Expedition 337 summary[1]

Expedition 337 Scientists[2]

Chapter contents

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[2]**Expedition 337 Scientists' addresses.**

Abstract

Integrated Ocean Drilling Program (IODP) Expedition 337 was the first expedition dedicated to subseafloor microbiology that used riser drilling technology. The site examined during this expedition (Site C0020) is located off the Shimokita Peninsula, Japan, at a water depth of 1180 m in a forearc basin formed by the subduction of the Pacific plate under the Okhotsk plate. Previously conducted seismic profiles suggested the presence of deep, coal-bearing horizons at ~2 km below the seafloor. Our primary scientific objectives during Expedition 337 were thus to study the relationship between these deep coalbeds and carbon cycling, as well as to explore the distribution of subseafloor life at the greatest depths that have ever been sampled by scientific ocean drilling. A key question that guided our research strategy was "Do deeply buried hydrocarbon reservoirs, such as coalbeds, act as geobiological reactors that sustain subseafloor life by releasing nutrients and carbon substrates?" To address this question and other objectives, we drilled through a 2466 m deep sedimentary sequence with a series of coal layers at ~2 km below the seafloor. Hole C0020A is thus the deepest borehole in the history of scientific ocean drilling, surpassing the previous maximum penetration depth by 355 m and providing the opportunity to extend the maximum depth of subseafloor life detection by >800 m. Site C0020 also provides the first geological record of a dynamically changing depositional environment in the former forearc basin off the Shimokita Peninsula during the late Oligocene and Miocene. This record comprises a rich diversity of lithologic facies reflecting environments ranging from warm-temperate coastal backswamps to cool-water continental shelf. The use of riser drilling technology in very deep sediment created both unique opportunities and new challenges in the study of subseafloor life. The continual use of drilling mud during riser drilling operations required implementation of a rigorous program dedicated to sample quality assurance and quality control. We successfully added chemical tracers to drilling mud to monitor levels of drilling mud contamination of samples and quantified levels of mud-derived solutes in interstitial fluid. Therefore, our data provide a framework for differentiating signals of indigenous microbes from those of contaminants. For the first time ever in scientific ocean drilling, we conducted downhole in situ fluid analysis and sampling as part of the logging operations. Logging operations yielded data of unprecedented quality that provide a comprehen-

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sive view of sediment properties at Site C0020. With an estimated temperature gradient of 24.0°C/km, temperatures in coal-bearing horizons are near 50°C and thus well within the known temperature range of life. We conducted gas analyses using a newly installed mud-gas monitoring laboratory. Gas chemistry and isotopic compositions provide the first indication of biological activity in deep horizons associated with the coalbed. Last but not least, this expedition provided a testing ground for the use of riser drilling technology to address geobiological and biogeochemical objectives and was therefore a crucial step toward the next phase of deep scientific ocean drilling. Potential benefits of deep riser drilling for the scientific community are enormous, but its implementation will require the adaptation and further fine tuning of riser drilling technology to the needs of basic research.

Introduction

Marine subsurface hydrocarbon reservoirs and the associated microbial life are among the least characterized ecosystems on Earth. Our understanding of the biological and abiotic processes associated with hydrocarbon production and consumption within these reservoirs is limited by the logistical challenge of obtaining microbial contamination-free samples from these systems. As a result, fundamental questions regarding deep subseafloor hydrocarbon systems have remained unanswered. These include

- What role does subsurface microbial activity play in the formation of hydrocarbons and hydrocarbon reservoirs?
- Do deeply buried hydrocarbon reservoirs, such as natural gas deposits and coalbeds, act as geobiological reactors that sustain subsurface life by releasing nutrients and carbon substrates?
- Do the conversion and transport of hydrocarbons and other reduced compounds influence biomass, diversity, activity, and function of deep subseafloor microbial communities?
- What are the fluxes of both thermogenically and biologically produced organic compounds, and how important are these for the carbon budgets in the shallower subsurface and overlying ocean?

To address these important scientific questions, Integrated Ocean Drilling Program (IODP) Expedition 337 aimed to drill into deeply buried coalbeds off the Shimokita Peninsula, Japan, in the northwestern Pacific using the riser drilling system of the D/V *Chikyu*.

The deep subseafloor biosphere

Subseafloor sediments harbor a vast microbial biomass (Parkes et al., 1994, 2000; Whitman et al., 1998; Lipp et al., 2008; Kallmeyer et al., 2012; Hinrichs and Inagaki, 2012). To date, microbial cells have been detected in sediment as old as the Cretaceous and as deep as 1626 meters below seafloor (mbsf) (Newfoundland margin, Ocean Drilling Program [ODP] Leg 210; Roussel et al., 2008). Diagenetic models of pore water chemical constituents and radioactive and stable isotope tracer incubation experiments show that metabolic activities of deep subseafloor microbes are extremely low because of the low supply of nutrient and energy substrates (D'Hondt et al., 2002, 2004). Though recent studies suggest a large fraction of microbial cells to be dormant or endospores (Lomstein et al., 2012; Langerhuus et al., 2012), experiments have demonstrated that most cells restore physiological activity when exposed to energy-replete conditions in the laboratory (Morono et al., 2009). Moreover, past studies have shown the subseafloor microbial activity to be stimulated at geochemical and/or lithologic interfaces such as porous ash layers and sulfate–methane transition (SMT) zones (Inagaki et al., 2003; Parkes et al., 2005; Biddle et al., 2006; Sørensen and Teske, 2006). The metabolic activities of subseafloor microbial communities are largely controlled by the flux of bioavailable electron donors and/or acceptors. These derive either from photosynthetic primary production in overlying seawater or terrestrial environments (D'Hondt et al., 2004, 2009; Lipp et al., 2008) or from fluids entering sedimentary habitat from the underlying Earth's crust (Cowen et al., 2003; Nakagawa et al., 2006; Engelen et al., 2008; Orcutt et al., 2011). Thus, sedimentological characteristics, such as sediment organic matter content, and lithologic characteristics, which influence fluid flow regimes, determine the energy available to subseafloor life and control habitability of the deep subseafloor environment.

Culture-independent molecular ecological surveys of 16S rRNA gene fragments reveal that microbial communities in continental margin sediment are predominantly composed of species lacking cultivated relatives, such as Bacteria within the candidate division JS1, Chloroflexi, and Planctomycetes, and Archaea within the Deep-Sea Archaeal Group, the Miscellaneous Crenarchaeotal Group, and the South African Gold Mine Euryarchaeotal Group (e.g., Inagaki et al., 2003, 2006b; Inagaki and Nakagawa, 2008). The carbon isotopic analysis of intact polar lipids and fluorescence in situ hybridization (FISH)-stained cells suggest that heterotrophic archaea sig-

nificantly contribute and sometimes even dominate microbial populations in organic-rich sediment (Lipp et al., 2008; Kubo et al., 2012) or in SMT zones, where the occurrence of anaerobic oxidation of methane (AOM) mediated by methanotrophic archaea and sulfate-reducing bacteria takes place (Biddle et al., 2006). Despite the significance of an organic-fueled microbial ecosystem in biogeochemical cycling within continental margin sediment, the metabolic pathways of organic matter degradation and fluxes of secondary metabolites remain largely unknown (e.g., Hinrichs et al., 2006; Onstott et al., 2010).

Coal diagenesis: microbiological significance for biogeochemical cycles

Within the generally energy-starved deep subseafloor biosphere, deeply buried immature coal (e.g., lignite) is a potential source of nutrients and energy for microbial communities. Previous studies of terrestrial coal deposits suggest that microorganisms play important ecological roles in coal diagenesis, producing substantial quantities of coalbed methane and secondary products (e.g., Brown et al., 1999; Detmers et al., 2001; Shimizu et al., 2007; Krüger et al., 2008; Strapoc et al., 2008; Jones et al., 2008; Fry et al., 2009; Glombitza et al., 2009; Orem et al., 2010; Ünal et al., 2012). The microbial communities in terrestrial coal habitats are phylogenetically diverse, despite often having low cell densities ($<10^6$ cells/cm^3). Methane-producing archaea (i.e., methanogens) such as the genera *Methanoculleus, Methanobacterium, Methanolobus,* and *Methanosarcina,* as well as potential acetate-producing bacteria (i.e., acetogens) such as *Acetobacterium,* were predominantly detected from a deep borehole aquifer directly connected to the coal deposits in the Hokkaido Island, Japan (Shimizu et al., 2007). Using incubation tracer experiments and FISH, active aceticlastic methanogenesis was found to occur even in a highly altered graphite deposit (Krüger et al., 2008). Fry et al. (2009) reported large cultivable populations of sulfate-reducing bacteria, methanogens, acetogens, and lignite-utilizing heterotrophs in the uplifted coaly sediment of northern New Zealand based on most probable number cultivations. In terrestrial deep subsurface shales, metabolic activities were stimulated at geologic interfaces between coal and sand/silt layers (e.g., Krumholz et al., 1997), and concentrations of organic acids were higher in coal than other layers, consistent with the co-occurrence of coal diagenesis and microbial processes.

Despite the microbiological and (bio)geochemical significance of coaly deposits for the global carbon cycle, there have been no studies of coal layers deeply buried underneath the subseafloor, mainly because of safety regulations applied to hydrocarbon gas–related hazards during riserless drilling. In continental margin sediment, large quantities of gaseous hydrocarbons and their derivatives (e.g., H_2 and organic acids) are generated by thermogenic and/or biogenic degradation processes of deeply buried organic matter such as lignite coal. All of these diagenetic compounds are potential nutrient and energy sources to deep subseafloor microbial communities. Hence, microbial activity associated with deeply buried coalbeds may influence characteristics of dissolved gases and organic matter, as well as the accumulation of gas hydrates in shallow sedimentary sequences. The relationship between deep subseafloor hydrocarbon systems and their microbial inhabitants is a frontier research theme in geochemistry and geobiology that can only be better understood through dedicated research initiatives such as Expedition 337.

Exploring the feasibility of CO_2 sequestration in deep offshore geological repositories

To date, CO_2 capture and sequestration (CCS) into deep subsurface environments, such as oil, gas, and porous aquifers, is considered a potential means to reduce anthropogenic release of CO_2 to the atmosphere and weaken the negative impact of anthropogenically induced climate change. CCS offshore in deep subseafloor environments has a number of advantages, including fewer risks than shallow-water CCS (House et al., 2006; Schrag, 2009). It has been predicted that CCS can potentially reduce future world emissions from fuel energy by ~20% (Dooley et al., 2006). In a terrestrial saline aquifer located in Germany, CO_2 injection has stimulated microbial growth and activity, indicating that deep microbial communities respond to drastic environmental change (Morozova et al., 2010; Krüger et al., 2011). In deep-sea CO_2 seeps, small microbial populations that mediate carbon cycling have been observed in sediment under high CO_2 and low pH conditions (Inagaki et al., 2006a; Yanagawa et al., 2013). By contrast, the behavior and stability of CO_2 and its geochemical and biological reactions in deep-marine subsurface repositories are still almost completely uncertain (Onstott, 2005; Kirk, 2011).

Using cored samples from Expedition 337, multiple scientific issues regarding geological CO_2 sequestration will be addressed through shore-based ex situ experiments. These include

- How does liquid or supercritical CO_2 penetrate into various lithostratigraphic settings?

- How does CO_2 react with minerals, organic matter, and life in the deep subsurface?
- What are the impacts of long-term CO_2 storage on biogeochemical carbon cycling and the subsurface biosphere?

By conducting various multidisciplinary ex situ experiments using cored materials and performing in situ logging characterizations of the deep-riser hole, Expedition 337 will significantly expand our knowledge of the coalbed subseafloor hydrocarbon system, including the physicochemical and biological factors that determine the potential for CO_2 sequestration.

Background

Geological setting

IODP Site C0020 is located in a forearc basin formed by the subduction of the Pacific plate (~8 cm/y, west-northwest plate motion vector; Seno et al., 1996) beneath northeastern Honshu, Japan (Fig. F1). The Hidaka Trough, a sedimentary basin formed by subsidence in the drilling area, originates just offshore, southwest of Hokkaido, and extends to the Japan Trench. Along the coastal area of the Shimokita Peninsula, both sedimentary and volcanic rocks younger than Late Cretaceous lie scattered on Triassic to Early Cretaceous sedimentary rocks or Cretaceous granites.

Several scientific drilling expeditions have been carried out off Shimokita Peninsula: Deep Sea Drilling Project–International Phase of Ocean Drilling (DSDP-IPOD) Legs 56 and 57 in 1977, DSDP-IPOD Leg 87 in 1982, and ODP Leg 186 in 1999. In addition, well data are available from hydrocarbon drilling exploration carried out between 1977 and 1999 (Japan Natural Gas Association and Japan Offshore Petroleum Development Association, 1992; Osawa et al., 2002). Seismic profiles around Site C0020 show pull-up blanking reflections below bottom-simulating reflectors at ~360 mbsf, suggesting the occurrence of methane hydrates in shallow sedimentary realms and a strong upward flux of free hydrocarbon gases from deep hydrocarbon reservoirs (Fig. F2). A thick and prominent Quaternary sedimentary unit onlaps a Pliocene unit and is thought to be composed mainly of alternating beds of mud and sand with intercalations of thin volcanic tephras and locally developed gravel/sand layers. The Pliocene unit consists primarily of alternating beds of mudstone and sandstone. Below these relatively recent formations, sedimentary deposits are believed to range from Cretaceous to Miocene in age and are cut by many landward-dipping normal faults. The presence of coal formations has been confirmed by natural gas drilling exploration at Site MITI Sanriku-Oki, ~50 km southward of Site C0020 (Fig. F1) (Osawa et al., 2002). Sonic logging data in the MITI Sanriku-Oki well showed that three major tuff layers involving coal layers with 30, 45, and 80 m thickness (40%–60% total organic carbon [TOC] in lignite coal layer and 0.5%–2% TOC in tuffs) are present in Eocene and Pliocene–Upper Cretaceous horizons, in which vitrinite reflection values (R_o) range between 0.5 and 0.7, indicating burial of relatively immature coals in the ocean (Osawa et al., 2002). In situ temperatures are expected to lie well within the range of the habitable zone of microbes, based on the previously reported thermal gradient of 22.5°C/km in that area (Osawa et al., 2002).

In 2002 and 2003, 2-D seismic surveys off Shimokita Peninsula were carried out by the Japan Agency for Marine-Earth Science and Technology (JAMSTEC) in a 15 km (north–south) × 30 km (east–west) area using the R/V *Polar Duke* and *Polar Princess*. During the NT04-01 cruise using R/V *Natsushima* in 2003, detailed bathymetry mapping was performed using a SeaBat 8160 multibeam echosounder with a frequency of 50 kHz (Taira and Curewitz, 2005) (Fig. F2). Site C0020, which is alternatively designated as Site C9001 by JAMSTEC, is located on the cross point of seismic Lines ODSR03–BS and ODSRW03–H81. During the *Chikyu* shakedown cruise (Expedition CK06-06) in 2006, 365 m of sediment core were recovered from the upper sedimentary section at Site C9001 (41°10.5983′N, 142°12.0328′E, 1180 m water depth), ~80 km off the coast of Shimokita Peninsula, Japan (Fig. F1) (Aoike, 2007). During the same cruise, riser drilling was tested to 647 mbsf without coring, 20 inch casing was installed to 511 mbsf, and the riser hole was suspended for a future riser drilling opportunity (i.e., Expedition 337).

Pilot studies of shallow subsurface sediment at Site C0020

The sediment cored from JAMSTEC Site C9001 during the *Chikyu* shakedown cruise was composed primarily of diatom-rich hemipelagic silty clay intercalated with volcanic tephra and sand layers. Preliminary biostratigraphic age models indicated very high sedimentation rates, ranging from 54 to 95 cm/k.y., and an approximate core-bottom age of 640 ka (Aoike, 2007; Aoike et al., 2010; Domitsu et al., 2010). During this shakedown cruise, core temperature anomalies were monitored immediately after recovery by Thermo-View infrared camera in order to identify and locate methane hydrates. Formation of methane hydrates, as well as metabolically active microbial aggregates that could be visualized using the FISH technique (F. Inagaki et al., unpubl. data), were observed in porous ash and sandy layers. Geochemi-

cal analyses of interstitial water consistently showed that chloride and other sea salts are depleted within the porous layers as a result of hydrate dissociation (Tomaru et al., 2009). Iodine concentrations and radioisotopic compositions (^{129}I/I) of deep pore waters suggest that the iodine and oldest hydrocarbon sources could be as old as 40 Ma (Tomaru et al., 2009). Acetate concentrations in pore waters were >100 µmol/L throughout the sediment column (maximum = 313 µmol/L; H. Yoshioka et al., unpubl. data), which was tentatively interpreted to be possibly linked to coal diagenesis in the deeper subsurface.

Microbial cell numbers in sediment from Site C9001 were evaluated by the fluorescent image-based automated cell count system, which showed that the sediments contain abundant microbial cells with counts >10^7 cells/cm^3 to 365 mbsf (Morono et al., 2009). The abundance of Bacteria and Archaea was studied by quantitative polymerase chain reaction and slot-blot hybridization techniques, suggesting a significant contribution of Archaea to subseafloor microbial biomass (average is ~40% at DNA level; Lipp et al., 2008). Phylogenetically diverse, reductive dehalogenase-homolog genes (*rdhA*) were detected, suggesting the occurrence of anaerobic microbial respiration using organohalides as electron acceptors (Futagami et al., 2009).

