Soil as a Resource
National Research Programme NRP 68

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Executive Stakeholder Summary

Project number:	406840_143144
Project title:	The role of land use in antibiotic resistance
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Contribution(s) to thematic synthesis:

☒ Soils and Food Production	☒ Soils and Environment	☐ Spatial Development	☐ Soil Data, Methods and Tools	☐ Soil Governance

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Background

The soil is a dynamic habitat for a wide range of interacting soil organisms, which are crucial for various soil functions, in particular for soil fertility, but also for human wellbeing. Long-term stability of the soil system is dependent on a careful and sustainable use of soil resources. Human influences on the soil ecosystem on a local and global scale can unintentionally alter the soil biodiversity – particularly of microorganisms - as an effect of land management. Thanks to modern gene sequencing technologies, researchers are able to study this effect.

On the other hand, **soil is thought to be a source of antimicrobial resistance (AMR) genes** which have been transferred from the environment to humans, and also a sink for antibiotics from agriculture and wastewater treatment plants. Soil therefore links the natural, agricultural and human microbiomes. Despite growing interest and research in the soil resistome (i.e., the total number of AMRs in soil), only a small amount of data is available to identify how the soil resistome is affected by different farming practices or soil conditions.

The use of antibiotics in agriculture has been severely restricted or banned due to the risk ofantimicrobial resistances (AMRs) being selected and spreading from food and the environment to human pathogens. In 2006, the use of antibiotics as animal growth promoters was banned within the European Union. However, the influence of anthropogenic effects and soil conditions on the selection and spread of the resistance mechanisms and resistant bacteria combined in soil has rarely been studied.

Aims

The "Antibiotic resistance" project aimed to describe the diversity of antibiotic resistance for Swiss agricultural soils, and to evaluate the effects of farming systems on the resistance dynamics. Field sites were chosen in collaboration with other NRP 68 project partners as part of the Soil Biology Cluster. The project aimed to find answers to the following key questions:

- What AMR mechanisms and resistant bacteria occur in Swiss soils?
- Which antibiotic resistance mechanisms and resistant bacteria are selected and spread by anthropogenic and ecological effects within soil?
- What is the relative proportion of specific soil processes (antibiotic resistance, N/P-cycles) in soil community metagenomes?

Results

The project team studied the long-term versus short-term effects of agricultural management strategies at two sites with comparable climatic conditions. One of chosen field sites, at the alpine Glasspass, offered a unique chance to study cultural history. Here the results show large differences in the soil microbial communities between the field sites having a 600-year history of manure application and those getting a sparse application by summering. These differences also indicated that bacteria potentially involved in the cycling of nitrogen compounds were present in larger numbers in the manured site, whereas other microorganisms dominated at the summering field. On the other hand, the effect of management was not visible when comparing the different treatments at the FAST field trial site at Agroscope, where separate management systems were only installed seven years ago. Although slight differences may be present, larger differences were discovered between root area (rhizosphere) and bulk soil microbial communities, indicating that, at this moment, the effect of the plant on the microbial community is larger than the effect of management.

Together with the NRP 68 partners, the “Antibiotic resistance” project also examined the microbial communities at several other field sites across Switzerland. All field sites were planted with wheat at the time of sampling, and all were sampled for rhizosphere and bulk soil. Here, the researchers also observed a strong difference between the microbial diversity in rhizosphere and bulk soil, which pointed towards a larger effect thereof than the differences in the soil parameters, including pH value, nutrient content or soil type.

In recent years, novel technologies have enabled the sequencing of the entire DNA extracted from samples (the so-called total DNA metagenomes). The project team applied this method to the samples from the FAST field site to study the presence of antimicrobial resistances AMR genes. Although they applied two different methods to analyse the data, there were still some methodological problems interfering with the quality of the results. The direct analysis of the sequence reads to different AMR databases yields only a low number of hits, indicating that the percentages of detectable AMR genes in the soil are very low. On the other hand, a similar analysis after read assembly indicated that, dependent on the AMR database, different results are obtained. This is probably mainly due to the maintenance strategies of these databases, that may lead to a different AMR gene content therein. Nevertheless, both analyses showed that the total number of AMR genes in the studied soil samples were very low, while clinically relevant AMR genes were at even lower numbers or not detectable.

