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Ecological assessment of groundwater trophic status by using artificial substrates to monitor biofilm growth and activity

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ABSTRACT

The European water legislation highlights the necessity of developing ecological criteria for assessing the quality of aquatic ecosystem. While a multitude of ecological indicators has been proposed and validated for streams, rivers and lakes, these indicators are not available for groundwater ecosystems. In the present study, we developed a method based on the measurements of the growth and activity of microorganisms developed on artificial substrates (glass beads) incubated in wells to characterize the trophic status of groundwater. Incubation sites were selected in an urban aquifer where previous works showed contrasting trophic conditions in groundwater due to artificial groundwater recharge practices. Total proteins, total carbohydrates, dehydrogenase and hydrolytic activities were measured on glass beads incubated in wells for two periods of 2 months (October–December 2010 and April–June 2011). Biofilm measured on glass beads was significantly more developed and active in wells where groundwater was enriched with dissolved organic matter due to artificial groundwater recharge. Indeed, most microbial variables (total proteins and dehydrogenase activities for the two incubation periods and hydrolytic activities for the second incubation period) were significantly and positively correlated with the concentrations of dissolved organic carbon (DOC) in groundwater. The availability of phosphorus also tended to influence biofilm growth (assessed by total carbohydrates) when PO_4^{3-} concentrations were lower than 50 μg/l. Overall, our study clearly demonstrated that artificial substrates acting as colonisable area for microorganisms could be used to efficiently monitor nutrient enrichment in aquifers.

1. Introduction

Groundwater constitutes the principal source of drinking water for Europeans as about 75% of the inhabitants of the EU rely on groundwater for their water supply (Gibert, 2001). Protection of groundwater quality and quantity is therefore recognized as a major challenge for industrial countries (EU Groundwater Directive 2006/118/EC). However, the protection of groundwater resources cannot be achieved without the development of efficient indicators of ecosystem health and functioning (Danielopol et al., 2004; Griebler et al., 2010). Historically, the assessment of groundwater status was based exclusively on chemical and hydrogeological parameters. Monitoring the chemical quality of groundwater may be a relatively simple task. Nevertheless, given the wide variety of pollutants (and their possible combinations) and possible occurrence of accidental contaminations, it is often a difficult, time consuming and expensive task. Therefore, biological indicators which can integrate groundwater status over time appear as promising tools for ecological monitoring (e.g., Steube et al., 2009). In the last two decades, descriptors based on microorganisms (biomass, activity and diversity, Goldscheider et al., 2006; Griebler et al., 2006) and fauna (invertebrate abundances and diversity, Mösslacher, 2000; Hahn, 2006) have been proposed to assess groundwater ecosystem health. If benthic invertebrates are commonly used to evaluate the ecological integrity of streams and rivers (e.g., Woodiwiss, 1964; Hawkes, 1997; Lafont, 2001; Smith et al., 2007; Peru and Dolédec, 2010), the usefulness of invertebrates for the ecological assessment of groundwater and especially of deep water-table unconsolidated aquifers (vadose zone thickness > 2 m) is questionable because the relationships between invertebrate communities and environmental conditions are often complex (e.g., Dumas et al., 2001). For example, the organic matter (OM) fluxes reaching groundwater systems can fuel the subterranean food web and increase the biomass and abundance of invertebrate assemblages (Datry et al., 2005) but, when OM fluxes exceed a certain threshold, they can lead to

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unfavorable conditions (e.g. low oxygenated waters) for subterranean invertebrates (Foulquier et al., 2011a). Adding to this, the low invertebrate abundances characterizing deep water-table aquifers makes it difficult to use invertebrate assemblages as pertinent indicators of groundwater ecosystem health. In contrast, microorganisms, which are omnipresent in aquifers and present rapid responses to environmental conditions, are promising integrative ecological indicators for groundwater (Griebler and Lueders, 2009; Steube et al., 2009). Indeed, microbial biomass, activity and diversity appear as ideal biological indicators of groundwater trophic status (Smith et al., 1986; Goldscheider et al., 2006; Griebler and Lueders, 2009; Williamson et al., 2012). For instance, Foulquier et al. (2011b) recently showed that the activity and the biomass of microorganisms attached to sediment were positively correlated to OM (dissolved organic carbon – DOC) fluxes in the aquifer. These results are promising for groundwater quality assessment, but the methods developed by Foulquier et al. (2011b) cannot be broadly applied because (1) they require the collection of fresh aquifer sediments by coring or pumping and (2) the characteristics of the sediment (sedimentary OM, mineralogy, colonisable surface for microorganisms) may have more influence on microorganisms than groundwater quality. To circumvent these problems, we developed a new method based on the incubation of artificial substrates (glass beads with a known colonisable area) in wells to assess the trophic status of alluvial aquifers by measuring the biomass and activity of microorganisms that develop on these substrates. Artificial substrates have been rarely used in subterranean environments but previous experiments performed in the hyporheic zone of the Rhône River (Claret, 1998a,b) and in an alluvial gravel aquifer system in New Zealand (Williamson et al., 2012) gave promising results.