The metabolic activity of organoclastic sulfate reduction (sulfate reduction coupled to AOM, aceticlastic methanogenesis, and autotrophic [CO_2 reducing] methanogenesis rates) investigated using ^{35}S and ^{14}C radiotracers, showed high AOM activity within and below the SMT zone and relatively low methanogenic activity throughout the core column (F. Inagaki et al., unpubl. data). Using a sediment sample from Site C9001, the carbon and nitrogen incorporation rate of deep subseafloor microbes was studied at the single cell level using nanoscale secondary-ion mass spectrometry (NanoSIMS) (Morono et al., 2011). A large fraction of subseafloor microbes was found to incorporate ^{13}C- and ^{15}N-labeled substrates into biomass, indicating that deeply buried microbial cells retain the potential to be physiologically alive.

Cultivation of aerobic and anaerobic microorganisms has been conducted, and a variety of microbes and their enzymatic activities were observed in the core sediments (Kobayashi et al., 2008). Using a continuous downflow bioreactor system, phylogenetically diverse anaerobic microbes were successfully activated, including methanogens, such as the genera *Methanobacterium, Methanoccoides,* and *Methanosarcina,* and uncultured archaeal and bacterial lineages (Imachi et al., 2011). Several attempts at traditional batch-type cultivations have led to the isolation of novel anaerobic subseafloor microbes, including *Geofilum rubicundum* (Miyazaki et al., 2012) and *Spinavirga faexivivus* (Takai et al., 2013), which are the first representatives of previously uncultivated genera. These successful cultivations and isolations confirmed the presence of metabolically and/or physiologically active microbial populations in deep subseafloor sediments off the Shimokita Peninsula.

During the first operation of riser drilling during the *Chikyu* shakedown cruise, the shift in microbial communities in the riser drilling mud tank and circulated mud fluid was examined through cultivation and cultivation-independent molecular ecological studies (Masui et al., 2008). Despite the high alkalinity of the mud circulation fluid (~pH 10), the predominance of *Xanthomonas* DNA and the potential growth of facultatively anaerobic and halophilic bacteria *Halomonas* suggest the potential utility of using molecular and microbiological signatures of these organisms as tracers of drilling mud contamination during Expedition 337.

Scientific objectives and hypotheses

An operational goal of Expedition 337 was to extend the riser drilling/coring depth at Site C0020 (i.e., JAMSTEC Site C9001) to at least 2200 mbsf (and possibly deeper if time and safety concerns permitted) in order to obtain samples from the terrigenous to shallow-marine coalbeds that were believed to be situated below marine sediments. This expedition was thus anticipated to provide the unique opportunity to examine geobiological and diagenetic processes associated with coal formation in deeply buried marine sediment. Moreover, microbial life or its activities had never been documented at the targeted burial depths in any marine environment. Expedition 337 was driven by three overarching testable hypotheses:

1. The deeply buried coalbeds act as geobiological reactors that release dissolved organic compounds such as methane, acetate, and other substances.
2. The conversion and transport of coalbed-derived organic substances influences microbial and diagenetic processes in the overlying, shallower strata.
3. Subsurface coalbeds have the potential to serve as a seal for CCS within the porous sand layers between the coal formation and can support biological conversion of CO_2 into biomass and or-

ganic compounds even at high CO_2 concentrations.

The following operational objectives addressed during Expedition 337 are tied to the above hypotheses and guided our research strategy:

- Constrain the impact of a thermally immature coalbed on the diagenetic and microbial processes at great burial depths;
- Quantify the upward fluxes of dissolved organic compounds, such as gaseous hydrocarbons and volatile fatty acids, out of the coalbed and evaluate their impact on microbial processes in shallower strata;
- Assess the limits of life and potential geochemical and geophysical constraints to microbial population, diversity, metabolic activity, and function in the deep subseafloor biosphere; and
- Examine whether distinct and active microbial communities inhabit the deeply buried coalbed, the overlying sediments of terrigenous origin, and the even shallower marine sediments and determine microbial response to high CO_2 concentrations.

We also addressed the following set of specific research questions:

- What is the ecological and biogeochemical relevance of deeply buried lignite in the natural hydrocarbon system offshore the Shimokita Peninsula?
- What are the fluxes of both thermogenically and biologically produced methane and other diagenetic products, such as organic acids, into shallower strata and how important are these for the carbon budget?
- How does coal diagenesis affect subseafloor microbial biomass, diversity, and metabolic activities?
- Does the presence of the low-maturity coalbed stimulate heterotrophic and autotrophic microbial communities?
- How do minerals and organic matter react in the coalbed formation, how will this change the physical and chemical characteristics, and how will it affect the microbial communities?
- What paleoenvironmental information and sedimentary regimes are recorded at Site C0020?
- What is the natural flux of CO_2 and CH_4 from the coalbed hydrocarbon system, and what is the potential for CO_2 sequestration in the deep subseafloor coal-sand formation?

In order to address these scientific objectives, we performed (1) spot coring of marine sediments and coalbed layers, (2) in situ wireline logging of various geophysical and geochemical properties, and (3) in situ sampling of formation fluids associated with coalbeds using a wireline fluid sampling tool. These materials and data were used for extensive microbiological, biogeochemical, geological, and geophysical analyses on board the ship and will be used in shore-based laboratories. Achieving these scientific objectives will expand our knowledge of geobiological and biogeochemical properties in the coalbed hydrocarbon system. Similar coaly environments are widely distributed along the western coast of the Pacific Ocean, and hence our results will be of great societal relevance. The examination of the effects of high CO_2 concentrations and the associated decrease in pH under conditions of hypothetical CO_2 sequestration into the deep coal/sand layers is an important objective. Therefore, shore-based laboratory experiments will include quantitative evaluation and modeling of fluid flow and biological systems in the subseafloor environment, including their response to high CO_2 concentrations. These applied scientific aspects will add an important new component to IODP.

Site C0020 site summary

Expedition 337 was the first expedition dedicated to subseafloor microbiology using riser drilling technology. Hole C0020A is the deepest hole in the history of scientific ocean drilling. On 9 September 2012, we terminated drilling at a total drilling depth of 2466 mbsf. Site C0020 is the extension of JAMSTEC Hole C9001D drilled during the *Chikyu* shakedown cruise in 2006 (Aoike, 2007), when drilling pilot holes by both riserless and riser systems was terminated at 647 mbsf and 36 and 20 inch casings were installed to 511 mbsf. During Expedition 337, we drilled from 647 mbsf to the final depth of 2466 mbsf (Table **T1**).

The use of riser drilling technology in very deep sediments created both unique opportunities and new challenges for the science party. Our experiences and results will be of great strategic value to future missions of deep scientific ocean drilling. Our scientific objectives focusing on the deeply buried coalbed strictly required the use of riser drilling technology. This technology enabled implementation of several operations of direct relevance to the Expedition 337 scientific objectives. For example, the use of a newly installed mud-gas monitoring laboratory provided the opportunity to monitor a range of biogeochemically relevant gases, including real-time measurements of isotopic compositions of methane. For the first time in scientific ocean drilling, we conducted downhole fluid analysis and sampling. Logging operations yielded data of unprecedented quality. At the

same time, the riser drilling technology, in particular the use of drilling mud, created substantial obstacles that posed considerable threats to the scientific success of Expedition 337. Riser drilling mud is saline, alkaline, contains a multitude of organic compounds, and, most importantly, high microbe concentrations. Our strategies to detect, quantify, and minimize contamination, as well as to deal with residual levels thereof, have consequently played an important role for the shipboard scientific program and will undoubtedly require careful interpretation of all future data. A substantial portion of this chapter will therefore be dedicated to quality assurance/quality control (QA/QC) of the sampled materials and data. Nevertheless, at the very least from an operational point of view, this expedition was successful: we carried out nearly all intended operations, we drilled and sampled several coalbed layers, and we were able to drill 266 m deeper than our initial target depth. The recovered samples hold the potential to extend the currently accepted evidence of deepest life below the seafloor (Roussel et al., 2008) by >800 m and provide the opportunities to address all major objectives related to the relationship of deep coalbeds and microbial life. In the following sections, we will provide principal results according to discipline followed by a synthesis of currently available information as pertaining to our scientific objectives.

Lithostratigraphy and biostratigraphic age constraints

Riser drilling during Expedition 337 provided an unprecedented record of dynamically changing depositional environments during the late Oligocene and Miocene in the former forearc basin off the Shimokita Peninsula. This record is composed of a rich diversity of lithologic facies reflecting environments ranging from warm-temperate coastal backswamps to cool-water continental shelf. Four distinct lithologic units were identified in Hole C0020A on the basis of combined analyses of cuttings and cores and assisted by inspection of X-ray computed tomography (CT) scan images and wireline logging data (Figs. **F3, F4, F5**).

Shipboard micropaleontology included identifications of diatoms, calcareous nannofossils, organic-walled dinoflagellate cysts (dinocysts), pollen, and spores. Through micropaleontological analyses, we were able to constrain an age of early Pliocene at the top of Hole C0020A at 647 mbsf and a probable age of late Oligocene–early Miocene at the base of the hole at 2466 mbsf (Fig. **F3**). Diatoms were recovered from the upper parts of Hole C0020A but were absent, or very poorly preserved, throughout most of lithologic Units II, III, and IV (defined below). The samples could be loosely dated, and to 1076.5 mbsf, all samples were consistent with Pliocene age. Diatoms were identifiable until the base of Unit I, where they appear to be Miocene in age; however, marker species were not identified and the boundary between the early Pliocene–late Miocene was not observed. Calcareous nannofossils were rare and poorly preserved. Hence, calcareous nannofossils did not inform the age model presented here:

- Unit I (647–1256.5 m mud depth below seafloor [MSF]) consists primarily of diatom-bearing silty clay. This unit resulted from sedimentation in an offshore marine environment. Diatoms were best and most abundantly preserved in Unit I, along with predominantly heterotrophic dinocysts. Diatom floras in Unit I are consistent with a Pliocene cool-water continental shelf succession. Heterotrophic dinocyst communities feeding off diatom blooms are suggestive of elevated marine productivity.
- Unit II (1256.5–1826.5 m MSF) consists mostly of silty shale with some interspersed intervals of sandstone and siltstone. Cuttings samples show a lower amount of sand and an increase of silt at the Unit I/II boundary. The abundance of biogenic siliceous material, glauconite, and plant remains also differentiate Unit II from the overlying unit. Unit II was divided into two different subunits: sandstone and siltstone associated with marine fossiliferous material (Subunit IIa; 1256.5–1500 m MSF) and organic-rich shale and sandstone associated with plant remains (Subunit IIb; 1500–1826.5 m MSF). The upper part of Unit II represents an offshore environment, possibly with a paleoposition close to the shelf margin; with increasing depth the paleoenvironment gradually changes into a shallow-marine setting. The bottom part of Unit II is situated in the intertidal zone. This shift is consistent with microfossil assemblages that exhibit few identifiable diatoms and poor dinocysts; reworked dinocysts in Unit II, as in deeper units, have Paleogene ages that broadly fall in the range of early middle Eocene–late Oligocene. Pollen and spores are moderately well represented but are abundant near the base of Unit II, which is consistent with increasing terrestrial influence in shallow-marine sediment.
- Unit III (1826.5–2046.5 m MSF) is dominated by several coal horizons, which we divided into coaly shale, siltstone, and sandstone. Almost all coal horizons consist of fine-detritic to xylodetritic coal with some layers of xylitic coal. Water content, color, and vitrinite reflectance measurements of the coal suggest that the coal has low maturity (see Fig. **F6**). Bioturbation and sedimentary fea-

tures like flaser bedding, lenticular bedding, or cross-bedding suggest a nearshore depositional environment with tidal flats and tidal channels. The presence of siderite bands at the bottom of this unit suggests a back-barrier marine environment in combination with wetlands (e.g., salt marsh or swamp). Small terrestrial influence might occur within sand bodies that overlie coal horizons. This could be due to channels from deltaic environments. Unit III contains excellent pollen and spore assemblages in the coal and associated terrestrial to coastal shallow-marine sediment. However, dinocysts are scarce and contain few useful biostratigraphic markers. The pollen floras tentatively suggest an age of early middle Miocene for Unit III.

- Unit IV (2046.5–2466 m MSF) is dominated by silty shales in the upper part, sandstone intercalated with siltstone and shale associated with sand in the middle part, and sandstone intercalated with silt and a thin coal layer in the lower part. Wireline logs and cuttings samples suggest a thick homogeneous shale layer between the Unit III boundary and Core 337-C0020A-27R (2200 m core depth below seafloor, Method B [CSF-B]). The depositional environment of Unit IV resembles that of Unit III, except that the former contains only one thin coalbed. Like Unit III, Unit IV experienced high-frequency fluctuations of the depositional environment. Within a few meters, there are sediments related to tidal flats and tidal channels, which are overlain by organic-rich material of a marsh that resulted in formation of peat. The pollen floras place a maximum age of late Oligocene for the base of Unit IV.

Physical properties

A series of physical properties measurements were performed on core and cuttings samples from Hole C0020A. Gamma ray attenuation density, magnetic susceptibility, natural gamma radiation, *P*-wave velocity, and noncontact electrical resistivity were measured with the multisensor core logger. Measurements of thermal conductivity were made mostly on working-half cores. Discrete samples taken from working-half cores and some whole-round core samples were applied to moisture and density (MAD) analyses to calculate porosity, bulk density, grain density, and water content. *P*-wave velocity analysis and electrical impedance analysis were made on cubic discrete samples. Cuttings samples were also applied to MAD analysis. Cuttings samples were separated into four categories: original bulk and sieved size categories of >4.0, 1.0–4.0, and 0.25–1.0 mm. Large-size fraction of the cuttings samples were cut off cubic samples and applied to the *P*-wave velocity analysis and the electrical impedance analysis. Anelastic strain recovery analysis was made on some whole-round cores. Vitrinite reflectance analysis was performed on some coaly samples, indicating generally low maturity of coal (R_o = 0.2–0.4) in Hole C0020A. Porosity of siltstone, sandstone, and shale gradually decreased with increasing depth (Fig. **F7**). Porosity corresponds to lithologic variation, with carbonate-cemented sandstone and siltstone having much lower values than noncemented sandstone and siltstone. The porosity of coal does not deviate from the major trend of the other lithologies, although we cannot exclude that the coal may have expanded after recovery. The cuttings also show a gradual decrease in porosity but have generally higher values than core samples. Discrete core samples are likely more representative of in situ porosity than cuttings.

Downhole logging

Because of the very good borehole condition and relatively simple lithology, logging data of excellent quality was obtained from Hole C0020A. The relatively simple lithology consists of sandstone, siltstone, shale, coal, and conglomerates, most of which show typical log response and are intercalated by a number of marker layers (i.e., coal and cemented sandstone). This resulted in straightforward interpretation of logging data with respect to lithology, compensated for our lack of cored materials in the majority of the drilled section, and ultimately led to the establishment of a database that fully reconstructs the sedimentation history at Site C0020 (Fig. **F5**).

Log characteristics suggest that Unit I lithology is similar to that of Unit II, which consists of alternation of relatively thick layers of massive sandstones and siltstones. Unit III is characterized by frequent coal layers in a few meters thickness of sandstone and siltstone alternation sequence. Based on correlations of log data and visual core descriptions, 7 coal layers, including the thickest 2, were acquired by coring from a total of 13 identified layers with thicknesses >30 cm. Unit IV consists of thick (~200 m) massive shale in the upper half; the lower half exhibits alternations of sandstone and shale of a few meters thickness each. One thin coal layer was observed in Core 337-C0020A-30R; log data suggest that this coal represents a single depositional event in Unit IV.

Resistivity borehole images suggest that the sandstones in Unit II are massive and include conglomerates, whereas those in Units III and IV consist of thin sandstones of centimeter thickness or lamina of this scale, suggesting a change in the sedimentary environment at the Unit II/III boundary. By combining

the logging data and core descriptions, sandstones that may be of high permeability were identified in Units II and III.

By using a formation-testing tool, fluid samples were acquired from six permeable sandstone layers. The 31 "pretest" measurements prior to fluid sampling indicate that formation pore pressure is hydrostatic or elevated by only a few percent of the hydrostatic value to depths of at least 2425 m wireline log matched depth below seafloor (WMSF) (the depth of the deepest reliable measurement).

Borehole temperature was measured with two types of logging tools. The maximum temperature at the bottom of Hole C0020A was estimated by examining the temperature build-up pattern during the logging operation. The estimated temperature gradient was 24.0°C/km or slightly lower (Fig. F8).

Preliminary log-seismic integration was carried out based on the time-depth curve derived from vertical seismic profile operation and synthetic seismogram calculation. The time-migrated seismic profile for seismic survey Line ODSR03-BS (Taira and Curewitz, 2005) was converted with the time-depth relationship and compared with logging data. Strong reflectors are well correlated with the abrupt change on the logging curves.

Geochemistry

Expedition 337 investigated the role of the Shimokita coalbed as a potential energy and carbon source for the deep subseafloor biosphere. In this context, geochemical studies are aimed at elucidating the cycling of carbon and nutrients, the conversion and transport of hydrocarbons, the flux of both thermogenically and biologically produced organic compounds, their utilization by the deep subseafloor biosphere, and the impact of deep hydrocarbon sources on the carbon budget of the shallower surface. To this end, geochemists investigated solid phase, gas, and fluid samples at Site C0020.

