

Decoding the Unifying Microbial Metabolic Controllers on Soil Carbon Cycling Across Freshwater Wetlands

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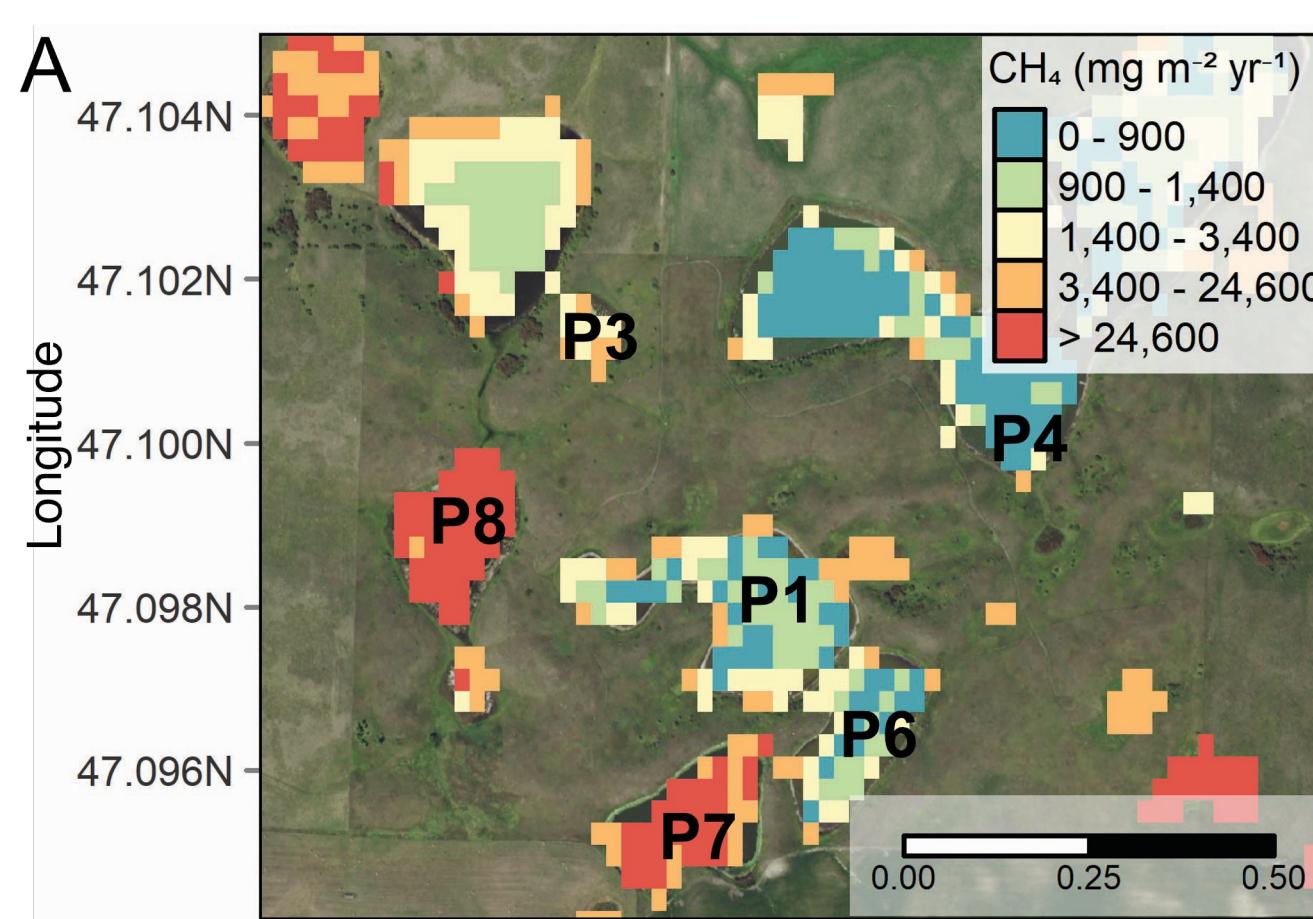
Project overview

KNOWLEDGE GAP: Despite their vital roles in transforming nutrients and controlling greenhouse gas (GHG) fluxes in wetlands, microbial knowledge is often limited to taxonomic identity alone and rarely includes cross-site comparisons.

