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Antimicrobials in constructed wetlands can cause in planta dysbiosis

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Background: Recent investigations have shown that wastewater treatment plants are typically not able to remove low concentrations of certain pollutants, known as organic micropollutants (OMPs). This class of pollutant is of emerging concern for ecotoxicologists because of their unknown toxic effects. To date, nothing is known about what consequences can arise for the plant-associated bacterial communities, mainly endophytes, upon exposure of antimicrobials.

Aims: To elucidate disturbances in the bacterial communities colonizing roots and shoots of the soft rush (*Juncus effusus*), a common wetland plant, after being repeatedly exposed to the two commonly found antimicrobials sulfamethaxazole (SMX) and trimethoprim (TMP) at low to moderate aqueous concentrations.

Methodology: The plant fitness was evaluated based on evapotranspiration rates, plant fluorescence signal, and by visual inspection. Changes of endophytic communities in roots and stems of exposed and unexposed plants were tracked via culture-dependent (CFU, biochemical characterization of the isolated strains) and culture-independent (qPCR, Illumina, FISH) approaches. The plant defense response was determined in terms of production of reactive oxygen species (ROS). An interplay of rhizo- and endophytic bacterial communities was deduced by manual interpretation of the abundant OTUs data followed by metaproteomics analysis.

Results: In two independent studies, repeated exposures of antimicrobials were found to decrease plant fitness in the model wetlands. Evapotranspiration of plants decreased upon exposure but recovered in the absence of the antimicrobials, albeit to lower values than prior exposure. After several exposures, plants became infested with insects, evapotranspiration was almost zero, and plant tissue turned necrotic. A decline in Fisher's alpha diversity index was observed up to the concentration of 50 µg/L SMX and 17 µg/L TMP, which was an obvious analogy of animal gut dysbiosis which affect the host health. Further increase in concentrations resulted in regain in the diversity, however, coordinate analyses revealed that this improvement was not due to the recovery of the previous microbiome but rather that a new community took over the system. Indigenous endophytic bacteria disappeared *in planta*, the flux of one-carbon compounds increased, and abundance of bacteria involved in iron cycle were rigorously high. High ROS and RNS were detected in the exposed roots, which suggested an invasion of detrimental bacteria. Fluorescent *in situ* hybridization (FISH) analysis further revealed the colonization of new community in the vascular bundles of the exposed plant roots.

Conclusions: Since helophytes are employed successfully in constructed wetlands, it is concluded that the presence of antimicrobials could disturb the beneficial endophytic community at environmentally relevant concentrations; thus can affect the system's performance.