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

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12 **Abstract**

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

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

abundant populations of denitrifying bacteria in groundwater. A significant correlation was found between *Methylobacterium mobilis* and the concentrations of NO₃ and SO₄, 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 $14000 \text{ m}^2 \cdot \text{d}^{-1}$ 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 (6500 m^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.

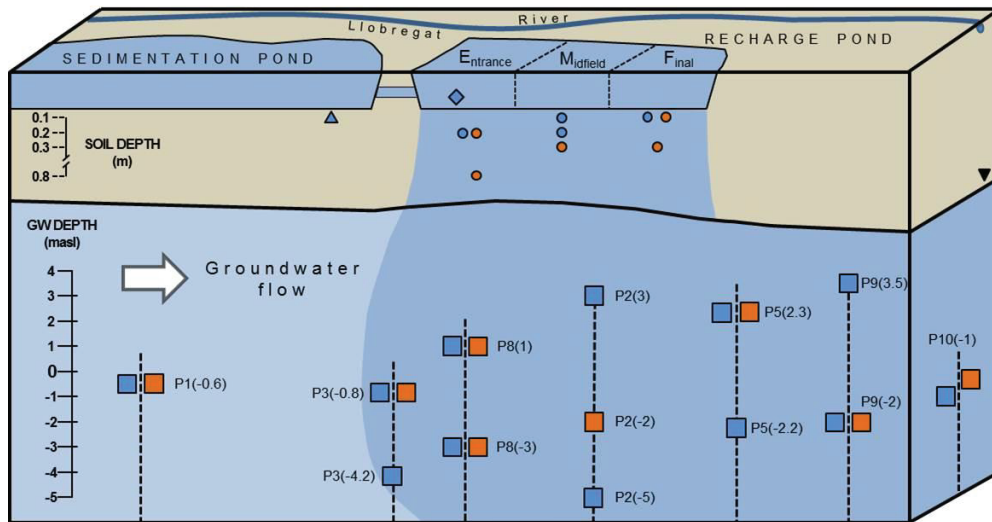
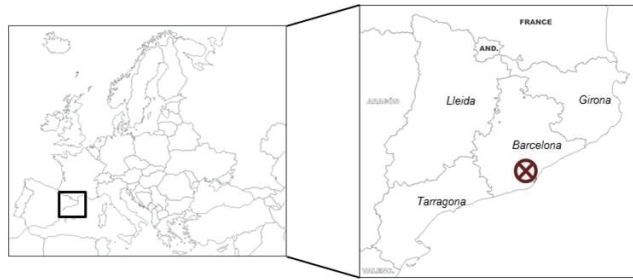


Figure 1 Geographical situation and cross-section of the Llobregat MAR System. Triangles and circles represent the location of soil samples. Diamond and squares represent those of water and groundwater samples, respectively. Blue symbols indicate samples taken in July 2014, after six months of continuous 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:

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

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, *Methylothermobacter mobilis*, *Vogesella indigofera*, *Stenotrophomonas*

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

271 **3. Results**

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

279 We present firstly global results of the eleven PCAs performed (Table 1). We indicate,
280 in the same table and for each statistical analysis performed, the extracted components
281 with their variables and the proportion of variance represented for each component.
282 Also, the measure of sampling adequacy (KMO test value) is reported for each
283 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: <i>Methylobacterium mobilis</i> _H , NO ₃ , SO ₄ , DOC-log	61.5	0.797
				C2: <i>Nitrospira</i> _sp _{2H} , EC, Na, V-log , Cu-log, Cl	15.4	
			PCA ₄	C1: Pontibacter _sp _H , <i>Dehalogenimonas</i> _sp _H , Richness	52.9	0.635
				C2: <i>Vogesella indigofera</i> _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: <i>Nitrospira</i> _2sp, Dryness, Shannon , <i>Pontibacter</i> _sp,	64.6	0.758
				C2: % Fine, <i>Subgroup3</i> _sp, CU	27.6	
			PCA ₈	C1: CC, D10, <i>Methylobacterium mobilis</i> , <i>Methylobacterium</i> sp,	59.5	0.640
				C2: % Fine, CU, <i>Dehalogenimonas</i> _sp, <i>Vogesella indigofera</i>	23.9	
Water and soil	Classes	2	PCA ₉	C1: Shannon, Richness, Actinobacteria _H , Type, Evenness	53.8	0.692
				C2: Others _H , Betaproteobacteria_H	16.4	
			PCA ₁₀	C3: Bacilli _H	12.5	0.696
				C1: Shannon, Evenness, Richness	44.0	
	Species	1	PCA ₁₁	C2: Dehalococcoides _H , Cytophagia_H , Acidobacteria _H	26.4	0.705
				C3: <i>Nitrospira</i> _H , Chlorobia_H	14.4	
			PCA ₁₁	C1: Shannon, Richness, Evenness, Type, <i>Nitrospira</i> _sp _{1H}	48.8	
				C2: Pontibacter _sp _H , <i>Dehalogenimonas</i> _sp _H , <i>Subgroup3</i> _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.

