Influence of hydrological conditions on bio-geochemical processes in a peatland

N. Bougon*[1, 2, 3], C. Auterives[1, 3], L. Aquilina[1, 3] , and P. Vandenkoornhuyse[2, 3]

[1]{Université Rennes 1-CNRS, CAREN Research federation - UMR 6118 Géosciences, Campus de Beaulieu, Avenue Leclerc, Rennes, France}

[2]{Université Rennes 1-CNRS, CAREN Research federation - UMR 6553 EcoBio, Campus de Beaulieu, Avenue Leclerc, Rennes, France}

[3]{Université Européenne de Bretagne, UEB (European University of Brittany)}

Correspondence to: L. Aquilina (**luc.aquilina@univ-rennes1.fr**)

Abstract:

Factors influencing the dynamics of nitrate and sulphate concentration observed in a south Normandy peatland were determined experimentally. The effects of high or low nitrate input, and oxic or anoxic conditions on microbial activity were investigated in bioreactors, using peat samples from field sites influenced by different hydrologic regimes. Site S, unlike site G, was characterized by the presence of hydrogeological gradients inducing water fluxes from stream to peat during most of the hydrological cycle. Peat samples from both sites were subjected to similar experimental conditions to distinguish between the chemical effects (NO_3^-, O_2 concentrations) and the physical effects (hydrologic regimes and peat soil moisture).

[Cl^-], [SO_4^{2-}] and [NO_3^-] were monitored for 240 hours. Nitrate was significantly reduced in most experiments: (1) Removal of 70% of the initial nitrate content after 51 hours under anoxic conditions; (2) Complete nitrate reduction after 240 hours in soil from the S site. This reduction was interpreted as heterotrophic denitrification. Sulphate monitoring revealed that 400 mg.L^{-1} were produced in peat from site S under aerobic conditions. Sulphate changes under anaerobiosis were not significant or, for samples from G, under any conditions. Clear differences in chloride content (deviance analysis, P<0.05), sulphate concentration and nitrate consumption dynamics (deviance analysis, P<0.0001) were observed between the G and S sites. Our results demonstrate that the rates of nitrate removal and sulphate production differ

between peat samples from sites subjected to different hydrological regimes, even under similar redox and nitrate conditions. This experimental approach highlights the effect of hydrological fluxes leading to modifications of microbial activity which are likely related to changes in microbial diversity.

1. Introduction

In wetlands, biologically active components (O, N, S, and Fe) are controlled by microbial processes, which in turn depend on redox conditions (Hedin et al. 1998; Bilanovic et al. 1999; Ostrom et al. 2002; Cannavo et al. 2004). Chemical conditions and the composition of microbial communities are thus intimately linked (Dassonville and Renault 2002; Stumm and Morgan 1996; Torsvik and Øvreås 2002). Abiotic factors such as pH, organic carbon and N-oxides content, temperature, bulk density, and soil textures can affect the activity and population dynamics of soil microorganisms (Clément et al. 2002; Cavigelli and Robertson 2000; Dommergues and Mangenot 1970; Knowles 1982; Nannipieri et al. 2003). The metabolic capabilities and interactions of microorganisms greatly influence biogeochemical processes. However, the complex interactions between microbial population ecology and abiotic factors are little understood at present.

Relationships between chemical conditions, microbial processes and physical parameters such as water fluxes have been addressed only recently (e.g. Chapelle 2000; Clément et al. 2002, 2003; Ginn et al. 2002; Vidon and Hill 2004). Such parameters are related to landscape physical structure and appear to be determinant in controlling the spatial variability of these biogeochemical processes (Burt and Pinay 2005; Clément et al. 2002, 2003; Duvall and Hill 2007; Fink and Mitsch 2007; Hedin et al. 1998; Hill et al. 2000; Packman et al. 2004; Sabater et al. 2003). Several hydrological models, focusing on the prediction of microbial transport and the effect of microbial mats on water fluxes in natural porous and high nutrient media, have been proposed (e.g. Ginn et al. 2002; Harvey et al. 1993; Murphy and Ginn 2000; Rockhold et al. 2004). However, few studies deal with unsaturated or variably saturated systems (Rockhold et al. 2004). Field studies on experimental (Phipps and Crumpton 1994), and natural wetlands (Day and Megonigal 1993) or associated to model analyses (Spieles and Mitsch 1999) highlighted the impact of hydrologic loads as flooding gradient on nitrate removal rates. Moreover even if highly water level fluctuation influenced temporary wetland system performing, the potential biogeochemical ability of the wetland will not be impaired (Ishida et al. 2006). To date, the effect of water fluxes on biological activities, independently

of redox conditions, has not been simultaneously addressed in both laboratory and field comprehensive studies.

Peatlands are specific ecosystems in which biological and chemical functioning is essentially maintained by hydrological aspects. Limited hydrological fluctuations can induce considerable modifications in the biotic composition, specific richness and productivity of the ecosystem (Mitsch and Gosselink 2000). Hydrological disturbances induced by human activities can have a severe impact on peatland functioning (e.g. Owen 1995). This may be important on a global scale since it can affect the key role of peatland as a carbon sink. Before the vulnerability of such wetlands can be assessed, the effects of hydrological fluctuations on the biogeochemical processes occurring in these environments need to be more thoroughly understood.

A previous study in peatland in western France (Auterives 2007; Auterives et al. 2009) revealed through field observations the sensitivity of biogeochemical cycles to hydrological fluxes and highlighted the potential influence of hydrologic conditions (soil moisture and water fluxes) on microbial activity. The aim of this study was thus to distinguish between physical effects induced by hydrological conditions (peat saturation, water fluxes) and chemical effects (nutrient availability) on microbial activity, and was focused on the activity of denitrifiers in peat soils. An experimental approach was used to understand the field observations, as regards S and N changes, and to isolate the physical and chemical factors which might influence the chemical trends. The overall reactivity of peat samples from field sites under distinct hydrologic regimes and different chemical dynamics was tested experimentally by subjecting these samples to similar nitrate and redox conditions. Our hypothesis was that the N and S changes observed during field monitoring (Auterives 2007) were not directly related to physical changes (moisture and water fluxes) but resulted from changes in microbial diversity which in turn induced modifications in bacterial activity. The experimental design is thus based on the following assumptions : (1) based on field hydrological monitoring, the peat samples from different sites corresponded to different hydrological conditions, especially water fluxes and peat moisture which were both higher in the site influenced by human activity. (2) The different hydrological regimes have modified the microbial communities. (3) These modifications have in turn induced changes in bacterial activity. In order to observe this potential different bacterial activity, peat samples from three different sites were subjected to similar chemical conditions (oxygen and/or nitrate addition).

The hydrological effect on peat reactivity was determined by statistical comparison of experiments performed under similar chemical conditions.

2. Material and methods

2.1. Field description

2.1.1. Geological setting

The peatland site, which developed after the last glacial period, is located in south Normandy, 49°15' N, 1°20'W, in the 'Marais du Cotentin et du Bessin' regional natural park. Peat thickness varies from 1 m close to the stream to 10 m in the center of the peatland. It is mainly covered by herbaceous plant communities. The peatland site is clearly located along the main streams, in relation to the discharge zones of a sand-aquifer. A thin (0.1 – 2.0 m) clay-rich layer is observed at the base of the peat. The aquifer consists of a sandy geological formation (Mio – Pliocene) filling an 80 m thick graben. This sand aquifer is highly permeable and the basin is pumped to supply drinking water. The hydrological conditions in the peat aquifer are locally modified by the wells used for pumping which indicates that the clay-rich layer does not acts as an impermeable barrier and that the wetland cannot be investigated without taking into account the sand-aquifer – wetland interactions.

2.1.2. Field monitoring

In order to investigate the sensitivity of wetlands to both climatic and anthropogenic influences, a complete hydrological monitoring was carried out from 2003 to 2005. The influence of hydrological conditions on peatland functioning was investigated by comparing wetland zones modified by pumping in the sand aquifer and non-modified zones. In this paper we highlight the major results from this previous study in order to compare the laboratory experiments to the field observations. Further details regarding hydrological or hydrochemical results and methodology can be found in Auterives (2007) and Auterives et al. (2009).

Two study sites were set up (Fig. 1). The first corresponded to a pumped site (site S), in front of a pumping station. The second was located one kilometer downstream, beyond the influence of the pumping station, and was used as a reference site (G site). The hydrological fluxes in the two sites were determined by monitoring the stream level by staff gauges and the groundwater levels with PVC piezometers which were set up at different depths (1.5 to 5 m) in the peat along a stream-peat transect and in the clay-rich layer at the base of the peat. The sand-aquifer piezometric monitoring was made with deep boreholes (80 – 100 m) which intersect the sandy aquifer in *pumping site S – left bank* and in *reference site G* (Fig.1).

Stream water level, sand, peat and clay groundwater level have been monitored along the 2-year period, from March 2003 to March 2005 with a time step of 2 or 3 weeks.

130 Peat hydraulic conductivity has been measured on field by the hydrostatic time lag method of Hvorslev (1951). 18 large permanent PVC pipes, 1.5 to 5 m depth, were tested once, 6 were tested twice and 26 temporary mini-piezometers covering the three sites were tested twice. The clay layer below the peat was tested independently using the same method but longer monitoring.

135 **2.1.3. Hydrologic budget**

A mass balance, corrected for water-level variations, was used to describe and quantify the peatland hydrological budget (Equation 1).

$$P - E = \pm Q_{stream} \pm Q_{sand} \pm Q_{peat} \qquad (1)$$

Precipitation (P) is an input and evapotranspiration (E) an output in the peatland water budget.
140 Q_{Stream} and Q_{Peat} are horizontal groundwater exchanged flows with the stream and the peatland respectively. Q_{Sand} is the vertical groundwater exchanged flow with the underlying aquifer through the basal clay layer. Q_{Stream}, Q_{Peat} and Q_{Sand} vary with time, they are either inputs or outputs depending on the hydrological conditions.

The mass balance was calculated on a peat section, from the stream and a more internal zone
145 of the peatland for the year 2004 (refer to Auterives et al. (2009) for further details). Each section is defined with one side along the stream and the others delimited along piezometers. This represents a section of 3150 m², 7320 m² and 3360 m² on the *pumping site S – left bank*, *pumping site S – right bank* and *reference site G* respectively. Groundwater flows, direction and rate, were calculated using Darcy´s law. QStream and QPeat, horizontal groundwater
150 flow, were calculated with the hydraulic conductivity K_{Peat} measured on field. Horizontal hydraulic gradient was deduced from the piezometric monitoring, water levels being measured on each side of the considered peat section. Q_{sand}, vertical groundwater flow through the clay layer, was calculated with the hydraulic conductivity of the clay-rich layer K_{Clay} from the field measurements and the vertical hydraulic gradient deduced from the
155 monitoring.

Meteorological data, precipitation and potential evapotranspiration were provided by MétéoFrance (Table 1). Precipitation data from Meteofrance are 914.6 mm in 2004 at the local meteorological station. Potential evapotranspiration from Meteofrance data in 2004 is 722.8 mm. According to the Turc formula, the actual E equals to 549.5 mm which is 76 % of

160   the potential E. Actual evapotranspiration estimation was controlled using a mass balance approach based on water table hydrograph (Freeze and Cherry 1979; Healy and Cook 2002). Both methods agreed within less than 10 %. This result was also confirmed using a chloride mass balance.

