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Millipedes cluster into distinct ecophysiological guilds based on their microbiome, with clear ecosystem implications

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Millipedes are among the largest and most important invertebrates, with over 12,000 identified and 80,000 expected species worldwide. Millipedes are detritivores living on leaf litter, deadwood, or soil. Because of the poor nature of their diets, millipedes compensate through high food consumption. Thanks to this, they are keystone species in many terrestrial ecosystems. In fact, in tropical and temperate zones, they rank the third most essential macrodetritivores after termites and earthworms and consume 10-36% of the annual litter. Thus, they contribute to soil formation and are essential forest ecosystem engineers. Despite their ecological importance, it remains unclear what role does their microbiome play in their diet.

We studied the gut microbiota of 11 millipede species and measured key physicochemical conditions (redox, pH and O₂ levels). We found that the bacterial and archaeal communities were phylogenetically conserved while the fungi matched the diet. Methanogenic millipedes had a distinct community dominated by fermenting and syntrophic microorganisms. Follow-up experiments on the methanogenic and non-methanogenic species *Epibolus pulchripes* and *Glomeris connexa*, respectively, showed that both could survive prolonged antibiotic treatment, although with some disruption of their digestion. Antibiotics treatment significantly reduced the faecal bacterial colony counts after seven days in both species. Additionally, methane production dropped by 74% in the group treated with antibiotics and 52%, in the group that received sterile feed without antibiotics.

Microbiome analysis of these groups showed major shifts of the community composition in response to antibiotics, but less so with sterile feed. Apart from the presence of methanogens, high methane production correlated with a high relative abundance of Bacteroidia, while Gammaproteobacteria dominated the guts of millipedes with low, or no, methane production.

By supplementing the millipedes' diet with BES, methane production could be suppressed entirely within 21 days. Microscopic analysis of the faeces (using CARD-FISH) revealed methanogens from the orders Methanobacteriales and Methanomassiliicoccales associated with ciliates. These methanogens persisted even in the absence of methane production.

Our results indicate a significant gut microbiome activity in cellulolytic, fermentative and methanogenic litter decomposition processes, however, unlike in ruminants and termites with a limited nutritional contribution to the host.