

Ratiometric gas reporting: a non-disruptive approach to monitor gene expression in soils

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ABSTRACT

Fluorescent proteins are ubiquitous tools that are used to monitor the dynamic functions of natural and synthetic genetic circuits. However, these visual reporters can only be used in transparent settings, a limitation that complicates non-disruptive measurements of gene expression within many matrices, such as soils and sediments. We describe a new ratiometric gas reporting method for non-disruptively monitoring gene expression within hard-to-image environmental matrices. With this approach, C₂H₄ is continuously synthesized by ethylene forming enzyme to provide information on viable cell number, and CH₃Br is conditionally synthesized by placing a methyl halide transferase gene under the control of a conditional promoter. We show that ratiometric gas reporting enables the creation of *Escherichia coli* biosensors that report on acylhomoserine lactone (AHL) autoinducers used for quorum sensing by gram-negative bacteria. Using these biosensors, we find that an agricultural soil decreases the bioavailable concentration of a long-chain AHL up to 100-fold. We also demonstrate that these biosensors can be used in soil to non-disruptively monitor AHLs synthesized by *Rhizobium leguminosarum* and degraded by *Bacillus thuringiensis*. Finally, we show that this new reporting approach can be used in *Shewanella oneidensis*, a bacterium that lives in sediments.

INTRODUCTION

Genetically-encoded reporters that fluoresce or bioluminesce enable visualization of gene expression in diverse organisms.^{1,2} These reporters transformed biology by providing fundamental insight into mechanisms underpinning cellular behaviors. Visual reporters are also critical in synthetic biology, enabling the evaluation of engineered genetic circuits over a range of conditions.³ However, visual reporters can only be used in transparent settings, a limitation that prevents non-disruptive measurements in many hard-to-image environmental matrices, such as soils and sediments, where $>10^{29}$ prokaryotes are estimated to live on Earth,⁴ and complex feedstocks used in bioreactors for the biological production of chemicals.⁵

Enzymes that synthesize rare volatile gases represent an alternative strategy for monitoring gene expression in hard-to-image materials.⁶ With gas reporters, the activity of the promoter controlling enzyme expression is evaluated using the concentration of the volatile product that diffuses out of the matrix.⁷ Gas reporting is appealing for diverse environmental and engineering applications because it has the potential to provide information on gene expression without requiring cell extraction, which can vary in efficiency across matrices.⁸

By enabling non-destructive monitoring of environmental microbiology, gas reporting could simplify studies examining the effects of matrix properties on the bioavailable levels of diverse molecules, such as pollutants,⁹ biological signals critical for agriculture,¹⁰ and organic matter,¹¹ whose processing contributes to both soil fertility and to greenhouse gas emissions. Unfortunately, gas reporters cannot yet accurately report on conditional gene expression, since it remains difficult to distinguish between

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3 gas accumulation resulting from promoter activation versus cell growth and metabolism.
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5 This problem also arises with visual reporters, where it has been overcome by coupling
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7 pairs of reporters to conditional and constitutive promoters (e.g., GFP and RFP) and
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9 evaluating the ratio of their signals (GFP/RFP).¹²
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12 We hypothesized that a ratiometric gas-reporting strategy could be developed for
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14 use in environmental matrices by normalizing the gas signal obtained from a
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16 conditionally-expressed methyl halide transferase (MHT) to the signal obtained by
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18 constitutively-expressed ethylene-forming enzyme (EFE). We tested this idea using
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20 *Batis maritima* MHT, which synthesizes methyl bromide (CH₃Br), and *Pseudomonas*
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22 *syringae* EFE, which produces ethylene (C₂H₄). These enzymes were chosen because
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24 they can be heterologously expressed as active enzymes in *Escherichia coli*.^{13,14}
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RESULTS AND DISCUSSION

Gas reporter characterization. To determine if EFE and MHT yield stable cellular signals across a range of microbial growth temperatures, we first evaluated gas production using *Escherichia coli* strains that constitutively express EFE, MG1655-efe and MHT, MG1655-mht (**Fig. 1a-b**). With MG1655-efe, the C₂H₄ production rate was similar across six hour incubations at 30 and 33°C (**Fig. 1c**). At 37°C, the C₂H₄ production rate was consistently lower across all time points, indicating that this enzyme is temperature sensitive. With MG1655-mht, in contrast, the CH₃Br production rate increased with time across all temperatures tested (**Fig. 1d**). MHT and EFE expression did not result in any apparent fitness burdens (**Fig. S1**). All subsequent gas measurements were performed at 33°C to maximize both gas signals.

To demonstrate that EFE and MHT can be used as reporters in matrices, we first evaluated their signals using an artificial soil with a well-defined matrix mimicking soil texture, and then transitioned to an agricultural soil (**Fig. S2**). For these incubations, we used environmentally-relevant hydration (75% water holding capacity) and constant nutrient levels. When MG1655-efe was incubated in the artificial soil, the C₂H₄ production rates changed by <25% over six hours (**Fig. 2a**). When MG1655-mht was incubated in the artificial soil, the rate of CH₃Br production was lower in the first hour and then increased to a constant level (**Fig. 2b**). Similar experiments performed using an agricultural soil from the NSF Kellogg Biological Station (KBS) Long Term Ecological Research (LTER) site (loamy Typic Hapludalf 0-5 cm Ap horizon) revealed that the C₂H₄ production rate stabilizes after two hours (**Fig. 2c**). In contrast, the CH₃Br production

rate required three hours to stabilize (**Fig. 2d**). MG1655-efe and MG1655-mht grew to a similar extent in both soils (**Fig. S3**).

Gas reporting of signaling compounds. We next used EFE and MHT to report on the output from synthetic genetic circuits that respond to acylhomoserine lactones (AHL) autoinducers used for quorum sensing in gram-negative bacteria.¹⁵ In these circuits (**Fig. 3a**), we constitutively expressed EFE, using C₂H₄ as an indicator of cell number (**Fig. S4**), while AHL regulators were used to control MHT expression. We built two biosensors. In MG1655-lux, MHT was regulated using *Vibrio fischeri* LuxR, a receptor for the short chain AHL 3-oxo-C6-HSL. In MG1655-las, MHT expression was regulated using *Pseudomonas aeruginosa* LasR, a receptor for the long chain AHL 3-oxo-C12-HSL.

With both AHL-sensing strains, C₂H₄ production in our standard loamy agricultural soil from KBS was not affected by AHL concentrations spanning five orders of magnitude (**Fig. 3b,c**), indicating that MHT expression does not influence EFE-dependent C₂H₄ production. In contrast, CH₃Br increased 14-fold in MG1655-lux (**Fig. 3d**) and ~200-fold in MG1655-las (**Fig. 3e**). We calculated the ratio of CH₃Br to C₂H₄ over all AHL concentrations. With this ratiometric signal, the highest concentration of 3-oxo-C6-HSL resulted in a 15.3-fold increase in signal with MG1655-lux (**Fig. 3f**), while 3-oxo-C12-HSL yielded an 81.5-fold increase in signal with MG1655-las (**Fig. 3g**). MG1655-las presented a half maximal response (EC₅₀) at a concentration of 3-oxo-C12-HSL ($2.6 \pm 0.4 \times 10^{-7}$ M) that was >100-fold larger than previous reports,¹⁶ while the response with MG1655-lux (EC₅₀ = $7.6 \pm 1.3 \times 10^{-8}$ M 3-oxo-C6-HSL) was more consistent with previous measurements.

