Bacterial community structure and biogeochemical activity in an aquifer contaminated with pesticides

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1. Introduction

Bacterial communities play a pivotal role in biogeochemical cycle, however there are still no consensus on the effect of the pesticide contamination on bacterial community function. Lower bacterial community ability to oxydize ammonium and nitrite has been observed in groundwater contaminated with atrazine (ATZ) (100 mg/L), leading to a possible risk of nitrate accumulation [1]. On the opposite, in ATZ-spiked soil or in soil with ATZ and metolachlor historically used to kill weeds, bacterial respiration, community abundance and composition were similar to the control soil, composition was only altered transitorily [2, 3].

Atrazine is an herbicide which have been widely used for weed control in corn, soja and sorgho cultures, until 2003 when it has been withdrawn in France. Desethylated atrazine (DEA) is among its metabolite the one most observed in soil and groundwater and it has been reported with higher effect to aquatic life, than ATZ [4]. Ten years after its withdrawn, ATZ concentrations exceeding the legal EU thresholds for groundwater and drinking waters (0.1 µg/L) are still reported [5].

Our objective was to assess the effect of different cocktail of pesticides on groundwater microbial abundance, community structure and their function in the nitrate reduction. This is, to our best knowledge, the first study on pesticide impact on groundwater microbial community diversity structure and function; it has the potential to provide sound-based arguments to be considered when improving the current strategy to manage water quality, as well as when proposing end points to monitor the microbial community in the biodiversity objective under the European water directive framework.

2. Materials and methods.

Two-year monitoring. The Ariège alluvial plain (France) is contaminated with several pesticides with concentations up to the ppm levels [5]. Water (1 L) was sampled from 17 selected springs on a monthly basis during 2 years (March 2012- March 2014, n = 50).
Microcosm. Water was sampled in July 2014 in two wells having different contamination profils. Water (700 mL) was placed in 1 L microcosm and ATZ, DEA or ATZ+DEA was spiked at 0, 1 and 10 µg/L. Units were sacrificed at the start and following 15-day and 30-day incubation (n = 58).
Bacterial analyses. Water was filtered through 0.22 µm filters and microbial DNA was extracted. Abundance of the universal marker (16S rRNA gene) and of nitrate-reducing bacteria (*nar*G and *nap*A genes) were assessed by quantitative PCR (qPCR). Diversity was assessed using fingerprinting technic, CE-SSCP (Capillary Electrophoresis-Single Strand Conformational Polymorphism) [6].
Chemical analyses. Water samples for pesticides were analyzed by LC-MS/MS following an on line-solid phase extraction, and samples for anions and cations were analyzed by ion chromatography [5].
Statistical analyses. Divergence between diversity profiles were analyzed with StatFingerprints software. Diversity indexes were also calculated (richness (**S**), Shannon-weaver index (**H**), eveness (**EH**)). Statistical differences were analyzed using two-way ANOVA with treatments, chemical or incubation time as factors ($p < 0.05$). Principal component analyses (PCA) were performed using XLSTAT Version 2011.2.02.

3. Results and discussion

3.1. Microcosm analyses

Biodiversity was higher thorough the experiment in the water **224** historically contaminated with various pesticides than in the water **261** where none of the 51 pesticides monitored was observed during 2 years (Figure 1). Pesticide concentrations in the water historically contaminated often exceeded the legal EU threshold for groundwater and drinking waters (2-year mean: 0.09 ± 0.01 µg ATZ /L, 0.43 ± 0.06 µg DEA /L (n = 23)). On the other side, during microcosm incubation, biodiversity decreased when spiked-chemical concentration increased from 1 to 10 µg/L. The chemical type (ATZ, DEA or ATZ+DEA) exhibited similar effect on the microbial diversity. In the water **261**, biodiversity also increased with the incubation duration

when exposed to 1 µg/L, while no increase was observed when the community was exposed to 10 µg/L, suggesting that adaptation to pesticides might occur and this process depends on the chemical concentration. In the water **224**, no effect of the incubation duration was noticed. Abundance of bacteria reducing nitrate among the total community drastically decreased during the experiment, but this was also observed in the control unit ($p < 0.05$). Total biomass was similar in both waters and during the whole experiment ($p > 0.05$). No biodegradation of ATZ and DEA was observed during the 1-month exposure.

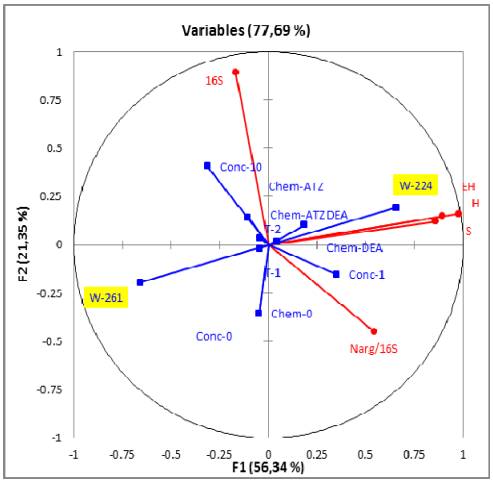

Figure 1. Principal component analysis with microbial end points as active variables (red) and chemophysical parameters as supplementary variables (blue) with microcosm dataset (n = 56)

3.2. Two-year monitoring at an agricultural catchment level

Analyses are undergoing; preliminary results suggests that biomass is similar in all samples while biodiveristy and nitrate-reducing bacteria show important differences between samples.

The undergoing analyses of the two-year monitoring at the catchment level will enable to refine boudaries within this complex relationship between biodiversity and pesticide contamination.

4. Conclusions

The undergoing analyses on natural waters monitored during two years at a catchment level will hopefully show boundaries within the positive relationship between biodiversity and chemical concentration observed in the microcosm experiment.

In microcosm ATZ, DEA or ATZ+DEA exhibited similar effect on the microbial community. Comparison at the catchment level of the effect of the 51 monitored pesticides taken individually or summed, on the microbial biodiversity will enable to assess the mixture effect on the microbial community.

Biomass was similar in all conditions, suggesting that this is not a sensitive endpoint to assess water quality.

Relationship between pesticide contamination and bacterial nitrate-reducing community will be assessed further to consider the risk of nitrate acumulation or of inhibition effect of nitrate on pesticide biodegradation.

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5. References

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