2.1.4. Field chemical monitoring

165   Water chemistry parameters were carried out every two months from 2003 to 2005 (Auterives 2007). Peat groundwater was collected from the PVC pipes. Physico-chemical parameters (pH, Eh, T°C) were measured on site with a field multiparameter WTW P4. Water was sampled, filtered (0.22 μm cellulose acetate filter capsule Sartorius) and analysed for Cl^-, SO_4^{2-}, and NO_3^- through ionic chromatography Dionex DX-120) in the Geosciences chemical

170   laboratory with an uncertainty below 5%.

2.2. Experimental batch design

Peat samples from three sites representing various hydrologic conditions (see the result section) were collected with an auger in March 2004 at the 2 sites, close to (2 meters) and distant from (100 m) the stream (Fig. 1). Samples were collected on both sides of the stream

175   (left and right banks) at pumping site S. The sampling depth varied from 50 to 80 cm. The chemical composition of the water used in the experiments was similar to that of the stream. Stream water, because of potential microbial content, was not used. The soil to water ratio ranged from 1/10 to 1/20. The same volume of water was added in each flask. The soil to water ratio is dependent on initial peat water content. The soil samples were stored at 4°C for

180   2 days before the experiment.

2.2.1. Peat sample characteristics.

The soil chemical profile shows that the variation in redox conditions was dependent on the distance to the stream (Table 2). During sampling, large roots were removed and the soil was

185   homogenized but not sieved in order to preserve soil microbial heterogeneity. pH was determined by AFNOR NF X 31-103 method (Table 2). C-N-S-O values were obtained with a CHNSO EA1108 Carlo-Erba apparatus.

2.2.2. Experimental procedure.

190   Changes in nitrate and sulphate concentrations were monitored for 240 hours under: *(i)* high and low nitrate input, *(ii)* oxic and anoxic conditions and *(iii)* biotic and abiotic control (Fig.

2). 30 g of wet soil was placed in serum flasks containing 100 ml of synthetic solution (40 mg.L^{-1} of Cl$^-$ as NaCl). No nitrate was added to the low nitrate samples. High nitrate concentrations were obtained by adding 30 mg.L^{-1} of NO$_3^-$ (as NaNO$_3$). The incubation conditions were either oxic (in an oxygen atmosphere) or anoxic (under a nitrogen atmosphere). Anaerobic conditions were ensured by flushing the ambient air in flasks 3 times with nitrogen. The flasks were shaken continuously throughout the experiment to ensure moderate homogeneity. One hour before sampling, the flasks were stirred to homogenize the water content and then left still for the particles to settle. Abiotic controls consisted of sterilized peat samples in which the bacterial enzymes had been metabolically inhibited by gamma ray irradiation so that the soil physical structure remained unaltered. Ionization, involving a 60 kGray or 6 mrads treatment, was carried out at the 'Commissariat à l'Energie Atomique' (CEA, Cadarache). The average dose of ionizing radiation required to inactivate a single colony forming unit is 30 Gray for *Escherichia coli* and around 6-7 kGray for *Deinococcus radiodurans* (Battista et al. 1999). Vegetative cells of *Bacillus* spp. cannot grow at 60 Gray and *Bacillus* spores show a 5-order-of-magnitude decrease in viability following acute exposure to 200 to 1,000 Gray (Thornley et al. 1965). All abiotic control samples were subjected to the same procedures as the other sample series (Fig.2). Potential variability related to peat heterogeneity was taken into account by performing triplicates for each experimental condition (nitrate addition, oxic or anoxic, sterilized…). All figures and tables from the batch experiment indicate the mean of the triplicate value and its standard error.

2.3. Chemical batch analyses

5 mL of solution was sampled from the flasks after 1, 9, 25, 76 and 240 hours of incubation. Three mL, sieved through 0.22 μm cellulose-acetate filters (Sartorius Minisart) were analyzed for major anions (Cl$^-$, SO$_4^{2-}$ and NO$_3^-$) by ionic chromatography (Dionex$^©$DX120) at the Caren-Geosciences Rennes laboratory. Uncertainty was less than 4%. The remaining 2 mL was used to determine microbial diversity (not addressed in this study; Bougon et al., 2009). Physico-chemical parameters were measured at the end of each experiment (Table 3). pH was measured with a precision of +/- 0.05 unit using a Sentix 50 electrode, calibrated with WTW standard solutions of known pH (4.01 and 7.00 at 25°C). Redox potential was determined using a platinum electrode (Mettler Pt 4805).

2.4. Statistical analysis

Nitrate, chloride and sulphate concentrations increased slightly in each bioreactor over the first 25 hours. Variation was related to soil pore water and added water equilibration

7

225   (solubilization effect). As the variations in nitrate and sulphate concentration were concomitant with those of chloride, biogeochemical mechanisms could be ruled out. This equilibration stage was therefore ignored in the following kinetic analyses and the zero time is henceforth defined as beginning after 25 hours, i.e. after equilibration. Furthermore, to permit comparison of the different experiments, which presented various concentrations after the first

230   25 hours, the variation of concentration is presented as the ratio to 'zero' concentration ($\Delta = C_t / C_0$ in %, with C_0 the concentration at hour 25) in all the figures from the batch experiment. Sulphate concentrations were corrected from chloride concentrations to distinguish the part of sulphate production from the diffusion one. The Δ SO_4^{2-}/Cl^- was obtained by the following equation: $\Delta = [((SO_4^{2-}{}_t - SO_4^{2-}{}_{t0}) / SO_4^{2-}{}_{t0}) / ((Cl^-{}_t - Cl^-{}_{t0})/Cl^-{}_{t0})]$;

235   with $[SO4^{2-}]_{t0}$ and $[Cl^-]_{t0}$ the concentrations at hour 25.

    The chloride, nitrate and sulphate concentrations for each site (reference site G and the S sites, near to and distant from the stream), for each set of conditions (added NO_3^- or not, oxic or anoxic), and for each time (25, 76 and 240 hours), were subjected to statistical analysis. As family-wise errors rate in the dataset followed a Poisson distribution, the linking function

240   used was the log transformation. Then, the data were analyzed using a Generalized Linear Model (GLM) implemented in *R*. A deviance analysis (effect on GLM when one term was removed) was performed to test *(i)* differences between the sites for each variable (Cl^-, SO_4^{2-} and NO_3^- concentrations) at each time; *(ii)* the possible effect of NO_3^- addition on the other variables, and *(iii)* the effect of oxic and anoxic conditions on the measured parameters.

245   Statistical analyses were performed using the chi-square test. When a term was significant, the contrasts (mean comparisons), i.e. interaction terms were subjected to *z* coefficient tests. This was feasible despite the normally asymptotic distribution of the beta coefficients. The significance of the results was confirmed by applying a Bonferroni correction. All these analyses were implemented using *R* (http://www.r-project.org/).

250 **3. Results**

3.1. Field observations

 The main results of the field hydrological and geochemical monitoring are presented here. Further details can be found in Auterives (2007) and Auterives et al. (2009).

3.1.1. Hydrological results

255 The two sites belong to the 'Marais du Cotentin' peatland which constitutes a large area (Fig. 1). This peatland presents an extremely flat surface which ranges between 4 to 5 meters above sea level. Therefore, no clear gradient parallel to the two main streams have been measured as can be seen in Fig. 3 which compares the water levels in two sites distant from 1.3 km. The upstream/downstream gradient remains negligible even during the low-flow period

260 (0.5/1300 m) as regards the stream/peat gradient (1/10-50 m). The peatland hydrology is thus mainly dominated by the exchanges between the peat and the main streams. The hydrogeological conditions exhibited by the two areas are clearly distinct (Fig.3).

Reference site G: Two hydrological regimes were observed. During high waters, the stream water level is above the peat groundwater level which decrease from the edge of the peatland

265 (close to the stream) to the inside part of the peatland. Stream water flows into the peat groundwater. During low water periods, the stream water level decreases but remains always above the peat groundwater level. However the peat groundwater level decreases from the inside part to the edge (close to the stream) of the peatland which makes fluxes from the stream to the peat impossible. Since groundwater flows cannot converge, stream and peat

270 groundwater systems appear locally disconnected. Such disconnection during the whole low-water period induces an important water table lowering in the peat (close to 1 m) (Fig. 3).

Pumping site S: The water table fell much less (less than 50 cm) in the S site, especially on the left bank (where pumping occurred) (Fig. 3). The water table is almost identical to the stream level during the high-water period. In contrast to site G, hydrogeological gradients

275 inducing water fluxes from stream to peat (water level into the peatland lower than close to the stream) were observed during most of the hydrological cycle (Fig. 3). S site is almost permanently supplied by stream surface water entering into the peatland.

Pumping in the sand aquifer resulted in lowering of the water table in the sand aquifer (almost 1.5 m). This decrease led to higher water fluxes from the peat to the sand aquifer especially

280 on the left bank of pumping site S. The relation between the peat aquifer and the sand-aquifer is confirmed by the permeability measurements. Peat permeability ranges from $2 \ 10^{-6} \ ms^{-1}$ to $1 \ 10^{-6}$ in the upper part of the peat to $2 \ 10^{-8}$ to $2 \ 10^{-9}$ in the lower part of the peat and the clay-rich layer. These values are high enough to allow fluid flow between the two aquifers.

The water budget for the peat in both sites, based on hydrogeological monitoring, was

285 computed (Table 1). We focus here on the water inputs of the peatland which can influence hydrological and/or hydrochemical conditions in the peatland. The peatland water inflow at pumping site S is related to precipitations ($0.915 \ 10^{-6} m^3$/year/m²) and to a permanent water

inflow ($0.128 \ 10^{-6} m^3$/year/m²) from the stream induced by vertical fluxes from the peat to the sand aquifer. The permanent inflow from the stream represents 9-12% of the total input. In contrast, the peatland water inflow at reference site G is mainly controlled by precipitations ($0.915 \ 10^{-6} m^3$/year/m²) which represent 99.3% of the total input. It was apparent from hydrological monitoring that the main differences in hydrological conditions were related to *i)* large fluxes from the stream through the peat at site S which maintains high peat water levels, and *ii)* limited fluxes from the stream which create long and considerable downward water movement and peat drying at site G.

3.1.2. Geochemical results

A two-year hydrogeological and hydrochemical monitoring programme (Auterives 2007) revealed that the peat groundwater was slightly acid (pH 5.5 - 7.5) and conductivity ranged from 200 to 600 $\mu S.cm^{-1}$. Redox potential varied according to the hydrological period, values exceeding 400mV/ESH throughout the high-water periods and indicating oxidized conditions. Oxygenation of peat groundwater is promoted by stream water flow into the peat, and water renewal. During the low-water periods, Eh fell below 200 – 300 mV/ESH and indicated moderately reduced conditions due to the slow flow of groundwater limiting oxygen renewal in the peat groundwater.

Chloride concentrations ranged from 15 to 40 $mg.L^{-1}$. A concentration gradient, dependent on the distance from the stream, was observed in site G with the highest concentration close to the stream.

Nitrate dynamics were dependent on hydrological conditions, NO_3^- concentrations being higher during high-water periods (5 to 25 $mg.L^{-1}$) than during low-water periods (0 to 10 $mg.L^{-1}$). A clear decrease in NO_3^- concentration was observed during low-water periods (Fig. 4).

SO_4^{2-} concentrations showed extremely high variations from 0 to 1200 $mg.L^{-1}$ (Fig. 4). Pulses of SO_4^{2-} related to pulses of H^+ (pH < 5) are observed after a desaturation / resaturation cycle. An increase in sulphate concentration was apparent after an increase in peat groundwater level. Such increases were located in two areas: on the left bank near the pumping well in site S and near the stream in reference site G. These areas correspond to maximum drying of the peat during low water. The SO_4^{2-} pulses result from a drying-rewetting effect (Devito and Hill 1999; Eimers et al. 2003; Auterives 2007). During washing out, the formerly reduced species which were oxidized during resaturation are again brought into solution.

