Evaluating the performance of water purification in a vegetated groundwater recharge basin maintained by short-term pulsed infiltration events

SUPPORTING INFORMATION

MATERIALS AND METHODS

Characterization of DOM

Qualitative evaluation of dissolved organic matter (DOM) was carried out by means of fluorescence spectroscopy (FA-256 SLM AMINCO Bowman Series 2). A 150 W Xenon lamp was used for excitation. A series of eight emission spectra was collected over a range of excitation wavelengths. The excitation wavelength range was from 260 to 400 nm. A wavelength step size of 20 nm was applied. Emission scans were recorded from 410 to 550 nm in reference mode. Scanning speed was set at 8 nm sec$^{-1}$. Filter WG 295 (Schott, Germany) was used to eliminate second order Rayleigh scatter. The main focus was to evaluate the content of fulvic acids (FA) and humic acids (HA) relative to each other, with FAs generally above 479 nm and HAs below. For further details see Senesi et al. ($2004$).

Quantification of bacteria

For determination of the total number of bacteria attached to particles, 2 mL of fine sediment was collected, preserved in 10 mL of a 2% paraformaldehyde solution buffered with 0.1% sodium pyrophosphate, and stored at 4 $^\circ$C in the dark. For the detachment of bacteria, sediment samples were shaken and sonicated following the protocol of Griebler et al. ($2001$). For direct counts of suspended bacteria, 25 mL of water was fixed with formaldehyde (2% final conc.). Subsequently, bacterial abundances were determined via flow cytometry (MoFlo, DakoCytomation). Sample aliquots of 5 and 50 $\mu$L were stained with SYBR Green I (Molecular Probes; final concentration 1×), then fluorescent microspheres (1 $\mu$m TransFluoSpheres 488/560, Molecular Probes) were added at a final concentration of 4.7 $\times$ 105 L$^{-1}$ as per internal standard. Quantifications were made with a water-cooled argon ion laser tuned at 488 nm (200 mW), plotting side scatter versus green fluorescence.

Bacterial community analysis

Bacterial community analysis based on T-RFLP fingerprinting. DNA from triplicate sediment subsamples was extracted, precipitated, and diluted in EB buffer (Qiagen) for storage ($-20$ $^\circ$C) as described in Winderl et al. ($2008$). Later, terminal restriction fragment length polymorphism (T-RFLP) analysis of bacterial 16S rRNA gene amplicons was done as previously described (Briellmann et al. 2009) applying the primers Ba27f-FAM and 907r. Primary electropherogram analysis was conducted using the GENEMAPPER 5.1 software (Applied Biosystems) excluding peaks $<50$ bp. Identification of baseline threshold of true peaks over noise and the alignment of terminal restriction fragments (T-RFs) were conducted with the T-REX software (Culman et al. 2009). The software implements a procedure where true peaks are iteratively identified as those whose height exceeds, in our case, one standard deviation computed over all peaks. This way, T-RFs with a relative fluorescence...
signal of #100 were excluded from further analysis. Peak alignment in T-REX was done automatically. A clustering threshold of ±0.5 bp was specified for the grouping of peaks into a common T-RF.

**Bacterial heterotrophic production**

The incorporation of [³H]leucine was used to estimate heterotrophic bacterial carbon production (BCP). Triplicates of 10 mL water samples plus one control (fixed prior to incubation with formaldehyde, 2.5% final conc.) were incubated with 150 nM [³H]leucine (83 Ci mmol⁻¹ specific activity, GE Healthcare) for 2 hours at in situ temperature in the dark. After incubation, samples were filtered (GE Healthcare) for 2 hours at in situ temperature in the dark. Triplicate [³H]leucine incubations plus two formaldehyde-killed (2.5% final conc.) controls were run for biological activity (respiration) with the highest values ranging from 13 to 19.7 °C in section 5B and from 15.7 to 19.2 °C in section 5D. Sediment porewater temperatures at 5B roughly reflected the temperatures in the surface water and showed no clear gradients with depth (Figure S1). No significant temporal and spatial trends between the two different sampling sites were detected for pH and electrical conductivity exhibiting values between 7.43 to 8.18 and 315 to 466 µS cm⁻¹, respectively (data not shown).

