^{14}C-Dead Living Biomass: Evidence for Microbial Assimilation of Ancient Organic Carbon During Shale Weathering

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Prokaryotes have been cultured from a modern weathering profile developed on a ~365-million-year-old black shale that use macromolecular shale organic matter as their sole organic carbon source. Using natural-abundance carbon-14 analysis of membrane lipids, we show that 74 to 94% of lipid carbon in these cultures derives from assimilation of carbon-14–free organic carbon from the shale. These results reveal that microorganisms enriched from shale weathering profiles are able to use a macromolecular and putatively refractory pool of ancient organic matter. This activity may facilitate the oxidation of sedimentary organic matter to inorganic carbon when sedimentary rocks are exposed by erosion. Thus, microorganisms may play a more active role in the geochemical carbon cycle than previously recognized, with profound implications for controls on the abundance of oxygen and carbon dioxide in Earth's atmosphere over geologic time.

The total global inventory of organic carbon (OC) contained in sedimentary rocks is estimated at roughly 10^{20} mol C (*1*), more than is present in other surface reservoirs, such as oceans, soils, and biomass. In most rocks, >95% of total organic matter (OM) is nonhydrolyzable, solvent-insoluble material termed kerogen. Kerogen composition is typically dominated by long chains of polymethylenic carbon, linked by carbon, sulfur, and oxygen bridging atoms and aromatic ring centers. As such, kerogen represents a natural form of refractory organic matter, comparable to synthetic polyalkylenes in composition and reactivity. However, given ample time, kerogen is susceptible to oxidation on Earth's surface, as revealed by OM loss (*2–4*) and CO_2 generation (*5*) upon exposure of shales to oxidizing conditions.

A wide variety of subsurface microbial communities have been recognized that use forms of ancient sedimentary OC. Most of these are anaerobes, because subsurface environments are largely anoxic. These include sulfate reducers living in deep subsurface shales and sandstones (*6*, *7*), anaerobes living in oil-field brines and oil pipelines (*8*), and hydrocarbon-degrading microorganisms living in petroleum-contaminated aquifers (*9*, *10*). To date, none of these anaerobic communities has been shown capable of degrading kerogen. However, there is evidence that kerogen loss and degradation is geologically rapid in well-oxygenated environments. Studies reveal sharp oxidation fronts in turbidites (*11*, *12*) and shales (*2*) after exposure to oxygen, by contrast with long periods of OM preservation under anoxic conditions in the subsurface.

^{14}C provides a useful tracer of carbon sources in many surficial environments. Atmospheric CO_2 is enriched in ^{14}C, which decays to ^{14}N with a half-life of 5730 years. Thus, any organic material greater than ~60,000 years in age, or any biomolecule assembled from an ancient carbon source, contains essentially no ^{14}C. This discrepancy between modern and ancient carbon provides evidence for kerogen oxidation in glacial tills (*5*). Accelerator mass spectrometry of extremely small masses of carbon allows measurement of the ^{14}C content of individual organic compounds. Such compound-specific radiocarbon analyses demonstrate addition of ancient hydrocarbons to modern soils (*13*), reburial of ancient OM in modern marine sediments (*14*), and chemoautotrophic fixation of ^{14}C-depleted deep-water CO_2 (aq) by Archaea in the modern ocean (*15*).

Phospholipids are integral components of the cell membrane lipid bilayers. Because phospholipids are rapidly degraded after cell death, these compounds are not found in ancient sedimentary rocks and instead indicate the presence of living organisms. Bacterial and eukaryotic cell membranes contain a wide diversity of ester-linked phospholipid fatty acids (PLFAs). Whereas the straight-chain $C_{16:0}$ (palmitic acid) and $C_{18:0}$ (stearic acid) PLFAs (*16*) are ubiquitous among bacteria and eukaryotes, odd-numbered, methyl-branched, and monounsaturated PLFAs are commonly assigned a purely bacterial origin (*17*, *18*). PLFAs are biosynthesized from common precursor biomolecules (e.g., acetyl–coenzyme A) and thus can be used for compound-specific ^{14}C analysis to trace carbon sources and pathways in microbial communities.

