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# Differences in the activity and bacterial community structure of drained grassland and forest peat soils

Luka Ausec, Barbara Kraigher, Ines Mandic-Mulec*

*Biotechnical Faculty, Department of Food Science and Technology, University of Ljubljana, Jamnikarjeva 101, 1000 Ljubljana, Slovenia*

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ABSTRACT

The microbial activity and bacterial community structure were investigated in two types of peat soil in a temperate marsh. The first, a drained grassland fen soil, has a neutral pH with partially degraded peat in the upper oxic soil horizons (16% soil organic carbon). The second, a bog soil, was sampled in a swampy forest and has a very high soil organic carbon content (45%), a low pH (4.5), and has occasional anoxic conditions in the upper soil horizons due to the high water table level. The microbial activity in the two soils was measured as the basal and substrate-induced respiration (SIR). Unexpectedly, the SIR ($\mu l\ CO_2\ g^{-1}$ dry soil) was higher in the bog than in the fen soil, but lower when $CO_2$ production was expressed per volume of soil. This may be explained by the notable difference in the bulk densities of the two soils. The bacterial communities were assessed by terminal restriction fragment length polymorphism (T-RFLP) profiling of 16S rRNA genes and indicated differences between the two soils. The differences were determined by the soil characteristics rather than the season in which the soil was sampled. The 16S rRNA gene libraries, constructed from the two soils, revealed high proportions of sequences assigned to the *Acidobacteria* phylum. Each library contained a distinct set of phylogenetic subgroups of this important group of bacteria.



## 1. Introduction

Marshes and other types of wetlands are globally important as reservoirs of soil organic carbon (SOC), and are locally important as hydro-regulators and biodiversity hotspots. These wetlands cover approximately 3% of the land-surface, store up to 30% of the Earth's terrestrial carbon and play a vital role in carbon cycling (Gorham, 1995). Their biomass production exceeds decomposition due to low pH and the anoxic conditions often found in the soil, caused by high ground water or flooding (Augustin et al., 1996). Despite the fact that bacteria play a major role in the turnover of energy and matter in the soil, there have been only a few attempts to study the bacterial diversity in the peat soils (Dedysh et al., 2006; Kraigher et al., 2006; Morales et al., 2006). Terminal restriction fragment length polymorphism (T-RFLP) analysis of 24 bogs in the USA revealed a high bacterial diversity with a marked similarity among the sites (Morales et al., 2006). The composition of the bacterial community was also studied in a Siberian peat bog (Dedysh et al., 2006). Data from that study showed that the largest number of sequences (24 out of 84) in the 16S rRNA gene library was from the recently described phylum, *Acidobacteria*. When counted by fluorescent *in situ* hybridization (FISH), *Acidobacteria* comprised 0.1–4.1% of the total bacterial cell number in nine different *Sphagnum*-dominated bogs in Northern Russia (Pankratov et al., 2008). Other studies on the bacterial diversity and activity in peat soil have focused on specific taxonomic (e.g., *Actinobacteria*, Rheims et al., 1996) or functional groups, e.g., methanogenic archaea (Basiliko et al., 2003; Horn et al., 2003) and methanotrophic bacteria (Dedysh et al., 2001). In addition to the northern peat soils, *Acidobacteria* were also dominant in the Alaskan acidic soils, representing approximately 40% and 30% of clones in the tussock and intertussock soil clone libraries, respectively. However, *Acidobacteria* were poorly represented in the shrub organic and mineral soils from the same area, which were dominated by *Proteobacteria* (Wallenstein et al., 2007).

Little is known about the microbial communities in the temperate bogs and fens. Usually these marshes have been subject to drainage and altered land use and therefore the microbial communities might not follow the patterns observed in the relatively undisturbed marshes of the high latitudes. The aim of this study was to investigate the microbial activity and bacterial community structure in a temperate marsh. These soils have been

* Correspondence to: Ines Mandic-Mulec, Biotechnical Faculty, Department of Food Science and Technology, University of Ljubljana, Chair of Microbiology, Vecna pot 111, 1000 Ljubljana, Slovenia. Tel.: +386 1 4233388; fax: +386 1 257 3390.
*E-mail address:* ines.mandic@bf.uni-lj.si (I. Mandic-Mulec).

