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A comparison of permanent and fluctuating flooding on microbial properties in an ex-situ estuarine riparian system

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ABSTRACT

Within natural floodplains, flood disturbances are one of the major events that control ecosystem development and functioning. Our objectives were to better understand the effect of alternate flooding–draining due to tides and/or flooding river regime on riparian soils in terms of bacterial community and nitrate removal. The effect of the frequency and duration of alternate flooding–draining events on redox potential, N removal and soil bacterial community composition was investigated under flooded soils mesocosms for 3, 7 and 14 days, using redox electrode, acetylene inhibition and quantitative real-time PCR techniques. We investigated the dynamics of total, denitrifying and sulfate-reducing bacteria by targeting bacterial 16S rRNA, *nosZ* and *dsrB* genes. Alternate flooding–draining conditions increased N removal efficiency compared to the permanently flooded soil and the non-flooded control soil. The redox potential decreased more slowly under alternate flooding–draining than under permanent flooding soils. We evidenced a significant response of the denitrification process to soil type and flood duration. Moreover, alternate flooding–draining had the greatest impact on the soil bacterial and functional groups abundance (*nosZ* and *dsrB* genes). This approach by molecular microbial ecology can be performed in addition to the usual soil descriptors and can be considered as a useful indicator.

1. Introduction

The functioning of riparian soils is largely determined by hydrological connectivity and exchange processes between a river and its floodplains (Amoros and Petts, 1993; Hohensinner et al., 2007). Nevertheless, human activities such as navigation and associated managements (e.g., embankments and channelization) greatly interfere with the natural flooding regime of riparian zones along rivers. In fluvial riparian zones, several studies have reported that flood disturbance regime through altered frequency, duration, and intensity of flooding directly affect N removal by controlling the duration of aerobic versus anaerobic phases (Pinay and Decamps, 1988; Song et al., 2010). Alternate flooding-draining, which controls the nitrification/denitrification balance, can thus lead to increased N losses in riparian zones (Hernandez and Mitsch, 2007; Pinay et al., 1995; Song et al., 2010). Denitrification is one of the important processes occurring in the wetlands, which uses nitrogen oxides as alternative electron acceptors when oxygen is limited. Denitrification process is mediated by denitrifying bacteria communities under anaerobic conditions (Henry et al., 2006; Mitsch and Gosselink, 2000). Denitrifying bacteria are frequently isolated from sediment, soil and aquatic environments, and the denitrifying ability is present in many phylogenetically diverse bacterial groups (Braker et al., 2000; Rösch et al., 2002).

In estuarine riparian zones, anoxic conditions rapidly occur and enhance the production of large amounts of sulfides by sulfate-reducing bacteria that play an important role in sulfur cycling in sediment and soils (Jorgensen, 1982; Ouddane et al., 2004). Elhottová et al. (2006), Mentzer et al. (2006) and Unger et al. (2009) showed that flooding regime in riparian zones modifies the oxic/anoxic characteristics of soils, and thus affects the microbial community structure. They studied the global microbial composition and activity by using phospholipid fatty acid (PLFA) analysis combined with enzyme activity assays. Molecular quantitative methods, such as qPCR and qRT-PCR, are particularly well

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suited for the quantification and study of the gene expression of sulfate-reducing and denitrifying bacteria (Besaury, 2012; Henry et al., 2006). Sulfate reduction and denitrification are not specific to any phylogenetic group (Joye and Hollibaugh, 1995; Wagner et al., 1998; Zumft, 1999). Therefore, to analyze sulfate reducer and denitrifier abundance, functional genes can be used, such as those coding for NO_2^- reductase (*nirK* and *nirS*) and N_2O reductase (*nosZ*) in the denitrification pathway (Henry et al., 2006; Kandeler et al., 2006; Scala and Kerkhof, 1999), and those coding for dissimilatory sulfite reductase (*dsrB*) and adenosine-5′-phosphosulfate reductase (apsA) in the sulfate reduction pathway (Leloup et al., 2005; Quillet et al., 2012).

Today, the restoration of biodiversity and its functions in human-disturbed floodplains is an important ecological topic. It is therefore necessary to better understand the influence of the disturbance regime (frequency and intensity of floods events) on the functioning of floodplain soils. Using soils from a riparian system of the Seine estuary (France), we experimentally assessed the effects of alternate flooding–draining conditions (due to daily water fluctuation related to tide) versus permanent floods on N removal, a microbially mediated soil process. We measured the effects of alternate flooding-draining events on redox potential, denitrification and soil bacterial community composition, using redox electrode, acetylene inhibition and quantitative real-time PCR methods, respectively. We used the functional genes *nosZ* and *dsrB* and real-time PCR molecular techniques to analyze the abundance and activity of denitrifying and sulfate-reducing bacteria. As the frequency of disturbance is more important in the fluctuating flooding regime than in the permanent one, we hypothesize that alternate flooding-draining events will have the strongest influence on soil bacterial, functional groups abundance (denitrifying versus sulfate-reducing bacteria) and N removal process.