The aim of the present study was therefore to develop a method in which artificial substrates were used to evaluate the trophic status of groundwater ecosystems. Artificial substrates were incubated below the water table of an urban aquifer in sites presenting contrasting fluxes of nutrients (due to stormwater recharge of the aquifer, Foulquier et al., 2010). The tests were performed at two periods (winter 2010 and spring 2011) to determine the potential influence of season on microbial growth and activities. We especially focused on microbial biomasses and activities that have been recognized as more pertinent indicators of trophic status in groundwater than variables assessing bacterial community structure (Foulquier et al., 2011a). Our main hypothesis was that growth and activities of microorganisms developed on artificial substrates efficiently responded and were positively correlated to trophic conditions (especially DOC availability) in groundwater.

2. Material and methods

2.1. Study sites

The sites influenced by artificial groundwater recharge (AGR sites) were located in the eastern aquifer of the Lyon metropolitan area, France (Fig. 1A). The aquifer (catchment area 314 km^2) consisted of three corridors separated by moraine hills of low hydraulic conductivity (10^{-5}–10^{-8} m/s) (Foulquier et al., 2009). Aquifer corridors consisted of highly permeable glaciofluvial sediments (hydraulic conductivity 10^{-3}–10^{-2} m/s) and were drained by the Rhône River. The aquifer was artificially recharged with stormwater at multiple sites to compensate for reduced natural recharge caused by the sealing of urban surfaces. Artificial groundwater recharge with stormwater represented at least 10% of the annual flux of groundwater which was estimated at 69.1 million of m^3 (Foulquier et al., 2009). The AGR sites consisted of a settling and an infiltration basin that collected stormwater from residential, commercial, and/or industrial areas.

2.2. Site instrumentation

Three AGR sites (IUT, Minerve and Grange Blanche) were instrumented with wells that allowed us to collect water and install artificial substrates at a depth of 30 cm below the groundwater table. The vadose zone thickness at AGR sites averaged 3.0 ± 0.9 m (Fig. 1B). At each AGR site, three replicate wells, located outside the area of influence of the stormwater plume induced by the infiltration basin, were used as control wells. Three other replicate wells were located in the immediate downstream vicinity of the basin and were used as recharge wells because they intersected the stormwater plume induced by the infiltration basins. The wells were made of transparent Plexiglas (inner diameter: 6 cm) and were screened (hole diameter: 0.5 cm) at their lower end over a height of 0.5 m (Foulquier et al., 2009). Previous experiments showed that water chemistry in wells (specific conductance, temperature, dissolved oxygen – DO–concentration) measured with probes did not differ from groundwater chemistry in the aquifer (Foulquier et al., 2010).

2.3. Artificial substrate incubations

Several materials have been used as artificial substrate for microbial studies: glass slides (Claret, 1998a,b), plastic slides (Morikawa, 1988) or glass beads (Bärlocher and Murdoch, 1989). To assess microbial growth into unconsolidated aquifers, we selected glass beads as artificial substrate because they offered a colonisable area mimicking glaciofluvial sediments (sand, gravel and cobbles). Glass beads (diameter of 4 mm, soda-lime glass, Carl Roth GmbH, Karlsruhe, Germany) were placed in wells in small plastic bags (5 cm × 4 cm) with a mesh size of 3 mm allowing the circulation of groundwater. Before their introduction into the small bags, glass beads were depolished to increase biofilm adhesion and then washed twice with demineralized water. For two periods of 2 months (first period: 20 October to 20 December 2010, and second period: 4 May to 7 July 2011), three replicate bags containing 120 glass beads each and corresponding to a total colonisable surface of 60.5 cm^2 were introduced in recharge and control wells of the three AGR sites.

2.4. Glass bead collection and water sampling

At three times during the period of glass bead incubation ($t = 0$, $t = 1$ month and $t = 2$ months), eight physico-chemical variables (temperature, specific conductance, pH, DO, NH_4^+, NO_3^-, PO_4^{3-} and DOC) were measured on water extracted from each well using a hand piston pump (Bou and Rouch, 1967). Temperature, specific conductance (LF92, WTW™, Weilheim, Germany), pH and DO concentrations (HQ20, HACH™, Dusseldorf, Germany) were measured in the field.

Water was collected in 0.5-l pre-combusted glass bottles for the determination of DOC and nutrients (NH_4^+, NO_3^- and PO_4^{3-}). Water samples were stored at 4 °C, brought within 4 h to the laboratory, and filtered through Whatman HAWP filters (pore size: 0.45-μm; Millipore, Billerica, MA, USA).

At the end of the incubation ($t = 2$ months), each glass bead bag was collected and immediately placed into a polypropylene box containing water extracted from the same well (see above for details on water sampling). Polypropylene boxes were stored at 4 °C and brought within 4 h to the laboratory.