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severely degraded in the past two centuries due to drainage, peat collection and agriculture. The area with neutral pH drained sedge peat soil (fen area) has primarily been converted into farmland (25%) and grasslands (65%), but a few fragments of the lowland bog with low pH *Sphagnum*-derived peat soil are still present (Hacin et al., 2001). While both of the soils are exposed to the same climate conditions, they differ most notably in SOC and pH. SOC was shown to be an important factor for microbial activity but not for the bacterial community structure of the two drained grassland fen soils of the European temperate region (Kraigher et al., 2006). Microbial activity was measured as the substrate-induced respiration (SIR) or dehydrogenase activity, and changed with the season and the soil organic matter content; in contrast, the bacterial community structure did not respond to these environmental factors.

Soil pH has been shown to have significant effects on microbial communities. It was found to be the best predictor of bacterial diversity and richness in 98 soil samples from a range of ecosystems in North and South America (Fierer and Jackson, 2006) and in temperate freshwater wetlands from two North American sites (Hartman et al., 2008). Here, the hypothesis that pH may influence the bacterial communities in drained marsh soils was tested by comparing the acidic pH peat soil (bog) and the neutral pH peat soil (fen). The bacterial community structure was evaluated by T-RFLP profiling. A comparative analysis of bacterial diversity and phylogenetic composition was performed on the bog 16S rRNA gene clone library obtained in this study and the fen soil clone library data from Kraigher et al. (2006). In addition, a comparative study of both of the soil types involved an assessment of microbial activity as represented by the basal and substrate-induced respiration. Based on the pH difference between the two soils, we hypothesized that both the soil activity and diversity would be lower in the acidic bog soil.

## 2. Materials and methods

### 2.1. Soil sampling

A sampling of the fen and bog soils for the basal and substrate-induced respiration measurements was performed in September 2008 at two nearby locations (less than 3 km apart) in the Ljubljana marsh, Slovenia (45°58′N, 14°28′E). The first site was a grassland sedge-derived peat fen soil with a neutral pH (Rheic Fibric Histosol). The peat had been degraded in the upper 30–50 cm due to drainage, peat collection and agriculture. The second site was an acidic (pH 4.5) bog soil (Dystric Rheic Fibric Histosol) in a swampy forest where the *Sphagnum*-derived peat had been better preserved. Three field replicates of both of the soil types were taken. For each field replicate, 5 individual soil cores (2.5 cm wide and 30 cm deep) were obtained, pooled and homogenized through a 4 mm sieve. The soil samples were refrigerated overnight prior to the experiments. T-RFLP analysis was performed on the soil sampled at the same sites in August 2003 and in March 2004, as described by Kraigher et al. (2006). For T-RFLP analysis, an additional fen soil sample was obtained in 2003 and 2004, approximately 50 m from the fen soil sampling site, and this sample had a markedly lower SOC (therefore the designation fenLC). Two field replicates (20 soil cores each) were obtained and pooled, passed through a 4 mm sieve, frozen and stored at −20 °C in several aliquots of 0.5 g. The fen soil sampling sites were located on a meadow that is mown twice a year but that has not been amended by fertilizer; both of the fen soils were previously described by Kraigher et al. (2006). The bog soil sample, obtained in August 2003, was used for the 16S rRNA gene library construction.