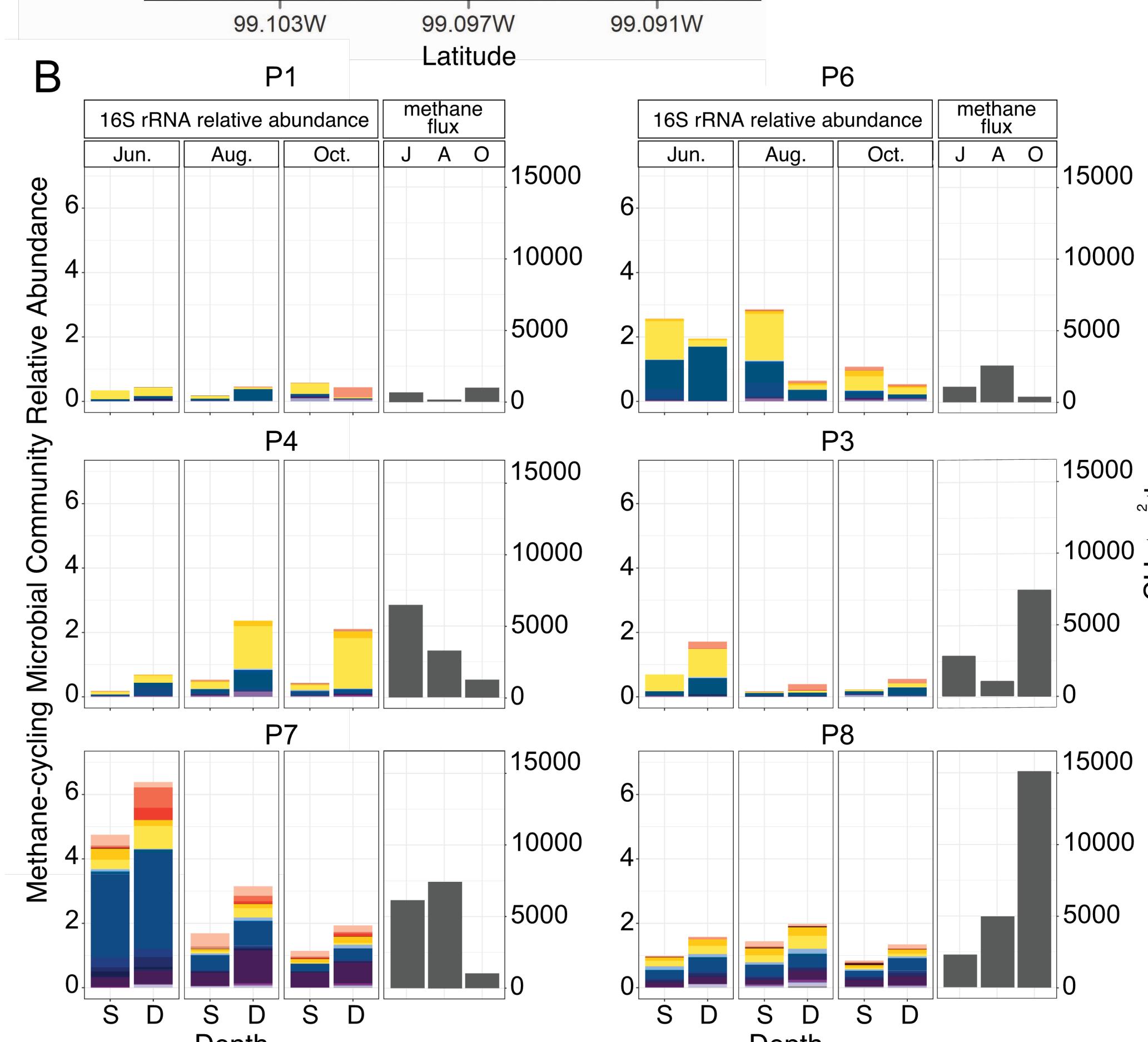
HYPOTHESIS: This project tests the overarching hypothesis that microbial genomic attributes are conserved across high methane-emitting wetlands.