285 3.1. Key parameters influencing microbial classes in surface water and
286 groundwater

287 Two PCAs were developed; the first one (PCA₁ in Table 1) included Shannon and
288 Richness indices, Cl, EC, Na, the log transformations of S, Cu, and V, as well as
289 Betaproteobacteria_H, Dehalococcoidia_H, Nitrospira_H and Others_H classes. The group
290 “Others” includes microbial classes that could not be classified. Results of this PCA
291 show that these twelve variables could be mostly explained with only two principal
292 components (combined they explained 74% of the total variance). The strength of the
293 analysis is supported by a value of 0.744 in the KMO test. Results of a 2D
294 representation of varifactors and components (Figure 2) show the significance of
295 hydrochemistry beyond microbial variables. All the chemical variables included in the
296 analysis are grouped in the first component, showing a high correlation amongst them;
297 Na, Cl, EC and Cu-log (“-log” indicates log-transformed) are positively correlated,
298 whereas V-log and S-log are situated in the opposite side of the plot (negative
299 correlation).

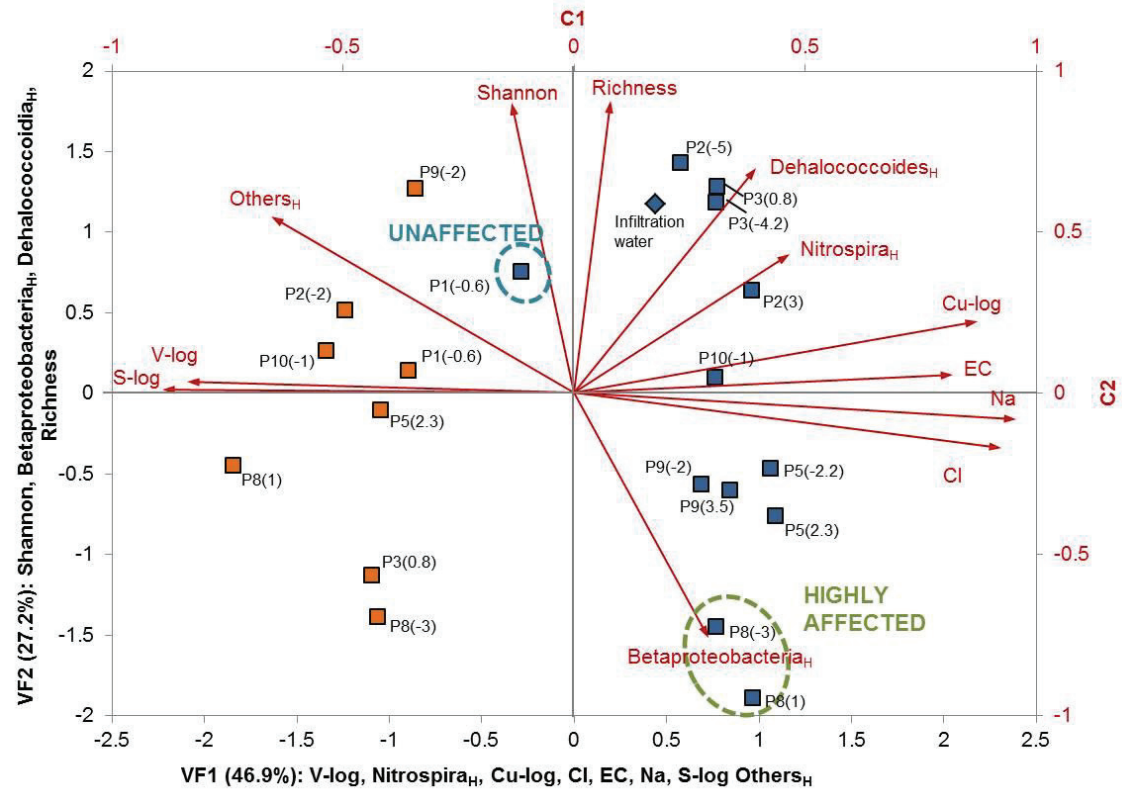


Figure 2 PCA₁ analysis including diversity indices, microbial classes and hydrochemical data from groundwater samples (squares) and one infiltration water sample (diamond) during the recharge period (blue) and no-recharge period (orange). The position of samples is scaled in VF1 and VF2 axes for visualization 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, PCA₂ 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

PCA₃ analysis (Table 1) included variables EC, Na, Cl, V-log, Cu-log, NO₃, SO₄, DOC-log, and two microbial species (as opposed to microbial classes, that were the objective of PCA₁ and PCA₂): *Methylobacterium 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.