### 2.2. Soil properties

Measurements were performed on the soil samples obtained from the upper 30 cm of soil. SOC was measured with a LECO CNS-2000 analyzer (LECO, USA) and $CaCO_3$ was subtracted. The organic soil nitrogen was determined by a standard Kjeldahl analysis (Bundy and Meisinger, 1994), using a Tecator 2012 digestion apparatus (Tecator AB, Sweden) and a micro Kjeldahl distillation apparatus. The soil pH was measured in a water slurry (1:5 soil:liquid ratio). The soil water holding capacity (WHC) was measured by packing the soil into soil sample rings (standard diameter 53 mm, volume 100 $cm^3$; Eijkelkamp, Giesbeek, NL) fitted with cloth at the bottom, and immersing the rings into water for 24 h. The soil moisture content (100% WHC) was measured after allowing the rings to drain freely on a funnel until a constant weight was reached (7 h). The soil water content was determined after drying at 105 °C for 24 h, and is expressed on the gravimetric basis. All of the results are expressed on an oven-dry (105 °C) weight basis and the characteristics of both the bog and fen soil types are presented in Table 1.

### 2.3. Basal and substrate-induced respiration (SIR)

The substrate-induced respiration was measured in 3 field replicates from the fen and bog soils, each with 3 methodological replicates, as described by Sparling (1995). 0.5 g glucose in the form of a 40% solution was added to 25 g of sieved fresh soil. $CO_2$ production at different soil moisture contents was measured prior to the experiment, and a water holding capacity (WHC) of 70% was determined to give a maximum response in all of the tested soil samples. This WHC was adopted for future analyses of the basal respiration and the SIR measurements. Sealed bottles with soil samples were incubated at room temperature for 3 h. The gas samples were taken at the beginning and end of incubation, and analyzed for $CO_2$ by gas chromatography (Network GC System 6890 N; Agilent Technologies, Palo Alto, CA, USA). The SIR is expressed as μl $CO_2$ $g^{-1}$ dried soil $h^{-1}$, and μl $CO_2$ $cm^{-3}$ dried soil $h^{-1}$, taking into account the soil bulk density. The basal respiration was measured in the same way, except that glucose was not added. The amount of added glucose proved to be sufficient to trigger a maximum SIR response in both of the soil samples since a higher amount of glucose did not result in a higher SIR response (data not shown).

### 2.4. Analysis of microbial community structure

Terminal restriction fragment length polymorphism (T-RFLP) was used to characterize the soil microbial community structure. The DNA was extracted from 0.5 g soil subsamples of a field replicate (two independent isolations for each type of soil, i.e. bog soil, fen soil

**Table 1**
Properties of the fen, fenLC and bog soils (values represent the mean ± standard deviation for the upper 30 cm of soil).

| | Fen | fenLC | Bog |
|---|---|---|---|
| $C_{org}$ content (%) | 16.3 ± 0.21 | 9.73 ± 0.1 4 | 45.4 ± 0.21 |
| $N_{org}$ content (%) | 1.40 ± 0.01 | 0.88 ± 0.01 | 2.75 ± 0.01 |
| C:N ratio | 11.7 ± 0.2 | 11.1 ± 0.25 | 16.5 ± 0.01 |
| pH | 7.55 ± 0.02 | 7.63 ± 0.01 | 4.58 ± 0.1 6 |
| WHC[a] ($g_{H_2O}/g_{soil}$) | 1.65 ± 0.21 | 6.99 ± 0.01 | 8.14 ± 0.29 |
| Bulk density (g $cm^{-3}$) | 0.59 ± 0.05 | 1.41 ± 0.20 | 0.16 ± 0.05 |
| Mean annual $T$(°C) | 17.8 ± 13.3 | 0.74 ± 0.04 | 12.6 ± 10.2 |
| Mean annual water table (cm) | −53.2 ± 22.0 | −17.2 ± 13.5 | −24.4 ± 13.8 |
| Soil classification[b] | Rheic Fibric Histosol | Mollic Gleysol (Thaptohistic) | Rheic Fibric Histosol (Dystric) |

[a] WHC – water holding capacity.
[b] Soils have been classified under the World Reference Base for Soil Resources (2006).