Comparing abundance, composition and environmental influences on prokaryotic ammonia oxidizers in two subtropical constructed wetlands

Lan-Feng Fan [a], Hsing-Ju Chen [a,b], Hwey-Lian Hsieh [a], Hsing-Juh Lin [b,1], Sen-Lin Tang [a,*]

[a] Biodiversity Research Center, Academia Sinica, No. 128 Academia Rd., Sec. 2, Nankang, Taipei 115, Taiwan
[b] Department of Life Sciences, National Chung Hsing University, Taichung 402, Taiwan

ABSTRACT

Microbial communities in wastewater treatment can be critical indicators for proper design and functional management of constructed wetlands (CWs). The aim of this study was to examine changes in abundance and composition of ammonia-oxidizing prokyotes (ammonium-oxidizing archaea and bacteria, AOA and AOB, respectively) and how these relate functionally to nutrient depletion, by comparing a series of treatment cells in two CWs in subtropical Taiwan. Abundance and composition of AOA and AOB were measured by amoA-gene targeted denaturing gradient gel electrophoresis (DGGE) and quantitative PCR, respectively. Prokaryotic composition (including archaea and bacteria) was also determined by 16S rRNA-gene targeted DGGE, while concurrently measuring environmental characteristics. Dominant AOA and AOB were related to uncultured *Nitrososphaera* and *Nitrosomonas* groups, respectively. Treatment cells of the two CWs had special clusters with regards to composition of bacteria and AOA, whereas composition of archaea and AOB were evenly distributed in the wetland enclosing a small treatment area. Composition of AOA reflected distinct environmental requirements compared to archaeal composition. Based on abundance analysis, there were more AOB in middle treatment cells, with abundance highly correlated to concentrations of ammonium and phosphate. However, AOA was distributed throughout most of the treatment cells, and was poorly correlated to measured environmental characteristics. With regards to distribution, community complexity, environmental affiliation and environmental demands, we inferred that AOA may be more critical than AOB for depleting nutrients in the CWs.

© 2016 Elsevier B.V. All rights reserved.

1. Introduction

Constructed wetlands (CWs) are often nutrient-enriched, with higher nutrient concentrations than natural wetlands (Kadlec and Wallace, 2009). A prominent function of CWs is nitrogen removal (Faulwetter et al., 2009; Villalobos et al., 2013). Inside CWs, nitrogen removal mainly occurs through microbial transformations, i.e. nitrification and denitrification (Ruiz-Rueda et al., 2009; Wang et al., 2011).

Ammonia-oxidizing archaea and bacteria (AOA and AOB) are commonly regarded as major drivers of nitrification in sediment.

Ammonia oxidation is the first step in nitrification; this reaction is a rate-limiting step for transforming ammonia to nitrite, linking mineralization of organic matter-derived nitrogen in estuarine and continental shelf sediments to its ultimate removal as gaseous products by denitrification or anammox (Fan et al., 2006). The key enzyme in the reaction is ammonia monooxygenase, which was discovered in AOBs in the late 19th century and used since 1997 as a molecular marker of AOB (Rotthauwe et al., 1997). Using metagenomics approaches, ammonia monooxygenase has also been identified in AOAs in recent years and a culturable marine AOA (categorized into an archaeal phylum, *Thaumarchaeota*; Treusch et al., 2005) known to have specific activity of ammonia oxidation.

Quantitative PCR (qPCR) of the *amoA* gene, which encodes subunit A of ammonia monooxygenase, was done to determine AOA and AOB abundance. The QPCR data reflected relative abundance of AOA and AOB in various environments, e.g. aquatic and terrestrial

environments including forest soil, estuarine sediment, mangrove, salt marsh, and other natural wetlands (Bernhard et al., 2007; Moin et al., 2009; Li et al., 2011; Sims et al., 2012).

Environmental characteristics are critical in shaping the ammonia-oxidizer community. Although AOA usually grow at a wide ammonium range, growth of AOB was prominent only at a higher concentration of 14.3 mM (Verhamme et al., 2011). Increasing oxygen concentrations enhanced growth of AOB, whereas growth of AOA was almost oxygen insensitive. In addition, AOA are detected over a wide pH range, whereas AOB are neutrophilic and their highest growth rate occurs at pH 7 to 7.5 (Prosser and Nicol, 2012). Biotic factors are also important for prokaryotes. For example, based on analysis of potential activity and diversity of ammonia-oxidizer communities in CWs, variation of plant species influences structure and function of ammonia oxidizers (Ruiz-Rueda et al., 2009; Wang and Gu, 2013).

To improve efficiency with which CWs remove redundant nutrients, a series of treatment cells were designed, namely depositional, heavily vegetated, open-water and ecological ponds (Faulwetter et al., 2009; Hsu et al., 2011). Varying the organization of treatment cells facilitates comparisons of their distinctive functionality and unique chemistry. Wetland hydrological conditions and dimensions are crucially important, both from engineering and ecological perspectives; to maintain treatment efficiency, surface flow design requirements are normally based either on hydraulic retention time or total wetland area (Economopoulou and Tshrintzis, 2004; Kadlec and Wallace, 2009).