Organic matter quantity and origin

Shipboard analysis of solid phase samples showed a strong lithologic control of the TOC contents in sediment: lignite layers had the highest average TOC contents (40.9 ± 9.9 wt%), followed by clayey (1.4 ± 1.0 wt%), silty (0.43 ± 0.29 wt%), and sandy (0.26 ± 0.18 wt%) materials (Fig. F9). The coal layers in Unit III were evident not only in the high TOC contents of visually identified coal layers in sediment cores but also in the elevated TOC contents of randomly sampled cuttings. In general, TOC contents in cuttings were slightly higher than in the corresponding cores, possibly because of mixing effects with the drilling mud. Since land plants contain less nitrogen than protein-rich marine plankton, the atomic ratio of TOC and total nitrogen (TN) is a useful first indicator for the origins of organic matter. Throughout cores taken form Hole C0020A, TOC/TN ratios ranged from 3 to 58, with higher values indicating a stronger contribution of higher land plant-derived organic matter to TOC. Like TOC contents, TOC/TN ratios were controlled by lithology. The highest TOC/TN ratios of ~58 were observed in coal. In general, clayey sediment had TOC/TN ratios of ~22, which are still indicative of terrigenous organic matter, whereas sandy and silty lithologies showed lower TOC/TN ratios of ~13. However, the clastic sediment had a large within-group variation in the TOC/TN ratio (1σ = 7–10), making it difficult to link the origins of organic matter (i.e., terrestrial or marine) to lithologic compositions. The total sulfur (TS) values were generally low (from below detection to 1.4 wt%) and showed no clear associations with lithology or specific trends with depth. The cluster of samples with low TS contents in Unit III contained samples of different lithology, including lignite coal. Rock-Eval pyrolysis (e.g., Tissot and Welte, 1984) provided some initial information on the type and maturity of organic matter. In Units II–IV, most of the core samples yielded maximum temperature (T_{max}) values in the range of 400°–440°C, indicating organic matter in a thermally immature to early mature state. The average values of parameters S_2, S_3, and hydrogen index (HI) decreased in the order of coal > clayey materials > silty or sandy materials, suggesting higher contents of hydrogen-rich organic matter in fine-grain sediment and coal (Fig. F9).

Biomarker evidence for origin and thermal maturity of organic matter

Further insights into the origin and thermal maturity of organic matter come from the analysis of lipid biomarkers. A large proportion of *n*-alkanes with an odd-over-even carbon number predominance in the C_{29} range and *n*-alkanoic acids with an even-over-odd predominance in the C_{26} range, as well as the general predominance of C_{29} sterenes derived from C_{29} sterols abundant in higher plants (Fig. F10), suggest that terrestrially derived organic matter substantially contributes to TOC (Huang and Meinschein, 1979). The extent of this contribution appears to reach a maximum close to the transition from Unit I to Unit II (~1200 m MSF), with another maximum near the coal-bearing horizons.

In addition, the degree of thermal alteration of sediment can be gauged from the conversion of sterenes to steranes and using biomarker data previously calibrated against temperature in the Hokkaido region

(Amo et al., 2007). A critical temperature threshold for this process in the subsurface is the range of 40°–60°C (Amo et al., 2007). The extent of sterene-to-sterane conversion for Site C0020 is consistent with previous work and the estimated geothermal gradient of 24°C/km, as well as the bottom-hole temperatures measured during Expedition 337 (Fig. **F11**).

Gases

For the composition of gases above, in, and below the Shimokita coalbed, about 1 million discrete data points could be recorded by mud-gas monitoring (i.e., the continuous extraction and online analysis of gases that are brought up from the formation to the *Chikyu* with the recycling of drilling mud during riser drilling). Mud-gas monitoring was supplemented by gas analysis in >100 samples from cuttings and cores. Analysis focused on the content and carbon isotopic composition of methane but also included higher hydrocarbon gases, N_2, O_2, Ar, H_2, and CO. Although the determination of absolute in situ concentrations is not possible, the relative ratio of gas species in the mud gas is very informative, particularly the C_1/C_2 ratio. Because the thermogenic generation of hydrocarbons is associated with production of higher hydrocarbon gases, low C_1/C_2 ratios indicate thermogenic methane formation. The downhole profile with its rather high C_1/C_2 ratios at Site C0020 points unambiguously to the predominance of biogenic methane sources (Fig. **F12**). Interestingly, the ratios found in mud gas of coal-bearing sediment horizons at ~2000 m MSF showed a strong positive inflection, suggestive of an active source of biologically produced methane.

O_2/Ar, N_2/Ar, and H_2/Ar ratios were used to monitor corrosive processes during drilling. O_2/Ar and N_2/Ar ratios resembled atmospheric values throughout the drilling process, and the lack of oxygen consumption suggests that only little corrosion occurred. Nevertheless, H_2 contents were distinctly elevated above atmospheric levels in mud gas and core samples, and the extent to which H_2 concentrations are impacted by drilling activities is not fully resolved at this point.

In summary, shipboard solid phase and gas analyses suggest that organic matter from predominantly terrigenous sources is available for the deep biosphere not only in coal but also in TOC-rich clay. Moreover, organic matter is apparently thermally relatively immature to 2466 mbsf and rich in hydrogen, which might be released as molecular H_2 over the course of diagenesis. Finally, C_1/C_2 ratios suggest that methane is predominantly formed by biogenic processes.

Fluid

Expedition 337 is the second riser drilling IODP expedition, and Hole C0020A is only the second hole from which interstitial water has been retrieved from riser drilled cores (following IODP Expedition 319 Hole C0009A; Expedition 319 Scientists, 2010). We recovered interstitial water from 2405 m CSF-B, extending the world record for deepest interstitial water recovered by 820 m (Expedition 319 Scientists, 2010). In addition, this is the first cruise to obtain large-volume formation water samples from specific lithologic intervals in the borehole using Schlumberger's Quicksilver device. A total of six formation water samples were sampled downhole, from 1279 to 1978 m WMSF.

Recovering fluids from such deep sediment is not without its challenges, however. Pressure changes during core recovery provoke phase changes in dissolved gases and lead to expansion of cores along fracture planes. Sediment cores have low porosity (see Fig. **F7**), are prone to fracturing along laminations, and thus are easily contaminated by drilling mud. Thus, among the objectives of shipboard inorganic chemists was assessing contamination of interstitial water and sampled formation water by the drilling process (see below). Because of low porosity and permeability, only 24 of the processed 48 whole-round cores yielded interstitial fluid through squeezing, with volumes of 0.2–33.5 mL despite squeezing up to 70 cm long whole-round cores. Of the cores that yielded pore water, 15 were from Unit II, 7 from Unit III, and 2 from Unit IV; the relationship between interstitial water yields and lithology is illustrated in Figure **F13.**

In addition to analyses on interstitial and formation fluids, for the first time the inorganic chemistry of both drilling mud and drilling cuttings data were analyzed in the context of sample contamination. Because there was no coring through Unit I (i.e., 647–1256.5 m MSF), the only geochemical information from this depth interval was from the drilling cuttings and mud-gas logging. Whereas the cuttings did not provide reliable information about the pore water from the depths at which they were obtained, they did provide information on interactions between drilling mud and sediment samples.

Microbiology

Expedition 337 was the first riser ocean drilling expedition to incorporate extensive shipboard microbiological and molecular biological analyses. These were performed with state-of-the-art equipment in the designated microbiology laboratory aboard the

Chikyu. Because the target sedimentary habitat is strictly anaerobic, all the cored materials were immediately transferred to anaerobic conditions where they were processed for shipboard and shore-based microbiological analyses.

Previous studies in continental margin sediment have shown that microbial populations and activity generally decrease with increasing sediment depth (e.g., Parkes et al., 2000). However, the various geophysical and geochemical factors that constrain the extent of the deep biosphere remain unknown. Therefore, one of the key objectives of the Expedition 337 microbiology program was the quantification of microbial cells and detection of molecular signatures of "indigenous" deep subseafloor life from sediment cored by riser drilling. In deeply buried horizons, microbial cell densities approach the detection limits of most established cell counting protocols (Expedition 329 Scientists, 2011), necessitating whole-cell extractions and cell concentration procedures and complementary use of different cell counting methods (e.g., manual and image-based microscopy and high performance flow cytometry). Results obtained by different methods can then be compared to evaluate method-inherent biases. To implement these ultra-sensitive assays for the detection of deep subseafloor life, QA/QC is extremely important. The high cell concentrations of >100 million cells/cm^3 in riser drilling mud required careful monitoring of core contamination by adding chemical tracers to drilling mud, DNA fingerprinting of contaminant microbes, and checking pore fluid for ionic species that had been added to drilling mud.

Critical steps such as microbiological sample processing, cell counts, cultivation, and molecular studies were performed aseptically in the microbiology laboratory on the *Chikyu*. Preliminary cell detections and enumerations showed extremely small cells to be present at very low concentrations in samples from as deep as 2457 m CSF-B (Fig. **F14**). These cells represent the deepest subseafloor life that has ever been studied through scientific ocean drilling. Complementary to gas compositional profiles suggesting biological activity in the deep coalbed, functional genes indicative of anaerobic microbial carbon cycling were consistently detected in strata near the coalbed. However, preliminary community-fingerprinting analysis based on polymerase chain reaction–amplified 16S rRNA genes showed that even very carefully collected samples are not free of signals from contaminant microbes; careful examination of all available lines of evidence is thus required for obtaining a comprehensive view of the potentially deepest subseafloor ecosystem ever studied.

To more fully address some of the primary scientific objectives, various types of microbiological and biogeochemical samples were prepared for shore-based studies. These include more than 1700 samples, which will be used for stable isotope probing combined with NanoSIMS and lipid-based experiments, quantitative functional gene surveys, whole-shot gun metagenomics and single cell genomics, batch-type and bioreactor cultivation experiments, and geobiological application of CO_2 capture and sequestration (see Fig. **F15**). In addition, biomineralogical studies are planned for some minerals that likely precipitated in the course of modern and/or past geomicrobial processes such as pyrite and siderite, which were found in the deeply buried coalbeds.

In conclusion, the goals of the shipboard microbiology program were successfully accomplished. Extensive research using samples and data collected during Expedition 337 will significantly expand our knowledge of the deep subseafloor biosphere and contribute to a better understanding of the biogeochemical carbon cycle.

Expedition-specific challenges, risks, and future perspectives

Quality assurance/Quality control

The riser drilling technology and the associated use of drilling mud had considerable impact on our scientific program. Whereas contamination control has become an integral measure of quality assurance in riserless ODP/IODP expeditions with focus on subseafloor life (Smith et al., 2000a, 2000b; House et al., 2003; Lever et al., 2006), the riser drilling procedure required a more rigorous QA/QC program during Expedition 337. Most severely affected by the contamination risk were the scientific objectives of the fluid chemistry and microbiology disciplines. Therefore, our sampling programs of both fluid chemistry and microbiology included routine monitoring of the integrity of samples selected for interstitial water and microbiology analysis by X-ray CT scanning (see Fig. **F16**). Heavily disturbed or fractured samples were returned into the normal core flow before processing, and alternative samples with lower risk of contamination were selected instead. An example of one such heavily disturbed, and thus contaminated, sediment interval is shown in Figure **F17.** Because drilling mud was highly divergent in chemical composition from sediment pore water, even minor contamination would complicate the accurate analysis of routinely measured interstitial water parameters such as sulfate, pH, or alkalinity, all of which are

directly relevant to the study of deep subseafloor life. Moreover, microbial cell concentrations in drilling mud were consistently >10^8 cells/cm^3. This is orders of magnitude higher than pelagic seawater used during past riserless drilling expeditions (Lever et al., 2006) and two to five orders of magnitude higher than expected concentrations of indigenous cells in sediment deeper than 1000 mbsf (Parkes et al., 2000), and thus increases the risk of contamination with nonindigenous cells substantially compared to previous riserless drilling operations.

The use of chemical and microbial contamination tracers was pioneered for riser scientific ocean drilling as part of an extensive QA/QC program. Contamination monitoring with perfluorocarbon (PFC) compounds as chemical tracers utilized a modified version of past protocols (Smith et al., 2000a, 2000b; House et al., 2003; Lever et al., 2006). PFC tracer was added daily to drilling mud tanks. Detailed sampling and analyses of drilling mud, sediment cuttings, and core samples provided valuable quantitative estimates of the volume of drilling mud and number of cells introduced into samples during riser drilling (Fig. **F16**). PFC concentrations monitored within drilling mud in tanks, core liners, and the mud ditch after recovery show consistently high values and low loss during drilling operations (mean concentration > 100 µg/L). PFC measurements within cores show a wide range of values, with high contamination near the core liner (exterior; typically 1–100 µL drilling fluid/g sediment) and low values in the core center (interior; 0.01–1 µL/g). DNA-based contamination tests targeting organisms associated with surface seawater, drilling mud viscosifiers, and sewage reveal drilling mud viscosifiers as the main source of drilling-induced microbial/DNA contamination.

Data obtained during Expedition 337 demonstrate the suitability of PFC tracers to monitor contamination during riser drilling operations and indicate that many core samples obtained have low to nondetectable levels of contamination at the core center. The successful detection of cells and DNA demonstrates that monitoring of microbial populations in cores obtained by riser drilling is possible on board the ship. Shore-based molecular analyses and cultivation experiments in the coming years will reveal the extent to which cells and DNA detected represent indigenous microbial communities, and, if so, what the metabolism of these microbes is. Cultivation assays will be carefully monitored for marker genes of potential contaminants and designed to specifically select for deep subseafloor rather than contaminant microbial populations (Fig. **F15**).

During fluid sampling and analyses of inorganic constituents, we sought to minimize contamination by relying on prior information of X-ray CT scan images and by thoroughly peeling off outer layers that were in contact with the core liner and thus drilling mud and avoiding fractures as much as possible. Nevertheless, residual levels of contamination were not avoidable, as illustrated in Figure **F18.** The fraction of drilling mud contamination in the total interstitial water was calculated assuming binary mixing of several major ions that are present in much greater concentrations in the drilling mud than in interstitial water. Likewise, we assumed that the degree of natural variation of these ions in the interstitial water composition was small compared to variation caused by contamination. The most contaminated samples were typically sandy mudstone at the top of Unit II. Results were variable based on the ion chosen but provide some indication of the degree of contamination. For example, the mud-derived water fraction of pore water in the most severely contaminated, shallowest whole-round sample (337-C0020A-1R-2, 0–65 cm; sandy; 1278 m CSF-B) varies from 65% to 88% depending on whether salinity, K^+, SO_4^{2-}, or Cl^- are considered as the interstitial water end-members in binary mixing. Consequently, corrections of other interstitial water constituents for their dilution with drilling mud is associated with inherent, residual uncertainties. For the majority of interstitial water samples obtained toward the bottom of Unit II and deeper, contamination is significantly lower. In these horizons, we estimate a range of contamination from 2% to 20%.

Considerable challenges were also posed on organic geochemical and micropaleontological analyses by contamination from drilling mud. TOC concentrations of cuttings were suspiciously elevated relative to core samples from nearby horizons. Lipid extracts of cuttings and some core samples showed molecular signatures of the asphalt-based drilling additive AS-TEX-S (trade name; also known as sulfonated asphalt sodium salt [SAS]); cuttings in shallower horizons of Hole C0020A showed other unidentified, highly concentrated contaminants that probably result from the drilling technology (Fig. **F19**). Moreover, micropaleontological analyses of diatom assemblages in core samples, core catchers, and especially cutting samples were affected by a "memory effect." For example, relatively young diatom assemblages representative of shallower late Miocene and Pliocene strata were abundant in drilling mud and complicated the use of cuttings for diatom-based chronostratigraphy. Focus on undisturbed core samples eliminated this problem but also substantially lowered the number of samples and thus the depth resolution that could be examined for age-relevant marker fossils.

Preliminary evidence for deep subseafloor microbial activity associated with coalbeds

The main goals of Expedition 337 were related to microbial life associated with deeply buried coalbeds. In order to tackle this set of goals within the Shimokita coalbeds, we had to drill deeper than any previous expedition of scientific ocean drilling. Both microbial activity and cellular concentrations are expected to decrease with sediment depth and age (e.g., Parkes et al., 2000; Jørgensen, 2012). Even if our samples were uncontaminated, the detection and examination of such deep subseafloor microbial life would not be trivial and would require both state-of-the-art methodology and utmost scientific scrutiny. With the added difficulty of contamination, we will need to build a case that integrates various lines of biological and chemical evidence for the presence and activity of microbes and the nature of microbially mediated processes.

This means it is necessary to assemble preliminary lines of evidence that are suggestive of microbial life associated with the coalbed and provide first answers to some of the expedition objectives. The most compelling evidence for microbially mediated methanogenesis is found in our gas compositional data. In particular, C_1/C_2 ratios (Fig. **F12**) analyzed during mud-gas monitoring are generally in the range suggesting biological methanogenesis as the major source of methane (e.g., Whiticar, 1999). The most striking result, however, is the strong positive inflection of this ratio associated with the major coal-bearing horizons at Site C0020 (i.e., Unit III at ~2 km subseafloor depth); this trend to higher values is consistent with coalbeds being an active source of methane to the deep formation. This interpretation of an important role of methanogenesis is supported by stable carbon isotopic compositions of methane that have been recorded in real-time mud-gas monitoring (data not shown). Further validation of isotopic relationships of different carbon pools and determination of stable hydrogen isotopic compositions of methane will provide more detailed information regarding the pathways and substrates utilized by methanogens.

Although further validation in shore-based laboratories is required, other lines of evidence such as extracted DNA or visual observation of intact cells are consistent with the presence of indigenous microbial populations at great burial depth at Site C0020. The sole detection of functional genes indicative of methane cycling in sediment samples (and not in drilling mud) is promising. Moreover, intact cells were detected in deep horizons (Fig. **F14**) and show generally very low cellular abundances. These very low concentrations in carefully cleaned samples are on the one hand encouraging as they suggest that contamination has not resulted in vastly elevated cellular counts; on the other hand we are faced with the relatively highest potential impact of contamination when in situ cell densities are already low. Additional molecular work on the single cell level to the system level is thus required to confidently assign these cells to indigenous populations.