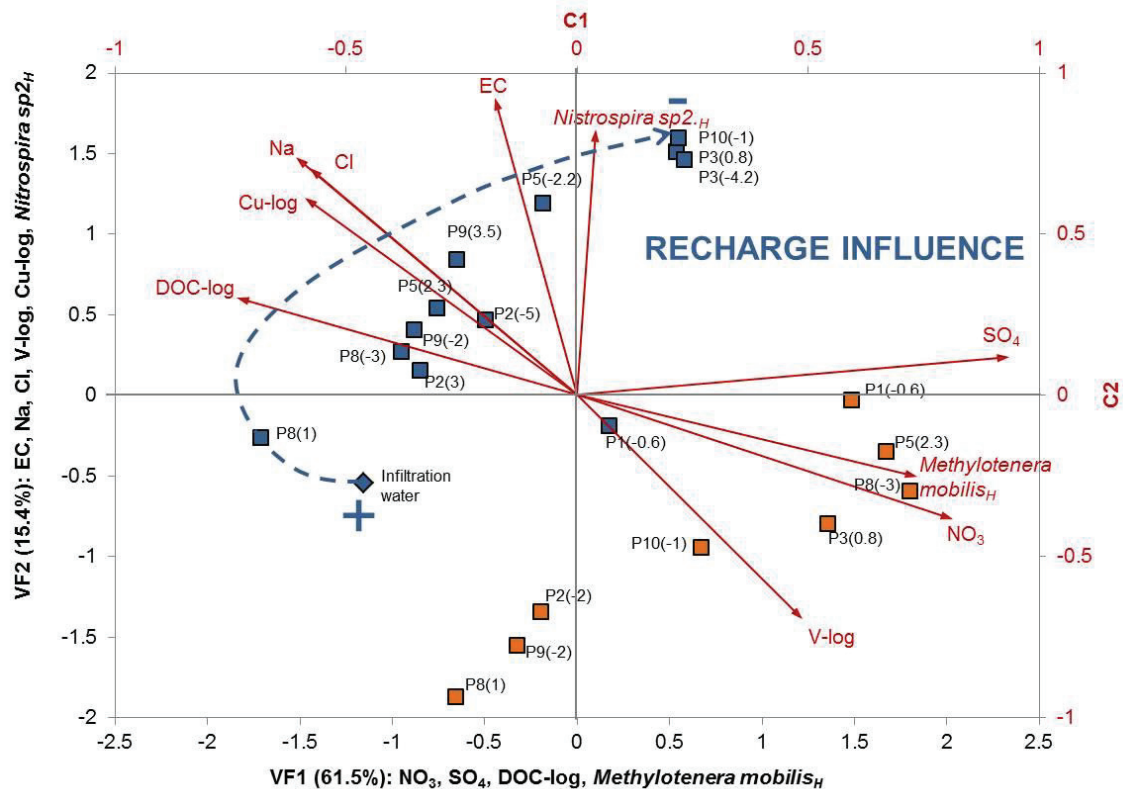


Figure 3 PCA₃ analysis including diversity indices, microbial species and hydrochemical data from groundwater samples (squares) and the infiltration water sample (diamond). The discontinuous blue arrow indicates the degree of disturbance caused by recharge.

Figure 3 also shows the main relations amongst variables. The first component groups mainly SO₄, NO₃ and DOC-log (inversely) with *Methylobacter mobilis*_H. The behavior of *Nitrospira* sp2_H and its high correlation with conductivity were explained by the second component.

PCA₄ 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

PCA₅ to PCA₈ 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).

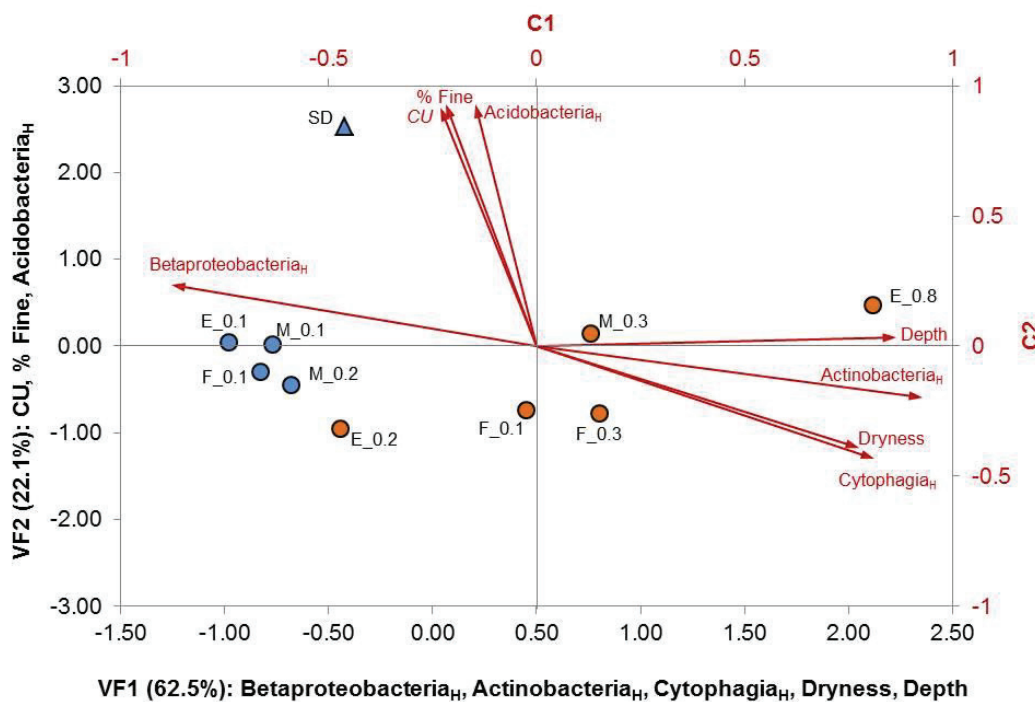


Figure 4 PCA₅ analysis including microbial classes, grain-size distribution curve parameters and depth from soil samples during the recharge period (blue) and no-recharge period (orange). Circles are related to the infiltration pond samples (E-Entrance, M-Midfield and F-Final stretch). Numbers indicate 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.

