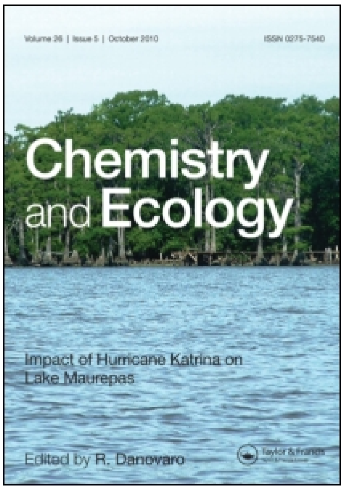

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Impact of invasive plant and environmental conditions on denitrification potential in urban riparian ecosystems

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Impact of invasive plant and environmental conditions on denitrification potential in urban riparian ecosystems

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One of the most serious problems involved in riparian restoration is the proliferation of invasive plants during or after a restoration project. Although many studies have assessed ecological influences of invasive plants, life history in particular, only a few have clarified the functional consequences of such changes. In this study, we aimed to determine the influences of an invasive plant, *Humulus japonicus*, and environmental conditions on riparian ecosystem functions, focusing on denitrification. Soil samples from five riparian ecosystems in Korea were collected on four occasions over a one-year period, and denitrification enzyme activity (DEA) was measured using an acetylene blocking method. DEA varied between 2.5 and $> 7000\,\mathrm{ng\,N_2O\,g^{-1}}$ soil $\mathrm{h^{-1}}$. Overall results suggest that DEA was fairly high in winter, but the influences of *H. japonicus* were minimal. The results suggest that water availability may be a more dominant controlling variable than the presence of *H. japonicus* for DEA.

Keywords: denitrification enzyme activity; wetland; microorganism; denitrifier; restoration

1. Introduction

Recent years have witnessed the wide application of ecological ideas to ecosystem restoration in many countries, including Korea. In particular, the restoration of urban streams is of great concern in both public interest and governmental policy. To restore the ecological integrity of streams, both the structure and functions of riparian zones should be considered, because they play a key role in various functions of streams [1,2]. First, most riparian vegetation can develop large root systems and endure rapid changes in water level. As such, riparian vegetation can secure the physical stability of the bank and reduce flow rates when a river is flooded [3]. Second, riparian systems are a ‘hot spot’ for many chemical and biological reactions in relation to nutrient removal [4]. For example, denitrification, sedimentation and the adsorption of various materials can occur at high rates, and play a key role in water-quality improvement. Finally, high biodiversity and productivity are often found in riparian systems, because of an ample supply of various nutrients and supplementary energy [5].

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One of the key aims in such a restoration project is to facilitate native species to inhabit restored riparian ecosystems. However, restoration projects often result in the proliferation of invasive plants in riparian ecosystems [6,7], which may reduce the biodiversity of an ecosystem [4,5,8,9], as reported for *Humulus japonicus* in Korea [10]. In general, modifications in environmental conditions (e.g. changes in flooding regimes, soil types and nutrient availability) or biological attributes (e.g. the absence of herbivores or presence of plants that are adapted to specific types of disturbances) may allow invasive plants to proliferate [8,11–15]. Such influences of invasive plants on ecosystem structure, particularly on biodiversity, have been well documented in many systems [5,6,8,16,17]. In Korea, *H. japonicas* S. et Z. is one of the most serious invasive plants found in riparian areas. Extensive areas of riparian ecosystems have been covered with *H. japonicus*, recently restored streams in particular. It is estimated that 20–60% of newly restored streams in and near Seoul are covered with it [17–19], and similar concerns of ecological risk have been raised in the USA [20]. Ecological studies on *H. japonicus* have recently been reported, but were limited to vegetation structure and litter decomposition rates [21,22].

