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Stimulation of Microbially Mediated Arsenic Release in Bangladesh Aquifers by Young Carbon Indicated by Radiocarbon Analysis of Sedimentary Bacterial Lipids

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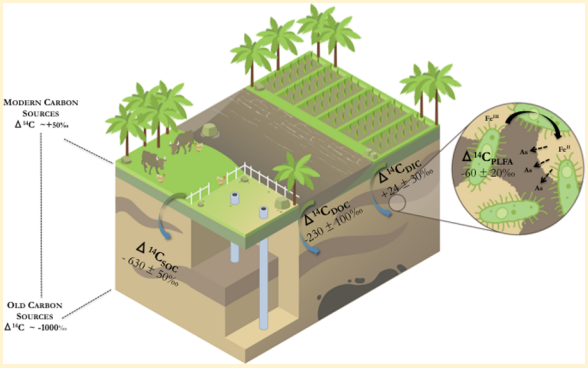
Supporting Information

ABSTRACT: The sources of reduced carbon driving the microbially mediated release of arsenic to shallow groundwater in Bangladesh remain poorly understood. Using radiocarbon analysis of phospholipid fatty acids (PLFAs) and potential carbon pools, the abundance and carbon sources of the active, sediment-associated, in situ bacterial communities inhabiting shallow aquifers (<30 m) at two sites in Araihazar, Bangladesh, were investigated. At both sites, sedimentary organic carbon (SOC) $\Delta^{14}C$ signatures of −631 ± 54‰ (n = 12) were significantly depleted relative to dissolved inorganic carbon (DIC) of +24 ± 30‰ and dissolved organic carbon (DOC) of −230 ± 100‰. Sediment-associated PLFA $\Delta^{14}C$ signatures (n = 10) at Site F (−167‰ to +20‰) and Site B (−163‰ to +21‰) were highly consistent and indicated utilization of carbon sources younger than the SOC, likely from the DOC pool. Sediment-associated PLFA $\Delta^{14}C$ signatures were consistent with previously determined $\Delta^{14}C$ signatures of microbial DNA sampled from groundwater at Site F indicating that the carbon source for these two components of the subsurface microbial community is consistent and is temporally stable over the two years between studies. These results demonstrate that the utilization of relatively young carbon sources by the subsurface microbial community occurs at sites with varying hydrology. Further they indicate that these young carbon sources drive the metabolism of the more abundant sediment-associated microbial communities that are presumably more capable of Fe reduction and associated release of As. This implies that an introduction of younger carbon to as of yet unaffected sediments (such as those comprising the deeper Pleistocene aquifer) could stimulate microbial communities and result in arsenic release.



INTRODUCTION

Across South and Southeast Asia, an estimated 100 million people regularly consume arsenic contaminated groundwater.[1] In Bangladesh, between 35 to 77 million inhabitants[2−4] use groundwater from shallow (<30 m) Holocene-aged-aquifers containing arsenic concentrations above the World Health Organization (WHO) water quality standards of 10 $\mu g/L$[5] for drinking, food preparation, and crop irrigation. Recently installed deeper tube-wells (usually >100 m) facilitate access to low arsenic groundwater in the underlying Pleistocene-aged aquifer.[6] There are concerns about whether this deep, Pleistocene aquifer also may be vulnerable to future arsenic contamination attributed to the same microbially mediated processes that have affected the shallow aquifer systems.[7−11] A fundamental understanding of the electron donors utilized during arsenic release in the shallow aquifers of Bangladesh is required to understand present day and future distributions of arsenic.

Strong evidence indicates that anaerobic micro-organisms mediate arsenic release in deltaic sediments throughout the Bengal Basin.[9,12−20] These organisms release arsenic by coupling the oxidation of organic carbon to the reductive dissolution of As-bearing Fe (oxy)-hydroxides.[12−18,20] The microbial reduction of sorbed arsenate (AsV) to arsenite (AsIII) can also enhance its mobility.[19,21] The abundance and metabolic activity of the microorganisms within these groundwater environments thus can control dissolved arsenic

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concentrations. Microbial abundance in such oligotrophic systems is often limited by nutrient availability. As such, fundamental controls on organic carbon cycling within Bangladesh aquifers should control microbial activity and subsequently arsenic release.[22]

The predominant source of organic carbon stimulating microbially driven arsenic release in Bangladesh aquifers remains controversial. A number of studies in Bangladesh and the surrounding regions have proposed that the bacteria are utilizing sedimentary organic carbon (SOC) present in the aquifers such as buried peat layers,[16,23−28] petroleum[29−32] or ambient carbon buried at the time of sediment deposition.[13,19,20,33,34] Conversely, it has been suggested that dissolved organic carbon (DOC) sources derived from human/animal waste in unsewered runoff water,[18,35] constructed ponds,[36,37] wetland and rice paddy environments,[33,38,39] and/or river-derived organic carbon[11] are transported downward from the surface to the Holocene-aged sediments in the aquifer and are the primary drivers of bacterial activity. Recently, radiocarbon analysis of DNA from filtered groundwater samples provided the first direct evidence of microbial carbon sources in Bangladesh at one site.[40] The relatively young $\Delta^{14}C$ contents of DNA derived from microorganisms present in groundwater samples indicated utilization of younger dissolved inorganic carbon (DIC) or DOC carbon sources rather than sedimentary sources. This is consistent with the proposal by Harvey et al.[18] that indigenous methanogens were primarily using younger DIC carbon sources based on the isotopic composition of DIC and methane in southern Bangladesh. However, neither study was able to assess the carbon sources being utilized by the sediment-associated microbial communities. Such assessment is of fundamental importance as sedimentary microbial communities are more likely to be involved in As release by virtue of their association with the solid phase iron oxides. Sediment-associated microbial communities are also expected to have much greater impact on subsurface biogeochemical cycling as they are generally present in greater abundances than groundwater-associated microorganisms.[41] Further, given their close association with sedimentary materials, these microbial communities have much greater potential to access organic carbon that is a component of, or sorbed to, solid matrix materials. These factors imply that there is the potential that sediment-associated microorganisms have a greater influence on As release in Bangladesh aquifer sediments but also that they may utilize different organic carbon sources that are not easily accessible to their groundwater-associated counterparts.