APPROACH: Here we use coordinated, reproducible field measurements collected across a wetland-methane continuum spanning geographical locations. First, we will use a cross-wetland approach to define the microbial membership, physiology, and interactions directly contributing to wetland methane production. Next, we will uncover the microbial decomposition network features that classify high methane emitting wetlands. Using this information, we will test the genomic resolution needed to make robust predictions of regional and global methane fluxes. These integrated field, laboratory, and modeling approaches will identify the unifying microbial properties governing soil carbon decomposition and methane fluxes, such that some level of biological representation in models will enhance predictions of soil methane fluxes.

Construction of an observatory wetland in the Prairie Pothole Region

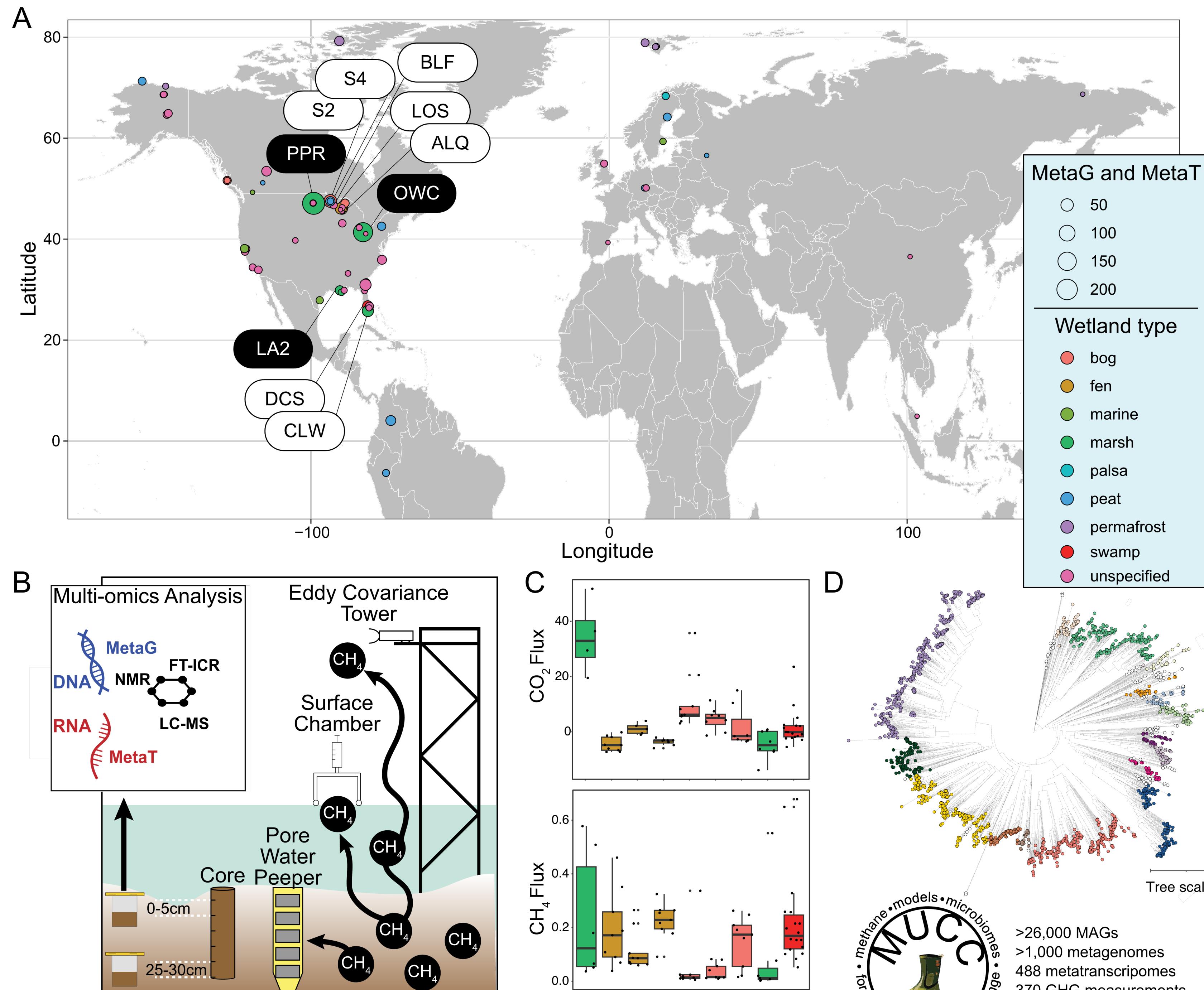


The Pothole Region (PPR) is comprised of thousands of depressional wetlands. Despite their geographic proximity, these wetlands exhibit diverse patterns of greenhouse gas emissions. We focus on six representative wetlands within PPR (A), investigating the relationship between geochemistry, microbial communities, and methane flux.



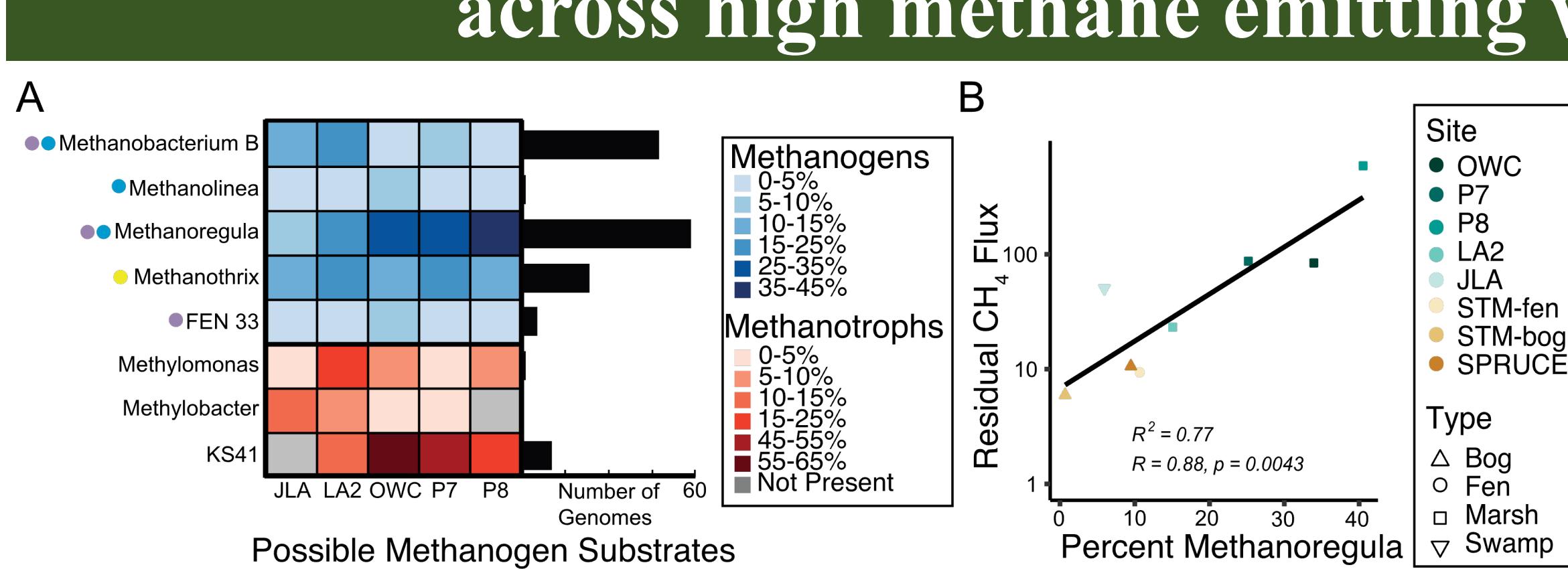
Data revealed diverse seasonal trends (June (J), August (A), October (O)) among different wetlands (B). Stacked bar charts denote 16S rRNA relative abundance of the methane-cycling microbial community members with colors denoting genome inferred metabolic potential. Methane flux along the same seasonal gradient is shown by grey bars.