320   The changes in nitrate and sulphate concentrations were clearly related to water table dynamics and reflected various redox conditions related to water saturation. However, the field results also showed an obvious variation between sites and with respect to the distance from the stream within each site (Fig. 4). An efficient nitrate removal in reference site G and pumping site S – right bank was observed. In pumping site S– left bank, above the abstraction

325   well, the nitrate removal was more limited than in the other sites. Sulphate was produced at high concentration (SO_4^{2-} > 100 mg.L^{-1}) throughout pumping site S – left bank, above the abstraction well. In reference site G, extremely high sulphate concentrations (> 1000 mg.L^{-1}) were observed at the beginning of high-water periods (peat-stream connection), close to the stream. At last, peat sampled close to the stream on the right bank of pumping site S also

330   presented relatively high sulphate concentrations (SO_4^{2-} ~ 20 mg.L^{-1}). The high sulphate concentrations on the right bank close to the stream were observed the chemical monitoring.

3.2. Batch results

3.2.1. Nitrate
A systematic decrease in nitrate concentration was observed under anaerobiosis (Fig. 5).

335   Nitrate reduction was complete at the end of the experiments in the S site samples. Maximal nitrate consumption occurred during the first 50 hours, reaching 70 % of the initial concentration under anaerobiosis. Nitrate consumption was more limited in samples from site G under aerobic conditions. Pumping site S (left bank – close to the stream) was still characterized by nitrate consumption even under aerobic conditions. However, nitrate

340   production was observed in aerobic conditions in pumping site S (left bank – distant from the stream). Nitrate consumption was greater in the nitrate-enriched solutions (Table 4, deviance analysis, P<0.0001). From 0 to 51 h, the estimated reduction in the bioreactor sample 'close to the stream, nitrate-enriched, under aerobic conditions' was 0.61 +/- 0.02 mg.L^{-1}.h^{-1} compared to 0.04 +/- 0.02 mg.L^{-1}.h^{-1} in the same soil without nitrate enrichment (Fig. 5). This is

345   representative of the mean of the difference observed between "with" and "without nitrate enrichment" in all the batches.

3.2.2. Sulphate
Increases in sulphate concentration were observed during several batch experiments (Figure 5). The sulphate increase was greater in samples collected close to the stream (Table 4,

350   deviance analysis, P<0.0001) and in samples subjected to aerobic conditions (deviance analysis, P<0.0001; Fig. 5, Fig. 6). Sulphate production in site S - left bank under aerobic

conditions was 300% (corresponding to Δ ($SO_4{}^{2-}{}_{t240h}$ - $SO_4{}^{2-}{}_{t0}$)= 400 mg.L^{-1}). This represents a sulphate concentration of 600 mg.L^{-1} at the end of the experiment. No significant sulphate production was observed during the same period under anaerobic conditions (Fig. 6) without nitrate addition.

3.2.3. Site comparison

A large increase in chloride concentration was observed at site G under aerobic conditions (deviance analysis, P<0.0001). More limited (from z coeff, P=0.03) and similar chloride concentration variations were respectively observed on the right and left banks of site S. Nitrate concentrations differed significantly between the S and G sites (deviance analysis, P<0.001; Table 5). Assuming that similar soil reactivities lead to similar reductions in nitrate concentration, the bacterial reaction kinetics at the S and G sites were different (Fig. 5). Without oxygen, the samples from the left and right banks of site S reacted more rapidly than those from site G. Although the observed variation during the experiment was not directly dependent on the peat sampling site (Table 5), the sulphate content also differed considerably between sites, the initial contents at reference site G being one order of magnitude lower than at pumping site S (Table: 4).

3.2.4. Distance from the stream

Differences in chloride content between peat sampled close to and distant from the stream were significant regardless of the site (Table 4). This was also true for the nitrate and sulphate contents. At the end of the experiment, samples 'close to' the stream in sites S had a higher sulphate concentration than those 'distant from' the stream whilst the reverse was observed in site G. Moreover, in the S sites at the beginning of the experiments, the concentrations in samples obtained 'close to' the stream were 3 times higher than in those 'distant from' the stream (Table 4). On the contrary, the sulphate concentrations in samples distant from the stream in reference site G, were higher than in those close to the stream.

3.3. Comparison of batch/field result

The batch experiment results accorded with the field observations. 1) The measured chloride variations were similar to the observed field concentrations; 2) Nitrate reduction was clearly reproducible, even under aerobic conditions, although this process was mainly expected under anaerobic conditions; 3) High sulphate concentrations were produced during some experiments; 4) Clear differences in chloride content (deviance analysis, P<0.05), sulphate concentration and nitrate consumption dynamics (deviance analysis, P<0.0001) were

observed between samples from the G and S sites; 5) Reactivity differed as a function of distance from the stream, as observed for chloride and sulphate concentrations; 6) Even under similar redox conditions and nitrate concentrations, nitrate removal and sulphate production rates differed between peat samples from sites subjected to different hydrological regimes.

4. Discussion

4.1. Nitrate removal

The observed reduction of nitrate concentration during batch experiments has already been reported in several studies. This phenomenon results from microbiological consumption, nitrate serving as electron acceptor (Correl 1997). The microbiological reduction of nitrates involves 3 types of processes: dissimilatory reduction, autotrophic and heterotrophic denitrification. Although nitrate-reducing microorganisms display a great plasticity to oxygen availability, most denitrifiers use nitrate as final electron acceptor under anoxic conditions (Florinski et al. 2004). The presence here of available dissolved organic carbon (> 30 mg.L^{-1}), moderately reduced redox conditions ($< 200 - 300$ mV) (Table 3), anoxic conditions and nitrate nutrients suggests a heterotrophic reduction process (Ingersoll and Baker 1998; Hedin et al. 1998; Hill et al. 2000; Vidon and Hill 2004). Nitrate removal is a major biological process. However part of this process may also interact with chemical reaction. The comparison of biotic and abiotic conditions (Fig. 7) indicates the importance of biological mediation in nitrate removal (deviance analysis, e.g. G site aerobic conditions: P<0.0001). It indicates however that the whole nitrate reduction cannot be assigned solely to biological activity.

Difference in nitrate removal could also be explained by DOC quantity and availability. Relationship between DOC quantity and quality/bio-availability is up to date a matter of debate (e.g. Jaffé at al. 2008; Cumberland and Baker 2007) and high DOC content might indicate low availability or high refractivity. Hydrological conditions may influence the DOC quantity and quality and oxygen availability (Sobczak and Findlay 2002; Vazquez et al. 2007; Peduzzi et al. 2008) and indirectly the nitrate respiration. It was shown that drought periods enhance a decrease in DOC concentration in peat waters (Clark et al. 2005) and probably an increase of aerobic conditions. Field monitoring showed differences in DOC quantity for similar peat soil material (Auterives 2007): (1) Site G presented higher DOC concentration (mean annual concentration: 77.2 +/- 12.6 mg.L^{-1}) than sites S (mean annual concentration: left bank: 34.5 +/- 17.8 mg. L^{-1} and right bank: 40.8 +/- 15.2 mg. L^{-1}). (2) DOC concentrations close to the stream were similar in sites S (sites S left bank: 19 +/- 5.5 mg.L-1 and right bank:

24.6 +/- 9.6 mg. L^{-1}) and highly variable in site G (site G: 34.4 +/- 19.4 mg. L^{-1}). The laboratory experiment showed slower nitrate removal with peat from site G. Assuming arbitrarily that DOC quantity is synonymous of DOC quality; we can hypothesize that peatland affected by alternating saturated to unsaturated conditions might show lower nitrate removal rates and higher NO_3 concentrations than permanently saturated peatland. Nitrate content was actually higher at the beginning of the experiment in site G than in site S (Table 4). Thus difference in nitrate removal can be explained by differences in microbial community and differences of carbon quality.

Denitrification was also observed under aerobic conditions. Various bacteria may activate this process (Chen et al. 2003) although denitrification is not as competitive as aerobic respiration in terms of energy produced. This phenomenon should be interpreted as an electron accepting mechanism that competes with aerobic respiration, providing an advantage in terms of fitness in a changing environment. Some oxygen-tolerant anaerobes are well adapted to survive oxygen stress, and are able to maintain a functional metabolism in presence of oxygen (Brune et al. 2000). Patureau and coauthors (2000) suggested that alternating aerobic-anoxic conditions can isolate new strains of aerobic denitrifiers, and that naturally aerobic denitrifiers may exist. Alternatively, the nitrate reduction observed under aerobic conditions could be due to localized development of micro-anaerobiosis even though the flasks were shaken.

4.2. Sulphates

Considerable sulphate production was observed (Fig. 5), especially under aerobic conditions. Some of these increases were related to chloride increases. The linear correlation between the S and Cl increases was interpreted as resulting from the diffusion of highly concentrated pore water during the experiments. Peat constitutes an excellent reservoir for chloride- and sulphate-enriched pore water. The effect of evapotranspiration on water derived from precipitation leads to higher concentrations in the pore water of the peat, especially in the upper layers. Furthermore, the drying-rewetting process (Devito and Hill 1999; Eimers et al. 2003) results in oxidation of the sulfur molecule and the accumulation of a sulphate pool within the peat matrix. The sulphate produced during the experiment and that diffused from the highly concentrated pore water, were distinguished by correcting the sulphate concentration for pore water sulphate content according to the chloride variations in Fig. 6. Sulphate concentrations were extremely high (close to 600 mg. L^{-1} at the end of the experiment, i.e. variation of 400 mg. L^{-1}; Fig. 5, Fig. 6) in peat samples collected from pumping site S close to the stream. Such concentrations are in good agreement with the

450 concentration observed *in situ* (up to 1200 mg. L^{-1}). Although a process of mixing with highly concentrated pore-water provided an important source of sulphate at the beginning of the batch experiments (Fig. 5), it is also apparent from Figure 6 that sulphate production occurred independently of pore water diffusion. The observed sulphate release in peat samples from site S close to the stream can result from (*i*) mineral and/or (*ii*) organic processes. Sulphates

455 released during the experiments were derived from the dissolution of mineral phases since the experiments performed under abiotic conditions indicated an important, non-biological sulphate-releasing process (Fig. 6). These results agree with other reports of sulphate release under oxidized conditions (Devito and Hill 1999; Eimers et al. 2003; Fenner et al. 2005). However, differences between the abiotic and biotic experiments could also be related to a

460 mediation of mineral sulfur dissolution by microorganisms, which could in turn affect the type of sulphates produced.

We conclude from the biotic/abiotic comparison that the release of sulphates cannot be attributed to a single process. The high sulphate concentrations result from the combination of chemical and biological processes.

4.3. Spatial variability
465

The inter- and intra-site variability observed in the field was reproduced in the laboratory under different experimental conditions. The importance of parameters such as the sampling site and distance from the stream was demonstrated statistically (Table 5).

Nitrate: Nitrate removal was clearly much more limited under aerobic conditions. This

470 conclusion agrees with the field observations which indicate nitrate removal after the high-water period, when more reducing conditions develop. However, under anaerobic conditions, peat from reference site G seemed to provide more efficient and rapid nitrate removal due to bacterial activity than peat from pumping site S. This effect was seen independently of nitrate concentrations or redox conditions which were similar in the different batchs (Tables 3 & 4).

