Are dominant microbial sub-surface communities affected by water quality and soil characteristics?

Carme Barba[a,b,*] (carme.barba@upc.edu), Albert Folch[a,b] (folch.hydro@gmail.com), Xavier Sanchez-Vila[a,b] (xavier.sanchez-vila@upc.edu), Maira Martínez-Alonso[c] (maira.martinez@uab.cat), Núria Gaju[c] (nuria.gaju@uab.cat)

[a]Department of Civil and Environmental Engineering, Universitat Politècnica de Catalunya (UPC), C/Jordi Girona 1-3, 08034 Barcelona, Spain

[b]Associated Unit: Hydrogeology Group (UPC-CSIC)

[c]Department of Genetics and Microbiology, Universitat Autònoma de Barcelona (UAB), 08193 Bellaterra, Spain

Abstract

Subsurface microorganisms must deal with quite extreme environmental conditions. The lack of light, oxygen, and potentially nutrients are the main environmental stresses faced by subsurface microbial communities. Likewise, environmental disruptions providing an unbalanced positive input of nutrients force microorganisms to adapt to varying conditions, visible in the changes in microbial community diversity. In order to test microbial community adaptation to environmental changes, we performed a study in a surface Managed Aquifer Recharge facility, consisting of a settlement basin (two-day residence time) and an infiltration pond. Data on groundwater hydrochemistry, soil texture, and microbial characterization was compiled from surface water, groundwater, and soil samples at two distinct recharge operation conditions.

Multivariate statistics by means of Principal Component Analysis (PCA) was the technique used to map the relevant dimensionality reduced combinations of input variables that properly describe the system behavior. The methodology selected allows including variables of different nature and displaying very different range values. Strong differences in the microbial assemblage under recharge conditions were found, coupled to hydrochemistry and grain-size distribution variables. Also, some microbial groups displayed correlations with either carbon or nitrogen cycles, especially showing

abundant populations of denitrifying bacteria in groundwater. A significant correlation was found between *Methylotenera mobilis* and the concentrations of NO_3 and SO_4, and also between *Vogesella indigofera* and the presence of DOC in the infiltrating water. Also, microbial communities present at the bottom of the pond correlated with representative descriptors of soil grain size distribution.

1. Introduction

Groundwater systems are perceived as relatively stable environments as compared to most aquatic ecosystems (Zhou et al., 2012). Despite that, investigations have shown that soil-aquifer systems support a wide diversity of organisms (e.g., Griebler and Lueders, 2009). Actually, the unsaturated zone, and more specifically the topsoil, supports the highest microbial activity and biomass of all compartments within the subsurface environment (Lapworth et al., 2012). Likewise, microorganisms are responsible for most biological processes in aquifers (Stein et al., 2010).

Several studies evidence microbial adaptation to groundwater extreme environments (thermal or hypersaline) (e.g., Rothschild and Mancinelli, 2001) or disturbed by human activities (Meckenstock et al., 2015). Human activities have caused disruption in aquifer dynamics to some extent (Griebler and Lueders, 2009; et al., 2017), with biological implications as indigenous microorganisms can acclimate (Pett-Ridge and Firestone, 2005) or even take advantage (Rezanezhad et al., 2014) of environmental disturbances.

In fact, the water treatment industry has benefited from the adaptability and metabolic capabilities of microorganisms to maximize the improvement of water quality. Several laboratory experiments and engineering applications have tested the effectiveness of microbial engineered techniques for water reclaim purposes. The former has been conducted aiming at (1) describing degradation pathways of specific pollutants and quantifying their degradation rates (Greskowiak et al., 2017; Regnery et al., 2015;

Rodriguez-Escales and Sanchez-Vila, 2016), (2) determining the physical and hydrochemical conditions that can govern the behavior of specific microbial groups (Alidina et al., 2014; Drewes et al., 2014; Freixa et al., 2015; Kolehmainen et al., 2008; Perujo et al., 2017), or (3) understanding the role of organic matter (dissolved organic carbon -DOC- or micropollutants), on the growth of microbial communities (Li et al., 2013, 2012). Regarding the engineered applications of microbial ecology designed to improve the quality of reclaimed water, examples are constructed wetlands (Faulwetter et al., 2009; Truu et al., 2009; Zhang et al., 2018) and sand filters (D'Alessio et al., 2015).

Natural and induced microbial attenuation have been studied and applied at the field scale concerning groundwater related environmental issues; this includes, e.g., landfill leakage affections (Röling et al., 2001; Staats et al., 2011), contaminant spills (Fahrenfeld et al., 2014; Haack et al., 2004; Martínez-Pascual et al., 2010; Nijenhuis and Kuntze, 2016), or nitrate polluted aquifers (Bellini et al., 2018, 2013).

In recent years, the number of soil aquifer treatment (SAT) facilities have increased worldwide. SAT is a particular case of the Managed Aquifer Recharge (MAR) family, that combines the replenishment of groundwater bodies with the treatment of water during infiltration, by taking advantage of the potential of the soil for the degradation of subsurface microbial communities (Bouwer, 2002). Studies testing the link between water quality and microbial communities in MAR systems depend on the system type, whether recharge wells (Ginige et al., 2013), riverbank areas (Schütz et al., 2009), or surface infiltration ponds (Barba et al., 2019; Reed et al., 2008; Regnery et al., 2016). Infiltration ponds are low-cost, low-tech, passive facilities compared to advanced water treatment methods (Drewes et al., 2003; San-Sebastián-Sauto et al., 2018); for these reasons, they are widely implemented, mostly in arid or semi-arid environments (Goren et al., 2014; Greskowiak et al., 2006; Rodríguez-Escales et al., 2018).

Biodegradation processes in surface infiltration ponds include aerobic oxidation of DOC (Maeng et al., 2011; Mermillod-Blondin et al., 2015), denitrification (Grau-Martínez et al., 2018), and removal of some emerging organic compounds (Hamann et al., 2016; Valhondo et al., 2015). Parameters modifying bioprocesses, such as DOC availability, dissolved oxygen content, redox conditions, temperature, nutrient concentrations, or soil moisture have been widely reported (Alidina et al., 2015; Bekele et al., 2011; Dutta et al., 2015; Goren et al., 2014; Greskowiak et al., 2005; Hellauer et al., 2017; Laws et al., 2011; Massmann et al., 2006; Rezanezhad et al., 2014). Apart from environmental parameters, properties of the porous media, such as porosity and the distributions of grain sizes and pore sizes, determine the spatial and temporal distributions of microorganisms in soils (Chau et al., 2011); they also influence hydraulic conductivity, and thus the access to nutrients, affecting in a distinct way the growth and activity of microorganisms (Perujo et al., 2018, 2017).

Subsequently, determining how much and to what extent physical, geochemical, biological and operational parameters influence a SAT system functioning is useful for managing purposes (Dutta et al., 2015; Grau-Martínez et al., 2018; Hellauer et al., 2017; Pedretti et al., 2012a; Rodríguez-Escales et al., 2017). Yet, it is difficult to find multidisciplinary research dealing with integrated approaches to improve understanding of infiltration problems. One such example is the Llobregat MAR surface infiltration system, where a number of experiments have been performed in the last decade. The system is, thus, well characterized in terms of natural and induced flow regime, including numerical modelling (Valhondo et al., 2016), DOC mapping (Valhondo et al., 2015) and the evaluation of nitrate attenuation (Grau-Martínez et al., 2018), and the fate of several emerging compounds (Valhondo et al., 2018) along the infiltration path. Finally, microbial fingerprinting was studied, and the spatial distribution of dominant microbial phylotypes was linked to the overall recharge processes (Barba et al., 2019). In that work, the microbial assemblage was characterized and discussed under an

ecological point of view. However paramount aspects, such as the effect of hydrochemical composition or the grain-size distribution, in water and soil samples, linked to their role in the presence of specific microbial signatures were not addressed.

We contend that a full analysis of processes occurring in any MAR facility should involve the simultaneous study of physical, hydraulic, geochemical and microbial data; therefore, it includes the joint analysis of continuous, discrete and categorical data, with different ranges of values and resolution windows, calling for the use of multivariate (MV) statistical techniques. Such techniques have been widely used in hydrogeology to provide process understanding and to accompany groundwater models (El Alfy et al., 2017; Menció et al., 2012). In the case of microbial ecology, the development of molecular analyses allowed the generation of large data sets, best treated using MV statistical techniques (see Paliy and Shankar, 2016 for a review).

Here, we aim at combining ecological, hydrochemical and hydrological approaches to understand the spatial distribution of subsurface microbial communities under changing recharge conditions, with significant consequences in the management of MAR facilities. For this purpose, we used a physical-bio-geochemical dataset from an existing infiltration facility, and then applied PCA, aiming at statistically discriminate the relationships among different parameters corresponding to microbial community structure, geochemical variables, soil grain size distribution, and operational conditions.

This work allows providing the most relevant microbial indicators present in the system and to correlate them with soil and groundwater local characteristics and feeding water. Furthermore, the existing data between microbial clades both in water and soil were also analyzed separately to obtain further relevant inter-clade correlations.

2. Material and methods

The present work is built on the dataset compiled and presented in Barba et al. (2019). This work describes in detail the fieldwork procedures and laboratory tasks performed to obtain data representative of the Llobregat MAR site regarding a large set of physical, geochemical and biological variables, and from two sampling campaigns involving different recharge conditions. In this section, we provide only the essential information to contextualize the statistical analysis, being the core of this work.

2.1. Study site

The field site is located in the Llobregat River Basin (Sant Vicenç dels Horts, Catalonia), 10 km SW of Barcelona. The recharge system replenishes the Lower Valley Aquifer, a strategic groundwater source for the Barcelona Conurbation. It is an alluvial aquifer, composed mainly by sands and gravels with small paleo-channels of clay (Pedretti et al., 2012b). Hydrogeologically, it is of high transmissivity that reaches 14000 $m^2 \cdot d^{-1}$ locally, with an average thickness of 15m.

The system is composed of two ponds. The first one (7300 m^2) is fed with river water, and acts as a sedimentation pond for fine particles, with 2-3 day residence time. Then, water passes through a pipe to the second pond (6500m^2), where it infiltrates to the aquifer through 4-10m of vadose zone. Being devised as a research facility, in 2011 an organic layer was placed at the bottom of the infiltration pond to test its efficiency for the removal of emergent pollutants by the addition of labile DOC to the inflow water.

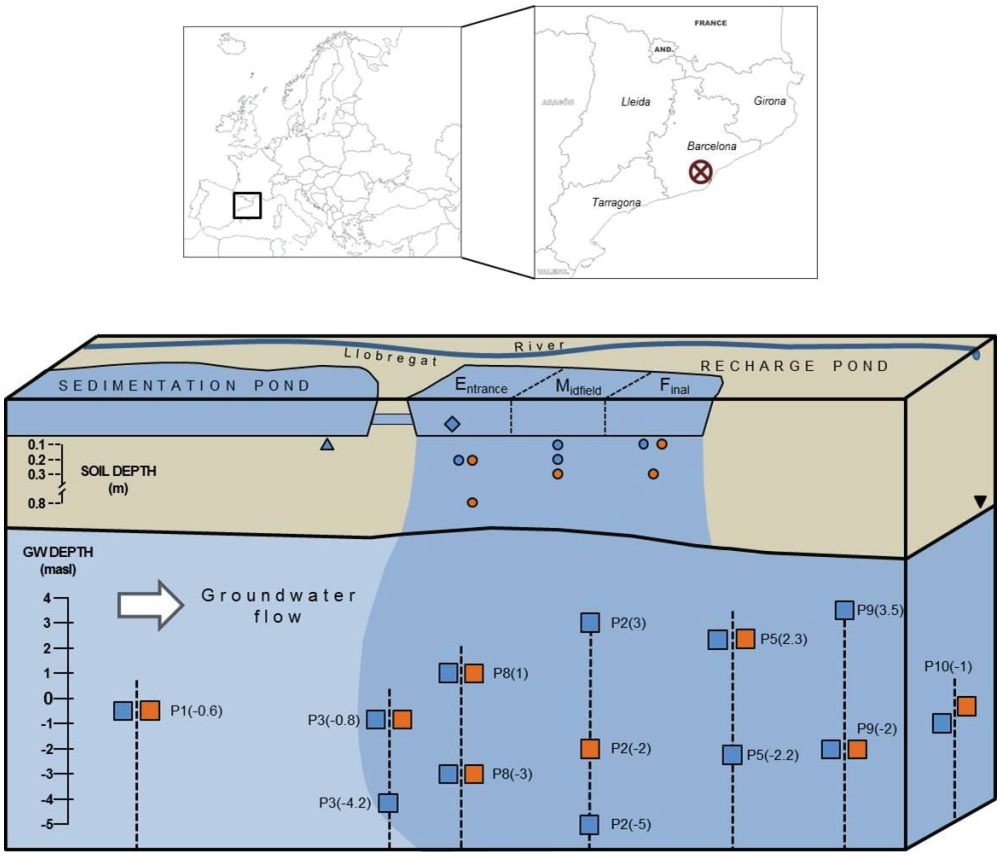

Figure 1 Geographical situation and cross-section of the Llobregat MAR System. Triangles and circles represent the location of soil samples. Diamond and squares represent those of water and groundwater samples, respectively. Blue symbols indicate samples taken in July 2014, after six months of continuous uninterrupted recharge. Orange samples were taken after recharge was discontinued for 4 months in March 2015.

The system includes a network of piezometers for monitoring and groundwater sampling corresponding to different travel times after recharge (Figure 1). Detailed information about the conceptual regional flow model and the local flow and transport models can be found in Barba et al. (2019) and Valhondo et al. (2016), respectively.

2.2. Input data for the multivariate statistical approach

Water and soil samples were taken in two different periods, July 2014 and March 2015, representing different recharge operation conditions. In the July campaign, sampling was performed after six months of uninterrupted recharge; in the March one, recharge had been discontinued for four months prior to sampling. In total, 21 water samples (20

for groundwater and 1 for recharge water) and 10 soil samples were subjected to molecular analyses, in duplicate (only average values are presented in this work). Furthermore, water samples were hydrochemically characterized, while granulometric curves were obtained for the soil samples. The sampling and analysis methodologies for chemistry, granulometry, DNA extraction, PCR, DGGE, diversity indices and relative abundances of the microbial clades, are reported in Barba et al. (2019).

The present study divides the statistical analyses according to the nature of the samples, treating those from water and soil separately, as different variables are included in each case. In the former, the statistical analysis accounted for (1) microbial diversity indexes, (2) relative microbial abundances at the taxonomical rank level of class and species, and (3) hydrochemical characterization (major, minor and trace elements). Instead, statistical analyses of soil samples contained (1) microbial diversity indexes, (2) relative microbial abundances at the taxonomical rank level of class and species, (3) grain-size distribution representative parameters, (4) depth, and (5) an operational binary variable indicating dry/wet conditions characterized by the presence or absence of recharge. Finally, a general statistical analysis was performed for all soil and water samples (31 in total) and the common variables (i.e., (1) and (2) from the previous lists), plus a binary variable indicating the sample nature (soil or water).

2.3. Rationale for the multivariate statistical approach: requirements for PCA

Multivariate statistics is a useful technique to treat large datasets involving different types of variables, allowing the inclusion of quantitative and categorical data together. In short, PCA transforms a set of data values of variables that in principle are correlated into a set of values of linearly uncorrelated variables called principal components (PCs). PCs are defined such that the first one accounts for as much of the variability in the data as possible, and each succeeding component, in turn, has the highest variance possible under the constraint that it is orthogonal to the preceding

ones. Thus, a multidimensional system in terms of variables is projected into a low dimensional map of components. Solutions were subjected to a varimax rotation of the original system corresponding to the directions of the largest variance in the dataset. Statistical analysis was done using software SPSS (IBM SPSS Statistics 24).