Analysis of in situ phospholipid fatty acid (PLFA) concentrations is often used to assess microbial bacterial abundances in subsurface systems and thus to identify zones of high levels of bacterial activity associated with increased cellular abundances.[42] PLFA degrade within days to weeks after cell death, thus they effectively represent the viable biological community.[43−45] PLFA concentrations can be converted to cellular abundances via conversion factors that are based on a consistent relationship between membrane PLFA concentrations and cell abundances.[42,43] In addition, compound specific radiocarbon analysis (CSRA) of phospholipid fatty acids (PLFA) can elucidate the carbon sources supporting in situ bacterial communities.[40,46−57] Comparing the $\Delta^{14}C$ signatures of PLFA to those of potential carbon sources (e.g., DIC, DOC, SOC) can directly identify the carbon pools being utilized by the bacterial community[40,46−57] assuming different sources of distinct signatures because $\Delta^{14}C$ signatures are normalized to remove biosynthetic fractionation effects during data processing.[58] This approach has been used to identify microbial carbon sources in a number of environments[40,46−57] including contaminated soils,[52] coastal environments,[48,51,56] and groundwater systems.[55,59]

The goal of this study was to elucidate the predominant carbon sources being used by the sediment-associated bacterial communities in Bangladesh aquifers, to extend our understanding beyond one location and to characterize the relationship of bacterial abundance to As concentrations. Two sites with distinct hydrogeologic and geochemical conditions, specifically having distinct aqueous arsenic distributions and ranges, were compared: Site F, a sandy site with faster recharge and lower arsenic, and Site B, a clay capped site with slower recharge and higher arsenic concentrations.[60] Concentrations of sediment-associated PLFAs were determined at both sites. In addition groundwater-associated PLFA concentrations were determined at Site B in order to assess relative abundances between the two communities. Carbon sources driving metabolisms by the sedimentary bacterial communities were then determined via CSRA of PLFA and comparison to potential carbon sources (SOC, DIC, DOC) in the aquifer.

METHODS

Field Sites. Field sampling for this study was focused in Araihazar Upazila, Bangladesh (Site F (Lashkardi Village), Site B (Baylakandi Village) (Figure S1), where geochemical and hydrological parameters have been well characterized over the past decade.[7,40,60−63] Additional samples that were collected from nearby locations (Site O, Site N, Site S, Site M, and Site T) were included in this study to provide a regional and depth profile context (Figure S1). Briefly, the study area is ∼25 km east of Dhaka and the shallow (Holocene-aged aquifer) groundwater commonly contains arsenic concentrations exceeding the WHO's drinking limit of 10 μg/L with local variation in the depth concentration gradients[7,60,61,63] (Figure S1). In the shallow groundwater wells tested in the area, ∼38% contain arsenic concentrations up to 10 μg/L, ∼53% contain up to 50 μg/L with the remainder above.[64] All site names within this study were kept consistent with the existing literature.[40,60,63,65] Site F and Site B were chosen as the focus of this study because while being relatively close in proximity (∼3.5 km), they contain distinct depth profiles of arsenic concentrations.[7,40,60,63] Groundwater at Site B contains significantly higher arsenic concentrations (as high as ∼500 μg/L) than Site F (as high ∼200 μg/L) and the highest arsenic concentrations at Site B occur at shallower depths (∼14 m) than Site F (∼20 m).[7,40,60,63] The arsenic concentrations are correlated with $^{3}H/^{3}He$ ages and Site B has slower recharge rates than Site F.[60] In addition, radiocarbon signatures from microbial DNA at Site F[40] enabled comparison of the two methods and potentially any differences or similarities between carbon sources of the sediment- and groundwater-associated bacterial populations.

Sediment and Groundwater Sampling. A detailed timeline outlining all sample collection events is included within the Supporting Information (SI) (Figure S2). Sediment samples for $\Delta^{14}C$ analysis of PLFA and SOC were taken in January 2013 from Site F (PLFA only) and Site B. SOC values for Site F were derived from values reported at the same site by Mailloux et al.[40] sampled in 2012. Sediment samples were taken in 2011 and 2012 from Sites O, N, M, S, and T (Figure S1).

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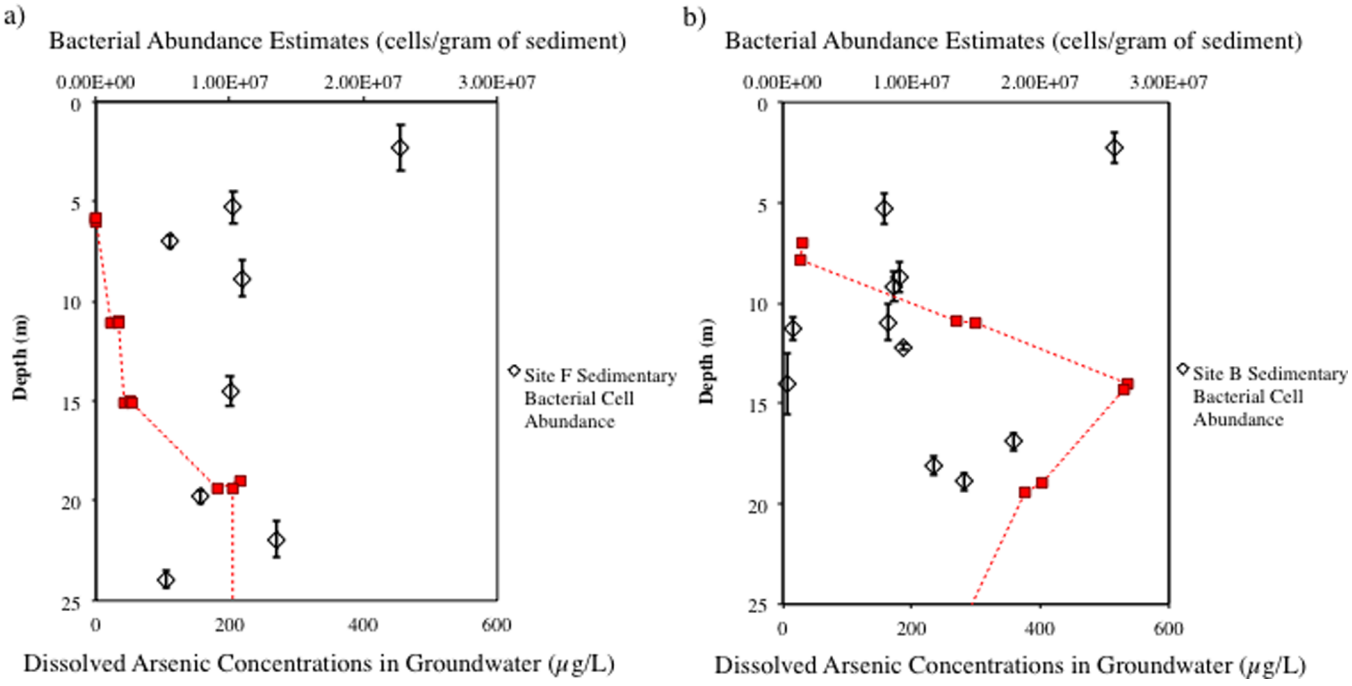