475 This difference, which agrees with the site observations, argues for the initial hypothesis linking bacterial activity to microbial communities, influenced by the hydrologic conditions.

Sulphate: Sulphate concentrations in batch experiments showed a general agreement with the field data although some differences could be observed. Much more important sulphate release were related to samples close to the stream in the S site (Table 4, Fig. 6), as observed

480 on site, especially for the left bank. No sulphate decrease was observed directly (Table 4), however after correction for chloride variation (Fig. 6), slight decrease is observed in the S site – right bank. This result agrees with the field observations. It also favors the initial

hypothesis since this sulphate decrease is observed only in the right bank (Fig. 6) whilst both banks present high sulphate concentrations (Table 4). Part of the evolution observed could be related to microbial activity, influenced by hydrologic conditions and not chemical changes.

Peat from site G had lower initial sulphate content and lower release rates than site S (Tables 3 and 4). The low sulphate concentrations observed in the batch samples from the G site agree with the field data which also showed relatively low concentrations except immediately close to the stream. The sulphate peaks in the G are localized in time and space. However, no sulphate increases could be observed in batch samples close to the stream as observed in batch samples from site S, although extremely high sulphate concentrations could be observed on site during high-water period. We assume that the sulphate peak observed on site was related to a drying/rewetting effect more important in site G. The lack of stream-peat connection created an important draw down of the water table and high amplitude of moisture variation. This effect was not reproduced in the batch experiments. However, the batch experiments also indicate that no labile-sulphur stock is present in the peat. This observation may also indicate a global bacterial activity difference between the S and G site.

The different hydrological regimes induced different water fluxes in the investigated sites. Pumping in the underlying aquifer resulted in a permanent flow from the stream into the peat at site S. The amplitude of water table fluctuation and peat drying were also controlled by the underlying aquifer and exchanges with the stream. High water tables were maintained in site S for much of the year, (Auterives 2007; Auterives et al. 2009). The observed differences in sulphate release within and between sites highlight the importance of hydrological fluxes in controlling sulphate dynamics through the introduction of oxygen and emphasis of biological processes. These results agree with previous reports (Devito 1995; Devito and Hill 1999, Warren et al. 2001; Eimers et al. 2003) that sulphate release can be predicted from hydrologic heterogeneity, especially during periods of drought.

The influence of the distance from the stream on the biological productivity and the observed lower biological productivity in reference site G may be explained by the temporary nature of the stream - peat connection. This zone, between terrestrial and aquatic ecosystems, represents a major mixing point for nutrients (Hedin et al. 1998; Hill et al. 2000; Mc Clain et al. 2003) which allows the production of dissolved organic carbon (Hill et al. 2000; Mitchell and Branfireun 2005) and thus enhances bacterial activity. The nutrient availability can be considered a mainly 'chemical' effect.

4.4. Potential mechanisms for physical influence on biological activity

The batch experiments indicated potential differences that were independent of the nutrient availability (nitrate and/or oxygen supply). The potential effect of hydrological regime on the ecosystem is based on soil moisture and diffusion/advection processes.

Water fluxes and high moisture might influence microbial activity by creating an open ecological ecosystem. An ecosystem includes a high degree of variation under conditions such as water fluxes, for example, and different soil structures. Potential interactions between micro-organisms could be increased by diffusion and advection processes. The ecosystem, by integrating a wide range of conditions, might increase the structural complexity of microbial communities. Through these processes, hydrological fluxes also influence microbial activity in terms of substrate availability (Ostrom et al. 2002; Sabater et al. 2003; Sánchez-Pérez and Trémolières 2003). The differences in the microbial ecosystem might be induced directly by the effects of 'physical parameters' such as water fluxes.

This study highlights the considerable effect of hydrological conditions on biological activity in peat. Hydrological fluxes, in addition to providing stimulating physico-chemical conditions for biotic activity, may also provide more diverse substrate availability which may also benefit from favorable physico-chemical conditions. The biochemical conditions created by a hydrological flow structure will facilitate the development of hot spots (Hill et al. 2000; Mc Clain et al. 2003). Thus the observed differences between sites and the spatial variability within sites may reflect the heterogeneous richness and diversity of microbial species in the ecosystem (Martin et al. 1999). Indeed, it can be seen from the experimental design that the observed differences between sites are not entirely controlled by variations in redox conditions and nutrient supply and that reactivity is also a result of the actual biological community structure. The observed differences between sites, even under similar redox and nutrient conditions, indicate that the distinct hydrological fluxes can control the structure of the associated microflora.

We conclude from the batch experiments that hydrological conditions can deeply and permanently influenced the structure, heterogeneity and diversity of microbial communities. A complementary survey of the *narG* gene by T-RFLP (Terminal Restriction Fragment Length Polymorphism) analyses, a method generating diversity signatures from environmental DNA samples (e.g. Liu et al. 1997, Vandenkoornhuyse et al. 2003), was also undertaken during the batch experiments. The nitrate reducers' communities were very similar at the beginning of the experiment whatever the peat-soil analyzed. A strong structuration and divergence within the nitrate reducers was evident after 76 hours of

incubation and was shown to be site dependent. This modification of community was attributed to the difference in peat saturation resulting from different hydrological regime. These clear differences in bacterial community structure confirm the biogeochemical interpretation (Bougon et al. 2009). Physical conditions such as hydrological regime should thus be considered as having a direct effect on biological communities and biological activities.

5. Conclusion

Monitoring of field peat presenting variable hydrological conditions revealed distinctly different chemical concentrations. The influence of hydrological factors on biogeochemical reactivity was investigated by experimental reproduction of various redox and nitrate concentrations in soil sampled from sites under different hydrological conditions. The experimental results confirmed the field observations. Comparisons performed under abiotic and biotic conditions to determine the origin of the observed processes, showed that nitrate reduction was related to heterotrophic denitrification. Extremely high sulphate concentrations (close to 600 mg. L^{-1}) observed in some experiments resulted from a combination of biological (peat mineralization) and chemical (mineral sulfur oxidation) processes.

The clear differences between the samples from the selected sites highlighted the effects of hydrological regime which likely impacted the development of specific ecosystem structures and diversity. The chemical variations observed in the field are not only controlled by physico-chemical conditions. Microbial reactivity also suggests changes within the microbial community structure which have been deeply modified by permanent hydrological fluxes.

Acknowledgments

References

Auterives, C.: Influence des flux d'eau souterraine entre une zone humide superficielle et un aquifère profond sur le fonctionnement hydrochimique des tourbières, Ph.D., *Mémoires du*

580   *CAREN*, 17, 261 pp., 2007. On line at the following address, http://tel.archives-ouvertes.fr/docs/00/13/77/57/PDF/Auterives_PhD.pdf

Auterives, C., Aquilina, L. and Davranche, M.: Hydrologic Sensitivity of a Peatland to the anthropogenic or climatic groundwater flow variations of a Regional Aquifer, (Submitted to Hydrological processes)

585   Battista, J.R., Earl, A.M. and Prak, M-J.: Why is Deinococcus radiodurans so resistant to ionizing radiation? Trends Microbiol., 7, 362-365, 1999.

Bilanovic, D., Battistoni, P., Cecchi, F., Pavan, P. and Mata-Alvare, J.: Denitrification under high nitrate concentration and alternating anoxic conditions, Water Res., 3, 3311 – 3320, 1999.

590   Bougon, N., Aquilina L., Briand, M.P., Coedel S. and Vandenkoornhuyse, P.: Influence of hydrological fluxes on the structure of nitrate-reducing bacteria communities in a peatland, Soil Biol. Biochem, 41, 1289 – 1300, 2009.

Brune, A., Frenzel, P. and Cypionka, H.: Life at the oxic-anoxic interface: microbial activities and adaptations, FEMS Microbial. Rev., 29, 691 – 710, 2000.

595   Burt, T.P. and Pinay, G.: Linking hydrology and biogeochemistry in complex landscapes, Prog. Phys. Geog., 29, 297 – 316, 2005.

Cannavo, P., Richaume, A. and Lafolie, F.: Fate of nitrogen and carbon in the vadose zone: in situ and laboratory measurements of seasonal variations in aerobic respiratory and denitrifying activities, Soil Biol. Biochem, 36, 463 – 478, 2004.

600   Cavigelli, M.A. and Robertson, G.P.: The functional significance of denitrifier community composition in a terrestrial ecosystem, Ecology, 81, 229 – 241, 2000.

Chapelle, F.H. : The significance of microbial processes in hydrogeology and geochemistry, Hydrogeol. J., 8, 41-46, 2000.

Chen, F., Xia, F. and Ju, L.K.: Aerobic denitrification of *Pseudomonas aeruginosa* monitored

605   by online NAD(P)H Fluorescence, Appl. Environ. Microb., 69, 6715 – 6722, 2003.

Clark, J.M., Chapman, P.J., Adamson, J.K. and Lane, S.N.: Influence of drought-induced acidification on the mobility of dissolved organic carbon in peat soils, Glob. Change Biol., 11, 791 – 809, 2005.

610 Clément, J.C., Pinay, G. and Marmonier, P.: Seasonal dynamics of denitrification along topohydrosequences in three different riparian wetlands, J. Environ. Qual.,31, 1025–37, 2002.

Clément, J.C., Aquilina, L., Bour, O., Plaine, K., Burt, T.P. and Pinay G.: Hydrological flowpaths and nitrate removal rates within a riparian floodplain along a fourth-order stream in Brittany (France), Hydrol. Process., 17, 1177-1195, 2003.

615 Correl, D.L.: Buffer zones and water quality protection: General principles. In: Naycock, N.E., Burt, T.P., Goulding, K.W., Pinay, G. (Eds.): Buffer Zones: Their Processes and Potential in Water Protection, Quest Environment, Hertfordshire, pp. 7–20, 1997.

Cumberland, S.A. and Baker, A.: The freshwater dissolved organic matter fluorescence-total organic carbon relationship, Hydrol. Processes, 21, 2093 – 2099, 2007.

620 Dassonville, F. and Renault, P.: Interactions between microbial processes and geochemical transformations under anaerobic conditions: a review, Agronomie, 22, 51–68, 2002.

Day, F.P. and Megonigal, J.P.: The relationship between variable hydroperiod, production allocation, and belowground organic turnover in forested wetlands, Wetlands, 13, 115 - 121, 1993.

625 Devito, K.J.: Sulphate mass balances of Precambrian Shield wetlands: The influence of catchment hydrogeology, Can. J. Fish Aqua Sci., 52, 1750–1760, 1995.

Devito, K.J. and Hill, A.R.: Sulphate Mobilization and Pore Water Chemistry in Relation to Groundwater Hydrology and Summer Drought in two Conifer Swamps on the Canadian Shield, Water Air Soil Poll., 113, 97-114, 1999.

630 Dommergues, Y. and Mangenot, F.: Ecologie microbienne du sol. Masson éditeur, Paris, 1970.

Duval, T.P. and Hill, A.R.:Influence of base flow stream bank seepage on riparian zone nitrogen biogeochemistry, Biogeochemistry, 85, 185 – 199, 2007.

Eimers, M.C., Dillon, P.J., Schiff, S.L. and Jeffries, D.S.: The effects of drying and re-wetting and increased temperature on sulphate release from upland and wetland material, Soil Biol. Biochem., 35, 1663-1673, 2003.

Fenner, N., Freeman, C. and Reynolds, B.: Hydrological effects on the diversity of phenolic degrading bacteria in a peatland: implications for carbon cycling, Soil Biol. Biochem., 37, 1277-1287, 2005.

Florinsky, I.V., McMahon S. and Burton D.L.: Topographic control of soil microbial activity a case study of denitrifiers, Geoderma, 119, 33 – 53, 2004.

