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Despite the critical role of soil microbial communities in carbon mineralization rates in forest soils, surprisingly little is known about the composition or size of these communities in pollution transects. This is mainly due to difficulties in determining the extent to which microbial communities adapt to pollution over time and in relating changes in microbial activities to changes in the abundance of specific bacterial taxa involved in decomposition of dead organic matter or nutrient cycling. Also our incomplete knowledge of microbial diversity in general (only a few percent of all microbial species is described to date) is a great handicap.

In this thesis I suggest that functional measurements plus the abundance of functional genes, in addition to analysis of community structure, may shed more light on the factors controlling nutrient cycling in response to elevated metal concentrations in long-term metal polluted soils. In addition, it is still poorly understood how stability of microbial parameters in pollution-adapted communities is affected by additional stressors. Therefore, in my thesis a comprehensive analysis is proposed, using a variety of functional as well as structural approaches, to reveal the effects of pollution on microbial communities.

Studying twelve sites located along two distinct gradients of metal pollution (Olkusz and Miasteczko Śląskie in Southern Poland), I demonstrated the occurrence of metal-tolerant communities along these gradients. A DNA microarray aimed at detection of functional genes (GeoChip 4.2) demonstrated an increased abundance of metal-resistance genes at the most polluted sites. My results illustrate that long-term exposure to metals has negatively influenced microbial biomass and basal respiration, and promoted metal resistance without large shifts in community composition across the gradients despite drastically diverging metal contamination levels. The community composition was established via MiSeq Illumina sequencing of 16S rRNA genes. This revealed
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that metal pollution significantly impacted microbial community structure, but not did reduce microbial diversity (bacterial taxon richness) in these forest soils.

In subsequent experiments on the same gradients, I examined how the exposure to four secondary stressors (arsenic, salt, benzo[a]pyrene and flooding) affected the functional and structural responses of microbial communities when field-contaminated soils were subjected to additional stress and incubated for 60 days in the laboratory. I found that basal respiration rate and community structure, as determined by 16S rRNA gene-based denaturing gradient gel electrophoresis (DGGE), in soils adapted to higher metal contamination levels were more resistant to arsenic and salt stress compared to less polluted soils. However, community structure and functioning were equally resistant to benzo[a]pyrene and flooding stress, independent of the position of soils in the field gradient. Our data indicate that functional and structural responses to arsenic and salt stress were clearly related to the level of historical pollution in the long-term contaminated forest soils.

In a final set of experiments, I used phospholipid fatty acid (PLFA) profiles to compare changes in field-adapted microbial communities upon exposure to additional stressors. These results showed that the historical level of metal pollution did not determine the stability of microbial biomass nor abundances of microbial groups to secondary stress. Apparently metal-tolerant communities, selected in the field over many years, some at very high pollution levels, have not become more susceptible to additional stress and so adaptation in these microbes has evolved without apparent costs.

The results presented in this thesis indicate that microbial responses to primary and additional stressors depend very much on the way in which the community is analysed. Total microbial biomass, substrate-induced respiration, Biolog profiling, DGGE, GeoChip-based functional analysis, Illumina sequencing and PLFA profiling all enlighten different aspects of the microbial communities. Also I showed that it is crucial to have background information about the microbial community such as functional gene structure before considering responses to additional stress as well as the interaction between
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secondary and initial stress. Knowledge about the community prior and after applying such stressors will benefit the integration of results.

Results of this thesis have expanded our knowledge on tolerance and adaptation of organic soil microbial communities to long-term metal pollution. Combining eco-physiological studies with modern molecular techniques will allow us to link microbial structure to ecosystem processes in polluted soils. Results of the stress-on-stress experiments improved our understanding of the stability of soil microbial communities in the face of additional disturbances. This thesis provides a scientific basis for predicting ecological responses to chronic pollution. In this way we also improve our understanding of the impacts of human-induced activities on microbial communities and crucial ecosystem functions.