To test whether our standard loamy agricultural soil affected the bioavailable levels of these autoinducers, AHL-induced gas production was also measured in liquid medium (**Fig. 3f,g**). With MG1655-lux, the 3-oxo-C6-HSL concentration that elicited a half maximal response was similar in liquid culture and in soil. In contrast, the 3-oxo-C12-HSL concentration required for a half maximal response ($EC_{50} = 2.1 \pm 0.6 \times 10^{-9} \text{ M}$) with MG1655-las was two orders of magnitude lower in liquid culture. This finding is consistent with previous measurements that have shown that matrices decrease the bioavailable 3-oxo-C12-HSL through sorption and hydrolysis.^{17,18}

To investigate the reliability and robustness of the signals obtained from our biosensors over the time required to run hundreds of samples, we measured the $\text{CH}_3\text{Br}/\text{C}_2\text{H}_4$ ratios following incubations of varying lengths with 1 μM AHL. With both biosensors, CH_3Br and C_2H_4 continuously increased with time (**Fig. 4a,b**), as did the number of colony forming unit (CFU) (**Fig. S5**). However, at all time points where CH_3Br could be detected, no significant change in the mean $\text{CH}_3\text{Br}/\text{C}_2\text{H}_4$ ratio was observed. A similar level of signal robustness was observed when an identical experiment was performed with a lower AHL concentration (**Fig. S6**).

To evaluate whether ratiometric gas reporting of AHLs can be used in an organism whose native habitat is an environmental sediment, we evaluated whether this approach can also be used with *Shewanella oneidensis* MR1.¹⁹ For these experiments, we constructed a plasmid (pHC01) that constitutively expresses EFE and uses 3-oxo-C12-HSL to regulate MHT expression (**Fig. 5a**). This plasmid was transformed into *S. oneidensis*, and the effect of different concentrations of 3-oxo-C12-HSL on gas production was measured in liquid medium and a soil slurry (**Fig. 5b**). As observed with

our *E. coli* biosensor, the AHL concentration required to achieve a half maximal response in liquid culture was lower than the concentration required for the same signal in the presence of soil (**Fig. 5c**). These finding provides evidence that ratiometric gas reporting method can be used in different microbes to study the effects of a matrix on the bioavailable level of an AHL autoinducer.

Monitoring biological signal synthesis and degradation. To test whether our ratiometric gas reporters are sufficiently sensitive to monitor biological degradation of an AHL, we measured the effect of adding a lactonase-synthesizing gram-positive bacterium (*Bacillus thuringiensis*, *Bt*)²⁰ on the stability of 3-oxo-C12-HSL in the loamy agricultural soil (**Fig. 6a**). A range of *Bt* titers (10^4 , 10^5 , and 10^6) were added to soil matrices that contained 3-oxo-C12-HSL. At different times following incubation of *Bt* in AHL-containing soil, MG1655-las was added to read out the effect of *Bt* on 3-oxo-C12-HSL. After 24 hours, MG1655-las presented a $\text{CH}_3\text{Br}/\text{C}_2\text{H}_4$ ratio that was inversely correlated with the titers of *Bt* added (**Fig. S7**). The highest titer of *Bt* (10^6 cfu) yielded a gas ratio that decreased exponentially with a 16.8 hour half-life (**Fig. 6b**). At the end of the incubations, the *Bt* CFUs correlated with incubation time and titer added (**Fig. S8**). A comparison of the individual gases at each time point attributed the signal change to a decrease in CH_3Br (**Fig. S9**). The C_2H_4 production remained constant across all measurements.

We next investigated whether our ratiometric biosensors could monitor the dynamic production of multiple AHL molecules (**Fig. 7a**) synthesized by the soil bacterium *Rhizobium leguminosarum* (*Rl*), a nitrogen-fixing bacterium that forms symbioses with agricultural crops. Among the AHLs produced by *Rl*¹⁰, MG1655-lux is specific for 3-oxo-

C8-HSL, while MG1655-las is activated by 3-OH-C14:1-HSL (**Figs. 7b,c and S10**). MG1655-las can also be activated by 3-oxo-C8-HSL, but this strain has a lower sensitivity than MG1655-lux.

To determine if *R/I* affects biosensor gas production, we incubated *R/I* with an *E. coli* strain that constitutively produces both gases (MG1655-mht-efe). With MG1655-mht-efe, the ratiometric signal was not affected by the presence of *R/I* (**Fig. S11**). We next used our biosensors to monitor dynamic AHL production by *R/I* in the loamy agricultural soil. Using the more specific MG1655-lux biosensor, we found that the CH₃Br/C₂H₄ ratio increased over a four-day incubation to a level that corresponds to 4×10^{-7} M 3-oxo-C8-HSL (**Fig. 7d**). This 3-oxo-C8-HSL concentration is not sufficient to activate MG1655-las (**Fig. S10**), indicating that the signal arising from our second biosensor results primarily from the accumulation of 3-OH-C14:1-HSL. Assuming that this ratiometric signal from MG1655-las is generated by 3-OH-C14:1-HSL, our measurements suggest that this longer chain AHL accumulated at a greater rate and to a higher concentration over the incubation, $\geq 5 \times 10^{-5}$ M (**Fig. 7e**).

Implications. Ratiometric gas reporting represents a simple method for monitoring the temporal activities of promoters, yielding a large dynamic range (>80 fold) when coupled to a conditional promoter in *E. coli* without presenting an apparent fitness burden. When used in an environmental matrix, this method was able to provide information on the bioavailable concentrations of different cell-cell signaling molecules. A comparison of the effects of the loamy agricultural soil on the bioavailable concentration of short- and long-chain AHLs revealed that the concentration of the long-chain AHL is decreased to by the matrix, while the short chain AHLs remain unaffected.

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3 This finding suggests that ratiometric gas reporting will be useful in future studies that
4 seek to evaluate the effects of different soils on a wide range of chemicals that regulate
5 promoters. One advantage of this approach is that it produces a consistent biosensor
6 signal for over half a day in capped vials, even if cells are growing in the matrix. This
7 finding is significant because it demonstrates that samples can be arrayed in a GC-MS
8 autosampler prior to performing analysis, and large numbers of samples (>100) can be
9 analyzed over half a day without altering the output signal.
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19 Our proof-of-concept ratiometric gas reporter will have immediate applications that
20 expand the utility of synthetic biology in environmental science. Our results show that
21 this approach can be used in short incubations that seek to examine how the
22 bioavailable concentration of chemicals within a soil or sediment differ from the
23 concentrations that are determined following extraction of molecules from a matrix.
24 Environmental scientists frequently use analytical chemistry and biosensors to quantify
25 the concentrations of chemicals in soils.^{9,21} However, these methods only provide
26 insight into the total chemical concentrations following extraction, rather than the
27 bioavailable concentrations *in situ*. Our ratiometric gas reporter overcomes these
28 limitations by enabling measurements without the need for extraction. In the case of
29 chemicals used for cell-cell signaling in soils, our approach will be useful for studying
30 how the composition of a matrix affects the fidelity of intercellular signaling. In the case
31 of toxic compounds in the environment, e.g., organic and inorganic pollutants, our
32 ratiometric reporter can be used to study how the composition of a matrix modulates the
33 bioavailability of toxic compounds. Beyond chemical sensing applications, our new
34 ratiometric reporter will also be useful for studying the effects of matrix composition on
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horizontal gene transfer,⁷ and it will be useful in studies with artificial soils that examine how matrix heterogeneity affects microbial behaviors.²²

When using ratiometric reporting to study the activity of conditional promoters, it is critical to consider how the rate of production of each gas is influenced by environmental conditions and the duration of the incubation. The concentration of each gas used to generate a ratiometric signal depends upon the expression and stability of the EFE and MHT, the availability of the EFE and MHT substrates, and gas partitioning out of the soil matrix following synthesis. In this work, we found that EFE activity decreases at 37°C as previously observed,²³ indicating that it is best to perform measurements at lower temperatures where both gases accumulate to level that can be easily detected. When ratiometric measurements were performed at 33°C, a robust gas ratio signal was obtained with our AHL biosensors that remained constant even when the signal was measured following different incubation lengths, ranging from three to thirteen hours in duration (**Fig. 4**). Thus, the relative accumulation of both gases remains constant when vials remain capped for different periods of time before GC-MS analysis, even though the absolute amount of each gas increases with time. We avoided performing longer incubations with our sensors because oxygen required for EFE activity²⁴ can become depleted in the capped vials used for these measurements.