Fink, D.F. and Mitsch, W.J.: Hydrology and nutrient biogeochemistry in a created river diversion oxbow wetland, Ecol. Eng., 30, 93 – 102, 2007.

Freeze, R.A. and Cherry, J.A.: Groundwater, Prentice-Hall, Englewood Cliffs, New Jersey, US, 1979.

Ginn, T.R., Wood, B.D., Nelson, K.E., Scheibe T.D., Murphy, E.M. and Clement, T.P.: Processes in microbial transport in the natural subsurface, Adv. Water Resour., 25, 1017-1042, 2002.

Harvey, R.W., Kinner, N.E., MacDonald, D., Metge, D.W. and Bunn, A.: Role of physical heterogeneity in the interpretation of small-scale laboratory and field observations of bacteria, microbial-sized microsphere, and bromide transport through aquifer sediments, Adv. Water Resour., 29, 2713-2721, 1993.

Healy, R.W. and Cook, P.G.: Using groundwater levels to estimate recharge, Hydrogeol. J., 10, 31-109, 2002.

Hedin, L.O., von Fischer, J.C., Ostrom, N.E., Kennedy, B.P., Brown, M.G. and Robertson, G.P.: Thermodynamic constraints on nitrogen transformations and other biogeochemical processes at soil – stream interfaces, Ecology, 79, 684 – 703, 1998.

Hill, A.R., Devito, K.J., Campagnolo, S. and Sanmugadas, K.: Subsurface denitrification in a forest riparian zone: interactions between hydrology and supplies of nitrate and organic carbon, Biogeochemistry, 51, 193–223, 2000.

Hvorslev, M.J.: Time lag and soil permeability in ground-water observations, U.S. Army Corps of Engineers Waterway Experimentation Station. Bull., 36, Vicksburg, Mississippi, 50 pp., 1951.

21

Ingersoll, T.L. and Baker, L.A.: Nitrate removal in wetland microcosms, Water Res., 32, 677 – 684, 1998.

Ishida, C.K., Kelly, J.J. and Gray, K.A.: Effects of variable hydroperiods and water level fluctuations on denitrification capacity, nitrate removal, and benthic-microbial community structure in constructed wetlands, Ecol. Eng., 28, 363-373, 2006.

Jaffé, R., McKnight, D., Maie, N., Cory, R., McDowell, W.H. and Campbell, J.L.:Spatial and temporal variations in DOM composition in ecosystems: The importance of long-term monitoring of optical properties, J. Geophys. Res., 113, G04032, doi:10.1029/2008JG000683, 2008.

Knowles, R.: Denitrification, Microbiol. Mol. Biol. R., 46, 43-70, 1982.

Liu, W.T., Marsh, T.L., Cheng, H. and Forney, L.J.: Characterization of microbial diversity by determining terminal restriction fragment length polymorphisms of genes encoding 16S rDNA, Appl. Environ. Microb., 63, 4516 – 4522, 1997.

Martin, T.L., Trevors, J.T. and Kaushik, N.K.: Soil microbial diversity, community structure and denitrification in a temperate riparian zone, Biodivers. Conserv., 8, 1057-1078, 1999.

McClain, M.E., Boyer, E.W., Dent, C.L., Gergel, S.E., Grimm, N.B., Groffman, P.M., Hart, S.C., Harvey, J.H., Johnston, C.A., Mayorga, E., McDowell, W.H. and Pinay, G.: Biogeochemical Hot Spots and Hot Moments at the Interface of Terrestrial and Aquatic Ecosystems, Ecosystems, 6, 301–312, 2003.

Mitchell, C.P.J. and Branfireun, B.A.: Hydrogeomorphic Controls on Reduction– Oxidation Conditions across Boreal Upland–Peatland Interfaces, Ecosystems, 8, 731-747, 2005.

Mitsch, W.J. and Gosselink, J.G.: Wetlands, John Wiley & Sons, Inc, New York, 920pp, 2000.

Murphy, E.M. and Ginn, T.R.: Modeling microbial processes in porous media, Hydrogeol. J., 8, 142-158, 2000.

Nannipieri, P., Ascher, J., Ceccherini, M.T., Landi, L., Pietramellara, G. and Renella, G.: Microbial diversity and soil functions, Eur. J. Soil Sci., 54, 655 – 670, 2003.

Ostrom, N.E., Hedin, L.O., von Fischer, J.C. and Robertson G.P.: Nitrogen transformations and NO3- removal at a soil stream interface: A stable isotope approach, Ecol. Appl., 12, 1027 – 1043, 2002.

22

Owen, C.R.: Water budget and flow patterns in an urban wetland, J. Hydrol., 169,171-187, 1995.

Packman, A.I., Salehin, M. and Zaramella, M.: Hyporheic exchange with gravel beds: basic hydrodynamic interactions and bedform-induced advective flows, J. Hydraul. Eng., 130, 647–656, 2004.

Patureau, D., Zumstein, E., Delgenes, J.P. and Moletta, R. :Aerobic Denitrifiers Isolated from Diverse Natural and Managed Ecosystems, Microb. Ecol., 39, 145–152, 2000.

Peduzzi, P., Aspetsberger, F., Hein, T., Huber, F., Kargl Wagner, S., Luef, B. and Tachkova, Y.: Dissolved organic matter (DOM) and bacterial growth in floodplains of the Danube River under varying hydrological connectivity, Fundam. Appl. Limnol., 171, 49 – 61, 2008.

Phipps, R.G. and Crumpton, W.G.: Factors affecting nitrogen loss in experimental wetlands with different hydrologic loads, Ecol. Eng., 3, 399-408, 1994.

Rockhold, M.L., Yarwood, R.R. and Selker, J.S.: Coupled microbial and transport processes in soil, Vadose Zone J., 3, 368-383, 2004.

Sabater, S., Butturini, A., Clement, J.C., Burt, T., Dowrick, D., Hefting, M., Maître, V., Pinay, G., Postolache, C., Rzepecki, M. and Sabater, F.: Nitrogen Removal by Riparian Buffers along a European Climatic Gradient: Patterns and Factors of Variation, Ecosystems 6, 20–30, 2003.

Sánchez-Pérez, J.M. and Trémolières, M. : Change in groundwater chemistry as a consequence of suppression of floods: the case of the Rhine floodplain, J. Hydrol., 270, 89– 104, 2003.

Sobczak, W.V. And Findlay, S.: Variation in bioavailability of dissolved organic carbon among stream hyporheic flowpaths, Ecology, 83, 3194 – 3209, 2002.

Spieles, D.J. and Mitsch, W.J.: The effects of season and hydrologic and chemical loading on nitrate retention in constructed wetlands: A comparison of low- and high-nutrient riverine systems. Ecol. Eng., 14, 77 - 91, 1999.

Stumm, W. and Morgan, J.J.: Aquatic chemistry, Chemical equilibria and rates in natural waters, John Wiley and sons, New York, USA, 1996.

Thornley, M.J., Horne, R.W. and Glauert, A.M.: The fine structure of ***Micrococcus radiodurans***, Arch. Mikrobiol., 51, 267-289, 1965.

725   Torsvik, V. and Øvreås, L.: Microbial diversity and function in soil: from genes to ecosystems, Curr. Opin. Microbiol., 5, 240–245, 2002.

Vandenkoornhuyse, P., Ridgeway, K., Watson, I.J., Fitter A.H. and Young, J.P.W.: Co-existing grass species have distinctive arbuscular mycorrhizal communities, Mol. Ecol., 12, 3085-3095, 2003.

730   Vázquez, E., Romaní, A.M., Sabater, F. and Butturini, A.: Effects of the Dry–Wet Hydrological Shift on Dissolved Organic Carbon Dynamics and Fate Across Stream–Riparian Interface in a Mediterranean Catchment, Ecosystems, 10, 239 – 251, 2007.

Vidon, P.G.F. and Hill, A.R.: Landscape controls on the hydrology of stream riparian zones, J. Hydrol., 292, 210–228, 2004.

735   Warren, F.J., Waddington, J.M., Bourbonniere, R.A. and Day, S.M.: Effect of drought on hydrology and sulphate dynamics in a temperate swamp, Hydrol. Process., 15, 3133-3150, 2001.

The R project for statistical computing: http://www.r-project.org/, access : march 2003.

Tables

740 **Table 1:** Hydrological budget of the two *pumping sites S* and the *reference site G for* the year 2004 (Auterives et al., 2009; Auterives, 2007).

Hydrologic component	Pumping site S - right bank	Pumping site S - left bank	Reference site G
	Absolute value $(10^{-6}m^3/year/m^2)$	Absolute value $(10^{-6}m^3/year/m^2)$	Absolute value $(10^{-6}m^3/year/m^2)$
INPUT			
Rainfall	0.915	0.915	0.915
Q_{stream}	0.092	0.128	0.005
Q_{peat}	0.001	0.027	0.001
Total Inflow	1.007	1.070	0.921
OUTPUT			
Evapotranspiration	0.550	0.550	0.550
Q_{stream}	0.003	0.006	0.000
Q_{peat}	0.012	0.007	0.001
$Q_{vertical\ flow}$	0.444	0.504	0.377
Total Outflow	1.008	1.067	0.928

Table 2 : Soil characteristics and CHNSO content

Sampling sites	Stream distance	Soil profile	pH	% C	% S	% N	% O
Pumping site S Right Side	Close	0 to 15 cm : soil 15 to 50 cm : ballast + 50 cm : peat	4.5	44.9 ± 1.7	1.0 ± 0.0	2.21 ± 0.11	31.8 ± 0.6
	Distant	0 to 15 cm : soil 15 to 50 cm : ballast 50 cm : peat	5	31.3 ± 2.	0.3 ± 0.1	2.2 ± 0.2	21.0 ± 1.6
Pumping site S Left Side	Close	0 to 15 cm : peaty soil 15 to 50 cm : clay loam oxidized 50 cm : peat	4.7	26.6 ± 1.18	0.3 ± 0.0	1.9 ± 0.0	18.9 ± 0.2
	Distant	0 to 10 cm : peaty soil 10 to 30 cm : clay loam oxidized 30 cm : peat	4.4	32.9 ± 0.8	0.5 ± 0.0	2.3 ± 0.0	22.1 ± 0.3
Reference G site	Close	0 to 15 cm : soil 15 to 50 cm : ballast 50 cm :peat	5.8	40.1 ± 3.1	0.5 ± 0.1	1.9 ± 0.0	15.4 ± 1.2
	Distant	0 to ∞ cm : peat	5.2	24.7 ± 1.5	ND	2.5 ± 0.2	27.6 ± 2.4

745

Table 3 : Physico - chemical parameters at the end of experiments.

Sampling Site	Oxygenation condition	Physico Chemical parameters			
		pH	T°C	Eh corrected (standard)	pε
Pumping site S Right bank					
Close					
	Anaerobiosis	5.1	25.9	175	0.306
	Aerobiosis	4.1	20.3	250	0.444
Distant					
	Anaerobiosis	5.4	26	171	0.299
	Aerobiosis	5.1	20.3	200	0.355
Pumping site S Left bank					
Close					
	Anaerobiosis	5.3	25.8	171	0.300
	Aerobiosis	3.7	20.3	179	0.318
Distant					
	Anaerobiosis	5	26.9	126	0.219
	Aerobiosis	4.5	20.3	204	0.363
Reference site G					
Close					
	Anaerobiosis	6.7	25.6	113	0.198
	Aerobiosis	6.1	20.3	208	0.371
Distant					
	Anaerobiosis	5.6	25.8	144	0.252
	Aerobiosis	5.3	20.3	167	0.297

750

Table 4 : Mean chloride, nitrate and sulphate concentrations after the mixing effect, at 25h and at the end of the experiment, at 240h. Each concentration indicated represents the mean of 3 replicates with the standard error.

