

2 ASTROBIOLOGY  
0 GRADUATE  
1 CONFERENCE



CHARLOTTESVILLE, VA

1  
00:00:00,790 --> 00:00:08,320

[Music]

2  
00:00:12,740 --> 00:00:11,360

this is kind of a side project that I

3  
00:00:14,749 --> 00:00:12,750

work on

4  
00:00:16,790 --> 00:00:14,759

I don't normally study salty systems

5  
00:00:20,000 --> 00:00:16,800

this is kind of like a side pet project

6  
00:00:24,800 --> 00:00:20,010

that we have going on until my other

7  
00:00:26,900 --> 00:00:24,810

samples come in next year so why do we

8  
00:00:28,880 --> 00:00:26,910

care about salt flats and astrobiology

9  
00:00:32,359 --> 00:00:28,890

because we know there are salt flats on

10  
00:00:37,340 --> 00:00:32,369

Mars these are the salt flats that were

11  
00:00:39,619 --> 00:00:37,350

studied in 2005 and they're thought to

12  
00:00:41,990 --> 00:00:39,629

be a remnant of a large body of water

13  
00:00:45,020 --> 00:00:42,000

that used to be in that area a long time

14

00:00:47,840 --> 00:00:45,030

ago and these are the Salt Flats in Utah

15

00:00:52,459 --> 00:00:47,850

that I'm currently working on that were

16

00:00:54,680 --> 00:00:52,469

also where a Lake Bonneville used to be

17

00:00:57,770 --> 00:00:54,690

and then when Lake Bonneville evaporated

18

00:01:00,049 --> 00:00:57,780

we are left with the Bonneville Salt

19

00:01:02,060 --> 00:01:00,059

Flats and so there's a couple very key

20

00:01:04,340 --> 00:01:02,070

difference things between these two

21

00:01:06,770 --> 00:01:04,350

places one is that the Salt Flats in

22

00:01:09,710 --> 00:01:06,780

Utah undergo these ephemeral wet/dry

23

00:01:11,810 --> 00:01:09,720

cycles and so right now and all winter

24

00:01:16,700 --> 00:01:11,820

long they were covered in a layer water

25

00:01:19,429 --> 00:01:16,710

and then as it heats up and warms up the

26

00:01:24,039 --> 00:01:19,439

water evaporates off and dries out and

27

00:01:26,270 --> 00:01:24,049

then you get the salt crust formation so

28

00:01:28,940 --> 00:01:26,280

there haven't been too many studies on

29

00:01:31,730 --> 00:01:28,950

the salt crust formation and what are

30

00:01:34,310 --> 00:01:31,740

the biophysical chemical properties that

31

00:01:36,140 --> 00:01:34,320

lead to it um there have been some

32

00:01:38,630 --> 00:01:36,150

geology studies where they've gone and

33

00:01:40,340 --> 00:01:38,640

drilled cores and looked at like kind of

34

00:01:42,679 --> 00:01:40,350

what are the mineral components on these

35

00:01:44,539 --> 00:01:42,689

salt flats um but the only microbiology

36

00:01:46,880 --> 00:01:44,549

studies that have been done to date our

37

00:01:49,249 --> 00:01:46,890

culture dependent one of our

38

00:01:51,530 --> 00:01:49,259

collaborators has gone out and tried to

39

00:01:53,870 --> 00:01:51,540

grow things from them and isolated your

40

00:01:55,969 --> 00:01:53,880

standard halo archaea some Salona back

41

00:01:57,289 --> 00:01:55,979

there things like that that we know are

42

00:02:02,810 --> 00:01:57,299

insult these systems and aren't very

43

00:02:05,030 --> 00:02:02,820

interesting from my perspective so when

44

00:02:07,280 --> 00:02:05,040

we culture things we only get like 1% of

45

00:02:09,790 --> 00:02:07,290

the microbial community so we're taking

46

00:02:13,070 --> 00:02:09,800

a culture independent approach and

47

00:02:13,760 --> 00:02:13,080

looking at what are the total anaerobic

48

00:02:16,460 --> 00:02:13,770

and aerobic

49

00:02:18,530 --> 00:02:16,470

microbe communities out there and then I

50

00:02:20,300 --> 00:02:18,540

want to dig a little bit deeper and sort

51  
00:02:22,820 --> 00:02:20,310  
of ask this question what are they doing

52  
00:02:24,590 --> 00:02:22,830  
and what are the species that are really

53  
00:02:27,410 --> 00:02:24,600  
responsible for the primary carbon

54  
00:02:30,380 --> 00:02:27,420  
fixation in the system and do they need

55  
00:02:33,730 --> 00:02:30,390  
hydrogen as the electron source and so

56  
00:02:36,770 --> 00:02:33,740  
this second question sort of comes more

57  
00:02:38,750 --> 00:02:36,780  
into play when we think about the

58  
00:02:41,300 --> 00:02:38,760  
anaerobic community members that could

59  
00:02:43,610 --> 00:02:41,310  
be fixing carbon in there and more

60  
00:02:46,070 --> 00:02:43,620  
specifically the methanogens and the

61  
00:02:48,770 --> 00:02:46,080  
asita jhin's that um might possibly be

62  
00:02:52,010 --> 00:02:48,780  
there because they both need hydrogen as

63  
00:02:57,010 --> 00:02:52,020

electron donor and so the Bonneville

64

00:02:59,300 --> 00:02:57,020

Salt Flats are also an interesting space

65

00:03:00,650 --> 00:02:59,310

aside from other salt flats because you

66

00:03:04,190 --> 00:03:00,660

have a lot of human activities that go

67

00:03:05,960 --> 00:03:04,200