Even though a consistent ratiometric gas signal was obtained with our AHL biosensors over a period of half a day, our measurements with strains that constitutively produce EFE and MHT at high levels suggested that the individual gas signals could be influenced by incubation time and environmental conditions. With strains that constitutively produced the individual gases, we measured the rate of gas accumulation

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3 by uncapping the vials following each measurement and then recapping for one hour
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5 before the next measurement. With this higher resolution temporal data (**Fig. 2**), the
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7 signal did not vary significantly from three to six hours for all conditions tested, which
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9 included a synthetic and real soil. However, the average CH_3Br production rate
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11 decreased over this time period. The underlying cause of this latter trend is not known.
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13 This trend could arise because of time-dependent changes in MHT substrate
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15 concentrations within the cell. The MHT used to synthesize CH_3Br requires SAM and
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17 bromide as substrates.¹³ To minimize substrate limitation issues, we supplied bromide
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19 at a concentration that is 10^4 higher than total CH_3Br produced in our experiments as
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21 previously described.⁷ SAM could not be similarly controlled since it is synthesized from
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23 ATP and methionine.²⁵
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28 With further development, our ratiometric gas reporter should have even wider
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30 applications. In the case of commensal bacteria that are being developed as human gut
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32 diagnostics and therapeutics,²⁶⁻²⁸ ratiometric gas reporting could be useful for studying
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34 how synthetic microbes behave in hard-to-image environmental matrices that they enter
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36 following their exit from host organisms. With further refinement, this new approach
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38 should be useful for monitoring dynamic changes in metabolites within bioreactors that
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40 use non-transparent feedstocks to produce green chemicals,⁵ and it should be valuable
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42 for studies that examine how soil amendments disrupt symbiosis that are important for
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44 agriculture.²⁹
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49 Current limitations of ratiometric gas reporting include a need for oxygen and halides
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51 as substrates and a minimum biosensor titer to obtain a reliable signal. The
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53 development of a pair of enzymes that produce rare volatile gases anaerobically would
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3 enable broader applications,³⁰ e.g., uses in anaerobic soils and fermenters, as would
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5 the application of more sensitive detection measurements.³¹ In the future, ratiometric
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7 gas reporting could be used for longer-term incubation studies. However, these longer
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9 incubations will require the use of microbes that grow more stably in matrices than *E.*
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11 *coli* and continuous gas sampling, similar to the gas sampling approached used by
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13 ecologists to study greenhouse gas production.³²
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METHODS

Materials. AHL autoinducers were purchased from Cayman Chemical, antibiotics were from Research Products International, and all other chemicals reagents were from Sigma-Aldrich, VWR, or BD Biosciences. Kits for DNA and plasmid purification were from Zymo Research and Qiagen. Vials for gas measurement were from Phenomenex.

Growth medium. Lysogeny Broth (LB) and LB-agar plates containing antibiotics were used to propagate plasmids and strains including *E. coli*, *Bt*, and *S. oneidensis* MR-1. Unless stated otherwise, all gas production assays were conducted using a modified M63 medium (referred to as M63) supplemented with 200 mM NaBr, which serves as substrate by MHT for the CH₃Br production. To generate our modified M63 medium, we generated 1 L of medium by mixing 50 mL 20% glucose, 50 mL 4 M NaBr, 1 mL 1 M MgSO₄, 100 µL 0.1% thiamin, 200 mL M63 salt stock, and water. The M63 salt stock contained 75 mM ammonium sulfate, 0.5 M potassium phosphate monobasic, and 10 µM ferrous sulfate. The salt stock and water were autoclaved, and all other components were sterile filtered. The M63 was adjusted to pH 7 prior to use. For simplicity, we refer to this modified M63 medium simply as M63 medium. Yeast Mannitol Broth (YMB) and Yeast Mannitol Agar (YMA) were used to culture *Rl*. In cases where *E. coli* biosensors were added to soils already harboring *Rl*, the *Rl* in soil contained YMB medium and the *E. coli* added to soil was in M63 medium. *S. oneidensis* MR1 experiments utilized LB medium containing 200 mM NaBr and 50 µg/mL kanamycin.

Bacterial strains and plasmids. *E. coli* XL1-Blue (Stratagene) was used for cloning and plasmid amplification, while *E. coli* MG1655 was used as a chassis for building a ratiometric gas biosensors. This latter strain was used to develop sensors because it

does not present detectable C₂H₄ or CH₃Br production. In a previous study, we integrated DNA containing the *Batis maritima* MHT¹³ into the 186 *attB* site in MG1655 to obtain MG1655-mht.⁷ In MG1655-mht, MHT expression is driven by the strong constitutive promoter/RBS pair P2.BCD2.³³ MG1655-efe was constructed by integrating *P. syringae* EFE³⁴ into the phage 186 *attB* site using Clonetegration.³⁵ This EFE has previously been expressed in *E. coli* as a functional enzyme.¹⁴ In MG1655-efe, EFE expression is controlled by the strong constitutive promoter/RBS pair P1.BCD2.³³ MG1655-mht-efe was modified from MG1655-mht by integrating P1.BCD2.EFE into P21 *attB* site using Clonetegration.

MG1655-lux and MG1655-las were constructed by integrating LuxR and LasR sensing modules into the λ attachment site in MG1655-efe using the λ recombination system.³⁶ The LuxR sensing module includes the gene encoding LuxR (BBa_C0062) under the control of the strong constitutive promoter/RBS pair P1.BCD2 and the MHT gene under control of the wildtype *P_{lux}* promoter harboring a C to T mutation (BBa_K658006). The LasR sensing module includes the gene encoding LasR (BBa_C0078) driven by the constitutive promoter *P_{laclq}* (BBa_J56015), and the MHT gene regulated by *P_{las}*. *Rl* was from ATCC (10004™).³⁷ *Bt* serotype israelensis AM65-52 was isolated by dissolving Mosquito Bits (Summit Chemical) in LB medium, streaking the slurry on LB plates, and isolating an individual colony. This strain was verified by PCR amplifying the AiiA gene encoding a lactonase that degrades AHL, and sequence verifying the amplicon.

Shewanella oneidensis MR-1 was kindly provided by Dr. Timothy Palzkill. We constructed a plasmid, pHC01, harboring the *lasR* sensing module and a bicistronic

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3 message encoding EFE and red fluorescent protein (RFP) under control of P_{tet} . This
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5 plasmid contains a ColE1 origin and kanamycin resistance marker. We transformed
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7 pHC01 into *S. oneidensis* MR-1 using electroporation³⁸ and selected for transformed
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9 cells on LB-agar plate containing 50 $\mu\text{g/mL}$ kanamycin.
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12 **Headspace gas analysis.** All gas analysis was performed using an Agilent 7820a gas
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14 chromatograph (GC) with PoraPLOT Q capillary column (24 m, 0.25 mm ID, and 8 mm
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16 film) and a 5977E mass spectrometer (MS). An Agilent 7693A liquid autosampler with a
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18 100 μL gastight syringe (Agilent G4513–80222) was used to inject 50 μL of headspace
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20 gas from sealed 2 mL vials containing soils and microbes into the GC. The autosampler
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22 is equipped with a cooling/heating tray (Agilent 7693A) that connects to a heated water
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24 bath with PID temperature controller (Thermo Scientific SC100-S7 4L). To quantify C_2H_4
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26 and CH_3Br , the oven temperature was programmed to ramp from 85°C to 105°C at
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28 12°C/min, followed by a fast increase (65°C/min) to 150°C and hold for 144 seconds.
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30 The total run time was five minutes. The 5977E was configured for selected ion
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32 monitoring mode, specifically to C_2H_4 (MW = 27 and 26 for C_2H_3 and C_2H_2 , respectively)
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34 and CH_3Br MW = 93.9 and 95.9 for $\text{CH}_3^{79}\text{Br}$ and $\text{CH}_3^{81}\text{Br}$, respectively). Agilent
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36 MassHunter Workstation Quantitative Analysis software was used to evaluate the
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38 amount of gas in each sample; the peak areas of the major ions (C_2H_3 and $\text{CH}_3^{79}\text{Br}$)
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40 were used to quantify the amount of each gas while the minor ions were used as
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42 qualifiers.
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49 The peak area from the GC-MS measurement was converted to the total mass of
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51 C_2H_4 and CH_3Br in each experimental vial using standard curves. Two CH_3Br standard
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53 curves generated. A standard curve for liquid studies was built using serial dilutions of
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an analytical standard (100 $\mu\text{g/mL}$ in methanol, purchased from Sigma-Aldrich) that had been diluted 100x into M63 medium. A standard curve for soil studies was generated by mixing the serial dilutions (10 μL) with M63 medium (190 μL) and soil (800 mg). Two C_2H_4 standard curves were produced using the decomposition of ethephon (Sigma-Aldrich) in alkaline solution. An ethephon stock solution was prepared at 100 $\mu\text{g/mL}$ in 0.1 M HCl. To build a C_2H_4 standard curves for liquid, serial dilutions of ethephon solution (100 μL) were added to M63 medium (800 μL), 6 M NaOH (100 μL) was added, and the vial was immediately crimped. To build a C_2H_4 standard for soil, serial dilutions of ethephon solution (100 μL) were added to soils (800 mg), 6 M NaOH (100 μL) was added to the mixture, and the vial was immediately crimped. All standards were incubated in 2 mL gastight vials (Phenomenex) for 2 hours at 45°C with shaking at 290 rpm and then moved to static 30, 33, or 37°C incubators for 2 hours to allow each gas to reach equilibration between the aqueous and gas phases prior to the GC-MS analysis. Standard curves were calculated in Excel using liner regression and a y intercept of zero.