Multi-omics for Understanding Climate Change (MUCC) database



Building off our previous work in Old Woman Creek (OWC), we have extended our sampling to 9 additional wetlands to construct the Multi-omics for Understanding Climate Change (MUCC) database (A). Points on world map denote wetland sampling locations by our team (10 labeled sites) and others (mined from the Joint Genome Institute Data base). Black labels denote observatory sites, sampled extensively over time points; while white labels denote satellite wetlands, with single time point sampling. Sampling of observatory and satellite sites resulted in highly-resolved view of methane flux (porewater and chamber measurements), geochemistry, and microbial activity (multi-omics analysis) in these wetland systems (B). Flux data paired to microbial sampling was collected across observatory and satellite sites, with CH₄ and CO₂ shown by boxplots in (C). Collectively, these analyses have been constructed into the MUCC database, totaling >26,000 MAGs, >1,000 metagenomes, 488 metatranscriptomes, and 370 GHG measurements.

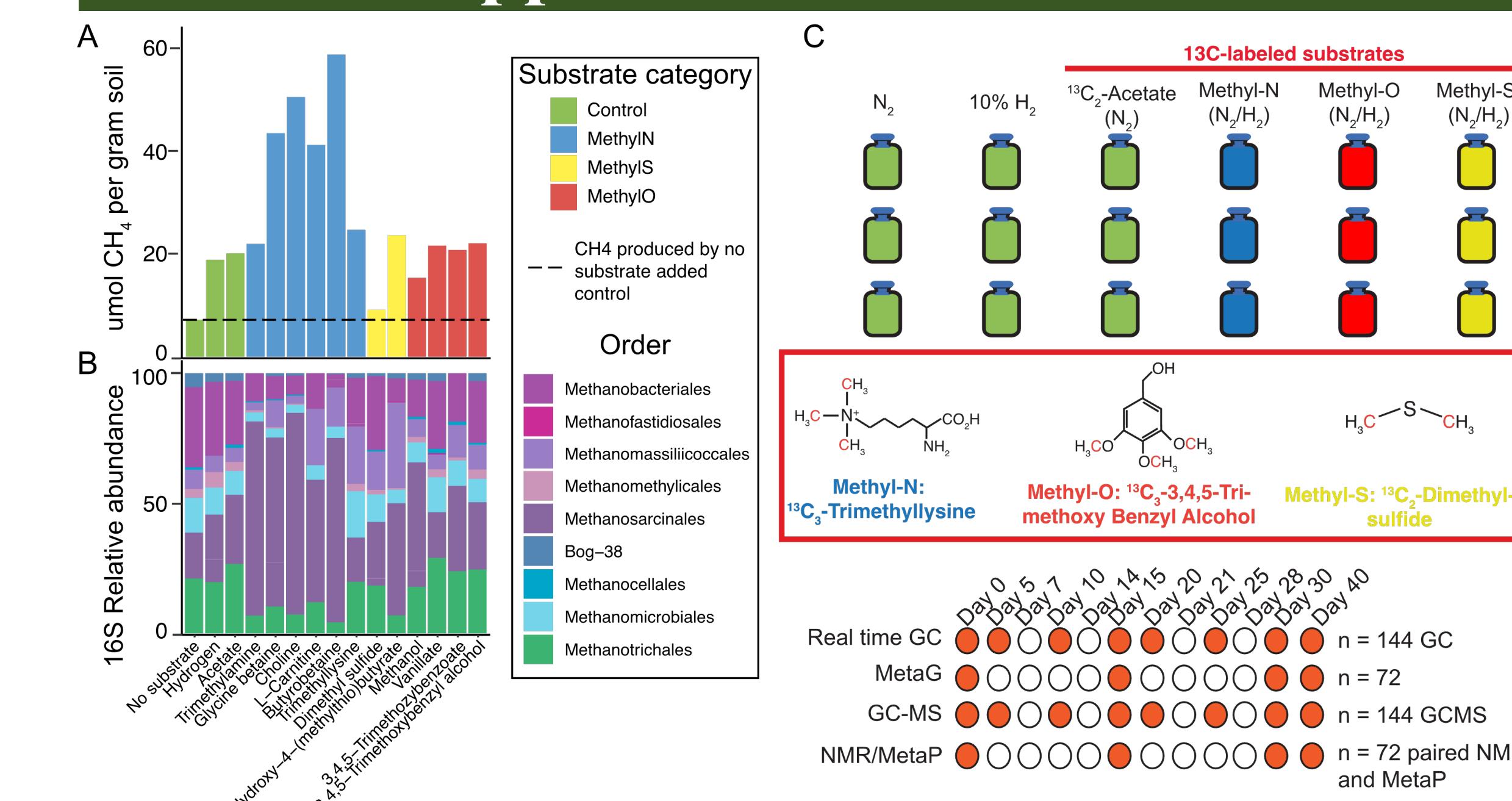
Cross-site comparison reveals core microbial membership across high methane emitting wetlands