We also have obtained the first indication related to the activity of the subseafloor biosphere in deep horizons associated with the coalbed. Whereas the compositions and concentrations of various gases suggest a stimulation of microbial activity through the coalbed, the relatively low levels of alkalinity suggest substantially lower rates of microbially mediated remineralization of carbon than in the upper few hundred meters of previously studied deep biosphere sites (e.g., Shipboard Scientific Party, 2003; Expedition 311 Scientists, 2006), including shallower sediment at this location (Aoike, 2007; Tomaru et al., 2009). Nevertheless, there is roughly a two-fold elevation in alkalinity within the coal-bearing Unit III, relative to the overlying Unit II. This supports other lines of evidence suggestive of stimulation of microbial activity through the coalbed. In conclusion, the coalbed at ~2 km subseafloor depth is probably not directly responsible for the presence of methane hydrates found in shallower layers at this site (Aoike, 2007); instead, it resembles a slow-paced bioreactor with sustained activity on geologic timescales as previously proposed for other organic-rich deeply buried layers such as Cretaceous black shales at Demerara Rise (Arndt et al., 2006).

Preliminary assessment of the sedimentation history at Site C0020

Our sedimentary analysis of the depositional environment, revealed through Hole C0020A, is that >700 m of intertidal and wetland sequences were deposited from late Oligocene/early Miocene through early/middle Miocene. This result is inconsistent with our expectations that such young sequences would not significantly thicken and that Eocene lignite layers would be present under an Oligocene unconformity layer. Our findings suggest that this sedimentary basin had been continuously subsiding in order to generate the accommodation space during this period without an abrupt faulting event and that the rate of basin subsidence had been in balance with the sedimentary input. For a better understanding of such basin dynamics and formation mechanisms, further investigation of the regional geology and tectonics is required.

Accomplishments and future perspectives

During Expedition 337, our major operational objectives (Inagaki et al., 2010) were successfully accomplished through use of the riser drilling system of the *Chikyu*. The bottom depth of Hole C0020A is 2466 mbsf, extending the previous maximum penetration depth in scientific ocean drilling by 355 m and providing the chance that our postcruise research will extend the widely accepted evidence of deepest subseafloor life by up to 800 m. The cored materials provide unprecedented opportunity to address fundamental scientific questions pertaining to the interactions between a deep coalbed hydrocarbon system and subseafloor life. New shipboard facilities on the *Chikyu* such as the mud-gas monitoring laboratory and the radioisotope laboratory were successfully implemented and strongly contributed to the mission achievement of Expedition 337. The core recovery through riser drilling was remarkably high (75.3% in average), often close to 100% (12 out of 32 spot cores were >90% recovery), even at great burial depths of 2000 mbsf and deeper. The cored materials include diverse lithologies (e.g., lignite coal, sandstone, silty mudstone, beach sand, carbonate minerals, and conglomerate). Extremely soft sand layers (i.e., beach sands), which had been almost impossible to retrieve in intact form by riserless drilling, were successfully recovered by riser drilling using high-density mud. The condition of the riser borehole was excellent, allowing close-to-perfect acquisition of downhole wireline logging data. The successful accomplishment of the aforementioned tasks required the technological capabilities of the riser drilling vessel *Chikyu*. Therefore, Expedition 337 has been an important step into a new era of scientific ocean drilling in which Earth and life scientists will jointly explore the deep realms of our planet that have never been studied before.

This first deep riser drilling expedition exploring deep life also had important strategic value in that this was the first time that the impact of commercially used drilling technology was rigorously tested by a large team of biologists, chemists, and geologists for its compatibility with the scientific goals. As a result, a number of recommendations related to the future use of this technology in scientific ocean drilling can be made. These relate to (1) coring technology, (2) drilling mud composition and sterilization, and (3) the use of deep riser holes for experiments:

1. During Expedition 337, we performed spot coring, instead of a conventional sequential coring strategy, using standard 8½ inch rotary core barrel (RCB) coring and 10⅝ inch industry-type large diameter coring (LDC) system. Both coring systems resulted in excellent quality cores, including very hard carbonate-cemented nodules and conglomerates, from scientifically important horizons. This spot coring strategy is essential for reducing the cost and time for riser drilling operation. LDC cores maximize the probability of obtaining uncontaminated massive core samples that are adequate for high recovery of pore water, allowing highly sensitive and specific biogeochemical and microbiological analyses. However, the use of an aluminum core liner required modification of the normal workflow and resulted in much longer time requirements for delivery of core material from the rig floor to the laboratory. Nevertheless, we processed LDC cores under anaerobic conditions and retrieved useful data and samples with relatively low levels of contamination for shipboard and shore-based analyses. Considering the high risk of drilling mud and microbial contamination of the standard RCB core, it would be desirable to explore the potential use of improved LDC-type coring systems with nonmetal core liner (e.g., carbon glass fiber or reinforced plastic liner) as the standard spot-coring tool for future deep scientific drilling on the *Chikyu*.
2. The use of riser drilling mud is essential for future deep scientific explorations. On the other hand, we need to address the causes of contamination to enhance detection of indigenous signatures of life and geochemical characteristics. The mud used during Expedition 337 contained ~10^8 contaminated cells per 1 cm^3, even though the fluid is alkaline and contains sterilizing chemicals. This high concentration of nonindigenous cells complicated precise detection of deep microbial life and its metabolic activities and influenced the chemical composition of pore water. To minimize the risk of drilling mud–related sample contamination during future scientific riser drilling expeditions, alternative drilling mud compositions should be considered. For example, are there feasible technologies for mud sterilization that could be implemented without conflicting with operational demands for deep drilling? Can the organic additives that appear to nourish microbial communities be substituted with inorganic components (e.g., silicon)? Can we develop in situ sampling devices for recovering noncontaminated and biologically pristine core and fluid samples?
3. A positive aspect of deep-riser drilling is the superior borehole stability supported by the use of high-viscosity mud that prevents possible collapse and flow down of rubbly horizons such as coal and fault layers. This is not only useful for

coring materials with high recovery rate, but also essential for successful completion of multiple deployments of logging tools, including downhole in situ fluid sampling and analysis. With the combined use of borehole observatory sensors and subseafloor laboratory equipment, the maintenance of stable deep-riser boreholes will be highly useful for advanced subseafloor research in short- to long-term projects.

Last but not least, this expedition also provided a testbed for the use of riser drilling technology to address geobiological and biogeochemical objectives and was therefore a crucial step toward the next phase of deep scientific ocean drilling. Since the riser system was originally developed by the petroleum industry, the *Chikyu* is equipped with a mature technology. However, the adaptation of this technology to the needs of basic science will be an important challenge that needs to be addressed as an integral component in plans for the next riser missions. Implementation of science-oriented deep-riser drilling in IODP would provide grand opportunities for Earth system sciences.

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Figure F1. Index map of Site C0020 off the Shimokita Peninsula of Japan with bathymetry, seismic survey track lines, and locations of existing drill holes. Inset map exhibits plate configuration around Japanese Islands and the location of the index map (red square).

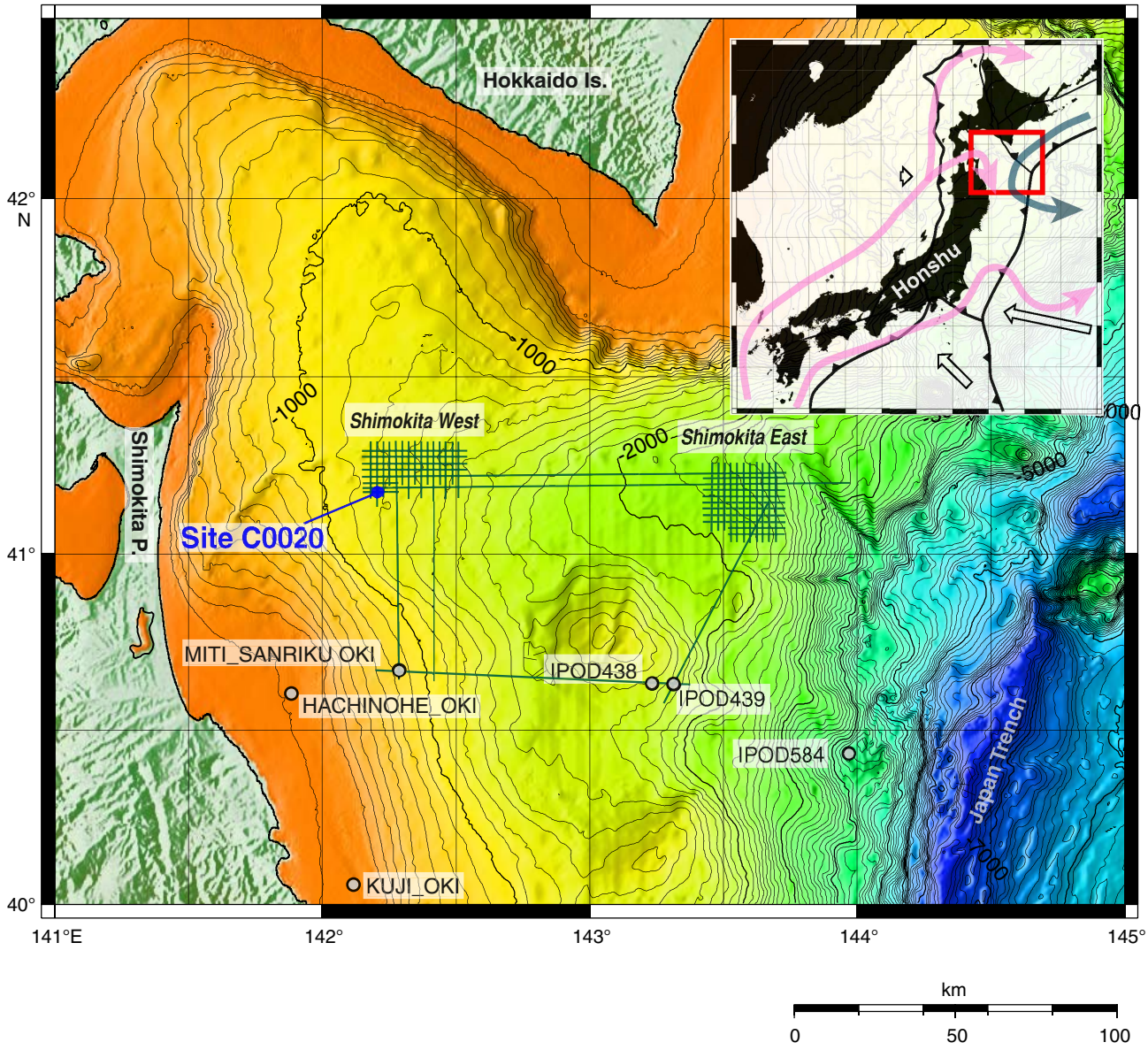

Figure F2. Seismic profile in the vicinity of Site C0020 off the Shimokita Peninsula. Gray zones = potential coal-bearing layers. WD = water depth, BSR = bottom-simulating reflector. Blue depths = estimate based on pre-expedition interpretation of seismic profile, black depths = corrected with vertical seismic profile check shot data.

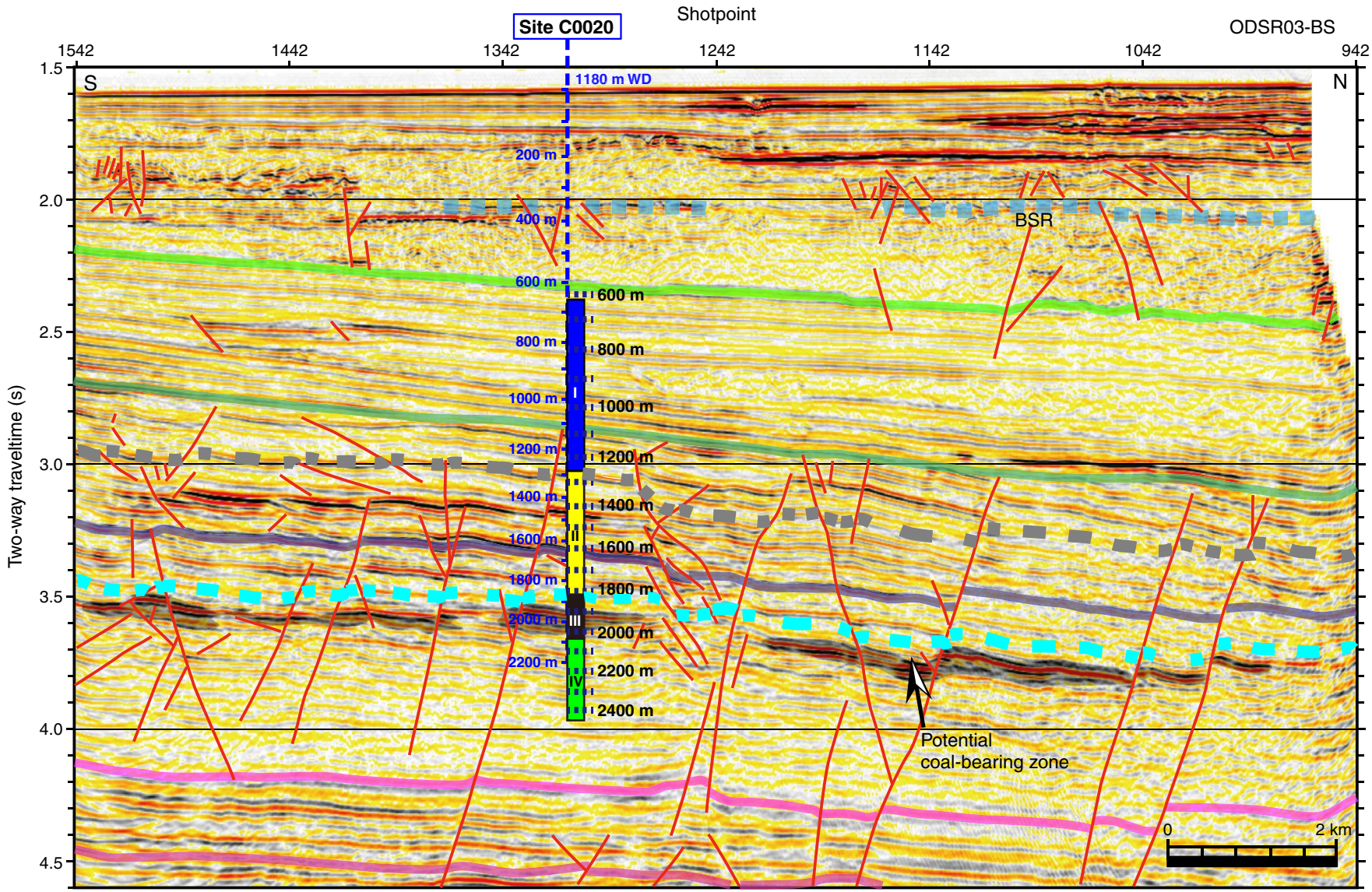

Figure F3. Lithostratigraphic profiles derived from macroscopic observation of cuttings samples and cores in Hole C0020A with ages; wood/lignite, glauconite, diatom, and sponge spicule relative abundance; depositional environment; and subdivision into different units.

