The effects of X-ray CT scanning on microbial communities in sediment cores

by
Erica Ewton

A THESIS

submitted to
Oregon State University
Honors College

in partial fulfillment of
the requirements for the
degree of

Honors Baccalaureate of Science in Microbiology
(Honors Scholar)

Presented November 26, 2019
Commencement June 2020
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Frederick Colwell

Using X-ray computed tomography (CT) scanning to characterize the physical characteristics of soil and sediment cores allows scientists to observe and analyze stratigraphy without destroying the integrity of different layers. Microbiologists often work with geologists to characterize the microbial communities in such cores; however, X-rays are known to be destructive to cells and this is not typically considered when cores are scanned. My objective was to determine whether X-ray CT scanning affects microbial community composition within the cores. Sediment cores were extracted from salt marshes in Netarts Bay, OR to examine CT scan effects on microbial communities in fine and coarse grain layers. We observed no apparent effect of X-ray CT scanning on microbial community composition in any of the sediment cores; however, other factors in the samples such as location in the marsh from which the samples were obtained and sediment type did have a marked effect on microbial community structure.

Key Words: Sediment core, X-ray Computed Tomography, microbial community structure.

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I understand that my project will become part of the permanent collection of Oregon State University, Honors College. My signature below authorizes release of my project to any reader upon request.

Erica Ewton, Author
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1. Introduction

X-ray computed tomography (CT) is commonly used as a medical practice to produce images of parts of the body that would otherwise not be visible to the naked eye. X-ray CT is the first imaging modality that allows accurate and non-destructive interior image reconstruction from X-ray projections. CT technology is very practical and useful for applications such as but not limited to examining the brain and confirming diagnostic tests, so much effort has been and will continue to be placed in advancement of biomedical and non-biomedical applications (Wang et al., 2008). Figure 1 serves to provide examples of X-ray effects at given doses to give context of X-ray dose range. The figure shows an approximate dose applied to experimental samples in my study compared to other commonly used doses for other purposes, including background dose per year of a person living in the United States (US EPA, 2015), the range of diagnostic X-ray imaging for medical practices (Radiologyinfo.org), and an amount that is considered fatal to humans within weeks (World Nuclear Association).
Figure 1: Relative X-ray doses for context. Approximate dose-received measured in milliSieverts (mSv) is calculated for my study and placed in the context of typical activities to provide baseline examples for comparison of X-ray dose. X-ray effect is indicated in boxes with arrows pointing to the approximate received dose, which is indicated at the bottom of the figure. My study delivered a dose slightly above the background dose of someone living in the United States, and within the range of diagnostic X-ray imaging. Dose data obtained from UA EPA, Radiologyinfo.org, and World Nuclear Association.

Geological cores are used to study earth’s geochemistry, and X-ray CT is often used to acquire images and conduct analyses of such cores (Orsi et al., 1994). X-ray CT scanning provides non-destructive, high-resolution, 3D views of sediment lithology and physical composition (e.g., Davey et al., 2011). Use of X-ray CT scanning to gain data about geology has been in use for decades (Petrovic et al., 1982). For example, sediment cores were used to study lithology, particle size, and micropaleontology to understand more about sediment accumulation, chronology, and history of the Atlantic and Caribbean (Ericson et al., 1961). A more recent study used sediment core analysis to help confirm numerous droughts in New Mexico and piece together the southwest monsoon history (Poore et al., 2005).
Microbiologists often work with geologists to analyze the microbial communities present in such cores. For example, X-ray CT scanning has been used to observe biomineralization by *Caulobacter crescentus* (Benzerara et al., 2004) and determine aspects of physical structures that microbial communities can form on the macro scale such as porosity and pore shape (Nunan et al., 2006). X-ray scanning has also identified physical features such as fractures in sediments that permit advection of methane-rich fluids that support methanotrophic microbial communities (Yao et al., 2018).