Cross wetland microbial community analysis uncovered core methane cycling members across distinct wetlands. (A) Heatmap shows the relative abundance of each genus within the methanogen (blue) or methanotroph (red) community across wetlands. To illuminate the metabolic features of these core taxa, we utilized the MUCC database, assigning 140 core MAGs

to methane-cycling metabolisms (methanotroph or methanogen, with methanogenic substrates noted). Genome counts per genus are shown in the bar chart (black). (B) Coupling data from Delwiche et al. (FLUXNET-CH₄) to microbial community data revealed that *Methanoregula* abundance could explain a significant portion of the deviation in CH₄ flux predicted from temperature alone, underlining the importance of biotic factors in methane predictions.

Methylotrophic methanogenesis is an underappreciated methane source



Wetland soil enriched with methanogenesis substrates showed that methylotrophic cultures yielded methane comparable to, or greater than, acetoclastic and hydrogenotrophic cultures (A). Paired microbial relative abundance - via 16S rRNA sequencing - highlighted enrichment of obligate or facultative methylotrophic methanogens (purple), relative to hydrogenotrophs (blue) and obligate acetoclasts (green) (B). Based on these results, we have built reactors using representative isotopically-labeled methanogenic substrates (red box), to quantify methylotrophic contributions to methane production in these soils (C).