Here, we used natural-abundance ^{13}C and ^{14}C analysis of PLFAs to establish that microorganisms enriched from a black shale weathering profile can assimilate shale OM into cellular carbon. The New Albany Shale is a Late Devonian black shale [5 to 15% total organic carbon (TOC)] exposed in Illinois, Indiana, and Kentucky, USA. An exposure near Clay City, Kentucky, has been well characterized by previous studies of black shale weathering. At this site, a recent roadcut has exposed a weathering profile, revealing the complete transition from subsurface, unweathered, high-OC shale to heavily weathered, low-OC regolith at the surface of the profile (Fig. 1).

Sampling and microbial cultures. A core into the Clay City weathering profile was obtained in January 2000 and sectioned on site (*19*) (Table 1). Samples for PLFA analysis were removed from six depths for PLFA characterization (*20*). Small masses were removed from the interior of the core at several depths and transferred to sterile glass culture tubes containing OC-free growth medium for enrichment culturing (*21*). Given the potentially wide diversity of metabolic types in the weathering profile samples, the goal of culturing was to enrich specifically for populations of microorganisms able to use shale OM as their carbon source, namely, aerobic heterotrophs that can assimilate kerogen. After 10 days, a small volume from each of these first-generation enrichments was added to fresh medium that contained shale substrate (*22*). Multiple subcultures over several months in fresh medium and substrate ensured that subsequent generations of cultures were accessing only substrate OM, and not OM entrained in the inoculum. The abundance and morphology of cells (Fig. 2) were monitored by examining stained samples by epifluorescence microscopy (*23*). A large-volume culture was initiated to generate sufficient biomass for PLFA ^{14}C analysis, inoculated with material enriched from the 50- to 60-cm core depth after six dilutions (*24*). After 90 days' growth, the large-volume culture was harvested for PLFA characterization (*25*). The large sample size afforded sufficient quantities of four classes of PLFA, which were submitted for ^{14}C analysis at the National Ocean Science Accelerator Mass Spectrometry Facility, Woods Hole Oceanographic Institution, after further sample preparation (*26*).

PLFA distributions in kerogen-degradation environments. Forty individual PLFAs were identified in core samples (Fig.

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3). In all core samples, $C_{16:0}$, $C_{18:0}$, $C_{18:1}$, and $C_{18:2}$ are the most abundant PLFAs. The presence of methyl-branched, monounsaturated, and cyclopropyl PLFAs revealed substantial bacterial contributions to the total PLFA pool. The highest concentration and greatest diversity of PLFAs were measured in the surface samples. Abundance of total PLFAs decreased downcore from 226 nmol/g at the surface to 12 nmol/g in the deepest sample, accompanied by a shift toward relatively greater contributions from branched, monounsaturated, and cyclopropyl PLFAs. Only seven PLFAs were identified in the large-volume culture, but total PLFA concentrations in culture were similar to those in deeper sections of the weathering profile core.

PLFAs isolated from microorganisms living on black shales provide a window into the diversity and metabolic activity of subsurface microbial communities. The abundance of total PLFA in the core examined in this study is lower than PLFA concentrations measured in typical forest soils (*27*) or marine sediments (*28*), but greater than that measured in anoxic subsurface environments (*7–10*). This suggests that even in environments where O_2 is available, kerogen degradation supports only a limited microbial population compared with environments where fresh terrestrial or marine OM is consumed. PLFAs in this weathering profile core also provided some taxonomic information. PLFA distributions suggest a community dominated by Bacteria with some Eukarya. Certain PLFAs detected in the core have been correlated with the presence of sulfate-reducing bacteria and other groups, e.g., $10Me_{16:0}$, which is a marker for Desulfobacter spp. (*29*), and cyclopropyl $C_{17:0}$ and $C_{19:0}$, which indicate microaerophilic Gram-negative bacteria (*30*). Whole-cell hybridizations with domain-specific probes confirm the presence of Bacteria and Eukarya (*31*); however, more-refined phylogenetic characterization of these samples is not possible with PLFA and, instead, will require DNA extracted from these samples.

PLFA abundance in the enrichment cultures provides an estimate of microbial carbon assimilation. After 90 days' growth, the 40-g culture supported a microbial population containing 200 μg C as PLFA. Given that dry-cell biomass is ~10% phospholipid, this approximates to 2 mg of total biomass carbon. The 40-g shale substrate contained 1.6 g TOC. Assuming a steady rate of carbon assimilation, this corresponds to 20 μg C/day in a population of $\sim 10^8$ cells/ml, given well-mixed conditions and ample substrate and growth medium. However, these organisms were probably actively respiring CO_2 during the course of the incubation, so the rate of kerogen degradation is probably much greater.

