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Links between sediment structures and ecological processes in the hyporheic zone: ground-penetrating radar as a noninvasive tool to detect subsurface biologically active zones

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ABSTRACT

At the scale of geomorphological units (riffles, pools, and gravel bars), the contribution of the hyporheic zone to the functioning of streams and rivers depends on the hydrological exchanges between surface water and groundwater. These exchanges are largely controlled by sediment structure and texture, which are difficult to assess with classical methods (shovelling and freeze coring). We aimed to evaluate the ability of a non-destructive method, ground-penetrating radar (GPR), to detect sediment structures associated with subsurface biologically active zones in a gravel-bed river. After GPR data acquisition and processing, a three-dimensional reconstruction of a gravel bar permitted the identification of two sediment facies: a cobble/gravel lithofacies denoted as 'coarse' and a sand/gravel lithofacies denoted as 'fine'. We installed piezometers along two longitudinal profiles (each corresponding to a lithofacies identified by GPR) and monitored hydraulic head and temperature for 20 days. Water and sediments were sampled along the two profiles to measure water physicochemistry, sediment characteristics, bacterial abundance and activity, and interstitial invertebrate assemblages. These measurements confirmed that the two profiles were characterized by distinct hydrological flow rates and associated biological activities. Rapid water transport in the coarse profile fuelled the hyporheic zone with organic matter, whereas water and organic matter supplies were lower in the fine profile. Consequently, the higher supply of organic matter in the coarse profile was associated with higher microbial activities and invertebrate density and diversity. Therefore, GPR could be an efficient tool to detect the sediment features playing a key role in hyporheic zone functioning.

KEY WORDS gravel bar; hyporheic hotspots; geophysics; lithofacies; hydrological processes; hyporheic fauna; microbial activity;

INTRODUCTION

The subsurface sediment of streams and rivers (the hyporheic zone, Orghidan, 1959) plays a key role in the hydrology, material cycling, and energy flow of lotic ecosystems (Jones and Holmes, 1996; Brunke and Gonser, 1997; Boulton *et al.*, 1998; Krause *et al.*, 2011). Many important biogeochemical processes have been shown to occur intensively in the hyporheic zone of streams, such as respiration (Pusch, 1996), nitrification (Jones *et al.*, 1995a; Butturini *et al.*, 2000), denitrification (Holmes *et al.*, 1996; Lefebvre *et al.*, 2004; Storey *et al.*, 2004), and

methanogenesis (Jones et al., 1995b). Its significance to hydrosystem function is largely dependent on the hydrological connections and associated organic matter supply between surface and interstitial habitats (Hendricks, 1993; Jones et al., 1995c; Fellows et al., 2001). For instance, Battin (2000) showed that the hydrolytic activity of hyporheic bacterial biofilms was positively linked to hydrological exchanges. Tonina and Buffington (2009) reported the main mechanisms driving hydrological connections between surface and interstitial habitats over multiple spatial scales. At the scale of geomorphological units (riffles, pools, and gravel bars), exchanges between surface water and groundwater are ultimately controlled by hydrology and sediment structure (Vervier et al., 1992; Claret et al., 1998; Battin et al., 2008). Monitoring hydraulic heads has been largely used to determine hydraulic gradients and infer water flows within geomorphological units

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(Bencala and Walters, 1983; Valett *et al.*, 1997; Poole *et al.*, 2006), but valid information about sediment structure and texture is more difficult to obtain because of disturbances associated with shovelling or freeze-coring sampling techniques (Marmonier *et al.*, 2004; Descloux *et al.*, 2010).

Ground-penetrating radar (GPR) is a powerful nondestructive tool for investigating fluvial deposits in detail up to a depth of 20 m (Leclerc and Hickm, 1997; Gourry et al., 2003; Neal, 2004; Mumphy et al., 2007) and is especially effective in coarse-grained (gravel and sand) environments (Best et al., 2003; Heinz et al., 2003; Lunt et al., 2004). GPR allows the documentation of the internal structure (bedding geometry) of active braided bars and channels under both water-saturated and unsaturated conditions (Lunt et al., 2004; Mumphy et al., 2007). It has also been used to estimate water content and porosity within the hyporheic zone (Brosten et al., 2009). This is also the unique geophysical method currently existing for the acquisition of shallow, high-resolution, continuous data (Neal, 2004; Schrott and Sass, 2008). Despite the many opportunities existing for employing this methodology to characterize the sediment habitats of fluvial environments and their expected influences on the distribution of biotic processes (Naegeli et al., 1996), GPR has not yet been used by aquatic ecologists to assess the links between subsurface biological processes and sediment structure within the hyporheic zone.

The present study aimed to fill this gap by evaluating the ability of GPR to detect sediment structures associated with distinct biological processes in the subsurface of a gravelbed river. We used high-resolution continuous GPR profiling to obtain the detailed sediment structure of a gravel bar. After data processing and interpretations, two distinct sediment structures were selected along the gravel bar: one dominated by cobbles and gravel (denoted as 'coarse') and one rich in sands and gravel (denoted as 'fine'). According to the literature (Wood and Armitage, 1997; Lefebvre *et al.*, 2005), we expected that a hydrological connection with the surface would be more pronounced within the coarse sediment structure than within the fine sediment structure. Consequently, the coarse sediment structure would be more biologically active in terms of microbial activity and hyporheic fauna than the fine sediment structure.