on out there so these high speed land

68

00:03:09,020 --> 00:03:05,970

aces take place every year that the

69

00:03:10,640 --> 00:03:09,030

salt crust thickness um is actually

70

00:03:13,250 --> 00:03:10,650

thick enough for them to occur and so

71

00:03:14,870 --> 00:03:13,260

you have like the highest speeds that

72

00:03:16,310 --> 00:03:14,880

have occurred on land have been out here

73

00:03:20,690 --> 00:03:16,320

and also the highest speeds on a

74

00:03:22,580 --> 00:03:20,700

barstool have taken place there and so

75

00:03:24,620 --> 00:03:22,590

we also have mining processes that are

76

00:03:27,530 --> 00:03:24,630

going on there they pump out the

77

00:03:31,040 --> 00:03:27,540

underground water and pump it into

78

00:03:33,020 --> 00:03:31,050

evaporating ponds and then sell that as

79

00:03:35,680 --> 00:03:33,030

table salt so you might be eating salt

80

00:03:40,400 --> 00:03:35,690

from Utah with your dinner tonight um

81

00:03:41,990 --> 00:03:40,410

okay so we went out and sampled some

82

00:03:44,600 --> 00:03:42,000

things as we do as environmental

83

00:03:46,699 --> 00:03:44,610

microbiologist so we paired up with our

84

00:03:49,430 --> 00:03:46,709

geology collaborators and the geology

85

00:03:52,640 --> 00:03:49,440

took samples all over here from every

86

00:03:55,280 --> 00:03:52,650

single site labeled they actually use

87

00:03:57,830 --> 00:03:55,290

this really cool drill and drilled them

88

00:03:59,630 --> 00:03:57,840

out and they got down there fairly deep

89

00:04:01,040 --> 00:03:59,640

however all of these samples were

90

00:04:03,410 --> 00:04:01,050

useless to us because they collected

91

00:04:06,440 --> 00:04:03,420

them in a way that was not aseptic there

92

00:04:10,820 --> 00:04:06,450

was no way to drill them cleanly um so

93

00:04:13,940 --> 00:04:10,830

we went out and instead dug pits um so

94

00:04:16,550 --> 00:04:13,950

we we sterilized our tools between sites

95

00:04:22,460 --> 00:04:16,560

as best as we could with ethanol and

96

00:04:24,440 --> 00:04:22,470

flame and these okay these are the sites

97

00:04:27,230 --> 00:04:24,450

that we sampled in red right here and so

98

00:04:29,480 --> 00:04:27,240

um this black line right here is the

99

00:04:31,400 --> 00:04:29,490

racetrack and so we tried to get a

100

00:04:33,950 --> 00:04:31,410

sample on and off the racetrack and sort

101  
00:04:37,610 --> 00:04:33,960  
of in this transect across the entire

102  
00:04:39,680 --> 00:04:37,620  
area and these are what our pits look

103  
00:04:43,370 --> 00:04:39,690  
like at each one of those sites um so

104  
00:04:44,570 --> 00:04:43,380  
you can see in in this site right here

105  
00:04:44,839 --> 00:04:44,580  
oh these aren't really coming up too

106  
00:04:49,969 --> 00:04:44,849  
well

107  
00:04:51,770 --> 00:04:49,979  
textural layers as you got down and so

108  
00:04:55,070 --> 00:04:51,780  
they looked different and we split them

109  
00:04:57,409 --> 00:04:55,080  
up according to those like visual and

110  
00:04:59,629 --> 00:04:57,419  
textural layers some of them had only

111  
00:05:04,059 --> 00:04:59,639  
two layers and some of them had three

112  
00:05:07,370 --> 00:05:04,069  
layers and one of them had four layers

113  
00:05:09,469 --> 00:05:07,380

and so we got back to the lab with all

114

00:05:11,870 --> 00:05:09,479

of these samples homogenized them

115

00:05:14,689 --> 00:05:11,880

extracted DNA from them sent a subset

116

00:05:18,710 --> 00:05:14,699

off to geology to do mineral analysis

117

00:05:21,589 --> 00:05:18,720

and then we sequence them using 16s

118

00:05:24,920 --> 00:05:21,599

sequencing and we will be doing

119

00:05:26,600 --> 00:05:24,930

metagenomic sequencing on a select few

120

00:05:28,370 --> 00:05:26,610

of these hits we haven't decided which

121

00:05:31,909 --> 00:05:28,380

ones to do that yet that's kind of the

122

00:05:34,459 --> 00:05:31,919

next step and I also set up these

123

00:05:35,450 --> 00:05:34,469

enriched carbon incubations try to get

124

00:05:37,219 --> 00:05:35,460

at this question of what are the

125

00:05:39,320 --> 00:05:37,229

microbes doing there so I wanted to dig

126

00:05:40,999 --> 00:05:39,330

a little bit deeper beyond who is there

127

00:05:45,350 --> 00:05:41,009

and try to answer this what are they

128

00:05:50,659 --> 00:05:45,360

doing and so I use stable isotope

129

00:05:53,240 --> 00:05:50,669

tracing with  $^{13}\text{C}$  carbon so these are all

130

00:05:55,999 --> 00:05:53,250

of the media that I set up incubations

131

00:05:57,499 --> 00:05:56,009

with I chose glucose to sort of get an

132

00:06:01,010 --> 00:05:57,509

idea of what the heterotrophic community

133

00:06:03,379 --> 00:06:01,020

is like so what are the organisms that

134

00:06:04,760 --> 00:06:03,389