Sampling sites	Stream distance	Oxygenation conditions	Nitrate inputs	Chloride concentrations (mg.L^{-1})		Nitrate concentrations (mg.L^{-1})		Sulphate concentrations (mg.L^{-1})	
				at 25h	at 240h	at 25h	at 240h	at 25h	at 240h
Pumping Site S - Right Side	Close	Anaerobiosis	with	37.8 ± 2.2	42.0 ± 3.4	23.6 ± 0.9	0.0 ± 0.1	176.4 ± 9.5	196.5 ± 4.3
			without	38.4 ± 0.6	47.5 ± 22.0	0.0 ± 0.0	0.0 ± 0.0	187.1 ± 5.4	225.1 ± 109.3
		Aerobiosis	with	45.6 ± 4.9	43.8 ± 1.6	25.5 ± 12.6	11.8 ± 2.1	201.6 ± 29.1	471.2 ± 38.1
			without	43.2 ± 1.5	46.6 ± 1.0	0.0 ± 0.0	0.0 ± 0.0	196.6 ± 5.4	581.4 ± 173.5
	Distant	Anaerobiosis	with	42.8 ± 1.0	46.9 ± 20.	29.5 ± 0.5	0.0 ± 0.0	63.31 ± 0.6	78.2 ± 3.2
			without	50.2 ± 6.8	43.4 ± 1.8	2.3 ± 0.5	0.0 ± 0.0	83.2 ± 11.6	81.7 ± 7.1
		Aerobiosis	with	45.8 ± 4.5	48.5 ± 1.4	31.4 ± 2.3	6.2 ± 1.0	68.2 ± 15.6	107.6 ± 30.1
			without	44.3 ± 3.3	51.1 ± 3.6	2.6 ± 0.2	1.6 ± 0.7	69.5 ± 4.35	136.7 ± 32.1
Pumping Site S - Left Side	Close	Anaerobiosis	with	44.9 ± 1.8	46.1 ± 3.6	24.7 ± 1.8	0.1 ± 1.2	194.6 ± 17.2	215.7 ± 19.2
			without	47.7 ± 2.3	49.5 ± 0.2	0.2 ± 0.4	0.0 ± 0.1	212.2 ± 6.8	241.5 ± 8.2
		Aerobiosis	with	54.7 ± 14.2	52.5 ± 4.6	38.9 ± 10.4	2.6 ± 1.9	152.7 ± 60.5	477.8 ± 37.6
			without	47.0 ± 0.7	51.9 ± 2.8	6.2 ± 1.7	0.0 ± 0.1	236.7 ± 9.9	680.3 ± 41.0
	Distant	Anaerobiosis	with	43.7 ± 1.4	41.2 ± 4.2	29.0 ± 1.6	0.0 ± 0.1	67.5 ± 3.9	75.1 ± 1.0
			without	42.3 ± 2.4	45.6 ± 3.0	0.6 ± 0.1	0.0 ± 0.0	73.8 ± 9.1	82.7 ± 7.7
		Aerobiosis	with	46.2 ± 6.9	49.2 ± 3.1	29.4 ± 0.6	37.1 ± 3.5	63.7 ± 4.3	78.4 ± 1.4
			without	43.9 ± 2.7	48.4 ± 0.9	1.6 ± 0.1	8.5 ± 2.3	75.3 ± 7.4	75.2 ± 24.7
Reference site G	Close	Anaerobiosis	with	44.9 ± 1.6	46.1 ± 1.3	40.2 ± 0.1	0.0 ± 0.0	7.1 ± 0.6	13.0 ± 1.0
			without	46.5 ± 2.0	48.9 ± 1.1	10.9 ± 0.4	0.0 ± 0.0	6.7 ± 0.4	12.2 ± 0.5
		Aerobiosis	with	54.7 ± 1.9	52.5 ± 3.9	44.6 ± 3.8	20.3 ± 11.2	5.6 ± 0.2	19.5 ± 3.8
			without	31.3 ± 0.3	60.5 ± 4.6	9.3 ± 8.0	10.0 ± 2.6	3.7 ± 3.2	18.9 ± 3.8
	Distant	Anaerobiosis	with	43.7 ± 0.7	41.2 ± 0.4	29.7 ± 0.6	0.0 ± 0.0	33.5 ± 0.4	39.7 ± 0.5
			without	40.1 ± 0.6	44.8 ± 0.9	0.8 ± 1.5	0.0 ± 0.0	33.1 ± 0.8	39.4 ± 0.2
		Aerobiosis	with	46.2 ± 7.4	49.2 ± 0.34	31.4 ± 5.8	12.5 ± 1.3	30.5 ± 5.1	45.5 ± 0.7
			without	38.9 ± 10.4	51.3 ± 1.8	4.4 ± 0.8	5.9 ± 0.9	30.5 ± 8.3	45.9 ± 2.5

755

Table 5 : Effects of experimental and site parameters on batch variation. The results of GLM (P-values and significance) for nitrate (NO_3^-), chloride (Cl^-) and sulphate (SO_4^{2-}) concentrations are presented.

Source	DF	P-values		
		NO_3^-	SO_4^{2-}	Cl^-
Treatment*	3	1.09×10^{-233} ***	2.217×10^{-69} **	-
Time*	4	1.64×10^{-56} ***	0 ***	3.99×10^{-26} ***
Site*	2	3.42×10^{-15} ***	0 ***	1.68×10^{-2} *
Distance*	1	0.01 **	0 ***	8.82×10^{-4} **

*Z<0.05, **Z<0.001, ***Z<0.0001; DF = degrees of freedom

760   * 'treatment' represents nitrate input and oxygenation condition; 'time' represents different times of sampling; 'site' represents the 3 sampling sites and distance represents the proximity to and distance from the stream
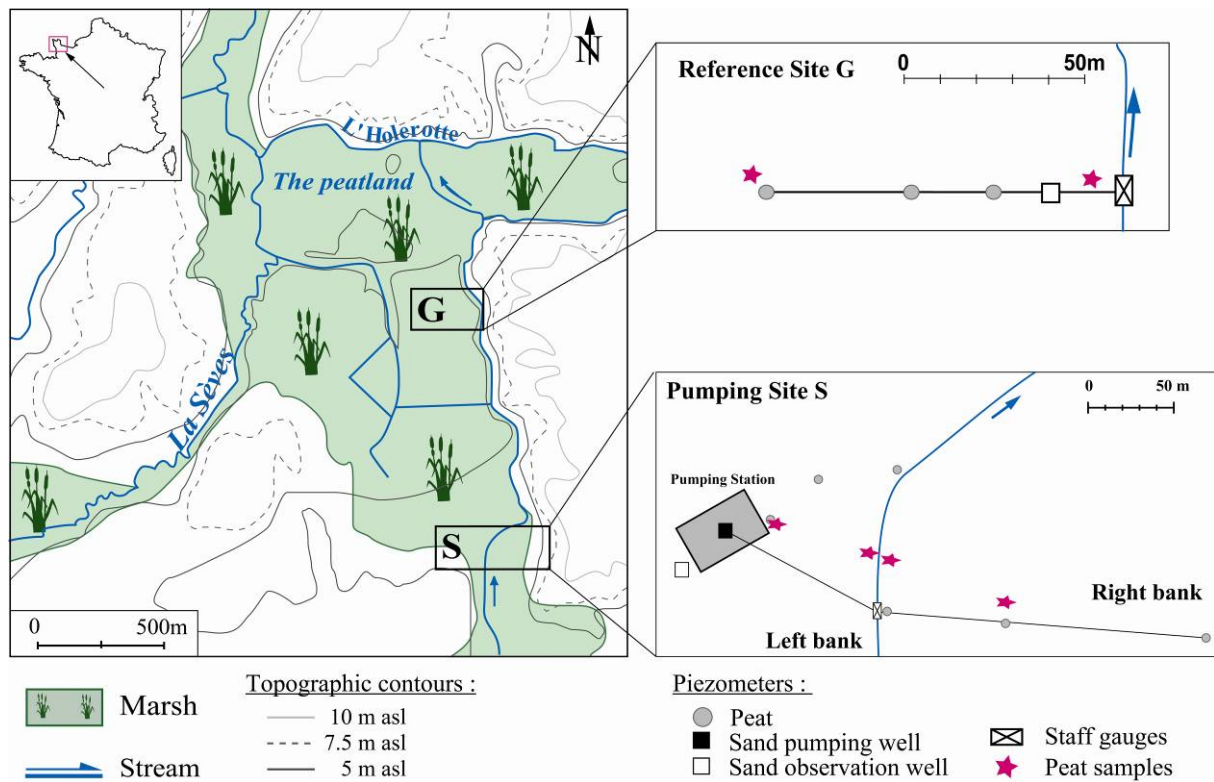
765

Figure captions

Figure 1 : Location of the Carentan site (modified from Auterives 2007) and piezometer map of the pumped site S, in the front of a pumping station, and the reference site G, one kilometer downstream, beyond thez influence of the pumping station.

770 **Figure 2 :** Experimental design. 'Anaerobic condition' indicates that the ambient atmosphere is changed to N_2. '+ nitrate' or '- nitrate' corresponding to addition or non addition of nitrate in the flask. 'Control' corresponds to sterilized samples. 3 samples are run for each experimental procedure to allow statistical analysis.

Figure 3 : Water level fluctuations during a 2-year period in reference site G, and pumping

775 site S - left bank (modified from Auterives et al. 2009). Schematic peatland profiles are an interpreted view of the peatland/stream water flow relationships (Auterives et al. 2009).

Figure 4 : Nitrate and sulphate concentration dynamics during a 2-year period according to the sites and the distance to the stream (modified from Auterives 2007). Blue-grey parts represent the high water period ranging over the period from October until February.

780 **Figure 5 :** Variation in nitrate and sulphate concentrations over time in peat samples under aerobic and anaerobic conditions in batch experiments. The values given for each sample correspond to the mean of the 3 replicates. The temporal variation is expressed as the difference from the zero concentration (see 3.3. statistical analysis). Bars indicate standard deviation. Only experiments with nitrate addition are represented.

785 **Figure 6 :** Variation in sulphate concentrations over time in peat samples under aerobic and anaerobic conditions in batch experiments. Sulphate concentration corrected for pore water sulphate content and chloride variations. Values given for each sample correspond to the mean of the 3 replicates. Bars indicate standard deviation.

Figure 7 : Comparison of biotic and abiotic changes according to nitrate and sulphate

790 concentrations throughout the batch experiment. Data correspond to experiments using samples distant from the stream and with nitrate addition.

