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Letter

Repression of Microbial Arsenite Uptake and Methylation by Dissolved Organic Carbon

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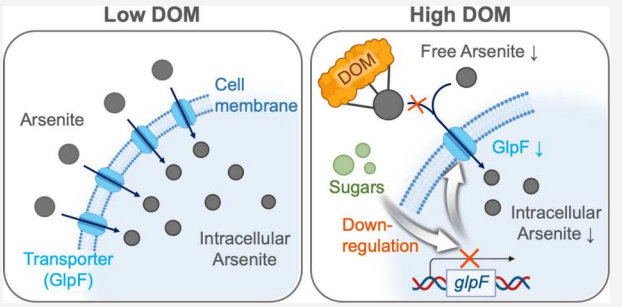
ABSTRACT: Arsenic methylation is the microbe-mediated transformation of inorganic As into methylated species, an important component of the biogeochemical arsenic cycle in rice paddies. Prior to methylation, arsenite is taken up into bacterial cells through GlpF, an aquaglyceroporin channel for uptake of glycerol and other low-molecular-weight organics. The uptake and subsequent biotransformation of arsenite are therefore linked to the bacterial utilization of organics. We hypothesized that increasing concentrations of carbon substrates will repress the uptake and methylation of arsenite through a carbon catabolite repression (CCR) mechanism. An arsenic biosensor assay demonstrated that arsenite uptake was repressed in the presence of glucose and environmental dissolved organic matter (DOM) isolates. RT-qPCR analysis of *glpF* expression linked the decrease in arsenite uptake at higher carbon concentrations to the repression of glycerol-transporting GlpF channels. Methylation of arsenite by *Arsenicibacter rosenii*, a rice paddy isolate, was repressed by the upper glycolytic substrates glucose, xylose, and mannose, but was not affected by pyruvate and succinate. This result is consistent with current CCR theories. Our findings provide a new perspective on the impacts of organic carbon on microbial arsenic transformations, and suggest that arsenic biotransformation can be repressed in systems that are rich in upper glycolytic carbon substrates.



KEYWORDS: *arsenic, microbial uptake, methylation, bioavailability, biosensor, dissolved organic carbon, catabolite repression, arsM, glpF*

INTRODUCTION

The microbial transformation of inorganic arsenic (As) into methylated species is an important biogeochemical process regulating As speciation in soil and aquatic environments.[1−3] Dimethylarsinic acid (DMA) is readily translocated to rice grains where it influences the toxicity of arsenic in rice,[4−7] and it is also phytotoxic and can decrease rice yields by causing straighthead disease.[8,9] These impacts have motivated intensive research into microbial As methylation in rice paddy soils.[10−13] Arsenic methylation is catalyzed by intracellular arsenite S-adenosylmethionine methyltransferase (ArsM) enzymes,[14] and *arsM* genes are widely distributed in functionally diverse microorganisms.[12,15−18] There has been significant research to identify microbial populations driving methylation reactions[15−20] and to characterize the biogeochemical controls on methylation efficiencies.[21−25] Despite these efforts, variations in methylation efficiencies between soils,[26] experimental treatments,[24] or geographic locations[7] often go unexplained.

Prior research from our group has highlighted effects of dissolved organic matter (DOM) on bioavailability of As to microorganisms, with implications for regulating As biotransformations.[27−29] Trivalent arsenite (pK_a = 9.2) is taken up into microbial cells through aquaglyceroporin channels, including the glycerol facilitator channel (GlpF) in *E. coli*.[30] These channels transport glycerol and other uncharged, low molecular weight (LMW) carbon compounds (e.g., glycine; xylitol) into cells.[30−32] This shared transporter for both arsenite and glycerol highlights potential couplings between bacterial utilization of organics and arsenite uptake. For example, the uptake of glycerol by *E. coli* is repressed by the uptake of glucose,[33,34] and the repression of glycerol uptake may impact arsenite uptake and subsequent transformation. This hierarchical utilization of carbon substrates is known as carbon catabolite repression (CCR).[35−37] Current conceptual models for CCR indicate that upper glycolytic substrates such as sugars repress the uptake of glycerol, while nonglycolytic substrates including LMW organic acids can be coutilized with glycerol and do not affect glycerol uptake.[33,34] In the environmental sciences, catabolite repression theories have been used to explain observations that transformations of

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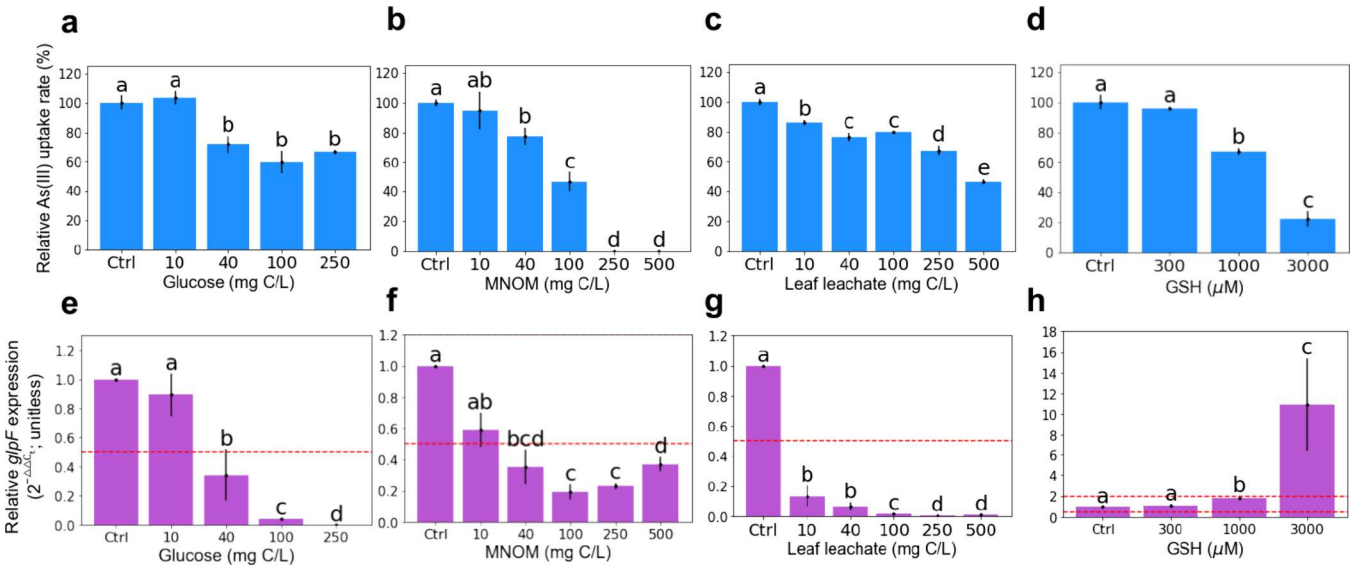

