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(Bio)degradation of glyphosate in water-sediment microcosms – A stable isotope co-labeling approach

Shizong Wang [a, b], Bettina Seiwert [c], Matthias Kästner [b], Anja Miltner [b], Andreas Schäffer [d], Thorsten Reemtsma [c], Qi Yang [a], Karolina M. Nowak [b, d, *]

[a] School of Water Resources and Environment, China University of Geosciences, Beijing 100083, PR China
[b] Helmholtz-Centre for Environmental Research – UFZ, Department of Environmental Biotechnology, Permoserstrasse 15, 04318 Leipzig, Germany
[c] Helmholtz-Centre for Environmental Research – UFZ, Department of Analytical Chemistry, Permoserstrasse 15, 04318 Leipzig, Germany
[d] RWTH Aachen University, Institute for Environmental Research, Worringerweg 1, 52074 Aachen, Germany

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ABSTRACT

Glyphosate and its metabolite aminomethylphosphonic acid (AMPA) are frequently detected in water and sediments. Up to date, there are no comprehensive studies on the fate of glyphosate in water-sediment microcosms according to OECD 308 guideline. Stable isotope co-labeled $^{13}C_3^{15}N$-glyphosate was used to determine the turnover mass balance, formation of metabolites, and formation of residues over a period of 80 days. In the water-sediment system, 56% of the initial $^{13}C_3$-glyphosate equivalents was ultimately mineralized, whereas the mineralization in the water system (without sediment) was low, reaching only 2% of ^{13}C-glyphosate equivalents. This finding demonstrates the key role of sediments in its degradation. Glyphosate was detected below detection limit in the water compartment on day 40, but could still be detected in the sediments, ultimately reaching 5% of $^{13}C_3^{15}N$-glyphosate equivalents. A rapid increase in $^{13}C^{15}N$-AMPA was noted after 10 days, and these transformation products ultimately constituted 26% of the $^{13}C_3$-glyphosate equivalents and 79% of the ^{15}N-glyphosate equivalents. In total, 10% of the ^{13}C label and 12% of the ^{15}N label were incorporated into amino acids, indicating no risk bearing biogenic residue formation from $^{13}C_3^{15}N$-glyphosate. Initially, glyphosate was biodegraded via the sarcosine pathway related to microbial growth, as shown by co-labeled $^{13}C^{15}N$-glycine and biogenic residue formation. Later, degradation via AMPA dominated under starvation conditions, as shown by the contents of ^{13}C-glycine. The presented data provide the first evidence of the speciation of the non-extractable residues as well as the utilization of glyphosate as a carbon and nitrogen source in the water-sediment system. This study also highlights the contribution of both the sarcosine and the AMPA degradation pathways under these conditions.

1. Introduction

Glyphosate (*N*-[phosphonomethyl] glycine) is one of the most widely used herbicides worldwide (Van Stempvoort et al., 2014). Following its application, glyphosate tends to sorb to minerals via its phosphonate group and undergo biodegradation (Borggaard and Gimsing, 2008). Although sorption rapidly inactivates glyphosate in soil and limits its transport to the aquatic environment, this herbicide and its main metabolite aminomethylphosphonic acid (AMPA) are often detected in surface and ground waters (Aparicio et al., 2013; Lupi et al., 2015; Peruzzo et al., 2008; Van Stempvoort et al., 2014). Surface water samples have been shown to contain up to 50 μg glyphosate L^{-1} (Europe) (Horth and Blackmore, 2009) and 427 μg L^{-1} (USA) (Scribner et al., 2007); ground water up to 24 μg L^{-1} (Europe) (Horth and Blackmore, 2009) and 4.7 μg L^{-1} (USA) (Scribner et al., 2007). These frequently detected levels are higher than the EU drinking water limit of 0.1 μg L^{-1} for glyphosate but are below the EPA maximum level of glyphosate protective of human health (700 μg L^{-1}). Due to the widespread occurrence of glyphosate and AMPA in aquatic and terrestrial environments, concern for their potential environmental impacts has recently been increasing (Lupi et al., 2015; Peruzzo et al., 2008; Van Stempvoort et al., 2014). However, fate analyses

* Corresponding author. RWTH Aachen University, Institute for Environmental Research, Worringerweg 1, 52074 Aachen, Germany
E-mail address: karolina.nowak@ufz.de (K.M. Nowak).

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with isotope labeled compounds are missing.

In general, microbial degradation of pesticides is the most important pathway of ultimate elimination from the environment and results in the formation of metabolites, microbial biomass, mineralization products and non-extractable residues (NER) (Kästner et al., 2014). Pesticides and their transformation products immobilized in soil or sedimentary organic matter (OM), defined as NER, often pose a toxicological hazard to living organisms (Barriuso et al., 2008). However, their exact composition and thus their hazard are unknown. For two other organic contaminants ($^{13}C_6$-2,4-D and $^{13}C_6$-ibuprofen), NER were primarily formed by incorporation of the ^{13}C label into microbial biomass components, e.g., amino acids (AA), and thus were nontoxic (Nowak et al., 2011, 2013). The organic contaminant-derived carbon and nitrogen thus can be used for the synthesis of microbial biomass and after the cells die, their constituents are incorporated into the non-living OM fraction and stabilized, ultimately forming hardly extractable biogenic residues (Kästner et al., 2014).

