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Effect of pesticides and metabolites on groundwater bacterial community

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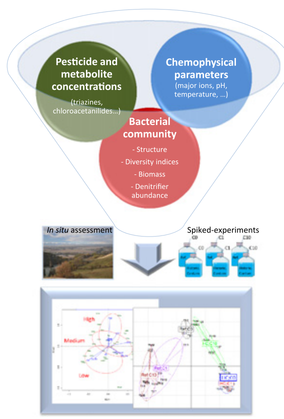
HIGHLIGHTS

- The first microbial ecotoxicology study of herbicides <10 μg/L in groundwater is presented.
- Combined experimental and in situ study allows strengthening triazine effect assessment.
- Triazine effects were observed at <10 μg/L, even in historically-contaminated water.
- Chloroacetanilides induce a slight increase of nitrate-reducing abundance.

GRAPHICAL ABSTRACT



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ABSTRACT

We assessed the effect of pesticides, especially commonly detected herbicides, on bacterial communities in groundwater. To this end, we used a combined approach with i) triazine-spiked experiments at environmentally relevant concentrations (1 and 10 μg/L) in waters with contrasting contamination histories, and ii) in situ monitoring in a rural aquifer, where many additional biotic and abiotic parameters also affect the community. Microbial community was characterized by fingerprinting techniques (CE-SSCP), gene presence (*atzA*/B/C/D/E/F and *amoA* genes) and abundance (16S RNA, *napA* and *narG genes*). During triazine-spiked experiments, the bacterial community structure in reference water was modified following an exposure to atrazine (ATZ) and/or its metabolite desethylatrazine (DEA) at 1 μg/L; in historically-contaminated water, the bacterial community structure was modified following an exposure to 10 μg/L ATZ/DEA. Similarly, biodiversity indices and biomass in the reference water appeared affected at lower triazine concentrations than in the historically-contaminated water, though these end-points are less sensitive than the community structure. Our results thus suggest that the history of contamination induced a community tolerance to the tested triazines. ATZ and DEA were not degraded during the experiment and this was consistent with the absence of *atz* genes involved in their degradation in none of the tested conditions. In field monitoring, triazines that represent a historical and diffuse contamination of groundwater, participate in the microbial community structure, confirming the triazine effect observed under laboratory conditions. Other herbicides, such as chloroacetanilides that are applied today, did not appear to affect the whole community structure; they however induced a slight, but significant, increase in the abundance of

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nitrate-reducing bacteria. To our best knowledge, this is the first study on the microbial ecotoxicology of pesticides and their metabolites at environmentally relevant concentrations in groundwater.

1. Introduction

Groundwater is an ecosystem in itself that should be protected (EU, 2006). In addition to the ubiquity of *Bacteria* and *Archaea* in groundwater, several novel phylogenetic lineages - including taxa with unusual chemoautotrophic pathways - appear to be restricted to groundwater (Gibert and Culver, 2009). There is increasing awareness of the importance of groundwater microbes for providing ecosystem services, especially the production of drinking water, the degradation of contaminants, and in nutrient cycles (Jacobsen and Hjelmso, 2014). It is therefore of great importance to establish if any of these microbial ecosystem services are hampered by the presence of contaminants. In addition, among groundwater systems, alluvial aquifers are particularly vulnerable as the thickness of the unsaturated zone commonly is reduced to only a few metres.

Exposure to pesticides has been reported to modify—transiently and chronically—microbial biomass, the abundance of pesticide degraders, and the entire microbial community dynamics in soil (two recent reviews: Imfeld and Vuilleumier, 2012; Jacobsen and Hjelmso, 2014) as well as in the periphyton community (e.g. Lorente et al., 2015; Pesce et al., 2010).

Subsurface materials are physically, chemically, and biologically different from surface soils. They have reduced concentrations and availability of oxygen, carbon, and inorganic nutrients, they are not exposed to light and harbor a 10^2 to 10^6 times lower bacterial density mainly dependent on allochthonous energy supply (Ghiorse and Wilson, 1988). Since pesticide is typically applied in the ppm range, soil microbial communities may also be naturally exposed to higher concentrations than groundwater communities. Results from near-surface soil environments cannot, thus, be readily extrapolated to subsurface aquifer conditions (Hose, 2005; Pereira et al., 2014).