Figure 1. Relative arsenite uptake rates (top row) and relative expression of *glpF* in biosensor cells (bottom row) in the presence of (a and e) glucose, (b and f) Upper Mississippi River natural organic matter (MNOM), (c and g) leaf leachate, and (d and h) glutathione (GSH). Ctrl is the control assay which contained 1 μM arsenite without additional carbon amendment. Arsenite uptake rates are biomass-normalized. An alphabetical letter over each bar denotes significant difference ($p < 0.05$) between bars determined using a one-way ANOVA with Tukey's honestly significant difference (HSD) post hoc testing. The red dashed line in (e)−(h) denotes a 2-fold change in *glpF* expression relative to the control. Note the different *y*-axis limits in panel (h).

organic pollutants are suppressed at higher labile carbon concentrations.[36,38−40] To our knowledge, impacts of catabolite repression on cellular uptake and subsequent methylation of arsenite due to repression of glycerol uptake have not been explored.

Here, we test the hypothesis that increasing concentrations of organic carbon—in particular glucose and other carbon substrates utilized in the upper glycolysis pathway—will repress the microbial uptake and methylation of arsenite, via a CCR-like process. We study the effects of model carbon substrates and environmental DOM isolates on the expression of GlpF transporters and uptake of arsenite in an *E. coli* arsenic biosensor, and on arsenic biomethylation using experiments with *Arsenicibacter rosenii*. This contribution connects arsenic methylation to the quality and quantity of dissolved organic carbon, and has the potential to provide mechanistic clarity and focus to ongoing lines of inquiry by the research community investigating biogeochemical controls on arsenic speciation in rice paddy soils.

METHODS AND MATERIALS

Preparation and Analysis of Carbon Sources and DOM Solutions. Glucose, xylose, and mannose were studied as examples of upper glycolytic substrates that are expected to repress glycerol uptake, while pyruvate and succinate were studied as nonglycolytic substrates that can be coutilized with glycerol and therefore not repress glycerol uptake.[34] Glycine and xylitol were studied as examples of carbon sources that are taken up into cells through GlpF channels, and glutathione (GSH) was studied as a thiol-containing model compound that can affect arsenite bioavailability through complexation.[41]

Upper Mississippi River natural organic matter (MNOM) and leaf leachate were studied as environmental DOM samples. We also performed measurements with a rice straw leachate, but because the leachate was found to contain 0.097 μg As/mg C (Table S1) we excluded this sample from further analysis (see Note S1 for more information). Liquid chromatography (Thermo Fisher Scientific Dionex UltiMate 3000) coupled to high-resolution/accurate mass spectrometry (Thermo Fisher Scientific Q Exactive quadrupole-Orbitrap hybrid MS) analysis was used to quantify LMW compounds including short-chain carboxylic acids, phenolic acids, amino acids, and nucleobases.[42] ^{1}H nuclear magnetic resonance spectroscopy was used to quantify glucose and xylose, which are the primary sugar monomers of cellulose and hemicellulose, respectively. All organic carbon samples were filtered (0.22 μm) before use.

Arsenic Biosensor Assay. The overall experimental workflow is shown in Figure S1. A whole cell *E. coli* NEB 10-beta biosensor assay[43] was used to quantify arsenite uptake in the presence of model organic carbon substrates and environmental DOM isolates. Biosensor fluorescence, induced by the presence of intracellular arsenite, provides a quantitative measure of cellular As uptake.[27,44] The biomass-normalized net arsenite uptake rate is proportional to $\sqrt{\frac{1}{N(t)}\frac{dF_{RFU}}{dt}}$, where $N(t)$ is the cell density at time t and dF_{RFU}/dt is the slope of the fluorescence curve over the first 5 h of the experiment. See Figure S2 and Yoon et al.[27] for additional details on the biosensor assay. Biosensor experiments were performed in two steps: First, cells were acclimated to different carbon concentrations (10−500 mg C/L) in an overnight preincubation. Then, biosensor cells were exposed to 1 μM arsenite and biosensor fluorescence and optical density at 600 nm (OD_{600}) were monitored for more than 10 h on a plate reader. The glycerol and LB broth used in the growth media were both diluted 10x from a standard *E. coli* growth media, to 300 mg C/L glycerol and 20 mg C/L in the LB broth, to create a lower background carbon concentration. Biomass-normalized biosensor results are reported as the arsenite uptake rate relative to a control without carbon addition.[27]

Dialysis Experiments. Float-A-Lyzer dialysis devices (Spectra-Por) with a membrane pore size of 0.1−0.5 kDa

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were used for dialysis equilibrium experiments[45] to quantify arsenite binding to DOM in conditions mimicking the arsenite-to-carbon ratios in biosensor experiments (see Note S3 and Yoon et al.[28] for details).