Figure 1. Depth profiles at (a) Site F and (b) Site B of sedimentary bacterial abundance (cells/g sediment) and dissolved arsenic concentrations measured in coinciding groundwater. Vertical error bars on PLFA indicate the depth range of composite sediment samples.

Sediment cores were taken using a gravity corer (see description in the SI), sectioned directly into whirl packs bags, immediately placed on ice and frozen at −20 °C at a local clinic. Sediments were kept frozen until further processing/analysis. Sediment samples were subsequently freeze-dried for 48−72 h and homogenized.

Groundwater samples (each 250 mL) for DOC and DIC analyses were taken from pre-existing well nests[60] at Site F and B from multiple depths in January 2015 with submerged pumps (methods outlined in Mailloux et al.[40]). PLFA in groundwater was sampled in 2013 from Site B by pumping large volumes (1800−8600 L) of groundwater from the three wells through glass-wool filters (poresize 0.7 μm, burnt at 400 °C overnight) from Site B and freezing at −20 °C on site for transport until being freeze-dried for 48−72 h prior to extraction. Groundwater samples for DOC radiocarbon analysis were acidified in 250 mL glass bottles with hydrochloric acid (HCl) on site until further processing.

DIC and DOC Groundwater Concentration Analysis. DOC concentrations were measured using a Shimadzu OC analyzer 5000A as nonvolatile organic carbon (NVOC) from acidified samples collected in baked glass vials (500 °C for 4 h) to avoid external contamination. DIC concentrations were measured in water fixed with $HgCl_2$, and the samples were shipped to National Ocean Sciences Accelerated Mass Spectrometry Facility (NOSAMS) at Woods Hole Oceanographic Institution (Maine, Massachusetts) using standard protocol described at www.whoi.edu/nosams/page.do?pid=40135.

Bacterial Phospholipid Extraction, Purification, and Analysis through Gas Chromatography−Mass Spectrometry (GC-MS). Preliminary extractions using ∼50 g of sediment were carried out to determine the amount of sediment required to obtain enough mass of PLFA for $\Delta^{14}C$ analysis. Sediments (ranging from ∼300 g to ∼1 kg depending on biomass) and glass-wool filters were extracted twice overnight using a modified Bligh and Dyer procedure,[43,66] and phospholipids were separated through silica gel chromatography (F_1 = dichloromethane (DCM), F_2 = acetone, F_3 = methanol (MeOH)). The phospholipid/methanol fraction was evaporated to dryness under N_2 and reacted to become fatty acid methyl esters (FAME) via the mild alkaline methanolysis reaction[43] allowing the compounds to be amenable to GC-MS analysis. FAMEs were purified through a secondary silica gel chromatography (F_1 = 4:1 hexane:DCM, F_2 = DCM (contains FAMEs) and F_3 = MeOH). All methanol used in methanolysis reactions are characterized for both its $\delta^{13}C$ and $\Delta^{14}C$ values to allow PLFA isotope values to be corrected for the addition of an extra methyl group.

All samples were analyzed for PLFA concentrations using gas chromatography on an Agilent 6890N GC (30 m × 0.32 mm DB-5 MS column, 0.25 μm film thickness) coupled to a 5973 quadrupole mass spectrometer monitoring for masses (50−450 m/z). Operating GC-MS conditions included a temperature program with an initial hold for 1 min at 40 °C ramped to 130 °C at 20 °C/min to 160 °C at 4 °C/min and finally to 300 °C at 8 °C/min. QA/QC; standards and reagents used for PLFA analysis are described in the SI.

Radiocarbon Analysis of PLFA, DOC, DIC, and SOC. PLFA extracts from sediments were run through a final purification procedure using a five fraction elution scheme (F_1 = hexane, F_2 = 3:1 hexane:DCM, F_3 = 5:1 hexane:DCM, F_4 = DCM (containing FAMEs), F_5 = MeOH) through ∼1 g of activated silica gel to remove non-PLFA carbon and purity was checked with GC-MS. DOC samples were obtained by filtering ∼250 mL of groundwater samples (Durpore PVDP 0.22 μm (Millipore), freeze-drying the filtered water and subsequent acid-treatment with HCl to liberate residual inorganic carbon. Sedimentary PLFA extracts, DOC, DIC, and freeze-dried sediment samples (SOC) were shipped to NOSAMS for radiocarbon analysis through accelerated mass spectrometry (AMS). All samples were analyzed using Oxalic Acid II and