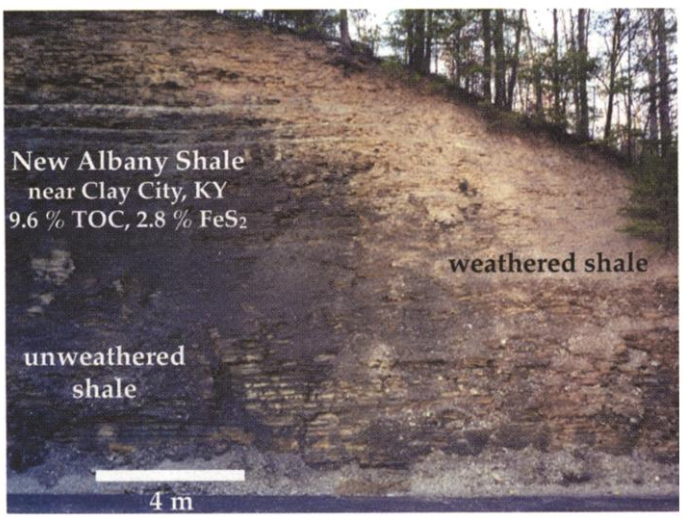

Fig. 1. Field site near Clay City, Kentucky, USA, where a weathering profile developed on New Albany Shale has been exposed by a recent roadcut. Unweathered shale (**left**) grades laterally into heavily weathered regolith (**right**). Evidence for weathering includes loss of color, increased fissility and friability, loss of organic carbon, and loss of pyrite.

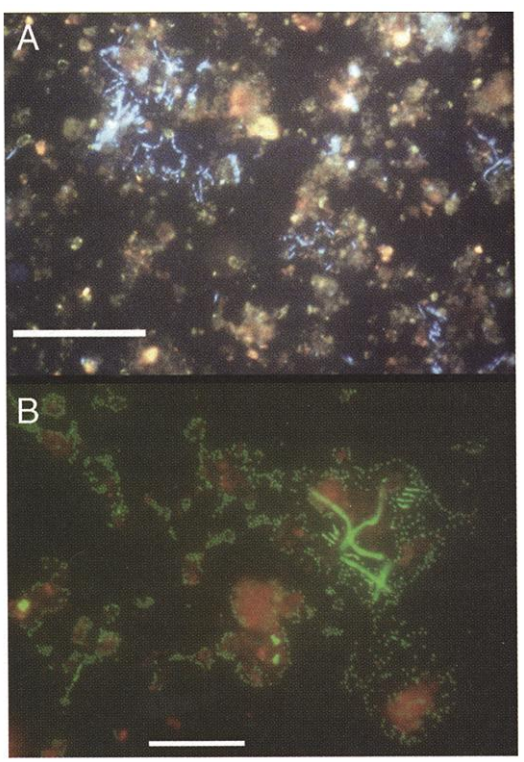

Fig. 2. Photomicrographs of stained microorganisms in association with shale particles. (**A**) Core sample from 50- to 60-cm depth, stained with DAPI. OM fragments fluoresce yellow-orange; stained cells fluoresce blue. Cells appear associated with clusters of mineral fragments and OM. (**B**) Large-volume enrichment culture after 90 days' growth, stained with acridine orange. Stained cells fluoresce green; OM fragments fluoresce red-orange. Cells are gathered along the surfaces of OM fragments. Most cells are prokaryotic rods and cocci, although several eukaryote filaments are visible. Bars, 25 μm.