The full dataset is provided as supplementary material. It also includes plots corresponding to some of the PCA analyses that are included for completeness but do not provide enough additional information to merit inclusion in the body of the text.

2.3.1. Selecting variables for the statistical analysis of water samples

Statistical parametric methods perform best when data follows a unimodal symmetric distribution (Paliy and Shankar, 2016). For this reason, some variables from the initial dataset were eliminated, grouped and/or transformed in order to conform better to the assumptions of PCA analysis. For example, hydrochemical variables with most values below the detection limit were eliminated (this is the case of Al, B, Cd, Co, Fe, Pb, and P). Also, Ba concentration values were rejected for data inconsistency. Second, hydrochemical variables that displayed a positive skewness were log transformed (Cu, DOC, Ni, S and V concentrations). Finally, sample depth, electrical conductivity (EC), temperature, and the concentrations of HCO_3, Ca, Cl, Li, Mg, Mn, Na, NO_3, pH, Si, SO_4, and Zn were added to the analysis without any data transformation.

With reference to variables of microbial abundances, they were all transformed into new variables. For ecological data, displaying a large number of zero values, Legendre and Gallagher (2001) recommend applying either the Chord or the Hellinger transformations to the data values. In our case, we applied the latter one, because after some preliminary analyses, the transformed variables presented higher correlation coefficients (r^2) than the non-transformed ones. Hellinger transformation (x'_{ij}) of a datum x_{ij} pertaining to the i -class and j -object is given as:

$$x'_{ij} = \sqrt{\frac{x_{ij}}{\sum x_{i+}}}$$; where *i*+ denotes all *i*'s.

Finally, in the case of microbial diversity indices, the input data for the PCA were the Shannon, Richness and Evenness index values for each sample.

The next step was to perform bivariate correlation analyses with all variables taken two by two, including hydrochemical variables (both raw and log-transformed; actually this was the way of selecting whether the log-transformation was finally applied or not) and Hellinger-transformed microbial variables in order to select the ones that would be used in the final PCA analyses. Since the objective was to emphasize the correlations with the microbial data, most redundant geochemical variables, as well as those displaying very low values of the Pearson r^2 coefficient to the biological ones, were removed. This was the case of As, HCO_3, Ca, Li, Mg, Mn, Si, Zn, and also depth, pH, and temperature. In the case of microbial phylotypes, some of them were eliminated because they did not show significant correlations with other variables; e.g., Bacilli, Acidobacteria, Actinobacteria, Chlorobia, Nitrospira, Gammaproteobacteria, Alphaproteobacteria, *Subgroup3 sp* (Acidobacteria), *Stenotrophomonas sp, Chryseomicrobium sp, Nitrospira1 sp, Methylobacterium sp,* and *Nitrospira2 sp*.

The selected variables could not be incorporated all together in a single PCA because the total variance was too high to allow proper discrimination of components. Then, different PCAs (each one involving a different subset of parameters) were performed, trying to maximize the amount of explained information. We performed two PCAs for microbial abundances at the class level and two more at the species level. The most informative set of variables were selected as the subset that maximized the measure of sampling adequacy reported by Kaiser-Meyer-Olkin test (KMO); the variables included in each analysis are provided in Table 1.

2.3.2. Selecting variables for the statistical analysis of soil samples

Soil samples were subjected to sieve analysis, obtaining grain-size distribution curves. For each sample, the uniformity coefficient (*CU*) and the coefficient of curvature (*CC*) were calculated as (Terzaghi et al., 1996): $CU = \frac{D_{60}}{D_{10}}$, $CC = \frac{(D_{30})^2}{D_{60} \cdot D_{10}}$, where D_{60}, D_{30} and D_{10} are the diameters so that 60%, 30% and 10% of the material (in weight) pass the corresponding sieve. D_{10} has also been used as one of the variables in the statistical analysis. Another variable incorporated in the analysis was the proportion (in weight) of fine material (<0.074 mm) for each sample. Soil sample depth and sampling campaign (operation) were also included.

However, here Hellinger transformation was not applied to relative abundances of microbial species, because correlations were high already for the raw relative abundances. Some microbial variables were removed for the final analyses, based on the low bivariate r^2 coefficients obtained (this was the case of Cyanobacteria, Nitrospira, Gammaproteobacteria, Alphaproteobacteria, *Stenotrophomonas sp, Chryseomicrobium sp,* and *Nitrospira1 sp).*

Here, the optimization between the number of PCAs, the measurement of sampling adequacy (KMO) and the number of considered variables was done following the same criteria indicated in the statistical analysis of the water samples.

2.3.3. PCA for microbial print in water and soil samples

Taking advantage of the shared variables by water and soil samples, a third statistical analysis was performed, now including all 31 samples (soil and water) together. Microbial classes and species were treated separately and were Hellinger transformed. In addition to microbial data, the type of sample (water or soil) was also included as a binary variable. From the bivariate correlation analysis, some variables were removed from the final statistical analysis: Cyanobacteria, Gammaproteobacteria, Alphaproteobacteria, *Methylotenera mobilis, Vogesella indigofera, Stenotrophomonas*

sp, Chryseomicrobium sp, Methylobacterium sp, and *Nitrospira2 sp*. The optimization criteria were the same as indicated in the two previous sections.

3. Results

As a consequence of the preliminary analysis, PCAs of water samples considered 10 hydrochemical variables, 2 diversity indices and 10 microbial variables (5 classes and 5 species). PCAs of soil samples took into account 1 operational variable, depth, 4 grain-size soil parameters, 3 diversity indices and 14 microbial variables (7 classes and 7 species). Finally, the statistical approach for the total of 31 samples (soil and water) included 3 diversity indices, the type of sample and 13 microbial variables (9 classes and 4 species).

We present firstly global results of the eleven PCAs performed (Table 1). We indicate, in the same table and for each statistical analysis performed, the extracted components with their variables and the proportion of variance represented for each component. Also, the measure of sampling adequacy (KMO test value) is reported for each analysis.

Type of sample	Microbial clade data treated	PCAs performed	PCA number	Extracted components	% of variance	KMO Test value
Water	Classes	2	PCA_1	C1: **V-log**, Cu-log, Cl, EC, **S-log**, Na, **Others$_H$**	46.9	0.744
				C2: Shannon, **Betaproteobacteria$_H$**, Dehalococcoidia$_H$, Richness	27.2	
			PCA_2	C1: Cyanobacteria$_H$, EC, Cl, Cu-log, Ni-log	52.6	0.701
				C2: **Cytophagia$_H$**, Richness	24.4	
	Species	2	PCA_3	C1: *Methylotenera_mobilis*$_H$, NO_3, SO_4, **DOC-log**	61.5	0.797
				C2: *Nitrospira_sp2*$_H$, EC, Na, **V-log**, Cu-log, Cl	15.4	
			PCA_4	C1: ***Pontibacter_sp$_H$***, *Dehalogenimonas_sp*$_H$, Richness	52.9	0.635
				C2: *Vogesella_indigofera*$_H$, Shannon, DOC-log	24.3	
Soil	Classes	2	PCA_5	C1: **Betaproteobacteria$_H$**, Actinobacteria$_H$, Dryness, Depth, Cytophagia$_H$	62.5	0.750
				C2: CU, % Fine, Acidobacteria$_H$	22.1	
			PCA_6	C1: Richness, Dehalococcoidia$_H$, Chlorobia$_H$	75.5	0.727
				C2: Shannon, Evenness, **Actinobacteria$_H$**, **Bacilli$_H$**	12.3	
	Species	2	PCA_7	C1: *Nitrospira_2sp*, Dryness, **Shannon**, *Pontibacter_sp*,	64.6	0.758
				C2: % Fine, *Subgroup3_sp*, CU	27.6	
			PCA_8	C1: CC, D10, *Methylotenera_mobilis*, *Methylobacterium sp*,	59.5	0.640
				C2: % Fine, CU, *Dehalogenimonas_sp*, *Vogesella indigofera*	23.9	
Water and soil	Classes	2	PCA_9	C1: Shannon, Richness, Actinobacteria$_H$, Type, Evenness	53.8	0.692
				C2: Others$_H$, **Betaproteobacteira$_H$**	16.4	
				C3: Bacilli$_H$	12.5	
			PCA_{10}	C1: Shannon, Evenness, Richness	44.0	0.696
				C2: Dehalococcoides$_H$, **Cytophagia$_H$**, Acidobacteria$_H$	26.4	
				C3: Nitrospira$_H$, **Chlorobia$_H$**	14.4	
	Species	1	PCA_{11}	C1: Shannon, Richness, Evenness, Type, *Nitrospira_sp1*$_H$	48.8	0.705
				C2: ***Pontibacter_sp$_H$***, *Dehalogenimonas_sp*$_H$, *Subgroup3_sp*$_H$	25.5	

Table 1 Relation to main correlations among variables obtained by PCA analysis (inverse correlations in bold). Subscript "H" in microbial variables indicates that Hellinger transformation had been applied to the data. Suffix "-log" in geochemical variables indicates that log transformation was performed.

284

3.1. **Key parameters influencing microbial classes in surface water and groundwater**

Two PCAs were developed; the first one (PCA_1 in Table 1) included Shannon and Richness indices, Cl, EC, Na, the log transformations of S, Cu, and V, as well as $Betaproteobacteria_H$, $Dehalococcoidia_H$, $Nitrospira_H$ and $Others_H$ classes. The group "Others" includes microbial classes that could not be classified. Results of this PCA show that these twelve variables could be mostly explained with only two principal components (combined they explained 74% of the total variance). The strength of the analysis is supported by a value of 0.744 in the KMO test. Results of a 2D representation of varifactors and components (Figure 2) show the significance of hydrochemistry beyond microbial variables. All the chemical variables included in the analysis are grouped in the first component, showing a high correlation amongst them; Na, Cl, EC and Cu-log ("-log" indicates log-transformed) are positively correlated, whereas V-log and S-log are situated in the opposite side of the plot (negative correlation).

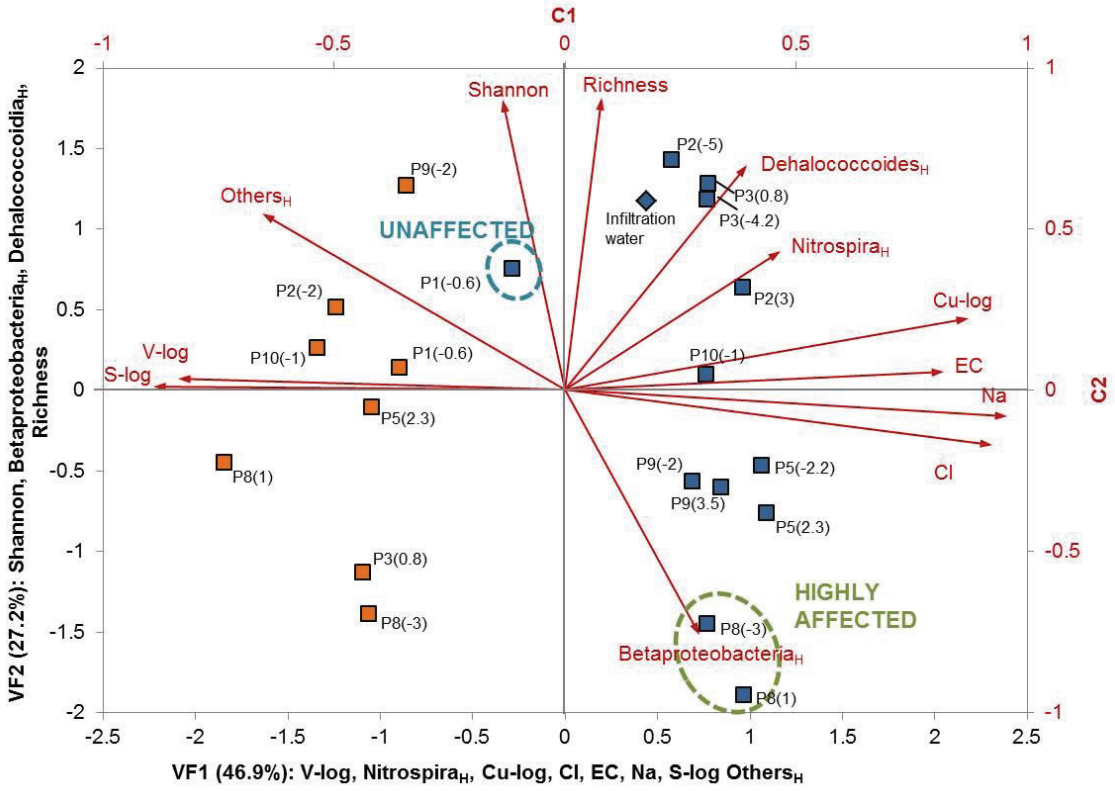

Figure 2 PCA$_1$ analysis including diversity indices, microbial classes and hydrochemical data from groundwater samples (squares) and one infiltration water sample (diamond) during the recharge period (blue) and no-recharge period (orange). The position of samples is scaled in VF1 and VF2 axes for visualization purposes. Red arrows represent the contribution of each variable projected into the varifactor plane. Samples are labeled corresponding to the sampling point and the height (meters above sea level, or masl) indicated in brackets. The symbols assignment follows the same criteria in all PCA plots.

The second component relates inversely the presence of Betaproteobacteria$_H$ to the Shannon and Richness indices. Furthermore, Betaproteobacteria$_H$ is most significant in the samples most affected by recharge (piezometer P8, located directly below the infiltration pond). Finally, the projection of sample data in the plane composed of the two varifactors (VF1, VF2) clearly separate data according to the operational period (recharge/no-recharge). Moreover, the sample not affected by recharge (P1, placed upstream) is displayed between the samples corresponding to the no-recharge and recharge periods.

The second PCA (Figure S1, PCA_2 in Table 1) also separates data corresponding to operational periods in the VF1-VF2 plane. Furthermore, two groups of variables can be distinguished. The first one relates $Cyanobacteria_H$, Cl, EC, Cu-log and Ni-log. Apart from hydrochemical differences that force the separation of samples between operational periods, Cyanobacteria are more abundant in the groundwater samples in the recharge period. Component 2 shows an inverse correlation between Richness and $Cytophagia_H$.

3.2. Key parameters influencing microbial species in water samples

PCA_3 analysis (Table 1) included variables EC, Na, Cl, V-log, Cu-log, NO_3, SO_4, DOC-log, and two microbial species (as opposed to microbial classes, that were the objective of PCA_1 and PCA_2): *Methylotenera mobilis*$_H$ and *Nitrospira sp2*$_H$. From the projection on the varifactor plane (Figure 3), samples corresponding to operational conditions clearly display on different areas within the plot. Furthermore, samples taken during active operation display a disturbance degree caused by recharge (indicated in qualitative terms by the blue arrow). On the other hand, the sample representing background conditions (P1) is located separated from the rest, indicating that in no recharge periods the areas that were recharged kept some memory of the events, i.e., the microbial communities did not go back to the pre-recharge conditions.

