Building a toolbox of synthetic microbes to study environmental processes in soils

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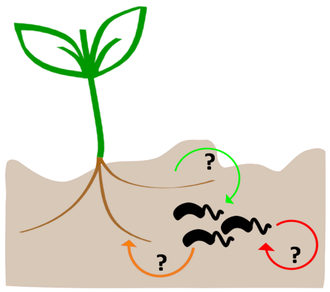
Biological processes in soil are dynamic



Many interactions between plants and bacteria depend on communication through extracellular signaling molecules, whose bioavailable concentrations and half-lives can vary with **soil conditions**:

- **Physical:** hydration, particle size and surface.
- **Chemical:** pH, nutrients, minerals and OM.
- **Biotic:** microbiome, invertebrates and viruses.

Environmental factors
Hydration and temperature.

Land Management
Fertilizer, soil amendment, tillage, etc.

TIME

Analytical tools do not capture time dependent processes such as production, dilution, and degradation of signals over long incubations.

Soil science
Net yield after chemical extraction
(emission and depletion by all microbes)

Metagenomics, Transcriptomics, Proteomics, Metabolomics
DNA, RNA, proteins and metabolites for all species
Snapshot only

Biosensors are microbes programmed to report on their environment

INPUT → BIOSENSOR → OUTPUT

The tools of synthetic biology have the potential to improve our understanding of the roles that microbes play in soil formation, water quality, crop yields, and greenhouse gas production.

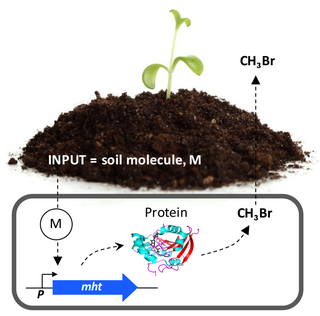
However, these tools have not yet seen many environmental applications because it is hard to monitor genetic circuit dynamic outputs in complex matrices.

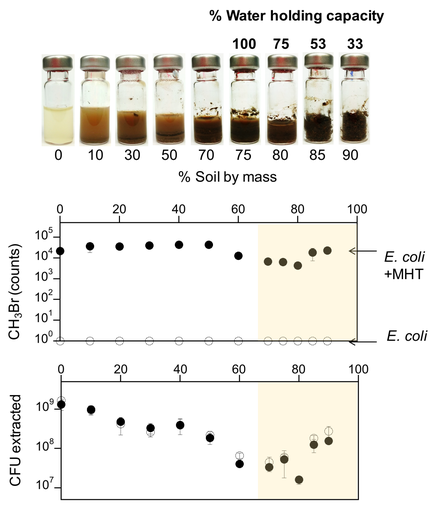
Genetic Expression

Visual reporters	Other reporters
Limited application in non-transparent matrices	e.g., ice nucleation protein **Arduous** sample preparation

MHT yield signals from soils hydrated at environmentally-relevant levels

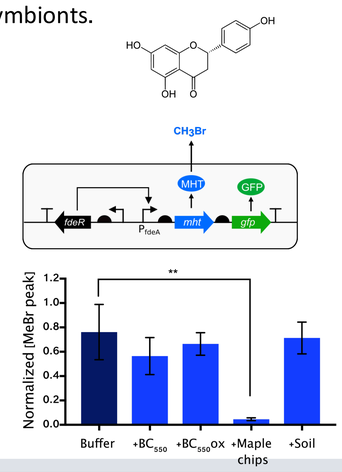
We harnessed a gas-production enzyme, methyl halide transferase **(MHT)** from *Batis maritima*, to build a series of biosensors that report by releasing an easily-detected gas that can be measured using GCMS.



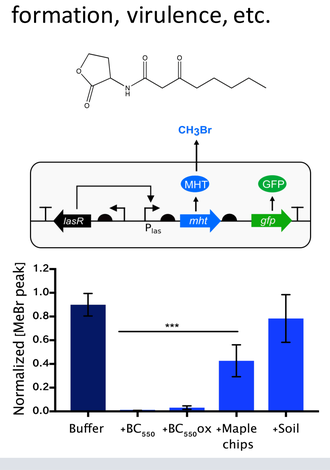


Bioavailability depends on the signal and soil amendment

Flavonoids coordinate nodule formation between legumes and symbionts.