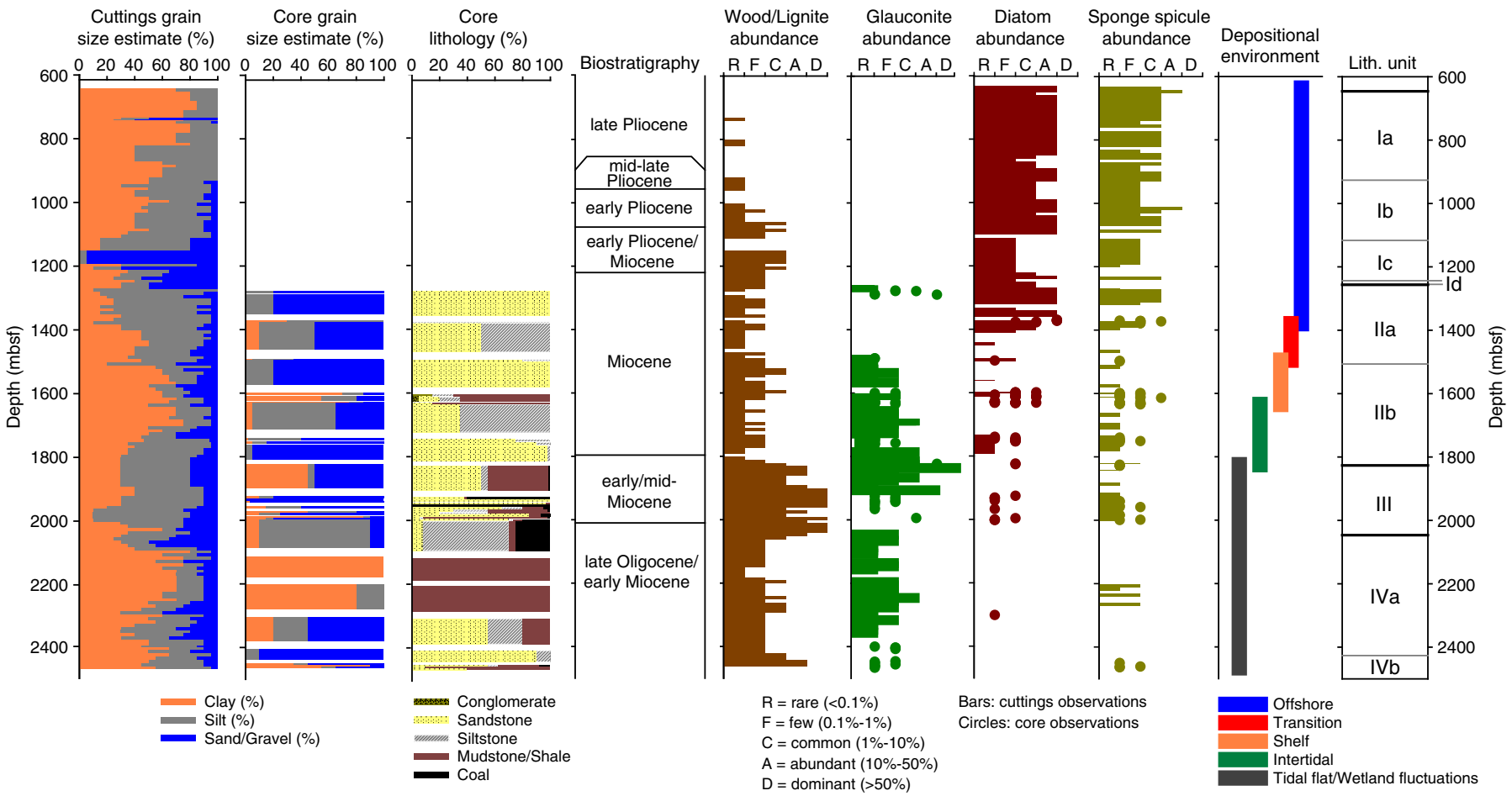

Figure F4. Section photos and X-ray CT scan images of selected intervals of different units. **A.** Sandstone with pebbles of igneous rocks and few cherts, Subunit IIa (Sample 337-C0020A-4R-2, 58–71 cm). **B.** Vertical burrows filled with medium sandstone in a silty shale, Subunit IIb (Sample 12R-2, 25–43.5 cm). Shell fragments appear in the lower part. **C.** Photo shows coaly shale with pyrite (upper part) in contact with coal interval (lower part), Unit III (Sample 19R-7, 98–115.5 cm). **D.** Light brown cemented (sideritic) mudstone and concretions, Unit III (Sample 24R-2, 24–39 cm). In the middle of this brown horizon, a thin coaly layer appears. **E.** Siltstone with siderite concretions and siderite band in silty shale, Unit IV (Sample 30R-1, 47.5–64 cm). Lenticular bedding is visible in this section.

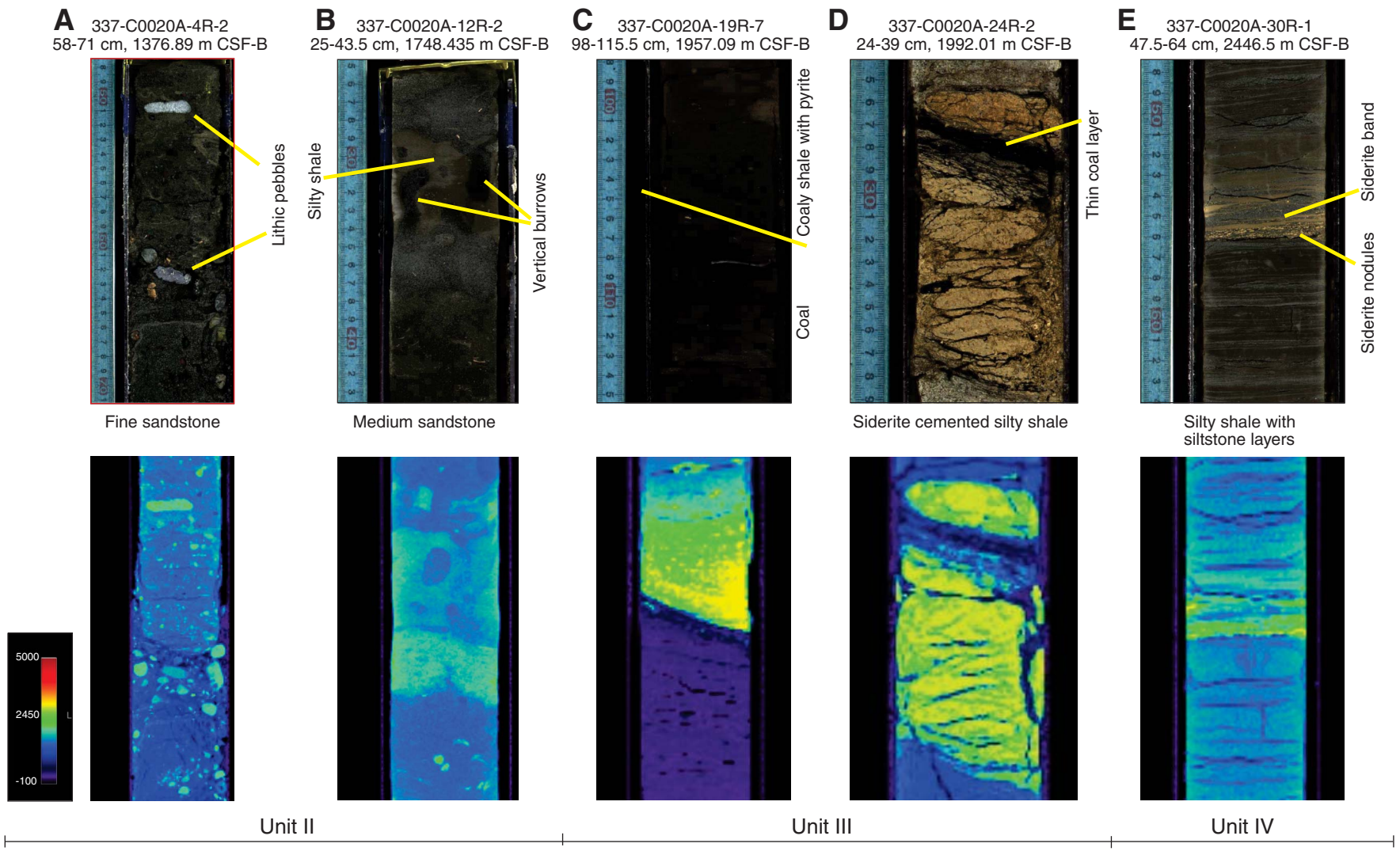

Figure F5. Site summary diagram of downhole logging operations for Site C0020 from 1200 to 2466 m WMSF, showing caliper (hole diameter), natural gamma ray, spontaneous potential, laterolog resistivity (RLA) of five different depths of investigation (RLA5 = deepest), neutron porosity, photoelectric factor, density, *P*- and *S*-wave velocities and Poisson's ratio calculated from these velocities, natural gamma ray from three different radioactive materials, a series of permeability and porosity measurements by nuclear magnetic resonance method together with their original data (combinable magnetic resonance [CMR] tool), and borehole resistivity image..

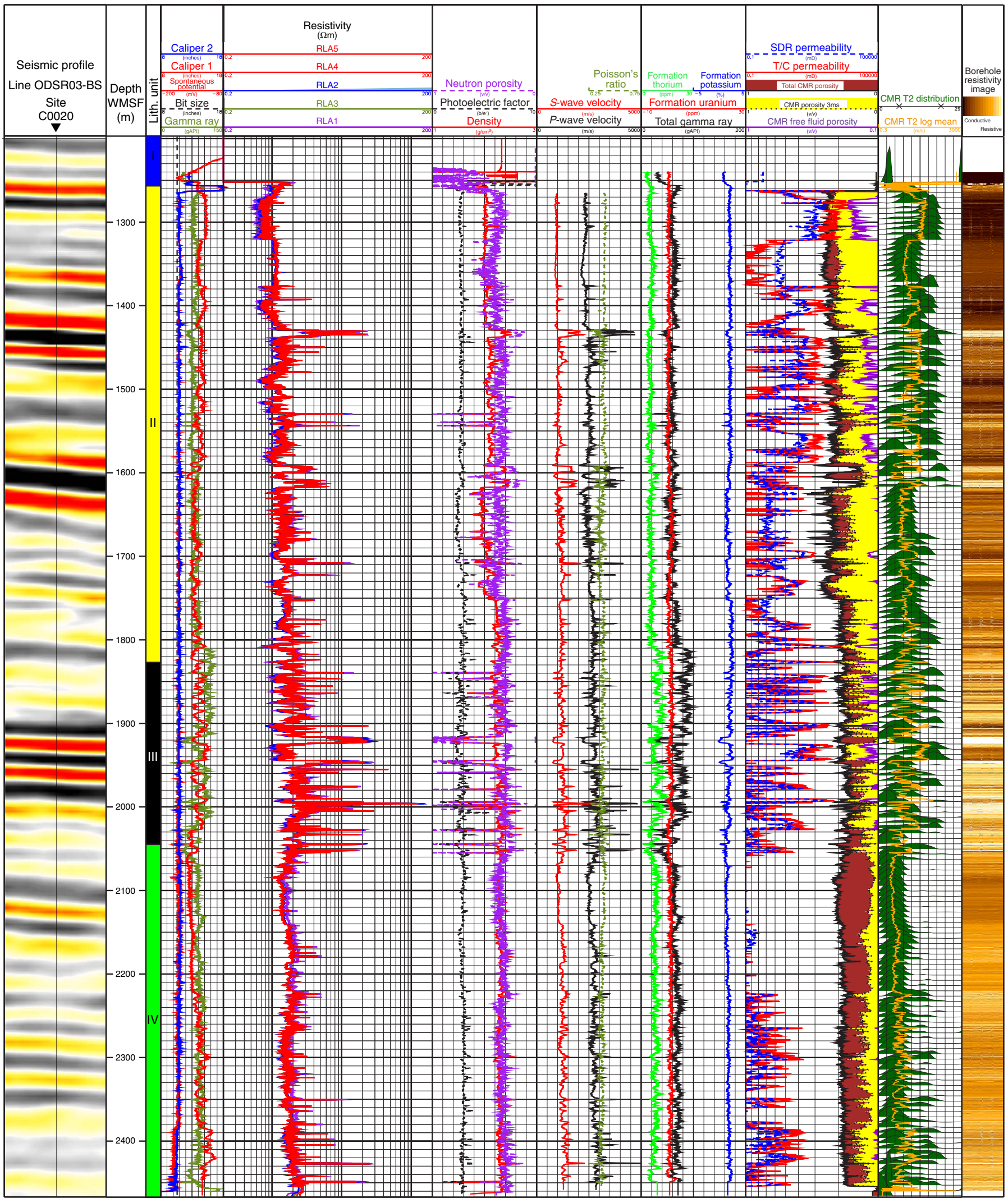

Figure F6. A. X-ray CT scan images from coal of Section 337-C0020A-18R-1. **B.** Top: SEM picture from a banded coal (xylitic and detritic bands) of Section 18R-1 with some pyrite veins (upper part). Middle: photomicrograph of coal from Section 19R-7; densinite with some resinite and phlobaphinite. Bottom: photomicrograph of coal from Section 30R-2; densinite with a large funginite in the lower part and ulminite in the upper third of the photomicrograph.

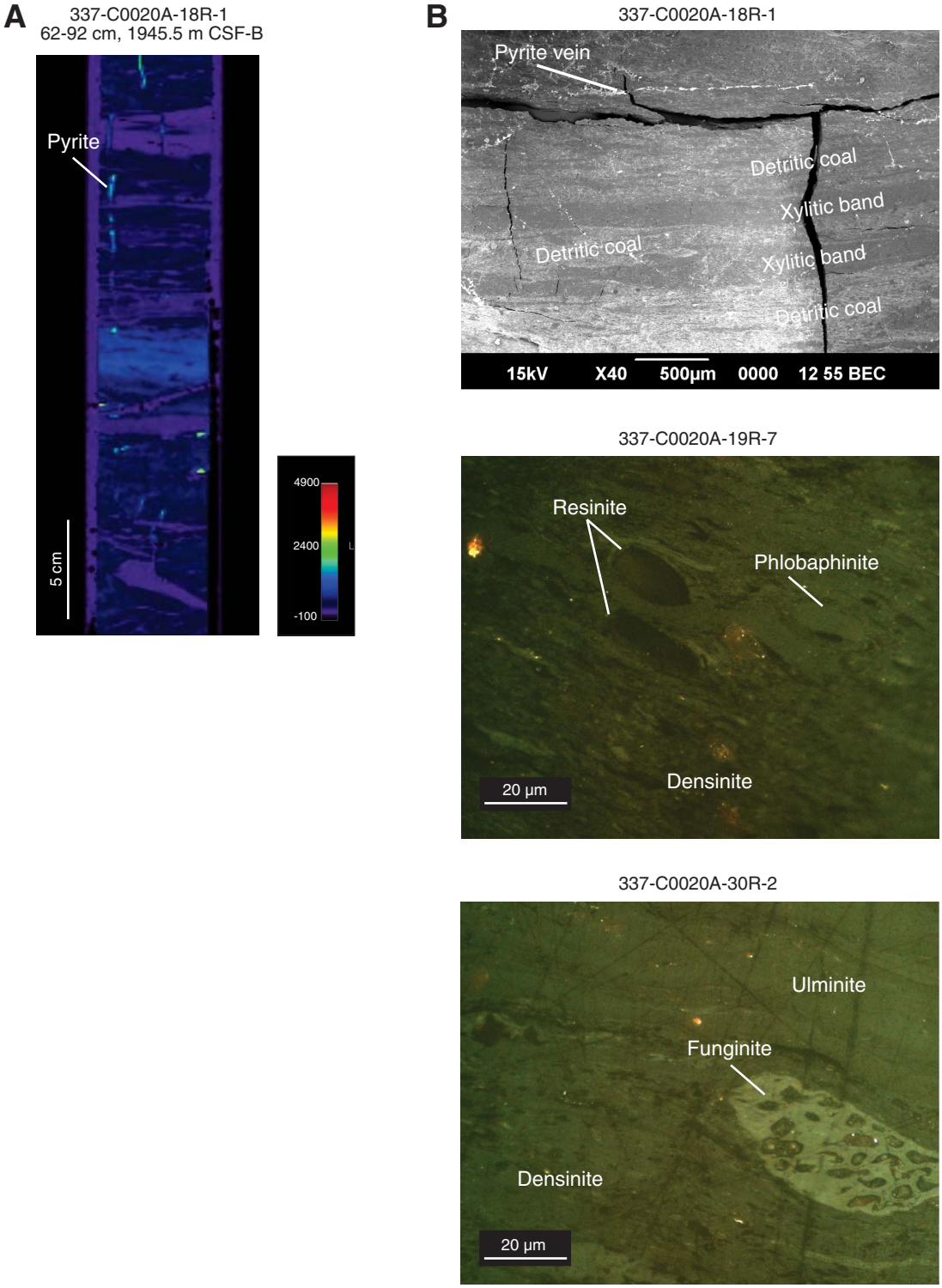

Figure F7. Distribution and lithologic variation of porosity in discrete core samples with comparison of cuttings, Site C0020. Porosity of sandstone and siltstone gradually decreased with depth, though carbonate cemented rocks deviate from the consolidation curve with remarkably low porosity. Higher porosity and slower reduction in porosity with depth is observed in cuttings samples. Discrete core samples can be more representative for in situ porosity than porosity of cuttings.

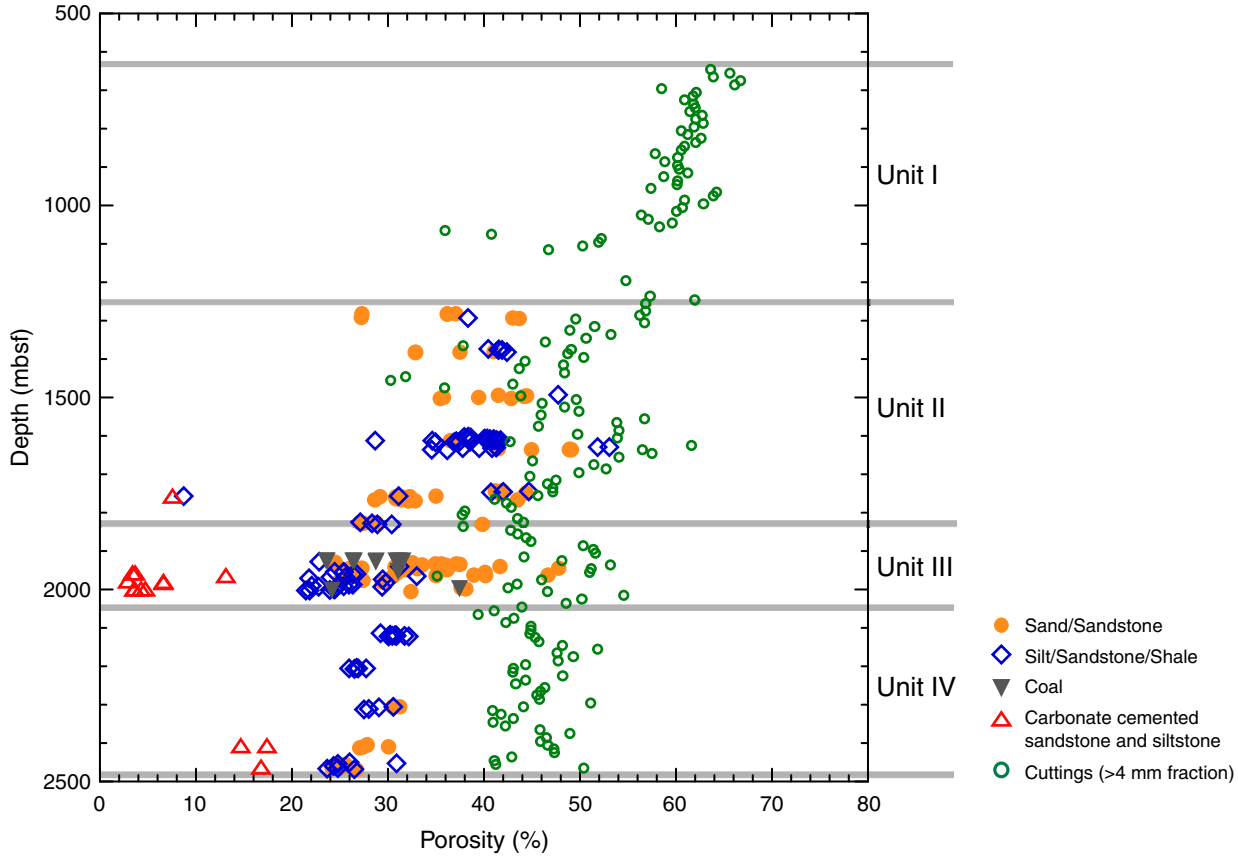

Figure F8. Summary of temperature measurements, Site C0020. Two types of logging tools (i.e., the environmental measurement sonde [EMS] and modular formation dynamics tester [MDT]) measured borehole temperature in situ. The MDT tool recorded temperature twice: during the pretests (MDT; yellow) and fluid sampling (Quicksilver [QS]; red). The maximum temperature at the bottom of the hole was estimated by temperature build-up pattern during logging operations (the Horner Plot method; green) (Dowdle and Cobb, 1975) that shows the maximum temperature gradient of 24.0°C/km.