PLFA carbon isotopic composition. Stable carbon isotope ($\delta^{13}C$) values for PLFAs isolated from the core samples indicated $\delta^{13}C$ variability among the PLFAs (Fig. 4). Measured $\delta^{13}C$ values for kerogen (−29.5‰) and the $C_{16:0}$ and $C_{18:0}$ PLFAs (−28 to −30‰) were similar, whereas the $C_{18:1}$ and $C_{18:2}$ were 3 to 5‰ depleted relative to kerogen. The $C_{16:0}$ and $C_{18:1}$ PLFAs had consistent $\delta^{13}C$ values throughout the core, whereas the $C_{18:2}$ and $C_{18:0}$ revealed depth-related trends in isotopic composition. For depths where those compounds are present, the unsaturated and methyl-branched PLFAs were between 3 to 6‰ depleted relative to kerogen. Leaf litter and humus collected from the core top measured –25.7‰, which is ~4‰ enriched relative to shale kerogen. Isotopic compositions for PLFAs from the culture share similarities with PLFAs from the core. The $C_{18:0}$ and $C_{16:0}$ PLFAs are most enriched, and the unsaturated and cyclopropyl PLFAs are more depleted. Overall, however, these PLFAs are enriched in ^{13}C relative to the kerogen substrate provided in the culture.

The ^{13}C depletion in PLFAs isolated from the core relative to kerogen can be understood as the mixed contribution from heterotrophy of kerogen and chemoautotrophy of weathering profile CO_2. Because the soil pore spaces are in limited exchange with the atmosphere, respiration results in accumulation of CO_2 derived from organic matter.

Thus, the CO_2 available for autotrophy is ^{13}C-depleted, and the resulting lipids will reflect this depletion.

The $\Delta^{14}C$ value of the kerogen substrate used for the culture measured –990‰, or essentially no ^{14}C. Four PLFA compound classes were isolated from the culture for ^{14}C analysis: (i) $C_{16:0}$, (ii) $C_{18:0}$, (iii) $C_{18:1}$ + $C_{18:2}$, and (iv) $cycC_{17:0}$ + $cycC_{19:0}$. The measured $\Delta^{14}C$ for these four PLFA classes ranged from –711‰ to –922‰ (Table 2). These $\Delta^{14}C$ values were used in a linear mixing model to estimate the relative contribution of kerogen (−990‰) and modern carbon (+100‰). The estimated fraction of carbon in these PLFAs derived from kerogen ranged from 74 to 94%. The remainder of the carbon probably derived from chemoautotrophic fixation of atmospheric CO_2 dissolved in the low-pH growth medium.

Carbon pathways in kerogen-degrading microorganisms. Taken together, the ^{13}C and ^{14}C data on PLFAs from the large-volume culture provide interpretation of carbon pathways in this microbial community. In culture, there are two carbon sources of distinct ^{13}C isotopic composition: kerogen (−29.5‰) and dissolved CO_2, which is itself a mixture of atmospheric CO_2 (−8‰), dissolved carbonate (~0‰), and CO_2 derived from degraded kerogen (−29.5‰). PLFA $\Delta^{14}C$ values require that these organisms obtain 74 to 94% carbon from a ^{14}C-depleted source. This excludes atmospheric CO_2 fixation as the dominant carbon pathway in this community. Other pathways include (i) kerogen assimilation by heterotrophs, (ii) remineralization followed by fixation of kerogen-derived CO_2, or (iii) dissolution of shale carbonate followed by fixation of carbonate-derived CO_2 (substrate measured <0.1% $C_{inorganic}$). Lipids of most autotrophic organisms are significantly (20 to 30‰) depleted in ^{13}C relative to their CO_2 source, whereas lipids of heterotrophic organisms measure generally ±4‰ relative to their carbon source. Culture PLFAs ranged from –24 to –27‰. These values are possible only under scenarios (i) and (iii) above; fixation of CO_2 derived from oxidized kerogen would be associated with substantial isotope depletion. Scenario (iii) is less likely than (i), because the growth medium was well mixed and in free exchange with the atmosphere, and during the 90 days of the experiment trended toward isotopic equilibrium with atmospheric CO_2. Subsequent culturing experiments have further excluded scenario (iii). Cultures grown with HCl-digested shale and demineralized kerogen as substrate have revealed that microorganisms enriched from the weathering profile are able to grow in the absence of carbonate and on pure OM without pyrite (a likely electron donor for chemoautotrophy) (*32*). Cell morphologies, generation times, and stationary-phase populations in these incubations were comparable to growth on shale substrate. Growth experiments with polished New Albany Shale blocks (*33*) as substrate have also revealed a strong affinity for microbial colonization along select, OM-rich microlaminae within the rock (Fig. 5). This association reveals that population growth is enhanced along surfaces rich in OM, suggesting that the organisms are actively using kerogen and not CO_2 released into solution by oxidation. The explanation consistent with ^{13}C and ^{14}C analysis and the various culturing experiments is that these organisms are heterotrophs that use kerogen.

