BRC Science Highlight
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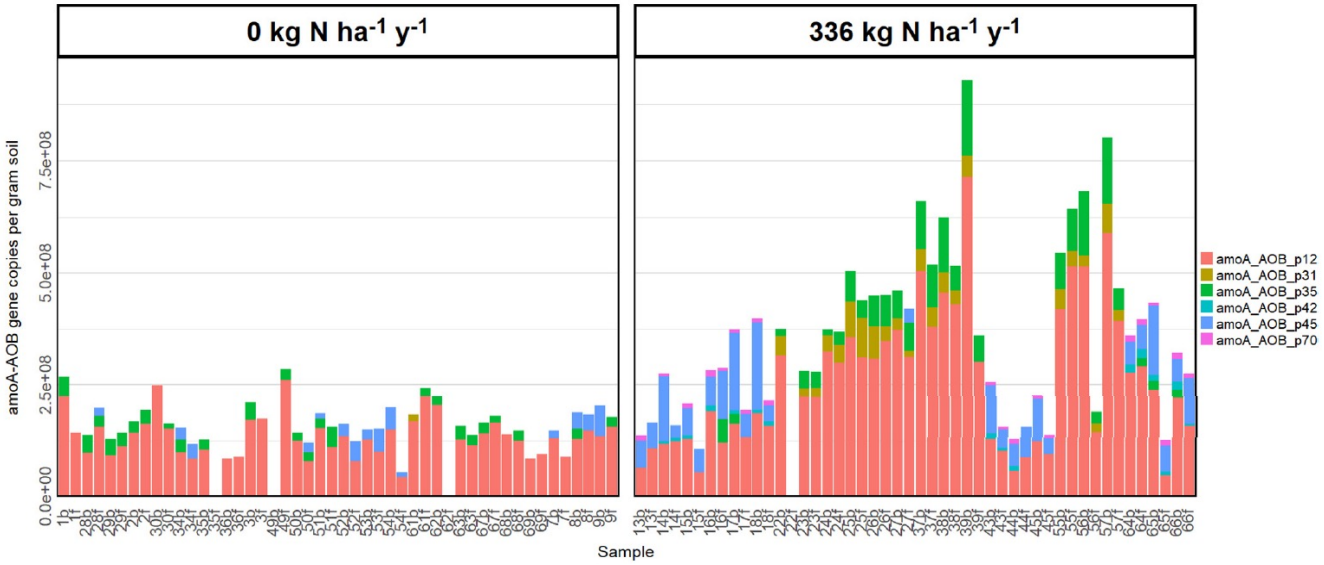
MetaFunPrimer: An Environment-Specific, High-Throughput Primer Design Tool for Improved Quantification of Target Genes

Objective

Microbial communities are key drivers of nutrient cycling. However, characterizing heterogeneous microbial communities responsible for key environmental functions has been hindered by the inability to design environment-specific primers for specific functional genes. To address this issue, researchers created and tested MetaFunPrimer, a bioinformatics pipeline for improved quantification of target genes.

Approach

- Developed MetaFunPrimer pipeline that performs high-throughput primer design for target genes which are abundant and widespread in user-provided metagenomes.
- Used MetaFunPrimer to design primer sets targeting bacterial ammonia monooxygenase subunit A (*amo*A-AOB) genes.
- Tested the newly designed primer sets *in silico* on metagenome reference sequences and also on soils from a long-term agricultural experiment.



Gene copy numbers of the *amo*A-AOB gene amplified by the six newly designed primer pairs identified as most relevant to the sampled environment. Abundance and diversity of *amo*A-AOB genes in fertilized samples were significantly higher than in unfertilized samples.

Results

- When tested *in silico* on 1,550 reference soil metagenomes, the 78 newly designed primer pairs showed 94% detection of *amo*A-AOB genes, as compared to 49% for the previously available primers.
- Experimental validation showed that the two-step quantification approach (i.e., absolute quantification using sample-specific primer pairs followed by screening step using dozens of environment-specific primer pairs) improved quantification of target genes.

Significance

MetafunPrimer high-throughput primer design software allows for more accurate quantification of environment-specific target genes and will serve as a valuable tool for characterizing relevant microbial players driving key environmental functions.

*Liu et al. 2021. "MetaFunPrimer: an Environment-Specific, High-Throughput Primer Design Tool for Improved Quantification of Target Genes." **mSystems**. DOI:10.1128/mSystems.00201-21..*

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