Science of the Total Environment 473–474 (2014) 63–71

Contents lists available at ScienceDirect

Science of the Total Environment

journal homepage: www.elsevier.com/locate/scitotenv

Characterisation of the soil bacterial community structure and composition of natural and constructed wetlands

Gemma Ansola [a,*], Paula Arroyo [b,1], Luis E. Sáenz de Miera [c,2]

[a] Departamento de Biodiversidad y Gestión Ambiental, Universidad de León. Campus de Vegazana s/n, CP: 24071, León, Spain
[b] Instituto de Medioambiente, Recursos Naturales y Biodiversidad, Universidad de León. Calle La Serna, no. 56, CP: 24071, León, Spain
[c] Departamento de Biología Molecular, Universidad de León. Campus de Vegazana s/n, CP: 24071, León, Spain

HIGHLIGHTS

- Pyrosequencing was used to characterise soil bacterial communities in two wetlands.
- Bacterial communities are mainly grouped by wetland type: natural or constructed.
- Communities are also grouped according to a gradient from flooded to dry–wet areas.

ARTICLE INFO

Article history:
Received 19 July 2013
Received in revised form 25 October 2013
Accepted 27 November 2013
Available online xxxx

Keywords:
Soil bacterial community
16S rRNA pyrosequencing
Natural wetlands
Constructed wetlands
Quality water

ABSTRACT

In the present study, the pyrosequencing of 16S ribosomal DNA was used to characterise the soil bacterial community composition of a constructed wetland receiving municipal wastewater and a nearby natural wetland. Soil samples were taken from different locations in each wetland (lagoon, zone with *T. latifolia*, zone with *S. atrocinerea*). Moreover, the water quality parameters were evaluated (pH, T^a, conductivity, dissolved oxygen, redox potential, nutrients and suspended solids), revealing that the organic matter and nutrient contents were significantly higher in the constructed wetland than in the natural one. In general, the bacterial communities of the natural wetland were more diverse than those of the constructed wetland. The major phylogenic groups of all soils included Proteobacteria, Verrucomicrobia and Chloroflexi, with Proteobacteria being the majority of the community composition. The Verrucomicrobia and Chloroflexi phyla were more abundant in the natural wetland than the constructed wetland; in contrast, the Proteobacteria phylum was more abundant in the constructed wetland than the natural wetland. Beta diversity analyses reveal that the soil bacterial communities in the natural wetland were less dissimilar to each other than to those of the constructed wetland.

1. Introduction

Wetlands are ecosystems distinguished by the presence of water, either at the surface or within the root zone, and they often have unique soil conditions (poorly aerated and/or water-saturated soil) that differ from adjacent uplands. Wetlands support vegetation adapted to wet conditions (hydrophytes) and, conversely, are characterised by an absence of flooding-intolerant vegetation (Mitsch and Gosselink, 2000).

The study of wetlands is critical because of their unique ecological roles in nutrient cycling, sediment accretion, pollution filtration and erosion control. In addition, their organic material is fundamental to our understanding of precedent landscapes (Qin and Mitsch, 2009).

Constructed wetlands are man-made ecosystems that simulate the ability of natural wetlands to remove pollutants from water. They have been designed and constructed to take advantage of many of the processes that occur in natural wetlands but within a more controlled environment (Vymazal, 2007). Currently a wide range of wastewater from domestic (Vymazal and Kröpfelova, 2011) and industrial (Arroyo et al., 2013a) sources, including motorway leachates (Terzakis et al., 2008) or agricultural non-point-source pollution (Braskerud, 2002), is treated in constructed wetland systems. The bacterial community in constructed wetlands consists of autochthonous (indigenous) and allochthonous (foreign) microorganisms (Truu et al., 2009). Autochthonous microbes exhibit adaptive features; they are able to possess metabolic activity, survive and grow in wetland systems participating in purification processes, while allochthonous microbes (including pathogens entering with the wastewater) usually do not survive or have any functional importance in the wetland environment (Vymazal, 2005).

* Corresponding author. Tel.: +34 987 291564; fax: +34 987 291563.
E-mail addresses: gemma.ansola@unileon.es (G. Ansola), parrh@unileon.es (P. Arroyo), luis.saenzdemiera@unileon.es (L.E. Sáenz de Miera).
[1] Tel.: +34 987 293135.
[2] Tel.: +34 987 293195.

0048-9697/$ – see front matter © 2013 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.scitotenv.2013.11.125

Information about microbial community structure and diversity has been noted as important for understanding the relationship between environmental factors and ecosystem functions (Sims et al., 2013; Peralta et al., 2013). An understanding of the bacterial community composition and structure in natural wetlands could increase the likelihood of successfully constructing a treatment wetland, as bacteria are key factors in many environmental processes.

The currently available high-throughput sequencing (using 16S rRNA genes) of environmental DNA allows the rapid analysis of microbial communities at a much higher throughput than has previously been possible (Inceoğlu et al., 2011). Recent use of this molecular tool has provided information about the soil bacteria community structure in different ecosystems, including studies on a continental scale (Roesch et al., 2007; Lauber et al., 2009) and studies of forest and grassland ecosystems (Uroz et al., 2010; Nacke et al., 2011; Lin et al., 2011), agricultural soil (Acosta-Martínez et al., 2010; Inceoğlu et al., 2011) and natural and created wetlands (Wang et al., 2012; Peralta et al., 2013; Ahn and Peralta, 2009). However, the existing knowledge of bacterial communities in constructed wetlands has mainly been obtained using DNA fingerprinting (Calheiros et al., 2010), cloning and sequencing (Arroyo et al., 2013a) and physiological profiles (Zhang et al., 2010). The use of pyrosequencing is revolutionary because it can provide a sufficient number of sequences of adequate length to enable extrapolations that estimate bacterial diversity based on its two components: richness and evenness or equitability (Acosta-Martínez et al., 2008).