Denitrification is a microbial process occurring under anaerobic conditions in which a certain group of microbes utilise NO_3^- as a terminal electron acceptor. This process results in the release of gaseous nitrogen (N_2O or N_2), by which inorganic nitrogen can be permanently removed from the water body into the atmosphere. Because of its importance in water-quality amelioration, denitrification rates have been extensively determined in riparian ecosystems. A field-scale study revealed that hydrology, NO_3^- concentration and carbon supply are the main controlling variables [23]. However, the vast majority of studies concerning riparian denitrification have been in agricultural or forested watersheds; few studies have assessed denitrification in urban riparian zones [24]. Further, the effects of invasive plants on denitrification in restored streams have rarely been reported. The objective of this study was to assess the influences of an invasive plant, *H. japonicus*, and soil environmental conditions on denitrification potential in urban riparian zones that have recently been restored in Korea. Specifically, we hypothesised that a riparian area with *H. japonicus* would exhibit higher denitrification rates because invasive plants have been reported to have a higher rate of nitrogen turnover [10,11,25,26]. To test this hypothesis, we collected soil samples from five riparian ecosystems and determined denitrification potential over a one-year period.

2. Materials and methods

2.1. *Study sites and soil sampling*

The study sites were urban riparian ecosystems in Amsa (two locations), Tan, Osan and Bam streams, which are located in or near Seoul in Korea. The mean annual temperature is 12.2 °C, and precipitation is 1344.3 mm. Since early this century, riparian zones of these sites have been ‘ecologically’ restored and natural riparian ecosystems recovered (Table 1). However, *H. japonicus* started to proliferate on completion of the restoration project, and a large proportion (20–60%) of the study sites are currently covered with it [17–19]. We selected 10 plots along riparian systems in each location: half of the plots are covered with *H. japonicus*, while *H. japonicus* is absent in the other half (control). In the control plots, we removed *H. japonicus* manually on a regular basis if expansion of it was observed. As such, it was not the effects of the vegetation itself (i.e. comparison between *H. japonicus* and bare ground), but the effects of the presence of *H. japonicus* that were assessed. A previous study reported that control plots have higher vegetation diversity than *H. japonicus*-covered plots [21].

In each plot, a soil sample was collected at 10 cm depth from the surface. Soils were transferred on ice to the laboratory, and biogeochemical analysis was completed within

Table 1. Descriptions of the study sites.

	Locations	Dominant species	Features
Amsa	37°32′ N 127°07′ E	*Phragmites communis* *Miscanthus sacchariflous* *Salix koreensis* *Humulus japonicas* (40%) [23]	Urban riparian Restoration completed in 2006
Osan	37°28′ N 127°07′ E	*Humulus japonicus* (20.4%) [19]	Urban riparian Restoration completed in 2006
Tan1	37°05′ N 127°02′ E	*Humulus japonicus* (31.4%) *Phragmites communis* *Miscanthus sacchariflous* [23]	Urban riparian Restoration completed in 2005
Bam	37°32′ N 126°55′ E	*Salix communis* *Artemisia selengensis* *Miscanthus sacchariflorus* *Phragmites communis* *Humulus japonicus* [24]	Naturally conserved

one week. The sampling was conducted four times per year to acquire information on seasonal variations.

2.2. *Physico-chemical factors*

Other physico-chemical factors of soils were determined by standard methods. Organic matter content, water content, soil pH, inorganic nitrogen (NH_4^+, NO_3^-) and phosphorus (PO_4^{3-}) were determined by standard methods [16]. In short, organic matter content was determined by loss-on-ignition, whereas water content was measured gravimetrically. Soil pH was determined using a pH electrode with 10:1 soil slurry. Cations and ions were measured by colorimetric methods using a spectrophotometer after extracting soils [9].

2.3. *DEA measurement*

Denitrification potential was determined by measuring denitrification enzyme activity (DEA), modified from Smith and Tiedje [27]. Denitrification potential rate provides a relative quantity of denitirifying enzymes exist in a soil. This method is based on the principle that the denitrification rate is proportional to the enzyme concentration when no other factors are limiting [28]. This may not represent ‘actual’ rates of denitrification under field conditions, but the method has been widely used to assess denitrification potential or activity in general [27–31]. In short, 10 g of soil was placed in a glass jar (100 cm^3) and 10 mL of DEA solution (0.45 g KNO_3, 0.1 g dextrose in 1 L ddH_2O) was added. The soil slurry was purged with N_2 gas followed by the addition of acetylene (10% in final volume). The slurry was incubated at 20 °C [29–31]. Gas samples from the head space of bottles were collected at 0, 40, 80 and 120 min using gas tight syringes. The collected gas was analysed by a GC (HP6890) equipped with an ECD detector.