Dissolved oxygen (DO) served as a sensitive indicator for biological activity (respiration) with the highest values measured in the surface water and a subsequent decline of about 75% from 0–10 cm to 110–120 cm sediment depth (Figure S1). Dissolved oxygen values measured in 110–120 cm sediment showed a mean concentration of 1.45 mg L⁻¹ at 5B with a decreasing trend from the first day of sampling (2.1 mg L⁻¹) to the last day (0.4 mg L⁻¹). At 5D, O₂ values ranged from 1.2–2.6 mg L⁻¹ with a mean of 1.75 mg L⁻¹ without exhibiting a temporal trend.

**RESULTS – PHYSICAL-CHEMICAL CONDITIONS**

**Hydraulic conductivity**

**Temperature and dissolved oxygen (DO)**

During the second sampling campaign, surface water temperatures varied depending on weather conditions and ranged from 13 to 19.7 °C in section 5B and from 15.7 to 19.2 °C in section 5D. Sediment porewater temperatures at 5B and 5D roughly reflected the temperatures in the surface water and showed no clear gradients with depth (Figure S1). No significant temporal and spatial trends between the two different sampling sites were detected for pH and electrical conductivity exhibiting values between 7.43 to 8.18 and 315 to 466 µS cm⁻¹, respectively (data not shown).

**RESULTS – MICROBIOLOGY**

**Distribution of bacteria, viruses and patterns of microbial activity in Pond 5B and 5D**

Numbers of bacteria in sediment porewater ranged from $1.57 \times 10^7$ cells mL⁻¹ (sampling day 1, 0–10 cm) to $2.45 \times 10^8$ (sampling day 3, 110–120 cm) in section 5B and from $1.9 \times 10^7$ cells mL⁻¹ (day 3, 0–10 cm) to $1.74 \times 10^6$ (day 4, 110–120 cm) in section 5D, respectively (Figure 3 in the main paper). Abundances of bacteria attached to the sediments ranged from $7.72 \times 10^6$ cells cm⁻³ (sampling day 1, 0–10 cm) to $8.23 \times 10^7$ (sampling day 3, 110–120 cm) at 5B and from $2.47 \times 10^9$ cells cm⁻³ (sampling day 4, 0–10 cm) to $6.57 \times 10^7$ (sampling day 4, 110–120 cm) at 5D, respectively (Figure 4 in the main paper).
The maximum number of viruses were found at section 5B with $12.8 \times 10^7$ ml$^{-1}$ (sampling day 4, 0–10 cm) and at 5D with $14.4 \times 10^7$ ml$^{-1}$ (sampling day 1, 0–10 cm). The minimum numbers accounted for $2.2 \times 10^7$ ml$^{-1}$ (sampling...
day 1, 110–120 cm) at 5B and 1.68 × 10⁷ mL⁻¹ (sampling day 1, 110–120 cm) at 5D, respectively (Figure 3).

The BCP of bacteria suspended in sediment porewater tended to decrease with depth, e.g. at 5B from the maximum of 1.43 (sampling day 1, 0–10 cm) to the minimum of 0.2 μg CL⁻¹ h⁻¹ (sampling day 1, 110–120 cm) compared to 5D, with a range from 0.69 (sampling day 3, 0–10 cm) to 0.1 μg CL⁻¹ h⁻¹ (sampling day 2, 110–120 cm) (Figure 3).

**Viruses and bacterial abundance in groundwater**

Groundwater from the up-gradient well EM 34 contained 0.6–1.2 × 10⁴ bacterial cells mL⁻¹, and groundwater from the down-gradient well EM 58 contained 1.2–3 × 10⁴ cells mL⁻¹.

The highest numbers of viruses in groundwater were found at the down-gradient well EM 58 which contained 21 ± 5.6 × 10⁴ mL⁻¹ at 0.5 m below the groundwater table. The lowest numbers were found in groundwater from the up-gradient and recharge unaffected well EM 54 with 1.4 (0.5 m BGWT) and 2.2 × 10⁴ mL⁻¹ (5 m bgwt) (Figure S2).

**Eubacterial community patterns**

Quantitatively important OTUs common for all sites included T-RF 137 with 8–12%, T-RF 147 with 6–12%, and T-RF 498 with 7–15% of relative abundance, respectively. The samples from 5D 11–120 cm were clearly dominated by T-RF 487, with almost 40% relative abundance, while other OTUs common at the other sites, i.e. T-RFs 120 and 158, were rare.

**Meio- and Macrofauna**

The organisms in the superficial sediment were sampled with a Bou-Rouch suction pump at 0–10, 30–40, 70–80, 10–120 cm intervals below the bottom of the basins and invertebrates were sorted from 3 litres of water and sediment samples (cf. method in Pospisil, 1992).