The relative patterns of PLFA $\delta^{13}C$ values between the core and culture samples are similar. $C_{16:0}$ and $C_{18:0}$ PLFAs are enriched in ^{13}C relative to cyclopropyl and to unsaturated and branched PLFAs, consistent with a previous study on bacterial PLFA isotopic composition variability (*34*). The overall enrichment in ^{13}C in culture samples relative to the core can be understood as a difference in the $\delta^{13}C$ of CO_2 available to chemoautotrophic organisms in these two environments.

^{14}C measurements of PLFAs from the weathering profile were not initially sought because resulting data are likely to be more ambiguous than the simple culture experiment owing to the need to account for multiple carbon sources. These include kerogen, atmospheric CO_2, soil OM, respired kerogen and soil OM, and carbonates.

Implications for global carbon and oxygen cycling. The presence of microorganisms capable of using kerogen may have significant implications for global-scale cycling of carbon and oxygen. The concentration of oxygen in Earth's atmosphere is controlled by a balance between the burial of OM in sediments and the subsequent weathering of ancient sedimentary rocks on the continents (*35*, *36*). Photosynthesis results in fixation of CO_2—chemical reduction

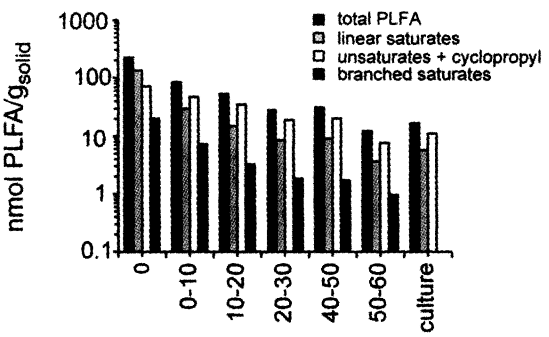

Fig. 3. Concentrations of total PLFA and compound classes for selected depths (in cm) within weathering profile core and large-volume culture. In the core surface sample, linear PLFAs were predominant. In all other samples, unsaturated and cyclopropyl PLFAs were predominant.

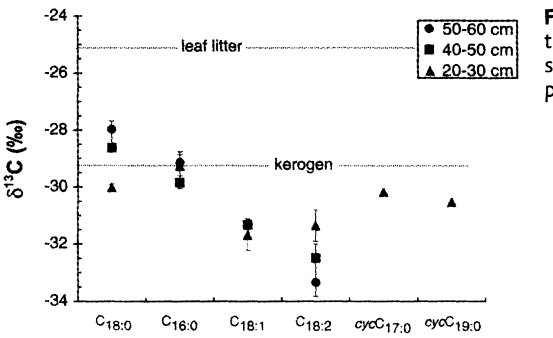

Fig. 4. Stable carbon isotopic composition of PLFAs from the three deepest sections of weathering profile core, reported in ‰ on the $\delta^{13}C$ scale.

Table 1. C_{org}, $\Delta^{14}C$, PLFA, $C_{16:0}$, and calculated biomass for core samples and enrichment culture. ND, not determined.

Core depth (cm)	% C_{org}	$\Delta^{14}C$* (‰)	Total PLFA† (nmol/g_{solid})	Biomass‡ (10^8 cells/g_{solid})	pH§
0	ND	ND	226.8	15.3	
0–10	ND	ND	84.3	5.3	
10–20	1.3	−151.6	53.2	3.1	
20–30	0.7	−472.9	29.9	1.7	2.2
40–50	4.3	−726.9	30.9	2.1	
50–60	7.7	−996.0	12.2	0.9	2.3
Culture	4.0	−990.1‖	16.8	1.9	3.0

*Values reported for TOC, measured as ^{14}C depletion in ‰ relative to 1950 HOxI standard. †Quantified with $C_{28:0}$ methyl ester internal standard. ‡Calculated with 5×10^8 cells/nmol $C_{16:0}$ (*18*). §pH measured on waters obtained from lysimeters. ‖Culture $\%C_{org}$ and $\Delta^{14}C$ reported for shale substrate used in incubations.