Even though X-rays are of great importance to geologists as they characterize subsurface materials, they are known to be destructive to certain components of microbial cells. X-rays delivered to soil can cause DNA mutations due to direct ionization of key macromolecules within cells. Additionally, indirect radiolysis of cell water can create free radicals in cells’ extra- and intracellular fluids which damage and kill cells by disrupting equilibrium through introduction of reactive oxygen species (Zappala et al., 2013). The amount of X-rays delivered to a sample is measured by dose, which is characterized by a number of controlled factors, including absorption coefficients of constituent atoms, number of molecules per unit, size, beam energy, flux, and irradiation time (Murray et al., 2005). Dose is a key factor in determining how much damage will be done to microbial communities, but is also variable based on sample composition. A sample with higher water content has more reactants that will readily form into free-radicals and therefore be more damaging to microbial communities than a sample with low water content (Zappala et al., 2013).

Despite the fact that X-rays damage microbial cells, the effect that CT scanning has on the microbial community composition within sediment cores has not been well researched, and deserves more attention because the method is used so frequently by geologists (Zappala et al.,
One study found that CT scanning causes small but significant decreases in microbial β-glucosidase and dehydrogenase activity when testing cores a day after irradiation (Bouckaert et al. 2013). This may indicate a drop in aerobic activity. Enzyme levels returned to normal after 21 days however, indicating that over a long period of time the enzyme activities in the different treatments could no longer be distinguished from each other (Bouckaert et al. 2013). A different study found slightly lower potential extracellular enzyme activity of β-glucosidase, chitinase and phosphatase in scanned versus unscanned control cores after approximately three weeks (Fischer et al. 2013). The study also found that retrievable DNA increased in the scanned cores, which suggests that more organisms were decaying and/or dying and leaving free DNA behind compared to the unscanned control groups. Scanned and unscanned cores showed differences in bacterial community structure, but these differences decreased seven days after scanning.

A metastudy published in 2013 compiled previously published experiments that measured impacts of X-rays on plant roots and microorganisms (Zappala et al., 2013). After a literature review, Zappala et al. conducted a study of their own involving rice roots growing in soil. They concluded that X-ray CT scanning does not impact plant growth or soil microbial populations at dose levels of 30 Gy or less. However, only 2.4% of publications used in the study explicitly stated dose estimations, so this study calculated using approximate values. My study aims to report delivered dose to align my findings with those reported by Zappala et al.

The objective of my study was to determine whether X-ray CT scanning alters microbial communities in sediment cores; more specifically, how microbial community structures shift when treated with ionizing radiation. This was accomplished by determining microbial community diversity in subsamples from scanned and unscanned replicate cores throughout an incubation period of three weeks. Several sediment layers that varied in key characteristics such as organic
matter content were used for comparison. *I hypothesized that microbial community structure would change immediately after CT scanning, then slowly return to conditions noted before scanning after several weeks, therefore reflecting the changes in enzyme activity reported previously* (Bouckaert et al. 2013). Findings from my study suggest that X-ray CT scanning at doses used by geologists does not affect microbial community structure.

2. Methods

2.1 Site description and coring method

To obtain geological materials for examination of the effect of X-ray CT scanning on microbial communities, 1.5 meter long, 10 centimeter diameter cores were collected from two areas in the Netarts Bay marsh on the Oregon coast (45.373778, -123.96489 and 45.372197, -123.964223) as reported previously (Peck, in prep). Netarts Bay estuary covers approximately 11.1 km$^2$, with a watershed of 363 km$^2$. This location was chosen because previous studies indicated the presence of different sediment types that could be accessed by short cores (Adamus, Larsen, & Scranton, n.d.). This marshland is broken up by tidal channels, and a beach to the west leads to the Pacific Ocean (see Figure 2). Sample locations were chosen based on a previous study which collected cores from the same location (Peck, in prep). Sample locations differed in their proximity to the waterline and their elevations; northern site elevation was 2.772 m and southern site elevation was 2.765 m.
Figure 2: Coring site map. This map shows sampling locations for each of the paired cores. To the west lies a beach that leads to the Pacific Ocean, and to the east is a creek that drains to the ocean and an access road. Cores 2 and 3 were collected from the location 45.373778, -123.96489. Cores 4 and 5 were collected from 45.372197, -123.964223. All coring locations are indicated in the figure with blue markers.
Each core was collected as follows: first, a PVC pipe with beveled edge was driven mostly into the ground using a sledgehammer; second, a mechanical test plug was placed in the PVC pipe above the sediment level to prevent sediment loss; third, a truck jack was attached to the PVC pipe via a pipe clamp to extract the cores from the ground. Cores were transported upright back to the laboratory, where they were split in half according to previously described methods (Peck, in prep). Upon splitting, distinct sedimentary features were observed including a sandy layer characteristic of tsunami deposits as described previously (Peck, in prep).