Arsenic Methylation Assay. *A. rosenii*, an aerobic bacterium isolated from an As-contaminated rice paddy soil,[46,47] was used in an As methylation assay. *A. rosenii* lacks an ArsB efflux transporter, allowing arsenite to accumulate inside cells and be efficiently methylated by ArsM.[17,47] *A. rosenii* was first acclimated to DOM isolates and model carbon compounds during an overnight preincubation, followed by exposure to 1 μM arsenite and monitoring of OD_{600} and As speciation. Arsenic speciation analysis in 0.22-μm filtered samples was performed with HPLC-ICP-MS (Agilent Infinity 1260 HPLC hyphenated to Agilent 7800 ICP-MS).[16] Samples were oxidized with 6% H_2O_2 to convert arsenite to arsenate and reveal the presence of trimethylarsine oxide (TMAO) that coelutes with arsenite.[16] The biomass-normalized As methylation efficiency was calculated as

$$\text{As methylation efficiency (\%)} = \frac{DMA_{24h} + TMAO_{24h}}{\sum As_{24h} \times OD_{600,24h}} \times 100\% \quad (1)$$

$DMA_{24\ h}$ and $TMAO_{24\ h}$ are the concentrations of DMA and TMAO, respectively, after 24 h of incubation, $\sum As_{24h}$ is the sum of As species at 24 h, and $OD_{600,24\ h}$ is the OD_{600} at 24 h. Monomethylarsonic acid (MMA) concentrations were below the limit of quantification, and we did not monitor volatile arsines. Methylation efficiencies were first tested using R2A media at 10%, 20%, 50%, and 100% strength,[17] and the 20% strength R2A (containing ~200 mg C/L) was chosen for the remainder of the methylation experiments to provide a low-carbon baseline.

Gene Expression Analysis. Reverse transcription (RT)-qPCR was used to monitor expression of *glpF* in *E. coli* biosensor cultures and *arsM* in *A. rosenii* cultures to evaluate physiological responses to variations in carbon. Expression of *glpF* is used as a direct measure of production of glycerol- and arsenite-transporting GlpF channels. Because the uptake channels for arsenite in *A. rosenii* are not known, we instead used the expression of *arsM* as a proxy for intracellular As because it is induced by intracellular As (among other factors).[14] Details on RT-qPCR methods are provided in Note S5 and Table S2.

RESULTS AND DISCUSSION

Biosensor Assay and *glpF* Expression. Relative to the control, cellular arsenite uptake decreased in the presence of glucose, GSH, MNOM, and leachates from leaves (Figure 1a-d). Uptake decreased by 40 to 60% when glucose and leaf leachate concentrations increased to 250 and 500 mg C/L, respectively, and complete inhibition of arsenite uptake was observed with MNOM (Figure 1a-c). 3,000 μM GSH led to an 80% decrease in arsenite uptake (Figure 1d). With the exception of GSH, the decrease in arsenite uptake was accompanied by a 1.3 to 2.4-fold increase in cell growth (Figure S3), indicating that environmental DOM isolates contained bioavailable carbon. The MNOM and leaf leachate samples contained a diverse range of LMW compounds that could serve as carbon sources (Figure S4). OD_{600} data showed no evidence of solution toxicity impeding cell growth (Figure S3). Interestingly, glycine and xylitol, two carbon substrates taken up through GlpF,[32] had no effect on arsenite uptake (Figure S5) and negligible effect on cell growth (Figure S6). Therefore, unlike the case of arsenate and orthophosphate where there is direct competition for uptake through the Pst phosphate transport system,[48] there was no evidence for direct competition between arsenite and organics for transport through GlpF.

Expression of *glpF* decreased by more than 80% in the presence of MNOM, and by more than 90% in the presence of glucose and leaf leachate (Figure 1e-g; and see Figures S7 and S8 for complete RT-qPCR data). This confirmed a physiological response to increasing carbon concentrations that suppressed production of arsenite-transporting GlpF channels.[34] Down-regulation of *glpF* in the presence of glucose (Figure 1e) is consistent with classical CCR theories for *E. coli*[37], where preferential uptake of glucose leads to a decrease in uptake of glycerol.[32−34,49] In contrast, *glpF* expression increased 1.8 to 10.9-fold with increasing GSH concentrations (Figure 1h), indicating that the decrease in arsenite uptake at high GSH concentrations cannot be explained by a physiological repression of GlpF channels. The reason for GSH stimulation of *glpF* expression remains unclear. While the biosensor data suggest a clear link between repressed *glpF* transporter expression and lower arsenite uptake with higher concentrations of glucose, MNOM, and leaf leachate, we cannot rule out the possibility that changes in other physiological processes such as expression of the *arsB* efflux permease may have also impacted net As uptake.

Effects of Arsenite-DOM Complexation on Repressed As Uptake. Between 10 and 22% of arsenite was bound to DOM in the environmental isolates, while there was no evidence of arsenite complexation with glucose (Figure S9). GSH, a thiol-containing complexing ligand for arsenite,[41] complexed 10 to 77% of the arsenite as GSH concentrations increased from 300 to 3000 μM. Comparison of biosensor measurements of repressed cellular uptake versus dialysis measurements of arsenite-DOM binding (Figure S10) indicates that decreased arsenite uptake in the presence of GSH can be explained by arsenite-GSH complexation in the media, while decreased arsenite uptake in the presence of glucose was unrelated to complexation and could instead be attributed to physiological changes. For MNOM and leaf leachate, arsenite uptake was affected by a combination of ligand complexation and physiological processes. There appeared to be a large physiological effect on arsenite uptake for 100 and 500 mg C/L MNOM and 500 mg C/L leaf leachate because uptake was inhibited by 50−100% while only ~20% of the arsenite was DOM-bound.