In groundwater, Sirisena et al. (2014) showed that the bacterial community structure assessed by terminal-restriction fragment length polymorphism (T-RFLP) is related to groundwater redox-sensitive substances, and that species composition showed minimal variation over time if these parameters remained unchanged; they thus stressed the potential of using bacterial communities as biological indicators for evaluating the health of groundwater ecosystems. Few studies have specifically dealt with the effects of pesticides on groundwater microbial communities; most reported on the effect of historical exposure on specific degrading species and showed that herbicides from 2 to 50 μg/L led to an enhanced degraders detection (Tuxen et al., 2002; de Lipthay et al., 2003; Liebich et al., 2009; Barra Caracciolo et al., 2010; Janniche et al., 2012).

A few studies reported on the effect of herbicides on indigenous non-target species of the bacterial groundwater community. Janniche et al. (2012) showed that in situ agricultural contamination of groundwater contributed to Microbial Community-Level Physiological Profiles (CLPP) clustering of the most affected groundwater samples in a tested catchment (Brévilles, France). de Lipthay et al. (2004) reported that in situ exposure of aquifer sediment to a mixture of herbicides (ca. 40 μg/L) did not have a significant effect on genetic diversity (denaturing gradient gel electrophoresis) and carbon substrate usage (EcoPlate).

The nitrogen cycle is an example of potential non-targeted function of pesticide action. In liquid culture and soil, it has been reported that an inorganic nitrogen source (nitrate, ammonium) can either reduce (Bichat et al., 1999; Guillen Garces et al., 2007; Sajjaphan et al., 2010), or stimulate (Wang et al., 2013), triazine herbicide degradation. However, the opposite, i.e. information on the impact of pesticides on the nitrogen cycle, is still limited. In soil, the adverse impact of pesticides on the genes and transcripts of the nitrogen cycle implies disturbance of the soil ecology (Chang et al., 2001; Guo et al., 2014; Singh et al., 2015). In aquifer sediments, Iker et al. (2010) reported that addition of atrazine (ATZ), a triazine herbicide, to historically-contaminated sediment reduced the relative abundance of ammonia-oxidizing and nitrite-oxidizing bacteria, while in uncontaminated sediments both bacterial types were initially absent and only appeared after ATZ addition. If the pesticide effect on the bacterial community results in an over-expression of genes involved in the nitrification process and an under-expression of the genes involved in denitrification, nitrate accumulation can occur. In an aquifer contaminated by pesticides and nitrates, the question for an integrated water-quality management is “Is herbicide presence one of the reasons why nitrates accumulate?”. Therefore, consideration of the history of contamination as well as community structure are required to predict how biogeochemical processes will respond to global change (Strickland et al., 2009).

In Europe, ATZ has been widely used for weed control, mainly on maize, soya and sorgho crops. Desethylatrazine (DEA), a major ATZ metabolite, has been reported to have a stronger effect than ATZ on aquatic life (Stratton, 1984). They both are particularly recalcitrant to biodegradation and have strong leaching properties (Vryzas et al., 2012a). Consequently, despite withdrawal of the active substance in 2003 in France, ATZ and DEA remain nation-wide among the most common molecules in groundwater (Lopez et al., 2015). The persistence of both molecules and the typical predominance of DEA over ATZ (occurrence and concentration) were also observed in England (Lapworth et al., 2015) and more generally in Europe (Loos et al., 2010; Vryzas et al., 2012b).

One of the most critical tasks, when assessing the effects of chemical substances on microbial communities in natural ecosystems, is to distinguish between chemical pressure and the other confounding factors resulting from physical, chemical or biological environmental variables and interactions. Field monitoring is informative for capturing the history of contamination though inertia (variance) in the community dynamic typically remains mainly background noise, probably because in situ communities are structured in response to multiple parameters, with as many interactions that, all together, are difficult to analyze and prioritize both analytically and statistically. Possible interactions with confounding factors are even more important under diffuse pollution conditions than point-source pollution, where the pollution can be seen as one of the dominant factors driving the community dynamic.

Our objective was thus to assess the effect of herbicides on groundwater microbial community dynamic. Community structure, biodiversity indices, biomass, abundance of nitrate-reducing bacteria, presence of bacteria involved in triazine degradation and in ammonia oxidation, were used as endpoints for characterizing the community dynamic. A combination of triazine-spiked experiments and microbial field-monitoring approaches served to reach firmer conclusions by partially addressing the upscaling challenge faced when laboratory-based outcomes are confronted to field conditions. In the context of this study, the term ‘dynamic’ is used to refer to changes in the abundance of various members in a community (Fuhrman et al., 2015). This is, to our best knowledge, the first study on the effects of pesticides and metabolites at environmentally relevant concentrations (1–10 μg/L) on the dynamic of a groundwater microbial community as a whole, including non-targeted micro-organisms, and organisms involved in biogeochemical, e.g. nitrogen, cycle.