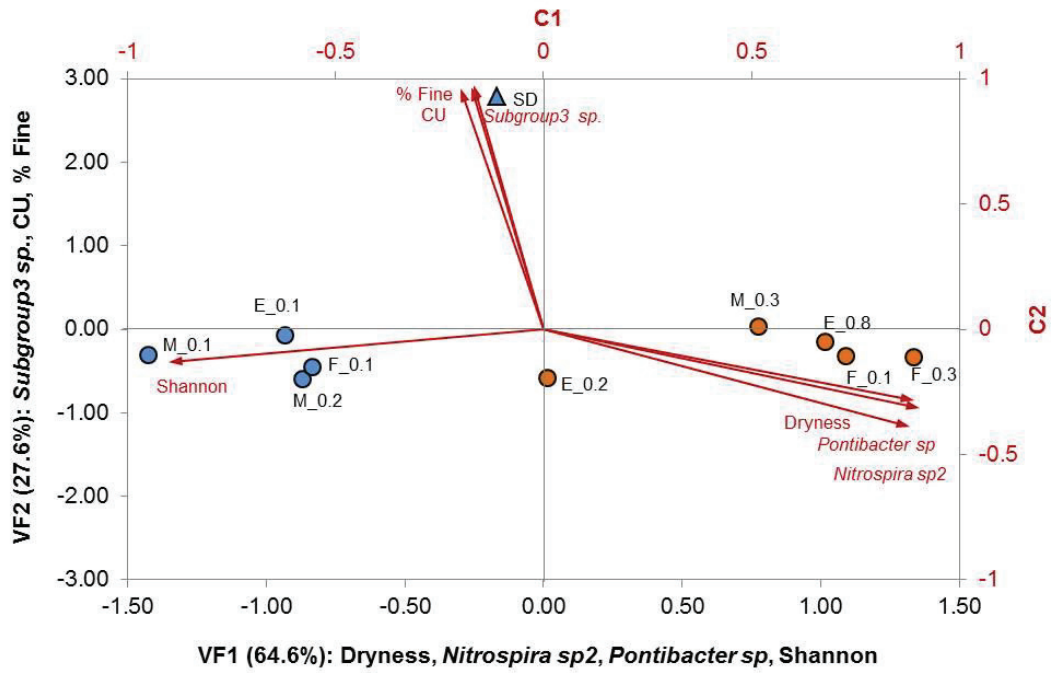
PCA₅ involved microbial soil classes (Acidobacteria_H, Betaproteobacteria_H, Actinobacteria_H, Cytophagia_H), Uniformity Coefficient (CU), fine material content (% Fine), Dryness (operation condition dry-wet) and Depth (Table 1). From Figure 4, Betaproteobacteria_H displays oppositely to Depth, Actinobacteria_H, 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). *PCA₆* 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 *Dehalococcoidia_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 *PCA₇* (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.

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389 **Figure 5** PCA₇ analysis including microbial species, grain-size distribution curve parameters and the
 390 operational period from sediment samples during the recharge period (blue) and no-recharge period
 391 (orange). Codes and symbols are reported in Figure 4.

392 PCA₈ indicates the affinity of *Vogesella indigofera* to silty soils (Figure S4). Despite
 393 *Dehalogenimonas sp.* behavior is explained by the second component, this species
 394 does not show correlation with *V. indigofera* or *CU*. However, the bivariate correlations
 395 matrix (not showed) revealed the correlation of *Dehalogenimonas sp.* with fine particles
 396 proportion. On the other hand, both *Methylothermobacter mobilis* and *Methylobacterium sp.*
 397 are correlated positively with two other soil characteristics, CC and D10.

398 3.5. Interclass and interspecies relationships

399 PCA₉ – PCA₁₁ involves the statistical analysis of all 31 samples together. In PCA₉, soil
 400 and water samples are distinctly located in the varifactor plane involving the first two
 401 components (explaining 70% of the total variance, Figure 6). From the analysis, soil
 402 samples are the most diverse, equally-distributed and rich environments sustaining
 403 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. Bacilli_H becomes an independent third component explaining 13% of the total variance (Table 1).