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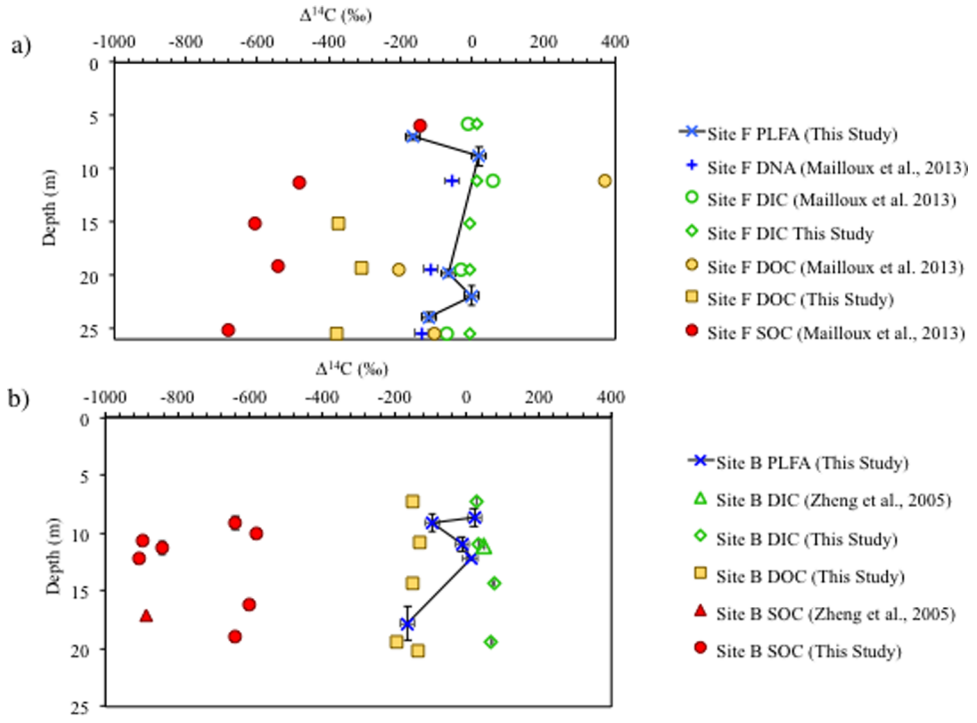

Figure 2. Radiocarbon signatures of microbial cellular components (sedimentary PLFA and groundwater microbial DNA) and carbon pools (dissolved inorganic carbon (DIC), dissolved organic carbon (DOC), sedimentary organic carbon (SOC)) in the shallow aquifer portion of (a) Site F and (b) Site B. Horizontal error bars of SOC, DIC, and DOC indicate instrumental error reported by NOSAMS and if not visible were smaller than the marker. Horizontal error bars on PLFA assume a conservative error estimate of ±20‰ considered appropriate for microscale $\Delta^{14}C$ measurements.[67] Vertical error bars on PLFA indicate the depth range of composite sediment samples.

Vienna Pee Dee Belemnite (VPDB) standards. An error ±20‰ was assumed for all PLFA $\Delta^{14}C$ values which is a conservative and appropriate estimate of error for microscale $\Delta^{14}C$ measurements.[67]

Statistical Analyses. All statistical analyses was carried out using SYSTAT software using 95% confidence intervals and detailed results of the statistical analysis are provided in the SI.

RESULTS

Sediment/Groundwater Bacterial Community Abundances and Arsenic Groundwater Concentrations. PLFA concentrations in sediments varied with depth and ranged from 20 to 1300 pmol/g of sediment at both Site F (540 ± 280 pmol/g) and Site B (520 ± 360 pmol/g) (SI Table S1 and S2). Corresponding bacterial cell abundances, calculated using a conversion factor of 2×10^4 cells/picomole PLFA,[42] averaged $1.1 \times 10^7 \pm 7 \times 10^6$ and $1.0 \times 10^7 \pm 6 \times 10^6$ cells/g of sediment (dry wt) for Site F (n = 8) and Site B (n = 11) respectively (Figure 1, Table S1 and S2). At Site B, the bacterial cell abundances in the groundwater at 7.3, 14.3, and 45.4 m were found to be approximately 5 orders of magnitude less abundant at 7.7×10^1, 1.3×10^2, and 1.0×10^2 cells/mL of groundwater respectively (Table S3). The sedimentary cell abundances are typical of nutrient/carbon limited aquifer sediments[68−70] and the groundwater-associated bacterial communities are consistent with a previously reported direct cell count of shallow Bangladesh groundwater.[71] No significant correlation was found between the total bacterial abundance estimates of the sediment and the average dissolved arsenic concentrations within the coinciding groundwater at either Site F (unparametric Spearman rank correlation coefficient ρ = 0.112) or Site B (unparametric Spearman rank correlation coefficient ρ = 0.217 (Figure 1)).

$\Delta^{14}C$ Signatures in Shallow Aquifer Carbon Pools. The average sedimentary organic carbon $\Delta^{14}C$ signature at the shallow depths corresponding to those analyzed for PLFA (<30 m) of Site F (Lashkardi Village)[40] and Site B (Baylakandi Village) was −631 ± 54 ‰ ($\Delta^{14}C$ range = −147‰ to −906‰, Figure 2a,b, Table 1). The youngest SOC signature (Site F, 7 m depth, $\Delta^{14}C$ = −147‰[40]) was a statistical outlier from all other SOC signatures found at Site B and Site F. Without this point, the mean is −675 ± 140 ‰. There was no significant trend with depth for these shallow sediments at Site F and B. These values were consistent with radiocarbon signatures of SOC at Sites M, N, O, S, and T (all within a ∼4 km radius of Site F and Site B) from depths ranging from 35 to 67 m (Table 1). $\Delta^{14}C$ of SOC at these deeper depths ranged from −613‰ to −989‰ with the most positive values occurring at the shallowest depths and overlapping with the ranges observed at Site B and F (Figure 3, Table 1). These SOC $\Delta^{14}C$ signatures are consistent with the expectations based on the sediment deposition history of rapid infilling of the Bengal Basin[72] after the last glacial maximum.