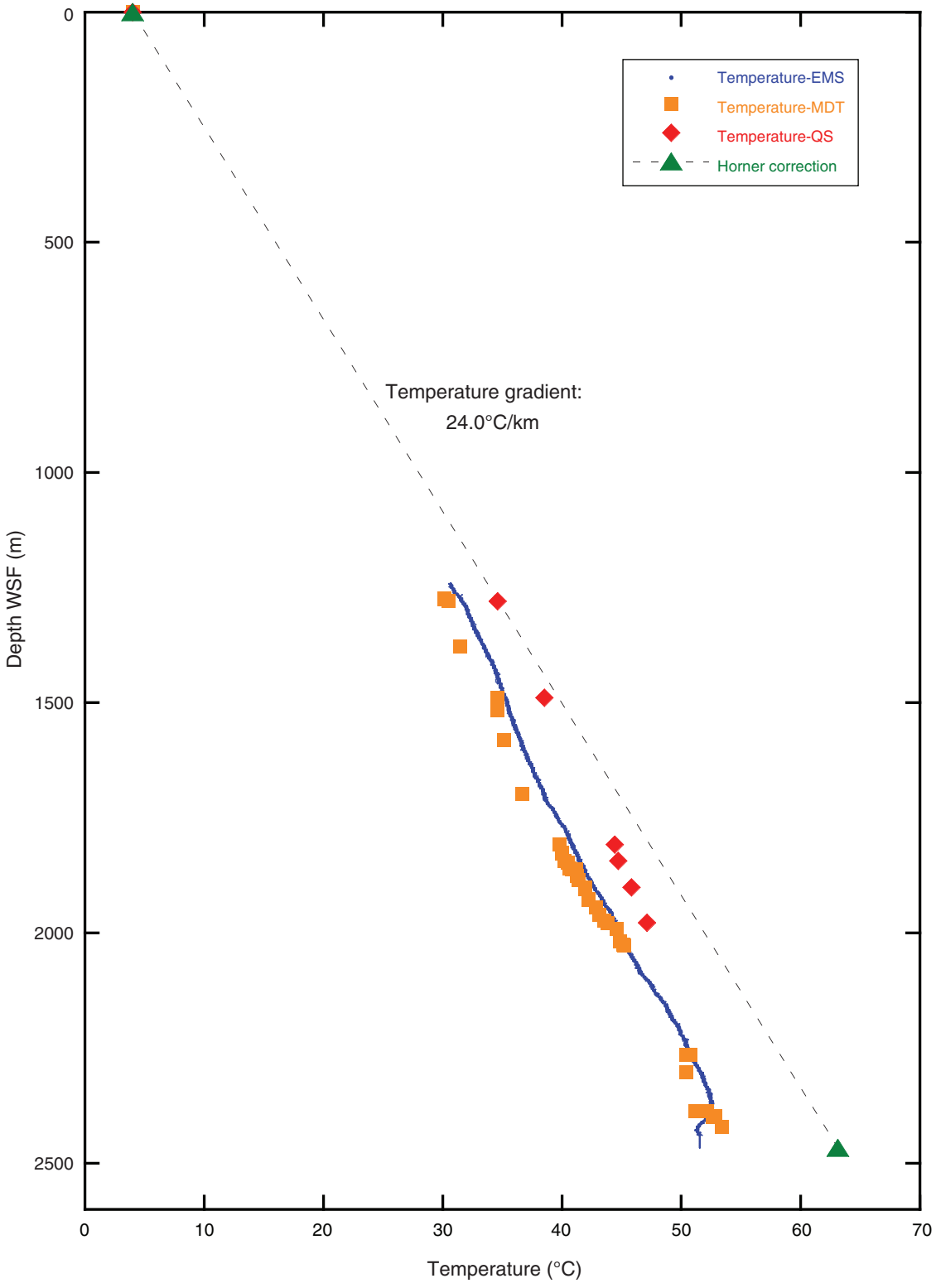

Figure F9. Depth profiles of total organic carbon (TOC), TOC to total nitrogen (TN) ratio, and hydrogen index (HI), Hole C0020A. Open symbols = data from cuttings samples, solid symbols = data from core samples. All three parameters showed a lithologic control, with values decreasing in the order of coal > clayey materials > silty and sandy materials.

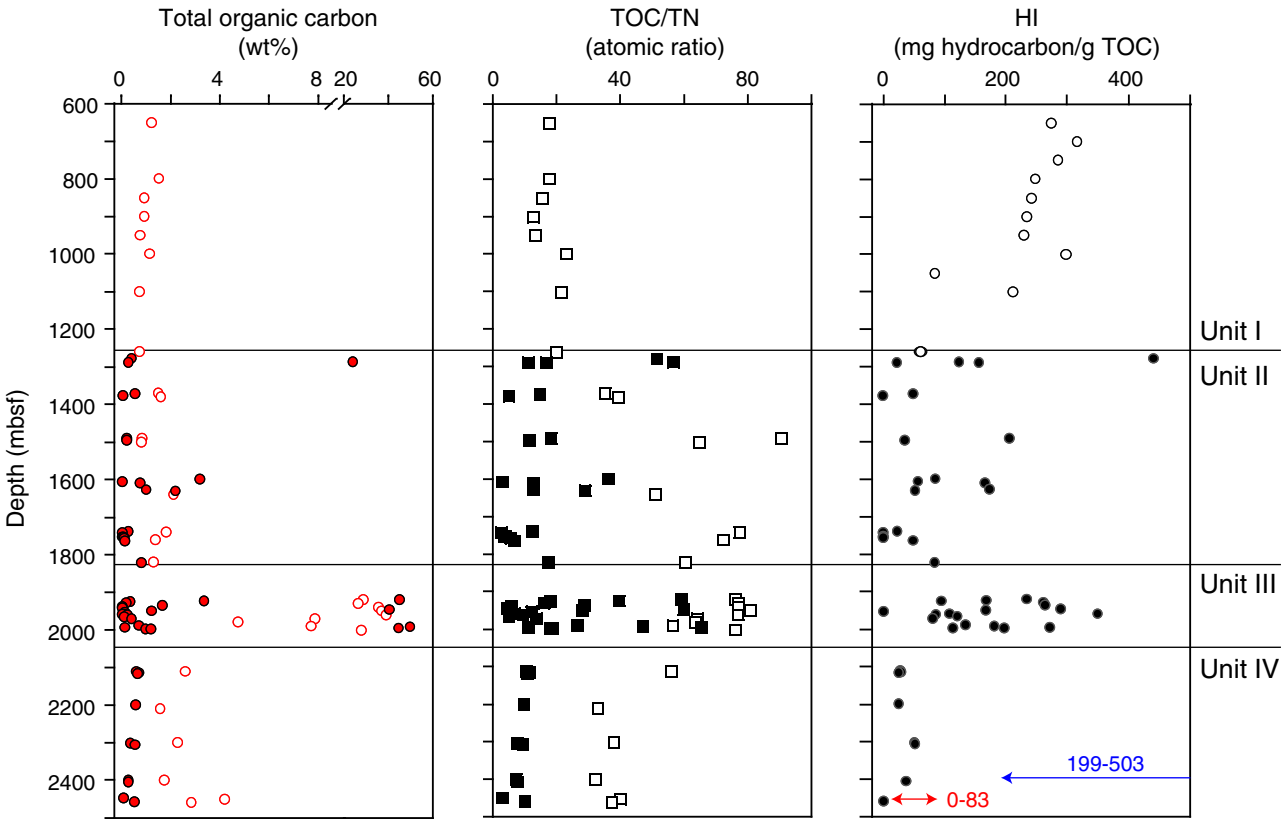

Figure F10. A. Occurrence of wood and lignite fragments in cuttings, Site C0020. **B.** Proportion of C_{29} Δ4 and Δ5 sterenes as a proportion of C_{27} to C_{29} Δ4 and Δ5 sterene-homologues (solid circles = coal, open circles = mudstone, x = cuttings, + = siltstone). **C.** *m/z* 215 ion chromatogram displaying doublets of Δ4 and Δ5 sterene. More detailed labeling provided in **"Organic geochemistry"** in the "Site C0020" chapter (Expedition 337 Scientists, 2013).

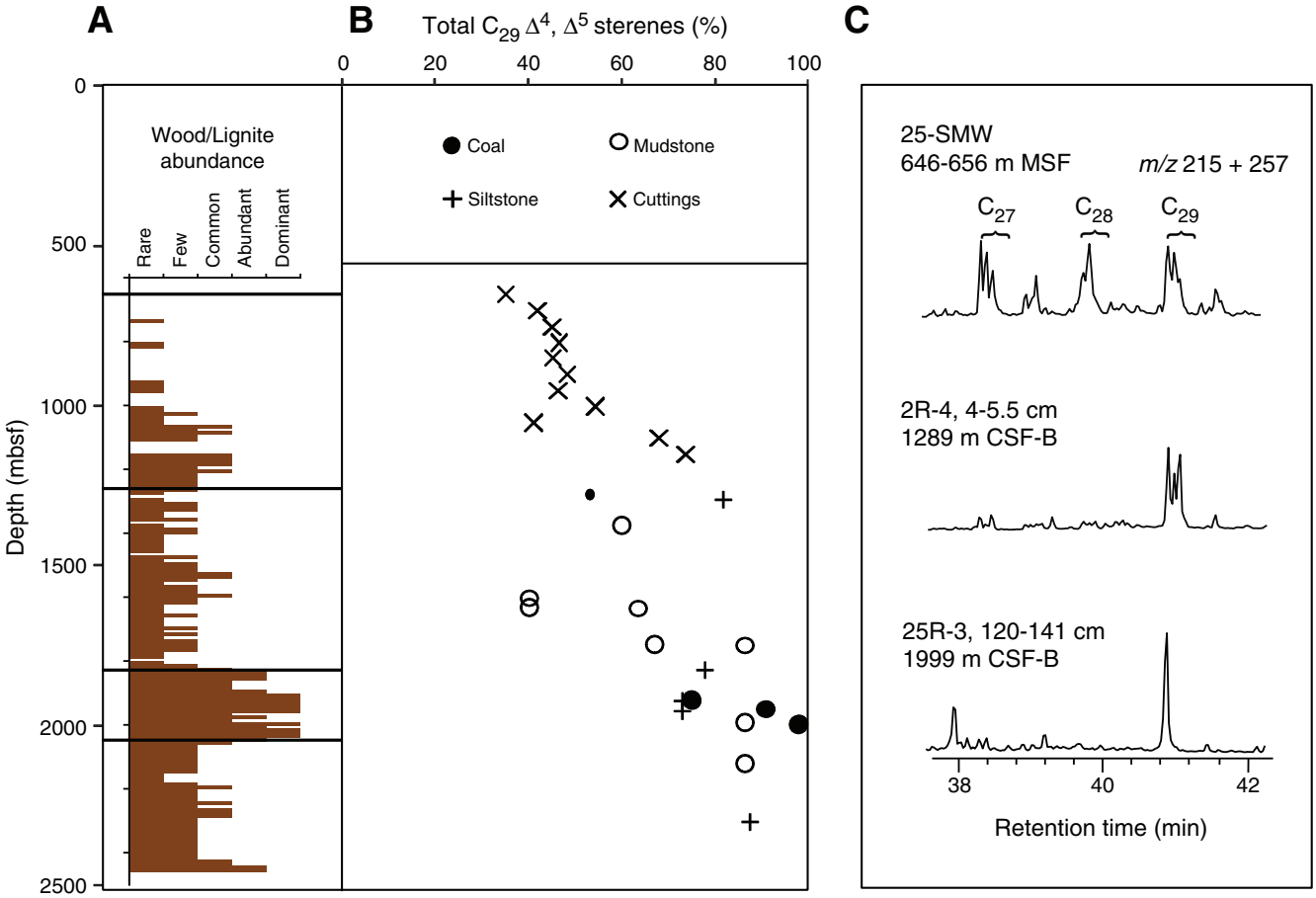

Figure F11. Ratio of C_{29} 5α,14α,17α(H)20R sterane to C_{29} Δ4 and Δ5 sterenes, Site C0020. Data plotted by sample type. Inset displays data from Hokkaido (Amo et al., 2007) used to calculate lines for 22°, 24°, and 26°C/km geothermal gradient.

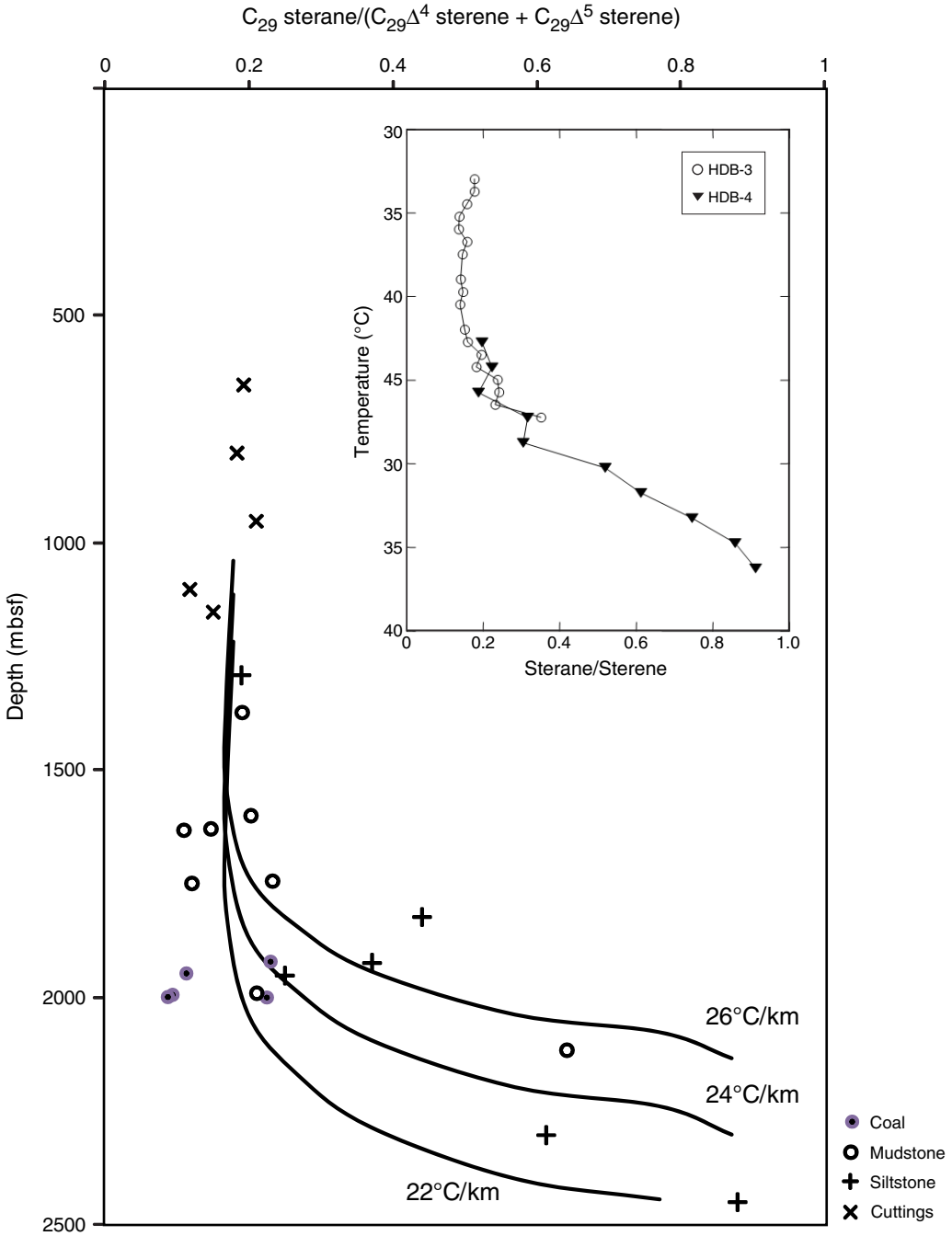

Figure F12. Downhole profile of C_1/C_2 ratios in mud gas analyzed by online mud-gas monitoring during the drilling of Hole C0020A. High C_1/C_2 ratios are characteristic for biogenic methane formation and low ratios indicate thermogenic sources because higher hydrocarbon gases are mainly formed by thermal alteration of organic matter. However, minor amounts of C_2 can also be generated during early diagenesis of organic matter. At Site C0020, C_1/C_2 ratios point altogether to biogenic methane sources. C_1/C_2 ratios generally decrease with depth as expected, but they show a distinct excursion toward higher values between 1840 and 2054 m MSF (i.e., the depth interval in which 12 coal layers were observed). The excursion of the C_1/C_2 ratio signals the presence of biogenic methanogenesis in coal-bearing horizons.

