SUMMARY

Treatment with synthetic anthelmintics (AHs), constitute the main strategy for prevention and treatment of gastrointestinal nematodes (GINs) in livestock. However, as many chemical substances AHs are not totally absorbed by the animals and as a consequence the are excreted with the faeces where they are detected at concentration levels ranging from μg Kg-1 to mg Kg-1. The subsequent application of contaminated manures in agricultural settings could lead to the contamination of soils with AHs and their further transport and pollution of natural water resources. Previous studies have demonstrated the undesirable effects of AHs on non-target organisms inhabiting pasture areas, like insects associated with fecal decomposition and other soil-dwelling organisms. However, little is currently known regarding their environmental fate in the soil and their effects on the soil microorganisms. Therefore, the main objective of this thesis was to investigate the interactions of synthetic AHs, with particular focus on the benzimidazoles (BZ) albendazole (ABZ) and the macrocyclic lactones (MLs) ivermectin (IVM) and eprinomectin (EPM) with soil microorganisms and to further evaluate the potential use of microbial degradation as a means for mitigating environmental exposure to AHs. To achieve this goal we (a) evaluated the role of soil microorganisms on the dissipation of AHs in soils, b) investigated the potential effects of AHs on the function and diversity of soil microorganisms with particular emphasis on toxicity effects on the soil microbiota or the potential acclimation of the soil microbiota towards the evolution of novel catabolic mechanisms against these compounds (c) explored the capacity of AH-degrading bacteria as bioaugmentation agents for the detoxification of contaminated manure, and d) isolated specialized soil bacteria capable to degrade the benzimidazole ABZ as a mean for the more efficient implementation of bioaugmentation of contaminated manures.

We first explored the degradation of the selected AHs in fumigated and non-fumigated soils collected from 12 sheep farms with a variable history of administration of albendazole (ABZ), ivermectin (IVM) and eprinomectin (EPM). From each farm, we collected soils from inside small ruminant barn facilities

(series A, high exposure) and the associated grazing pastures (series B, low exposure). We asked the following questions: (a) What is the role of soil microorganisms in AH dissipation? (b) Does repeated exposure of soils to AHs lead to their accelerated biodegradation? (c) Which soil physicochemical properties control AH dissipation? Our results provided answers to all these scientific questions. First, soil fumigation significantly retarded ABZ (DT50 1.9 and 4.33 days), IVM (34.5 and 108.7 days) and EPM dissipation (30 and 121 days) suggesting a key role of soil microorganisms in AHs dissipation. No significant acceleration in AH dissipation was evident in soils from farms with a history of administration of the studied AHs or in soil series A vs series B, suggesting that the level of prior exposure in our experimental setting was not adequate to induce enhanced biodegradation of AHs. Transformation of ABZ to its transformation products ABZ-SO and ABZ-SO2 was observed in both fumigated and non-fumigated soil samples. Significant positive and negative correlations of soil total organic carbon (TOC) and ABZ and IVM dissipation, respectively, were observed. Soil adsorption of AHs increased in the order IVM > ABZ > EPM. TOC controlled soil adsorption of IVM and EPM, but not of ABZ, in support of the contrasting effect of TOC on IVM and ABZ dissipation.

Following up our first soil dissipation study, we further tried to further shed light into the complex interactions, beneficial or detrimental, between the studied AHs and the soil microbiota. In this quest two soils selected from the soils used in our first dissipation survey (see paragraph above) which were identified as «fast» or «slow», regarding the degradation of ABZ, IVM and EPM, were subjected to repeated applications at two dose rates (1, 2 mg kg-1 and 10, 20 mg kg-1). We hypothesized that this application scheme will lead to enhanced biodegradation of AHs in «fast» soils and accumulation of AH residues and toxicity in the «slow» soils. Repeated application of ABZ resulted in different transformation pathways in the two soils and a clear acceleration of its degradation in the «fast» soil only. In contrast residues of IVM and EPM accumulated in both soils. In addition, we evaluated the effects of ABZ, IVM and EPM on activity, abundance, and diversity of functional microbial group of ammonia oxidizing microorganisms (AOMs) as well of broad microbial groups (bacteria, fungi, crenarchaeota and protists). ABZ was the sole AH that induced a consistent reduction in the abundance of total fungi and crenarchaea. In addition, inhibition of nitrification and of the abundance of ammonia-oxidizing bacteria (AOB) and archaea (AOA) by all AHs was observed, while commamox bacteria were less responsive. Amplicon sequencing analysis showed dose-depended shifts in the diversity of bacteria, fungi, and protists in response to AHs application. ABZ presented the most consistent effect on the abundance and diversity of most microbial groups. These results provided first strong evidence for the potential adverse effects of AHs on the soil microbiota at levels expected to be found in soils and hence could be useful in a forthcoming revision of the regulatory framework regarding the environmental risk assessment of AHs.

Considering the harmful effects of AHs on the soil microbiota we explored means to alleviate the dispersal of AHs in soil. In this respect bioaugmentation of contaminated manures with microorganisms able to degrade AHs would be a promising, biobased mitigation measure. In this frame we investigated the potential capacity of a thiabendazole (TBZ)-degrading bacterial consortium to degrade other AHs belonging to the same benzimidazole group like ABZ and its transformation product, albendazole sulfoxide (ABZ-SO, also called ricobendazole, RBZ), fenbendazole (FBZ), mebendazole (MBZ) and flubendazole (FLU). Preliminary liquid culture tests showed that the consortium was more efficient in the degradation of AHs with smaller benzimidazole substituents (TBZ, ABZ, RBZ), rather than benzimidazoles with bulky substituents (FBZ, FLU, MBZ). We further explored the bioaugmentation capacity of the consortium under realistic conditions, in sheep feces fortified with 5 and 50 mg kg-1 of TBZ, ABZ and FBZ. Bioaugmentation enhanced the degradation of TBZ and ABZ, and its efficiency was accelerated upon fumigation of feces, in the absence of the indigenous fecal microbial community which competes with the exogenous inoculum.

In order to further optimize the efficiency of our bioaugmentation approach we aimed to isolate specialized microorganisms able to rapidly and specifically degrade ABZ. Enrichment cultures led to the isolation of two bacterial strains which were able to rapidly degrade ABZ. Sequencing of their 16S rRNA gene and phylogenetic analysis showed that both isolates belonged to the genus Acinetobacter.

Overall, our findings lead to several important conclusions for the environmental fate of AHs ABZ, IVM and EPM in soils and the mechanisms driving their interactions with the soil microbiota. Briefly, this thesis highlighted the important role of soil microorganisms in the dissipation of AHs. In addition, we showed the exposure of the soil microbiota to synthetic AHs is expected to have strong effects on the soil microbiota, both at diversity and functional level, with more characteristic example the negative effects of all AHs, but mostly of ABZ, on the function, diversity, and abundance of AOM. These data could be used as benchmarcks for the future adjustment of the regulatory framework regarding the environmental risk analysis of AHs which is currently not considering the soil microbiota as protection goal. Finally, we verified the potential of bioaugmentation of manures as a mitigation mean to alleviate the dispersal of AH residues in the soil environment, although further optimization is needed most probably with the use of tailored-made microbial inocula per compound.