are consuming carbon produced by other

135

00:06:06,950 --> 00:06:04,770

species there

136

00:06:10,300 --> 00:06:06,960

I chose acetate because I'm interested

137

00:06:12,670 --> 00:06:10,310

in the interplay between asita jhin's

138

00:06:16,520 --> 00:06:12,680

organisms that make acetate methanogens

139

00:06:18,260 --> 00:06:16,530

organisms to make methane and that have

140

00:06:19,580 --> 00:06:18,270

trophic community so can the

141

00:06:23,420 --> 00:06:19,590

heterotrophic community consume the

142

00:06:27,439 --> 00:06:23,430

acetate made by the citizens and I set

143

00:06:29,180 --> 00:06:27,449

up these bicarbonate incubations also to

144

00:06:31,010 --> 00:06:29,190

sort of get at this play between

145

00:06:35,120 --> 00:06:31,020

methanogens and its citizens they both

146

00:06:37,519 --> 00:06:35,130

usually compete for  $\text{CO}_2$  as well as even

147

00:06:39,589 --> 00:06:37,529

sometimes you have sulfate reducers that

148

00:06:42,019 --> 00:06:39,599

can use all of these compounds

149

00:06:43,429 --> 00:06:42,029

and so sulfate reducers will generally

150

00:06:45,709 --> 00:06:43,439

out-compete both the citizens and

151  
00:06:49,100 --> 00:06:45,719  
methanogens for things there's much

152  
00:06:52,519 --> 00:06:49,110  
better at grabbing these things and they

153  
00:06:53,839 --> 00:06:52,529  
it takes some much less energy to do

154  
00:06:58,010 --> 00:06:53,849  
what they're doing then the methanogens

155  
00:06:59,419 --> 00:06:58,020  
and these heated ins um so so that's why

156  
00:07:02,559 --> 00:06:59,429  
I did all of these and then we had one

157  
00:07:05,419 --> 00:07:02,569  
with hydrogen and one without hydrogen

158  
00:07:07,339 --> 00:07:05,429  
again this is because the citizens and

159  
00:07:09,469 --> 00:07:07,349  
the methanogens specifically need the

160  
00:07:11,179 --> 00:07:09,479  
hydrogen as electron donor as well as

161  
00:07:14,269 --> 00:07:11,189  
bicarbonate to grow um

162  
00:07:16,189 --> 00:07:14,279  
and so these are the incubations right

163  
00:07:19,939 --> 00:07:16,199

here they hung out on my bench for about

164

00:07:23,059 --> 00:07:19,949

sixty days and then I extracted all of

165

00:07:27,230 --> 00:07:23,069

the headspace um inside of these EXA

166

00:07:29,149 --> 00:07:27,240

Taner tubes and split that up into two

167

00:07:31,999 --> 00:07:29,159

samples for the glucose and acetate

168

00:07:33,920 --> 00:07:32,009

samples one to be sent off for thirteen

169

00:07:38,839 --> 00:07:33,930

co2 analysis one to be sent off for

170

00:07:40,429 --> 00:07:38,849

thirteen ch4 analysis um and so the

171

00:07:43,159 --> 00:07:40,439

reason we did this is because if you

172

00:07:46,309 --> 00:07:43,169

have something consuming glucose that is

173

00:07:48,769 --> 00:07:46,319

labeled with this 13 C they'll respire

174

00:07:50,779 --> 00:07:48,779

it out as 13 co2 and so you can catch

175

00:07:52,609 --> 00:07:50,789

that in the headspace and likewise with

176

00:07:54,049 --> 00:07:52,619

methane if you have active methanogens

177

00:07:57,319 --> 00:07:54,059

in your community that are using any of

178

00:07:59,959 --> 00:07:57,329

these urgency car pot compounds um they

179

00:08:04,399 --> 00:07:59,969

will you can see that as a tracer in the

180

00:08:05,749 --> 00:08:04,409

headspace gases so this kind of answers

181

00:08:07,309 --> 00:08:05,759

the question of what is the total

182

00:08:09,889 --> 00:08:07,319

microbial community doing but doesn't

183

00:08:12,290 --> 00:08:09,899

get at the individuals that are doing it

184

00:08:15,949 --> 00:08:12,300

and so I'm also doing these single cell

185

00:08:17,480 --> 00:08:15,959

Raman sorting experiments um using a

186

00:08:20,329 --> 00:08:17,490

microfluidic device that I'm currently

187

00:08:22,429 --> 00:08:20,339

in the process of designing and I'm not

188

00:08:24,259 --> 00:08:22,439

going to talk too much about this um

189

00:08:26,119 --> 00:08:24,269

because I don't actually have any data

190

00:08:28,489 --> 00:08:26,129

or results to show you guys or even a

191

00:08:31,759 --> 00:08:28,499

device to do them with but the idea is

192

00:08:34,279 --> 00:08:31,769

basically you can shine a Raman laser on

193

00:08:36,800 --> 00:08:34,289

a single cell and if that single cell

194

00:08:40,759 --> 00:08:36,810

has incorporated  $^{13}\text{C}$  into its biomass

195

00:08:43,040 --> 00:08:40,769

you'll see a shift in the spectra so you

196

00:08:45,829 --> 00:08:43,050

can then take that single cell sort it

197

00:08:48,079 --> 00:08:45,839

off to the side sequence it and answer

198

00:08:50,350 --> 00:08:48,089

the question of what cell was using

199

00:08:54,770 --> 00:08:50,360

these  $^{13}\text{C}$  carbon compounds

200

00:08:56,450 --> 00:08:54,780