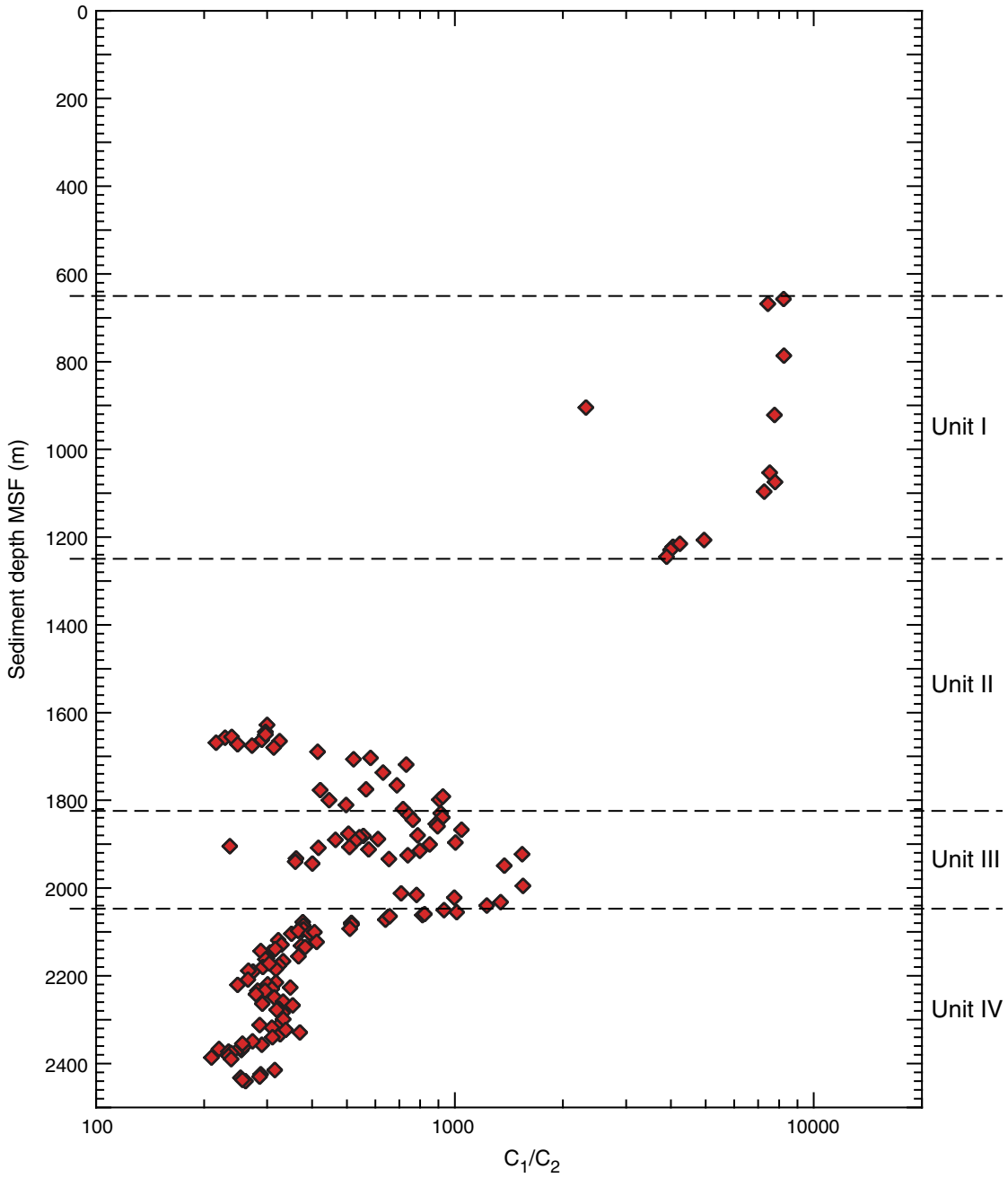

Figure F13. The yield of interstitial water squeezed from whole-round cores in relation to lithology.

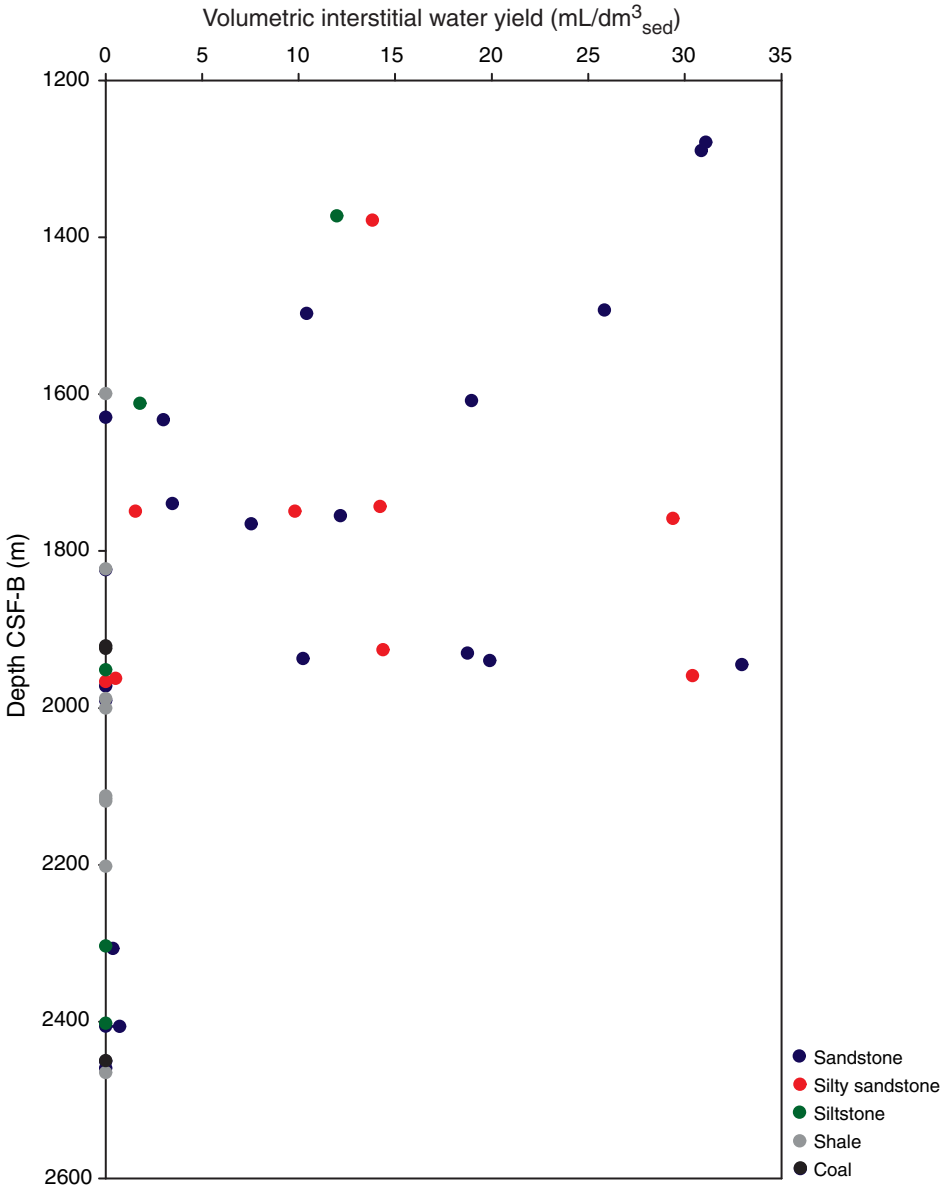

Figure F14. Fluorescent microscopic images of microbial cells from core samples. Each picture shows SYBR Green I-stained microbial cells observed in Sections (**A**) 337-C0020A-1R-2 (1278 m CSF-B), (**B**) 15R-2 (1920 m CSF-B), (**C**) 25R-2 (1997 m CSF-B), (**D**) 26R-7 (2117 m CSF-B), and (**E**, **F**) 32R-1 (2457 m CSF-B). Scale bars = 3 µm.

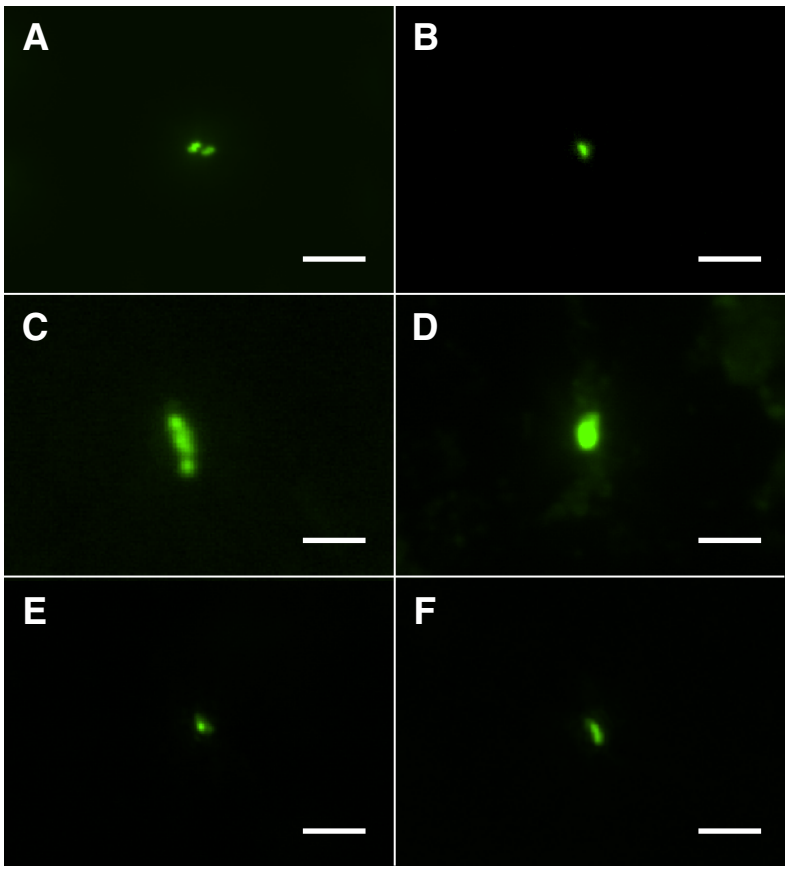

Figure F15. Sampling scheme for microbiology sampling and analyses flow, including whole-round core (WRC) distribution to specific analyses procedures, Expedition 337. Green = shipboard experimental processes, blue = shore-based experiments and analyses. MBIO = microbiology, IW = interstitial water, Sed. = sedimentology, PFC = perfluorocarbon, pSRR = potential sulfate reduction rate, H_2ase = hydrogenase, NanoSIMS = nanoscale secondary ion mass spectrometry, GC-ECD = gas chromatography-electric conductivity detection, FCM = flow cytometry, FISH = fluorescence in situ hybridization, PCR = polymerase chain reaction, qPCR = quantitative polymerase chain reaction, T-RFLP = terminal-restriction fragment length polymorphism, subst. = substrate, cont. = contaminated.

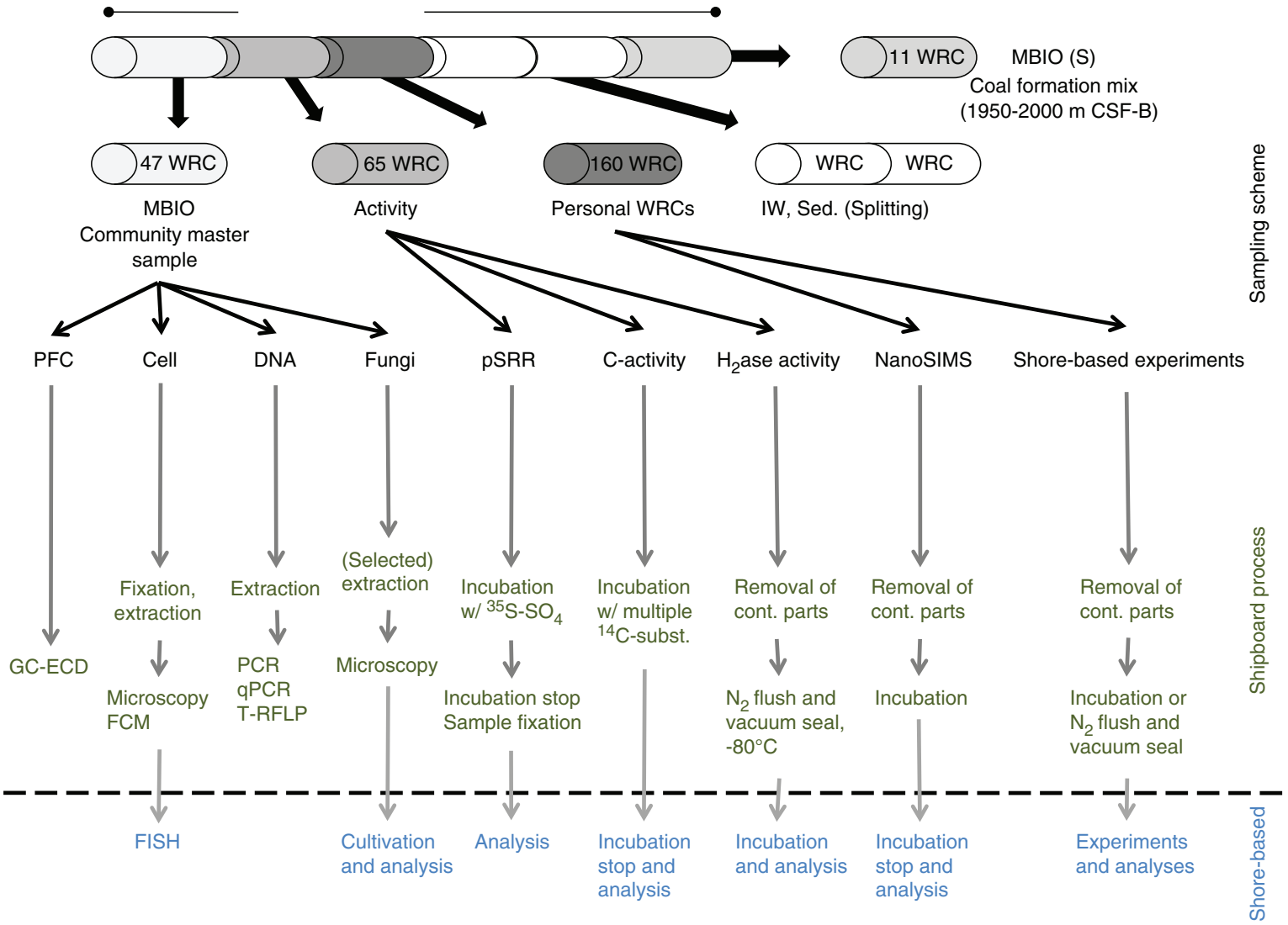

Figure F16. Change in drilling fluid contamination and predicted number of drilling-induced contaminant cells, from the outer centimeter of cores (X), to halfway between the core liner and core center (H), to the innermost part (IN), using Sample 337-C0020A-26R-7, 84–101 cm (Code: MBIO-COM) as an example. PFC concentrations were below detection (BD) in the innermost part. The empty column shown illustrates the expected contamination at the detection limit and should thus be treated as a maximum value. Only the innermost parts of cores were used for cell counts and DNA extractions on board the ship. The boxed photos show X-ray CT scan images of Core 26R-7, 84–101 cm.