MATERIALS AND METHODS

Study site

The study was carried out in a channel (Vieux Rhône) of the Rhône River located about 2 km upstream from Lyon (France) and upstream from the pumping well field of 'Crépieux-Charmy' (Figure 1), which provides drinking water for the Lyon metropolitan area. The bed sediments are made of a coarse substrate mainly composed of cobbles and gravels (Poinsart *et al.*, 1989). We selected a gravel bar located on the right bank of the Vieux Rhône channel. The Vieux Rhône channel is a regulated channel, and its discharge remained constant along most of the experiment duration but decreased by 60 cm on the last day of the experiment.



Figure 1. Location of the Rhône River, Lyon, France (A), aerial photograph indicating the location of the studied gravel bar (B), and layout of the 5 m×5 m rectilinear ground-penetrating radar (GPR) acquisition grid with GPR profile designations (C). Positions of sediment samples for grain size analyses are indicated by A1, A2, B1, and B2.

GPR method

Ground-penetrating radar is a non-invasive geophysical technique that detects electrical discontinuities in the shallow subsurface. This technique is based on the generation, transmission, propagation, reflection, and reception of discrete pulses of high-frequency (MHz) electromagnetic energy. It allows the assessment of spatial structures of sediments to a depth of 10-20 m over extended areas (Huggenberger et al., 1994; Beres et al., 1995; Bridge and Lunt, 2006). Sediment geologists widely used this technique to reconstruct depositional environments and document historic sediment processes (Schrott and Sass, 2008). Indeed, alluvial deposits are characterized by a textural heterogeneity derived from erosion, transport, and sedimentation phases that have led to the current configuration of lithological units. In the present paper, the term lithofacies has been used as a reference to lithological units of fluvial deposits, each lithofacies corresponding to a genetic unit (i.e. formed by a homogeneous process of transport and sedimentation, Miall, 1999).

The GPR measurements were carried out with the GSSI SIR 3000 system (Geophysical Survey System Inc., Salem, USA), operated with a shielded antenna at a central frequency of 400 and 200 MHz, running in monostatic mode. The data processing was performed using the GSSI Radan 6 software. This processing consisted of a distance normalization, a static time shift (to align direct ground wave arrival to 0 ns), a background removal (to eliminate the high-amplitude direct ground wave), and a Kirchhoff migration. The electromagnetic wave velocity was determined by common midpoint with two 400-MHz antennas. This velocity ranged from $0.12 \,\mathrm{m}\,\mathrm{ns}^{-1}$ in the unsaturated zone to $0.06 \,\mathrm{m}\,\mathrm{ns}^{-1}$ in the saturated zone. Accordingly, a velocity of $0.06 \,\mathrm{m\,ns^{-1}}$ was selected to convert two-way travel time into actual depth, a velocity that is comparable with those measured in the saturated zone of analogous alluvial deposits using the common midpoint method (Woodward et al., 2003; Neal, 2004; Mumphy et al., 2007; Lindhorst et al., 2008). In addition, a topographic survey of the bar using a D-GPS Trimble station was performed to localize the acquisition grid, the topography of the gravel bar, the position of the gravel bar relative to the Vieux Rhône channel, and the position of sediment samples that were used to validate GPR interpretations. Topographic corrections were applied to the data using transect elevation data collected during this survey.

The 400- and 200-MHz profiles were collected in a $5 \text{ m} \times 5 \text{ m}$ grid pattern (Figure 1C) positioned in the north of the gravel bar. The dimensions of the acquisition grid were $60 \text{ m} \times 40 \text{ m}$, and the grid was oriented 350°N , parallel to the Vieux Rhône channel. The spacing between each grid line was set at 5 m. GPR measurements (400 and 200 MHz) were performed on all acquisition grid lines.

The GPR data set was interpreted using radar facies analysis (Regli et al., 2002; Jol and Bristow, 2003; Mumphy et al., 2007; Bridge, 2009) in conjunction with the classification of seismic reflections developed by Mitchum et al. (1977). During interpretation of the profiles, constant gain was applied to the data to note the amplitude of reflections. The GPR profiles were exported into an image file format (.bmp), and major reflections were traced out and coloured using Illustrator CS6 drafting software (Adobe, Inc.). The radar facies packages were positioned in a three-dimensional (3D) context to view spatial relationships between profiles using RockWorks16 geological software (RockWare, Inc.). As GPR profiles acquired an immense amount of interpreted sedimentological information, only major radar reflection packages were described with the profiles of 200-MHz antennae. The profiles of 400-MHz antennae (not presented in this paper) were used to confirm the interpretation of profiles obtained with the 200-MHz antennae.

To validate the interpretation of GPR results, grain size analyses (NF P94-056 standard, Association Française de Normalisation, 1996) were performed on four samples collected at the surface of the gravel bar (Figure 1C). Briefly, around 51 of sediment was collected per sampled point, dried, and sieved on a set of sieves with mesh sizes varying from 0.1 to 50 mm. The dry sediment was weighted for each retained size fraction and reported as a percentage of the total dry mass of the sediment sample.