Two cores from each location were collected, one to be CT scanned and the other to serve as a control that would remain unscanned. Subsample collection depths were chosen at 5 cm above, within, and 5 cm below a sandy tsunami deposit to capture different sediment types, thereby increasing the types of geological, and presumably microbiological, materials that could be tested.

2.2 Experimental design, X-ray CT scanning, and core analysis

After splitting, one half-core from each location was X-ray CT scanned using a Toshiba Aquilion 64-slice CT unit at Oregon State University’s Veterinary Hospital. The following parameters were used: 120 kV peak, 400 mA, 0.5 pitch, 200 mAS, 41.0 HP, and 0.641 pitch factor. Dose delivered for each half-core was 66.60 mGy. Figure 3 shows images of Core 05 taken with a cell phone camera and CT scanner. Core 03 had similar lithology to core 05. After scanning, cores were stored at 14 degrees C in the dark to maintain approximate in situ conditions. Three subsamples from each of the three layers described above were collected from each half core on days 0, 7, 14, and 21 after collection of the cores. During the 21 days, sediment was covered in plastic wrap to minimize oxygen exposure and drying, but even so the open surfaces of clay turned red after the
first day, apparently due to oxidation of reduced iron. Subsamples were taken from one cm depth within the sediment to minimize the impact of oxygen exposure on community structure.

Figure 3: Images of Core 05. The left image was taken with a regular camera. The right image was created by CT-scanning using a Toshiba Aquilion 64-slice CT unit at Oregon State University’s Veterinary Hospital. The following parameters were used: 120 kV peak, 400 mA, 0.5 pitch, 200 mAS, 41.0 HP, and 0.641 pitch factor. Depth increases from top to bottom of the figure. Loose soil and root fragments constitute the top ‘layer’, gradually transitioning to a more clay-like texture. Following, a light grey stripe is a tsunami deposit from a tsunami in 1770 (Peck, in prep). Further down lies sediment similar to that at the top, but is statistically significantly different in microbial community structure and organic matter content (see Figures 4 and 6).
Additional subsamples were taken from above, within, and below the tsunami deposit in each core on day 21 and freeze-dried for two days in the OSU Core Lab to eliminate water content. Freeze-dried samples were then examined for organic carbon weight percentage by loss on ignition, following published protocols (Heiri et al., 2001).

**2.3 DNA extraction, purification, amplification, and sequencing**

DNA from each subsample was extracted using a MoBio PowerSoil DNA Isolation kit following manufacturer’s instructions. A purified water sample was used as a sediment-free extraction control. Bacterial and archaeal 16S rRNA genes were amplified in triplicate following the Earth Microbiome Protocol, using 515-forward and 806-reverse universal primers targeting the V4 hypervariable region (Caporaso et al., 2011). Primers also contained dual-indexed Illumina sequencing adaptors (Kozich et al., 2013). Amplification was verified with gel electrophoresis, and pooled amplicons were purified using a QIAQuick PCR Purification kit. Illumina MiSeq V2 paired-end 250 bp sequencing was performed at the Center for Genomic Research and Bioinformatics (CGRB) at Oregon State University.