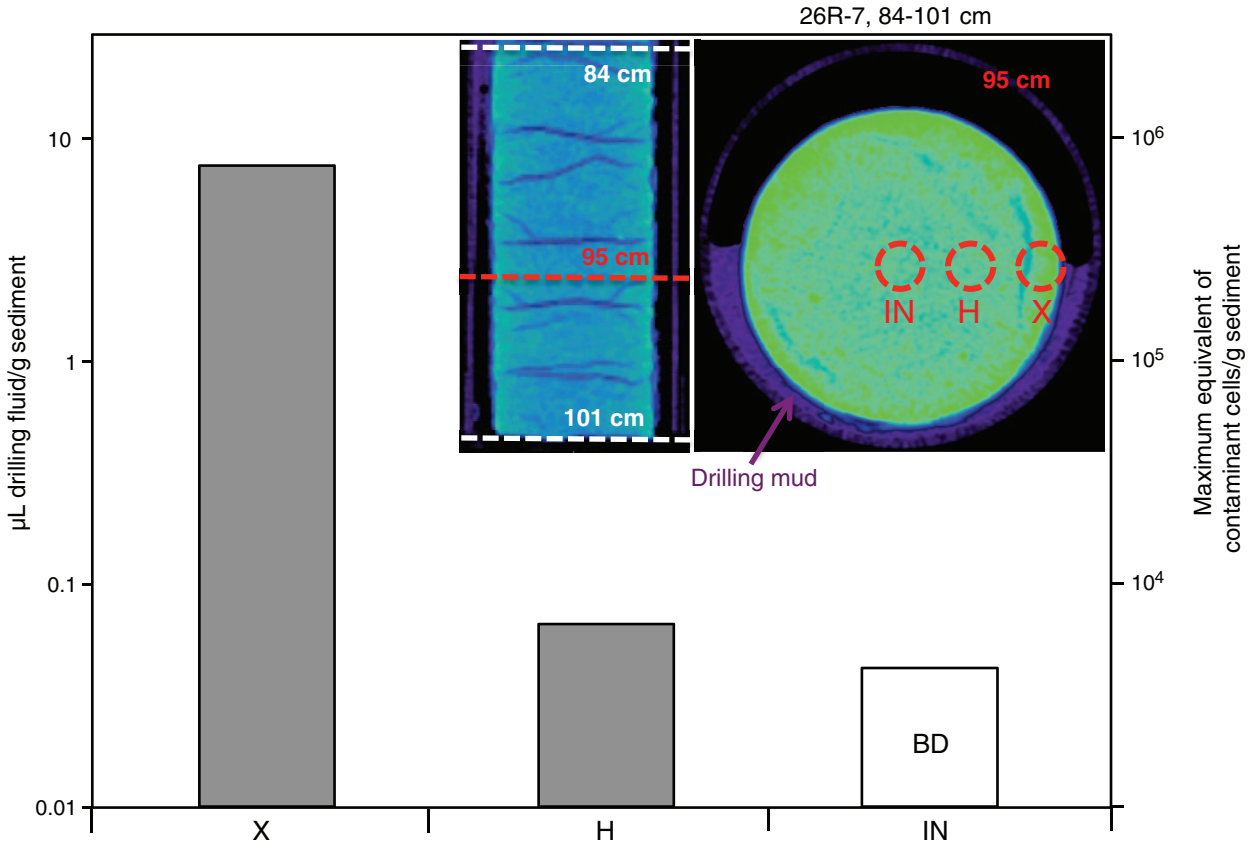

Figure F17. Section photo and X-ray CT scan images. **A.** Sample 337-C0020A-1R-CC, 0–14.5 cm. Drilling mud injection is causing some lamination in semiconsolidated sandstone. **B.** Partial Section 19R-3. An example of "nugget structures" caused by drilling mud injections is shown.

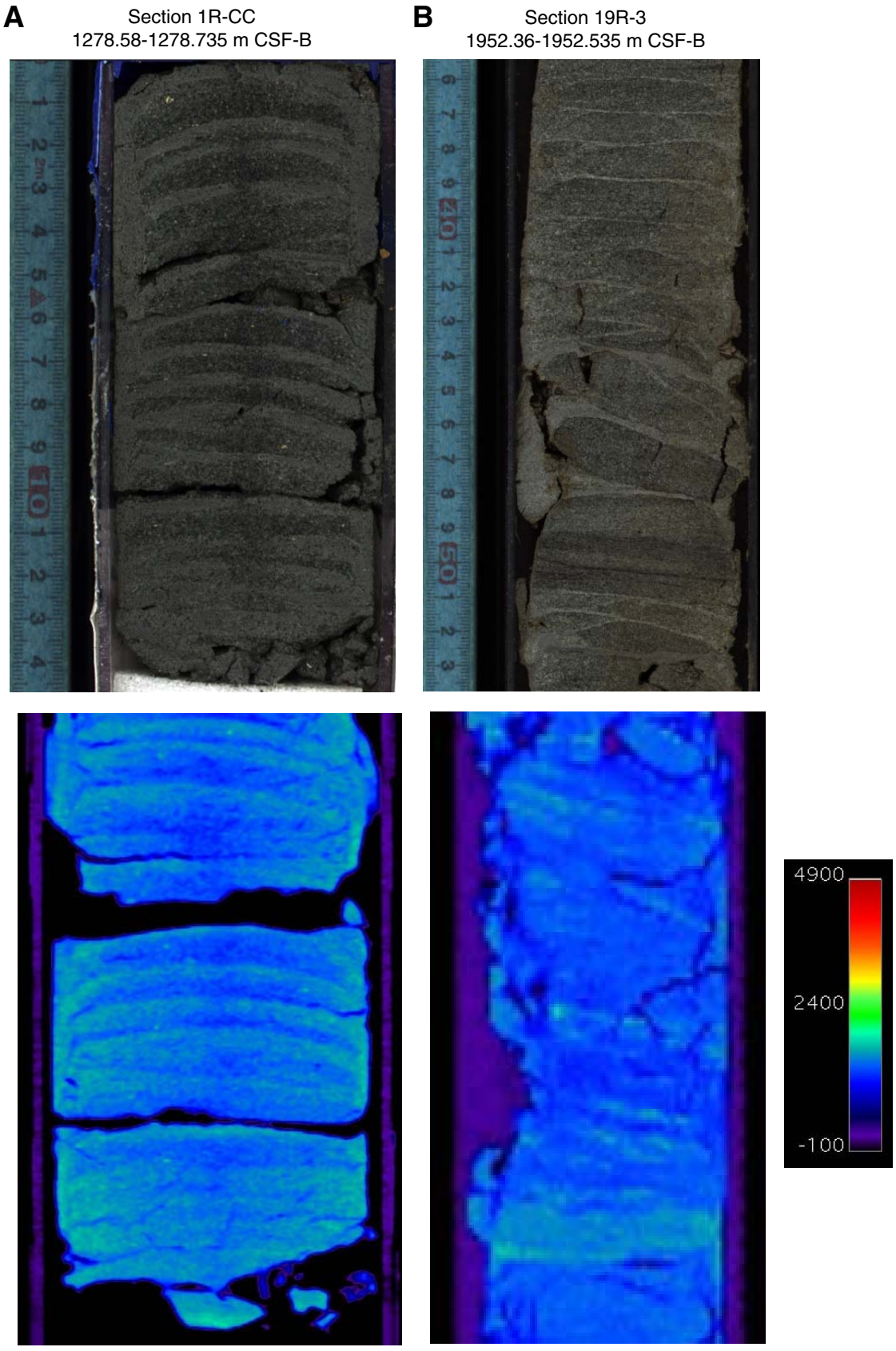

Figure F18. Estimates of fraction drilling mud liquid (X_{DML}) incorporated into interstitial water samples, Site C0020. X_{DML} was estimated based on concentrations of sulfate, potassium, and salinity. Shaded area represents the –1σ and +1σ region of the average X_{DML} values (not shown) calculated for the pore waters. Open symbols are for X_{DML} in the formation water collected in Single-Phase Sample Bottles (SSBs); the color is the same as the corresponding mud fractions based on sulfate, potassium, and salinity that were applied to the pore water. With the exception of the first SSB sample in Unit II, all of the other samples showed mud fractions that exceeded those of the pore water.

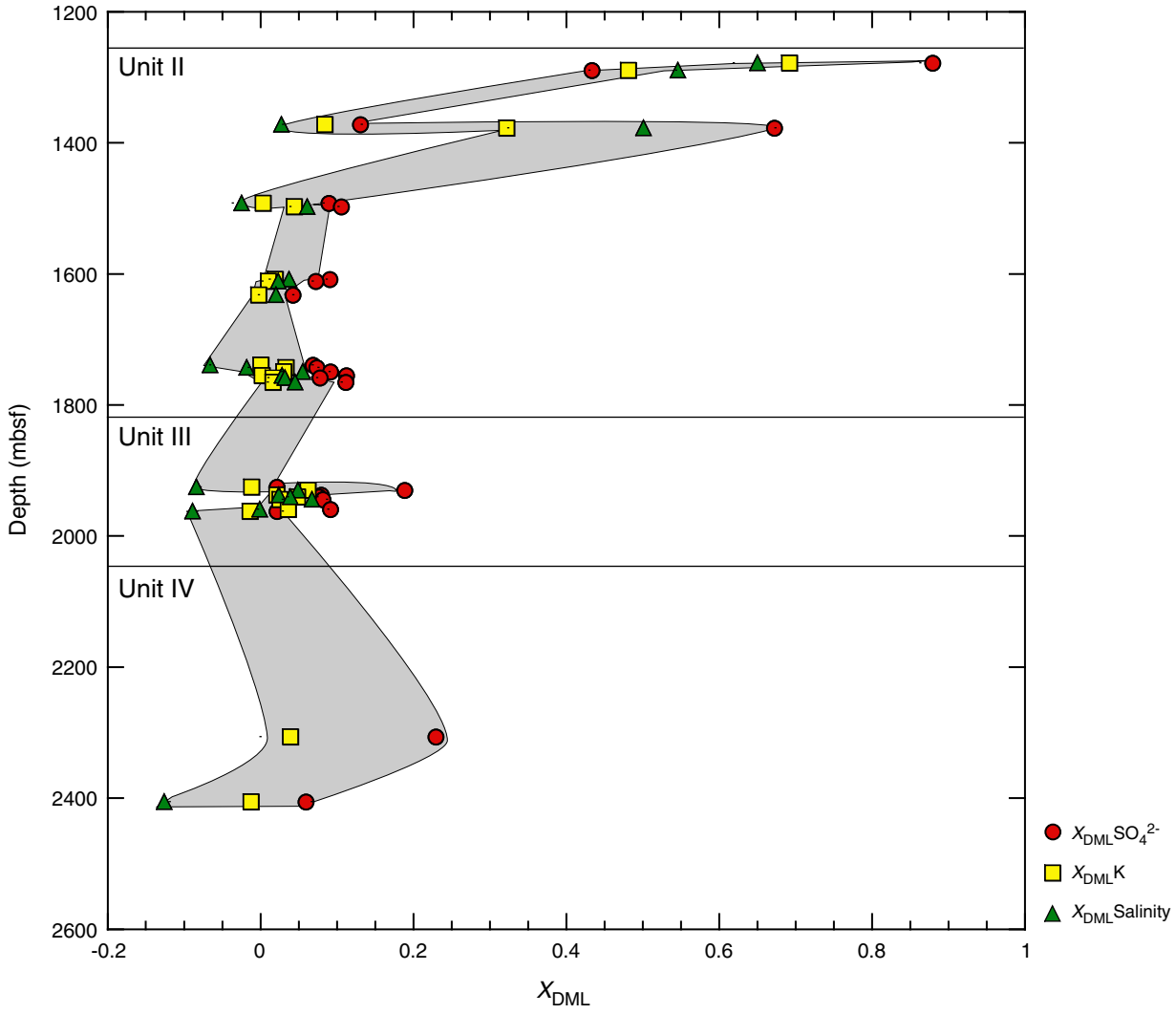

Figure F19. A. Biomarker contamination 2: *m/z* 191 ion chromatograms for ASTEX-S SAS (a drilling additive), the extract from a sandstone (Sample 337-C0020A-16R-2, 12–45 cm) and from a siltstone (Sample 15R-2, 0–21 cm). ASTEX-S SAS contains thermally mature hopanes; C_{29} and C_{30} αβ hopanes (hopanes with a 17α, 21β [H] configuration) and a distinctively high proportion of C_{35} 22S and 22R hopanes relative to the C_{34} 22S and 22R hopanes. A sample from a more contamination resistant lithology (a siltstone from Section 15R-2) contains C_{30} and C_{31} hopanes with the thermally immature 17β, 21β (H) configuration. The sandstone from Section 16R-2 contains a mixture of hopanes and has a hopane-fingerprint that is a mix of the indigenous formation and the infiltrated ASTEX-S SAS—both thermally mature and immature hopanes that would not be expected to be found together. (Continued on next page.)

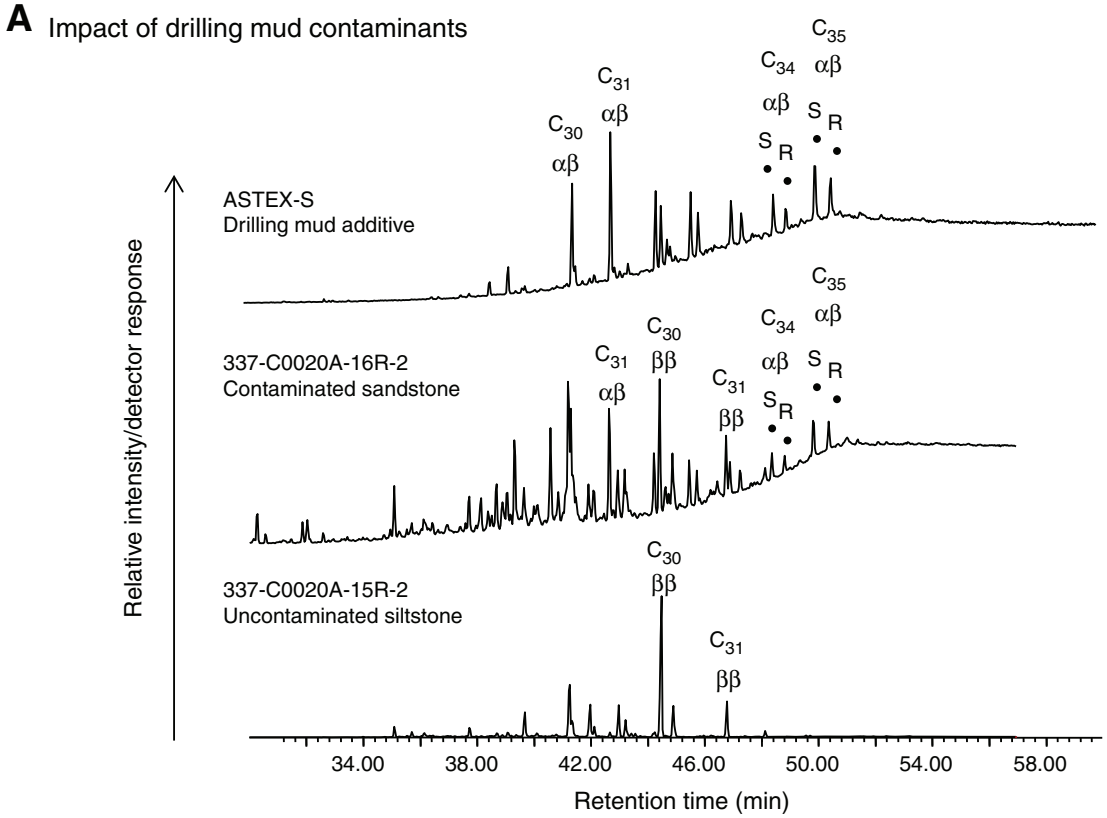

Figure F19 (continued). B. Total ion chromatograms for sequential extraction of drilling cuttings. The extract from the exterior is dominated by drilling mud components (marked by *). After cleaning by sonication in solvent, a repeat extraction (second extract) yields far less organic matter, suggesting that the surface has been cleaned. The solvent extract obtained from the now clean sample after it has been crushed contains far less drilling components (Accelerated Solvent Extractor [ASE]-extract for cuttings Sample 337-C0020A-25-SMW). is = internal standard.

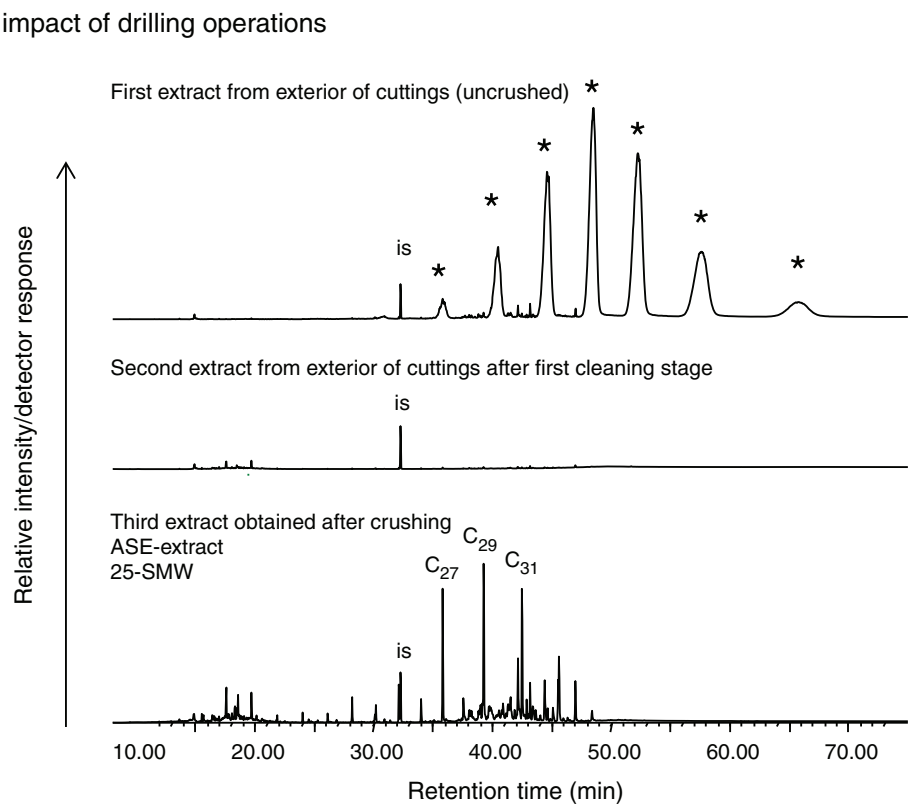

Table T1. Expedition 337 coring summary.

Hole	Latitude	Longitude	Water depth (mbsl)	Cores (*N*)	Cored (m)	Recovered (m)	Recovery (%)	Drilled (m)	Penetration (m)	Time on site (days)
337-C0020A	41°10.5983′N	142°12.0328′E	1180	32	263.5	198.4	75.3	1555.5	1819	60