CAN NOVEL APPROACHES FOR CHARACTERIZING THE EFFECTS OF CHEMICALS ON THE SOIL MICROFLORA PROVIDE AN ADDED VALUE IN THE SCOPE OF REGULATION?

Kerstin Hund-Rinke[1], Anna Huemmler[1,2], Karsten Schlich[1], Franziska Wege[1], Gabriele Broll[2]

[1]Fraunhofer Institute for Molecular Biology and Applied Ecology IME, Schmallenberg, Germany;[2] Osnabrück University, Institute of Geography, Osnabrück, Germany

Contact: kerstin.hund-rinke@ime.fraunhofer.de

Introduction & objectives

The current regulation imposes tests investigating side effects of chemical substances on the soil microflora that focus on the determination of nitrogen transformation. According to EFSA (EFSA Journal 2017;15(2):4690) a more comprehensive risk assessment is necessary. The MicroResp™ is mentioned as potential approach. We performed studies to investigate whether a combination of test approaches addressing various microbial fractions results in a better under-standing of the fluctuation of the soil microflora after a threat and in a more comprehensive risk assessment.

Conclusion

1. Time course and sensitivity of the test systems differ since different microbiological fractions are included.
   ➔ Informative value of the various fractions have to be considered for the interpretation of an effect.
2. ***Potential ammonium oxidation (POA)***: Sensitive, concentration-dependent indicator for toxic effects since a small microbial population is detected; results highly reproducible; standard deviation usually <10%.
   ➔ Sensitive indicator for the presence of toxic compounds. The importance of function preservation should not be overestimated here.
3. ***MicroResp™***: Determination of respiration activity for a wide range of microbial cells in the presence of various C-sources; results on day 56 are questionable; partly high standard deviation due to low amount of soil and filling procedure for the 96 well plates.
   ➔ Suitability for hazard assessment has to be investigated further.
4. ***Exoenzymes***: Sensitivity depends on the enzyme and its protection/stabilization in soil; standard deviation usually <10%.
   ➔ Results difficult to interpret. So far knowledge on whether stabilized and protected enzymes are active comparably to the free ones is not available. Considered less suitable for a risk assessment.
5. ***Next generation sequencing (NGS)***: Sensitivity comparable to PAO; no link to microbial activities.
   ➔ Indicates that chemical broadly affects microflora; indicates an adaptation of soil microflora and supplements activity measurements; Shannon index, evenness, … lower sensitivity.

Approach

1. Test chemical: spherical silver nanomaterial (⌀ 15 nm).
2. Methods:
   - Potential ammonium oxidation (ISO 15685)
   - MicroResp™: glucose, cellobiose, L-alanine, cysteine
   - Exoenzymes (ISO/DIS 20130): phosphatase, arylamidase, arylsulfatase, ß-glucosidase
   - NGS (16S rRNA → DNA)
3. Soil: loamy sand; Corg: 1.1 %, pH ($CaCl_2$): 5.0

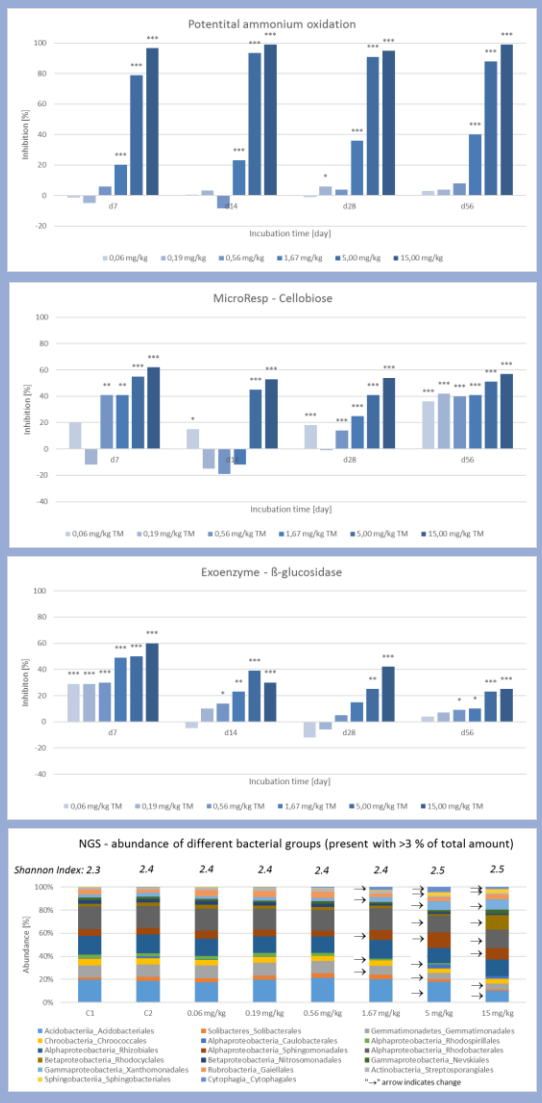

Figure 1: Effect of a silver nanomaterial on the soil microflora - examples for results determined with the four approaches.

Session: Advances in Soil Ecotoxicology and Risk Assessment of Terrestrial Ecosystems
Poster / Exhibition Hall, Thursday May 17th, 2018, 08.30 a.m., ID: TH154

Can approaches beyond the traditional ones characterizing the effects on soil microflora provide an added value in the scope of regulation?

Kerstin Hund-Rinke[1], Anna Huemmler[1,2], Karsten Schlich[1], Franziska Wege[1], Gabriele Broll[2]

[1]Fraunhofer IME, Auf dem Aberg 1, 57392 Schmallenberg, Germany
[2]University of Osnabrueck, Institute of Geography, Seminarstraße 19a/b, 49074 Osnabrück, Germany
E-mail contact: kerstin.hund-rinke@ime.fraunhofer.de

According to the current regulation, side effects of chemical substances on the soil microflora focus on the determination of the nitrogen transformation (OECD 216). However, according to EFSA a more comprehensive risk assessment is required.

We investigated whether a combination of several test approaches addressing various microbial aspects results in a better understanding of the fluctuation of the soil microflora after a threat and a more comprehensive risk assessment taking the new requirement regarding the consideration of ecosystem services and the protection of the biodiversity into account.

We used a silver nanomaterial as example and applied three functional approaches to get information on the functional microbial diversity: (i) potential ammonium oxidation activity (PAO) addressing a small, mainly autotrophic bacterial community with comparable low diversity; (ii) respiration activity of the heterotrophic microflora as indicator for the C-transformation with basal respiration activity and activity in the presence of carbon sources (glucose, cellobiose) as well as sulfur or nitrogen containing organic substances (cysteine, alanine); (iii) activity of selected exoenzymes (selection based on the carbon sources used in the second approach). The second and third approach were performed in microwell plates.

The three functional approaches (i) PAO, (ii) C-transformation, (iii) exoenzymes seem to be a suitable assessment tool and can provide a benefit in the assessment of chemicals. The combination of results was dependent on the test concentration. The exoenzymes were the most sensitive indicator and seem to be a suitable early warning indicator. An increased concentration of the chemical responsible for the initial effect or a further impact can severely affect the microbial population. Additionally affected nitrifiers indicated a stronger damage. Effects in all three approaches indicated a severe impact. The high sensitivity of the exoenzymes in contrast to the respiration activity of the microbial cells could be due to their location outside the microbial cell and a lower protection level. However, also the small size of the ions as affecting substance has to be considered. In further experiments, the combination of results obtained with the three functional approaches have to be determined with additional chemicals. Due to the use of microwell plates the additional work load seems to be acceptable also for testing in the scope of regulation.