Figure 1 : Location of the Carentan site (modified from Auterives 2007) and piezometer map of the pumped site S, in the front of a pumping station, and the reference site G, one kilometer downstream, beyond the influence of the pumping station. Asl : Above Sea Level
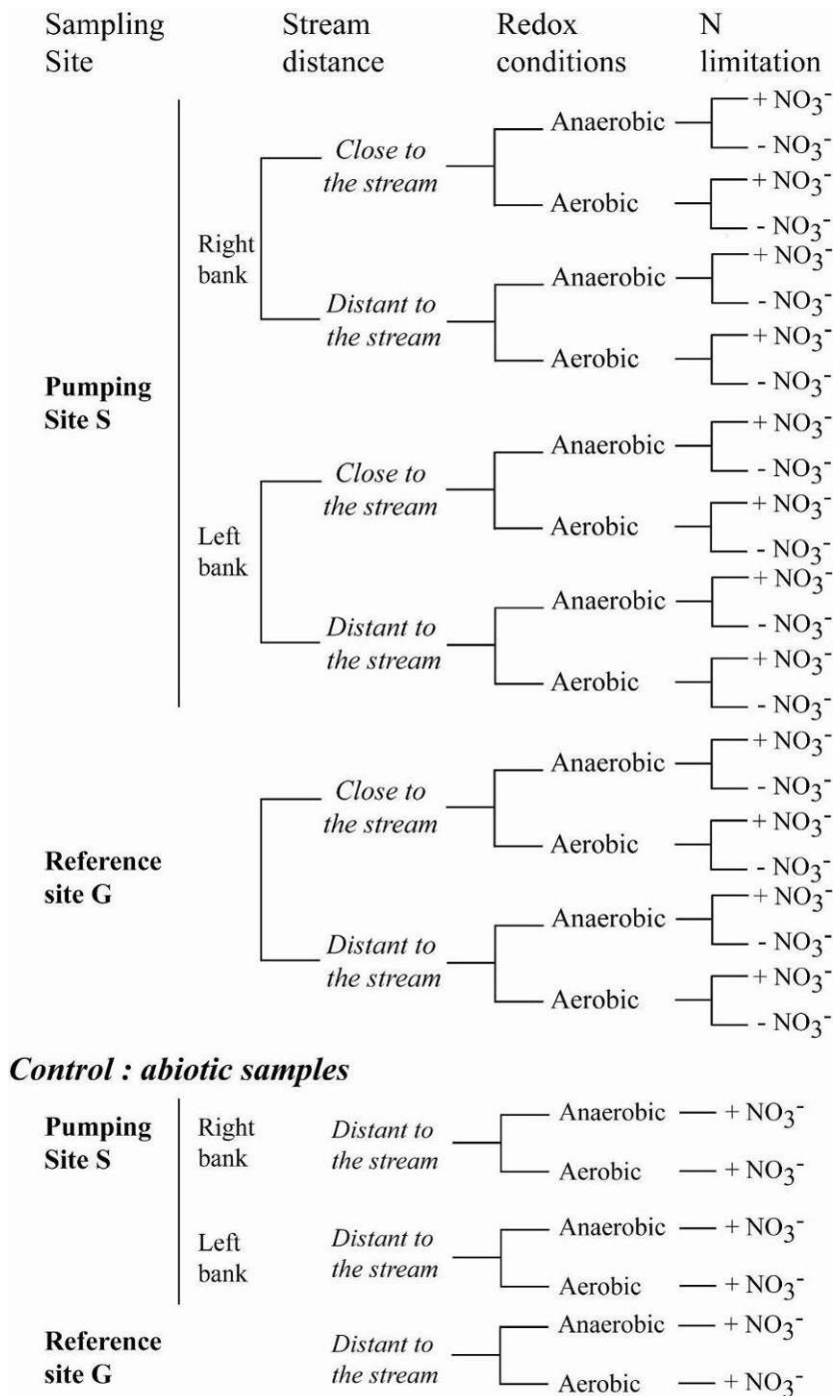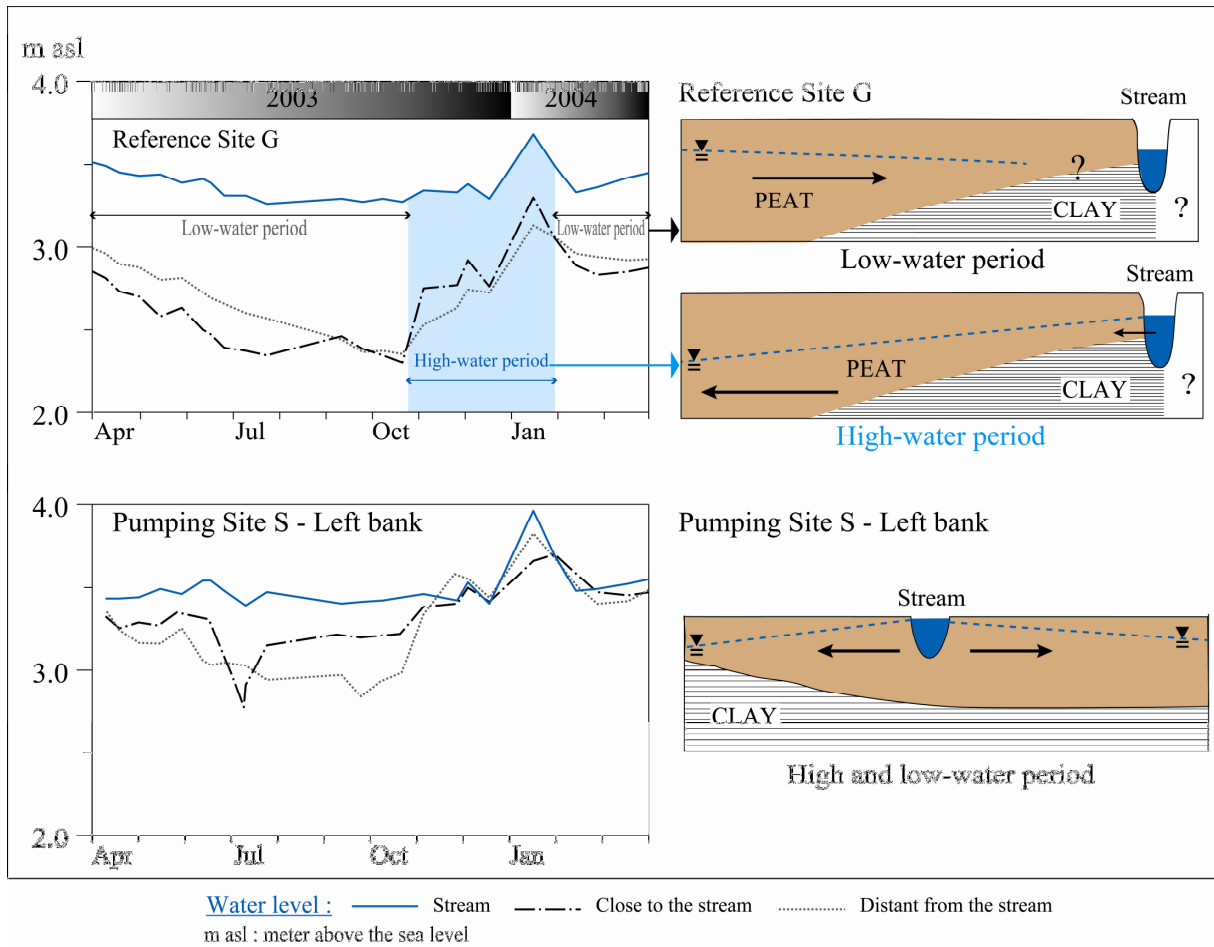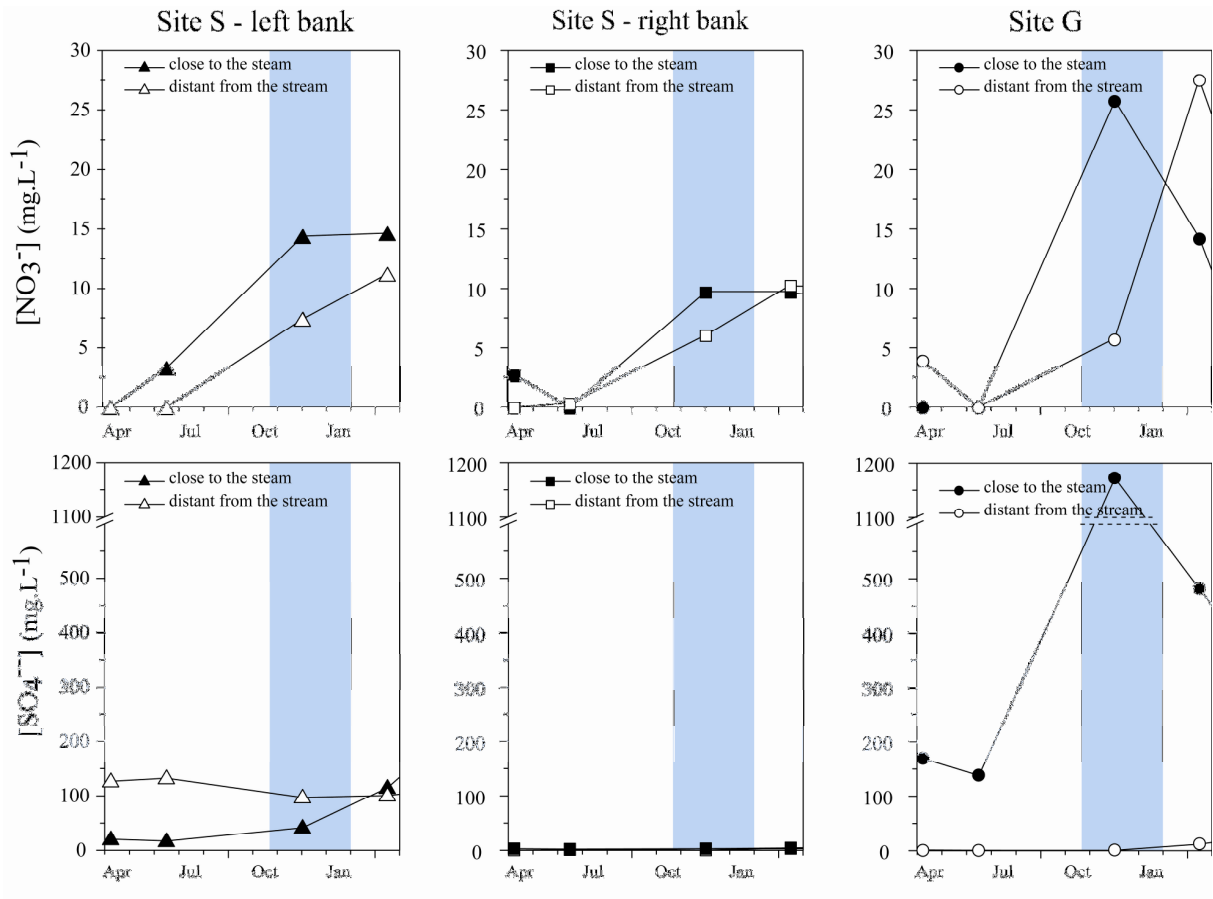
Figure 2: Experimental design. 'Anaerobic condition' indicates that the ambient atmosphere is changed to N_2. '+ nitrate' or '- nitrate' corresponding to addition or non addition of nitrate in the flask. 'Control' corresponds to sterilized samples. 3 samples are run for each experimental procedure to allow statistical analysis.
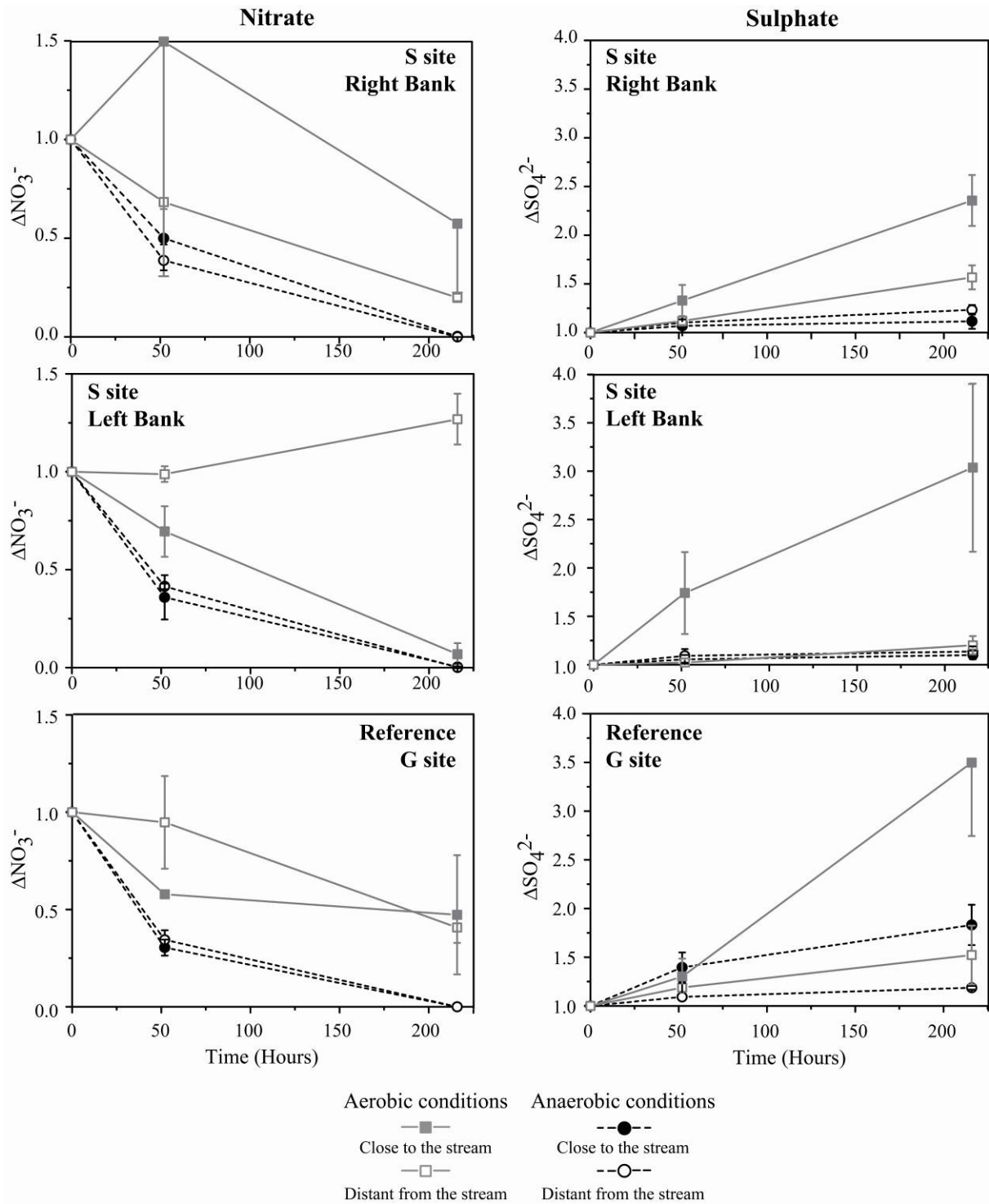
800