okay um so I will talk to you about the

201  
00:09:00,740 --> 00:08:56,460  
headspace data because I have some of

202  
00:09:02,750 --> 00:09:00,750  
that back so this is all of the data

203  
00:09:05,000 --> 00:09:02,760  
that I have back in a really complicated

204  
00:09:09,020 --> 00:09:05,010  
chart um I will just kind of go through

205  
00:09:11,480 --> 00:09:09,030  
it step by step so um we'll look at the

206  
00:09:16,220 --> 00:09:11,490  
methane data first so as I told you

207  
00:09:19,120 --> 00:09:16,230  
methanogens need hydrogen to grow um so

208  
00:09:21,500 --> 00:09:19,130  
we would expect methanogens to be only

209  
00:09:24,020 --> 00:09:21,510  
producing methane in this control right

210  
00:09:26,630 --> 00:09:24,030  
here with hydrogen however we can see

211  
00:09:29,720 --> 00:09:26,640  
that there is some methane production in

212  
00:09:35,180 --> 00:09:29,730  
these other incubation x' without

213  
00:09:37,580 --> 00:09:35,190

hydrogen so this kind of indicates that

214

00:09:38,960 --> 00:09:37,590

methanogens might be using hydrogen but

215

00:09:43,820 --> 00:09:38,970

they could also be using something else

216

00:09:46,670 --> 00:09:43,830

for their electron donor and so we did

217

00:09:49,940 --> 00:09:46,680

aerobic and anaerobic incubations were

218

00:09:51,920 --> 00:09:49,950

not really expecting any sort of methane

219

00:09:53,420 --> 00:09:51,930

production in these aerobic incubations

220

00:09:56,120 --> 00:09:53,430

because methanogens or anaerobic

221

00:09:58,430 --> 00:09:56,130

organisms and oxygen generally kills

222

00:10:01,250 --> 00:09:58,440

them if they're exposed to it for a

223

00:10:02,600 --> 00:10:01,260

really long time so this is kind of what

224

00:10:07,430 --> 00:10:02,610

we expected to see across the board

225

00:10:09,290 --> 00:10:07,440

either in one of these two however in

226

00:10:11,630 --> 00:10:09,300

this one pit fifty-six

227

00:10:14,030 --> 00:10:11,640

we did get some methane production in

228

00:10:18,590 --> 00:10:14,040

the aerobic incubations and not the

229

00:10:21,200 --> 00:10:18,600

anaerobic incubations um so this is very

230

00:10:22,970 --> 00:10:21,210

strange and I don't really have too many

231

00:10:26,000 --> 00:10:22,980

ideas of what's going on here um

232

00:10:29,900 --> 00:10:26,010

especially because you can see in layer

233

00:10:32,900 --> 00:10:29,910

four you have a lot of it and maybe they

234

00:10:35,030 --> 00:10:32,910

were consuming all of the oxygen that

235

00:10:36,530 --> 00:10:35,040

was in the - or maybe other my grew new

236

00:10:38,420 --> 00:10:36,540

members were consuming all oxygen in the

237

00:10:40,490 --> 00:10:38,430

tube really quickly and so you get to

238

00:10:43,100 --> 00:10:40,500

that anaerobic state fairly fast

239

00:10:45,890 --> 00:10:43,110

um I didn't set up anaerobic incubations

240

00:10:48,130 --> 00:10:45,900

for that layer because our koi chamber

241

00:10:51,310 --> 00:10:48,140

sort of crapped out on us that night and

242

00:10:54,140 --> 00:10:51,320

refused to work because it had no gas um

243

00:10:56,810 --> 00:10:54,150

so I wasn't able to set up once for that

244

00:10:59,360 --> 00:10:56,820

layer unfortunately but also I had a

245

00:11:03,620 --> 00:10:59,370

little bit of methane production right

246

00:11:06,140 --> 00:11:03,630

here in this layer too

247

00:11:08,480 --> 00:11:06,150

as you'll note up here we also had

248

00:11:10,850 --> 00:11:08,490

methane production from glucose which is

249

00:11:13,130 --> 00:11:10,860

very strange because methanogens can't

250

00:11:16,520 --> 00:11:13,140

use glucose they can't even transport it

251  
00:11:19,250 --> 00:11:16,530  
inside of their cell and so because this

252  
00:11:21,920 --> 00:11:19,260  
occurred in aerobic incubations and not

253  
00:11:23,300 --> 00:11:21,930  
the anaerobic incubations this kind of

254  
00:11:25,610 --> 00:11:23,310  
suggests that there's some sort of

255  
00:11:27,380 --> 00:11:25,620  
partnering going on with the aerobic

256  
00:11:34,940 --> 00:11:27,390  
microbial community members and the

257  
00:11:37,910 --> 00:11:34,950  
methanogens and so we can kind of extend

258  
00:11:39,250 --> 00:11:37,920  
this into pit 12v where I also saw the

259  
00:11:41,660 --> 00:11:39,260  
same thing

260  
00:11:43,700 --> 00:11:41,670  
there was methane production from

261  
00:11:46,790 --> 00:11:43,710  
glucose going on under aerobic

262  
00:11:49,400 --> 00:11:46,800  
conditions but not anaerobic conditions

263  
00:11:52,580 --> 00:11:49,410

down here and so this was kind of a

264

00:11:54,500 --> 00:11:52,590

strange result but it also kind of makes

265

00:11:58,370 --> 00:11:54,510

sense when you look at the co2 data so

266

00:12:02,270 --> 00:11:58,380