To verify the numbers of AMR genes, the project team examined samples taken from the FAST site over a 6-month period that included two applications of liquid manure to half of the treatments, as well as the manure itself for the presence and quantity of specific, clinically relevant AMR genes. Whereas six out of eleven examined genes were not detected in any of the samples, it was possible to detect the same set of five AMR genes both in the manure and in the soil samples, showing that manure can be regarded as a source of AMR genes in soil. It was shown that the relevant ratio of AMR genes to the total community increased significantly after addition of manure, while returning to normal values within a short time. However, the treatments having no manure showed a tenfold lower level of these AMR genes, indicating that the treatments receiving manure lead to a higher baseline of AMR gene levels.

Implications for research

Although it is known that AMR genes are naturally present in soil, in this project, only low amounts of AMR genes in the different soil samples were found, while the number of clinically relevant AMR genes was too small to be detected. The absolute numbers obtained for the detected genes appear relatively low. This could be due to the choice of cattle manure from organic farming, which should not contain manure from antibiotic-treated cattle, according to the definition of organic farming used in Switzerland. Additionally, it is quite difficult to compare the results of this project with those of other studies, as some of the primer sets were never used for environmental studies. It is not sure whether the obtained numbers of AMR genes are even dependent on the type of soil, the field crop and the exact management strategy. This will need to be investigated in future studies.

The researchers involved in the “Antibiotic resistance” project collaborated closely with researchers working on the NRP 68 projects “Soil bacteria” (M. Maurhofer/C. Keel, University of

Lausanne) and “Mycorrhiza” (M. vand der Heijden, Agroscope). Collaborative efforts to describe the biodiversity of soil microbial communities in joint field trials offered a good opportunity to understand qualitatively and quantitatively the effects of land-management practices on key functional groups (such as *Pseudomonas*) in the context of the total microbial communities. Scientific publications with the different project partners are currently in preparation.

Practical significance

The results of the “Antibiotic resistance” project provide evidence contradicting widely held notions that human activity including agriculture has a primarily adverse impact on soil ecosystms. Microbial community analysis revealed dramatic restructuring of soil microbial communities in exceptionally long-term transition to Alpine pasture land-use. These stable, diverse communities are characterised by bacterial groups suited to recycling of nutrient rich manure inputs that contribute to the productivity of this sustainable ecosystem. This natural diversity in Swiss soil microbial communities contributes to a healthy, effective and beneficial nutrient cycling that is resilient across agricultural systems.

The “Antibiotic resistance” project provides evidence supporting the safe use of animal waste in agriculture. Manure application to soil as solid or slurry is a cornerstone of building healthy, productive soils and is also a critical recycling means for the sanitation of animal production. The results show that manure application has a very transient influence on AMR gene levels in soil that disappears within several days or weeks. Moreover, we found that antibiotic resistance genes in Swiss soils are largely general resistance mechanisms, such as effux pumps, and do not pose a reasonable risk for transfer to clinical settings in the frame of agricultural land-usage.

Recommendations

Microbial diversity studies are used widely in science. The results obtained in the “Antibiotic resistance” project indicate, however, that the soil chemistry may not have as large an effect on the microbial diversity as previously reported. The influence of the flora and its rhizosphere may be more pronounced than currently accepted. This means that more work should be done to characterise the effect of different plants on microbial community studies. Direct correlations between soil parameters and microbial communities, in particular, should now include information on the plants growing at the sample site. Additionally, the sampling strategy should be adapted to exclude as much as possible the effects of the rhizosphere on bulk samples taken from the site.

Moreover, the results indicate that for the tested samples, there is **only a small risk of AMR gene transfers from the soil to the direct environment** (ground or surface water) as only low numbers of clinically relevant AMR genes were detected in soil. Although the addition of manure temporarily increased the number of AMR genes in the samples at the FAST site, the fate of the organisms containing them and the risk of transfer from manure to ground or surface water due to runoff were not quantified. Quantification and modeling of the transfer of AMR genes through the soil column, dependent on the amount of rainfall after the manure application, will be a topic of our application to NRP 72. These results could have an effect on defining the time point for manure application depending on weather predictions.

The low numbers of AMR genes in Swiss soils can be regarded as a good sign for the Strategy on Antibiotic Resistance Switzerland (StAR). However, we examined only a few field sites, which are not representative for all soil types in Switzerland for AMR gene quantities. It would be an important task for initiatives like the Swiss Soil Monitoring Network (NABO) to study the AMR gene content of a much wider diversity of Swiss soils to obtain a more conclusive statement on the presence and quantities of AMR genes in Swiss soils.

The finding that manure application has only a transient impact on the load of antibiotic resistance genes in soils is important in terms of refining regulations and policy for the beneficial use of animal waste in agriculture. Quantitative measurements of genes in soil after manure application can be used to develop suitable guidelines to minimise farm workers' and other people's exposure without creating any unneccessary economic burdon.