2. Materials and methods

2.1. Study site

Soils were sampled in April 2011 in the site of Petiville, near the Seine River in the North-West of France (49° 26′ 08′ N; 00°°35′ 73″ E) under a temperate oceanic climate. In the absence of dykes, the site is directly connected to the river by water flows due to the tidal regime and/or floods. The soils are typical hydric soils classified as "REDUCTISOL fluvique" according the "Référentiel Pédologique" (Association Française pour l'Etude du Sol AFES, 2009), also referred to as Gleysol fluvic (IUSS Working Group WRB, 2006). Their morphological characteristics are as follows: a water-table close to the surface, a G horizon (named Go) that reflects temporarily re-oxidized conditions (corresponding to the fluctuations of the water table) and another G horizon (named Gr) that reflects permanent anaerobic conditions (corresponding to the permanent level of the water table).

Vegetation in the study site was typical of riparian zones and was composed of *Phalaris arundinacea*, willows (*Salix alba*) and poplar (*Populus* sp.) stands. Riparian vegetation distribution in the site is largely controlled by periodic flooding varying in magnitude and soil properties. Therefore, vegetation and soil morphological characteristics indicate that the study site is still active and directly connected to the Seine River.

2.2. Soil sampling

Three soil sampling sites were taken (0–10 cm depth) along a landscape gradient perpendicular to the Seine River at the commune of Petiville in the North-West of France (49° 26′ 08′ N; 00° 35′ 73″ E). At each site, three replicate were taken. The sites were selected based on elevation and distance from the river channel, with each soil type having distinct geomorphical properties found under varying depths, frequency and duration of flooding, and vegetation. Soils were sampled to a 10 cm depth by using metal spade. The samples were passed through a 5-mm mesh sieve, homogenized and stored at 5 °C.

The three soils classified by the French system were: (1) Mudflat (REDUCTISOL fluvique from mudflat in the intertidal zone), (2) Salix (REDUCTISOL fluvique in the high-frequency flooding zone <30 m from the channel), and (3) Populus (REDUCTISOL fluvique under, low-frequency flooding within the riparian zone, >100 m from the channel). Each soil had distinct soil properties as shown in Table 1. For the following soil properties, organic carbon, total nitrogen, cation exchange capacity (CEC), total iron and clay contents all increased in the following order: Mudflat < Salix < Populus. Populus had the highest organic matter and clay content and visibly the most aggregated, which can be attributed to greater in litter inputs from the forest stand and reduced erosion by flood events.

2.3. Experimental design

The experiment was conducted outdoors at the ECODIV laboratory of the Normandie University, Rouen (France) under similar climatic conditions as the sampling site. The experimental design of the mescosm study was a 3 × 3 factorial of three soil types (Mudflat, Salix, and Populus) and three flooding conditions as follows:

(1) Control no flood water added (C).
(2) Daily alternating of 12 h flooding and 12 h draining (DD).
(3) Permanently flooded (PF).

These treatment intervals were based on flood durations typically found on the Seine River floodplain (Programme Scientifique Seine-Aval, 2010). For the flooding treatments 6 cm of water above the soil was maintained by adding water as needed. Deionized water used for the flooding and was assumed that it would be chemically in equilibrium with soil solution after 3 days.

The treatments were carried out in a mescosm study. Approximately 2500 g of field moist soil were placed in polyethylene pots (24 cm diameter at the top and 20 cm diameter at the bottom × 32 cm depth) which resulted in a 17-cm deep soil profile. The bottom of the pot was constructed of polypropylene geotextile (PAILLAGE HORS-SOL TISSE, Intermas-Celloplast, Mayenne, France). These pots were placed in a larger pot (32 cm diameter at the top and 22 cm at the bottom × 42 cm depth) which had solid and non-draining bottoms to create an ex situ mescosm. The mescosms were destructively sampled at 3, 7, or 14 days. After each sampling period the mesocosms were taken out and allowed to drain for about 12 h and subjected to destructive sampling.

2.4. Soil functioning

Three replicates of each treatment were sampled on days 0, 3, 7 and 14. Just before destruction of soil samples, redox potential measurements (Eh in mV) were made 5-cm deep in the soil profile, using a Pt 5900 redox electrode. An aliquot of each soil was then used after 0, 3, 7 and 14 days of incubation for soil analysis (NH_4^+–N and NO_3^-–N) and soil water content measurements (gravimetric moisture measurements). All analyses were completed as quickly as possible and within no more than 6 days after sample collection. In the laboratory, a sub-sample (∼30 g) of each soil was oven-dried at 105 °C for 48 h to determine moisture content. For mineral N (NO_3^-–N and NH_4^+–N) measurements, a sub-sample of 20 g of fresh soil from each soil sample was extracted with 100 ml of 0.5 M K_2SO_4 and analyzed by flow injection (Anderson and Ingram, 1993).

Soil cores were taken after 0, 3, 7 and 14 days of incubation for denitrification rate measurements that were conducted using the