in these same tubes right here where I

267

00:12:04,700 --> 00:12:02,280

have methane production from glucose we

268

00:12:06,410 --> 00:12:04,710

know that there's labeled co2 in there

269

00:12:09,430 --> 00:12:06,420

so I kind of have even more evidence

270

00:12:12,140 --> 00:12:09,440

that these aerobic community members are

271

00:12:14,090 --> 00:12:12,150

aerobic lee respiring glucose turning it

272

00:12:16,130 --> 00:12:14,100

into 13 co2 and then the most antigens

273

00:12:18,950 --> 00:12:16,140

are able to use that 13 co2 and making

274

00:12:22,610 --> 00:12:18,960

methane and I think a similar story is

275

00:12:27,290 --> 00:12:22,620

happening with acetate right here in pit

276

00:12:29,720 --> 00:12:27,300

12b and so some methanogens can directly

277

00:12:31,520 --> 00:12:29,730

use acetate to make methane however I

278

00:12:37,820 --> 00:12:31,530

don't think that's going on because if

279

00:12:40,940 --> 00:12:37,830

we look at the whoops okay um so if we

280

00:12:47,420 --> 00:12:40,950

look at the anaerobic ones down here

281

00:12:49,670 --> 00:12:47,430

with acetate and uh yeah so if we look

282

00:12:51,470 --> 00:12:49,680

at the the ones down here that are

283

00:12:55,310 --> 00:12:51,480

anaerobic with acetate there is no

284

00:12:57,020 --> 00:12:55,320

methane production so methanogens are

285

00:12:58,580 --> 00:12:57,030

anaerobic if they were able to use

286

00:13:00,130 --> 00:12:58,590

acetate directly we would expect to see

287

00:13:04,300 --> 00:13:00,140

methane production in the anaerobic

288

00:13:07,280 --> 00:13:04,310

controls also but we only see it in the

289

00:13:11,480 --> 00:13:07,290

aerobic ones so this is further evidence

290

00:13:13,370 --> 00:13:11,490

that there is in fact some sort of

291

00:13:15,040 --> 00:13:13,380

interaction between the aerobic

292

00:13:16,790 --> 00:13:15,050

community members and the methanogens

293

00:13:25,840 --> 00:13:16,800

especially in

294

00:13:31,250 --> 00:13:25,850

layer two right here okay so um whoops

295

00:13:32,509 --> 00:13:31,260

let me go on to what's going on Oh okay

296

00:13:37,730 --> 00:13:32,519

so that was what I just mentioned where

297

00:13:39,290 --> 00:13:37,740

you have um no so you have no methane

298

00:13:44,660 --> 00:13:39,300

production right here from bicarbonate

299

00:13:46,670 --> 00:13:44,670

also uh in layer one all right right

300

00:13:48,920 --> 00:13:46,680

here yeah so in layer one you have no

301  
00:13:52,519 --> 00:13:48,930  
methane production in the anaerobic

302  
00:13:54,410 --> 00:13:52,529  
controls from bicarbonate but we kind of

303  
00:13:57,500 --> 00:13:54,420  
see it as we go deeper in the pits and

304  
00:13:59,630 --> 00:13:57,510  
so this is a result that sort of makes

305  
00:14:01,430 --> 00:13:59,640  
sense because you would expect

306  
00:14:03,590 --> 00:14:01,440  
methanogens to be deeper down in the

307  
00:14:07,910 --> 00:14:03,600  
soil where they're kind of isolated from

308  
00:14:10,460 --> 00:14:07,920  
that oxygen that's getting to them okay

309  
00:14:13,940 --> 00:14:10,470  
and then lastly we'll kind of look up

310  
00:14:16,310 --> 00:14:13,950  
here at what was going on in the aerobic

311  
00:14:18,410 --> 00:14:16,320  
bicarbonate incubations and so you can

312  
00:14:23,030 --> 00:14:18,420  
see there's a little bit of methane

313  
00:14:24,079 --> 00:14:23,040

production in that first layer um so for

314

00:14:26,150 --> 00:14:24,089

some reason there were a little bit of

315

00:14:28,400 --> 00:14:26,160

methanogens that maybe we're hanging out

316

00:14:30,170 --> 00:14:28,410

in here but they can't really do

317

00:14:31,430 --> 00:14:30,180

anything because they're not they're not

318

00:14:32,510 --> 00:14:31,440

doing anything right here when it's in

319

00:14:34,069 --> 00:14:32,520

aerobic they can't really do anything

320

00:14:38,840 --> 00:14:34,079

unless they have their aerobic partners

321

00:14:40,610 --> 00:14:38,850

um so this pit right here definitely

322

00:14:43,670 --> 00:14:40,620

there's some interesting things going on

323

00:14:45,079 --> 00:14:43,680

and I don't really have too much else to

324

00:14:47,870 --> 00:14:45,089

say about that other than there's some

325

00:14:50,000 --> 00:14:47,880

evidence that it's happening um but I

326

00:14:51,440 --> 00:14:50,010

don't really know too much more until I

327

00:14:55,940 --> 00:14:51,450

dig a little bit deeper into this story

328

00:14:59,210 --> 00:14:55,950

um okay so who are the microbial

329

00:15:03,620 --> 00:14:59,220

community members so this is our total

330

00:15:06,350 --> 00:15:03,630

16s sequencing of all of the archaea

331

00:15:08,750 --> 00:15:06,360

unity members and from sample to sample

332

00:15:12,350 --> 00:15:08,760