EFE and MHT temperature dependence. MG1655-eфе and MG1655-mht were used to inoculate M63 medium and incubated at 37°C while shaking at 250 rpm. At $\text{OD}_{600} = 0.5$, cultures were spun down and resuspended at an $\text{OD}_{600} = 0.1$ (2×10^7 cell/ml) using fresh M63 medium. These cultures (1 mL) were added to 2 mL gastight vials and incubated on the autosampler tray that was programmed to either 30, 33, or 37°C. Every hour, the gas in the headspace of each vial was measured using the GC-MS, the culture was transferred to a cuvette, and an OD measurement was performed using a Cary 50 spectrophotometer. Following the OD measurement, the culture was

transferred back to the vial, re-capped, and returned to the tray for the next incubation period. OD₆₀₀ readout was converted to CFU using a fit to a standard curve that revealed $CFU = OD_{600} \times 2 \times 10^8$ (*data not shown*).

Soil matrices. Two soils were used for measurements. First, we used a soil from the NSF Kellogg Biological Station Long-term Ecological Research station (KBS LTER) located in Hickory Corners, Michigan. We collected the 0–5 cm depth from the Ap horizon of a Typic Hapludalf (USDA soil taxonomic nomenclature) that was part of a switchgrass biofuels trial experiment. The soil particle distribution was measured using chemical dispersion followed by gravity precipitation³⁹ and found to be $64.9 \pm 0.6\%$ sand (2 to 0.05 mm particles), $27.2 \pm 0.5\%$ silt (0.05 to 0.002 mm), and $7.9 \pm 0.1\%$ clay (<0.002 mm). With the KBS soil, the water holding capacity (WHC), defined as the amount of water held after saturation and free drainage for 6 hours, was 0.33 g water/g soil. All the soil incubation experiments in this work were conducted at 0.25 g water/g soil (75.7% WHC) to represent agriculturally-relevant hydration conditions.

We used the particle size distribution of the KBS soil to guide the creation of an artificial soil by mixing varying sizes of quartz particles (U.S. Silica) corresponding to the sizes of sand (NJ 2, 1.19 mm to 1.68 mm), silt (Min-U-Sil 40, median size 8.71 μ m), and clay (Min-U-Sil 5, median size 1.7 μ m). Aggregates were created for artificial soil to mimic soil structure of natural soil using multiple wet-dry cycles. Briefly, artificial soil was wet to its water holding capacity and dried in oven at 105 °C overnight. The dried material was gently crushed and passed through 2 mm sieve. The step was repeated 3 times. All soils were sieved to 2 mm and autoclaved twice prior to incubation measurements.

Constitutive gas production in soils. To ensure that the amount of nutrient per cell was the same across experiments, we inoculated soil matrices with the same cell density as the liquid culture experiments (2×10^7 cells in 1 mL). Thus, with 200 μ L of the medium volume in soil matrices, we used 5-fold lower cells for inoculation (4×10^6 cells). For each sample, dry KBS or quartz soil (800 mg) was weighed and transferred to a 2-mL vial. M63 medium (200 μ L) containing 2×10^7 CFU of MG1655, MG1655-e_{fe} or MG1655-m_{ht} was added into the vial, and a sterile toothpick was used to homogenize the mixture. Vials were sealed with Parafilm to prevent evaporation and incubated on the autosampler tray set at 33°C for up to six hours. For each condition, 18 identical vials were prepared. After different durations (0, 60, 120, 180, 240, and 300 minutes), three vials were crimped for an hour before C₂H₄ and CH₃Br was measured by GC-MS. After each GC-MS measurement, cells from these samples were extracted to determine CFU following the incubation.

Soil bacteria extraction from soil. After GC-MS measurements, LB (800 μ L) was added to each vial, which increased the total liquid to 1 mL. Each vial was covered with Parafilm, vortexed for 30 seconds, and allowed to stand for 10 seconds so that the larger particles settled to the bottom. The liquid-soil mixture was serially diluted into LB medium, dilutions were spread on LB agar plates, and colonies were counted after incubating 16 hours at 37°C.

Measuring AHL following addition to soils. Experiments evaluating AHL effects on biosensor functions were performed by adding M63 medium (100 μ L) containing the various AHLs to KBS soil (800 mg), and adding M63 medium (100 μ L) containing 4×10^6 biosensor cells (MG1655-l_{as} or MG1655-l_{ux}) into the soil. The final soil water

content of each sample was 0.25. Samples were incubated statically at 33°C for three hours prior to using GC-MS to measure C₂H₄ and CH₃Br.

AHL degradation by *Bacillus*. Overnight cultures of *Bt* were used to inoculate fresh LB, and cells were grown to OD₆₀₀ = 0.5 at 30°C with 250 rpm shaking. The *Bt* culture was spun down and resuspended in fresh M63 supplemented with 0.01% casamino acid to support *Bt* growth⁴⁰ and ZnSO₄ (10 μM) to support lactonase activity.⁴¹ Varying titers of *Bt* (0, 10⁴, 10⁵, or 10⁶ CFU) were mixed with 3-oxo-C12-HSL (1 μM) in modified M63 (100 μL) and immediately added to 800 mg of KBS soil in 2 mL vials. Twelve vials were prepared for each *Bt* titer. Vials were sealed with Parafilm and incubated at 30°C for up to 36 hours. After different durations, Parafilm was removed from three vials in each group and M63 (100 μL) containing MG1655-las (4 x 10⁶) was added to the soil. The vials were immediately crimped and incubated at 33°C for 3 hours prior to the gas measurement. The number of MG1655-las and *Bt* were measured after each incubation by extracting cells, diluting the extracted cells, and spreading dilutions on LB-agar plates. After 16 hours at 37°C, *Bt* formed white colonies with rough edge while MG1655 formed colonies with smooth edges that were more yellow in color. This colony-morphological difference allowed us to enumerate the titer of each cell type following the gas measurements. Under the experimental conditions, *Bt* alone did not produce detectable C₂H₄ and CH₃Br.