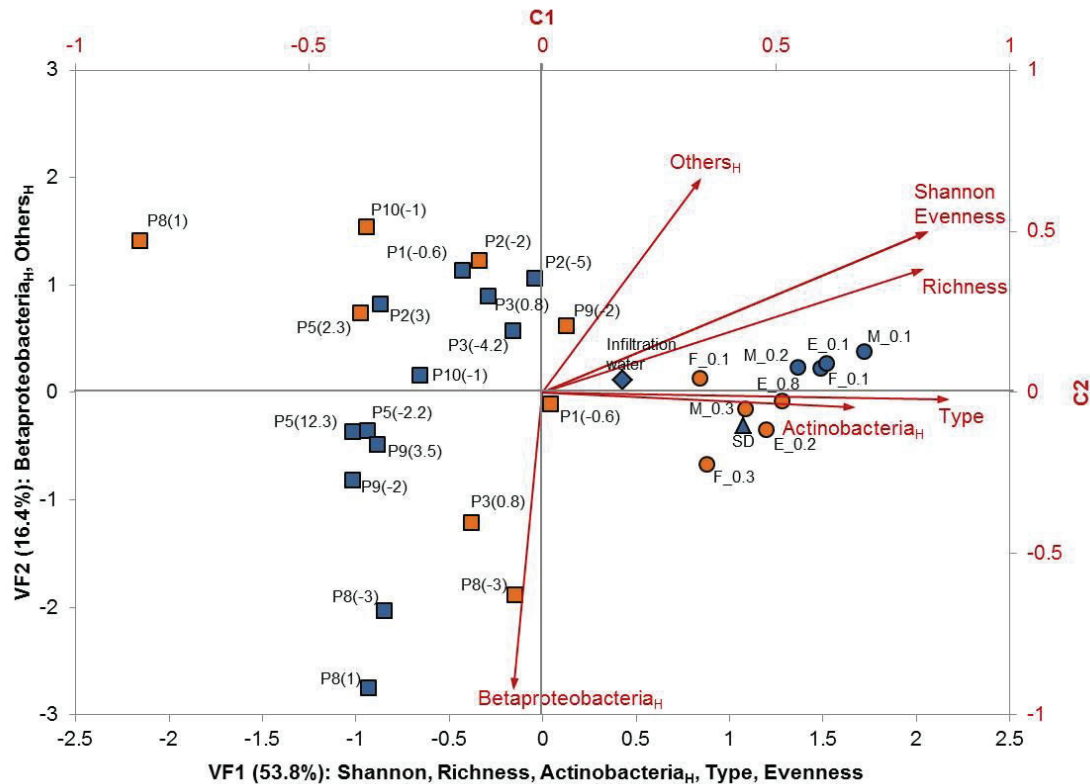


Figure 6 PCA₉ analysis including diversity indices, microbial classes and sample type from soil (circles), groundwater (squares) and surface water (diamonds and triangle) samples during the recharge period (blue) and no-recharge period (orange).

PCA₁₀ 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, PCA₁₁ emphasizes microbial species. Similar to PCA₁₀, the first component groups all three diversity indices, and is representative of soil samples in the varifactor plane (Figure 7). *Nitrospira* sp_{1H} 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.

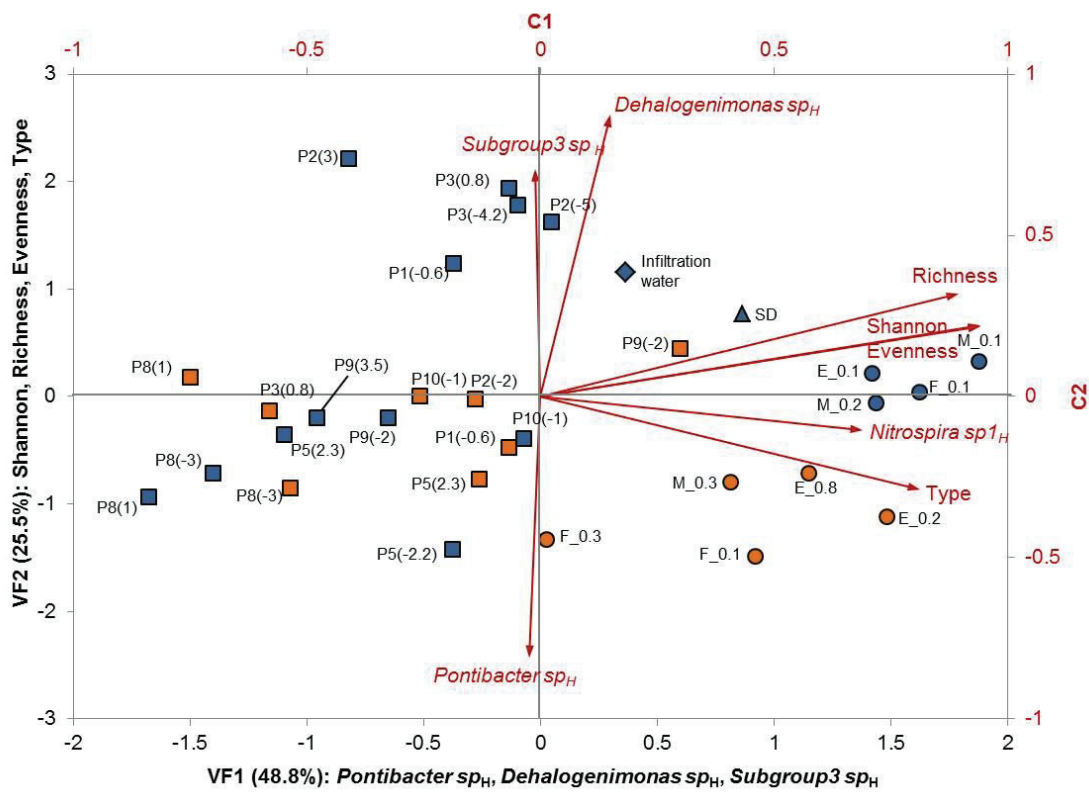


Figure 7 PCA₁₁ analysis including diversity indices, microbial species, and type of sample from soil (circles), groundwater (squares) and surface water (diamond and triangle) during the recharge period (blue) and no-recharge period (orange).