layer to layer it all pretty much looked

333

00:15:15,530 --> 00:15:12,360

like this and so it's mainly dominated

334

00:15:18,350 --> 00:15:15,540

by hey Leo Beck bye halo bacteria AC II

335

00:15:21,290 --> 00:15:18,360

which isn't too surprising they're from

336

00:15:24,410 --> 00:15:21,300

Salt Flats so layer 1 to layer 3 you

337

00:15:26,900 --> 00:15:24,420

just get a bunch of halo back t racy and

338

00:15:29,600 --> 00:15:26,910

these are the heterotrophic halo archaea

339

00:15:32,069 --> 00:15:29,610

that we just heard to talk about

340

00:15:33,600 --> 00:15:32,079

except they're not cold-adapted they're

341

00:15:37,350 --> 00:15:33,610

kind of hanging out in normal

342

00:15:40,980 --> 00:15:37,360

temperatures okay so I looked at the

343

00:15:42,059 --> 00:15:40,990

similarity between these samples um even

344

00:15:43,319 --> 00:15:42,069

though they kind of looked all the same

345

00:15:45,989 --> 00:15:43,329

when I just look at who's there you can

346

00:15:47,819 --> 00:15:45,999

this is an MDS plot and so the Axis

347

00:15:49,379 --> 00:15:47,829

don't really matter too much they're

348

00:15:51,110 --> 00:15:49,389

largely arbitrary it's sort of like a

349

00:15:53,730 --> 00:15:51,120

PCA plot if you're familiar with that

350

00:15:55,590 --> 00:15:53,740

and it's really just the distance

351  
00:15:57,780 --> 00:15:55,600  
between each of these points that have

352  
00:15:59,429 --> 00:15:57,790  
any meaning on here and so some

353  
00:16:02,129 --> 00:15:59,439  
interesting trends sort of come out when

354  
00:16:04,590 --> 00:16:02,139  
you look at the Archaea unity in this

355  
00:16:09,090 --> 00:16:04,600  
way and you can see all of these circles

356  
00:16:11,009 --> 00:16:09,100  
on here are layer 1 all of the triangles

357  
00:16:13,470 --> 00:16:11,019  
on here are layer 2 and all of the

358  
00:16:15,660 --> 00:16:13,480  
squares are layer 3 so you kind of have

359  
00:16:18,019 --> 00:16:15,670  
like this general shift in your

360  
00:16:21,299 --> 00:16:18,029  
microbial community as you go down and

361  
00:16:23,040 --> 00:16:21,309  
just to kind of show you I don't have

362  
00:16:25,259 --> 00:16:23,050  
these labeled I just told you what was

363  
00:16:27,150 --> 00:16:25,269

in there these are all just down to you

364

00:16:30,179 --> 00:16:27,160

the genus level different halo bacteria

365

00:16:32,040 --> 00:16:30,189

a see um but it's not really shifting

366

00:16:33,629 --> 00:16:32,050

in who's there it's just the abundances

367

00:16:34,910 --> 00:16:33,639

that are shifting as we go down in

368

00:16:41,160 --> 00:16:34,920

layers

369

00:16:43,829 --> 00:16:41,170

okay so bacteria so bacteria mostly

370

00:16:45,720 --> 00:16:43,839

Salona bacter and some unclassified

371

00:16:47,249 --> 00:16:45,730

bacteria where the 16s sequences didn't

372

00:16:50,160 --> 00:16:47,259

really line up to anything in the

373

00:16:52,079 --> 00:16:50,170

database but it's pretty much dominated

374

00:16:54,030 --> 00:16:52,089

by cilona vector again not too

375

00:16:56,699 --> 00:16:54,040

surprising we would completely expect

376

00:16:58,199 --> 00:16:56,709

this um you can't see it too well but we

377

00:17:00,360 --> 00:16:58,209

have some sulfate reducers right here

378

00:17:03,480 --> 00:17:00,370

and we do actually find some sea urchins

379

00:17:05,059 --> 00:17:03,490

the acetyl Darma right here um oh I

380

00:17:07,860 --> 00:17:05,069

forgot to point it out on the air keel

381

00:17:10,559 --> 00:17:07,870

graph but we did find a tiny amount of

382

00:17:13,199 --> 00:17:10,569

methanogens um so this is really

383

00:17:15,569 --> 00:17:13,209

interesting because as I was hoping we

384

00:17:18,569 --> 00:17:15,579

do have an environment where a citizen's

385

00:17:22,079 --> 00:17:18,579

methanogens and sulfate reducers are all

386

00:17:24,569 --> 00:17:22,089

coexisting and so I want to dig into a

387

00:17:26,460 --> 00:17:24,579

little bit deeper why they're not out

388

00:17:29,130 --> 00:17:26,470

competing each other and why they're all

389

00:17:33,060 --> 00:17:29,140

able to coexist in these sort of lower

390

00:17:34,350 --> 00:17:33,070

abundances in this community but I don't

391

00:17:36,299 --> 00:17:34,360

really have an answer to that yet that's

392

00:17:40,260 --> 00:17:36,309

future work we'll be looking into that a

393

00:17:41,860 --> 00:17:40,270

little bit more and so we can look at if

394

00:17:43,870 --> 00:17:41,870

we have this same

395

00:17:45,070 --> 00:17:43,880

shift that's going on in the bacterial

396

00:17:47,590 --> 00:17:45,080

community like we did with the archaea

397

00:17:51,970 --> 00:17:47,600

unity and again we have circles our

398

00:17:56,019 --> 00:17:51,980

layer one and the triangles or layer two

399