In the present study, we characterise the soil bacterial community composition in a constructed wetland receiving municipal wastewater and a nearby natural wetland and performed a detailed comparison of the bacterial diversity in the constructed and natural ecosystems. We hypothesised that the bacterial community structures would differ by wetland type.

2. Materials and methods

2.1. Research sites

In this study, two wetlands located in a rural area in northwest Spain were selected. The first one is an endorheic wetland of natural origins. The second one has been constructed to treat the municipal wastewater of Bustillo de Cea, a small village in which farming and small livestock holdings have been the main economic ventures for some time. These wetlands have been chosen because of their similarities in terms of morphological characteristics and plant species and their proximity (6 km).

The natural endorheic wetland, named "Laguna Corrillos", presents a lagoon zone (12,000 m^2) partially surrounded by an area amply dominated by *Typha latifolia* (680 m^2) and an area featuring the only occurrence of *Salix atrocinerea* (530 m^2) besides the area with *T. latifolia*.

The constructed wetland was developed as a Hierarchical Mosaic of Artificial Ecosystems (HMAE®) by applying the ecological adaptations of hydrophyte plants to flooded and polluted conditions (Ansola et al., 2003). The system is comprised of a pre-treatment followed by three basins. The first basin consists of a lagoon (230 m^2) of up to 2 m in depth where the inflow enters and 1.5 m where the out-flow exits. The next basin is a constructed wetland planted with *T. latifolia* (210 m^2) and operated with freewater flow. The last basin is divided into two areas: the first is planted with *Iris pseudacorus* (87.5 m^2) and also operated with freewater flow, whereas the second is a gravel bed system planted with *S. atrocinerea* (362.5 m^2). The plants were obtained from the nearby natural wetlands. The outflow is used for agricultural irrigation. Further details on this process can be found elsewhere (Ansola et al., 2003; Arroyo et al., 2010, 2013b).

2.2. Wetland soil sampling and analyses

Field sampling was conducted in the natural and constructed wetlands described above. Soil samples were collected using a push core sampler (Ø 5.3 cm, length of 100 cm). Soil sampling was conducted twice in the summer and twice in the winter for soil characterisation and once in the summer and once in the winter for bacterial community characterisation. At each wetland, soil was taken from the top layer (0–5 cm) in the three different locations: the lagoon zone, the area with *T. latifolia* and the area with *S. atrocinerea*. When sampling vegetated environments, the root zones were selected. Three replicate samples were taken at each position. The samples were stored in bags and placed on ice immediately after sampling. At the laboratory, each bag was homogenised manually to mix all three replicates for each environment. Any visible root or plant material was manually removed prior to homogenisation. Once mixed, a subsample was taken from each bag and transferred to a 10-mL tube for bacterial community analysis, and the reminder was used for soil characterisation.

The redox potential was measured *in situ* in each soil using a multiparameter probe (PCE-228-R redox meter). Each measurement was repeated three times at each site to ensure repeatability. To determine SOM and pH, the soils were air-dried and homogenised, after which the large constituents (e.g., plant material and rocks) were removed. At the laboratory, SOM analysis sub-samples (5 g of air-dried soil) were oven-dried at 105 °C for 24 h, weighed and heated at 375 °C for 16 h. SOM (%) was measured using the weight-loss-on-ignition method (Wilson and Sander, 1996). For pH determination, 10 g of each air-dried soil sample was combined with 50 mL of deionised water, mixed manually and allowed to stabilise for 10 min prior to measurement (Thomas, 1986).

2.3. Water sampling and analyses

The water samples were collected from the same locations as the soil samples in the lagoon zone and in the area with *T. latifolia*. Samples were stored in sterile plastic bottles. *In situ* measurements of pH, conductivity, oxidation-reduction potential, temperature and dissolved oxygen were conducted using a field multiparameter instrument (YSI 556 MPS) comprising a glass combination electrode, a four-electrode cell, a platinum button and a polarographic sensor. Measurements were repeated three times at each site to ensure repeatability.

All water samples were transferred immediately to the lab and stored at 4 °C before analysis. Water samples were tested for nutrients (nitrogen and phosphorus), organic matter (biochemical and chemical oxygen demand) and solids (total suspended solids). All of the analyses were performed according to standard methods (APHA, 2005).

Water sampling was conducted four times at the natural and constructed wetlands. It should be highlighted that water quality in the constructed wetland has also been analysed repeatedly since 2000, showing a high buffer capacity to direct changes (annual concentration fluctuations) and indirect changes (environmental conditions) (Arroyo et al., 2013b).

2.4. Microbial analyses

2.4.1. DNA extraction, PCR and pyrosequencing

DNA was extracted from the 12 wetland soil samples (0.25 g) using the Power Soil DNA isolation kit (MoBio Laboratories, Inc., Carlsbad, CA, USA), following the manufacturer's protocols. The DNA concentrations were determined spectrophotometrically using a Nanodrop instrument (Thermo Scientific, Wilmington, DE, USA).

Twelve different barcoded forward primers were composed of sequencing adaptor A of Roche 454 pyrosequencing, sample-specific 8 bp keys and the bacterial primer "AYTGGGYDTAAAGNG" (*E. coli* positions 563–577). The reverse primers were composed of four variants targeting the same 16S rRNA region, "TACNVGGGTATCTAATCC", "TACCRGGGTHTCTAATCC", "TACCAGAGTATCTAATTC" and "CTACDSRG GTMTCTAATC" (*E. coli* positions 785–802), added to sequencing adaptor B of Roche 454 pyrosequencing. These primers amplified the 16S rRNA