***Rhizobium* AHL production dynamics.** YMB (2 mL) was inoculated with a single colony of *Rl* grown on a YMA plate at 30°C for four days. The *Rl* culture was incubated at 28°C with 250 rpm shaking for 48 hours until it reached an OD₆₀₀ = 0.4. The culture was diluted 10x in fresh YMB and then incubated for 24 hours while shaking at 28°C. At

the end of the incubation the OD₆₀₀ was ~0.5. This culture was spun down and resuspended in a modified YMB which had NaCl substituted by NaBr (0.2 g/L). *Rl* (10⁷ cells) in 150 µl of the modified YMB were added into the KBS soil (800 mg) in 2 mL vials. Vials were sealed with Parafilm, and incubated at 30°C. A YMB only control group was also set up in the same manner. After varying incubation times (0, 12, 24, 48, 72, and 96 hours), MG1655-las, MG1655-lux, or MG1655-mht-efe (4 x 10⁶ CFU each) were added into separate vials in triplicates. Both C₂H₄ and CH₃Br were measured following a 3 hour incubation at 33°C. *Rl* did not produce detectable amount of C₂H₄ and CH₃Br.

AHL-sensing by *Shewanella oneidensis* MR-1. An overnight culture of *S. oneidensis* transformed with pHC01 was used to inoculate fresh LB medium containing NaBr, and the resulting culture was grown in a shaking incubator at 30°C to an OD₆₀₀ = 0.5. The culture was pelleted, resuspended in fresh medium, and used for sensing in liquid culture and soil slurries. To evaluate AHL detection in liquid culture, 2 mL gastight vials containing 1 mL of LB medium containing NaBr and varying concentration of 3-oxo-C12-HSL were inoculated with 2 x 10⁷ *S. oneidensis* cells. To evaluate AHL in soil slurries, 150 µL of LB medium containing NaBr and varying concentrations of 3-oxo-C12-HSL was first mixed with 700 mg of KBS soil. Then, 150 µL of medium containing 6 x 10⁶ cells was mixed into the matrix. Vials were incubated at 33°C without shaking for 4 hours and 8 hours, respectively, prior to the gas measurement.

Statistical analysis. GraphPad Prism[®] 7 software was used to conduct ANOVA and AHL dose-response curve fitting. The gas production data was fitted to a dose-response curve (Equation 1):

$$y = b + \frac{x^h(a-b)}{x^h + EC50^h} \quad \text{Eq.1}$$

where y is the gas production of a biosensor, x is the AHL concentration, a represents the maximal response, and b is the basal response. h indicates the hill slope, which represents the steepness of a response curve and $EC50$ is the concentration of AHL that results in the half-maximum response of a system. An exponential model was used to describe the half-life of AHL co-culture with *Bt* (Equation 2):

$$y = (y_0 - G)e^{-kx} + G \quad \text{Eq. 2}$$

where y is the gas production of a biosensor, x is the incubation time, y_0 is the y value at x is zero, G is the gas production at infinite times (the value must be greater than zero), and k is the rate constant. Student t -test and the linear regression of the C_2H_4 and CH_3Br standard curves were calculated in Excel.

SUPPORTING INFORMATION

The Supporting Information is available free of charge on the ACS Publications website at DOI:

Data showing growth of microbes at different temperatures and in soils, extracted CFU from soils, constitutive gas production, and effect of chemical inputs on microbial gas production (PDF)

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CITATIONS

- (1) Tsien, R. Y. (1998) The green fluorescent protein. *Annu. Rev. Biochem.* 67, 509–544.
- (2) Contag, C. H., and Bachmann, M. H. (2002) Advances in *in vivo* bioluminescence imaging of gene expression. *Annu Rev Biomed Eng* 4, 235–260.
- (3) Purnick, P. E. M., and Weiss, R. (2009) The second wave of synthetic biology: from modules to systems. *Nat. Rev. Mol. Cell Biol.* 10, 410–422.
- (4) Kallmeyer, J., Pockalny, R., Adhikari, R. R., Smith, D. C., and D'Hondt, S. (2012) Global distribution of microbial abundance and biomass in subseafloor sediment. *Proc Natl Acad Sci USA* 109, 16213–16216.
- (5) Sánchez, O. J., and Cardona, C. A. (2008) Trends in biotechnological production of fuel ethanol from different feedstocks. *Bioresour. Technol.* 99, 5270–5295.
- (6) Bayer, T. S., Widmaier, D. M., Temme, K., Mirsky, E. A., Santi, D. V., and Voigt, C. A. (2009) Synthesis of methyl halides from biomass using engineered microbes. *J. Am. Chem. Soc.* 131, 6508–6515.
- (7) Cheng, H.-Y., Masiello, C. A., Bennett, G. N., and Silberg, J. J. (2016) Volatile gas production by methyl halide transferase: an *in situ* reporter of microbial gene expression in soil. *Environ. Sci. Technol.* 50, 8750–8759.
- (8) Dai, Z., Webster, T. M., Enders, A., Hanley, K. L., Xu, J., Thies, J. E., and Lehmann, J. (2017) DNA extraction efficiency from soil as affected by pyrolysis temperature and extractable organic carbon of high-ash biochar. *Soil Biology and Biochemistry* 115, 129–136.
- (9) van der Meer, J. R., and Belkin, S. (2010) Where microbiology meets

- microengineering: design and applications of reporter bacteria. *Nat. Rev. Microbiol.* 8, 511–522.
- (10) Danino, V. E., Wilkinson, A., Edwards, A., and Downie, J. A. (2003) Recipient-induced transfer of the symbiotic plasmid pRL1JI in *Rhizobium leguminosarum* bv. *viciae* is regulated by a quorum-sensing relay. *Mol. Microbiol.* 50, 511–525.
- (11) Schmidt, M. W. I., Torn, M. S., Abiven, S., Dittmar, T., Guggenberger, G., Janssens, I. A., Kleber, M., Kögel-Knabner, I., Lehmann, J., Manning, D. A. C., Nannipieri, P., Rasse, D. P., Weiner, S., and Trumbore, S. E. (2012) Persistence of soil organic matter as an ecosystem property. *Nature* 478, 49–56.
- (12) Hentzer, M., Riedel, K., Rasmussen, T. B., Heydorn, A., Andersen, J. B., Parsek, M. R., Rice, S. A., Eberl, L., Molin, S., Høiby, N., Kjelleberg, S., and Givskov, M. (2002) Inhibition of quorum sensing in *Pseudomonas aeruginosa* biofilm bacteria by a halogenated furanone compound. *Microbiology (Reading, Engl.)* 148, 87–102.
- (13) Ni, X., and Hager, L. P. (1999) Expression of *Batis maritima* methyl chloride transferase in *Escherichia coli*. *Proc. Natl. Acad. Sci. U.S.A.* 96, 3611–3615.
- (14) Digiaco, F., Girelli, G., Aor, B., Marchioretti, C., Pedrotti, M., Perli, T., Tonon, E., Valentini, V., Avi, D., Ferrentino, G., Dorigato, A., Torre, P., Jousson, O., Mansy, S. S., and Del Bianco, C. (2014) Ethylene-producing bacteria that ripen fruit. *ACS Synth Biol* 3, 935–938.
- (15) Papenfort, K., and Bassler, B. L. (2016) Quorum sensing signal-response systems in Gram-negative bacteria. *Nat. Rev. Microbiol.* 14, 576–588.
- (16) Winson, M. K., Swift, S., Fish, L., Throup, J. P., Jørgensen, F., Chhabra, S. R., Bycroft, B. W., Williams, P., and Stewart, G. S. (1998) Construction and analysis of

- luxCDABE-based plasmid sensors for investigating N-acyl homoserine lactone-mediated quorum sensing. *FEMS Microbiol. Lett.* 163, 185–192.
- (17) Masiello, C. A., Chen, Y., Gao, X., Liu, S., Cheng, H.-Y., Bennett, M. R., Rudgers, J. A., Wagner, D. S., Zygourakis, K., and Silberg, J. J. (2013) Biochar and Microbial Signaling: Production Conditions Determine Effects on Microbial Communication. *Environ Sci Technol* 47, 11496–11503.
- (18) Gao, X., Cheng, H. Y., Del Valle, I., Liu, S., Masiello, C. A., and Silberg, J. J. (2016) Charcoal disrupts soil microbial communication through a combination of signal sorption and hydrolysis. 1, 226–233.
- (19) Nealson, K. H., and Saffarini, D. (1994) Iron and manganese in anaerobic respiration: environmental significance, physiology, and regulation. *Annu. Rev. Microbiol.* 48, 311–343.
- (20) Dong, Y.-H., Gusti, A. R., Zhang, Q., Xu, J.-L., and Zhang, L.-H. (2002) Identification of quorum-quenching N-acyl homoserine lactonases from *Bacillus* species. *Appl. Environ. Microbiol.* 68, 1754–1759.
- (21) Bereza-Malcolm, L. T., Mann, G., and Franks, A. E. (2015) Environmental sensing of heavy metals through whole cell microbial biosensors: a synthetic biology approach. *ACS Synth Biol* 4, 535–546.
- (22) Vogel, C., Babin, D., Pronk, G. J., Heister, K., Smalla, K., and Kögel-Knabner, I. (2014) Establishment of macro-aggregates and organic matter turnover by microbial communities in long-term incubated artificial soils. *Soil Biology and Biochemistry* 79, 57–67.
- (23) Ishihara, K., Matsouka, M., Inoue, Y., Tanase, S., Ogawa, T., and Fukuda, H.

