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The Biodegradation of the Pharmaceutical Diclofenac over a Range of Redox Conditions in Agricultural Soils and the Identification of the Microorganisms Involved

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Background/Objectives. It is now widely recognized that pharmaceuticals and personal care products (PPCPs) are not fully eliminated in wastewater treatment plants (WWTPs). This is a cause for concern because of the growing use of wastewater effluents for irrigation and the application of biosolids as nutrient amendments to agricultural soils. The persistence of PPCPs in soils poses a risk for water contamination or uptake into crops and eventual unintended human consumption. PPCP biodegradation by soil microorganisms is a potential removal mechanism; however, the specific bacteria and pathways involved are generally unknown. The occurrence of these degraders across different WWTP biosolid samples or soils is also unknown. Such information is critical to protect natural systems from long-term PPCP contamination. This study investigated the biodegradability of diclofenac (2-(2,6-dichloranilino) phenylacetic acid) (DCF), a pharmaceutical commonly found in WWTP effluents and biosolids. Specifically, the microorganisms and functional genes associated with DCF biodegradation in agricultural soils were investigated. The objective was to determine which genes were associated with DCF biodegradation over a range of redox conditions.

Approach/Activities. The experiments were performed with laboratory microcosms with four agricultural soils (herein A, B, C, and D). For this, soil samples (5 g) were added to serum bottles, and a range of electron accepting conditions (oxygen, nitrate, sulfate or no electron acceptor). Abiotic controls were established by autoclaving the microcosms for three consecutive days. Following the establishment of each redox condition, DFC (50 ng/g soil) was added to live samples and the abiotic controls. In addition, live controls were treated in the same manner, except DFC was not added. At different times following the addition of DFC, DFC was extracted using a modified QuECHERS approach followed by purification by solid phase extraction. Liquid chromatography electrospray tandem mass spectrometry was used to measure the concentration of DFC in the soil extract. To determine the effect of DFC on the microbial community, extracted DNA was submitted for high throughput sequencing using the Illumina MiSEQ platform. The sequencing data were analyzed using Mothur, PICRUSt and STAMP.

Results/Lessons Learned. The results demonstrated that DCF readily biodegraded in all soils under aerobic conditions. DCF was significantly removed in soil A under nitrate-reducing conditions on day 50 only. Similarly, significant biological removal was noted in soil C under methanogenic conditions on day 7 only. In contrast, no significant removal was noted for soils A and C under sulfate-reducing conditions over the 50-day exposure. A statistically significant difference between DCF amended soils and their controls was found only under aerobic conditions for several pathways (e.g., Valine, leucine, isoleucine, and lysine). Several microorganisms, including *Actinobacteria, Proteobacteria*, *Bacteroidetes*,*Gemmatimonadetes*, and *Firmicutes,* illustrated a large increase in abundance following DCF aerobic degradation.