2.4. *Statistical analysis*

The relationship between environmental factors and DEA was sought by a correlation analysis and multiple linear regressions. Differences between control and *H. japonicus* sites were compared by a t-test and significant differences were reported at $p < 0.05$. All procedures were conducted with SPSS v. 17.0.

Table 2. Physico-chemical properties of the riparian soils.

		Water content (%)	Organic matter (%)	pH	Ammonium ($ng \cdot g\ soil^{-1}$)	Nitrate ($ng \cdot g\ soil^{-1}$)	Phosphate ($ng \cdot g\ soil^{-1}$)
Amsa1	Control	27.8–48.1	7.8–12.7	5.7–6.9	1.2–28.2	1.0–18.8	17.1–95.3
	Humulus	29.6–50.8	7.8–14.3	6.2–7.1	5.5–30.9	8.5–25.7	27.6–65.3
Amsa2	Control	30.8–42.5	9.4–12.1	5.5–6.6	0.9–30.0	9.6–17.8	39.0–70.9
	Humulus	28.3–55.6	9.0–14.8	5.9–6.9	0.9–82.5	8.7–24.9	41.7–99.5
Osan	Control	6.3–11.4	1.5–4.1	4.9–6.4	1.3–41.3	1.3–6.5	54.2–126.5
	Humulus	8.0–14.7	1.8–4.8	5.0–7.1	0.8–43.1	3.2–9.8	42.9–68.8
Tan1	Control	14.1–34.3	7.3–14.6	5.7–6.9	0.4–70.9	4.6–13.3	26.3–61.6
	Humulus	12.3–40.7	7.5–16.0	5.9–7.1	0.1–48.6	5.3–13.7	27.9–68.4
Bam	Control	20.1–38.1	9.6–14.3	5.9–6.8	4.5–29.2	7.2–12.9	31.7–52.3
	Humulus	19.7–35.9	7.8–30.9	5.9–7.0	4.9–32.0	7.1–20.0	31.4–54.7

Note: The values are ranges determined four times a year.

3. Results and discussion

Soil chemical properties are presented in Table 2. There were substantial differences in chemical properties among different streams, but differences between control and *H. japonicus*-dominated sites were not discernible in most cases.

Variations in DEA are presented in Figure 1. DEA varied between 2.5 and $> 7000\,ng\,N_2O\,g^{-1}$ soil h^{-1}. Overall, DEA was highest in the Amsa 1 stream and lowest in the Osan stream. The activity in the Osan stream exhibited a range similar to values reported in the eastern USA [32], where the mean DEA value was $2.63\,ng\,N_2O\,g^{-1}$ soil h^{-1}. However, activities in other sites were much higher than seen in the eastern USA. The concentration of soil-extractable NO_3^- and the organic matter content did not differ substantially between the sites in Korea (Table 2) and those in the eastern USA [24]. As such, it appears that differences in vegetation type (e.g. herbaceous vs. forest) and consequent differences in the quality of soil organic matter are the reason for the differences in DEA. It is noteworthy that herbaceous sites exhibited much higher DEA values than forest sites in the urban areas, as reported by Groffman and Crawford [24].