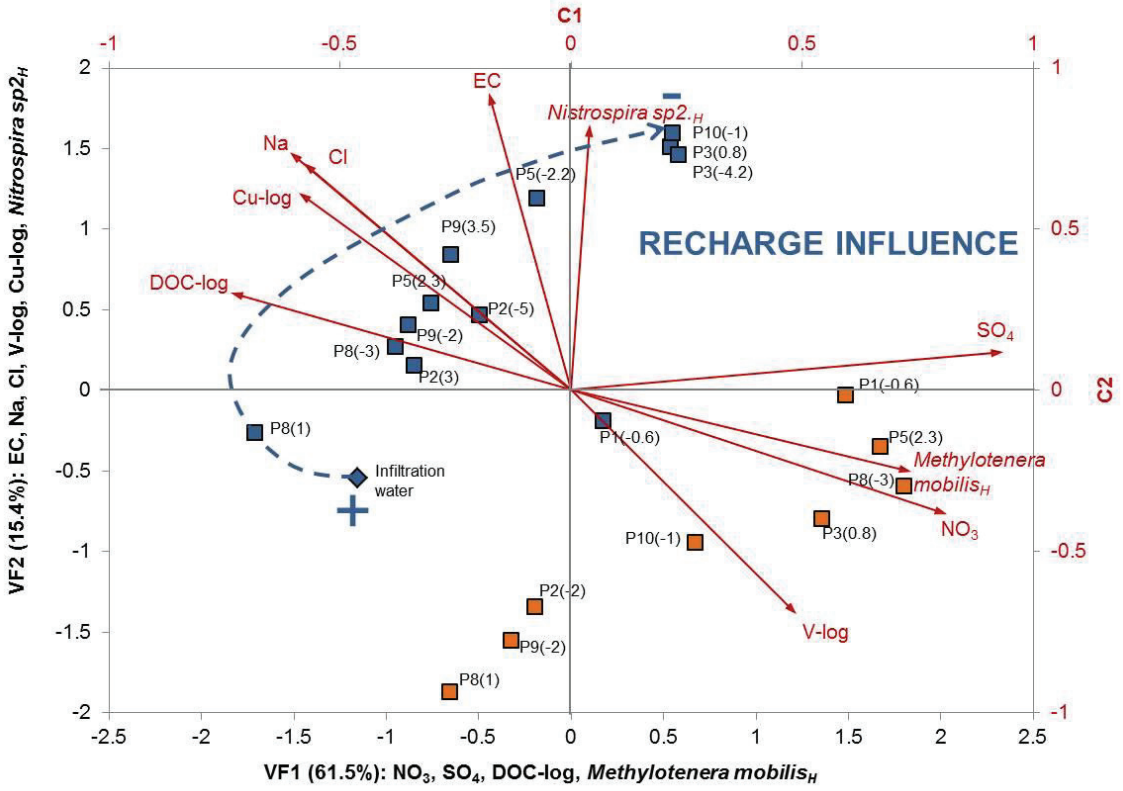

Figure 3 PCA_3 analysis including diversity indices, microbial species and hydrochemical data from groundwater samples (squares) and the infiltration water sample (diamond). The discontinuous blue arrow indicates the degree of disturbance caused by recharge.

Figure 3 also shows the main relations amongst variables. The first component groups mainly SO_4, NO_3 and DOC-log (inversely) with *Methylotenera mobilis*$_H$. The behavior of *Nitrospira sp2*$_H$ and its high correlation with conductivity were explained by the second component.

PCA_4 indicated a relationship between *Dehalogenimonas sp.*$_H$, *Pontibacter sp.*$_H$ and *Vogesella indigofera*$_H$ with some hydrochemical and biological indicators (Figure S2). *Dehalogenimonas sp.*$_H$ appears to be a key contributor to Richness and Diversity indices and in turn, is inversely correlated with *Pontibacter sp.*$_H$. However, *Vogesella indigofera*$_H$ *is* correlated with DOC-log (see Figure S2), placed in the vicinity of the P8 samples projection, indicating the effect of recharge on both variables.

3.3. Key parameters influencing microbial classes in MAR soils

PCA_5 to PCA_8 provide statistical analyses for samples taken from soils. Here, the parameters extracted from the grain-size distribution curves are included, together with microbial variables, depth and the operational variable. In all cases, the position of samples is scaled in VF1 and VF2 axes. Red arrows represent the contribution of each variable projected into the plane defined by the two varifactors (Figure 4).

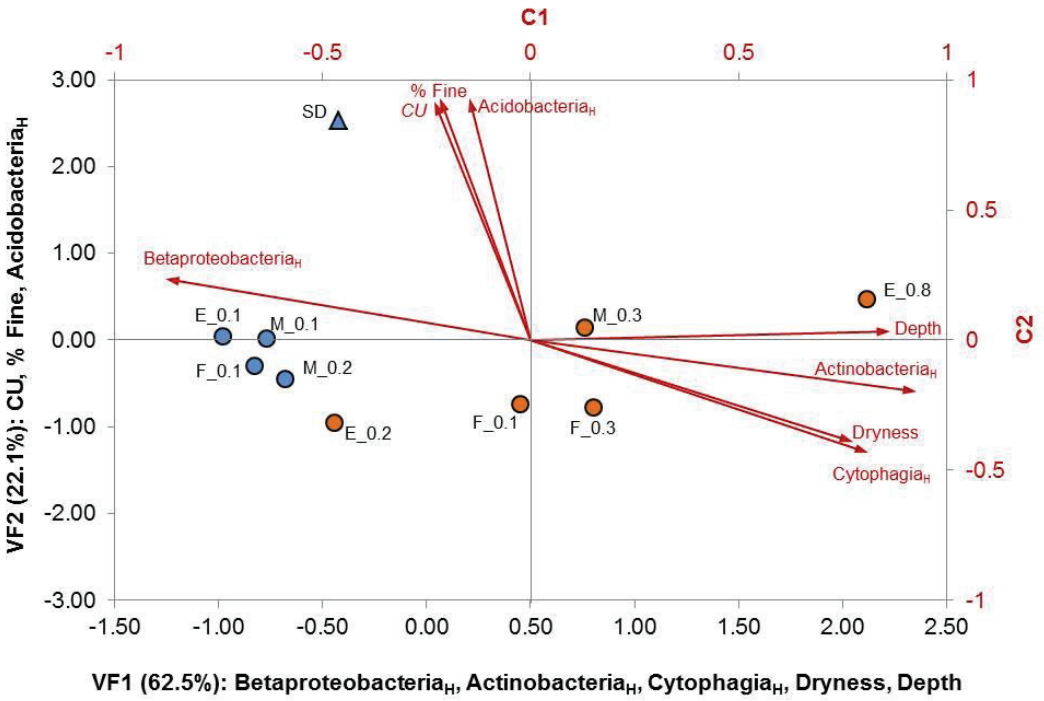

Figure 4 PCA_5 analysis including microbial classes, grain-size distribution curve parameters and depth from soil samples during the recharge period (blue) and no-recharge period (orange). Circles are related to the infiltration pond samples (E-Entrance, M-Midfield and F-Final stretch). Numbers indicate the depth the sample was taken (in m below surface). Triangle corresponds to the sedimentation pond sample. Symbols follow the same criteria in the following PCA charts.

PCA_5 involved microbial soil classes ($Acidobacteria_H$, $Betaproteobacteria_H$, $Actinobacteria_H$, $Cytophagia_H$), Uniformity Coefficient (*CU*), fine material content (% Fine), Dryness (operation condition dry-wet) and Depth (Table 1). From Figure 4, $Betaproteobacteria_H$ displays oppositely to Depth, $Actinobacteria_H$, $Cytophagia_H$ and Dryness, suggesting that $Betaproteobacteria_H$ is more favorable to live in wet and shallow soils, while $Actinobacteria_H$ and $Cytophagia_H$ are preferably found in dry and

deep soils. It should also be noted that when projected over the first two varifactors plane, wet samples (blue circles) are clustered, whereas dry samples (orange circles) are more scattered in space, indicating that they are affected by depth gradients. Finally, $Acidobacteria_H$ is positively correlated with *CU* and proportion of fines, well represented by the sample taken in the sedimentation pond (blue triangle).

PCA_6 clusters microbial classes with diversity indices (Shannon, Richness and Evenness), $Actinobacteria_H$, $Dehalococcoidia_H$, $Chlorobia_H$, $Bacilli_H$ and $Others_H$ (Figure S3). Whereas $Bacilli_H$ behavior is basically explained by the second component, Shannon, Evenness and $Actinobacteria_H$ are partially included in both components. Same as shown in a previous analysis, $Actinobacteria_H$ is negatively correlated with Shannon and Evenness indices. $Chlorobia_H$ and $Dehalococoidia_H$ are explained by the first component and contribute visibly to the microbial print of the wet soil samples.

3.4. Key parameters influencing microbial species in MAR soils

In PCA_7 (Figure 5, Table 1), the first two components explain 92% of the total variance. Projection of samples on varifactor space provides a clear separation of samples according to the operational period (during recharge and no-recharge periods), while the sample from the sedimentation pond remains separated. This last sample (SD in Figure 5) is explained mainly by the proportion of fines, CU and notably, the presence of Subgroup_3 Acidobacteria. Moreover, Shannon Index and Dryness are displayed in opposite sides of the plot, while the latter is placed in the same side as *Pontibacter sp* and *Nitrospira sp2*, highlighting that both species have a high affinity to dry conditions.

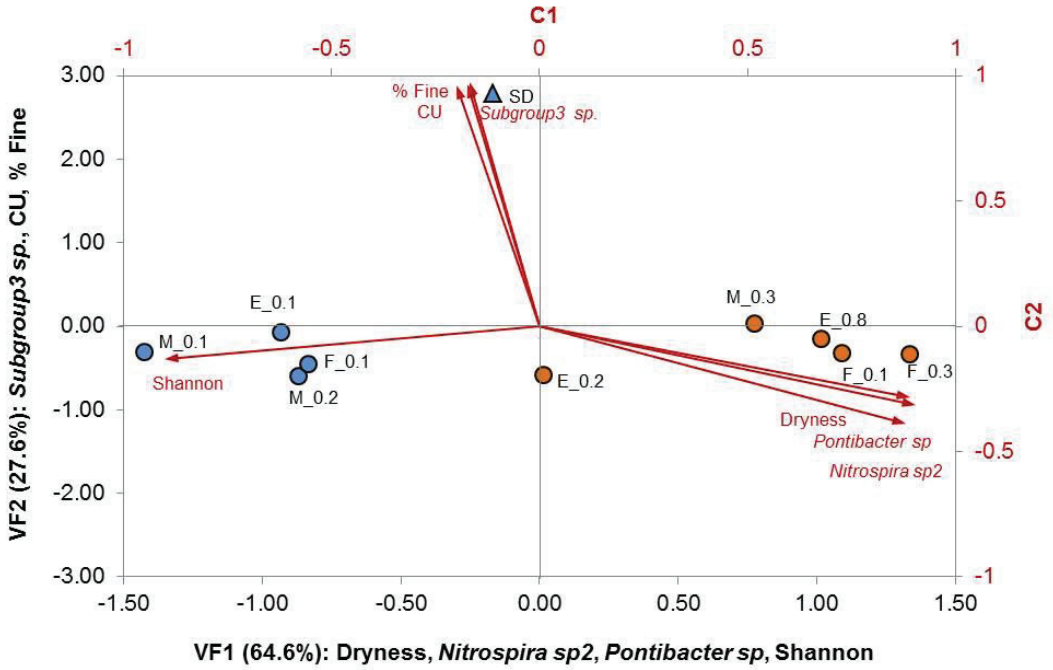

Figure 5 PCA_7 analysis including microbial species, grain-size distribution curve parameters and the operational period from sediment samples during the recharge period (blue) and no-recharge period (orange). Codes and symbols are reported in Figure 4.

PCA_8 indicates the affinity of *Vogesella indigofera* to silty soils (Figure S4). Despite *Dehalogenimonas sp.* behavior is explained by the second component, this species does not show correlation with *V. indigofera* or *CU*. However, the bivariate correlations matrix (not showed) revealed the correlation of *Dehalogenimonas sp.* with fine particles proportion. On the other hand, both *Methylotenera mobilis* and *Methylobacterium sp.* are correlated positively with two other soil characteristics, *CC* and D10.

3.5. Interclass and interspecies relationships

PCA_9 – PCA_{11} involves the statistical analysis of all 31 samples together. In PCA_9, soil and water samples are distinctly located in the varifactor plane involving the first two components (explaining 70% of the total variance, Figure 6). From the analysis, soil samples are the most diverse, equally-distributed and rich environments sustaining microbial growth, as the location of Shannon, Evenness and Richness indices indicate

in the PCA. On the other hand, Betaproteobacteria$_H$ shows higher affinity for water samples. Bacilii$_H$ becomes an independent third component explaining 13% of the total variance (Table 1).

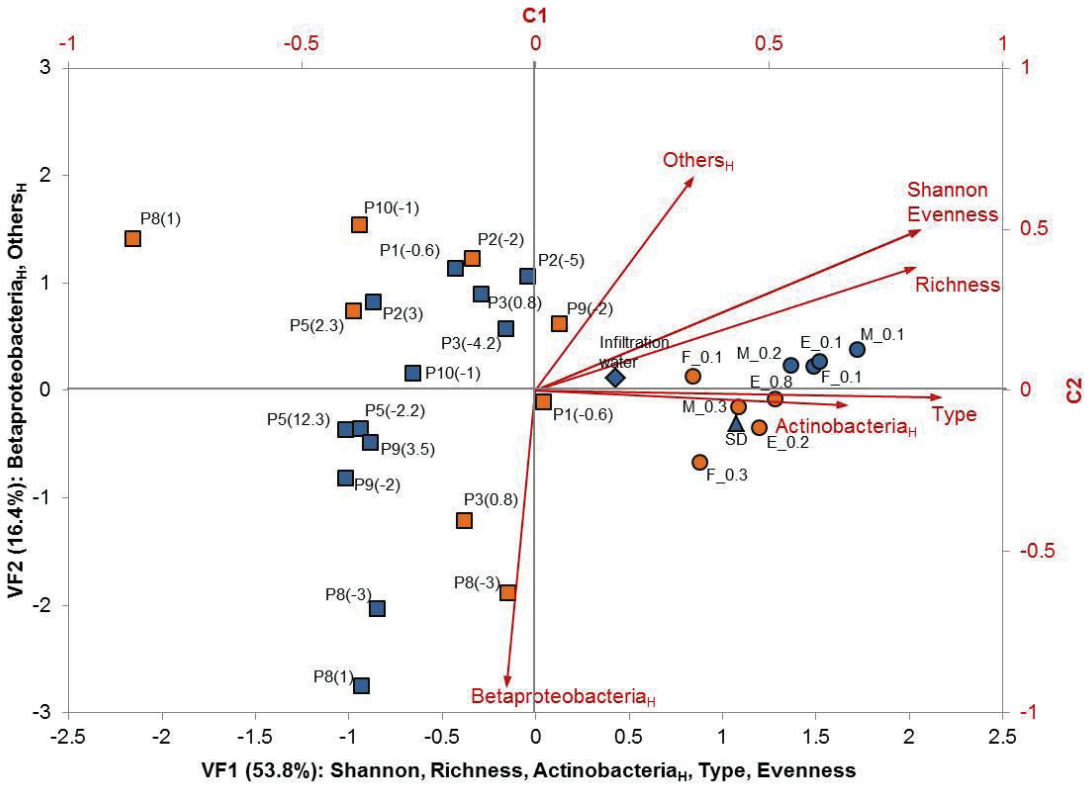

Figure 6 PCA$_9$ analysis including diversity indices, microbial classes and sample type from soil (circles), groundwater (squares) and surface water (diamonds and triangle) samples during the recharge period (blue) and no-recharge period (orange).

PCA$_{10}$ was performed to analyze the intra-relationships among microbial classes. Three components were needed to explain 85% of the total variance. The first one clusters the three microbial diversity indices. The second one explains the positive correlation between Dehalococcoidia$_H$ and Acidobacteria$_H$ and the negative one with Cytophagia$_H$. Finally, the third component showed a negative correlation between Nitrospira$_H$ and Chlorobia$_H$. Samples distribution in VF axes did not show any relevant association among samples (Figure S5).

Finally, PCA_{11} emphasizes microbial species. Similar to PCA_{10}, the first component groups all three diversity indices, and is representative of soil samples in the varifactor plane (Figure 7). *Nitrospira sp1*$_H$ was also positively correlated with Shannon and Evenness indices. Regarding the second component, *Pontibacter sp.*$_H$ is inversely correlated with *Dehalogenimonas sp.*$_H$ and in turn, the latter correlated positively with *Subgroup3 sp*$_H$, pointing out that these two species seem to be more associated to water and groundwater samples during active recharge period.