Sampling procedure

Following the 3D GPR interpretations, two longitudinal profiles of the bar were selected: one profile with a sediment structure dominated by cobbles and gravel (denoted as coarse) and a second profile associated with sand and gravel (denoted as fine). For each longitudinal profile, three positions were selected at increasing distances from the Vieux Rhône channel along the flow path: 1, 15, and 35 m. For each of these three positions, a pipe (30-mm internal diameter, with holes of 5-mm diameter around the base) was inserted into the bar sediments to reach a depth of 30 cm below the water table. These pipes (n=6, 3)positions within the gravel $bar \times 2$ sediment structures) were equipped with probes (Mini-Diver, Schlumberger Water Services, Waterloo, Canada) to monitor pressure and temperature dynamics during the experiment (from 20 May to 9 June 2011). They were closed with caps to prevent any contamination from the surface. At the end of the monitoring period (9 June 2011), three replicate samples of water and sediments were collected using a mobile pipe at each position (1, 15, or 35 m) in the two sediment structures to measure water physicochemistry (pH, electrical conductivity, dissolved oxygen, dissolved organic carbon, nitrate, ammonium, and phosphate concentrations),

characteristics of collected sediments (grain size distribution, total nitrogen, and total organic carbon), bacterial abundance and activity (respiration and hydrolytic activity), and interstitial invertebrate assemblages. At each position, the three replicate samples were spaced about 1.5 m apart to form an equilateral triangle around the pipe equipped with probes. These replicate samples allowed comparisons between sediment structures (coarse structure vs fine structure) along the flow paths. We also collected surface water at three points in the Vieux Rhône channel upstream of the gravel bar.

Sampling technique

Water and sediment were collected using the Bou-Rouch method (Bou and Rouch, 1967; Bou, 1974). A mobile steel standpipe (25 mm in internal diameter, openings of 5 mm) was inserted to a depth of 30 cm below the water table. Interstitial water was sampled with a syringe to measure electrical conductivity (LF92, WTWTM, Weilheim, Germany), pH, and dissolved oxygen (O₂) concentration (HQ20, HACHTM, Dusseldorf, Germany). Afterwards, 101 of interstitial water mixed with fine sediment was extracted using a hand pump and collected in a plastic box, pre-washed with acid and deionized water, for chemical (in interstitial water and sediments) and bacterial (on sediments) measurements. From each sample, 50 ml of water was filtered through 0.7-µm-pore-size Whatman GF/F filters (Millipore, Billerica, MA, USA) for subsequent analyses of nitrate, ammonium, and phosphate, and 25 ml of water was filtered through a 0.45-µm-pore-size Millipore HAWP filters (Millipore, Billerica, MA, USA) for subsequent analyses of dissolved organic carbon. The same procedure was followed for surface water samples collected from the Vieux Rhône channel. After collection of water samples, the remaining mix of interstitial water and sediment was elutriated three times to separate interstitial fauna from the denser sediment particles. As the Vieux Rhône channel was large and not surrounded by dense riparian vegetation, we did not collect significant proportions of leaf litter debris in hyporheic samples, and most of the particulate organic matter collected was associated with sediments. During elutriation, interstitial fauna was collected using a 200-µm-mesh net and preserved with 96% ethanol. The remaining sediment was collected in a sterile glass vial for physicochemical and microbial analyses. Water and sediment samples were kept in an isotherm box during the 30-min journey back to the laboratory.

Water analyses for nutrients and dissolved organic carbon

 NH_4^+ , NO_x , $(NO_3^- + NO_2^-)$, and PO_4^{3-} concentrations were measured on water samples filtered in the field following standard colorimetric methods (Grasshoff *et al.*, 1983) using a sequential automatic analyser (Easychem Plus, Systea, Anagni, Italy). The concentration of dissolved organic carbon in filtered water samples was measured with a total carbon analyser (multi-N/C 3100, Analytik Jena, Jena, Germany) based on combustion at 900 °C after removal of dissolved inorganic C with HCl and CO_2 stripping under O_2 flow.

Sediment analyses

Each sediment sample was wet-sieved at $1600 \,\mu\text{m}$ to perform grain size distribution analyses and total organic carbon, total nitrogen, and microbial measurements on particles with sizes lower than $1600 \,\mu\text{m}$. This procedure was chosen to homogenize sediment particle sizes in order to reduce the potential influence of grain size distribution on microbial activities (as observed in hyporheic sediments by Rulík and Spáčil, 2004).

Grain size volumic proportions of sediment samples were measured by laser diffractometry (Malvern Mastersizer 2000G) on particles ranging between 0.02 and 1600 μ m. Three measurements per sample were carried out. These data were analysed using GRADISTAT version 8.0 (Blott and Pye, 2001) to determine the volumetric percentages of sand (63–1600 μ m), silt (2–63 μ m), and clay (<2 μ m) in each sample.

For total organic carbon and total nitrogen analyses, sediment samples were freeze dried for at least 48 h and then crushed using a mortar and pestle. For each sample, about 100 mg of dry sediments was placed in a silver capsule and acidified with 2 M HCl to remove calcite. The acidified sample was then dried at 50 °C for 24 h and placed in tin capsules for C and N analysis using an elemental analyser (FlashEA, Thermo Fisher Scientific, Waltham, MA, USA).

Bacterial abundance was enumerated after 4',6diamidino-2-phenylindol staining (Porter and Feig, 1980). Wet sand (1 g) fixed with formaldehyde (final concentration 4%) was diluted with 20 ml of a solution with pyrophosphate (0.1%). Bacteria were detached from sediment by sonication and aliquots were spotted onto slides and stained with 4',6-diamidino-2-phenylindol solution (Schönholzer *et al.*, 2002). The slides were examined at 1000× magnification with a microscope fitted for epifluorescence. Bacteria were counted in 30 fields per sample with up to 20 cells per field and were expressed as numbers of bacteria per gramme of dry sediment.