Biomethylation Assay and *arsM* Expression. *A. rosenii* methylated arsenite into a combination of DMA and TMAO (Figures S11 and S12). The biomass-normalized methylation efficiency of *A. rosenii* decreased by 66% when the R2A media strength increased from 10% to 100% (Figure S13), clearly showing that methylation was repressed on a per-cell basis in the carbon-rich full-strength media. While production of DMA and TMAO was roughly two and 4-fold greater in the 100% strength R2A compared to the 10% strength R2A, respectively, the more than 6-fold increase in OD_{600} from 10% to 100% strength R2A led to a sharp decrease in biomass-normalized methylation efficiencies (Figure S11). This result is similar to recent findings in Qiao et al.,[50] where biomass-normalized arsenic methylation by *Paraclostridium bifermentans* strain EML was inhibited at higher media concentrations.

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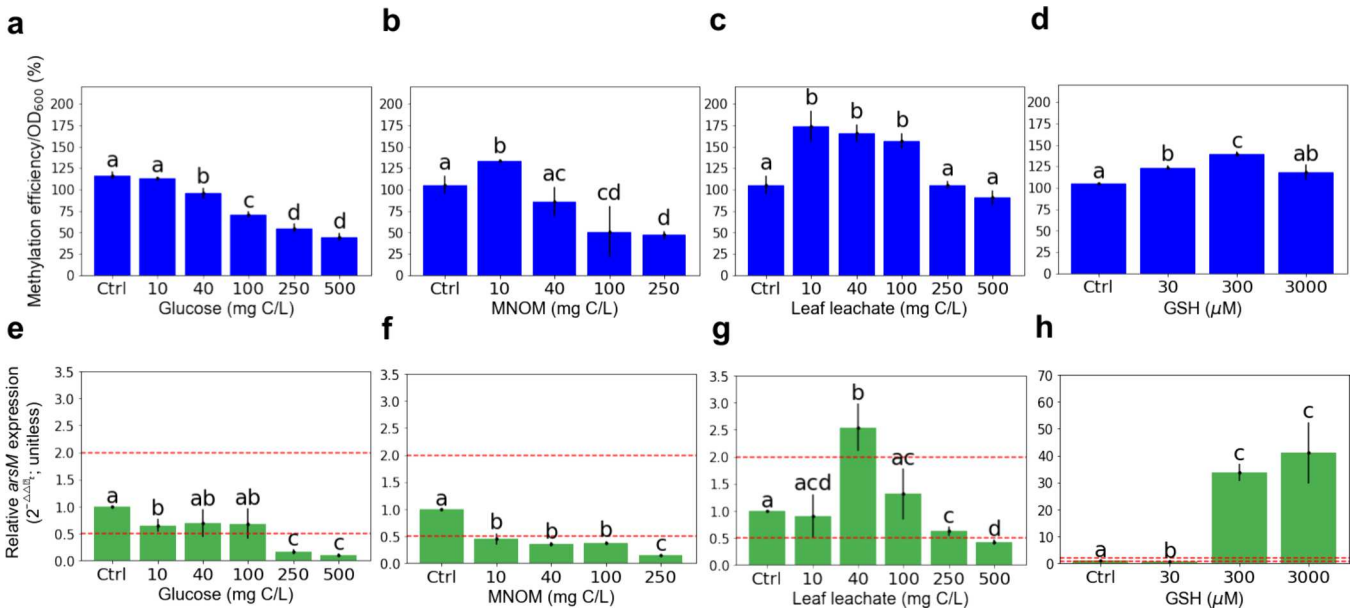

Figure 2. Biomass-normalized arsenic methylation efficiencies (top row) and relative *arsM* expression (bottom row) in *A. rosenii* cultures in the presence of (a and e) glucose, (b and f) Upper Mississippi river natural organic matter (MNOM), (c and g) leaf leachate, and (d and h) glutathione (GSH). Ctrl is the control assay with 1 μM arsenite but no additional carbon. An alphabetical letter over each bar denotes significant difference ($p < 0.05$) between bars based on one-way ANOVA with Tukey's honestly significant difference (HSD) post hoc test. Methylation efficiencies can be greater than 100% because they are normalized by OD_{600}. The red dashed line in (e)−(h) denotes either a 2-fold increase or decrease from the control. Data for 500 mg C/L MNOM are not shown because cell growth was inhibited by greater than 50% in that condition. Note the different *y*-axis limits in panel (h).

Adding glucose at concentrations of 10 to 500 mg C/L to the 20% strength R2A media caused a consistent decrease in As methylation, with biomass-normalized efficiencies decreasing by approximately 58% at the highest glucose concentration (Figure 2a). A clear decrease in methylation efficiencies was observed even without biomass-normalization (Figure S14). In the presence of MNOM, methylation efficiencies initially increased by 26% at 10 mg C/L before decreasing by more than 50% relative to the control at 100 and 250 mg C/L. With leaf leachate, methylation efficiencies increased from 10 to 100 mg C/L before decreasing at 250 and 500 mg C/L to levels similar to the no-DOM control.