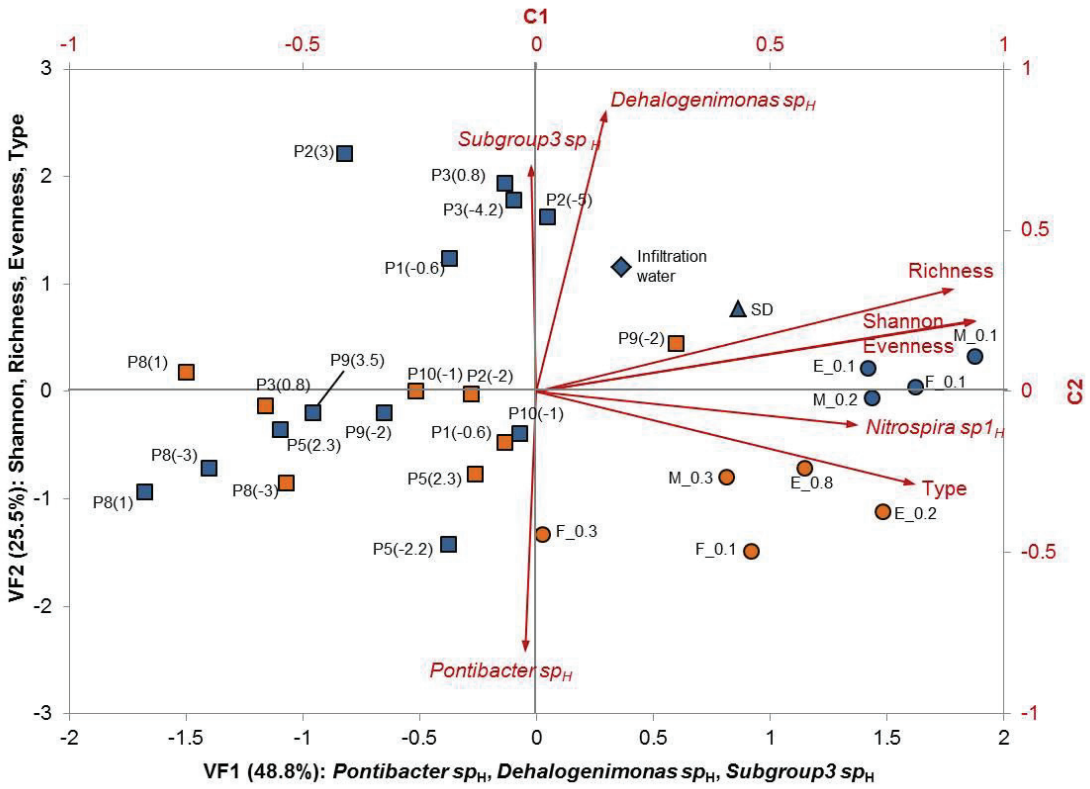

Figure 7 PCA_{11} analysis including diversity indices, microbial species, and type of sample from soil (circles), groundwater (squares) and surface water (diamond and triangle) during the recharge period (blue) and no-recharge period (orange).

4. Discussion

The most significant result from the statistical analysis is that the projection of samples on the planes defined by the principal components shows a clear influence of the operational conditions, this being the parameter that is subject to optimization in a MAR facility management scheme. On one hand, most of the figures show the polarization of samples according to the recharge conditions (blue and orange marks); on the other hand, the variable “Dryness”, indicating soil samples during non-recharge conditions, remains always beside the samples obtained in dry conditions. These results together redundantly imply that recharge operation drives significant changes in microbial communities. Recharge results in shifts in the microbial community structure, in terms of relative abundance and composition of dominant taxonomic groups.

Cyanobacteria, the only diazotrophs that produce oxygen as a by-product of the photosynthetic process, were found most abundantly in the surface water, and the measured concentrations correlated with several hydrogeochemical variables (EC, Cl, Cu-log and Ni-log) (recall Figure S1). Correlations among EC and cyanobacteria abundance have been already reported, for example, in a Neotropical urban lake (Frau et al., 2018) or in a eutrophicated reservoir in Brazil (Chellappa and Mederios Costa, 2003). Cu-log is positively correlated with cyanobacteria; this follows Dwivedi et al. (2006), who described the simultaneous presence of cyanobacteria and some metals (including Cu) in river water samples enriched by fly-ash originated in a coal thermal power station. Indeed, some cyanobacterial species have the ability to immobilize metals, and this is used in some water treatment industries (de-Bashan and Bashan, 2010).

The highest relative abundance of Betaproteobacteria was found in the groundwater samples most affected by recharge (e.g., conforming more than 50% of the total microbial abundance in piezometer P8, located directly below the infiltration pond). This microbial group correlates inversely with Richness and Shannon indices (recall Figure 2) suggesting that members of the Betaproteobacteria class could have an

opportunistic behavior, displacing other microbial populations when recharge is active, and becoming the leading microorganisms degrading organic matter.

We also evaluated the role of DOC, nitrate and sulfate on the abundance of *Methylotenera mobilis* in surface and subsurface water environments (see PCA_3). *M. mobilis* may carry out heterotrophic denitrification by using different substrates, such as methanol (Kalyuhznaya et al., 2009; Sun et al., 2016) or methylamine (Kalyuzhnaya et al., 2006) in aerobic environments. In fact, *M. mobilis* is the first methylotroph using nitrate as an electron acceptor, thus being a potential explanation of the positive correlation observed between *M. mobilis* abundance and nitrate concentration in water. Denitrification is an anaerobic process, but in some cases occurs in the presence of oxygen. In these situations, anoxic microenvironments (called microsites) may develop due to the heterogeneity in flow paths that would lead to local denitrification (Knowles, 2005; Modin et al., 2007) even in a zone with overall aerobic conditions. One surprising finding from PCA_3 is that correlation of *M. mobilis* and nitrate seems associated with the non-recharge period. Therefore, denitrification conditions are not completely reversed even after recharge has been discontinued for a significant amount of time.

On the other hand, it is not surprising that abundance of *M. mobilis* correlates with *Methylobacterium sp.* presence in the soil environment (see PCA_8); since they belong to different proteobacterial classes, they are both obligate methylotrophs (Kumaresan et al., 2018). Indeed, correlations between both species of methylotrophs have been reported by Wright et al. (2017) in a groundwater dichloromethane contaminated site in the US. Unlike *M. mobilis*, *Methylobacterium sp.* is a dichloromethane degrader (Gisi et al., 1998; Vuilleumier et al., 2009), capable to dechlorinate in aerobic conditions. Following Wright et al. (2017), this suggests that *M. mobilis* could take advantage by using the byproducts of dichloromethane degradation carried out by *Methylobacterium sp.* However, methane has not been measured at the site, so we are not assuming its presence *via* dichloromethane degradation.

In any case, it is not clear whether *M. mobilis* abundance correlates with some redox species; while the bivariate correlation coefficients with DOC-log and SO_4 were not significant (data not shown), *M. mobilis* grouped in the same component with NO_3 and SO_4. Regarding this last point, there is no evidence that SO_4 might be part of the metabolic pathway of *M. mobilis*.

Vogesella indigofera positively correlates with DOC-log (PCA_5). Members of this genus are important denitrifiers in groundwater systems (Bellini et al., 2018), capable of oxidizing few monosaccharides in low-oxygen concentration conditions (Grimes et al., 1997). Contrarily, DOC-log was negatively correlated to NO_3 concentration (PCA_3). In fact, the isotopic study performed by Grau-Martínez et al. (2018) at the same facility and on the same dates confirmed that recharge induced denitrification.

Dryness (a binary dry/wet variable) is the most significant one controlling the distribution of microbial communities in soils. Similar to what was observed in water samples, soil samples taken during recharge periods displayed largest diversity and highest Betaproteobacteria abundance. Recharge of river water ensured the continuous supply of nutrients and DOC to the system, favoring Betaproteobacteria growth, as it is one of the main bacterial groups biodegrading DOC (Li et al., 2013). In fact, in a work carried out in column models (Li et al., 2012), Betaproteobacteria were found mainly close to the inlet, associated to the high DOC concentrations fed; they also found that water content in soils (and extrapolating, recharge conditions) contribute to microbial diversity in soils. Along the same line, decreasing proportions of Betaproteobacteria were found in dry soils (as compared to wet ones) in an experiment performed with streambed fresh sediments (Pohlon et al., 2013).

Distribution of microbial populations in soils is also strongly affected by granulometry (PCA_8). *M. mobilis* and *Methylobacterium sp.* correlated with *CC* and D_{10}. There is not much literature on this subject. The work performed by Madhaiyan et al. (2007)

associated the variability in the distribution of pink-pigmented facultative metylotrophs, such as *Methylobacterium sp*, with soil type and moisture among other environmental variables. In fact, soil texture conditions is of paramount importance for nutrient transference into flooded soils (Perujo et al., 2017), also influencing methane consumption rates (Jäckel et al., 2001). In the same way, the presence of *Vogesella indigofera* and *Subgroup3 sp* (Acidobacteria) in soils is linked to *CU* (an indicator of grain-size heterogeneity) and proportion of fine particles, resulting in these two populations being most abundant in sandy-clayey soils.

Members of the phylum Acidobacteria, such as *Subgroup 3 sp* are widespread abundant in soils and sediments, and capable to tolerate moisture fluctuations (Ward et al., 2009). This tolerance can be related to the capability to form biofilms, highly hydrated structures (Kielak et al., 2016), allowing them to survive under stressful dryness conditions (Ward et al., 2009); this could be the reason why they are found regardless the recharge operational conditions. Moreover, it is also important to know the interactions of this group with other microbial classes present in the soil. In this regard, we found a significant statistical positive correlation between *Subgroup3 sp* (Acidobacteria) and *Dehalogenimonas sp* (Dehalococcoidia class); we associate this correlation to both groups being environmentally important, as they are involved in different contaminant degradation processes (Chen et al., 2018; Song et al., 2016).

In the soil samples, the relative abundance of Actinobacteria was directly correlated with dry conditions (PCA_5), similar to the observations of Pohlon et al. (2013). Different members of this phylum have the ability to grow developing mycelia structures and forming spores (Bhatti et al., 2017), providing them an advantage in the colonization of soil with limited water availability. Moreover, the combined analysis of soil and water samples showed the widespread presence of this phylum, especially abundant in soil samples, and could be associated to their important role in the cycling of organic matter due to their decomposition capabilities (Bhatti et al., 2017; Polkade et al., 2016). This

behavior is consistent with results observed in our system (PCA_9), where Actinobacteria is linked to microbial diversity indexes and dryness conditions. These last results are not surprising, since soils probably are one of the most diverse and rich environments supporting microbial life in the world (Curtis et al., 2002; Or et al., 2007; Torsvik et al., 2002; Young and Crawford, 2004).

Characterization of microbial communities may have strong implications in MAR facilities management when better understood. A particular combination of soil and inflow water characteristics at the infiltration pond would result in a particular microbial population signature. Such population would degrade preferably some particular organic compounds. The spatial distribution of microorganisms can be used for mapping the area influenced (even partially) by recharge. From another point of view, such detailed mapping can be eventually used in the future for optimal design of biofilm growth and composition in order to promote degradation of different target compounds. Furthermore, indicator species could be included in monitoring networks in order to characterize environmental conditions that take place at longer temporal scales than the one represented by punctual hydrochemical data.

5. Conclusions

The work presented in this paper led us to draw conclusions regarding both the statistical analysis proposed and the intrinsic results obtained.

Multivariate statistical methods allow the simultaneous treatment of interdisciplinary data (physical, chemical, and biological), from different nature (categorical, binary and continuous) and spanning different ranges, in a formal statistical way. This is potentially very useful for many experimental studies in soil science or freshwater systems as a way to integrate data in a subset of statistical components that despite being small in dimensional terms, explains a large amount of the variance of the dataset.

The application of this methodology in the dataset obtained in the Sant Vicenç recharge facility enabled to conclude that MAR influences significantly microbial communities in soil, surface and subsurface water. Therefore, they can be used to assess the area of the soil and the volume of the aquifer influenced by recharge. Microbial communities are directly influenced by a combination of biohydrogeochemical processes and vice versa. Aquifer recharge *via* infiltration ponds has a significant impact upon carbon and nitrogen cycles in the topsoil, since most of the microbial species detected in this study have a role in the aerobic degradation of organic matter and in denitrification processes. Thus, a strong correlation was found between hydrochemical compounds and some microbial communities, both in the soil and groundwater samples; two examples are the correlation between *Vogesella indigofera* with DOC and *Methylotenera mobilis* with NO_3.

The statistical analysis of both groundwater and soil samples clearly discriminate operational conditions. Recharge drives distinct populations to become dominant (Betaproteobacteria, Dehalococcoidia, and Nitrospira). Moreover, groundwater samples can be graphically represented according to the degree of disturbance (in terms of the proportion of infiltrating water versus the native one in the corresponding water samples).

In the case of soil samples, there are two most set of significant variables affecting the distribution of microbial communities: (1) grain-size distribution (affecting *Vogesella indigofera, Subgroup3 sp* (Acidobacteria), *Methylotenera mobilis*, *Dehalogenimonas sp* and *Methylobacterium sp*),; and (2) operation conditions, as wet soils contain more microbial diversity than dry ones. However, there are some classes (e.g., Acidobacteria) that are less affected by recharge conditions. In the future, this could provide ideas for the affection of the climate change consequences in stream-flows, wetlands, and other drought-vulnerable aquatic systems.

Multivariate statistical analysis indicates that DOC was another determinant factor shaping microbial community structure. Among them, members of Betaproteobacteria, Dehalococcoidia and Acidobacteria are involved in pollutant bioremediation and can be considered as potential bioindicators for recharge monitoring.

Acknowledgments

This investigation was financially supported by the European Union project MARSOL FP7-ENV-2013-WATER-INNO-DEMO, Generalitat de Catalunya via FI scholarship program (FI-DGR 2014) and the Spanish Ministry of Economy and Competitiveness and EU (project ACWAPUR PCIN-2015-239). The authors would like to acknowledge Comunitat d'Usuaris d'Aigües de la Vall Baixa i del Delta del Riu Llobregat (CUADLL) for their cooperation.

References

Alidina, M., Li, D., Drewes, J.E., 2014. Investigating the role for adaptation of the microbial community to transform trace organic chemicals during managed aquifer recharge. Water Res 56, 172–180. doi:10.1016/j.watres.2014.02.046

Alidina, M., Shewchuk, J., Drewes, J.E., 2015. Effect of temperature on removal of trace organic chemicals in managed aquifer recharge systems. Chemosphere 122, 23–31. doi:10.1016/j.chemosphere.2014.10.064

Barba, C., Folch, A., Gaju, N., Sanchez-Vila, X., Carrasquilla, M., Grau-Martínez, A., Martinez-Alonso, M., 2019. Microbial community changes induced by Managed Aquifer Recharge activities: Linking hydrogeological and biological processes. Hydrol Earth Syst Sci., 23, 139-154, doi: 10.5194/hess-23-139-2019

Bekele, E., Toze, S., Patterson, B., Higginson, S., 2011. Managed aquifer recharge of treated wastewater: Water quality changes resulting from infiltration through the vadose zone. Water Res 45, 5764–5772. doi:10.1016/j.watres.2011.08.058

Bellini, M.I., Gutiérrez, L., Tarlera, S., Scavino, A.F., 2013. Isolation and functional analysis of denitrifiers in an aquifer with high potential for denitrification. Syst Appl Microbiol 36, 505–16. doi:10.1016/j.syapm.2013.07.001

Bellini, M.I., Kumaresan, D., Tarlera, S., Murrell, J.C., Fernández-Scavino, A., 2018. Identification of active denitrifiers by DNA-stable isotope probing and amplicon sequencing reveals Betaproteobacteria as responsible for attenuation of nitrate contamination in a low impacted aquifer. FEMS Microbiol Ecol 94, fix181-fix181.