Hydrolytic activity of biofilm was estimated using fluorescein diacetate (FDA) as a substrate for hydrolases. Within 24 h of sampling, FDA was added directly to 1 g of wet sediment in 3 ml of pH 7.6 buffer solution. Incubation was performed at the experimental temperature ($15^{\circ}C$) until a green fluorescein colouration appeared (between 30 and 40 min). The reaction was stopped by freezing the sediment after an addition of 3 ml of acetone following Battin (1997). The optical density of the supernatant was read at 490 nm after filtration on a 0.7-µm glass fiber filter. Results were expressed in micromoles of FDA hydrolysed per gramme of dry sediment per hour (µmole FDA g⁻¹ h⁻¹).

Respiration rates of micro-organisms attached to sediment were measured on fresh sediments in a thermostated room at 15 °C using 60-ml respiration chambers. Five grammes of sediment was introduced into respiration chambers filled with filtered water from the Vieux Rhône channel. Control chambers without sediment were also prepared to determine background respiration in the experimental system. An oxygen sensor (UNISENSE, Denmark) coupled with a peristaltic pump was adapted to the respiration chambers to measure the O_2 concentrations in the water every hour for 12 h. The peristaltic pump also mixed water to ensure dissolved oxygen homogeneity in respiration chambers during the incubation. The rate of linear decrease of oxygen concentration with time (from 0 to 12 h) was used to calculate the oxygen respiration for each sediment sample in microgrammes of O₂ consumed per hour. The respiration rates were corrected for respiration measured in control chambers and are reported relative to grammes of dry sediment.

Statistical analyses

Cross-correlation analysis was used to compare temperature patterns of surface and hyporheic waters (Box and Jenkins, 1976; Malard *et al.*, 2001). The cross-correlation corresponds to the correlations of the time series of surface water temperature with the time series of hyporheic water temperature, shifted by a particular number of observations performed every 10 min (i.e. k = lag). The crosscorrelogram indicates the values of the cross-correlation coefficient (*r*) between surface water and hyporheic water temperature for increasing values of *k* (*x*-axis shifted forwards) and -k (*x*-axis shifted backwards). Based on the number of k corresponding to the highest r between temperatures of surface and hyporheic waters, we calculated a travel time (in hours) from surface to each hyporheic point in the gravel bar (Foulquier *et al.*, 2009).

For each day of temperature recording, we also calculated the daily thermal amplitude (maximal temperature – minimal temperature) at each point (n = 21 days). The relation between daily thermal amplitudes of surface water and those of hyporheic water was examined using Pearson's correlation coefficient. Damping of daily thermal amplitude with distance within the gravel bar was evaluated in relation with the significance of these correlations in daily thermal amplitude between surface and hyporheic waters.

Two-way ANOVAs were used to evaluate the influence of distance within the gravel bar (1, 15, and 35 m) and sediment structure (fine and coarse sediment structures) on physicochemical, microbial, and faunal variables with distance and sediment structures as main effects. The homoscedasticity and the normality of the data set were verified using Levene's and Shapiro's tests, respectively. Statistical analyses were performed with the Statistica software package (Statsoft Inc., Tulsa, OK, USA). Significance for all statistical tests was accepted at $\alpha < 0.05$.

RESULTS

Gravel bar characteristics

The subaerial portion of the bar during the GPR survey had a length of 65 m and a cross-channel dimension of 45 m (Figure 1C). The bar exhibited typical geometry with a high-angle slip face at its tail and a more gently tapered head. The flanks of the bar had a curved, concave-up shape. The presence of the slip face suggested that the bar was actively migrating downstream. Flow was deflected around



Figure 2. Particle size distribution (cumulative) of the four surface samples obtained from the study site. Photographs illustrate the characteristics of the sediment samples.

the bar at the bar head and wraps around the bar tail, where flow direction was parallel to the slip face during low-flow periods. The bar top was mainly characterized by small dunes (crest height 0.5 m). The observations showed two lithofacies: bimodal gravel (A1 and A2) and open framework gravel (B1 and B2) (Figure 2).

Radar facies

Figure 3 showed two radar profiles (GPR2 and GPR5) interpreted with references to specific areas of interest. Interpreted radar facies reflections represented the macroform bounding surfaces because small-scale (centimetre-scale) internal cross stratifications could not be detected with the resolution of the 200-MHz antennae (\sim 0.20 m).

The radar facies R1 is long, sub-horizontal, and continuous. It was easily distinguishable from other

reflectors and was at an elevation of approximately 172.5 m on the 20 GPR profiles. Its location was at the same elevation as the surface of the Vieux Rhône channel, meaning that reflectors indicated the presence of the water table.

The radar facies R2 consisted of sub-horizontal to wavy continuous to discontinuous reflections observed at several depths within the gravel bar. Although some reflections were discontinuous, many of them extended laterally for the entire length of the GPR profiles. In many places, these radar facies reflections serve as bounding for the other radar facies (R3) described in this section. The regular, subhorizontal to horizontal nature of these layers was a characteristic of sand and gravel deposition by upward stacking. This interpretation of radar facies into lithofacies was confirmed by the grain size analyses of sediment samples A2 at the surface of GPR2 (Figures 1 and 2) and A1 at the surface of GPR1 (Figure 2).