- (1995) Overexpression and *In Vitro* Reconstitution of the Ethylene-Forming Enzyme from *Pseudomonas syringae*. *Journal of Fermentation and Bioengineering* 79, 205–211.
- (24) Fukuda, H., Ogawa, T., Tazaki, M., Nagahama, K., Fujii, T., Tanase, S., and Morino, Y. (1992) Two reactions are simultaneously catalyzed by a single enzyme: the arginine-dependent simultaneous formation of two products, ethylene and succinate, from 2-oxoglutarate by an enzyme from *Pseudomonas syringae*. *Biochem. Biophys. Res. Commun.* 188, 483–489.
- (25) Fontecave, M., Atta, M., and Mulliez, E. (2004) S-adenosylmethionine: nothing goes to waste. *Trends Biochem. Sci.* 29, 243–249.
- (26) Kotula, J. W., Kerns, S. J., Shaket, L. A., Siraj, L., Collins, J. J., Way, J. C., and Silver, P. A. (2014) Programmable bacteria detect and record an environmental signal in the mammalian gut. *Proc Natl Acad Sci USA* 111, 4838–4843.
- (27) Landry, B. P., and Tabor, J. J. (2017) Engineering Diagnostic and Therapeutic Gut Bacteria. *Microbiol Spectr* 5.
- (28) Riglar, D. T., Giessen, T. W., Baym, M., Kerns, S. J., Niederhuber, M. J., Bronson, R. T., Kotula, J. W., Gerber, G. K., Way, J. C., and Silver, P. A. (2017) Engineered bacteria can function in the mammalian gut long-term as live diagnostics of inflammation. *Nat. Biotechnol.* 35, 653–658.
- (29) LeCroy, C., Masiello, C. A., Rudgers, J. A., Hockaday, W. C., and Silberg, J. J. (2013) Nitrogen, biochar, and mycorrhizae: Alteration of the symbiosis and oxidation of the char surface. *Soil Biology and Biochemistry* 58, 248–254.
- (30) Schulz, S., and Dickschat, J. S. (2007) Bacterial volatiles: the smell of small

- organisms. *Nat Prod Rep* 24, 814–842.
- (31) Archbold, M. E., Redeker, K. R., Davis, S., Elliot, T., and Kalin, R. M. (2005) A method for carbon stable isotope analysis of methyl halides and chlorofluorocarbons at pptv concentrations. *Rapid Commun. Mass Spectrom.* 19, 337–342.
- (32) Studer, M. S., Künzli, R., Maier, R., Schmidt, M. W. I., Siegwolf, R. T. W., Woodhatch, I., and Abiven, S. (2017) The MICE facility - a new tool to study plant-soil C cycling with a holistic approach. *Isotopes Environ Health Stud* 53, 286–297.
- (33) Mutalik, V. K., Guimaraes, J. C., Cambray, G., Lam, C., Christoffersen, M. J., Mai, Q.-A., Tran, A. B., Paull, M., Keasling, J. D., Arkin, A. P., and Endy, D. (2013) Precise and reliable gene expression via standard transcription and translation initiation elements. *Nature Methods* 10, 354–360.
- (34) Nagahama, K., Ogawa, T., Fujii, T., Tazaki, M., Tanase, S., Morino, Y., and Fukuda, H. (1991) Purification and properties of an ethylene-forming enzyme from *Pseudomonas syringae* pv. *phaseolicola* PK2. *J. Gen. Microbiol.* 137, 2281–2286.
- (35) St-Pierre, F., Cui, L., Priest, D. G., Endy, D., Dodd, I. B., and Shearwin, K. E. (2013) One-step cloning and chromosomal integration of DNA. *ACS Synth Biol* 2, 537–541.
- (36) Diederich, L., Rasmussen, L. J., and Messer, W. (1992) New cloning vectors for integration in the lambda attachment site attB of the *Escherichia coli* chromosome. *Plasmid* 28, 14–24.
- (37) Gray, K. M., Pearson, J. P., Downie, J. A., Boboye, B. E., and Greenberg, E. P. (1996) Cell-to-cell signaling in the symbiotic nitrogen-fixing bacterium *Rhizobium*

- 1
2
3 *leguminosarum*: autoinduction of a stationary phase and rhizosphere-expressed
4 genes. *J. Bacteriol.* 178, 372–376.
5
6
7
8 (38) Myers, C. R., and Myers, J. M. (1997) Replication of plasmids with the p15A origin
9 in *Shewanella putrefaciens* MR-1. *Lett. Appl. Microbiol.* 24, 221–225.
10
11
12 (39) Kettler, T. A., Doran, J. W., and Gilbert, T. L. (2017) Simplified method for soil
13 particle-size determination to accompany soil-quality analyses. *Soil Science*
14 *Society of America Journal* 1–5.
15
16
17 (40) Nickerson, K. W., and Bulla, L. A. (1974) Physiology of sporeforming bacteria
18 associated with insects: minimal nutritional requirements for growth, sporulation,
19 and parasporal crystal formation of *Bacillus thuringiensis*. *Appl Microbiol* 28, 124–
20 128.
21
22
23 (41) Thomas, P. W., Stone, E. M., Costello, A. L., Tierney, D. L., and Fast, W. (2005)
24 The quorum-quenching lactonase from *Bacillus thuringiensis* is a metalloprotein.
25 *Biochemistry* 44, 7559–7569.
26
27
28
29
30
31
32
33
34
35
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FIGURE LEGENDS

Figure 1. Effect of temperature on volatile gas production rates. (a) In MG1655-efe, EFE uses α -ketoglutarate (AKG) and oxygen as substrates to produce C_2H_4 . (b) In MG1655-mht. MHT uses S-Adenosyl Methionine (SAM) and bromide (Br^-) as substrates to synthesize CH_3Br and S-Adenosyl Homocysteine (SAH). (c) The C_2H_4 and (d) CH_3Br production rates of MG1655-efe and MG1655-mht in M63 liquid medium at 30°C, 33°C, and 37°C. Rates were calculated as the gas produced per hour per CFU following a 1 hour incubation. At 33°C, the C_2H_4 production rate did not significantly change across the different time points (one-way ANOVA, $p > 0.05$). Error bars represent $\pm 1\sigma$ from three experiments and are only plotted when larger than symbols.

Figure 2. Gas production rates in matrices. (a) MG1655-efe and (b) MG1655-mht incubated at 33°C in an artificial quartz soil (80% matrix and 20% M63 medium) yielded C_2H_4 and CH_3Br that could be detected in the headspace. The C_2H_4 production rate showed no significant difference across all time points. With the CH_3Br production rates, only the first time point is significantly lower than the other time points. (c) MG1655-efe and (d) MG1655-mht grown in the loamy agricultural soil held at environmentally-relevant hydration (80% soil and 20% M63 medium) also yielded strong C_2H_4 and CH_3Br production rates. The C_2H_4 production rates presented no significant differences from 120 to 360 minutes, while the CH_3Br production rate showed no significant differences between 180 and 360 minutes. Error bars represent $\pm 1\sigma$ from three experiments and are only plotted when larger than symbols. n.s., not significant (one-way ANOVA followed by Tukey multiple comparison test, $p > 0.05$).