of CO_2 to carbohydrates—in the process releasing O_2 to the atmosphere and ocean surface waters. Although most biomass carbon is respired or otherwise remineralized to CO_2 within months after fixation, a small fraction of biomass becomes buried in sediments. Organic carbon buried in sediments represents a net addition of O_2 to the atmosphere, understood as total oxygen produced by photosynthesis less the oxygen consumed by respiration. Eventually, sediments become lithified to sedimentary rocks, and processes of uplift and exhumation result in exposures of ancient sedimentary rocks on Earth's continents. Weathering and erosion expose chemically reduced organic carbon in sedimentary rocks to the O_2-rich environment of the atmosphere. At sites of weathering of ancient sedimentary rocks, oxidation and loss of OM in sedimentary rocks, as a result of chemical weathering, have been shown. This oxidation consumes O_2 by mechanism(s) that, on a global scale, to a large degree balance the net oxygen release associated with OM burial. This study shows that one of these mechanisms is biological assimilation of kerogen. Remarkably, this near balance between O_2 production and consumption has been maintained for at least the past ~560 million years. Small imbalances between global rates of OM burial in sediments and OM oxidation during weathering have resulted in modest changes in atmospheric O_2 during Earth's past (*37*, *38*). However, controls on OM oxidation during weathering remain poorly understood, and thus understanding of the evolution of Earth's atmosphere remains limited.

Relict, ancient OM has been detected in modern sediments (*14*), suggesting that OM oxidation is not 100% efficient in all environments during weathering. The presence of this residual OM after weathering implies that quite commonly, oxidative weathering of ancient OM occurs more slowly than rates of erosion and mass transport. This suggests a kinetic barrier to OM oxidation in shale weathering profiles in spite of abundant available oxygen. ^{14}C-depleted bacterial phospholipids demonstrate that kerogen is or becomes to some extent bioavailable and able to support growth of some microorganisms during weathering. Thus, biological processes may play a previously unrecognized pivotal role in controlling the rate of oxidation of OM during weathering of sedimentary rocks. The limited taxonomic and metabolic information obtained from PLFA analysis indicates that this community contains a consortium of heterotrophs and autotrophs, and aerobes and anaerobes. Thus, it is unclear what effect variations in O_2 concentration would play on the rate of kerogen degradation by this community. Further study is required before any possible feedback between atmospheric oxygen concentration and rates of microbial kerogen degradation can be established. Biological activity has long been acknowledged as a key component of the geochemical carbon cycle, owing to the role that organisms play in OM production and subsequent degradation during sediment burial and diagenesis. Discovery of ^{14}C-depleted living organisms growing on ancient shale OM indicates that remineralization of ancient organic matter during weathering of sedimentary rocks may likewise be controlled by microbial activity.

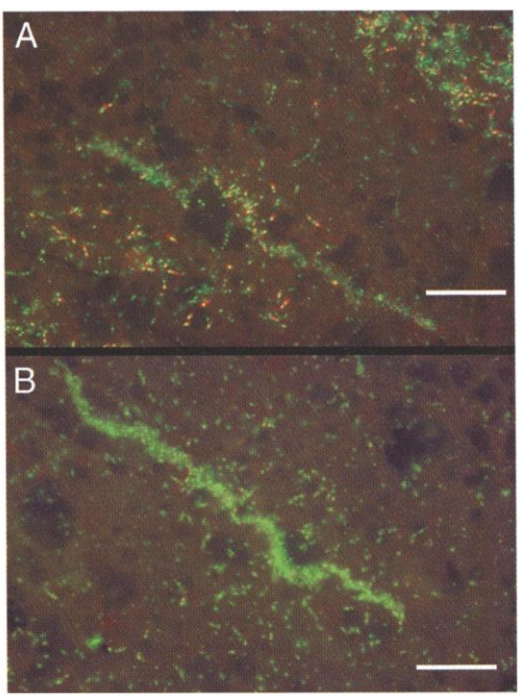

Fig. 5. (**A** and **B**) Photomicrographs of microbial colonies attached to a polished surface of New Albany Shale. Polished blocks were colonized for 96 hours, rinsed in growth medium, stained with acridine orange, and viewed by epifluorescence microscopy. Colonies did not grow radially, but instead followed fine, dark laminae within the shale that are rich in OM. Colonies appear to have grown around large mineral grains, indicating selective attachment to the shale surface. Bars, 25 μm.