Glyphosate degradation has been studied extensively in soil systems. The herbicide is biodegraded rapidly in soils with an exposure history (Mamy et al., 2005). Biodegradation can occur either through the AMPA pathway or the sarcosine pathway (Borggaard and Gimsing, 2008). Although biodegradation of glyphosate via the AMPA pathway is well known, the sarcosine pathway has been documented only in pure culture experiments with single or several degraders' strains isolated from soils (Shinabarger and Braymer, 1986; Pipke and Amrhein, 1988). In addition, glyphosate was demonstrated to be degraded either co-metabolically or as a source of phosphorus, carbon and nitrogen in microbiological cultures (Obojska et al., 1999; Ternan et al., 1998). There is no clear evidence yet for glyphosate utilization as a source of carbon or nitrogen and for the contribution of the sarcosine pathway in the heterogeneous environments like water-sediment systems (Borggaard and Gimsing, 2008; Singh and Walker, 2006). In particular, detailed studies on the fate of glyphosate-derived carbon and nitrogen under controlled conditions using (co)-labeled glyphosate are missing. For example, the study on the degradation of glyphosate in a water-sediment system by Zaranyika and Nyandoro (1993) was conducted without isotope tracers and under fluctuating temperature and in the sunlight. In addition, it is not clear whether glyphosate residues in freshwater sediments present an environmental risk. Therefore, detailed knowledge on glyphosate turnover, including xenobiotic or biogenic residue formation in freshwater and sediments according to the OECD guideline 308, is needed to properly assess the risk and the environmental fate of this herbicide.

The purpose of this study was to investigate the environmental fate of glyphosate in the water-sediment system with a particular focus on its microbial metabolization as a source of carbon and nitrogen. Co-labeled stable isotope tracers (^{13}C and ^{15}N) were used to determine the glyphosate turnover mass balance in the water-sediment system and to identify the different biodegradation pathways. Biogenic residue formation was also examined by studying the incorporation of ^{13}C and ^{15}N labels into amino acids during the biodegradation of $^{13}C_3^{15}N$-glyphosate and observing their fate over a period of 80 days in order to identify the speciation of NER.

2. Methods

2.1. Chemicals

All the chemicals used were analytical or reagent grade and were obtained from the Carl Roth Company (Karlsruhe, Germany) if not specified otherwise. Resin for amino acid purification (Dowex 50W-X8, 50–100 mesh) was purchased from VWR/Merck (Darmstadt, Germany). Methanol and ammonium acetate for ultra-performance liquid chromatography-mass spectrometry (UPLC/MS) measurements were provided by Biosolve (Valkenswaard, Netherlands). Labeled $^{13}C_3^{15}N$-glyphosate was purchased from Iso-Sciences Company (Trevose, PA, USA). The isotopic enrichment of the labeled glyphosate was 99 at % for ^{13}C and 98 at % for ^{15}N; the chemical purity was 98%.

2.2. Sediments and water

The sediments and associated water were collected from the Getel creek (51°45′25.02 °N, 11°17′50.25°E) located in the north-eastern rim of the Harz Mountains in Saxony-Anhalt, Germany. The sampling site was located in the Ballenstedt city district. The catchment of this creek comprises agricultural lowlands with continuous crop rotation and pesticide application. It is thus a high-risk area for exposure to pesticides. The sediments contained 38% (±0.7%) sand (>0.05 mm), 62% (±0.7%) silt + clay (<0.05 mm), 85 mg g^{-1} (±2 mg g^{-1}) total organic carbon and 15 mg g^{-1} (±1 mg g^{-1}) total nitrogen. The pH of the sediments and creek water was 7.1 and 8.8, respectively. The content of total organic carbon of the suspended matter in the creek water was 8 mg L^{-1} (±1 mg L^{-1}), and the content of total nitrogen was 3 mg L^{-1} (±0.6 mg L^{-1}). Neither glyphosate nor AMPA were detected in the sediments or creek water.

Sediments and associated water were taken from the upper layer (up to 5 cm) of the Getel creek sediment. The sediments were separated from the water by filtration, wet sieved through a 2 mm screen, and gently homogenized.

2.3. Incubation experiment

Degradation experiments were conducted according to the OECD guideline 308 in biometer flasks to address the transformation in aquatic sediment systems (Test, 2002). Six incubations were performed: 1) water-sediment without glyphosate (non-amended control), 2) water-sediment with unlabeled glyphosate (unlabeled control), 3) water-sediment with labeled glyphosate (biotic system), 4) water with unlabeled glyphosate (unlabeled control), 5) water with labeled glyphosate and 6) sterilized water-sediment with labeled glyphosate (abiotic system). The two controls without glyphosate and unlabeled glyphosate were used to correct for the natural abundances of ^{13}C (~1.1 at %) and ^{15}N (~0.37 at %) in the sediment, and water systems without sediment were prepared to test the effect of sediment on the microbial degradation of glyphosate. Abiotic controls were incubated to distinguish between abiotic and biotic degradation of $^{13}C_3^{15}N$-glyphosate. In these controls, sediment and water were sterilized by autoclaving three times at 120 °C for 20 min prior to incubation.

Fifty grams (dw) of sediment and 90 mL of creek water containing either unlabeled or labeled glyphosate were added to 250 mL Duran glass bottles. The initial concentration of glyphosate was 50 mg L^{-1} in water and water-sediment systems, except in the blanks containing no glyphosate. This concentration is well above environmentally relevant levels, but it was required to obtain reliable isotopic enrichment results in the water-sediment systems given the limited sensitivity of $^{13}C/^{15}N$ isotope analytical methods and the high background due to natural abundance of the heavy isotopes in the controls. To assess the overall fate and turnover at lower concentrations that were closer to environmentally relevant concentrations, additional water-sediment experiments at 3 mg L^{-1} (minimum ^{13}C and ^{15}N label detection limit) were prepared. Incubation experiments were conducted in the dark and at constant temperature (20 °C) for 80 days. The bottles were sampled