Figure 3. Using ratiometric reporting to quantify AHLs in soil. (a) Scheme illustrating the genetic circuits used to program *E. coli* to report on 3-oxo-C6-HSL (MG1655-lux) and 3-oxo-C12-HSL (MG1655-las). In these circuits, EFE is expressed using a constitutive promoter (P_1), MHT is regulated by either the lux (P_{lux}) or las (P_{las}) promoters, and LuxR/LasR are constitutively expressed (P_{con}). Varying concentrations of 3-oxo-C6-HSL and 3-oxo-C12-HSL were added to a loamy agricultural soil, soils were incubated for 3 hours at 33°C with identical titers of (b, d) MG1655-lux and (c,e) MG1655-las, and the amounts of C₂H₄, (blue symbols) and CH₃Br (green symbols) were measured in the headspace of sample vials. This data was used to calculate the ratiometric signal (CH₃Br/C₂H₄) generated by (f) MG1655-lux and (g) MG1655-las in the loamy agricultural soil (black symbols). Identical experiments performed using cells in M63 medium lacking soil (grey symbols) are shown for comparison. Error bars represent $\pm 1\sigma$ calculated using three replicates and are only shown when larger than the symbol. Dashed lines represent a dose-response curve fit.

Figure 4. Robustness of the ratiometric signals in soil. The relationship between the total time where the gas was allowed to accumulate following biosensor addition and C₂H₄, CH₃Br, and the ratiometric signals for (a) MG1655-lux and (b) MG1655-las that had been added into loamy agricultural soil at 33°C containing 1 μ M of 3-oxo-C6-HSL and 3-oxo-C12-HSL, respectively. The ratios of CH₃Br to C₂H₄ present no significant differences across all incubation times (one-way ANOVA, $p > 0.05$). Error bars represent $\pm 1\sigma$ calculated using three replicates.

Figure 5. Ratiometric gas reporting can be used in *Shewanella oneidensis* MR1. (a) A ratiometric biosensor plasmid, pHCO1, was constructed that constitutively

expresses a bicistronic EFE/RFP cassette and uses LasR to regulate MHT expression. This plasmid was transformed into *S. oneidensis* MR-1. (b) Gas production was measured in 2 mL vials containing liquid medium or a soil slurry at 33°C. The slurry was 70% soil and 30% LB medium. (c) *S. oneidensis* MR-1 transformed with pHCO1 was added into liquid medium and the soil slurry that had been pre-inoculated with various concentration of 3-oxo-C12-HSL, and C₂H₄ and CH₃Br were measured 4 hours and 8 hours, respectively. The gas ratio was calculated, normalized to the highest value obtained, and a dose-response curve was fit to the data. Error bars represent $\pm 1\sigma$ calculated using three replicates and are only shown when larger than the symbol.

Figure 6. Monitoring *Bt* degradation of AHL in soil. (a) Scheme illustrating how the *E. coli* MG1655-las biosensor (cyan) was used to spy on *Bt* (yellow) degradation of 3-oxo-C12-HSL in soil. *Bt* synthesizes an AHL-degrading lactonase. Soils containing 3-oxo-C12-HSL and *Bt* were incubated for the indicated durations at 30°C, MG1655-las was added and the temperature was shifted to 33°C, and the gas ratio was measured 3 hours following MG1655-las addition to assess the level of 3-oxo-C12-HSL remaining in the soil. (b) The ratiometric signal generated by MG1655-las that had been added to soils containing 1 μ M 3-oxo-C12-HSL and either 0 or 10⁶ cfu of *Bt*. The incubation time represents how long *Bt* and 3-oxo-C12-HSL were allowed to incubate in the loamy agricultural soil prior to MG1655-las addition. Error bars represent $\pm 1\sigma$ calculated using three replicates. The line represents a fit of the data to a single exponential model.

Figure 7. Dynamics of *Rhizobium* AHL synthesis in soil. (a) Scheme showing how *E. coli* MG1655-lux and MG1655-las (cyan and blue, respectively) were used to spy on *Rl* (orange) synthesis of 3-oxo-C8-HSL and 3-OH-C14:1-HSL. Following different

incubations of *R/I* in soil at 30°C, the biosensors were added, the temperature was shifted to 33°C, and the ratiometric signal was used to read out the accumulation of each AHL. **(b)** Ratiometric signals arising from adding MG1655-lux and MG1655-las to the loamy agricultural soil containing different concentrations of 3-oxo-C8-HSL and **(c)** 3-OH-C14:1-HSL. A fit of a dose response curve to the MG1655-lux data yielded an $EC_{50} = 3.3 \pm 1.9 \times 10^{-7}$ M for 3-oxo-C8-HSL, while a fit of the curves for MG1655-las data produced EC_{50} values of $2.6 \pm 0.8 \times 10^{-6}$ and $1.1 \pm 0.2 \times 10^{-5}$ for 3-oxo-C8-HSL and 3-OH-C14:1-HSL, respectively. **(d)** The ratiometric signals arising from adding MG1655-lux and **(e)** MG1655-las to the loamy agricultural soil at different times after addition of medium containing *R/I* (shaded circles) or lacking *R/I* (open circles). AHL concentrations produced by *R/I* were estimated by comparing these ratiometric signals to the standard curves generated in soil. The concentration of 3-oxo-C8-HSL detected by MG1655-lux is not sufficient to activate CH₃Br production in MG1655-las, indicating that the signal obtained from MG1655-las arises solely from 3-OH-C14:1-HSL, even though this sensor can be activated by 3-oxo-C8-HSL. Error bars represent $\pm 1\sigma$ calculated using three replicates and are only shown when larger than the symbol.

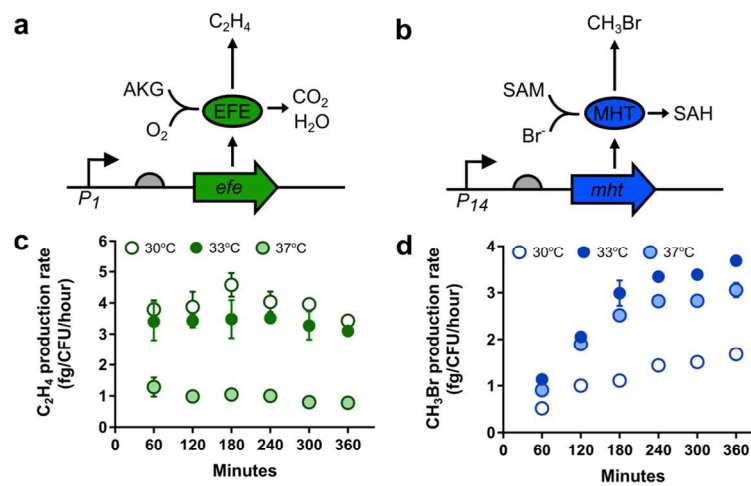


Figure 1

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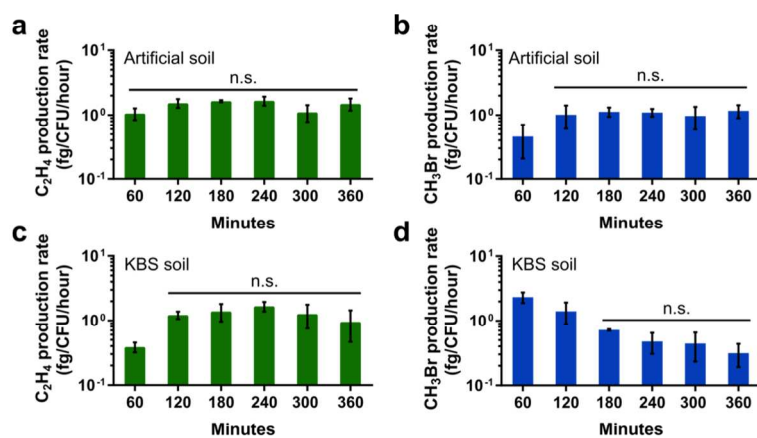