Relative *arsM* expression decreased monotonically with higher levels of glucose and MNOM, and was repressed by more than 80% at 250 mg C/L (Figure 2e and f). In contrast, leaf leachate caused an initial increase in *arsM* expression at intermediate carbon levels, followed by a significant decrease of more than 50% relative to the control at 500 mg C/L (Figure 2g). Effects of organic carbon on *arsM* expression were thus largely consistent with patterns observed in As methylation efficiencies (Figure S15), except at the highest leaf leachate concentrations where *arsM* expression was repressed but there was no change in the methylation efficiency relative to the control. Overall, there was agreement between *E. coli* biosensor and *A. rosenii* results around the repressing effects of organics on both uptake and methylation of arsenite (Figure S16). For the leaf leachate, biosensor results and *arsM* expression analysis both indicated a decrease in arsenite uptake at the highest carbon concentrations, but methylation efficiencies at the highest leaf leachate concentrations were similar to the control (Figure 2c). See Note S7 for further discussion of comparison of results between *E. coli*- and *A. rosenii*-based methods.

We additionally tested the effects of xylose, mannose, pyruvate, and succinate on As methylation in order to further assess effects of upper glycolytic (xylose, mannose) vs nonglycolytic (pyruvate, succinate) substrates on repressed As methylation. Xylose and mannose both had similar effects as glucose, causing a continuous decrease in biomass-normalized methylation efficiencies as carbon concentrations increased, with decreases of 62% and 81%, respectively, at 500 mg C/L (Figure 3a-b). These sugars all increased cell growth relative to the control (Figure S17e and f). In contrast, neither pyruvate nor succinate had a systematic effect on As methylation efficiencies or cell growth (Figures 3c-d, S17g and h). These results are consistent with contemporary CCR models[33,34] in which uptake of upper glycolytic substrates such as sugars represses glycerol (and therefore arsenite) uptake, while LMW organic acids can be coutilized with glycerol and do not impact coupled uptake of glycerol and arsenite.

Arsenic methylation efficiencies increased by 10 to 30% in the presence of 300 to 3,000 μM GSH (Figure 2d), despite the fact that 77% of arsenite was complexed in the 3,000 μM GSH condition (Figure S9). Additional GSH may have facilitated methylation because GSH-complexed arsenite is the substrate for methylation by ArsM.[14,51] Extracellular As methylation can occur with ArsM, GSH, and the methyl donor S-adenosylmethionine released from cells,[17] but we determined that this extracellular methylation was negligible in our experiments by exposing filtered cell lysate to 3,000 μM GSH and 1 μM arsenite (Figure S18). We concluded that increased media GSH stimulated *in vivo* bacterial methylation, as corroborated by higher *arsM* expression with increasing GSH (Figure 2h), providing an example of how some carbon compounds may enhance methylation rather than repressing it.

Effects of Carbon Quantity and Quality on Arsenic Methylation. The arsenic biogeochemistry literature suggests that organic carbon should stimulate arsenic methylation rather than repress it.[13,52,53] Our results add nuance to that

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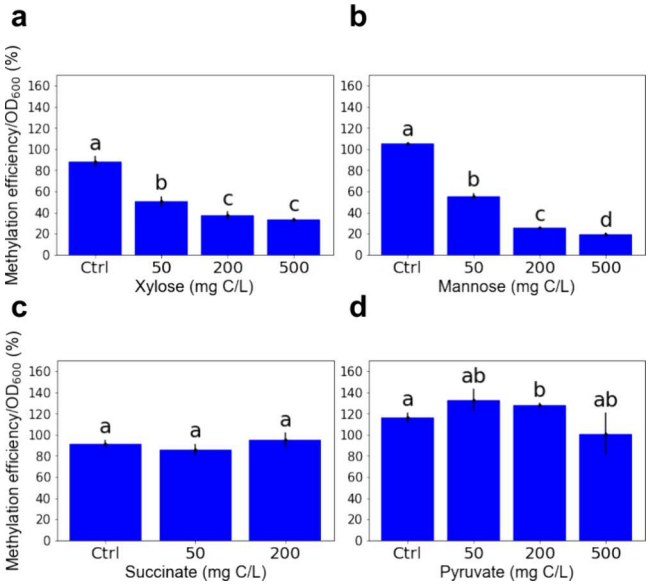

Figure 3. Biomass-normalized arsenic methylation efficiencies in the presence of (a) xylose, (b) mannose, (c) succinate, and (d) pyruvate. Ctrl is the control assay with 1 μM arsenite but no additional carbon. An alphabetical letter over each bar denotes significant difference ($p < 0.05$) between bars based on one-way ANOVA with Tukey's honestly significant difference (HSD) post hoc test. Methylation efficiencies can be greater than 100% because they are normalized by OD_{600}. Data for 500 mg C/L succinate are not shown because cell growth was inhibited by greater than 50% in that condition.

understanding and show that upper glycolytic substrates such as the sugars glucose, xylose, and mannose repress As methylation, while LMW organic acids such as succinate and pyruvate have no effect (Figures 2a and 3). Results with the *E. coli* biosensor, including a significant decrease in *glpF* transporter expression with higher glucose concentrations (Figure 1e), are consistent with a CCR mechanism where upper glycolytic substrates repress the coupled uptake of glycerol and arsenite. The repressing effects of carbon compounds on methylation were most clear when methylation efficiencies were expressed on a per-cell basis (Figures S11 and S13). Studies on CCR of organic pollutant biodegradation have also observed that enhanced biomass growth on labile carbon can compensate for the effects of repression on a per-cell basis.[38] However, repressed methylation was apparent on a total population basis for the sugars and MNOM (Figure S14).