Average DIC groundwater concentrations at Site F and Site B of 5 ± 2 and 9 ± 2 mmol/L were orders of magnitude higher than corresponding DOC concentrations (0.05 ± 0.02 and 0.2 ± 0.02 mmol/L) (Figure 4; Table S4). DIC $\Delta^{14}C$ signatures from all measured samples <30 m depth were significantly more modern than SOC ranging from −8‰ to +12‰ at Site F (+1 ± 10‰) and from +27 ‰ to +75 ‰ at Site B (+48 ± 20) (Figure 2a,b; Tables S5−S7). DOC $\Delta^{14}C$ signatures were likewise more modern than SOC but significantly more depleted than DIC. $\Delta^{14}C$ DOC ranged from −379‰ to

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Table 1. Radiocarbon Values of Sedimentary Phospholipid Fatty Acids (PLFAs) and Sedimentary Carbon Sources (SOC) at Study Sites in the Araihazar Region, Bangladesh

Bangladesh site	sample description	PLFA depth interval (m)[a]	PLFA depth midpoint (m)	PLFA $\Delta^{14}C$ (‰) ± 20[b]	SOC depth (m)	SOC $\Delta^{14}C$ (‰) ± error[c]
Site F (Lashkardi Village)	sediment	6.7−7.3	7	−167		
Site F (Lashkardi Village)	sediment	7.9−9.8	8.8	20		
Site F (Lashkardi Village)	sediment	19.5−20	19.8	−67		
Site F (Lashkardi Village)	sediment	21−22.9	21.9	−1		
Site F (Lashkardi Village)	sediment	23.5−24.4	23.9	−119		
Site B (Baylakandi)	sediment	7.6−9.8	8.7	21	9.1	−640 ± 1.6
Site B (Baylakandi)	sediment	8.5−9.8	9.1	−96	10.7	−896 ± 1.4
Site B (Baylakandi)	sediment	10−11.9	11	−14	10.1	−580 ± 1.5
Site B (Baylakandi)	sediment	12−12.4	12.2	10	11.3	−842 ± 1.5
Site B (Baylakandi)	sediment	16.5−19.2	17.8	−163	12.2	−906 ± 1.5
Site B (Baylakandi)	sediment				16.2	−604 ± 1.6
Site B (Baylakandi)	sediment				18.9	−640 ± 1.6
Site M	buried peat				35.4	−664 ± 1.5
Site M	sediment				35.4	−663 ± 1.8
Site M	charcoal				37.2	−613 ± 1.8
Site M	buried peat				37.2	−652 ± 2.2
Site M	sediment				37.2	−654 ± 1.5
Site M	buried peat				37.8	−658 ± 1.5
Site M	sediment				50	−777 ± 1.3
Site M	sediment				50.6	−754 ± 1.4
Site M	sediment				73.8	−883 ± 0.9
Site M	sediment				78	−989 ± 2.2
Site N	sediment				50.3	−710 ± 1.5
Site O	sediment				56.4	−843 ± 1.6
Site S	sediment				33.5	−663 ± 1.5
Site S	sediment				44.2	−950 ± 1.6
Site S	sediment				44.2	−923 ± 2.6
Site S	sediment				68.6	−973 ± 1.4
Site S	sediment				73.2	−884 ± 1.5
Site T	sediment				42.7	−642 ± 1.7
Site T	sediment				57.9	−805 ± 1.6
Site T	sediment				67.1	−955 ± 1.6

[a]PLFA depth intervals represent the range of depths in the composite sediment samples for PLFA. [b]A conservative error of ±20‰ was applied to each PLFA radiocarbon measurement which is considered appropriate for <100 μg sample size run with AMS.[67] [c]Error provided for the sedimentary organic carbon is the instrumental error reported by NOSAMS.

−370‰ at Site F (−353 ± 40‰) and −194‰ to −131‰ at Site B (−151 ± 25‰) (Figure 2a,b, Table S5−S7). At both sites, the $\Delta^{14}C$ of DIC was found to be statistically more positive than that of the DOC (Site F: p = 0.002, Site B: p = 0.039) (Table S6 and S7). Notably, the DOC $\Delta^{14}C$ signatures measured in groundwater collected for this study in 2015 from Site F were significantly lower and had a smaller range than the DOC measured in 2012 samples ($\Delta^{14}C$ = +19 ± 308) from the same depths by Mailloux et al.[40]

$\Delta^{14}C$ Signatures of PLFA Relative to Available Carbon Pools. $\Delta^{14}C$ signatures of PLFA extracted from sediment of both Site F and Site B ranged from −167‰ to +20‰ and −163‰ to +21‰ respectively, varying with depth (Figure 2a,b; Table 1). When a comparison between $\Delta^{14}C$ of PLFA and the $\Delta^{14}C$ of DOC/DIC was possible for a given depth (sample depths were within 1.5 m of each other) (n = 7), the $\Delta^{14}C$ of PLFA were in agreement (within error (±20‰)) with the $\Delta^{14}C$ of DIC for three sample sets (Site B at 8, 11, and 12 m). In the remaining four samples, the $\Delta^{14}C$ of PLFA fell within an intermediate range between the DOC and the DIC. At Site F where Mailloux et al.[40] had previously measured the $\Delta^{14}C$ of DNA from the groundwater microbial community, the $\Delta^{14}C$ signatures of PLFA correlated (Pearson R = 0.995) with those measured in microbial DNA[40] but had a slightly younger signature (average difference of +50‰, just outside of analytical precision). Given that the samples were collected two years apart and may have been affected by slight variation in the age of the microbial carbon sources between 2013 and 2015 or differences between sediment-associated (this study) vs groundwater[40] bacteria (Figure S3, Table S8), this agreement between the two methods is remarkably good. To examine which carbon source the indigenous sedimentary bacteria were utilizing, posthoc Tukey pairwise statistical comparisons of $\Delta^{14}C$ between all of the PLFA, DOC, DIC and SOC at each site from this study combined with the results from Mailloux et al.[40] (Site F: DOC, DIC, SOC) were carried out. The DOC and DIC by Mailloux et al.[40] were specifically included within the statistical analysis with the 2015 DIC and DOC values to