Figure 2

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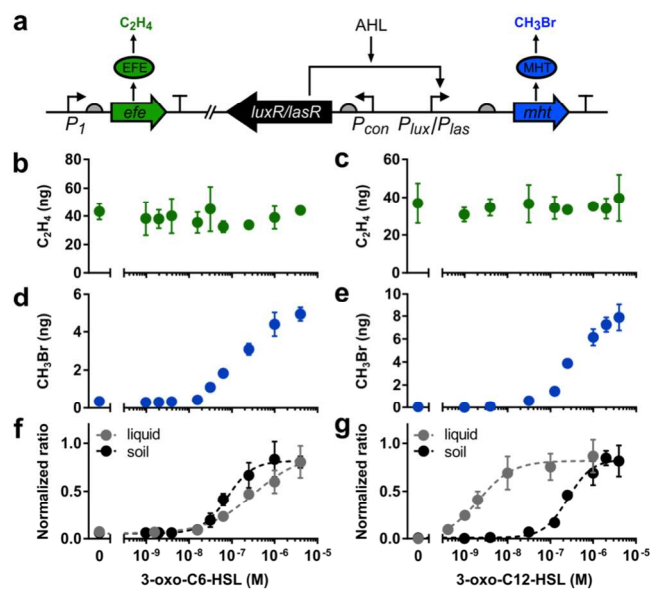


Figure 3

529x527mm (72 x 72 DPI)

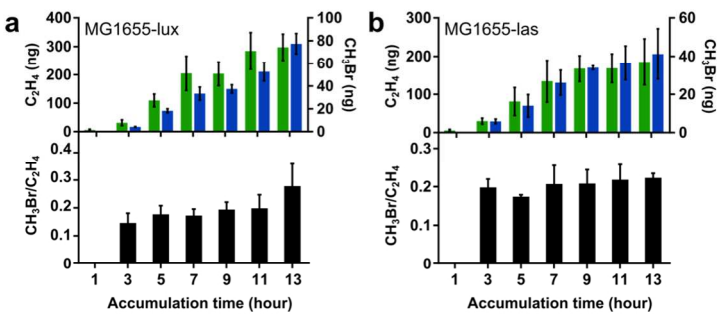


Figure 4

529x527mm (72 x 72 DPI)

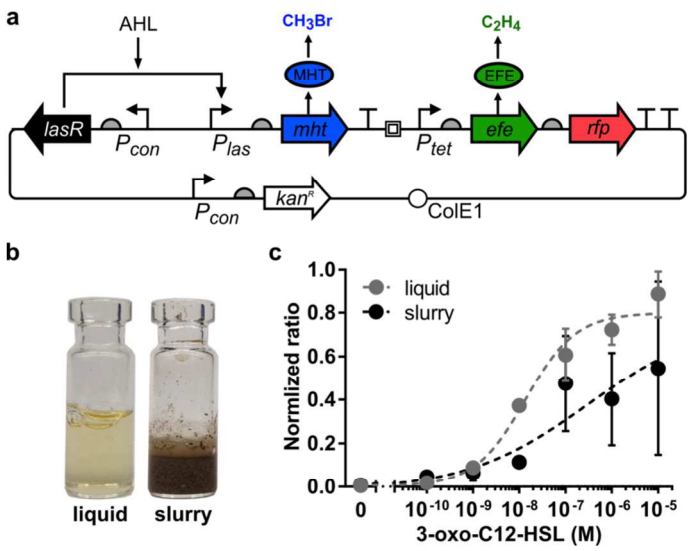


Figure 5

529x527mm (72 x 72 DPI)

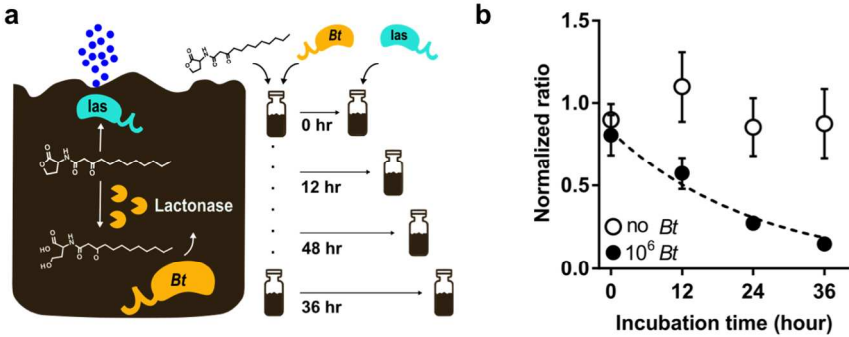


Figure 6

529x527mm (72 x 72 DPI)

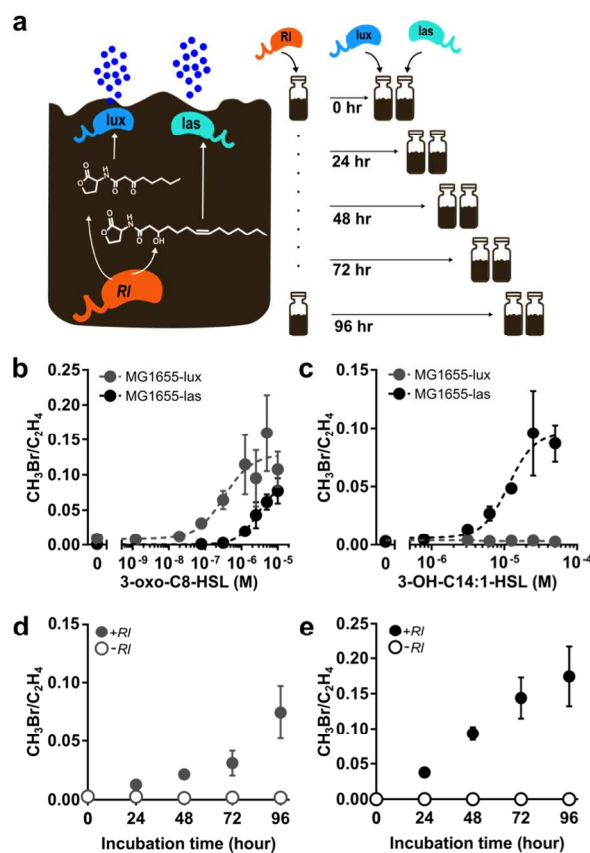
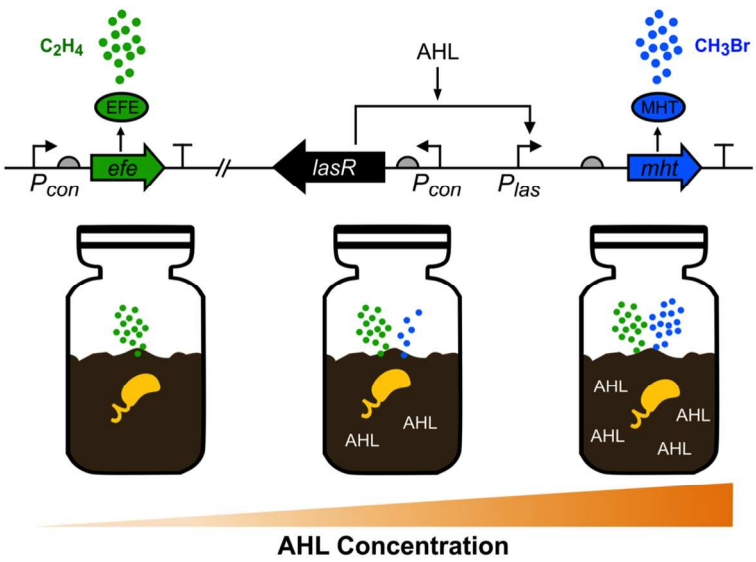


Figure 7

529x527mm (72 x 72 DPI)



TOC Art

529x527mm (72 x 72 DPI)