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Contributions of biogeochemical cycling to pharmaceutical biotransformation in an open-water engineered wetland biomat

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Interconnected biogeochemical reactions influence the biotransformation of emerging pollutants in natural and engineered treatment systems, convoluting the contributions of specific microbial players and metabolisms. Yet, understanding which microbes and/or enzymes catalyze biotransformation is crucial for improving water treatment and the efficacy of predictive models. In open-water treatment cells at the Prado Wetlands complex (Corona, CA), a benthic photosynthetic biomat naturally colonizes due to design parameters of a shallow (~20 – 30 cm) water column, geotextile liner preventing the growth of macrophytes, and wastewater-impaired influent chemistry. Similar to benthic stream biofilms, the biomat is oxic within the surficial millimeters and transitions to anoxia within ~5 to 10 mm of the mat-water interface. Photosynthesis, nitrification, denitrification, and methane oxidation co-occur over diel cycles in this dynamic biomat environment, however their contributions to emerging pollutant biotransformation remain unknown. We used genome-resolved metatranscriptomes derived from field-collected biomat cores to inform a series of microcosm experiments aimed at de-coupling these metabolisms. Microcosms were inoculated with biomat, wetland water, and a suite of pharmaceuticals (atenolol, metoprolol, emtricitabine, trimethoprim, sulfamethoxazole, and carbamazepine). Biogeochemical processes were isolated through oxic or anoxic incubations subject to the presence and absence of (i) light, (ii) methane, and (iii) enzyme inhibitors specific to nitrification, denitrification, and/or methane oxidation. Results indicated that metabolisms which occur simultaneously in the field could broadly be decoupled and associated with pharmaceutical biotransformation in the laboratory. In oxic incubations, for example, the persistent antibiotic sulfamethoxazole was biotransformed only in the presence of methane oxidation; genome-resolved metatranscriptomics from the field and amplicon sequencing of microcosm enrichments implicated gammaproteobacterial methanotrophs as the primary drivers. In anoxic incubations, as much as ~88% of the biotransformation of the anti-viral pharmaceutical emtricitabine was associated with nitrate reduction catalyzed by membrane-bound nitrate reductases, likely expressed by denitrifiers within the Burkholderiales. Additional findings include associations between biotransformation and photosynthesis (atenolol, metoprolol), ammonia oxidation (trimethoprim, emtricitabine) and nitrous oxide reduction (trimethoprim). Our results shed light on benthic microbes involved in emerging pollutant biotransformation and highlight the importance of coupled field and laboratory studies when investigating the microbial contributions to pharmaceutical biotransformation.