4. Discussion

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

441 Cyanobacteria, the only diazotrophs that produce oxygen as a by-product of the
442 photosynthetic process, were found most abundantly in the surface water, and the
443 measured concentrations correlated with several hydrogeochemical variables (EC, Cl,
444 Cu-log and Ni-log) (recall Figure S1). Correlations among EC and cyanobacteria
445 abundance have been already reported, for example, in a Neotropical urban lake (Frau
446 et al., 2018) or in a eutrophicated reservoir in Brazil (Chellappa and Mederios Costa,
447 2003). Cu-log is positively correlated with cyanobacteria; this follows Dwivedi et al.
448 (2006), who described the simultaneous presence of cyanobacteria and some metals
449 (including Cu) in river water samples enriched by fly-ash originated in a coal thermal
450 power station. Indeed, some cyanobacterial species have the ability to immobilize
451 metals, and this is used in some water treatment industries (de-Bashan and Bashan,
452 2010).

453 The highest relative abundance of Betaproteobacteria was found in the groundwater
454 samples most affected by recharge (e.g., conforming more than 50% of the total
455 microbial abundance in piezometer P8, located directly below the infiltration pond). This
456 microbial group correlates inversely with Richness and Shannon indices (recall Figure
457 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 *Methylothera mobilis* in surface and subsurface water environments (see PCA₃). *M. mobilis* may carry out heterotrophic denitrification by using different substrates, such as methanol (Kalyuzhnaya 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 PCA₃ 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 PCA₈); 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.

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485 In any case, it is not clear whether *M. mobilis* abundance correlates with some redox
486 species; while the bivariate correlation coefficients with DOC-log and SO₄ were not
487 significant (data not shown), *M. mobilis* grouped in the same component with NO₃ and
488 SO₄. Regarding this last point, there is no evidence that SO₄ might be part of the
489 metabolic pathway of *M. mobilis*.

490 *Vogesella indigofera* positively correlates with DOC-log (PCA₅). Members of this genus
491 are important denitrifiers in groundwater systems (Bellini et al., 2018), capable of
492 oxidizing few monosaccharides in low-oxygen concentration conditions (Grimes et al.,
493 1997). Contrarily, DOC-log was negatively correlated to NO₃ concentration (PCA₃). In
494 fact, the isotopic study performed by Grau-Martínez et al. (2018) at the same facility
495 and on the same dates confirmed that recharge induced denitrification.

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

508 Distribution of microbial populations in soils is also strongly affected by granulometry
509 (PCA₈). *M. mobilis* and *Methylobacterium* sp. correlated with CC and D₁₀. There is not
510 much literature on this subject. The work performed by Madhaiyan et al. (2007)

associated the variability in the distribution of pink-pigmented facultative methylotrophs, 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 Pohlen 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 (PCA₉), 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 biogeochemical 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 *Methylobacterium mobilis* with NO₃.

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), *Methylobacterium 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.

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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 PCA₂ analysis with microbial classes and hydrochemical data from groundwater samples and infiltration water sample

Figure S2 PCA₄ analysis with microbial species and hydrochemical data from groundwater samples and infiltration water sample

Figure S3 PCA₆ analysis with microbial classes data from soil samples

Figure S4 PCA₈ analysis with microbial species data from soil samples

Figure S5 PCA₁₀ analysis with microbial classes data from soil and water samples

1. Input data for Principal Component Analyses

[illegible]

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
Alphapaproteobacteria	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 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	