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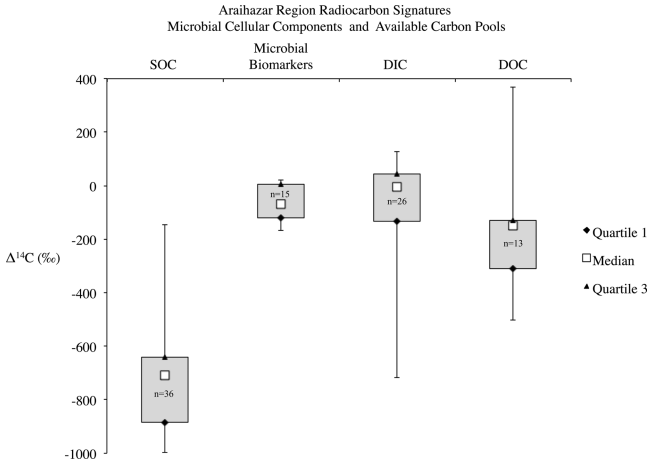

Figure 3. Box-plot summary of available radiocarbon signatures in the Araihazar Region in Bangladesh from sedimentary organic carbon (SOC), microbial biomarkers (PLFA and DNA), dissolved inorganic carbon (DIC), and dissolved organic carbon (DOC) reported in this study, Mailloux et al.,[40] and Zheng et al.[7] Error bars indicate range of $\Delta^{14}C$ signatures.

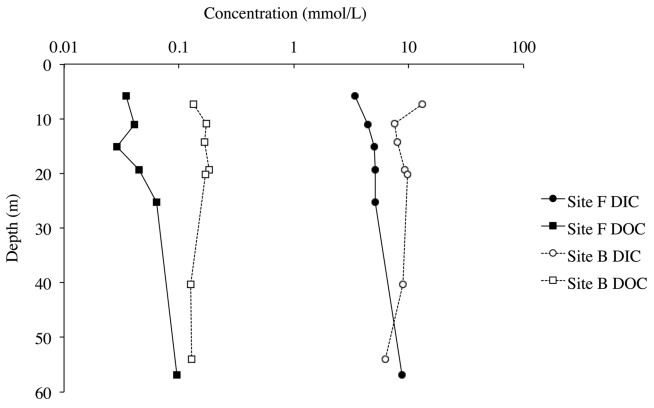

Figure 4. Depth profile of dissolved inorganic and organic carbon concentrations (mmol/L) measured in groundwater at Site F (black symbols) and Site B (open symbols).

ensure the analysis was representative as the sediments for PLFA were collected at an intermediate time point (2013). This analysis revealed no significant difference between the bulk DOC and PLFA at Site B (p = 0.38) but did reveal a statistical difference at Site F between the bulk DOC and PLFA (p = 0.04) (Table S6 and S7). At either site, no statistical difference was observed between the radiocarbon signatures between the bulk DIC and the PLFA (Site F: p = 0.78, Site B: p = 0.67) (Table S6 and S7). The PLFA values are significantly more enriched than the SOC values for Site B (p = <0.001) and Site F (p = 0.0003) (Figure 2a,b; Table S6 and S7) or the region (Figure 3, Table 1).

Carbon Source Age and Bacterial Community Abundance. If Site F is considered in isolation, a positive correlation (R = 0.91, p = 0.032) between the bacterial community abundance (calculated using conversion factors from total PLFA concentrations) and the $\Delta^{14}C$ of PLFA is observed. When Site B is examined independently, no significant positive correlation was observed. However, this lack of correlation is largely controlled by a single sample where the indigenous populations with the highest bacterial abundance (~1.5 × 10^7 cells/g of sediment) also had the most depleted $\Delta^{14}C$ signature at the site. Exclusion of this sample's data and a combined Site F and Site B regression analysis between bacterial cell abundance estimates and radiocarbon signatures gives a positive correlation of Pearson R = 0.72 (p = 0.028) (Figure 5).

DISCUSSION

Arsenic Groundwater Concentrations and Sediment Bacterial Community Abundance. The observed lack of correlation between arsenic concentrations in the groundwater and the sedimentary bacterial community abundances indicates that it is not an increase in the total bacterial population size that is responsible for the observed high As concentrations (Figure 1). While the sedimentary bacterial community abundances are not changing coincident with the occurrence of high levels of As, an increased proportion of the active community carrying out Fe and/or As reduction has been observed previously in the areas of high arsenic.[14,40] However,

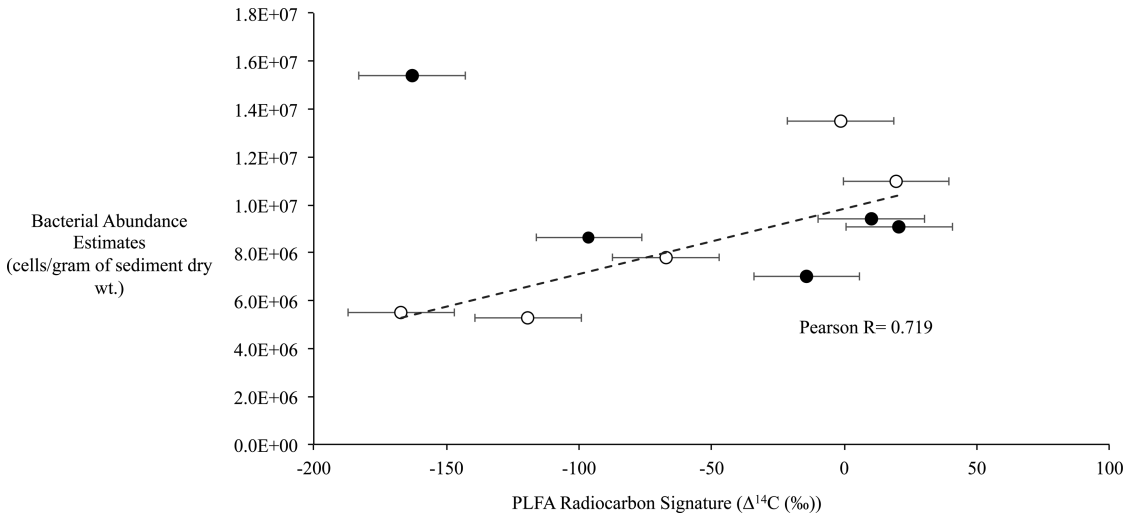

Figure 5. Regression plot of sedimentary bacterial abundance estimates (cells/g of aquifer sediments (dry)) and corresponding PLFA radiocarbon signature at Site F (open circles) and Site B (filled circles). Regression analysis (Pearson R = 0.719) excluded a single outlier point from Site B ($\Delta^{14}C$ = −163‰, bacterial abundance =1.54 × 10^7 cells/g dry sediment). Horizontal error bars on PLFA assume a conservative error estimate of ±20‰.[67]