Bhatti, A.A., Haq, S., Bhat, R.A., 2017. Actinomycetes benefaction role in soil and plant health. Microb Pathog. doi:10.1016/j.micpath.2017.09.036

Bouwer, H., 2002. Artificial recharge of groundwater: hydrogeology and engineering. Hydrogeol J 10, 121–142. doi:10.1007/s10040-001-0182-4

Chau, J., Bagtzoglou, A., Willig, M., 2011. The effect of soil texture on richness and diversity of bacterial communities. Environ Forensics 12, 333–341. doi:10.1080/15275922.2011.622348

Chellappa, N.T., Mederios Costa, M.A., 2003. Dominant and co-existing species of Cyanobacteria from a Eutrophicated reservoir of Rio Grande do Norte State, Brazil, in: Acta Oecologica. Elsevier Masson, pp. S3–S10. doi:10.1016/S1146-609X(03)00005-5

Chen, J., Wang, P.-F., Wang, C., Liu, J.-J., Gao, H., Wang, X., 2018. Spatial distribution and diversity of organohalide-respiring bacteria and their relationships with polybrominated diphenyl ether concentration in Taihu Lake sediments. Environ Pollut 232, 200–211. doi:10.1016/J.ENVPOL.2017.08.124

Curtis, T.P., Sloan, W.T., Scannell, J.W., 2002. Estimating prokaryotic diversity and its limits. Proc Natl Acad Sci 99, 10494–10499. doi:10.1073/pnas.142680199

D'Alessio, M., Yoneyama, B., Kirs, M., Kisand, V., Ray, C., 2015. Pharmaceutically active compounds: Their removal during slow sand filtration and their impact on slow sand filtration bacterial removal. Sci Total Environ 524–525, 124–135. doi:10.1016/j.scitotenv.2015.04.014

de-Bashan, L.E., Bashan, Y., 2010. Immobilized microalgae for removing pollutants: Review of practical aspects. Bioresour Technol 101, 1611–1627. doi:10.1016/j.biortech.2009.09.043

Drewes, J.E., Li, D., Regnery, J., Alidina, M., Wing, A., Hoppe-Jones, C., 2014. Tuning the performance of a natural treatment process using metagenomics for improved trace organic chemical attenuation. Water Sci Technol 69, 628–633. doi:10.2166/wst.2013.750

Drewes, J.E., Reinhard, M., Fox, P., 2003. Comparing microfiltration-reverse osmosis and soil-aquifer treatment for indirect potable reuse of water. Water Res 37, 3612–3621. doi:10.1016/S0043-1354(03)00230-6

Dutta, T., Carles-Brangarí, A., Fernàndez-Garcia, D., Rubol, S., Tirado-Conde, J., Sanchez-Vila, X., 2015. Vadose zone oxygen (O2) dynamics during drying and wetting cycles: An artificial recharge laboratory experiment. J Hydrol 527, 151–159. doi:10.1016/j.jhydrol.2015.04.048

Dwivedi, S., Tripathi, R.D., Rai, U.N., Srivastava, S., Mishra, S., Shukla, M.K., Gupta, A.K., Sinha, S., Baghel, V.S., Gupta, D.K., 2006. Dominance of algae in ganga water polluted through fly-ash leaching: Metal bioaccumulation potential of selected algal species. Bull Environ Contam Toxicol 77, 427–436. doi:10.1007/s00128-006-1083-y

El Alfy, M., Lashin, A., Abdalla, F., Al-Bassam, A., 2017. Assessing the hydrogeochemical processes affecting groundwater pollution in arid areas using an integration of geochemical equilibrium and multivariate statistical techniques. Environ Pollut 229, 760–770. doi:10.1016/j.envpol.2017.05.052

Fahrenfeld, N., Cozzarelli, I.M., Bailey, Z., Pruden, A., 2014. Insights into Biodegradation Through Depth-Resolved Microbial Community Functional and Structural Profiling of a Crude-Oil Contaminant Plume. Microb Ecol 68, 453–462. doi:10.1007/s00248-014-0421-6

Faulwetter, J.L., Gagnon, V., Sundberg, C., Chazarenc, F., Burr, M.D., Brisson, J., Camper, A.K., Stein, O.R., 2009. Microbial processes influencing performance of treatment wetlands: A review. Ecol Eng 35, 987–1004. doi:10.1016/j.ecoleng.2008.12.030

Frau, D., Pinto, P.D.T., Mayora, G., 2018. Are cyanobacteria total, specific and trait abundance regulated by the same environmental variables? Ann Limnol 54, 3. doi:10.1051/limn/2017030

Freixa, A., Rubol, S., Carles-Brangarí, A., Fernàndez-Garcia, D., Butturini, A., Sanchez-Vila, X., Romaní, A.M., 2015. The effects of sediment depth and oxygen concentration on the use of organic matter: An experimental study using an infiltration sediment tank. Sci Total Environ 540, 20–31. doi:10.1016/j.scitotenv.2015.04.007

Ginige, M.P., Kaksonen, A.H., Morris, C., Shackelton, M., Patterson, B.M., 2013. Bacterial community and groundwater quality changes in an anaerobic aquifer during groundwater recharge with aerobic recycled water. FEMS Microbiol Ecol 85, 553–567. doi:10.1111/1574-6941.12137

Gisi, D., Willi, L., Traber, H., Leisinger, T., Vuilleumier, S., 1998. Effects of bacterial

host and dichloromethane dehalogenase on the competitiveness of methylotrophic bacteria growing with dichloromethane. Appl Environ Microbiol 64, 1194–1202.

Goren, O., Burg, A., Gavrieli, I., Negev, I., Guttman, J., Kraitzer, T., Kloppmann, W., Lazar, B., 2014. Biogeochemical processes in infiltration basins and their impact on the recharging effluent, the soil aquifer treatment (SAT) system of the Shafdan plant, Israel. Appl Geochemistry 48, 58–69. doi:10.1016/j.apgeochem.2014.06.017

Grau-Martínez, A., Folch, A., Torrentó, C., Valhondo, C., Barba, C., Domènech, C., Soler, A., Otero, N., 2018. Monitoring induced denitrification during managed aquifer recharge in an infiltration pond. J Hydrol 561, 123–135. doi:10.1016/j.jhydrol.2018.03.044

Greskowiak, J., Hamann, E., Burke, V., Massmann, G., 2017. The uncertainty of biodegradation rate constants of emerging organic compounds in soil and groundwater – A compilation of literature values for 82 substances. Water Res 126, 122–133. doi:10.1016/j.watres.2017.09.017

Greskowiak, J., Prommer, H., Massmann, G., Johnston, C.D., Nützmann, G., Pekdeger, A., 2005. The impact of variably saturated conditions on hydrogeochemical changes during artificial recharge of groundwater. Appl Geochemistry 20, 1409–1426. doi:10.1016/j.apgeochem.2005.03.002

Greskowiak, J., Prommer, H., Massmann, G., Nützmann, G., 2006. Modeling Seasonal Redox Dynamics and the Corresponding Fate of the Pharmaceutical Residue Phenazone During Artificial Recharge of Groundwater. Environ Sci Technol 40, 6615–6621. doi:10.1021/es052506t

Griebler, C., Lueders, T., 2009. Microbial biodiversity in groundwater ecosystems. Freshw Biol 54, 649–677. doi:10.1111/j.1365-2427.2008.02013.x

Grimes, D.J., Woese, C.R., MacDonell, M.T., Colwell, R.R., 1997. Systematic study of the genus Vogesella gen. nov. and its type species, Vogesella indigofera comb. nov. Int J Syst Bacteriol 47, 19–27. doi:10.1099/00207713-47-1-19

Haack, S.K., Fogarty, L.R., West, T.G., Alm, E.W., McGuire, J.T., Long, D.T., Hyndman, D.W., Forney, L.J., 2004. Spatial and temporal changes in microbial community structure associated with recharge-influenced chemical gradients in a contaminated aquifer. Env Microbiol 6, 438–448. doi:10.1111/j.1462-2920.2003.00563.x

Hamann, E., Stuyfzand, P.J., Greskowiak, J., Timmer, H., Massmann, G., 2016. The fate of organic micropollutants during long-term/long-distance river bank filtration. Sci Total Environ 545–546, 629–640. doi:10.1016/j.scitotenv.2015.12.057

Hellauer, K., Karakurt, S., Sperlich, A., Burke, V., Massmann, G., Hübner, U., Drewes, J.E., 2017. Establishing sequential managed aquifer recharge technology (SMART) for enhanced removal of trace organic chemicals: Experiences from field studies in Berlin, Germany. J Hydrol. doi:10.1016/j.jhydrol.2017.09.044

Jäckel, U., Schnell, S., Conrad, R., 2001. Effect of moisture, texture and aggregate size of paddy soil on production and consumption of CH4. Soil Biol Biochem 33, 965–971. doi:10.1016/S0038-0717(00)00248-0

Kalyuhznaya, M.G., Martens-Habbena, W., Wang, T., Hackett, M., Stolyar, S.M., Stahl, D.A., Lidstrom, M.E., Chistoserdova, L., 2009. Methylophilaceae link methanol oxidation to denitrification in freshwater lake sediment as suggested by stable isotope probing and pure culture analysis. Environ Microbiol Rep 1, 385–392. doi:10.1111/j.1758-2229.2009.00046.x

Kalyuzhnaya, M.G., Bowerman, S., Lara, J.C., Lidstrom, M.E., Chistoserdova, L., 2006. Methylotenera mobilis gen. nov., sp. nov., an obligately methelamine-utilizing

bacterium within the family Methylophilaceae. Int J Syst Evol Microbiol 56, 2819–2823. doi:10.1099/ijs.0.64191-0

Kielak, A.M., Barreto, C.C., Kowalchuk, G.A., van Veen, J.A., Kuramae, E.E., 2016. The ecology of Acidobacteria: Moving beyond genes and genomes. Front Microbiol. doi:10.3389/fmicb.2016.00744

Knowles, R., 2005. Denitrifiers associated with methanotrophs and their potential impact on the nitrogen cycle. Ecol Eng 24, 441–446. doi:10.1016/J.ECOLENG.2005.01.001

Kolehmainen, R.E., Tiirola, M., Puhakka, J.A., 2008. Spatial and temporal changes in Actinobacterial dominance in experimental artificial groundwater recharge. Water Res 42, 4525–4537. doi:10.1016/j.watres.2008.07.039

Kumaresan, D., Stephenson, J., Doxey, A.C., Bandukwala, H., Brooks, E., Hillebrand-Voiculescu, A., Whiteley, A.S., Murrell, J.C., 2018. Aerobic proteobacterial methylotrophs in Movile Cave: genomic and metagenomic analyses. Microbiome 6, 1. doi:10.1186/s40168-017-0383-2

Lapworth, D.J., Baran, N., Stuart, M.E., Ward, R.S., 2012. Emerging organic contaminants in groundwater: A review of sources, fate and occurrence. Environ Pollut. doi:10.1016/j.envpol.2011.12.034

Laws, B. V, Dickenson, E.R. V, Johnson, T.A., Snyder, S.A., Drewes, J.E., 2011. Attenuation of contaminants of emerging concern during surface-spreading aquifer recharge. Sci Total Environ 409, 1087–1094. doi:10.1016/j.scitotenv.2010.11.021

Legendre, P., Gallagher, E.D., 2001. Ecologically meaningful transformations for ordination of species data. Oecologia 129, 271–280. doi:10.1007/s004420100716

Li, D., Alidina, M., Ouf, M., Sharp, J.O., Saikaly, P., Drewes, J.E., 2013. Microbial community evolution during simulated managed aquifer recharge in response to

different biodegradable dissolved organic carbon (BDOC) concentrations. Water Res 47, 2421–2430. doi:10.1016/j.watres.2013.02.012

Li, D., Sharp, J.O., Saikaly, P.E., Ali, S., Alidina, M., Alarawi, M.S., Keller, S., Hoppe-Jones, C., Drewes, J.E., 2012. Dissolved organic carbon influences microbial community composition and diversity in managed aquifer recharge systems. Appl Env Microbiol 78, 6819–6828. doi:10.1128/aem.01223-12

Madhaiyan, M., Poonguzhali, S., Sa, T., 2007. Influence of plant species and environmental conditions on epiphytic and endophytic pink-pigmented facultative methylotrophic bacterial populations associated with field-grown rice cultivars. J Microbiol Biotechnol 17, 1645–1654.

Maeng, S.K., Sharma, S.K., Lekkerkerker-Teunissen, K., Amy, G.L., 2011. Occurrence and fate of bulk organic matter and pharmaceutically active compounds in managed aquifer recharge: A review. Water Res 45, 3015–3033. doi: 10.1016/j.watres.2011.02.017

Martínez-Pascual, E., Jiménez, N., Vidal-Gavilan, G., Vinas, M., Solanas, A.M., 2010. Chemical and microbial community analysis during aerobic biostimulation assays of non-sulfonated alkyl-benzene-contaminated groundwater. Appl Microbiol Biotechnol 88, 985–995. doi:10.1007/s00253-010-2816-8

Massmann, G., Greskowiak, J., Dünnbier, U., Zuehlke, S., Knappe, A., Pekdeger, A., 2006. The impact of variable temperatures on the redox conditions and the behaviour of pharmaceutical residues during artificial recharge. J Hydrol 328, 141–156. doi: 10.1016/j.jhydrol.2005.12.009

Meckenstock, R.U., Elsner, M., Griebler, C., Lueders, T., Stumpp, C., Aamand, J., Agathos, S.N., Albrechtsen, H.J., Bastiaens, L., Bjerg, P.L., Boon, N., Dejonghe, W., Huang, W.E., Schmidt, S.I., Smolders, E., Sørensen, S.R., Springael, D., Van

Breukelen, B.M., 2015. Biodegradation: Updating the Concepts of Control for Microbial Cleanup in Contaminated Aquifers. Environ Sci Technol 49, 7073–7081. doi:10.1021/acs.est.5b00715

Menció, A., Folch, A., Mas-Pla, J., 2012. Identifying key parameters to differentiate groundwater flow systems using multifactorial analysis. J Hydrol 472–473, 301–313. doi:10.1016/j.jhydrol.2012.09.030

Mermillod-Blondin, F., Simon, L., Maazouzi, C., Foulquier, A., Delolme, C., Marmonier, P., 2015. Dynamics of dissolved organic carbon (DOC) through stormwater basins designed for groundwater recharge in urban area: Assessment of retention efficiency. Water Res 81, 27–37. doi:10.1016/j.watres.2015.05.031

Modin, O., Fukushi, K., Yamamoto, K., 2007. Denitrification with methane as external carbon source. Water Res 41, 2726–2738. doi:10.1016/j.watres.2007.02.053

Nijenhuis, I., Kuntze, K., 2016. Anaerobic microbial dehalogenation of organohalides—state of the art and remediation strategies. Curr Opin Biotechnol 38, 33–38. doi:10.1016/j.copbio.2015.11.009

Or, D., Smets, B.F., Wraith, J.M., Dechesne, A., Friedman, S.P., 2007. Physical constraints affecting bacterial habitats and activity in unsaturated porous media – a review. Adv Water Resour 30, 1505–1527. doi: 10.1016/j.advwatres.2006.05.025

Paliy, O., Shankar, V., 2016. Application of multivariate statistical techniques in microbial ecology. Mol Ecol. doi:10.1111/mec.13536