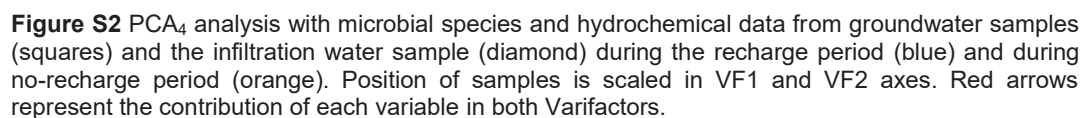
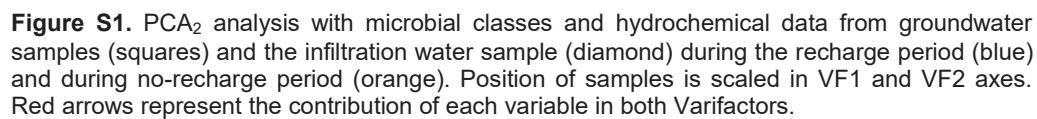
Table S6 Summary of soil parameters from grain-size distribution curves												
Operating conditions		Recharge (July 2014)					No Recharge (March 2015)					
Sample	SD	E_0.1	M_0.1	M_0.2	F_0.1	E_0.2	E_0.8	M_0.3	F_0.1	F_0.3		
CC	0.04	2.01	1.43	0.84	0.53	3.31	0.54	0.93	0.55	1.27		
CU	159.39	53.52	28.32	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			
Belaproteobacteria	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	
<i>Methylobacter mobilis</i>	0.00	0.00	0.60	0.00	0.00		2.64	0.00	0.00	0.00	0.91	
<i>Pontibacter sp</i>	0.85	2.45	0.60	3.29	4.72		4.53	13.21	10.51	13.99	11.77	
<i>Dehalogenimonas sp</i>	4.40	1.77	2.10	2.97	3.37		0.00	1.93	0.00	0.00	0.00	
<i>Subgroup 3 sp (Acidobact.)</i>	9.80	2.31	1.95	0.99	1.69		0.00	2.09	2.83	0.00	0.00	
<i>Vogesella indigofera</i>	8.10	7.49	4.80	4.94	4.22		4.53	2.42	2.83	3.57	0.00	
<i>Stenotrophomonas sp</i>	2.13	3.95	1.80	0.00	4.05		4.34	2.26	3.97	0.00	0.00	
<i>Chryseomicrobium sp</i>	6.39	5.17	4.35	8.07	7.25		7.74	7.56	4.53	6.85	21.50	
<i>Nitrospira sp1</i>	2.70	0.54	5.56	3.62	2.53		14.72	7.41	1.39	4.17	0.00	
<i>Methylobacterium sp</i>	0.99	3.81	2.10	1.65	7.76		0.57	3.06	6.71	6.43	7.88	
<i>Nitrospira sp2</i>	2.70	1.36	1.50	3.13	1.52		0.00	3.06	3.68	1.49	4.53	

2. Supplementary figures



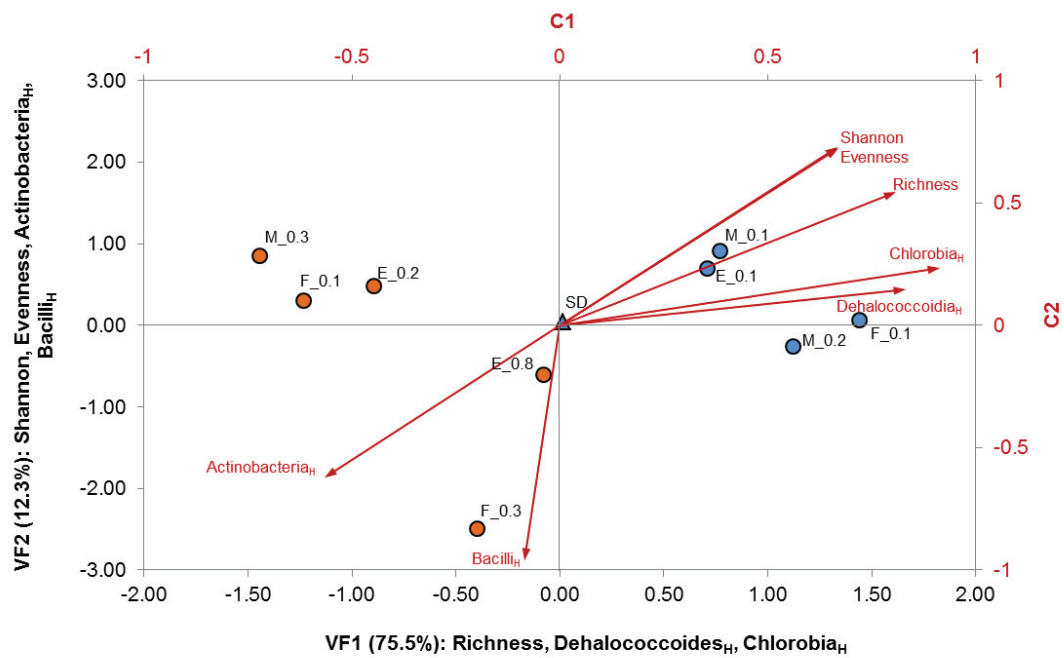


Figure S3 PCA₆ analysis with microbial classes data from soil samples during the recharge period (blue) and during no-recharge period (orange). Circles are related to the infiltration pond samples (Entrance, Midfield and Final strecht according the capital letter close to each symbol). Triangle is referred 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.