Figure 3. Representative ground-penetrating radar (GPR) profiles and interpretative sketches of the radar facies and major GPR reflection patterns. Location, length, and direction of GPR profile sections are illustrated on the GPR acquisition grid map indicated on the right.

The radar facies R3a and R3b consisted of a set of obliqueparallel reflectors. They were more visible on the east side than on the west side of the gravel bar. These reflectors presented a dip angle of around 30° and were mainly obtained from the transverse profiles (GPR9–GPR18). The occurrence of a relatively steep slip face has been recognized as a characteristic of braided bars (Mumphy *et al.*, 2007). The clear, high-amplitude nature of radar facies reflections was indicative of gravel (high-flow conditions) because the occurrence of sand would have caused, at some degree, signal attenuation. This lithofacies was confirmed by grain size analysis of sediment samples B1 and B2 at the surface of GPR2 and GPR5 (Figures 1 and 2).

The radar facies R4 was a long reflector visible on numerous profiles, which was curvilinear or horizontal. This reflector separated sub-horizontal reflectors R2 from oblique reflectors R3. We presume that this reflector was a limit between the old sediment of the river (former river bed) and the recent sediment deposited during high-flow events.

Grid analysis and positioning of piezometers along longitudinal profiles

Based on the determination of the lithofacies on twodimensional profiles, we obtained a 3D reconstruction of the facies in the gravel bar (Figure 4) by creating a fence diagram of the GPR profiles. The 3D model was useful in determining the interface between the 'old' sediment characterized by cobble/gravel lithofacies and the 'recent' sediment characterized by sub-horizontal structure and sand/gravel lithofacies. On the basis of this 3D interpretation, two longitudinal profiles that were sub-parallel to the water flow were selected on the gravel bar (Figure 4): one in the sand and gravel sediments (fine sediment structure, corresponding to A1 and A2 in Figure 2) and the other in the cobbles and gravel (coarse sediment structure corresponding to B1 and B2 in Figure 2). For each longitudinal profile, three positions were determined and equipped with piezometers at increasing distances from the Vieux Rhône channel along the flow path: 1, 15, and 35 m.

Hydraulic head and temperature monitoring

Hydraulic head measured in piezometers decreased from the head to the centre of the gravel bar in the two sediment structures (Supplementary Figure S1). Comparable hydraulic gradients were observed in the fine (Δ 35 cm from 1 to 35 m within the gravel bar) and coarse (Δ 31 cm from 1 to 35 m within the gravel bar) sediment structures. Hydraulic heads responded quickly to the decrease of water level in



Figure 4. Location of the six piezometers at the surface of the gravel bar (A). Diagram of interpretative sketches of the 20 ground-penetrating radar profiles in the acquisition grid and three-dimensional representation of the two lithofacies with the positions of the piezometers (B).

the Vieux Rhône channel observed on 9 June (Supplementary Figure S1). On the last week of monitoring, we could also note greater variations of water level measured in piezometers located at 15 and 35 m within the gravel bar in comparison with measurements performed in the Vieux Rhône channel and at 1 m within the gravel bar.

Results of the 0.5-h moving-average smoothing of surface water and hyporheic water temperature series recorded from 20 May to 9 June 2011 (n=985, Figure 5) showed that daily fluctuations of water temperature in the Vieux Rhône channel were rapidly transferred to hyporheic water collected at the three points of the coarse sediment structure. Although a thermal buffering was observed at 15 and 35 m within the gravel bar (Figure 5), daily thermal amplitudes measured in hyporheic water of the coarse sediment structure were significantly correlated with those

of the Vieux Rhône channel (Table I). In contrast, the daily thermal fluctuations monitored in the Vieux Rhône channel were only observed at 1 m within the fine sediment structure, no correlation being obtained between daily thermal amplitudes in surface and hyporheic water for points located at 15 and 35 m within the gravel bar (Table I). The cross-correlations between surface and hyporheic water temperatures indicated that the calculated time of water transport between surface and hyporheic points were more than threefold higher in the fine sediment structure than in the coarse sediment structure for the three monitored positions in the gravel bar (Table I).

Water chemistry

Mean water temperature, pH, and concentrations of NH₄⁺ and dissolved organic carbon were not significantly



Figure 5. Temperature records for the Vieux Rhône channel (in grey and reported on all panels) and hyporheic habitats (in black) in the two sediment structures and at the three positions within the gravel bar.

Table I. Daily amplitude of water temperature.

Position	Distance from the Vieux Rhône channel (m)	Amplitude (°C)	r	Lag time (hours)
Vieux Rhône channel	0	1.71 (±0.51)		
Coarse sediment structure	1	$1.60 (\pm 0.49)$	0.956**	1.33
	15	$1.63 (\pm 0.37)$	0.807**	3.67
	35	$1.13 (\pm 0.44)$	0.754**	5.33
Fine sediment structure	1	$1.38 (\pm 0.32)$	0.627*	4.83
	15	$0.62 (\pm 0.59)$	0.276	16.67
	35	0.48 (±0.52)	0.286	15.00

r, Pearson's correlation coefficient between daily amplitude of surface water and hyporheic water temperatures; lag time between surface and hyporheic temperature patterns.

p < 0.01, p < 0.001

influenced by sediment structure and distance within the gravel bar (Tables II and III). Electrical conductivity and NO_3^- concentrations were significantly higher in water collected within the fine sediment structure compared with the coarse sediment structure. Dissolved oxygen and PO_4^{3-} concentrations showed the opposite pattern, with significantly lower concentrations in the fine sediment structure. We did not detect significant influence of the distance within the gravel bar on water physicochemistry (Table III) except in interaction with sediment structure effect for dissolved oxygen and phosphate concentrations.