In the water column planktonic crustaceans like daphnids (Cladocera) and copepods (Calanoïda and Cyclopoïda species) were found. At the bottom, between the accumulation of decaying organic matter, oligochaetes and chironomid larvae dominated.

A higher taxonomic diversity of invertebrates was present in section 5B with maximal densities in the first 10 cm layer and a drastic decrease of abundance in the following 3 depth layers; 35 invertebrate species occurred in the 5B basin compared to 29 in the 5D (Danielopol et al., 2006). The most diverse and abundant groups were the Nematoda, Oligochaeta and Cyclopoïda, followed by insect larvae (mainly Chironomidae) and Cladocera. The density of invertebrates in the sediment increased with flooding time in all layers from the 1st to the 4th sampling day due to the continuous water seepage into the subsurface. As an example, the abundance of surface dwelling cyclopoids in the upper 10 cm sediment layer at 5B exhibited less than 200 individuals in the first day while in the deeper layers only isolated copepods occurred, and in the 4th sampling round, 8 days later, we caught more than 250 specimen in the 1st layer, and between 10 and 86 individuals in deeper zones. This trend was visible for other groups too, like the benthic cladoceran *Chydrorus sphaericus* which increased in abundance in the upper sediment layer from about 100 individuals to more than 1,500. At the site 5B we found 11 cyclopoid species in the sediments, while at site 5D, only 6 species occurred, which all have been epi-gean taxa. Large-size crustaceans, which are normally restricted to the open water column (e.g. the cyclopoid *Mesocyclops leuckarti* and the cladocerans *Ceriodaphnia reticulata* and *Bosmina longirostris* occurred in large numbers only in the upper 10 cm of sediment at 5B.

Especially interesting are the oligochaetes, the chironomids and the nematodes, because of their well-known ability to stimulate the bacterial growth and their capacity to rework fine sediments, through feeding and movement activities. The oligochaetes were represented by small-sized species belonging to *Aeolosomatidae, Naididae* and *Enchytraeidae*, known to live in both terrestrial and aquatic habitats. There were 9 species in the 5B section, with the highest abundance in the 1st sediment layer (*Aeolosoma quaternarium, Nais elinguis* and *Marionina riparia*), dominated while in 5D only 5 species were found in lower densities (*Nais pseudoobtusus* and *Marionina argentea* were the most abundant species). The density of oligochaetes, even if low (maximal 105 ind. 5 L⁻¹ sample in the 5B sediment), correlated positively with the microbial biomass. The chironomids showed low densities

<table>
<thead>
<tr>
<th>Sampling site Depth [cm]</th>
<th>5B Hydraulic conductivity [m s⁻¹]</th>
<th>5D Hydraulic conductivity [m s⁻¹]</th>
</tr>
</thead>
<tbody>
<tr>
<td>0–10</td>
<td>1.2</td>
<td>7.3 × 10⁻¹</td>
</tr>
<tr>
<td>50–40</td>
<td>8.7 × 10⁻²</td>
<td>5.6 × 10⁻⁴</td>
</tr>
<tr>
<td>70–80</td>
<td>3.0 × 10⁻²</td>
<td>3.0 × 10⁻⁵</td>
</tr>
<tr>
<td>110–120</td>
<td>6.7 × 10⁻²</td>
<td>3.2 × 10⁻²</td>
</tr>
</tbody>
</table>

Table S1 | Hydraulic conductivity values (K, values in mm s⁻¹) at two different sampling sites in Pond 5. Results are means of three measurements at 5B and two measurements at 5D.
in the 1st sediment layer of both basins. Their abundance varied between 1–19 specimens at the 5B site and 2–16 at 5D.

Nematoda showed the highest abundance in the sediments of the 5D basin, up to 3,120 specimens, were recorded in the upper first sediment layer. The numbers of nematodes in the 5B sediments were lower, varying mainly between 25–288 individuals. However, in section 5D nematodes showed a lower species diversity than in 5B. At both sites the secernetean species of the family Hemicycliophoridae, which feed by suction of the plant roots, dominated numerically (Figure 3). The other species which feed on sediments or which are epistrate-feeders ingesting bacteria, as well as those which chew their food, represent only a small fraction (about 15% of the total nematode assemblage). In 5D, which is covered with trees, more than 80% Hemicyclophora species have been found while these accounted for only about 50% of the community at site 5B.

**REFERENCES**