The effects of environmental DOM isolates on As methylation were more complex than the effects of model carbon sources. While the biosensor data clearly show that both leaf leachate and MNOM decrease *glpF* expression and cellular As uptake consistent with a CCR-like process (Figure 1), for the leaf leachate this did not translate into a decrease in As methylation relative to the control (though methylation efficiencies were roughly 40% lower at 250 and 500 mg C/L than at 10−100 mg C/L). These divergent responses may be related to the complexity of the environmental DOM isolates (see Note S8 for further discussion on results from environmental DOM samples).

Environmental Implications. Our results suggest that microbial arsenite uptake and methylation will be repressed in environments that are rich in sugars, due to the role of sugar uptake in repressing the coupled uptake of glycerol and arsenite. Root exudation and decomposition of plant residues are important sources of sugars in rice paddy soils. To our knowledge this is the first report to link carbon source quantity and quality to the control of As methylation through a CCR-like mechanism, but a close examination of recent studies reveals findings that are consistent with this theory. For example, Qiao et al.[50] showed that biomass-normalized As methylation by *P. bifermentans* decreased with higher media carbon concentrations, while Leon Ninin et al.[23] reported a plateau followed by a decrease in methylated As in paddy soil incubations as DOC concentrations increased. We acknowledge that the literature generally suggests that organic matter stimulates As methylation,[13,52,53] but these prior observations may be caused by enhanced biomass growth in carbon-rich systems that obscures the repression of methylation on a per-cell basis. Our results suggest that the greatest levels of repressed As methylation will occur when dissolved organic matter pools are rich in sugars, but there is a need for future field-based research to further test these theories under real-world conditions.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acs.estlett.4c00400.

Preparation of DOM solutions, As biosensor assay, dialysis equilibrium experiments, As methylation assay, gene expression analysis, and metabolomics analysis (PDF)

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Notes

The authors declare no competing financial interest.

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■ REFERENCES

(1) Maguffin, S. C.; Kirk, M. F.; Daigle, A. R.; Hinkle, S. R.; Jin, Q. Substantial contribution of biomethylation to aquifer arsenic cycling. *Nature Geoscience* **2015**, *8* (4), 290−293.

(2) Zhu, Y.-G.; Xue, X.-M.; Kappler, A.; Rosen, B. P.; Meharg, A. A. Linking genes to microbial biogeochemical cycling: lessons from arsenic. *Environ. Sci. Technol.* **2017**, *51* (13), 7326−7339.

(3) Zhu, Y.-G.; Yoshinaga, M.; Zhao, F.-J.; Rosen, B. P. Earth abides arsenic biotransformations. *Annual Review of Earth and Planetary Sciences* **2014**, *42*, 443.

(4) Reid, M. C.; Asta, M. P.; Falk, L.; Maguffin, S. C.; Pham, V. H. C.; Le, H. A.; Bernier-Latmani, R.; Le Vo, P. Associations between inorganic arsenic in rice and groundwater arsenic in the Mekong Delta. *Chemosphere* **2021**, *265*, 129092.

(5) Zavala, Y. J.; Duxbury, J. M. Arsenic in rice: I. Estimating normal levels of total arsenic in rice grain. *Environ. Sci. Technol.* **2008**, *42* (10), 3856−3860.

(6) Zavala, Y. J.; Gerads, R.; Gürleyük, H.; Duxbury, J. M. Arsenic in rice: II. Arsenic speciation in USA grain and implications for human health. *Environ. Sci. Technol.* **2008**, *42* (10), 3861−3866.

(7) Zhao, F.-J.; Zhu, Y.-G.; Meharg, A. A. Methylated arsenic species in rice: geographical variation, origin, and uptake mechanisms. *Environ. Sci. Technol.* **2013**, *47* (9), 3957−3966.

(8) Limmer, M. A.; Wise, P.; Dykes, G. E.; Seyfferth, A. L. Silicon decreases dimethylarsinic acid concentration in rice grain and mitigates straighthead disorder. *Environ. Sci. Technol.* **2018**, *52* (8), 4809−4816.

(9) Tang, Z.; Wang, Y.; Gao, A.; Ji, Y.; Yang, B.; Wang, P.; Tang, Z.; Zhao, F.-J. Dimethylarsinic acid is the causal agent inducing rice straighthead disease. *Journal of Experimental Botany* **2020**, *71* (18), 5631−5644.

(10) Zhao, F.-J.; Harris, E.; Yan, J.; Ma, J.; Wu, L.; Liu, W.; McGrath, S. P.; Zhou, J.; Zhu, Y.-G. Arsenic methylation in soils and its relationship with microbial *arsM* abundance and diversity, and As speciation in rice. *Environ. Sci. Technol.* **2013**, *47* (13), 7147−7154.

(11) Zhao, F.-J.; McGrath, S. P.; Meharg, A. A. Arsenic as a food chain contaminant: mechanisms of plant uptake and metabolism and mitigation strategies. *Annual Review of Plant Biology* **2010**, *61*, 535−559.

(12) Jia, Y.; Huang, H.; Zhong, M.; Wang, F.-H.; Zhang, L.-M.; Zhu, Y.-G. Microbial arsenic methylation in soil and rice rhizosphere. *Environ. Sci. Technol.* **2013**, *47* (7), 3141−3148.

(13) Afroz, H.; Su, S.; Carey, M.; Meharg, A. A.; Meharg, C. Inhibition of microbial methylation via arsM in the rhizosphere: arsenic speciation in the soil to plant continuum. *Environ. Sci. Technol.* **2019**, *53* (7), 3451−3463.

(14) Qin, J.; Rosen, B. P.; Zhang, Y.; Wang, G.; Franke, S.; Rensing, C. Arsenic detoxification and evolution of trimethylarsine gas by a microbial arsenite S-adenosylmethionine methyltransferase. *Proc. Natl. Acad. Sci. U. S. A.* **2006**, *103* (7), 2075−2080.