**2.4 Analysis of sequence data**

16S rRNA gene sequence data was processed using Mothur version 1.39.3 (Schloss et al., 2009) following an established pipeline (Kozich et al., 2013). Assembled reads were clustered into operational taxonomic units (OTUs) at the 97% similarity level and classified using the SILVA taxonomic database version 128 (Quast et al., 2013). Communities were rarefied to 1,311 reads per sample, and indices of alpha diversity were then calculated. To assess differences in microbial community composition, a tree file containing the most abundant sequence from each OTU was
constructed with Clearcut (Evans et al., 2006). Weighted Unifrac distances (Lozupone et al., 2007) were used to calculate a dissimilarity matrix for non-metric multidimensional scaling (NMDS) ordination. ANOSIM was used to test for differences in community structure (Clarke, 1993), and Metastats was used to determine whether individual OTUs showed differences in percent abundance between communities derived from different samples (White et al., 2009). Raw fastq sequences were uploaded into the NCBI Sequence Read Archive (SRA) under the Bioproject number PRJNA533633.

3. Results

The objective of this study was to build on previous findings by identifying whether microbial community structure is altered by CT scanning, an analysis technique commonly used for characterization of geological cores. According to the experimental procedure that we used, we observed no evidence that CT scanning altered either the microbial communities or relative abundances of taxa when using DNA extraction and sequencing methods commonly used by microbiologists.

Microbial communities from all spatial and temporal subsamples of scanned and unscanned cores from the two sites are shown in the ordination (Figure 4). ANOSIM tests of paired samples showed that CT scanning did not affect microbial community structure in this study (p=0.946). Communities stored at 14 °C were likewise unaffected by storage times of up to 22 days (p=0.995), and thus storage times are not indicated in Figure 4. Communities showed differences in composition according to original location of the samples (i.e., northern or southern coring site; p<0.001), and depth within the soil profile (above-below-within, p=0.002; above-below, p= 0.008; above-within, p=0.008; below-within, p=0.028).
Figure 4: Nonmetric multidimensional scaling (NMDS) plot. This plot shows relative similarity of microbial communities in subsamples obtained from three depths in each of the scanned and unscanned split cores during the three-week study. “North” and “South” refer to the two sampling sites in the marsh. Numbers in the plot refer to the depth in cm below the surface from which each respective subsample was acquired. Sample storage times are not indicated in the figure.
The experiment was designed to test a wide range of sample types including Northern vs Southern coring site, sediment type (above/within/below tsunami deposit), and beginning vs end of study (to examine a storage effect). Percent abundances of dominant bacterial and archaeal classes (each >1% relative abundance) among paired subsamples are shown in Figure 5. At the class level, microbial communities present in scanned cores were not notably different than those present in comparable core materials that were not scanned. This suggests that certain bacterial and archaeal classes clearly dominate these samples and their abundances are not altered by the X-ray CT treatment of the cores. Additionally, no differences were seen based on storage time. Subsamples taken from the same coring site tended to have statistically insignificant differences in the percent abundance of bacterial and archaeal classes.

Instead, the most notable differences in percent abundances were observed between coring locations. *Deltaproteobacteria* and *Gammaproteobacteria* were in highest abundance of all subsamples from the Northern coring site. Slight but significant differences were seen when comparing sediment type within each core. This was most notable in Southern cores; for example, RGB-1 accounts for 14.3% of reads within the tsunami deposits as compared to 4.6% of subsamples from above or below (p=0.0028), and *Nitrospira* comprises 12.3% of above-tsunami deposit communities but only 2.9% of those from within or below (p=0.0011). Though percent abundances shifted slightly, the same taxonomic classes were nearly equally abundant at each coring site. *Zixibacteria* and *Epsilonproteobacteria* were the taxa that fluctuated the most between northern and southern coring sites. *Zixibacteria* are likely iron oxidizers/reducers (Castelle & Banfield, 2018), and *Epsilonproteobacteria* are sulfide detoxifiers (Grote et al., 2011). Slight differences in taxa percent abundances likely reflect slightly differing nutrient concentrations between sites, due to tidal influence/macofauna cover/etc.
Figure 5: Bubble plot. This plot indicates the highest percent abundant archaeal and bacterial classes for scanned and unscanned cores for factors including location relative to tsunami deposit, coring location, and period of storage of the subsample following core collection. Bubble (circle) area is proportional to percent abundance. Classes are indicated at the bottom. Storage time is indicated on the left side. Colors blue, yellow, and red refer to depth of the sample relative to the tsunami deposit, referencing above, within, and below the tsunami deposit, respectively. A 1% cutoff rate, calculated using the dataset as a whole, was used in determining which taxa are presented.