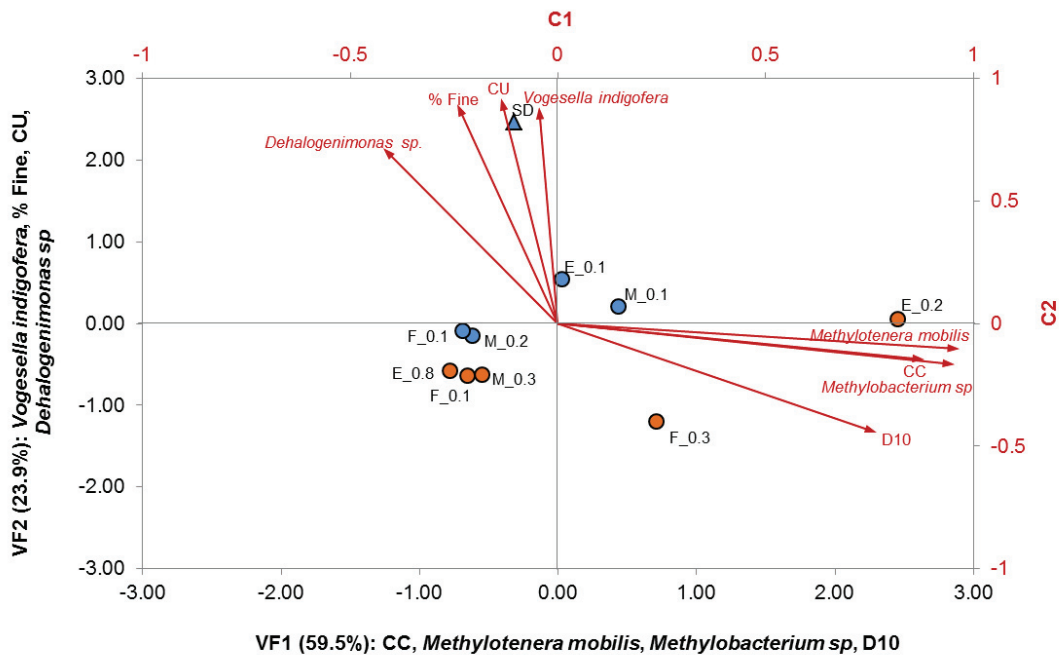


Figure S4. PCA₈ analysis with microbial species data from soil samples during the recharge period (blue) and during no-recharge period (orange). Circles are related to the infiltration pond samples (Entrance, Midfield and Final strecht according the capital letter close to each symbol). Triangle is referred 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.

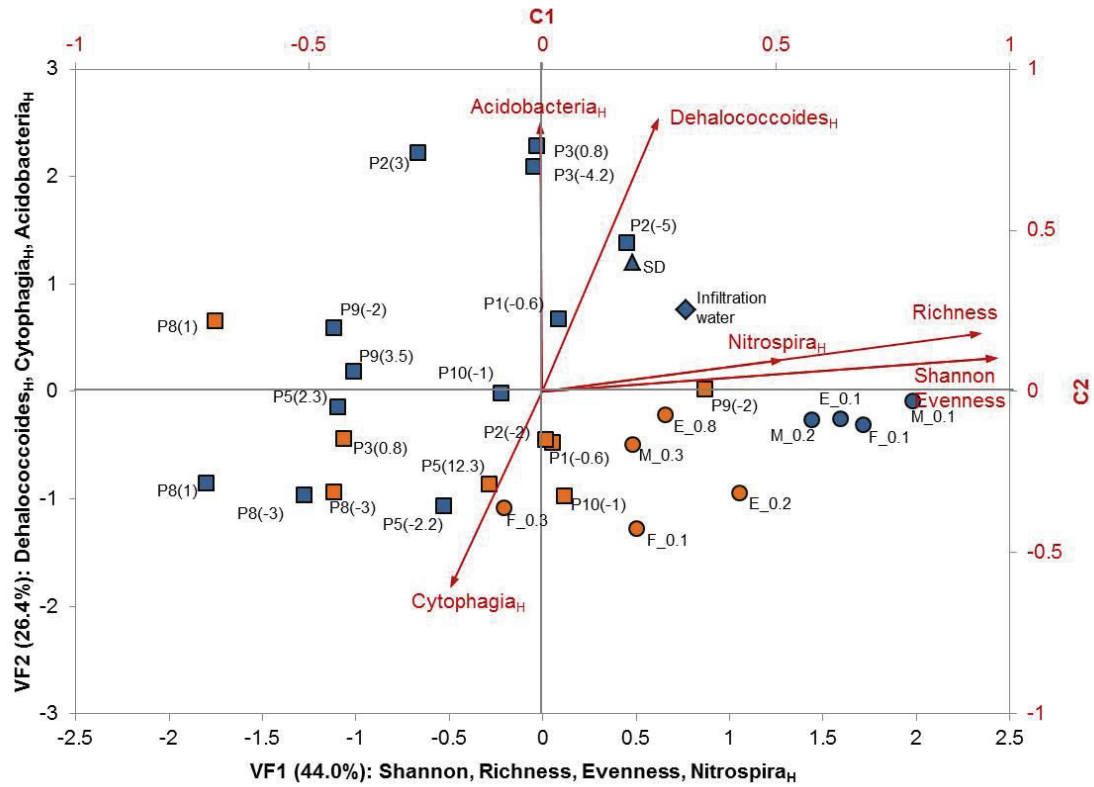


Figure S5. PCA₁₀ analysis with microbial classes data from soil and water samples during the recharge period (blue) and during no-recharge period (orange). Circles are related to soil samples (Entrance, Midfield and Final stretch according the capital letter close to each symbol). Triangle is referred 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.