Pedretti, D., Barahona-Palomo, M., Bolster, D., Fernàndez-Garcia, D., Sanchez-Vila, X., Tartakovsky, D.M., 2012a. Probabilistic analysis of maintenance and operation of artificial recharge ponds. Adv Water Resour 36, 23–35. doi: 10.1016/j.advwatres.2011.07.008

Pedretti, D., Barahona-Palomo, M., Bolster, D., Sanchez-Vila, X., Fernàndez-Garcia, D., 2012b. A quick and inexpensive method to quantify spatially variable infiltration capacity for artificial recharge ponds using photographic images. J Hydrol 430–431, 118–126. doi: 10.1016/j.jhydrol.2012.02.008

Perujo, N., Romaní, A.M., Sanchez-Vila, X., 2018. Bilayer Infiltration System Combines Benefits from Both Coarse and Fine Sands Promoting Nutrient Accumulation in Sediments and Increasing Removal Rates. Environ Sci Technol 52, 5734–5743. doi:10.1021/acs.est.8b00771

Perujo, N., Sanchez-Vila, X., Proia, L., Romaní, A.M., 2017. Interaction between Physical Heterogeneity and Microbial Processes in Subsurface Sediments: A Laboratory-Scale Column Experiment. Environ Sci Technol 51, 6110–6119. doi:10.1021/acs.est.6b06506

Pett-Ridge, J., Firestone, M.K., 2005. Redox fluctuation structures microbial communities in a wet tropical soil. Appl Environ Microbiol 71, 6998–7007. doi:10.1128/AEM.71.11.6998-7007.2005

Pohlon, E., Fandino, A.O., Marxsen, J., 2013. Bacterial community composition and extracellular enzyme activity in temperate streambed sediment during drying and rewetting. PLoS One 8, e83365. doi:10.1371/journal.pone.0083365

Polkade, A. V, Mantri, S.S., Patwekar, U.J., Jangid, K., 2016. Quorum sensing: An under-explored phenomenon in the phylum Actinobacteria. Front Microbiol. doi:10.3389/fmicb.2016.00131

Reed, D.A., Toze, S., Chang, B., 2008. Spatial and temporal changes in sulphate-reducing groundwater bacterial community structure in response to Managed Aquifer Recharge. Water Sci Technol 57, 789. doi:10.2166/wst.2008.172

Regnery, J., Li, D., Roberts, S., Higgins, C., Sharp, J.O., Drewes, J.E., 2016. Linking

Trace Organic Chemical Attenuation to Microbiome metabolic Capabilities: Insights from Laboratory- and Full-scale Managed Aquifer Recharge Systems, in: ACS Symposium Series. pp. 163–187. doi:10.1021/bk-2016-1241.ch011

Regnery, J., Wing, A.D., Alidina, M., Drewes, J.E., 2015. Biotransformation of trace organic chemicals during groundwater recharge: How useful are first-order rate constants? J Contam Hydrol 179, 65–75. doi:10.1016/j.jconhyd.2015.05.008

Rezanezhad, F., Couture, R.M., Kovac, R., O'Connell, D., Van Cappellen, P., 2014. Water table fluctuations and soil biogeochemistry: An experimental approach using an automated soil column system. J Hydrol 509, 245–256. doi:10.1016/j.jhydrol.2013.11.036

Rodríguez-Escales, P., Canelles, A., Sanchez-Vila, X., Folch, A., Kurtzman, D., Rossetto, R., Fernández-Escalante, E., Lobo-Ferreira, J.-P., Sapiano, M., San-Sebastián, J., Schüth, C., 2018. A risk assessment methodology to evaluate the risk failure of managed aquifer recharge in the Mediterranean Basin. Hydrol Earth Syst Sci 22, 3213–3227. doi:10.5194/hess-22-3213-2018

Rodríguez-Escales, P., Fernàndez-Garcia, D., Drechsel, J., Folch, A., Sanchez-Vila, X., 2017. Improving degradation of emerging organic compounds by applying chaotic advection in Managed Aquifer Recharge in randomly heterogeneous porous media. Water Resour Res 53, 4376–4392. doi:10.1002/2016WR020333

Rodriguez-Escales, P., Sanchez-Vila, X., 2016. Fate of sulfamethoxazole in groundwater: Conceptualizing and modeling metabolite formation under different redox conditions. Water Res 105, 540–550. doi:10.1016/j.watres.2016.09.034

Röling, W.F., van Breukelen, B.M., Braster, M., Lin, B., van Verseveld, H.W., 2001. Relationships between microbial community structure and hydrochemistry in a landfill leachate-polluted aquifer. Appl Environ Microbiol 67, 4619–29.

Rothschild, L.J., Mancinelli, R.L., 2001. Life in extreme environments (nature).PDF. Nature 409, 1092–1101. doi:10.1038/35059215

San-Sebastián-Sauto, J., Fernández-Escalante, E., Calero-Gil, R., Carvalho, T., Rodríguez-Escales, P., 2018. Characterization and benchmarking of seven managed aquifer recharge systems in south-western Europe. Sustain Water Resour Manag 1–23. doi:10.1007/s40899-018-0232-x

Schütz, K., Nagel, P., Vetter, W., Kandeler, E., Ruess, L., 2009. Flooding forested groundwater recharge areas modifies microbial communities from top soil to groundwater table. FEMS Microbiol Ecol 67, 171–182. doi:10.1111/j.1574-6941.2008.00608.x

Song, M., Jiang, L., Zhang, D., Luo, C., Wang, Y., Yu, Z., Yin, H., Zhang, G., 2016. Bacteria capable of degrading anthracene, phenanthrene, and fluoranthene as revealed by DNA based stable-isotope probing in a forest soil. J Hazard Mater 308, 50–57. doi:10.1016/J.JHAZMAT.2016.01.009

Staats, M., Braster, M., Röling, W.F.M., 2011. Molecular diversity and distribution of aromatic hydrocarbon-degrading anaerobes across a landfill leachate plume. Environ Microbiol 13, 1216–1227. doi:10.1111/j.1462-2920.2010.02421.x

Stein, H., Kellermann, C., Schmidt, S.I., Brielmann, H., Steube, C., Berkhoff, S.E., Fuchs, A., Hahn, H.J., Thulin, B., Griebler, C., 2010. The potential use of fauna and bacteria as ecological indicators for the assessment of groundwater quality. J Environ Monit 12, 242–254. doi:10.1039/B913484K

Sun, Y., Shen, D., Zhou, X., Shi, N., Tian, Y., 2016. Microbial diversity and community structure of denitrifying biological filters operated with different carbon sources. Springerplus 5, 1752. doi:10.1186/s40064-016-3451-3

Terzaghi, K., Peck, R.B., Mesri, G., 1996. Soil mechanics in engineering practice, Third

ed. ed. Wiley-Interscience.

Torsvik, V., Øvreås, L., Thingstad, T.F., 2002. Prokaryotic diversity - Magnitude, dynamics, and controlling factors. Science (80-). doi:10.1126/science.1071698

Truu, M., Juhanson, J., Truu, J., 2009. Microbial biomass, activity and community composition in constructed wetlands. Sci Total Environ 407, 3958–3971. doi: 10.1016/j.scitotenv.2008.11.036

Valhondo, C., Carrera, J., Ayora, C., Tubau, I., Martinez-Landa, L., Nödler, K., Licha, T., 2015. Characterizing redox conditions and monitoring attenuation of selected pharmaceuticals during artificial recharge through a reactive layer. Sci Total Environ 512–513, 240–50. doi:10.1016/j.scitotenv.2015.01.030

Valhondo, C., Martinez-Landa, L., Carrera, J., Ayora, C., Nödler, K., Licha, T., 2018. Evaluation of EOC removal processes during artificial recharge through a reactive barrier. Sci Total Environ 612, 985–994. doi:10.1016/j.scitotenv.2017.08.054

Valhondo, C., Martínez-Landa, L., Carrera, J., Hidalgo, J.J., Tubau, I., De Pourcq, K., Grau-Martínez, A., Ayora, C., 2016. Tracer test modeling for local scale residence time distribution characterization in an artificial recharge site. Hydrol Earth Syst Sci Discuss 1–17. doi:10.5194/hess-2016-197

Vuilleumier, S., Chistoserdova, L., Lee, M.C., Bringel, F., Lajus, A., Yang, Z., Gourion, B., Barbe, V., Chang, J., Cruveiller, S., Dossat, C., Gillett, W., Gruffaz, C., Haugen, E., Hourcade, E., Levy, R., Mangenot, S., Muller, E., Nadalig, T., Pagni, M., Penny, C., Peyraud, R., Robinson, D.G., Roche, D., Rouy, Z., Saenempechek, C., Salvignol, G., Vallenet, D., Zaining, W., Marx, C.J., Vorholt, J.A., Olson, M. V, Kaul, R., Weissenbach, J., Médigue, C., Lidstrom, M.E., 2009. Methylobacterium genome sequences: A reference blueprint to investigate microbial metabolism of C1 compounds from natural and industrial sources. PLoS One 4.

doi:10.1371/journal.pone.0005584

Ward, N.L., Challacombe, J.F., Janssen, P.H., Henrissat, B., Coutinho, P.M., Wu, M., Xie, G., Haft, D.H., Sait, M., Badger, J., Barabote, R.D., Bradley, B., Brettin, T.S., Brinkac, L.M., Bruce, D., Creasy, T., Daugherty, S.C., Davidsen, T.M., DeBoy, R.T., Detter, J.C., Dodson, R.J., Durkin, A.S., Ganapathy, A., Gwinn-Giglio, M., Han, C.S., Khouri, H., Kiss, H., Kothari, S.P., Madupu, R., Nelson, K.E., Nelson, W.C., Paulsen, I., Penn, K., Ren, Q., Rosovitz, M.J., Selengut, J.D., Shrivastava, S., Sullivan, S.A., Tapia, R., Thompson, S., Watkins, K.L., Yang, Q., Yu, C., Zafar, N., Zhou, L., Kuske, C.R., 2009. Three genomes from the phylum Acidobacteria provide insight into the lifestyles of these microorganisms in soils. Appl Environ Microbiol 75, 2046–2056. doi:10.1128/AEM.02294-08

Wright, J., Kirchner, V., Bernard, W., Ulrich, N., McLimans, C., Campa, M.F., Hazen, T., Macbeth, T., Marabello, D., McDermott, J., Mackelprang, R., Roth, K., Lamendella, R., 2017. Bacterial Community Dynamics in Dichloromethane-Contaminated Groundwater Undergoing Natural Attenuation. Front Microbiol.

Young, I.M., Crawford, J.W., 2004. Interactions and self-organization in the soil-microbe complex. Science (80-). doi:10.1126/science.1097394

Zhang, Y., Sun, R., Zhou, A., Zhang, J., Luan, Y., Jia, J., Yue, X., Zhang, J., 2018. Microbial community response reveals underlying mechanism of industrial-scale manganese sand biofilters used for the simultaneous removal of iron, manganese and ammonia from groundwater. AMB Express 8, 2. doi:10.1186/s13568-017-0534-7

Zhou, Y., Kellermann, C., Griebler, C., 2012. Spatio-temporal patterns of microbial communities in a hydrologically dynamic pristine aquifer. FEMS Microbiol Ecol 81, 230–242. doi:10.1111/j.1574-6941.2012.01371.x

Zhu, B., Wang, X., Rioual, P., 2017. Multivariate indications between environment and ground water recharge in a sedimentary drainage basin in northwestern China. J Hydrol 549, 92–113. doi:10.1016/j.jhydrol.2017.03.058

Supplementary material

1. Input data for Principal Component Analyses

Table S1 Summary of physico-chemical variables of water samples (values in bold represent concentrations under detection limit)

Table S2 Summary of diversity indices of water samples

Table S3 Summary of relative abundance (%) of microbial community classes of water samples

Table S4 Summary of relative abundance (%) of microbial community species of water samples

Table S5 Summary of diversity indices and depth of soil samples

Table S6 Summary of soil parameters from grain-size distribution curves

Table S7 Summary of relative abundance (%) of microbial community classes of soil samples

Table S8 Summary of relative abundance (%) of microbial community species of soil samples

2. Supplementary figures

Figure S1 PCA_2 analysis with microbial classes and hydrochemical data from groundwater samples and infiltration water sample

Figure S2 PCA_4 analysis with microbial species and hydrochemical data from groundwater samples and infiltration water sample

Figure S3 PCA_6 analysis with microbial classes data from soil samples

Figure S4 PCA_8 analysis with microbial species data from soil samples

Figure S5 PCA_{10} analysis with microbial classes data from soil and water samples

1. Input data for Principal Component Analyses

Table S1 Summary of physico-chemical variables of water samples (values in bold represent concentrations under detection limit)