No general patterns of seasons were discernible, but it is interesting to note that DEA in winter was the highest or comparable with other seasons at all sites (Figure 1). This result indicates that temperature would not be the key controlling variable for denitrification potential in our study sites. Previous studies have suggested that carbon availability, water level and nitrate availability are the key controlling variables for DEA [23,33]. Our study sites are relatively dry, except for a monsoon season and in winter when soils are covered with snow. It is speculated that water availability would be the main controlling variable for DEA at our sites. This hypothesis may explain the high DEA value in winter; the sites were under snow when soil samples were collected and hence ample water was available to surface soil. Apart from water content, pH and organic matter content exhibited positive correlations with DEA, but the correlation coefficients were lower than for water content (Table 3). Similarly, a multiple linear regression (a step-wise procedure) between DEA and chemical properties of riparian soils indicates that water content and pH are the key controlling variable as presented below:

$$\text{DEA} = 53.9 \times \text{Water content} + 401.6 \times \text{pH} - 2927.3\ (N = 213,\ r^2 = 0.295,\ p < 0.001).$$

A repeated measure analysis of variance (ANOVA) exhibited that seasonal variations in DEA were significant at three sites (Table 4). However, the influences of *H. japonicus* on DEA were not substantial, except for a few cases in which *H. japonicus* increased DEA in soils (Figure 1). A similar increase in denitrification potential by an invasive plant has been reported in river wetland [32]. It was found that *H. japonicus* decreased plant biodiversity at the sites, but did

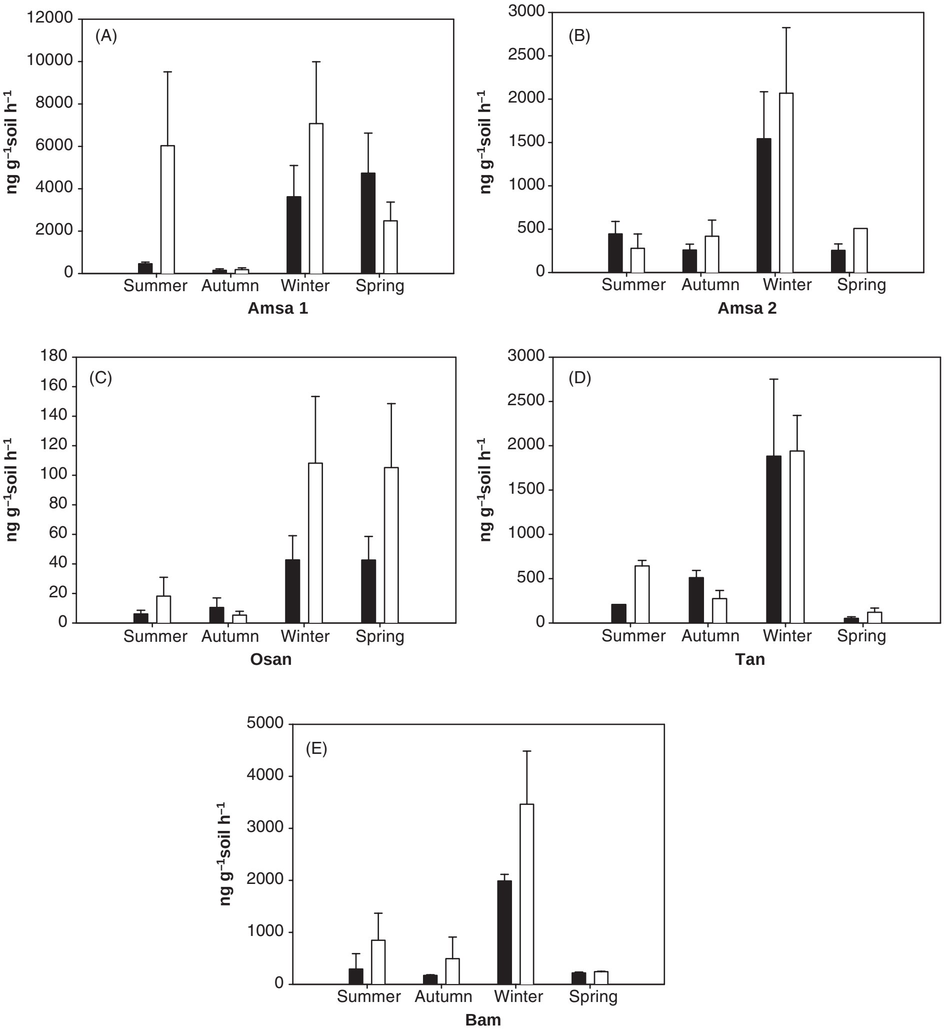

Figure 1. Denitrification enzyme activities (DEA) in Amsa 1 stream (A), Amsa 2 stream (B), Osan stream (C), Tan stream (D) and Bam island (E) in Korea. Black bars represent data from control sites (without *Humulus japonicus*), and open bars represent those from sites with *H. japonicus*. Values with different letters are significantly different between a control site and a *H. japonicus*-invaded site $(p < 0.05, n = 5)$.