Sediment analyses

Analyses of grain size distributions showed that all collected sediment samples were dominated by sand with volumetric fractions ranging between 94.9% and 100%. A small proportion of silt was measured at 1 m in the coarse sediment structure (5.1% in volumetric fraction) and at 1, 15, and 35 m within the fine sediment structure (volumetric fractions: 4.4% at 1 m, 2.2% at 15 m, and 0.5% at 35 m). Both total nitrogen and organic carbon concentrations measured on collected sediments significantly decreased from 1 to 35 m within the gravel bars in the two sediment structures (Figure 6 and Table III). We detected around threefold higher nitrogen and organic carbon concentrations on fine sediments collected at 1 m in the coarse sediment structure compared with those collected in the fine sediment structure at the same distance from the Vieux Rhône channel (Figure 6). This effect was also observed at 15 m within the gravel bar but did not occur at 35 m within the gravel bar (significant interaction effect between sediment structure and distance within the gravel bar, Table III).

Microbial analyses

Bacterial abundances varied between 7×10^9 cells and 17×10^9 cells per gramme of dry sediment (Figure 6). For each sediment structure, the abundances were the highest at

the head (1 m) of the gravel bar (distance effect, Table III). Bacteria were also more numerous on sediments collected in the fine sediment structure compared with sediments sampled from the coarse sediment structure (sediment structure effect, Table III). An opposite pattern was obtained for microbial activities with higher O₂ consumption and hydrolytic activity on sediments collected in the coarse sediment structure (Figure 6 and Table III). As observed for bacterial abundance, there was a clear decrease of microbial activity between 1 and 15 m along the flow path for the two sediment structures (Table III).

Interstitial fauna

We clearly observed a strong decrease in taxa richness and abundance from 1 to 15 m within the gravel bar for the two sediment structures (Figure 6 and distance effect, Table III). Invertebrate assemblages were also more abundant and diverse in samples collected in the coarse sediment structure compared with samples from the fine sediment structure (sediment structure effect, Table III). For instance, it is interesting to note that the common surface-dwelling amphipods of the genus Gammarus and the isopod Asellus aquaticus were collected in the coarse sediment interstitial habitat but were absent from all hyporheic samples from the fine sediment structure (Table IV). The distribution of the groundwater amphipods of the genus Niphargus differed according to the sediment grain size. These animals were collected at 15 and 35 m within the gravel bar in the coarse sediment structure, but only at 1 m in the fine sediment structure.

DISCUSSION

Our results were in accordance with previous studies (e.g. Naegeli *et al.*, 1996; Brosten *et al.*, 2009), which showed that GPR was an efficient tool to determine the geometry of sediment texture and sediment stratigraphy in gravel bars. Four major radar facies were discerned from

			-				1		
osition	Distance from the Vieux Rhône channel (m)	Temp. (°C)	EC $(\mu S cm^{-1})$	Hq	DO (mg l ⁻¹)	$NH_4^+~(\mu gl^{-1})$	$NO_{3}^{-} (mg l^{-1})$	$PO4^{3-} (\mu g l^{-1})$	DOC (mg l ⁻¹)
/ieux Rhône channel	0	22.65 (±0.21)	361 (±2)	8.0 (±0.1)	9.35 (±0.07)	21 (±1)	$4.1 (\pm 0.3)$	27 (±1)	2.56 (±0.04)
Coarse sediment structure	1	23.67 (±0.21)	362 (±3)	8.2 (±0.1)	8.88 (±0.53)	16 (±6)	$4.8(\pm 0.3)$	23 (±13)	$3.24 (\pm 0.33)$
	15	23.77 (±0.64)	365 (±2)	8.0 (±0.1)	7.61 (±0.44)	26 (±7)	5-2 (±0-3)	30 (±4)	2·71 (±0·17)
	35	23.23 (±0.31)	363 (±1)	8.0 (±0.1)	8·21 (±0·10)	19 (±12)	$4.5(\pm 0.6)$	37 (±1)	2.98 (±0.97)
ine sediment structure	1	23.63 (±0.59)	374 (±4)	8.0 (±0.1)	6·04 (±0·96)	35 (±14)	$5.4(\pm 0.1)$	$30 (\pm 1)$	$3.39 (\pm 0.15)$
	15	23.73 (±0.15)	373 (±4)	8.1 (±0.1)	6.73 (±0.26)	24 (±2)	5.5 (±0.2)	18 (±7)	2.42 (±0.28)
	35	23.53 (±0.68)	373 (±3)	8.0 (±0.1)	6·15 (±0·61)	25 (±1)	5·7 (±0·1)	17 (±3)	3.60 (±0.74)
			-	-	:	-	• • •	:	-
emp., mean temperature; Eu	C. electric conductivity: DO. diss	solved oxygen: D	UC, dissolved orga	unic carbon. I	or all parameters	, mean values±st	andard deviation (<i>i</i>	i = 3 replicates) are	indicated.

Table II. Physicochemical characteristics of surface water and groundwater collected during the sediment and fauna sampling (on 9 June 2011).