Table 2. ^{14}C and ^{13}C analysis of PLFA compound classes isolated from enrichment culture grown on New Albany Shale, and calculated fraction of PLFA carbon derived from ancient kerogen.

PLFA	Mass (nmol/g)	$\Delta^{14}C$ (‰)	$F_{ancient\ carbon}$	$\delta^{13}C$ (‰)
$C_{16:0}$	3.91	−711.3 ± 14.4	0.744	−25.56
$C_{18:0}$	1.78	−773.9 ± 13.8	0.802	−26.19
$C_{18:1} + C_{18:2}$	5.08 ($C_{18:1}$) 1.16 ($C_{18:2}$)	−882.2 ± 6.0	0.901	−26.50
$cycC_{17:0} + cycC_{19:0}$	1.65 ($cycC_{17:0}$) 2.87 ($cycC_{19:0}$)	−921.6 ± 7.8	0.937	−26.94
Total mass PLFA	16.45			
Kerogen in shale substrate		−990.1 ± 0.8		−29.5
Modern atmospheric CO_2		~+100		

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16. PLFAs are designated by the ratio of the number of carbon atoms to the number of double bonds. A *cyc* prefix indicates a cyclopropyl ring.
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19. The 60-cm core was obtained with a hand-powered coring auger equipped with presterilized stainless-steel core sleeves. Material from the bottom of the core was dark in color, blocky, and unfractured and gave off a petroliferous odor. Mid-depths of the core exhibited progressive lightening in color and increased friability. Iron staining was visible along several partings in the shale. The top 10 cm of the core was characterized by light brown shale rubble interspersed with roots and leaf litter. Further core data are provided in Table 1. Lysimeters were installed in the weathering profile and were sampled in June 2000 to measure soil-water pH.

20. Core samples were maintained at −70°C until ultrasonically extracted with 2:1 CH_3OH/CH_2Cl_2 in an ice bath. Total lipid extracts were separated by silica gel column chromatography into hydrocarbon, diglyceride, and phospholipid fractions. PLFAs were transesterified with $NaOCH_3$ to methyl esters. PLFA quantification and structural confirmations were achieved by gas chromatography and mass spectrometry with octacosanoic acid methyl ester as an internal standard. PLFA stable-carbon isotopic compositions were determined by gas chromatography and isotope ratio monitoring mass spectrometry. ^{13}C data are reported with $\delta^{13}C$ notation as ‰ deviation from the Pee Dee Belemnite standard and were corrected for the addition of methyl carbon during transesterification.
21. Growth medium contained 7.5 mM Na_2PO_4, 5.9 mM KH_2PO_4, 0.8 mM $MgSO_4$, 15 mM $(NH_3)_2SO_4$, and HCl (to pH 3).
22. Shale substrate was prepared by powdering, autoclaving, and ultrasonically extracting unweathered New Albany Shale (6.8% C_{org}, 3.2% FeS_2) in 2:1 CH_2Cl_2/CH_3OH. The extracted shale was then dried at 40°C for 18 hours to drive off solvent.
23. To monitor cells, 2 to 10 μl were removed from cultures and either dried in air onto microscope slides or filtered onto black polycarbonate membranes (0.2 μm). Slides and filters were stained with DAPI (4′,6′-diamidino-2-phenylindole), which binds to DNA, or acridine orange, which binds to DNA and RNA. Slides and mounted filters were observed with a Zeiss Axiovert S100 equipped with a Hg vapor lamp.
24. Inoculum (200 μl) was added to 10 ml of growth medium and 1 g of shale substrate. Additional substrate and medium were added over 10 days to a final volume of 200 ml of medium and 40 g of shale. The culture was incubated at 25°C in the dark until harvested for PLFA analysis. Total DAPI-stained microbial population immediately before harvesting was ~10^8 cells/ml, extrapolated from 20 counts conducted on three separate filters.
25. The culture was centrifuged to separate solids from growth medium, and then extracted as in (*20*).
26. PFLA methyl esters from the culture were separated by preparative scale gas chromatography into four PLFA compound classes. Purified PLFAs were sealed in evacuated quartz tubes with CuO and combusted to CO_2 at 850°C. CO_2 was reduced to graphite targets over a Co catalyst and analyzed for ^{14}C content by the National Ocean Science Mass Spectrometry facility at Woods Hole Oceanographic Institution.
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31. Fluorescent in situ hybridizations were performed on core samples and cultures with established hybridization protocols and fluorescent-labeled probes (universal and domain-level bacterial, archaeal, and eukaryotic probes). Positive hybridizations and negative controls showed that in culture and deepest core samples, the cell population was predominantly Bacteria, with smaller numbers of Archaea and Eukarya.
32. Carbonate-free shale was prepared by digesting powdered, solvent-extracted shale in 12 M HCl at 25°C with stirring for 24 hours, followed by multiple washes in an excess of distilled water until the pH of washes reached 5. Kerogen was isolated from carbonate-free shale by digestion in 48% HF at 25°C for 18 hours in Teflon centrifuge tubes. HF-digested shale was rinsed in an excess of distilled water, then suspended in a 60% $ZnBr_2$ solution (density, 1.6 g/cm^3) and centrifuged at 4500 rpm for 30 min to separate pyrite from OM. Floated material was collected; rinsed in distilled H_2O, CH_3OH, and CH_2Cl_2; and dried at 40°C to remove solvent.
33. Polished blocks of unweathered New Albany Shale were sterilized by autoclaving at 120°C for 20 min, and then introduced into existing cultures. Blocks were removed after 24, 48, and 96 hours. Blocks were rinsed in sterile growth medium to remove loosely attached cells, stained with acridine orange, and viewed by epifluorescence microscopy to examine attachment of microbial cells to the shale surface.
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REPORTS