00:17:58,210 --> 00:17:56,029

and the squares are layer three and so

400

00:17:59,830 --> 00:17:58,220

again we see sort of a little bit more

401  
00:18:01,870 --> 00:17:59,840  
muddled than the layer two and layer 3

402  
00:18:04,120 --> 00:18:01,880  
with the bacterial sequences but there

403  
00:18:07,690 --> 00:18:04,130  
is still like a general shift sort of a

404  
00:18:10,080 --> 00:18:07,700  
way from layer 1 2 as you go deeper into

405  
00:18:15,639 --> 00:18:10,090  
the more anaerobic community members and

406  
00:18:19,090 --> 00:18:15,649  
here's another plot and so this is genus

407  
00:18:20,950 --> 00:18:19,100  
level again and it's just kind of

408  
00:18:23,560 --> 00:18:20,960  
showing that it doesn't really change

409  
00:18:26,639 --> 00:18:23,570  
the same colors are between all of the

410  
00:18:29,260 --> 00:18:26,649  
sequences it's really just the sizes of

411  
00:18:30,880 --> 00:18:29,270  
what's there so the abundances of the

412  
00:18:33,070 --> 00:18:30,890  
bacterial community are changing not

413  
00:18:35,500 --> 00:18:33,080

exactly the individual sequences

414

00:18:37,930 --> 00:18:35,510

themselves ok

415

00:18:40,360 --> 00:18:37,940

so lastly I'm going to talk a little bit

416

00:18:42,760 --> 00:18:40,370

at how I looked at the human impact on

417

00:18:47,049 --> 00:18:42,770

these microbial communities so I

418

00:18:50,200 --> 00:18:47,059

classified all of the all of the pits as

419

00:18:54,430 --> 00:18:50,210

such these ones are on the racetrack and

420

00:18:58,240 --> 00:18:54,440

these ones were off the racetrack and so

421

00:19:00,250 --> 00:18:58,250

then you can look at this you look at

422

00:19:03,399 --> 00:19:00,260

this all using a multivariate analysis

423

00:19:05,769 --> 00:19:03,409

and the everything so if you cut it off

424

00:19:09,730 --> 00:19:05,779

at zero right here every little dot

425

00:19:14,409 --> 00:19:09,740

represents a sequence and all of these

426

00:19:17,649 --> 00:19:14,419

ones that are negative belong to one of

427

00:19:20,909 --> 00:19:17,659

those pits that were on the racetrack

428

00:19:26,139 --> 00:19:20,919

whereas all of these ones up here are

429

00:19:29,970 --> 00:19:26,149

sequences from those pits that were off

430

00:19:33,240 --> 00:19:29,980

the racetrack and so I did this using

431

00:19:35,860 --> 00:19:33,250

sequences from each layer so I split I

432

00:19:37,450 --> 00:19:35,870

go back one so I split all of the

433

00:19:39,580 --> 00:19:37,460

sequences from these pits up into layer

434

00:19:43,240 --> 00:19:39,590

1 layer 2 and layer 3 and did the same

435

00:19:45,610 --> 00:19:43,250

thing layer 1 and 3 didn't have anything

436

00:19:48,850 --> 00:19:45,620

that was significant so these ones in

437

00:19:52,000 --> 00:19:48,860

red right here are the only sequences

438

00:19:54,120 --> 00:19:52,010

that are significantly different from

439

00:19:57,990 --> 00:19:54,130

any sequences up here

440

00:20:01,770 --> 00:19:58,000

um so then all of these red dots right

441

00:20:06,180 --> 00:20:01,780

here indicate a sequence from some

442

00:20:08,400 --> 00:20:06,190

microbe that is similar to everything

443

00:20:13,830 --> 00:20:08,410

that is off the racetrack that isn't

444

00:20:17,550 --> 00:20:13,840

really um on the racetrack so then you

445

00:20:20,640 --> 00:20:17,560

can say what are those sequences and um

446

00:20:24,210 --> 00:20:20,650

this isn't really that exciting it's

447

00:20:37,680 --> 00:20:24,220

just halo back to here AC again um which

448

00:20:39,300 --> 00:20:37,690

is an archaeological primers aren't

449

00:20:41,910 --> 00:20:39,310

really acting in a domain-specific way

450

00:20:43,320 --> 00:20:41,920

there's known issues with that I have to

451  
00:20:45,030 --> 00:20:43,330  
look into this a little bit more and

452  
00:20:47,520 --> 00:20:45,040  
actually go in and pull out these exact

453  
00:20:48,960 --> 00:20:47,530  
sequences and see if they where they

454  
00:20:51,300 --> 00:20:48,970  
line up to the primers and see if

455  
00:20:53,400 --> 00:20:51,310  
they're actually were hitting the primer

456  
00:20:56,610 --> 00:20:53,410  
of it's some like weird sequencing error

457  
00:20:57,480 --> 00:20:56,620  
um okay so anyways I don't have too much

458  
00:21:02,520 --> 00:20:57,490  
to say about that

459  
00:21:03,840 --> 00:21:02,530  
other than that we found a little bit of

460  
00:21:05,520 --> 00:21:03,850  
differences that might indicate human

461  
00:21:08,250 --> 00:21:05,530  
impact and I'm going to look into it a

462  
00:21:09,750 --> 00:21:08,260  
little bit further as well as do some of

463  
00:21:12,030 --> 00:21:09,760

these other things like make some more

464

00:21:14,970 --> 00:21:12,040

comparisons to are there differences in

465