GPR profiles (200 MHz). The radar facies R1 with long, sub-horizontal, and continuous reflectors highlighted the water table. The facies R2 with regular, sub-horizontal to horizontal layers was characteristic of sand and gravel deposition by upward stacking, while the facies R3 with oblique-parallel reflectors was characteristic of braided bars and was indicative of the downstream advancement of bars (Regli et al., 2002; Mumphy et al., 2007; Bridge, 2009). This R3 structure was observed in zones where sediment accumulated during high-flow events. Finally, the facies R4 was probably indicative of a limit between the former river bed and more recently deposited sediment. The 3D model obtained allowed us to position piezometers along two longitudinal profiles: one in the 'old' sediment characterized by cobble/gravel lithofacies (denoted as 'coarse sediment structure') and another in the 'recent' sediment characterized by sand/gravel lithofacies (denoted as 'fine sediment structure').

This description of sediment structures by GPR associated with hydraulic head measurements was precise enough to determine zones of high and low hydrological flow rates within the hyporheic habitat. Indeed, we could delimitate two longitudinal profiles with distinct hydrological properties and ecological functioning. The coarse sediment structure was characterized by rapid water transport and associated fluxes of dissolved organic matter and nutrients in the hyporheic zone, leading to an enrichment of fine sediments with total organic carbon and total nitrogen. In contrast, the water and nutrient transports were low in the fine sediment structure. As a consequence, the higher flux of organic matter and nutrients in the coarse sediment structure compared with the fine sediment structure was associated with higher microbial activities. Indeed, respiration rates and hydrolytic activities were up to threefold higher in hyporheic sand collected in the coarse sediment structure compared with sand collected in the fine sediment structure.

We can note that these differences in microbial activities between sediment structures were not associated with differences in sediment grain sizes as sand fraction dominated all sediment samples (>94% in volume). Moreover, microbial activities were not tightly linked with bacterial abundances because bacteria were the most numerous per gramme of dry sand in the fine sediment structure. In our experimental conditions, the low quantity of coarse particulate organic matter (leaf litter) did not favour the occurrence of fungi, which could contribute significantly to the measured microbial activities (Romani et al., 2006). Indeed, Findlay et al. (2002) reported that most of the microbial biomass associated with sediments and very fine organic matter was due to bacteria rather than fungi. Therefore, the differences of microbial activities measured between the two sediment structures supposed that bacteria were more dormant and/or less active in

	Sediment	ary structure	Distance in	the gravel bar	Inte	raction
Variable	<i>F</i> (1, 12)	<i>p</i> -value	<i>F</i> (2, 12)	<i>p</i> -value	F(2, 12)	<i>p</i> -value
Temperature	0.11	0.751	0.95	0.413	0.25	0.782
Electric conductivity	49.09	< 0.001***	0.22	0.807	0.65	0.537
pH	0.50	0.493	1.50	0.262	3.50	0.063
DO	54.30	< 0.001***	0.53	0.603	4.75	0.030*
NH4 ⁺	3.69	0.079	0.30	0.746	2.35	0.138
NO ₃ ⁻	22.05	< 0.001***	1.25	0.321	3.15	0.080
PO_4^{3-}	7.65	0.017*	0.38	0.692	7.07	0.009**
Dissolved organic carbon	0.40	0.539	3.78	0.053	1.08	0.371
Total organic carbon	59.03	< 0.001***	28.71	< 0.001***	15.81	< 0.001***
Total nitrogen	51.48	< 0.001***	38.56	< 0.001***	17.53	< 0.001***
Bacterial abundance	26.87	< 0.001***	75.6	< 0.001***	7.82	< 0.01**
Hydrolytic activity	92.54	< 0.001***	57.65	< 0.001***	37.40	< 0.001***
Respiration rate	48.10	< 0.001***	45.12	< 0.001***	24.61	< 0.001***
Invertebrate diversity	19.10	< 0.001***	12.41	< 0.005**	0.32	0.733
Invertebrate abundance	15.32	<0.005**	20.15	< 0.001***	1.17	0.345

Table III. Results of two-way analyses of variance for testing the effects of sediment structure and distance within the gravel bar on physicochemistry, micro-organisms (bacterial abundance and microbial activities) and invertebrate diversity and abundance.

*****p* < 0.001

thefine sediment structure than bacteria collected from the coarse sediment structure. We can suggest that the physiological states of bacteria differed in the two sediment structures. The percentage of active bacteria in the hyporheic zone has been shown to vary greatly (2-50% of active bacteria, Fischer et al., 1996; Claret and Fontvieille, 1997) depending on water chemistry and organic matter availability (Fischer et al., 2002; Findlay and Sinsabaugh, 2003; Clinton et al., 2010). In the present study, differences in microbial activities measured between the coarse and fine sediment structures were apparently not related to drastic contrast in the availability of electron acceptors between the two sediment structures. Indeed, mean dissolved oxygen concentrations were higher than 6 mg l^{-1} in all hyporheic water samples and did not indicate constraining conditions for micro-organisms in both sediment structures (e.g. Sun et al., 2002). Similarly, variation of mean nitrate concentrations (from 4.1 to 5.7 mg l^{-1} of N-NO₃⁻) could not explain the significant differences in microbial activities measured between coarse

and fine sediment structures (e.g. Bowen *et al.*, 2009). We can therefore conclude that the threefold differences in microbial activities between sediment samples collected from the coarse

and fine sediment structures were more likely related to differences in the availability of organic matter, higher flux of water, and associated dissolved organic matter measured in the coarse sediment structure, probably leading to an increased supply of organic matter available for micro-organisms (Foulquier *et al.*, 2011).

