Environ Sci Pollut Res
DOI 10.1007/s11356-017-0217-6

RESEARCH ARTICLE

Proteomic analysis of the soil filamentous fungus *Aspergillus nidulans* exposed to a Roundup formulation at a dose causing no macroscopic effect: a functional study

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Received: 24 May 2017 / Accepted: 13 September 2017

Abstract Roundup® is a glyphosate-based herbicide (GBH) used worldwide both in agriculture and private gardens. Thus, it constitutes a substantial source of environmental contaminations, especially for water and soil, and may impact a number of non-target organisms essential for ecosystem balance. The soil filamentous fungus *Aspergillus nidulans* has been shown to be highly affected by a commercial formulation of Roundup® (R450), containing 450 g/L of glyphosate (GLY), at doses far below recommended agricultural application rate. In the present study, we used two-dimensional gel electrophoresis combined to mass spectrometry to analyze proteomic pattern changes in *A. nidulans* exposed to R450 at a dose corresponding to the no-observed-adverse-effect level (NOAEL) for macroscopic parameters (31.5 mg/L GLY among adjuvants). Comparative analysis revealed a total of 82 differentially expressed proteins between control and R450-treated samples, and 85% of them (70) were unambiguously identified. Their molecular functions were mainly assigned to cell detoxification and stress response (16%), protein synthesis (14%), amino acid metabolism (13%), glycolysis/gluconeogenesis/glycerol metabolism/pentose phosphate pathway (13%) and Krebs TCA cycle/acetyl-CoA synthesis/ATP metabolism (10%). These results bring new insights into the understanding of the toxicity induced by higher doses of this herbicide in the soil model organism *A. nidulans*. To our knowledge, this study represents the first evidence of protein expression modulation and, thus, possible metabolic disturbance, in response to an herbicide treatment at a dose that does not cause any visible effect. These data are likely to challenge the concept of "substantial equivalence" when applied to herbicide-tolerant plants.

Keywords Roundup® · NOAEL · *Aspergillus nidulans* · Proteomics · Metabolism · Herbicide tolerance · Substantial equivalence

Responsible editor: Philippe Garrigues

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Introduction

The effects of intensive agricultural practices on biogeochemical flows have been defined as critical for earth-system functioning (Steffen et al. 2015). A common feature of biochemical cycles is that soil microorganisms are key agents in the maintenance of soil quality and resilience. Sustainability at this level can be affected by pesticide application, such as by glyphosate-based herbicides (GBH), which are currently the most widely used pesticides worldwide. GBH are used exponentially, especially since 80% of genetically modified (GM) plants commercially grown are designed at least to tolerate Roundup® (James 2011): glyphosate (GLY) represented

Published online: 23 September 2017