00:21:16,950 --> 00:21:14,980

these pits in mineralogy elemental

466

00:21:19,590 --> 00:21:16,960

concentrations like heavy metals and

467

00:21:21,390 --> 00:21:19,600

sulfur as I mentioned before we're going

468

00:21:24,930 --> 00:21:21,400

to metagenomic Li sequence some of these

469

00:21:26,580 --> 00:21:24,940

samples to sort of get an idea of what

470

00:21:29,640 --> 00:21:26,590

genes are present and link that back to

471

00:21:31,590 --> 00:21:29,650

the incubation data and then I have some

472

00:21:33,330 --> 00:21:31,600

additional headspace results that are

473

00:21:36,810 --> 00:21:33,340

going to come in from all of those

474

00:21:38,460 --> 00:21:36,820

incubations and I'm going to do the

475

00:21:42,480 --> 00:21:38,470

Raman single cell sorting of these

476  
00:21:45,480 --> 00:21:42,490  
samples and then right now or earlier

477  
00:21:47,160 --> 00:21:45,490  
this week I guess um my lab was going

478  
00:21:49,650 --> 00:21:47,170  
out and sampling wells that are located

479  
00:21:51,450 --> 00:21:49,660  
on the Salt Flats so we have shallow

480  
00:21:53,040 --> 00:21:51,460  
wells and deep wells and we'll be

481  
00:21:54,780 --> 00:21:53,050  
sequencing those and sort of comparing

482  
00:21:57,450 --> 00:21:54,790  
them to the surface communities to see

483  
00:21:59,670 --> 00:21:57,460  
if we can see if some of the shallow

484  
00:22:03,420 --> 00:21:59,680  
wells in the surface communities share

485  
00:22:05,130 --> 00:22:03,430  
similarities um and with that I would

486  
00:22:07,800 --> 00:22:05,140  
like to acknowledge my lab the browser

487  
00:22:10,440 --> 00:22:07,810  
sin lab um as well as our clabber

488  
00:22:14,220 --> 00:22:10,450

readers in the geology department Brenda

489

00:22:16,800 --> 00:22:14,230

Bowen and Betsy Club WA who was the lead

490

00:22:20,010 --> 00:22:16,810

microbiologist who was at the University

491

00:22:30,110 --> 00:22:20,020

of Westminster but has since changed to

492

00:22:36,960 --> 00:22:34,290

what kind Oh what kind of salt is on the

493

00:22:39,000 --> 00:22:36,970

on this salt flat is it just mostly

494

00:22:40,800 --> 00:22:39,010

sodium chloride or I think it's got more

495

00:22:43,830 --> 00:22:40,810

magnesium than sodium in it

496

00:22:50,850 --> 00:22:43,840

I think magnesium is more than potassium

497

00:22:53,340 --> 00:22:50,860

magnesium chloride or okay um real quick

498

00:22:55,470 --> 00:22:53,350

I looked at um it looked like the soil

499

00:22:57,030 --> 00:22:55,480

below the salt was really dark is with

500

00:22:59,130 --> 00:22:57,040

is that true when you're there is it

501  
00:23:01,080 --> 00:22:59,140  
really dark yeah there's a lot of

502  
00:23:03,180 --> 00:23:01,090  
organics I guess that are below the salt

503  
00:23:04,920 --> 00:23:03,190  
yes so I'm really excited to get some of

504  
00:23:07,500 --> 00:23:04,930  
the mineral data back from the

505  
00:23:08,910 --> 00:23:07,510  
geologists um they have a undergrad

506  
00:23:11,220 --> 00:23:08,920  
who's gonna process all those all summer

507  
00:23:14,430 --> 00:23:11,230  
long for me um but yeah some of these

508  
00:23:16,440 --> 00:23:14,440  
pits you would dig down and you you

509  
00:23:18,900 --> 00:23:16,450  
would hit this like weird interface

510  
00:23:21,750 --> 00:23:18,910  
between two salt layers that was just

511  
00:23:23,370 --> 00:23:21,760  
really black and sulfur II smelling like

512  
00:23:25,430 --> 00:23:23,380  
all you just as soon as you hit it

513  
00:23:31,050 --> 00:23:25,440

you're just like oh god that's sulfur

514

00:23:35,340 --> 00:23:31,060

cool all right thank you all right we

515

00:23:37,610 --> 00:23:35,350

have time for one last question so were

516

00:23:40,230 --> 00:23:37,620

you able to remove the community members

517

00:23:44,520 --> 00:23:40,240

that were predominantly present on the

518

00:23:47,130 --> 00:23:44,530

racetrack from the rest of the data um I

519

00:23:50,760 --> 00:23:47,140

don't know if I understand let me go

520

00:23:54,360 --> 00:23:50,770

back here okay so from all of these guys

521

00:23:57,420 --> 00:23:54,370

right cuz you would assume that those

522

00:23:59,850 --> 00:23:57,430

are due to human impacts well the

523

00:24:04,230 --> 00:23:59,860

problem with this is that none of these

524

00:24:06,390 --> 00:24:04,240

were really significantly different from

525

00:24:09,330 --> 00:24:06,400

ones that were off the racetrack so it

526

00:24:12,060 --> 00:24:09,340

seemed like we had sequences that were

527

00:24:13,500 --> 00:24:12,070

different off the racetrack that weren't

528

00:24:18,270 --> 00:24:13,510

found on the racetrack so is the other

529

00:24:21,160 --> 00:24:18,280

way around got it okay thanks

530

00:24:28,140 --> 00:24:21,170

all right thank you very much