Operating conditions	Recharge (July 2014)													No Recharge (March 2015)							
Sample	Infiltration water	P1(-0.6)	P2 (3)	P2 (-5)	P3 (0.8)	P3 (-4.2)	P5 (2.3)	P5 (-2.2)	P8 (1)	P8 (-3)	P9(3.5)	P9(-2)	P10(-1)	P1(-0.6)	P2 (-2)	P3 (0.8)	P5 (2.3)	P8 (1)	P8 (-3)	P9(-2)	P10(-1)
Relative depth (m)	14.00	-0.59	4.80	-5.00	0.80	-3.20	2.31	-2.19	0.96	-3.04	3.53	-1.91	-0.90	-0.59	-2.00	0.80	2.31	0.96	-3.04	-1.97	-0.90
Temperature (^{0}C)	25.10	16.87	22.72	18.66	17.77	17.71	24.84	22.05	18.00	17.50	18.86	16.99	15.56	16.50	18.50	17.10	24.50	16.30	17.80	14.50	13.70
Conductivity (µS/cm	1.28	1.56	1.51	1.52	1.65	1.68	1.56	1.54	1.48	1.54	1.57	1.55	1.65	1.33	1.27	1.29	1.37	1.24	1.31	1.22	1.32
DOC (mg/l)	6.60	1.65	3.17	4.70	3.22	3.15	3.61	3.76	13.65	8.02	16.31	11.71	2.57	1.93	1.80	2.33	1.53	3.96	1.65	1.74	1.61
pH	8.47	7.05	7.30	7.28	7.15	7.15	7.39	7.42	7.45	7.39	7.30	7.32	7.28	7.36	7.34	7.15	6.98	7.13	7.27	7.12	7.35
Cl- (ppm)	245.86	224.75	267.00	212.00	241.00	241.66	274.62	269.68	267.52	262.78	256.58	249.53	265.49	212.39	211.80	193.19	191.65	186.14	193.46	192.34	189.11
NO3- (ppm)	6.14	5.02	3.13	3.64	6.06	8.73	3.41	4.08	2.75	2.88	4.29	6.57	5.88	17.79	12.90	18.20	17.70	2.82	15.49	11.54	16.28
SO4-2 (ppm)	123.43	173.04	140.79	138.37	168.60	168.66	133.80	147.84	120.04	152.06	152.89	131.52	179.98	181.48	139.62	177.03	200.31	141.23	199.54	137.00	174.81
HCO3- (mg/l)	144.50	354.30	261.20	272.60	399.00	385.80	256.10	255.20	241.30	253.80	301.00	293.90	327.10	382.60	314.20	294.30	408.10	379.30	408.10	333.10	396.40
Ca (ppm)	67.59	135.23	94.24	103.49	144.15	141.27	97.40	96.73	92.46	99.88	102.87	105.21	123.29	142.63	121.41	133.29	141.88	122.96	72.31	114.70	132.74
Fe (ppm)	**0.20**	**0.20**	**0.20**	**0.20**	**0.20**	**0.20**	**0.20**	**0.20**	**0.20**	**0.20**	**0.20**	**0.20**	**0.20**	**0.20**	**0.20**	**0.20**	**0.20**	**0.20**	**0.20**	**0.20**	**0.20**
Si (ppm)	0.12	3.28	1.86	1.99	4.14	4.27	1.73	1.73	1.64	1.89	1.94	1.94	2.10	3.00	2.64	3.49	3.21	3.85	3.30	2.85	2.46
B (ppm)	**0.20**	0.26	0.23	0.22	0.22	0.21	**0.20**	**0.20**	0.21	0.21	0.23	0.22	0.22	**0.20**	**0.20**	**0.20**	**0.20**	**0.20**	**0.20**	**0.20**	**0.20**
Mg (ppm)	22.99	33.73	27.44	29.81	35.99	35.15	29.06	28.77	26.67	28.47	29.13	29.33	31.44	36.28	31.86	33.02	38.17	30.81	38.34	31.85	37.22
Na (ppm)	147.89	138.91	153.85	158.19	142.48	143.92	163.36	165.02	159.96	161.18	158.53	157.98	162.79	116.28	113.52	108.76	118.03	110.09	116.74	117.43	117.49
S (ppm)	42.22	52.21	41.72	42.55	47.64	47.90	42.93	42.78	40.45	47.19	46.72	46.27	51.40	63.51	58.08	58.73	63.37	64.34	51.23	53.88	59.89
K(ppm)	28.01	19.63	30.15	30.99	24.18	24.41	38.60	31.86	29.97	29.39	33.13	30.60	26.32	23.76	22.90	23.92	24.44	23.02	20.73	23.57	27.52
P (ppm)	0.38	1.14		0.96	1.63	0.71	**0.50**	**0.50**	0.66	**0.50**	**0.50**	**0.50**	**0.50**	**0.50**	**0.50**	**0.50**	**0.50**	**0.50**	**0.50**	**0.50**	**0.50**
Ba (ppb)	50.00	74.89	70.28	70.62	59.40	58.68	66.94	66.11	74.87	74.62	58.33	656.87	56.72	76.99	56.13	71.99	74.42	58.55	39.87	46.10	60.64
Li (ppb)	14.54	46.28	23.96	34.39	23.78	23.26	24.15	24.57	20.88	21.89	25.22	24.84	22.80	15.64	15.46	16.27	18.62	16.49	18.54	16.69	19.00
Mn (ppb)	4.76	28.93	10.79	8.94	3.67	1.74	2.17	1.46	2.46	2.77	4.46	2.26	1.58	0.87	5.51	0.81	**0.80**	145.30	**0.80**	3.90	**0.80**
Zn (ppb)	10.67	57.28	69.22	19.92	23.94	22.56	15.62	9.70	24.55	30.71	18.21	12.78	15.73	53.76	15.13	24.75	13.36	17.37	6.61	32.25	13.68
Al (ppm)	10.09	12.08	18.17	86.17	4.11	3.70	3.96	99.48	4.93	8.20	2.36	10.52	0.50	**0.20**	**0.20**	**0.20**	**0.20**	**0.20**	**0.20**	**0.20**	**0.20**
As (ppb)	3.51	3.18	2.64	2.43	1.20	1.29	3.56	3.47	3.77	3.64	1.49	1.52	1.46	3.28	1.78	1.56	3.00	2.02	1.89	1.70	1.67
Pb (ppb)	**0.80**	1.29	1.12	**0.80**	**0.80**	**0.80**	**0.80**	**0.80**	**0.80**	**0.80**	**0.80**	**0.80**	**0.80**	2.09	**0.80**	**0.80**	**0.80**	**0.80**	**0.80**	**0.80**	**0.80**

Cu (ppb)	4.95	6.94	8.28	5.22	4.91	5.16	5.05	4.50	6.47	6.93	3.82	3.70	3.17	1.32	1.61	1.14	1.13	**0.80**	**0.80**	1.96	1.11
U (ppb)	1.23	1.80	1.11	1.27	2.18	2.11	1.01	1.04	0.91	1.08	1.44	1.45	1.81	2.32	2.02	2.49	2.35	1.46	1.97	2.07	2.42
Cd (ppb)	9.08	6.49	17.04	**0.80**	10.06	4.44	1.45	0.65	**0.80**	13.41	**0.80**	**0.80**	**0.80**	**0.80**	**0.80**	**0.80**	**0.80**	**0.80**	**0.80**	**0.80**	**0.80**
V (ppb)	2.70	29.78	2.40	2.50	2.17	2.20	2.77	2.68	2.51	2.63	1.71	2.07	2.10	8.76	8.64	8.50	8.99	9.47	9.80	9.30	9.06
Ni (ppb)	10.62	35.34	6.97	5.51	6.16	5.37	6.24	4.63	5.66	8.26	4.48	4.20	4.50	2.53	1.89	2.00	1.91	2.59	1.32	1.62	1.82
Co (ppb)	**0.80**	**0.80**	**0.80**	**0.80**	**0.80**	**0.80**	**0.80**	**0.80**	**0.80**	**0.80**	**0.80**	**0.80**	**0.80**	**0.80**	**0.80**	**0.80**	**0.80**	**0.80**	**0.80**	**0.80**	**0.80**

Table S2 Summary of diversity indices of water samples

Operating conditions	Recharge (July 2014)													No Recharge (March 2015)							
Sample	Infiltration water	P1(-0.6)	P2 (3)	P2 (-5)	P3 (0.8)	P3 (-4.2)	P5 (2.3)	P5 (-2.2)	P8 (1)	P8 (-3)	P9(3.5)	P9(-2)	P10(-1)	P1(-0.6)	P2 (-2)	P3 (0.8)	P5 (2.3)	P8 (1)	P8 (-3)	P9(-2)	P10(-1)
Shannon	2.78	2.57	2.29	2.8	2.65	2.65	1.91	2.22	1.43	1.69	2.18	2.07	2.51	2.73	2.67	2.01	2.41	1.73	2.03	3.01	2.49
Richness	29.00	22.00	19.00	23.00	21.00	20.00	15.00	13.00	10.00	11.00	11.00	13.00	17.00	19.00	18.00	11.00	14.00	11.00	10.00	23.00	16.00
Evenness	0.65	0.6	0.54	0.66	0.62	0.62	0.45	0.52	0.34	0.4	0.51	0.49	0.59	0.64	0.63	0.47	0.57	0.41	0.48	0.71	0.59

Table S3 Summary of relative abundance (%) of microbial community classes of water samples

Operating conditions	Recharge (July 2014)													No Recharge (March 2015)							
Sample	Infiltration water	P1(-0.6)	P2 (3)	P2 (-5)	P3 (0.8)	P3 (-4.2)	P5 (2.3)	P5 (-2.2)	P8 (1)	P8 (-3)	P9(3.5)	P9(-2)	P10(-1)	P1(-0.6)	P2 (-2)	P3 (0.8)	P5 (2.3)	P8 (1)	P8 (-3)	P9(-2)	P10(-1)
Betaproteobacteria	13.81	9.48	7.29	9.09	7.24	10.49	21.88	22.88	69.94	54.76	33.71	37.80	23.50	22.69	4.83	38.52	14.29	3.02	57.02	19.60	2.69
Cyanobacteria	12.85	11.13	6.25	2.86	13.57	13.64	9.09	7.63	5.83	8.44	8.57	6.71	21.31	8.40	4.42	5.74	7.94	1.51	0.00	3.71	8.31
Bacili	1.53	23.92	17.53	12.63	10.86	6.29	42.90	11.02	1.84	1.73	21.71	0.61	6.56	8.40	2.00	18.03	15.87	0.00	11.57	2.47	4.64
Dehalococcoidia	3.74	4.54	29.86	14.98	17.19	16.43	1.42	0.00	0.00	0.00	2.86	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	9.28	0.00
Acidobacteria	7.57	4.95	9.03	4.55	11.31	9.09	1.99	0.00	0.00	0.00	2.29	9.15	4.92	4.20	3.64	0.00	0.00	13.57	0.00	0.00	0.00
Actinobacteria	8.53	1.24	0.52	4.21	3.17	5.24	0.00	0.00	0.92	1.08	0.00	0.00	0.00	3.36	8.14	6.15	0.00	0.00	7.44	2.20	1.81
Cytophagia	0.19	0.00	0.00	0.00	0.00	0.00	12.22	18.64	6.75	3.90	12.00	6.71	9.29	16.81	4.91	0.00	4.76	5.53	7.02	1.51	0.00
Chlorobia	13.52	10.52	6.77	2.53	0.00	0.00	2.84	0.00	2.76	10.61	0.00	0.00	0.00	0.00	4.09	0.00	0.00	5.03	0.00	0.00	17.18
Nitrospira	4.03	0.00	0.00	4.38	9.50	8.39	1.14	11.02	0.00	0.00	2.86	7.32	8.20	2.52	0.00	0.00	3.17	0.00	0.00	4.27	0.00
GAmmaproteobacteria	5.08	0.82	4.17	5.89	0.00	0.00	4.55	20.34	5.83	7.79	3.43	6.71	0.00	11.76	5.65	13.93	18.25	0.00	0.00	1.86	11.61
Alphaproteobacteria	4.89	1.44	1.74	0.17	2.26	0.00	0.28	1.69	0.61	0.65	0.00	0.00	2.73	2.52	0.00	1.64	9.52	0.00	0.00	8.03	7.15
Others	24.26	31.96	16.84	38.72	24.89	30.42	1.70	6.78	5.52	11.04	12.57	25.00	23.50	19.33	62.32	15.98	26.19	71.36	16.94	47.08	46.61

Table S4 Summary of relative abundance (%) of microbial community species of water samples

Operating conditions	Recharge (July 2014)													No Recharge (March 2015)							
Sample	Infiltration water	P1(-0.6)	P2 (3)	P2 (-5)	P3 (0.8)	P3 (-4.2)	P5 (2.3)	P5 (-2.2)	P8 (1)	P8 (-3)	P9(3.5)	P9(-2)	P10(-1)	P1(-0.6)	P2 (-2)	P3 (0.8)	P5 (2.3)	P8 (1)	P8 (-3)	P9(-2)	P10(-1)
Methylotenera mobilis	0.48	0.00	0.17	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	10.92	0.00	32.79	14.29	0.00	31.40	0.00	0.00
Pontibacter sp	0.19	0.00	0.00	0.00	0.00	0.00	12.22	18.64	6.75	3.90	12.00	6.71	9.29	16.81	4.91	0.00	4.76	5.53	7.02	1.51	0.00
Dehalogenimonas sp	3.74	4.54	29.86	14.98	17.19	16.43	1.42	0.00	0.00	0.00	2.86	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	9.28	0.00
Subgroup 3 sp (Acidobact.)	7.57	4.95	9.03	4.55	11.31	9.09	1.99	0.00	0.00	0.00	2.29	9.15	4.92	4.20	3.64	0.00	0.00	13.57	0.00	0.00	0.00
Vogesella indigofera	3.36	9.48	4.17	2.86	3.17	3.50	2.84	1.69	60.12	48.70	13.14	32.93	0.00	4.20	3.23	2.46	0.00	0.00	6.20	0.00	2.69
Stenotrophomonas sp	5.08	0.82	4.17	5.89	0.00	0.00	4.55	20.34	5.83	7.79	3.43	6.71	0.00	11.76	5.65	13.93	18.25	0.00	0.00	1.86	11.61
Chryseomicrobium sp	1.53	23.92	17.53	12.63	10.86	6.29	42.90	11.02	1.84	1.73	21.71	0.61	6.56	8.40	2.00	18.03	15.87	0.00	11.57	2.47	4.64
Nitrospira sp1	3.36	0.00	0.00	0.84	1.81	2.80	0.00	4.24	0.00	0.00	0.00	5.49	4.37	0.00	0.00	0.00	3.17	0.00	0.00	4.27	0.00
Methylobacterium sp	4.89	1.44	1.74	0.17	2.26	0.00	0.28	1.69	0.61	0.65	0.00	0.00	2.73	2.52	0.00	1.64	9.52	0.00	0.00	8.03	7.15
Nitrospira sp2	0.67	0.00	0.00	3.54	7.69	5.59	1.14	6.78	0.00	0.00	2.86	1.83	3.83	2.52	0.00	0.00	0.00	0.00	0.00	0.00	0.00

Table S5 Summary of diversity indices and depth of soil samples

Operating conditions	Recharge (July 2014)					No Recharge (March 2015)				
Sample	SD	E_0.1	M_0.1	M_0.2	F_0.1	E_0.2	E_0.8	M_0.3	F_0.1	F_0.3
Depth (cm)	0.10	0.10	0.10	0.20	0.10	0.20	0.80	0.30	0.10	0.30
Shannon	2.89	3.22	3.36	3.14	3.29	2.93	2.86	2.90	2.81	2.40
Richness	25.50	35.50	37.00	30.50	34.00	25.00	24.00	21.00	19.50	16.00
Evenness	0.68	0.76	0.79	0.74	0.77	0.69	0.67	0.68	0.66	0.57

Table S6 Summary of soil parameters from grain-size distribution curves

Operating conditions	Recharge (July 2014)					No Recharge (March 2015)				
Sample	SD	E_0.1	M_0.1	M_0.2	F_0.1	E_0.2	E_0.8	M_0.3	F_0.1	F_0.3
CC	0.04	2.01	1.43	0.84	0.53	3.31	0.54	0.93	0.55	1.27
CU	159.39	53.52	28.32	20.03	13.28	17.72	19.23	32.59	19.69	8.13
% Fine	10.12	2.17	2.92	0.85	2.60	0.54	1.67	1.78	1.94	0.43
D10	0.07	0.27	0.54	0.20	0.31	1.08	0.25	0.28	0.28	1.42

Table S7 Summary of relative abundance (%) of microbial community classes of soil samples

Operating conditions	Recharge (July 2014)					No Recharge (March 2015)				
Sample	SD	E_0.1	M_0.1	M_0.2	F_0.1	E_0.2	E_0.8	M_0.3	F_0.1	F_0.3
Betaproteobacteria	23.01	21.37	16.97	18.29	18.89	21.32	8.06	14.28	11.31	14.01
Cyanobacteria	8.66	5.31	8.26	6.26	3.54	11.09	8.36	3.40	4.76	2.72
Bacili	6.39	5.17	4.35	8.07	7.25	7.74	7.56	4.53	6.85	21.50
Dehalococcoidia	4.40	1.77	2.10	2.97	3.37	0.00	1.93	0.00	0.00	0.00
Acidobacteria	9.80	2.31	1.95	0.99	1.69	0.00	2.09	2.83	0.00	0.00
Actinobacteria	3.27	2.18	4.35	3.79	2.36	4.96	15.63	9.49	7.89	16.11
Cytophagia	0.85	2.45	0.60	3.29	4.72	4.53	13.21	10.51	13.99	11.77
Chlorobia	0.00	2.31	2.55	2.97	4.22	0.00	0.32	0.00	0.00	0.00
Nitrospira	5.40	1.91	7.06	6.75	4.05	14.72	10.47	5.07	5.65	4.53
GAmmaproteobacteria	2.13	3.95	1.80	0.00	4.05	4.34	2.26	3.97	0.00	0.00
Alphaproteobacteria	0.99	3.81	2.10	1.65	7.76	0.57	3.06	6.71	6.43	7.88
Others	35.09	47.47	47.90	44.98	38.11	30.74	27.04	39.21	43.13	21.50

Table S8 Summary of relative abundance (%) of microbial community species of soil samples