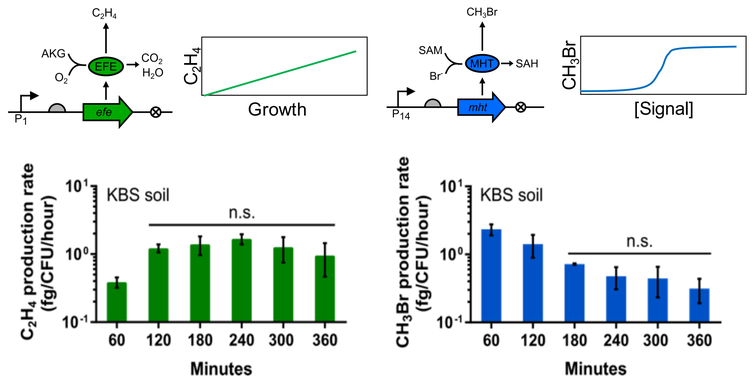


Acyl-homoserine lactones (AHL) coordinate symbiosis, biofilm formation, virulence, etc.

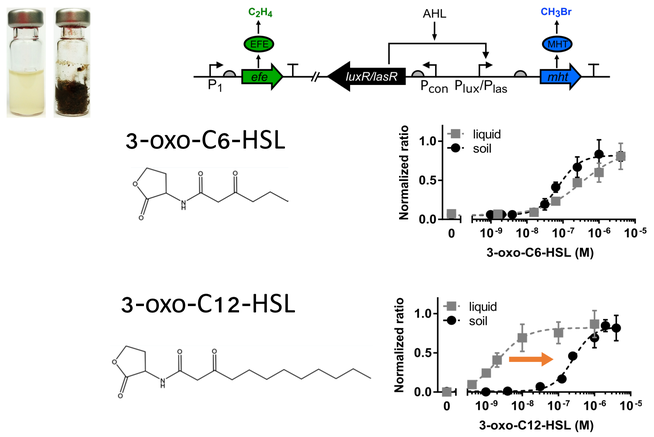


Improving signal robustness using a ratiometric approach

Ethylene forming enzyme (**EFE**) and **MHT** were used in the two gas biosensor prototype. The first gas report on **GROWTH**, the second gas to report on the **[SIGNAL]**.



Using the ratiometric biosensor to generate AHL transfer functions in liquid and soil



Using artificial matrices to study specific soil parameters

AS experimental matrix design considers 18 variations.

Q2a
Silt loam
Quartz
No EPS

M2a
Silt loam
Montmorillonite
No EPS

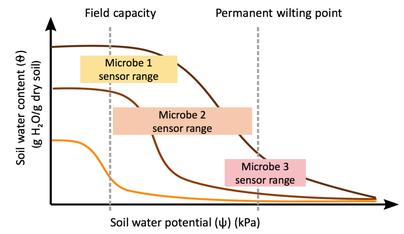
Q2Xa
Silt loam
Quartz
EPS

1. Soil texture	Sand (%)	Silt (%)	Clay (%)
Sand	90	5	5
Silt loam	20	60	20
Clay	20	20	60

2. Mineralogy
Quartz
1:1 Kaolinite
2:1 Montmorillonite

3. Aggregation
Wetting-drying cycles
0.5% w/w Xanthan gum

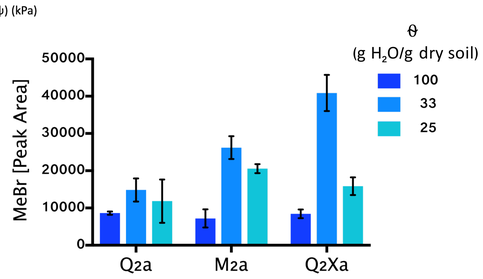
Evaluating how soil water potential affects biosensor function



Engineered soil microorganisms

Bacillus subtilis
Sinorhizobium meliloti
Shewanella oneidensis
Pseudomonas stutzeri

E. coli preliminary data shows effect of EPS on microbial activity.



Take home messages

1. Gas reporting **non-disruptively** provides information on gene expression in soil.
2. Signaling bioavailability depends upon both the **chemical characteristics** of the signal and **soil properties.**
3. Ratiometric reporting provides a more **robust signal** for use in soils by accounting for microbial growth.
4. Artificial soils should be useful as a **simplified matrix** to study how microbial gene expression relates to soil composition.

References

1. H.Y. Cheng, , C.A. Masiello, G.N Bennett and J. J. Silberg, ***Environ. Sci. Technol.*** (2016).
2. X. Gao, H.Y. Cheng, I Del Valle, S. Liu, C.A. Masiello, aand J. J. Silberg, ***ACS Omega.*** (2016).

Acknowledgments

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