(15) Qiao, J.; Liu, J.; Palomo, A.; Bostick, B. C.; Phan, K.; Zheng, Y.; Li, F. Prevalence of methylated arsenic and microbial arsenic methylation genes in paddy soils of the Mekong Delta. *Environ. Sci. Technol.* **2023**, *57* (26), 9675−9682.

(16) Reid, M. C.; Maillard, J.; Bagnoud, A.; Falquet, L.; Le Vo, P.; Bernier-Latmani, R. Arsenic methylation dynamics in a rice paddy soil anaerobic enrichment culture. *Environ. Sci. Technol.* **2017**, *51* (18), 10546−10554.

(17) Viacava, K.; Meibom, K. L.; Ortega, D.; Dyer, S.; Gelb, A.; Falquet, L.; Minton, N. P.; Mestrot, A.; Bernier-Latmani, R. Variability in arsenic methylation efficiency across aerobic and anaerobic microorganisms. *Environ. Sci. Technol.* **2020**, *54* (22), 14343−14351.

(18) Viacava, K.; Qiao, J.; Janowczyk, A.; Poudel, S.; Jacquemin, N.; Meibom, K. L.; Shrestha, H. K.; Reid, M. C.; Hettich, R. L.; Bernier-Latmani, R. Meta-omics-aided isolation of an elusive anaerobic arsenic-methylating soil bacterium. *ISME journal* **2022**, *16*, 1740.

(19) Chen, C.; Li, L.; Huang, K.; Zhang, J.; Xie, W.-Y.; Lu, Y.; Dong, X.; Zhao, F.-J. Sulfate-reducing bacteria and methanogens are involved in arsenic methylation and demethylation in paddy soils. *ISME journal* **2019**, *13* (10), 2523−2535.

(20) Chen, C.; Li, L.; Wang, Y.; Dong, X.; Zhao, F.-J. Methylotrophic methanogens and bacteria synergistically demethylate dimethylarsenate in paddy soil and alleviate rice straighthead disease. *ISME Journal* **2023**, *17*, 1851.

(21) Zhang, X.; Reid, M. C. Inhibition of methanogenesis leads to accumulation of methylated arsenic species and enhances arsenic volatilization from rice paddy soil. *Science of The Total Environment* **2022**, *818*, 151696.

(22) Wang, L.; Guo, Q.; Wu, G.; Yu, Z.; Ninin, J. M. L.; Planer-Friedrich, B. Methanogens-driven arsenic methylation preceding formation of methylated thioarsenates in sulfide-rich hot springs. *Environ. Sci. Technol.* **2023**, *57* (19), 7410−7420.

(23) Hussain, M. M.; Bibi, I.; Niazi, N. K.; Shahid, M.; Iqbal, J.; Shakoor, M. B.; Ahmad, A.; Shah, N. S.; Bhattacharya, P.; Mao, K.; et al. Arsenic biogeochemical cycling in paddy soil-rice system: Interaction with various factors, amendments and mineral nutrients. *Science of the total environment* **2021**, *773*, 145040.

(24) Li, L.; Zhang, N.; Zhang, Y.; Zhang, Y.; Shi, Q.; Sun, Y.; Huo, L.; Zeng, X.; Su, S. Selective utilization of organic carbon molecules promotes arsenic methylation by increasing methyltransferase activity in arsM-harboring microbes of paddy soils. *Soil Biology and Biochemistry* **2024**, *190*, 109305.

(25) Dykes, G. E.; Chari, N. R.; Seyfferth, A. L. Si-induced DMA desorption is not the driver for enhanced DMA availability after Si addition to flooded soils. *Science of The Total Environment* **2020**, *739*, 139906.

(26) León Ninin, J. M.; Muehe, E. M.; Kölbl, A.; Mori, A. H.; Nicol, A.; Gilfedder, B.; Pausch, J.; Urbanski, L.; Lueders, T.; Planer-Friedrich, B. Changes in arsenic mobility and speciation across a 2000-year-old paddy soil chronosequence. *Science of The Total Environment* **2024**, *908*, 168351.

(27) Yoon, H.; Giometto, A.; Pothier, M. P.; Zhang, X.; Poulain, A. J.; Reid, M. C. Time-Dependent Biosensor Fluorescence as a Measure of Bacterial Arsenic Uptake Kinetics and Its Inhibition by Dissolved Organic Matter. *Appl. Environ. Microbiol.* **2022**, *88* (16), No. e00891-22.

(28) Yoon, H.; Stenzler, B.; Abu-Ali, L.; Asta, M. P.; Poulain, A. J.; Reid, M. C. Effects of iron and dissolved organic matter on bioavailability of arsenite under anaerobic conditions. *ACS ES&T Water* **2023**, *3* (11), 3676−3686.

(29) Abu-Ali, L.; Yoon, H.; Reid, M. C. Effects of organic sulfur and arsenite/dissolved organic matter ratios on arsenite complexation with dissolved organic matter. *Chemosphere* **2022**, *302*, 134770.

(30) Meng, Y.-L.; Liu, Z.; Rosen, B. P. As(III) and Sb(III) uptake by GlpF and efflux by ArsB in *Escherichia coli*. *J. Biol. Chem.* **2004**, *279* (18), 18334−18341.

(31) Rosen, B. P.; Liu, Z. Transport pathways for arsenic and selenium: a minireview. *Environ. Int.* **2009**, *35* (3), 512−515.