Operating conditions	Recharge (July 2014)					No Recharge (March 2015)				
Sample	SD	E_0.1	M_0.1	M_0.2	F_0.1	E_0.2	E_0.8	M_0.3	F_0.1	F_0.3
Methylotenera mobilis	0.00	0.00	0.60	0.00	0.00	2.64	0.00	0.00	0.00	0.91
Pontibacter sp	0.85	2.45	0.60	3.29	4.72	4.53	13.21	10.51	13.99	11.77
Dehalogenimonas sp	4.40	1.77	2.10	2.97	3.37	0.00	1.93	0.00	0.00	0.00
Subgroup 3 sp (Acidobact.)	9.80	2.31	1.95	0.99	1.69	0.00	2.09	2.83	0.00	0.00
Vogesella indigofera	8.10	7.49	4.80	4.94	4.22	4.53	2.42	2.83	3.57	0.00
Stenotrophomonas sp	2.13	3.95	1.80	0.00	4.05	4.34	2.26	3.97	0.00	0.00
Chryseomicrobium sp	6.39	5.17	4.35	8.07	7.25	7.74	7.56	4.53	6.85	21.50
Nitrospira sp1	2.70	0.54	5.56	3.62	2.53	14.72	7.41	1.39	4.17	0.00
Methylobacterium sp	0.99	3.81	2.10	1.65	7.76	0.57	3.06	6.71	6.43	7.88
Nitrospira sp2	2.70	1.36	1.50	3.13	1.52	0.00	3.06	3.68	1.49	4.53

2. Supplementary figures

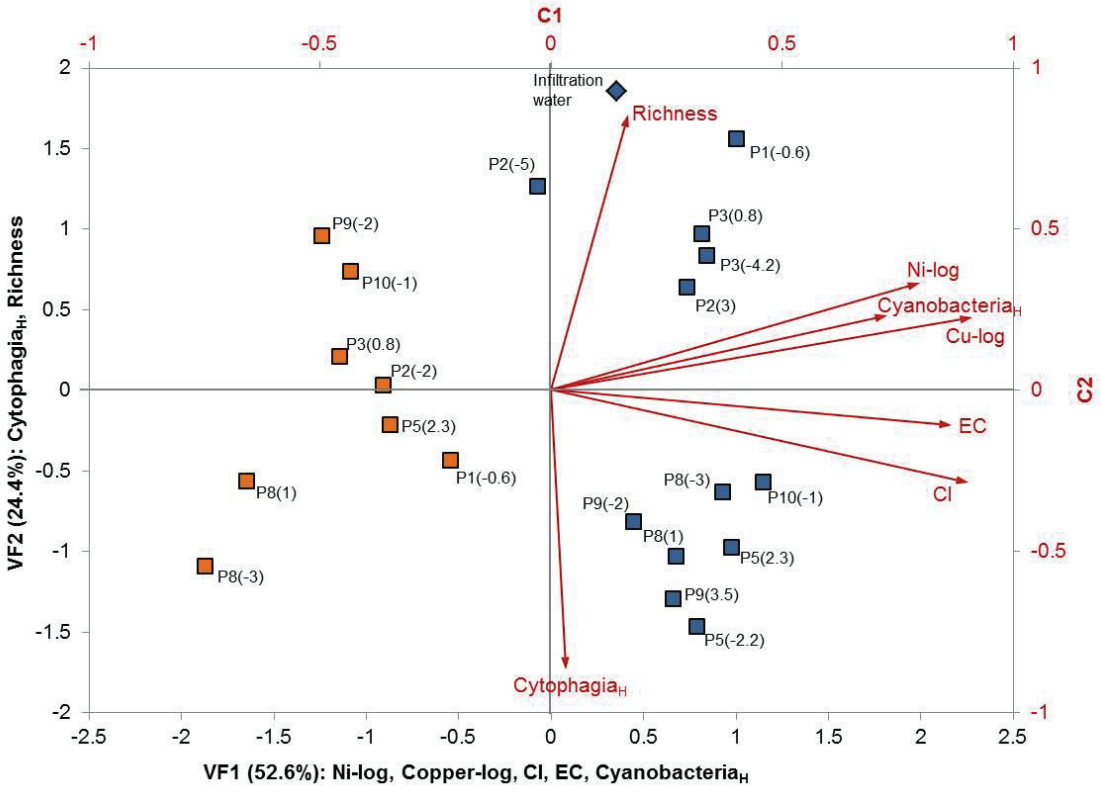

Figure S1. PCA_2 analysis with microbial classes and hydrochemical data from groundwater samples (squares) and the infiltration water sample (diamond) during the recharge period (blue) and during no-recharge period (orange). Position of samples is scaled in VF1 and VF2 axes. Red arrows represent the contribution of each variable in both Varifactors.

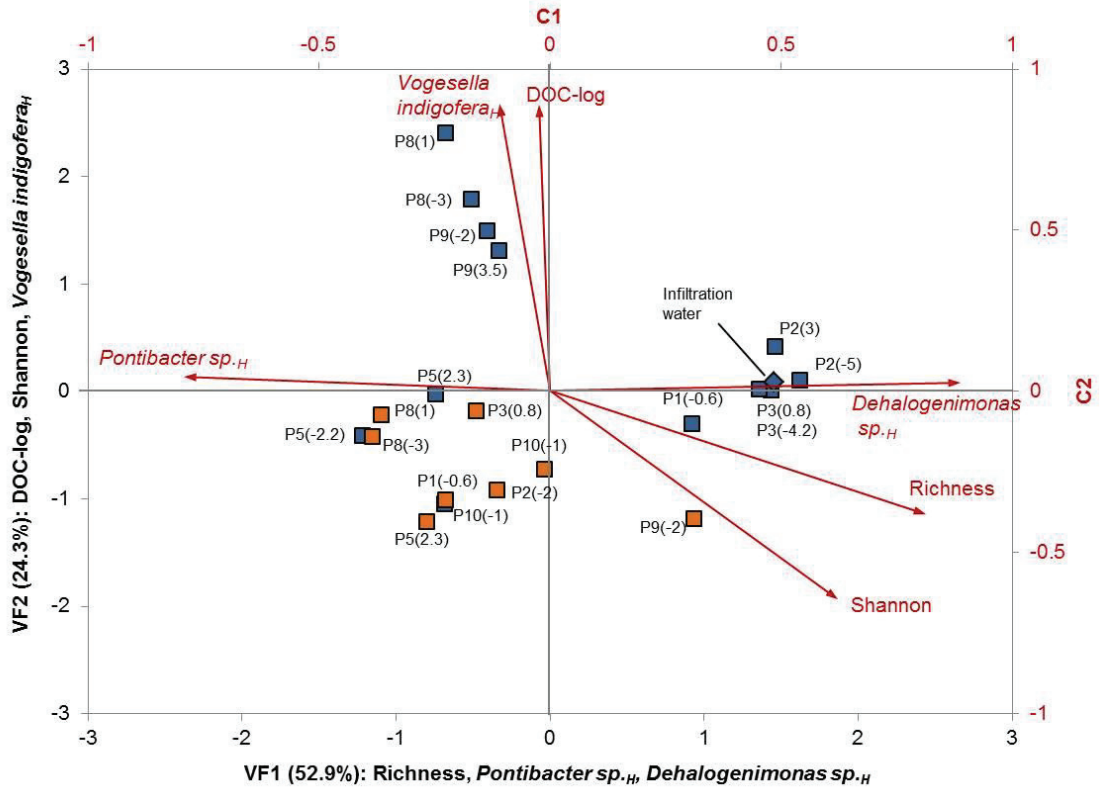

Figure S2 PCA_4 analysis with microbial species and hydrochemical data from groundwater samples (squares) and the infiltration water sample (diamond) during the recharge period (blue) and during no-recharge period (orange). Position of samples is scaled in VF1 and VF2 axes. Red arrows represent the contribution of each variable in both Varifactors.

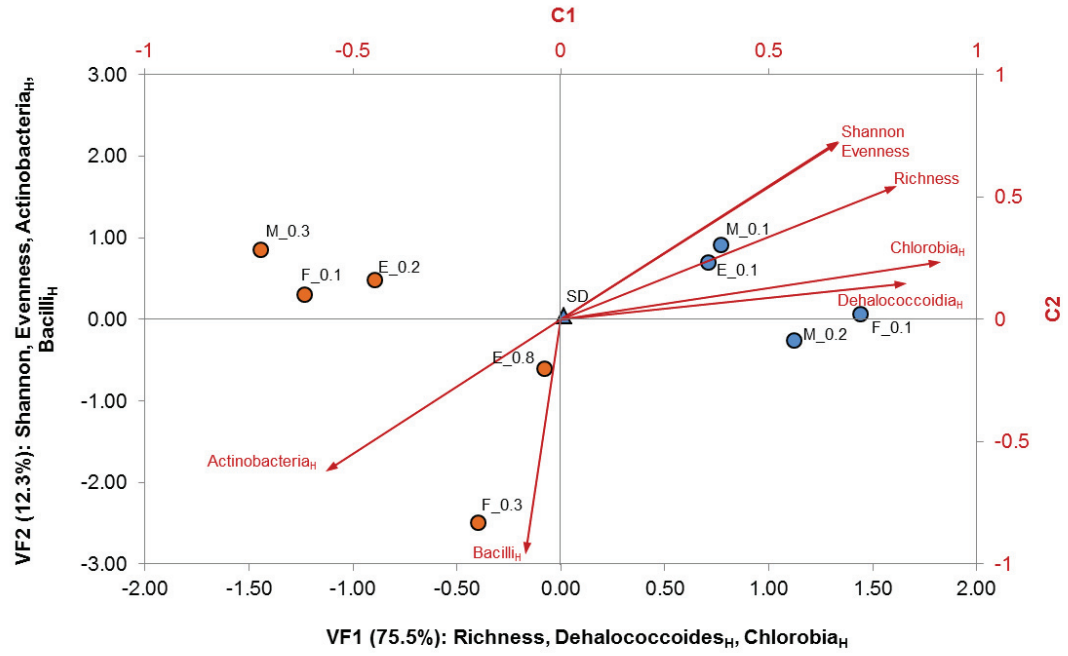

Figure S3 PCA_6 analysis with microbial classes data from soil samples during the recharge period (blue) and during no-recharge period (orange). Circles are related to the infiltration pond samples (Entrance, Midfield and Final strecht according the capital letter close to each symbol). Triangle is referred to the sedimentation pond sample. Position of samples is scaled in VF1 and VF2 axes. Red arrows represent the contribution of each variable in both Varifactors scaled by components.

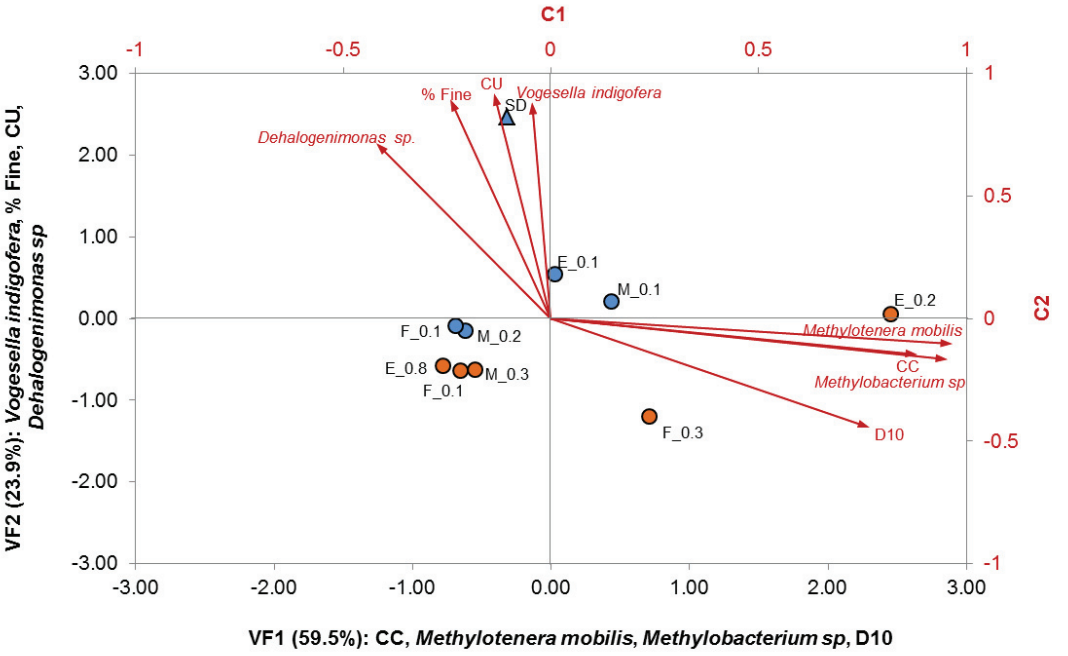

Figure S4. PCA_8 analysis with microbial species data from soil samples during the recharge period (blue) and during no-recharge period (orange). Circles are related to the infiltration pond samples (Entrance, Midfield and Final strecht according the capital letter close to each symbol). Triangle is referred to the sedimentation pond sample. Position of samples is scaled in VF1 and VF2 axes. Red arrows represent the contribution of each variable in both Varifactors scaled by components.

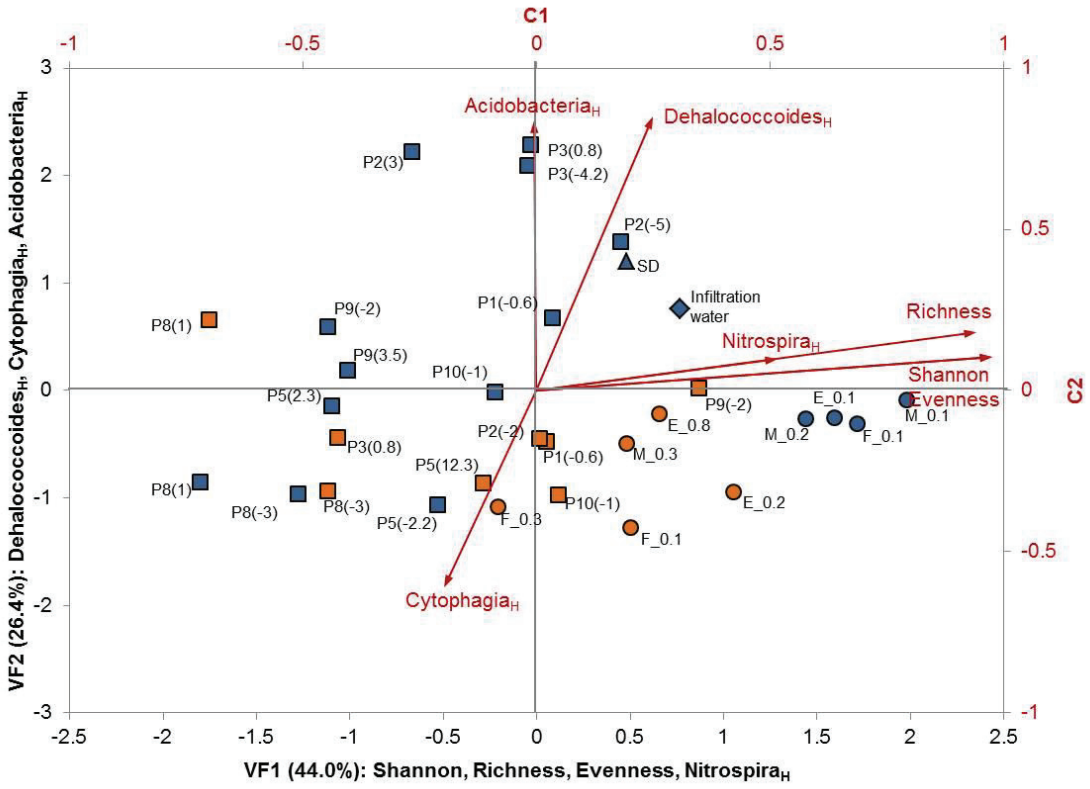

Figure S5. PCA_{10} analysis with microbial classes data from soil and water samples during the recharge period (blue) and during no-recharge period (orange). Circles are related to soil samples (Entrance, Midfield and Final stretch according the capital letter close to each symbol). Triangle is referred to the sedimentation pond soil sample. Squares represent groundwater samples and the diamond is for infiltration water. Position of samples is scaled in VF1 and VF2 axes. Red arrows represent the contribution of each variable in both varifactors.