The Hsin-Hai II Constructed Wetland (HS2) and Daniaopi Constructed Wetland (DN) are free-water-surface integrated wetlands in the riparian zone of the Danshuei River system in northern Taiwan. Although both are effective for nutrient removal,

performance of these two CWs varies, as does that of various sub-cells within each CW (Hsu et al., 2011; Hsueh et al., 2014). Therefore, we hypothesized that the abundance and composition of prokaryotic ammonium-oxidizers differs between the two constructed wetlands and among treatment cells of each constructed wetland. The 16S rRNA- and *amoA*-genes were targeted in denaturing gradient gel electrophoresis (DGGE) as a proxy to describe composition of prokaryotes and ammonium oxidizers. Relative abundance of archaeal- and bacterial-ammonium oxidizers was measured by *amoA*-gene targeted qPCR. In addition to categorizing compositional variation of ammonium oxidizers in the two CWs, environmental characteristics were also measured to correlate these with possible factors influencing the presence of ammonium-oxidizing communities and their relative abundance.

2. Materials and methods

2.1. Study sites

The Hsin-Hai II Constructed Wetland (HS2) and Daniaopi Constructed Wetland (DN) are located in the riparian zone on the eastern bank of the Da-Han River, one tributary of the Danshuei River in northern Taiwan (Fig. 1a). With <6 km between site, these two CWs have less difference than channel versus riparian features. Both CWs are five-cell, free-water-surface integrated wetlands and were constructed in September and November 2006, respectively. The five sequential cells in HS2 consist of a depositional pond, heavily vegetated pond I, an open-water pond, heavily vegetated pond II, and an ecological pond (cells HS2-1–HS2-5, Fig. 1b). In the DN, the depositional pond (DN1) was followed by an open-water pond (DN2). The heavily vegetated pond I (DN3) was divided into
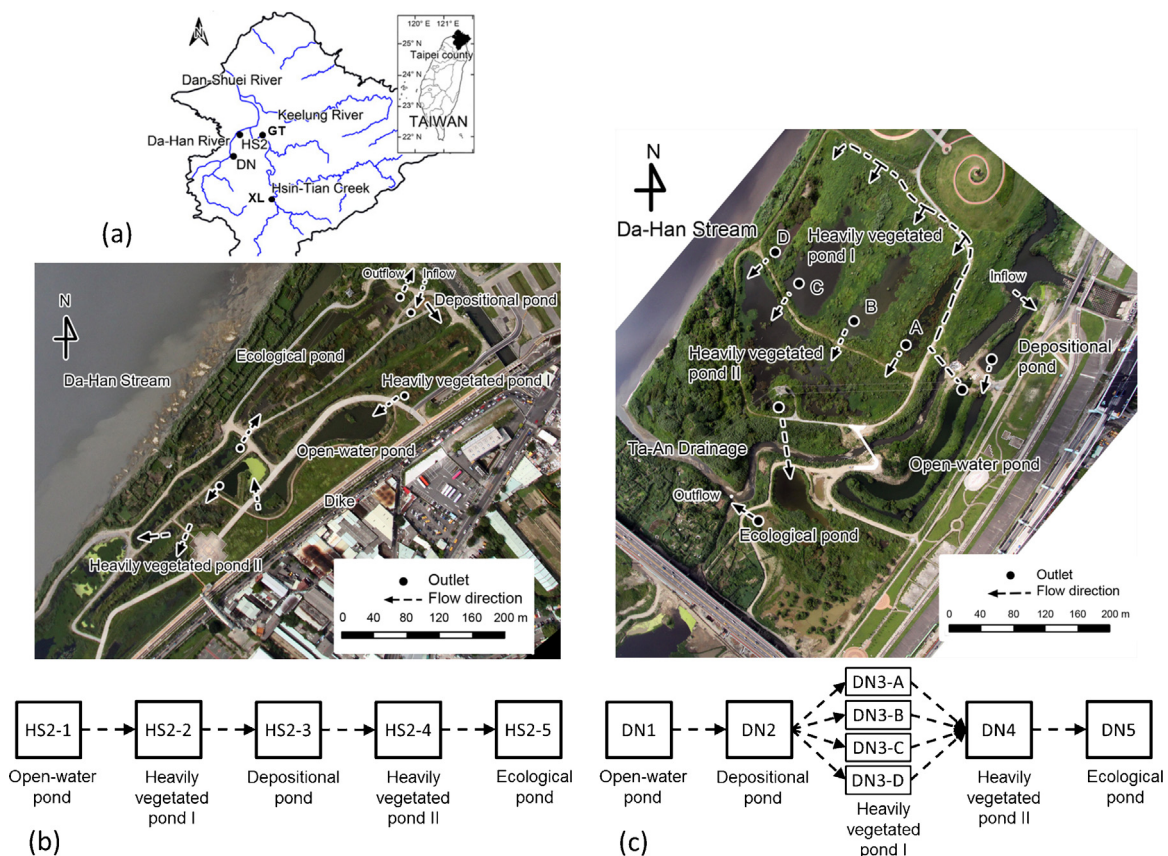

Fig. 1. Locations (a) and configurations of the Hsin-Hai II Constructed Wetland (HS2) (b) and Daniaopi Constructed Wetland (DN) (c), and two natural wetlands (NW): Gu-Ting (GT) and Xiu-Lang (XL).