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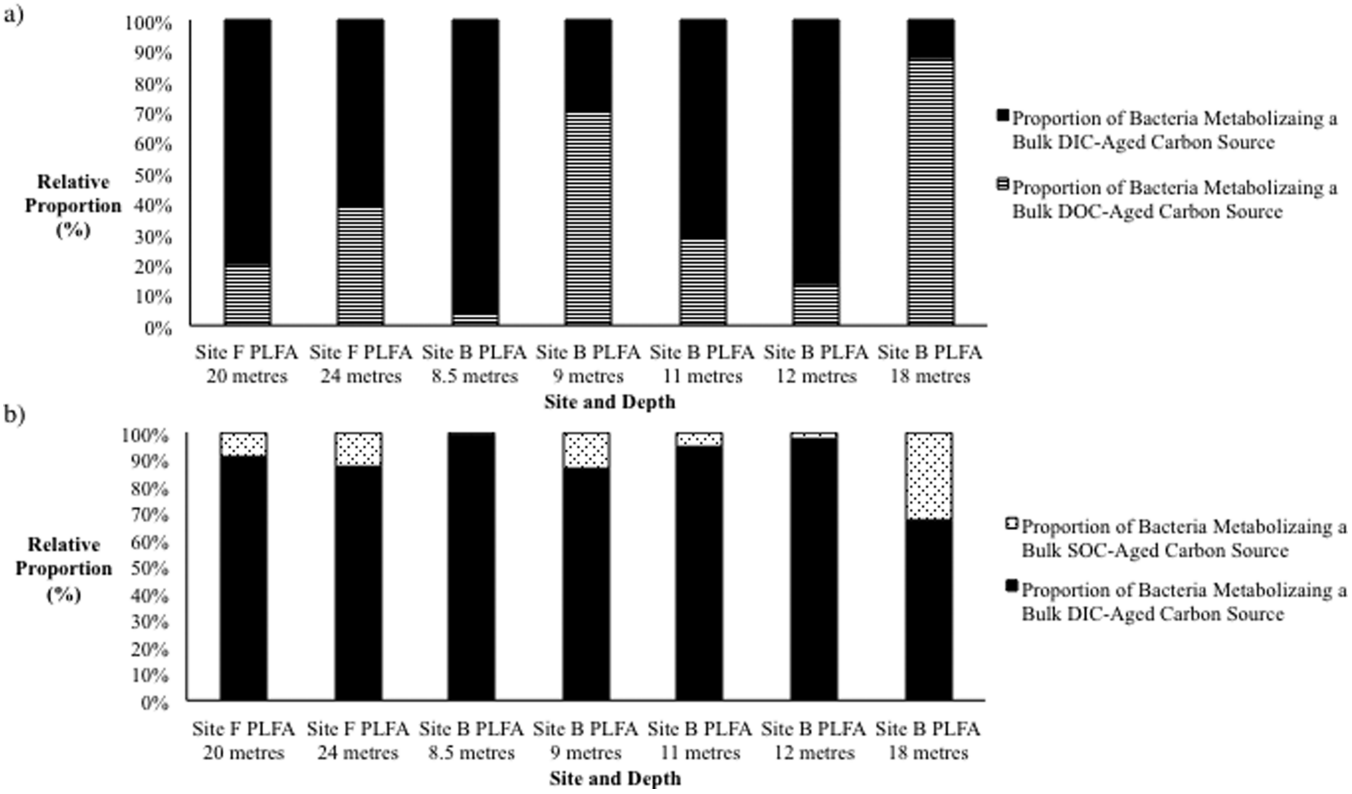

Figure 6. Mass balance approach using PLFA radiocarbon signatures with (a) DOC and DIC-aged carbon sources as two predominant sources at Site F and Site B and (b) SOC and DIC-aged carbon sources as two predominant sources at Site F and Site B.

this observation would also be consistent with As release occurring close to recharge points and being transported with water as has been suggested recently.[39] The approaches used within this research study are unable to differentiate between these two possibilities and thus warrants future research.

$\Delta^{14}C$ Shallow Aquifer Carbon Pools and Carbon Cycling. The modern DIC values were consistent with the expectations based on tritium dating[60] indicating that this DIC is partially atmospherically derived and has undergone vertical transport with the water and/or is the product of mineralization of modern organic carbon being transported vertically. The more positive range observed at Site B, including points above the current $\Delta^{14}C$ of the atmosphere, is consistent with the presence of DIC influenced by atmospheric weapons testing ("bomb carbon") and the slower infiltration rates at this site.[60] DOC is likely a mixture of relatively modern components derived from vertical recharge cotransported with the DIC and dissolution/mobilization of some SOC carbon from shallower depths (~<30 m) during transport. The cause of temporal fluctuation observed in DOC at Site F compared to Mailloux et al.,[40] while the $\Delta^{14}C$ of DIC at Site F remained consistent, is not known. At Site F, more depleted $\Delta^{14}C$ values of DOC suggest a higher proportion of SOC dissolution may be occurring in 2015 compared to 2012. At Site B, SOC is not the predominant source of carbon contributing to the bulk DOC pool in 2015. Variations in the proportions of these sources may be responsible for the observed $\Delta^{14}C$ variations.