Coring site locations and sediment layers differed according to the quantity of organic matter present (OM, Figure 6) and the dry bulk density (DBD). OM variation between samples may contribute to differences in microbial community structure (Priha et al., 2001). DBD trends
follow those of OM (data not shown). Both cores in each pair had similar OM and DBD levels, independent of whether the core had been CT scanned.

Figure 6: Percent organic matter (OM). Samples were obtained from three depths, measured in centimeters, in each of two cores (“North” and “South”) in the Netarts Bay marsh. Sample depths are indicated as being above, within, or below tsunami deposits in each core in colors blue, yellow, and yellow, respectively. Error bars indicate data from a previously published study (Peck, in prep).

CT scanning treatments did not significantly alter OTU richness or the inverse Simpson indices among all paired samples (p-values 0.989 and 0.180, respectively). OTU richness refers to the number of OTUs in a sample, indicating the α-diversity of the microbial community. Inverse Simpson index is another method to measure biodiversity of a sample with uniform evenness that would have the same level of diversity. These metrics of α-diversity did not change between scanned or unscanned subsamples stored up to 22 days, as one-way ANOVA tests of OTU richness and inverse Simpson indices resulted in p-values of 0.785 and 0.644, respectively. Therefore, we
concluded that community α-diversity does not change over time during storage for up to 21 days after CT scanning.

4. Discussion

The objective of my study was to identify whether microbial community structure in sediment cores was altered by CT scanning, an analysis technique commonly used for characterization of geological cores. Our findings suggest that for the samples I tested there was no discernible difference in microbial community structure based on whether the cores were scanned.

My findings support those discussed in previous literature. Bouckaert et al. (2013) and Fischer et al. (2013) found decreased enzyme activity in cells subjected to CT scanning. In my study, subsamples were taken every seven days in an attempt to capture any changes in community structure, modeled this way because Bouckaert et al. found slightly decreased levels of β-glucosidase and dehydrogenase, which indicate a drop in aerobic activity, when testing cores a day after they had been irradiated. They concluded that enzyme levels between treatments were statistically insignificantly different when measured over the duration of the study, however they did note a drop in aerobic activity of irradiated samples when measuring one day post-scan. Results from my study suggest that storage time may not be an influential factor over timescales of several weeks in determining microbial community structure in both scanned and unscanned cores. It is possible that X-rays may reduce enzymatic activity in a way that does not preferentially select for certain members of a microbial community within the time frame tested, hence the results from my study.
A previously conducted study found a decrease in \( \alpha \)-diversity of scanned cores seven days after scanning because there was a strong increase in microbial biomass (Bouckaert et al., 2013). They found more retrievable DNA in scanned cores, however, which is a possible indicator that there were more dead and/or dying organisms present than in unscanned cores. It is possible that some of the DNA that was extracted from subsamples in this study was from dead and/or dying cells. Turnover times of extracellular DNA from surficial marine sediments may range from 29 to 93 days (Dell’Anno & Corinaldesi, 2004), and extracellular DNA pools are known to persist over millennial timescales in frozen and/or anoxic conditions (Torti et al, 2015).

