in order to prevent any gaseous leakages during the collection of sediment. Previous studies showed that about 90% denitrification