



How do Biostimulators Enhance Soil Remediation and Methanogenesis: Insights from Microbial Community and Their Function

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The remediation of underground petroleum hydrocarbon (PHC)-contaminated soil relies

heavily on anaerobic bacterial metabolism.

Methanogenesis, occurring as the final stage of soil remediation, involves methanogenic bacteria utilizing intermediate products (e.g., fatty acids, alcohols) from degraded PHCs to produce methane. High methanogenic activity can serve as an indicator of active soil remediation, although this activity is closely linked to soil physicochemical properties such as permeability, nutrient levels, and pH. To enhance anaerobic bacterial activity, biostimulators—combinations of critical nutrients and diverse electron acceptors—are often injected into the soil. While the positive effect of biostimulators on enhancing microbial activity is acknowledged, understanding microbial responses to biostimulators at compositional, structural, and functional levels remains limited. In this study, soil cores were sampled from PHC-contaminated areas and split into depths of 0-1.5 m, 1.5-3.0 m, and 3.5-4.5 m. Four 20 cm soil cores were prepared at each depth. Each depth section was treated with four different biostimulator formulations for 60 days, alongside untreated controls. After treatment, 10 g of soil from each core were collected into 160 ml sterilized serum bottles. Serum bottles were purged with N₂:CO₂:H₂ (80:10:10) until no oxygen detected. Gas samples were collected periodically to measure methane concentrations using Gas Chromatography. DNA extraction from soil samples was performed for shotgun metagenomic sequencing. Methane generation rates were significantly higher in soil treated with biostimulator formulations compared to controls, with decreasing rates at greater depths, indicating increased methanogenic bacteria activity in surface soil. The effect of biostimulator formulations on methanogenic bacteria activity varied between depths and replicates, highlighting soil heterogeneity. Preliminary results of metagenomic sequencing data showed significant variation in bacterial community composition between depths and biostimulator formulations. However, Pseudomonadota (> 55%) and Actinomycetota (> 5%) were dominant bacterial phyla across all samples, while Euryarchaeota (> 66%) was the dominant archaeal phyla. Biostimulator treatments enriched soil with over one hundred soil remediation-related bacteria species, such as Anaeromyxobacter sp., Variovorax sp., Acidovorax sp.,

Comamonas sp., Desulfosporosinus sp., Diaphorobacter sp., Hydrogenophaga sp., and Geobacter sp. Furthermore, specific soil remediation-related enzymes and Clusters of Orthologous Groups (COG) were enriched in biostimulator-treated samples, including pyruvate synthase, malonate-CoA ligase, nitrogenase, phosphotransferases, and Fe-S-cluster-containing dehydrogenase component. This exploration of bacterial community responses to biostimulators enhances understanding of soil remediation mechanisms, aiding microbial engineering strategies for improved soil treatment.

Peter Li

The study of microbiomes—communities of microorganisms like bacteria, fungi, and viruses—is crucial due to their significant roles in health and the environment. In humans, microbiomes aid digestion, bolster the immune system, and protect against pathogens. In soil, they decompose organic matter, cycle nutrients, and support plant health. Understanding microbiomes helps develop targeted therapies for health issues, promotes sustainable agriculture by reducing chemical use, and enhances environmental conservation through microbial bioremediation of pollutants. Dr. Peter (Yunliang) Li has dedicated the past decade to microbiome research, applying amplicon and shotgun metagenomic sequencing methods to explore plant-soil-microbiome interactions and the role of anaerobic bacterial communities in soil remediation. Dr. Li's research examines how farming practices like fertilization, bioinoculants, and crop rotation, as well as plant genotype, interact with root-associated microbiomes to enhance agricultural productivity. Additionally, his work investigates how biostimulator formulations affect anaerobic bacterial community composition and function in petroleum hydrocarbon-contaminated soil remediation. Through these studies, Dr. Li has uncovered how microbial community composition, structure, and function respond to experimental factors, identifying marker microbes correlated with crop yield and subsets of anaerobic bacteria that promote soil remediation. This work continues to pave the way for innovative strategies in harnessing microbial communities to benefit agricultural systems and ecosystem health.