not affect overall productivity [21]. Increased DEA under *H. japonica* on a few occasions can be explained by two mechanisms. First, by its growth pattern: *H. japonicus* can grow over other vegetation and cover soils. This provides shade beneath, which may maintain relatively higher humidity compared with control sites. Because we did not measure evapotranspiration *per se*, it is not clear that higher humidity is directly related to higher soil moisture. Continuous measurements of soil moisture over a one-day period are warranted to confirm this hypothesis. This is probably connected to the higher DEA values in winter when water availability was high. Second, litter from *H. japonicus* may be of higher quality than that of other native vegetation so that it may supply enough carbon sources to microbes in soil. Similarly, previous studies on the decomposition rates of invasive plant have observed faster turnover of organic matter and nutrients, in particular nitrogen [10,11,25,26,34,35].

Table 3. Correlation coefficients between denitrifying enzyme activity (DEA) and chemical properties of riparian soils.

	Water content	pH	OM	NH_4^+	NO_3^-	PO_4^{3-}
DEA	0.753**	0.580*	0.543*	0.140	0.048	0.018

Note: Mean values from each sampling occasion for each location were composited ($n = 40$, $^{**}p < 0.01$; $^{*}p < 0.05$).

Table 4. *p*-Values of repeated measure ANOVA for denitrifying enzyme activity (DEA) data.

Sites	Season	Presence/absence of *H. japonicus*	Interaction
Amsa 1	0.979	0.466	0.057
Amsa 2	**0.045**	0.446	0.497
Osan	**0.020**	0.182	0.151
Tan	**0.018**	0.060	**0.015**
Bam	0.763	0.465	0.687

Note: Values < 0.05 are given in bold.

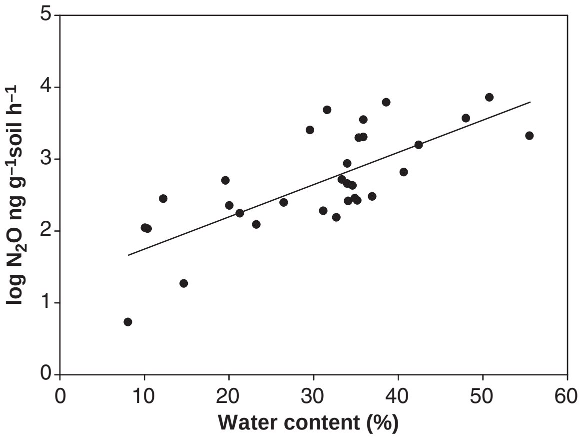

Figure 2. Relationship between denitrifying enzyme activity (DEA) and soil water content. Each point is a mean value of five replicates ($r = 0.753$, $p < 0.001$, $n = 40$).

Overall, the results of our study suggest that *H. japonicus* slightly increases DEA depending on the site, and invasion of *H. japonicus* might not interfere with ecosystem function, namely denitrification potential. It appears that water content rather than *H. japonicus* is the key controlling variable for DEA in our study sites, which is in accordance with a report from the USA [24]. A similar result was reported from a peatland manipulation experiment, where water level drawdown induced a >95% decline in nitrous oxide emission [36]. Our hypothesis is further supported by a significant positive correlation between DEA and water content in soils (Table 4 and Figure 2).

In conclusion, denitrification potential in our sites was extremely high, and hence restored riparian may function for N removal from surface run-off. Influences of an invasive plant on denitrification potential are minimal. Rather, water availability and hence hydrological conditions may be a critical factor for denitrification potential in our sites.

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