Microbial cells can be affected by radiation at levels as low as 30 Gy, but this is dependent on conditions such as metabolic activity, organism size and complexity, and lifecycle stage (Zappala et al., 2013). X-ray dose absorbed is a function of the absorption coefficients of constituent atoms in the sediment type, number of molecules per unit, and size (Murray et al., 2005). Sediment type is likely a factor in determining dose because different sediment types have different nutrient concentrations (Kachi & Hirose, 1983). Additionally, grain size was visibly larger in tsunami deposits, which is also a contributing factor of microbial settlement (DeFlaun & Mayer, 1983). Tsunamis wash sandy sediment up over the coast and into estuaries, carrying with it microbes that live there (Somboona et al., 2014). This likely happened with the tsunami of 1700 in Netarts Bay, leaving a microenvironment similar to the ocean floor from which it came.

Though X-rays can be detrimental to cells (Meo et al., 2006) it is likely that the dose given by a CT scan is low enough to not kill microbial cells, and therefore may not change the microbial community structure. Zappala et al. (2013) compiled results from numerous studies on the effects of X-ray irradiation on plant roots and microorganisms and concluded that too few papers report
X-ray dose, and called for more reports of such information in future studies. Among the studies they considered, most used doses below the 33 Gy threshold where no effect was seen on post-germinal plant growth. They sampled soil for microbial activity after irradiating samples with X-ray CT following these parameters: 120kV, 100 µA, 0.1 mm Cu filter, 5.5 cm distance to sample, 33 min scan time, and 12.38 µm voxel size. Scanning parameters were similar to those used in my study, however the total dose received by each sample was 23 Gy over 6 scans, versus 66 mGy reported in my study. They concluded that there was no significant difference between scanned and unscanned samples after 24 weeks using the reported doses (P=0.975) (Zappala et al., 2013).

While I found no evidence that CT scanning altered the observable microbial communities in the samples, sediment type and core location were quite clearly important factors in determining the microbial community structure in the samples that I examined. The coring sites and sediment layers have differing levels of organic carbon, providing habitats for organisms with different nutrient needs (Priha et al., 2001). The Northern coring site is closer to estuarine waters and therefore more likely to be tidally influenced. This could determine differences in the flux of water and nutrients as well as different macroflora at the respective core locations (Yu et al., 2012). However, depth and coring location are the principle variables which drive differences in community structure for my study.

Other factors might be important to consider to fully understand the implications of X-ray CT scanning of geological cores. For example, dead cells may contain amplifiable DNA for up to three weeks even if stored at 4°C (Josephson et al., 1993). In a similar way, CT scanning may damage or kill cells in sediment cores while leaving their DNA extractable and amplifiable.
Future studies should consider whether DNA from damaged cells might be a factor in such studies and could use techniques that target extracellular DNA (Patel et al. 2010; Alawi et al. 2014).

5. Conclusion
According to the experimental procedure that I used, I observed no evidence that CT scanning altered either the microbial communities or relative abundances of taxa when using DNA extraction and sequencing methods commonly used by microbiologists. My findings suggest that current approaches for analyzing microbial community structure after CT scanning to discern key geological or sedimentological features may be possible without significantly altering the structure of the microbial community.

6. Significance of study
This study examined the possible effects of X-ray CT scanning on microbial populations in sediment cores. With the knowledge gained from my study I was able determine how CT scanning affected microbial community data in geological cores I used, and whether physical and chemical properties of the cores played a role in how CT scanning impacted the microbial communities. I also determined that, according to the conditions used in my study, different approaches will not be needed to preserve microbial features in geological cores that are scanned using X-ray CT, and I therefore concluded that current approaches are appropriate for continued use. The findings from my work will be implemented on upcoming research expeditions (e.g., a 2020 expedition to core methane hydrate-bearing sediments in the Gulf of Mexico) where microbiologists will work closely with geologists to characterize native microbial communities in drilled cores.
I expect that my results will inform treatment of cores for programs like the International Ocean Discovery Program and the International Continental Drilling Program that actively encourage interdisciplinary research between microbiologists and geologists.
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