Higher abundances of hyporheic invertebrates in the coarse sediment structure compared with the fine sediment structure suggest that organic matter availability and also porosity had major influences on invertebrate distribution in the hyporheic zone. The distribution of groundwater organisms in gravel bars is generally linked to groundwater pathways (Dole-Olivier and Marmonier, 1992), but organic matter availability may explain the spatial patterns of groundwater fauna observed in the present study. For instance, the occurrence of Niphargus spp. at 15 and 35 m in the coarse sediment structure and at only 1 m in the fine sediment structure might have been due to the occurrence of fine particulate organic matter (FPOM) partially processed during its transport inside the gravel bar. Indeed, FPOM has been recognized as a significant food source for the species Niphargus rhenorhodanensis (Navel et al., 2011). Sediment features likely influenced the physical habitat availability of the hyporheic zone for invertebrates. Field studies (Maridet et al., 1992, 1996; Strayer et al., 1997; Olsen and Townsend, 2003) and a laboratory experiment (Navel et al., 2010) showed that pore volume determined the suitability of sediment habitats for invertebrates. Our results were in accordance with this relationship between sediment habitat and invertebrate abundances: the coarse sediment structure allowed the occurrence of large interstitial voids suitable for large crustaceans such as gammarids or asellids, while the fine sediment structure limited the hyporheic habitat availability for large organisms. Therefore, information on the sediment structures obtained by GPR was very efficient to localize biological hotspots in the hyporheic zone although we could not discern the respective influences of organic matter availability and interstitial porosity on fauna distribution in the present study.

^{*}p < 0.05

p < 0.01



Figure 6. Total nitrogen (TN), total organic carbon, bacterial abundance, microbial activities (oxygen consumption and hydrolytic activity), diversity, and abundance of invertebrates measured in the two sediment structures at three positions within the gravel bar. FDA, fluorescein diacetate.

Several field studies (Danielopol *et al.*, 1992; Mermillod-Blondin *et al.*, 2000; Deforet *et al.*, 2009; Nogaro *et al.*, 2010; Maazouzi *et al.*, 2013) reported high heterogeneity of hyporheic processes at a decametre scale within/between geomorphological units. For instance, Mermillod-Blondin *et al.* (2000) showed that two riffles located 80 m apart in a small French river exhibited functional differences: one riffle acted as a trap for organic matter whereas the other did not. Using an experimental procedure to increase water flow rates, Maazouzi *et al.* (2013) obtained contrasted responses in nutrient dynamics in the hyporheic zone for two gravel bars located in the same river. In most studies reporting such kind of variability, there is an expected significant influence of local heterogeneities in sediment characteristics (grain size, porosity, and hydraulic conductivity) on groundwater flow paths. The present study highlights the feasibility and usefulness of GPR to test the potential influences of sediment heterogeneities on the location of biological hotspots in the hyporheic zone. The application of GPR would greatly improve our description of the sediment structures in the underground without perturbation by subsurface sediment sampling. Moreover, future applications

					Coarse	sedim	ent stru	acture					Fin	e sedi	ment	struc	ture		
				1 m			15 m		37	2 m		1 m			15	Е		35	в
Phylum/class/order	Family	Lowest identification	1	5	ю	1	7	ю	1	2	-	2	ю		7	3		5	3
Nematoda/Adenophorea	Mermithoïdae	I	15	4	7	6			-		56	_							
Oligochaeta			16	21	24	34	-	ю			37	. 15	Ξ	0			-		
Bivalvia	Sphaeriidae	Pisidium sp.	6	4	0														
Gastropoda	Valvatidae	Valvata sp.	0	4	7				0		5								
Gastropoda	Hydrobiidae	Potamopyrgus antipodarum	6	0	9														
Copepoda			200	287	98	4	٢	Ξ		4	23(21		0					
Ostracoda	Candonidae	Pseudocandona zschokkei	б	-		1	-												
Cladocera	Chydoridae	Alona guttata		Э							7	-							
Cladocera	Chydoridae	Chydorus sp.																	
Amphipoda	Gammaridae	Gammarus sp.	9	٢	15					-									
Amphipoda	Niphargidae	Niphargus sp.					Ś		6	-	4	_		ŝ					
Isopoda	Asellidae	Asellus aquaticus		4	9														
Arachnida/Acari								-	×										
Ephemeroptera	Baetidae								-										
Plecoptera	Leuctridae		0		1														
Coleoptera	Dytiscidae		-		ω		0												
Coleoptera	Elmidae	Esolus sp.	Э	0															
Diptera	Chironomidae	Orthocladinae sp.					14												
Diptera	Chironomidae	Chironomini sp.					`												
Diptera	Chironomidae	Tanytarsıni sp.					9		ı										
Collembola			4						2										

Table IV. Abundances of invertebrate taxa (for 101 of collected mixture of water and sediment) collected in the hyporheic zone at three replicated points (1, 2, and 3) per sediment

of GPR in river systems could include (1) identifying the hyporheic areas acting as main water purification zones in a river and (2) visualizing the preferential water pathways that could act as productive water catchments or vulnerable areas for pollutions.

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