Radiocarbon Signatures of Bacterial PLFA and Available Carbon Pools. $\Delta^{14}C$ values of the bacterial lipids (PLFA) generally fell between DIC and DOC and trended closer to the age of the DIC pool. Recent research has demonstrated the presence of autotrophic bacterial genes in the subsurface[73,74] which may indicate that DIC utilization could be occurring, likely in combination with more predominant heterotrophy. The agreement between the PLFA and DIC $\Delta^{14}C$ signatures could also in part be explained through heterotrophic bacteria mineralizing a younger component of the DOC, adding to the DIC pool. However, with the DOC far less abundant than the DIC, the DIC signatures are unlikely to be produced predominantly from DOC mineralization. To examine microbial metabolization of a mixture of potential carbon sources (Figure 2a,b), a mass balance approach (example in eq 1) was carried out twice assuming in each case only two major carbon pools (DIC versus DOC aged carbon (eq 1, Figure 6a) and DIC versus SOC aged carbon (Figure 6b) contributing to the PLFA $\Delta^{14}C$ signature (Table S9).

$$\Delta^{14}C_{PLFA} = \Delta^{14}C_{DOC}(f) + \Delta^{14}C_{DIC}(1 - f) \quad (1)$$

where (f) equals the proportion of sedimentary bacteria metabolizing DOC aged carbon and ($1 - f$) equals proportion of sedimentary bacteria using DIC aged carbon. The mass balance results between DIC and DOC (Figure 6a) indicate that in this scenario the bacterial community would be predominantly (>60%) using the DIC or alternatively, the microbes could be using a component of the DOC pool of equivalent $\Delta^{14}C$ age to the DIC. Utilization of a component of the DOC pool is consistent with the idea that heterotrophic communities dominate subsurface aquifer systems. In this scenario, DOC potentially comprised of more polar, bioavailable organics is transported with surface recharge and heterotrophic bacteria utilized this subcomponent of DOC to respire CO_2 contributing some younger carbon to the DIC pool.

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The results of the mass balance between the $\Delta^{14}C$ signatures of DIC and SOC at both Site F and Site B suggested that negligible (<10%) utilization of SOC derived carbon was occurring. (Figure 6b, Table S9). The PLFA were consistently more modern than the DOC and SOC, so a mass balance approach using the bulk $\Delta^{14}C$ DOC and SOC signatures could not result in the observed PLFA values. These results imply that sedimentary bacterial communities in Bangladesh aquifers are not primarily utilizing older sedimentary derived organic carbon sources such as petroleum-derived carbon,[29−32] peat,[16,23,27,28] or carbon buried at the time of sediment deposition[33] to drive their metabolisms. These results do not preclude the possibility of minor amounts of SOC being utilized as suggested previously.[11,40] At the shallowest depth measured at Site F (~7 m), PLFA $\Delta^{14}C$ values were within the 20‰ error of the sedimentary-derived carbon, and therefore metabolization of younger peat layers or other similarly aged sedimentary carbon sources cannot be ruled out for this depth. Overall, these findings strongly suggest a predominant source of carbon that is significantly younger than the SOC.

Rather than a primary carbon source, peat and/or other sedimentary derived carbon sources may contain humic substances acting as electron shuttling substrates. This would allow SOC to facilitate enhanced microbial reduction and iron dissolution[27,28,75−79] while more modern carbon sources in the dissolved phase serve as the carbon and electron donors for the microbial community. This indirect role for SOC rather than as the carbon source might explain the co-occurrence of high arsenic concentrations and peat layers reported at some sites,[16,23−28] but arsenic release occurs in the absence of peat layers indicating this is a secondary requirement.[15,18,80]

Preferential Microbial Utilization of Younger Carbon Sources by Sedimentary Bacteria. A preferential utilization of younger dissolved carbon pools over older sedimentary derived carbon by the in situ bacterial communities may be explained by younger carbon pools still containing higher proportions of labile carbon compounds that are more bioavailable than older and more recalcitrant mixtures present in the sedimentary carbon pool.[48] There is also a possibility the older sedimentary pool is labile but somehow protected in environmental settings from microbial degradation. The latter is supported through recent microcosm experiments with Bangladesh sediments, where Neumann et al.[81] reported a promoted mobilization of SOC followed by microbial utilization of sedimentary organic carbon after sampling and homogenization of the sediment. Preferential degradation of younger carbon sources by bacteria in soils and sediments has been reported in other environments through radiocarbon analysis.[48,82,83] The correlation between the age of metabolized carbon sources and the overall size of the sedimentary bacteria communities (Figure 5) supports that younger carbon sources are more labile and may support a larger (more active) bacterial community. Preferential microbial metabolization of younger-aged DOC over older SOC is in agreement with a recent study by Al Lawati et al.[84] Al Lawati et al.[84] reported that no correlational relationship was found between the carbon species distribution within sedimentary carbon and arsenic release in a microcosm experiment using Southeast Asian aquifer sediments (from Taiwan). The authors inferred that an additional electron donor (such as dissolved carbon sources) is providing the electron donors facilitating iron reduction and arsenic release. The single outlier from Site B where the sedimentary bacteria with most depleted $\Delta^{14}C$ also had the highest abundance is a reminder that carbon sources and/or controls on bacterial community abundance can vary locally due to numerous ecosystem factors such as nutrient limitation, organic carbon availability and/or characteristics, changing redox conditions, predatory microeukaryotic populations, etc.

Implications of Carbon Cycling Effects on Arsenic Contamination in Bangladesh. The hydrologic system within Bangladesh has been rapidly changing including increases in irrigation pumping, water withdrawal for municipal pumping causing large scale drawdowns, communities switching to deeper community wells to avoid arsenic exposure, and the installation of local piped water supplies. All of these changes are increasing the demand for groundwater and will increase flow rates while decreasing residence times. The results of this study and those of Mailloux et al.[40] and Harvey et al.[18] all suggest that utilization of relatively modern carbon is driving microbial metabolism in the Holocene-aged sediments and that this carbon can be advected through the aquifer sediments. The changes in the hydrologic regime could redistribute the reactive organic carbon pools throughout both the shallow and deep aquifer sediments and could lead to changes in the microbial communities, geochemistry of the groundwater and the distribution of As.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.est.6b00868.

Field site photograph and map, chemicals and reagents, PLFA data, DOC and DIC concentrations, $\Delta^{14}C$ data, mass balance results, and statistical analyses (PDF)

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Notes

The authors declare no competing financial interest.

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