32

Figure 3 : Water level fluctuations during a 2-year period in reference site G, and pumping site S - left bank (modified from Auterives et al. 2009). Schematic peatland profiles are an interpreted view of the peatland/stream water flow relationships (Auterives et al. 2009). The peatland water level is represented by the dash blue line. Black arrows represent the water flow direction. The question-marks represent unknown condition.
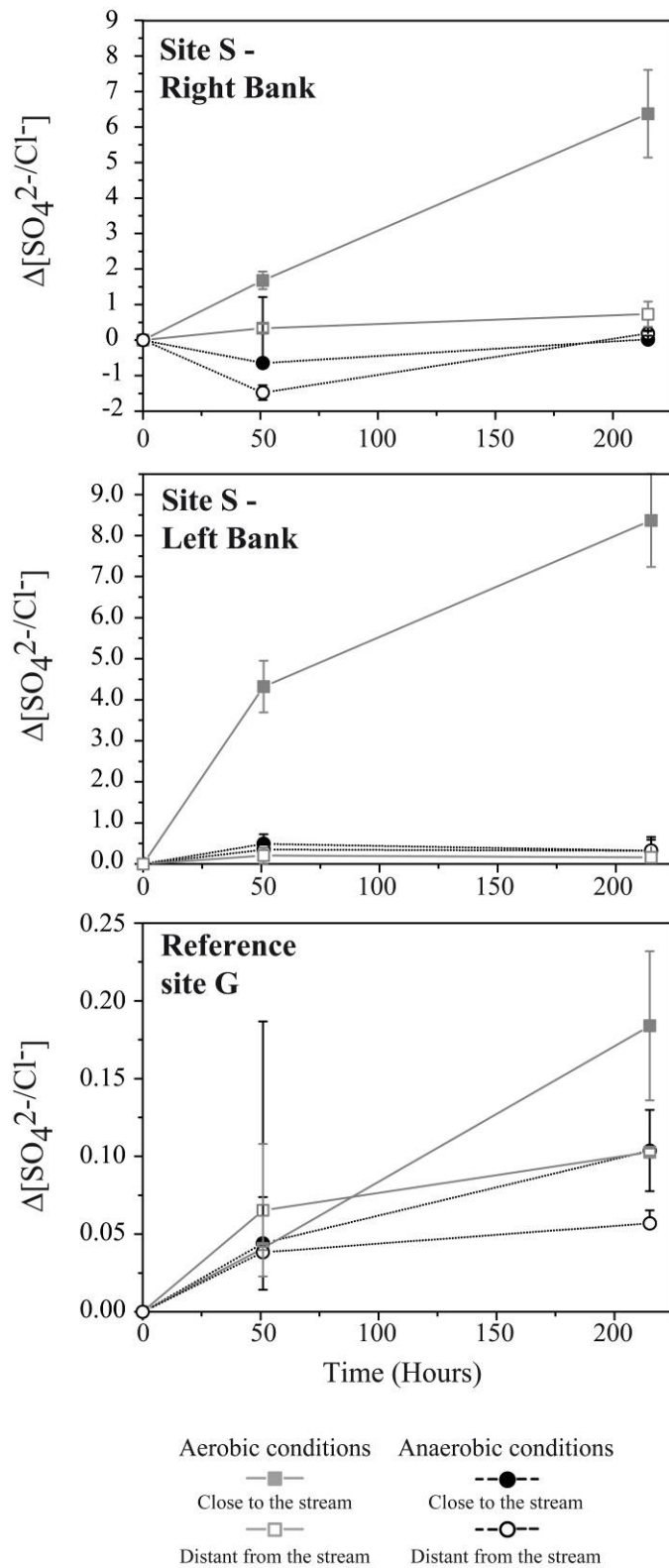
810

Figure 4 : Nitrate and sulphate concentration dynamics during a 2-year period according to the sites and the distance to the stream (modified from Auterives 2007). Blue-grey parts represent the high water period ranging over the period from October until February.
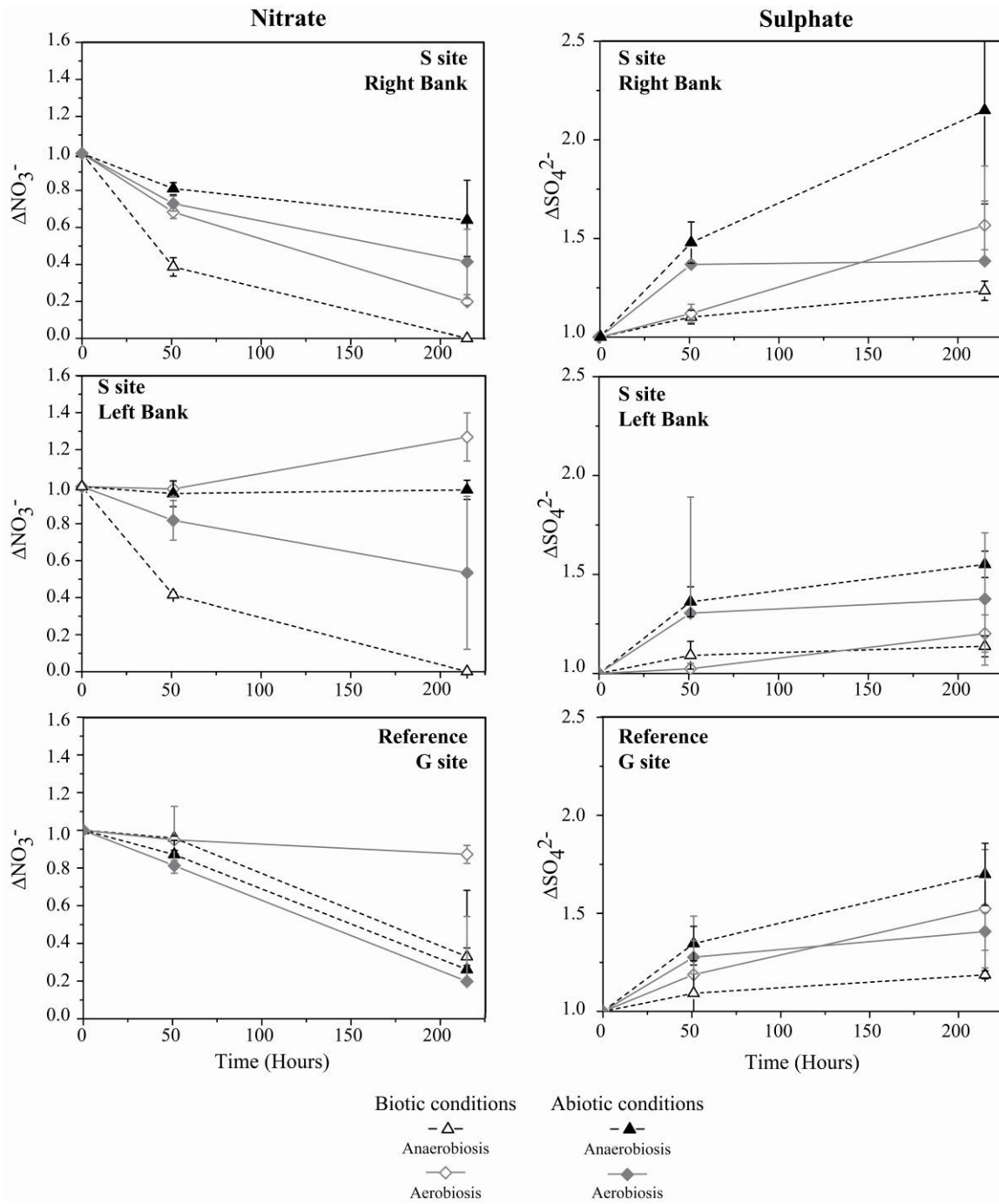
Figure 5 : Variation in nitrate and sulphate concentrations over time in peat samples under aerobic and anaerobic conditions in batch experiments. The values given for each sample correspond to the mean of the 3 replicates. The temporal variation is expressed as the difference from the zero concentration (see 3.3. statistical analysis). Bars indicate standard deviation. Only experiments with nitrate addition are represented.

Figure 6 : Variation in sulphate concentrations over time in peat samples under aerobic and anaerobic conditions in batch experiments. Sulphate concentration corrected for pore water sulphate content and chloride variations. Values given for each sample correspond to the mean of the 3 replicates. Bars indicate standard deviation.

820

825

Figure 7 : Comparison of biotic and abiotic changes according to nitrate and sulphate concentrations throughout the batch experiment. Data correspond to experiments using samples distant from the stream and with nitrate addition.