Quantum Electronic Stability of Atomically Uniform Films

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We have studied the structural stability of thin silver films with thicknesses of N = 1 to 15 monolayers, deposited on an Fe(100) substrate. Photoemission spectroscopy results show that films of N = 1, 2, and 5 monolayer thicknesses are structurally stable for temperatures above 800 kelvin, whereas films of other thicknesses are unstable and bifurcate into a film with $N \pm 1$ monolayer thicknesses at temperatures around 400 kelvin. The results are in agreement with theoretical predictions that consider the electronic energy of the quantum well associated with a particular film thickness as a significant contribution to the film stability.

Structures and devices of nano-dimensions have useful and possibly unique properties (*1*), and an important issue for practical applications is structural stability. As the physical size of a system approaches atomic dimensions, quantum effects are likely to play a significant role. Here we demonstrate that quantum electronic effects can be the dominant factor for the stability of thin films. We used thin Ag films prepared on Fe(100), which can be made with atomic-layer precision up to ~100 monolayers (ML) and over a macroscopic area (*2*). Angle-resolved photoemission from these films shows quantum-well states corresponding to confined electrons. Cutoff of the occupancy of these states at the Fermi level gives rise to monolayer-by-monolayer variations in total electronic energy. The predicted stability for films with $N > 1$ agrees well with an experiment in which each film is ramped up in temperature until its geometry changes. Films with thicknesses of N = 1, 2, and 5 ML are unusually stable, and remain intact to temperatures above 800 K. Other films with thicknesses of N up to 15 begin to evolve at temperatures around 400 K and bifurcate into adjacent-integer-monolayer thicknesses $N \pm 1$. In our experiment, no specific "magic thicknesses" (*3*–*5*) are ultimately produced. Recent work on Au nanowires has concluded that atomic packing is the deciding factor underlying structural stability, and that electronic effects are less important because of the lack of *d*-electron participation at the Fermi level (*6*, *7*). Ag is electronically similar to Au, and the present results constitute a counter example.

To determine the film thickness and its evolution, we used angle-resolved photoemission. Quantum-well states arising from electron confinement in a film have energies uniquely related to the film thickness (*8*, *9*). Photoemission probes these energy levels directly and is thus a sensitive tool for absolute film thickness measurement. Atomically uniform films of Ag of thicknesses N = 1 to 15 were prepared, and the temperature of each film was then ramped up slowly from a base temperature of ~110 K (also the initial Ag deposition temperature), while spectra were taken repetitively to monitor any spectral changes. Results for initial film thicknesses of 6 and 3 ML (Fig. 1) illustrate the general behavior. The spectra taken at low temperatures (top spectrum in each panel) show

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