(32) Heller, K. B.; Lin, E.; Wilson, T. H. Substrate specificity and transport properties of the glycerol facilitator of Escherichia coli. *J. Bacteriol.* **1980**, *144* (1), 274−278.
(33) Okano, H.; Hermsen, R.; Kochanowski, K.; Hwa, T. Regulation underlying hierarchical and simultaneous utilization of carbon substrates by flux sensors in Escherichia coli. *Nature microbiology* **2020**, *5* (1), 206−215.
(34) Wang, X.; Xia, K.; Yang, X.; Tang, C. Growth strategy of microbes on mixed carbon sources. *Nat. Commun.* **2019**, *10* (1), 1279.
(35) Egli, T. How to live at very low substrate concentration. *Water Res.* **2010**, *44* (17), 4826−4837.
(36) Helbling, D. E. Bioremediation of pesticide-contaminated water resources: the challenge of low concentrations. *Curr. Opin. Biotechnol.* **2015**, *33*, 142−148.
(37) Stülke, J.; Hillen, W. Carbon catabolite repression in bacteria. *Curr. Opin. Microbiol.* **1999**, *2* (2), 195−201.
(38) Rentz, J. A.; Alvarez, P. J.; Schnoor, J. L. Repression of Pseudomonas putida phenanthrene-degrading activity by plant root extracts and exudates. *Environmental microbiology* **2004**, *6* (6), 574−583.
(39) Horemans, B.; Breugelmans, P.; Hofkens, J.; Springael, D. Carbon catabolite repression and cell dispersal affect degradation of the xenobiotic compound 3, 4-dichloroaniline in *Comamonas testosteroni* WDL7 biofilms. *FEMS Microbiology Ecology* **2017**, *93* (3), fix004.
(40) Horemans, B.; Hofkens, J.; Smolders, E.; Springael, D. Biofilm formation of a bacterial consortium on linuron at micropollutant concentrations in continuous flow chambers and the impact of dissolved organic matter. *FEMS Microbiology Ecology* **2014**, *88* (1), 184−194.
(41) Spuches, A. M.; Kruszyna, H. G.; Rich, A. M.; Wilcox, D. E. Thermodynamics of the As(III)− thiol interaction: arsenite and monomethylarsenite complexes with glutathione, dihydrolipoic acid, and other thiol ligands. *Inorg. Chem.* **2005**, *44* (8), 2964−2972.
(42) Aristilde, L.; Reed, M. L.; Wilkes, R. A.; Youngster, T.; Kukurugya, M. A.; Katz, V.; Sasaki, C. R. Glyphosate-induced specific and widespread perturbations in the metabolome of soil Pseudomonas species. *Frontiers in Environmental Science* **2017**, *5*, 34.
(43) Pothier, M. P.; Hinz, A. J.; Poulain, A. J. Insights into arsenite and arsenate uptake pathways using a whole cell biosensor. *Frontiers in microbiology* **2018**, *9*, 2310.
(44) Stocker, J.; Balluch, D.; Gsell, M.; Harms, H.; Feliciano, J.; Daunert, S.; Malik, K. A.; Van der Meer, J. R. Development of a set of simple bacterial biosensors for quantitative and rapid measurements of arsenite and arsenate in potable water. *Environ. Sci. Technol.* **2003**, *37* (20), 4743−4750.
(45) Buschmann, J.; Kappeler, A.; Lindauer, U.; Kistler, D.; Berg, M.; Sigg, L. Arsenite and arsenate binding to dissolved humic acids: influence of pH, type of humic acid, and aluminum. *Environ. Sci. Technol.* **2006**, *40* (19), 6015−6020.
(46) Huang, K.; Xu, Y.; Zhang, J.; Chen, C.; Gao, F.; Zhao, F.-J. Arsenicibacter rosenii gen. nov., sp. nov., an efficient arsenic methylating and volatilizing bacterium isolated from an arsenic-contaminated paddy soil. *International Journal of Systematic and Evolutionary Microbiology* **2017**, *67* (9), 3186−3191.
(47) Huang, K.; Chen, C.; Zhang, J.; Tang, Z.; Shen, Q.; Rosen, B. P.; Zhao, F.-J. Efficient arsenic methylation and volatilization mediated by a novel bacterium from an arsenic-contaminated paddy soil. *Environ. Sci. Technol.* **2016**, *50*, 6389−6396.
(48) Willsky, G. R.; Malamy, M. H. Effect of arsenate on inorganic phosphate transport in *Escherichia coli*. *Journal of bacteriology* **1980**, *144* (1), 366−374.
(49) Sanno, Y.; Wilson, T.; Lin, E. Control of permeation to glycerol in cells of *Escherichia coli*. *Biochem. Biophys. Res. Commun.* **1968**, *32* (2), 344−349.
(50) Qiao, J.; Sallet, H.; Meibom, K. L.; Jacquemin, N.; Bernier-Latmani, R., The effect of growth substrate availability on anaerobic arsenic methylation by *Paraclostridium bifermentans* strain EML. *bioRxiv* 2023.
(51) Hayakawa, T.; Kobayashi, Y.; Cui, X.; Hirano, S. A new metabolic pathway of arsenite: arsenic−glutathione complexes are substrates for human arsenic methyltransferase Cyt19. *Arch. Toxicol.* **2005**, *79*, 183−191.
(52) Huang, H.; Jia, Y.; Sun, G.-X.; Zhu, Y.-G. Arsenic speciation and volatilization from flooded paddy soils amended with different organic matters. *Environ. Sci. Technol.* **2012**, *46* (4), 2163−2168.
(53) Yan, M.; Zeng, X.; Wang, J.; Meharg, A. A.; Meharg, C.; Tang, X.; Zhang, L.; Bai, L.; Zhang, J.; Su, S. Dissolved organic matter differentially influences arsenic methylation and volatilization in paddy soils. *Journal of Hazardous Materials* **2020**, *388*, 121795.

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