Genome-scale metabolic modeling of MUCC

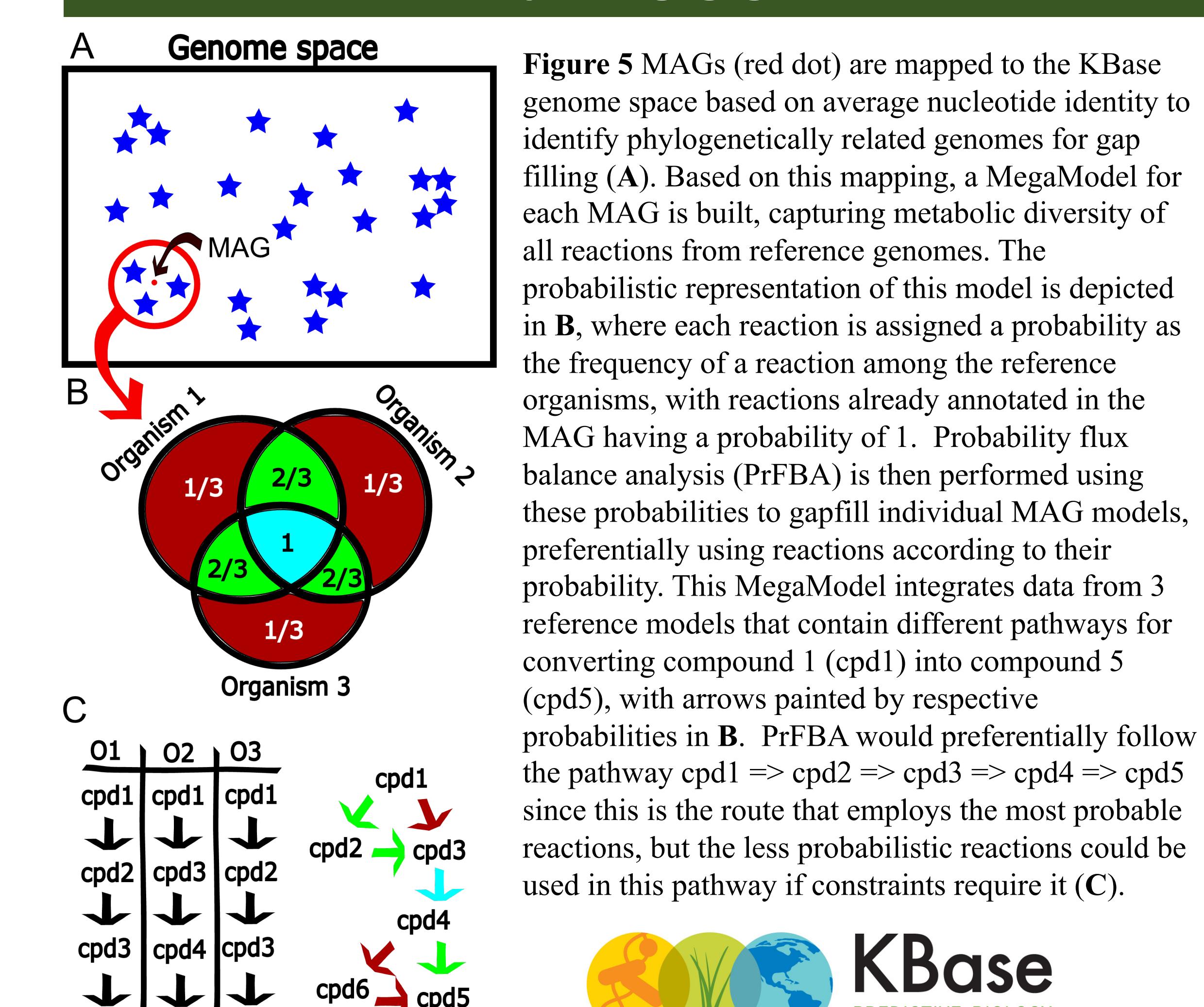


Figure 5 MAGs (red dot) are mapped to the KBase genome space based on average nucleotide identity to identify phylogenetically related genomes for gap filling (A). Based on this mapping, a MegaModel for each MAG is built, capturing metabolic diversity of all reactions from reference genomes. The probabilistic representation of this model is depicted in B, where each reaction is assigned a probability as the frequency of a reaction among the reference organisms, with reactions already annotated in the MAG having a probability of 1. Probability flux balance analysis (PrFBA) is then performed using these probabilities to gapfill individual MAG models, preferentially using reactions according to their probability. This MegaModel integrates data from 3 reference models that contain different pathways for converting compound 1 (cpd1) into compound 5 (cpd5), with arrows painted by respective probabilities in B. PrFBA would preferentially follow the pathway cpd1 => cpd2 => cpd3 => cpd4 => cpd5 since this is the route that employs the most probable reactions, but the less probabilistic reactions could be used in this pathway if constraints require it (C).



Funding and support

