Community Structure in Contaminated Habitats: the dynamic tension between selective forces and environmental heterogeneity

Allan Konopka and Cindy Nakatsu
Purdue University

Analysis of bacterial diversity

How diverse should microbial communities be?

"Everything is everywhere, the environment selects"

"Niche-assembly perspective"

Ecological communities are limited-membership assemblages that coexist at <u>equilibrium</u> under strict <u>niche partitioning</u> of limiting resources

Gause's Principle of Competitive Exclusion:

What constitutes a microbe's "niche?" Individual organic substrate? (But microbes often simultaneously utilize >1 substrate)

Analysis of bacterial diversity

How diverse should microbial communities be?

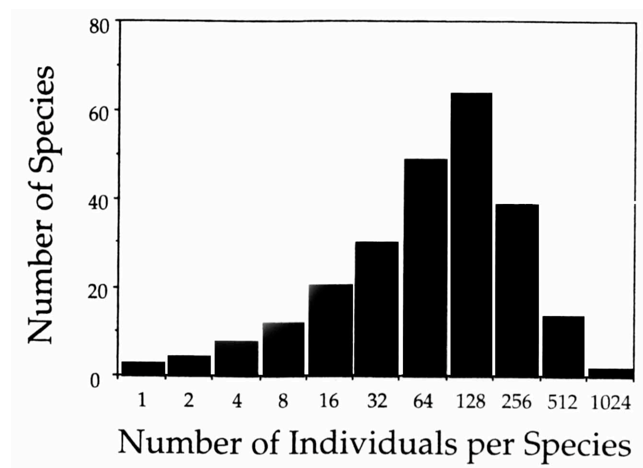
“Dispersal-assembly perspective”

Ecological communities are open, nonequilibrium assemblages of species. The dynamics are governed by random speciation and dispersal, ecological drift, and extinction.

Macarthur and Wilson: Island biogeography theory

Stephen Hubbell. (2001) *Unified Neutral Theory of Biodiversity and Biogeography*. Princeton University Press

Theory is modified by including speciation, and assuming neutrality operates at individual level rather than species level

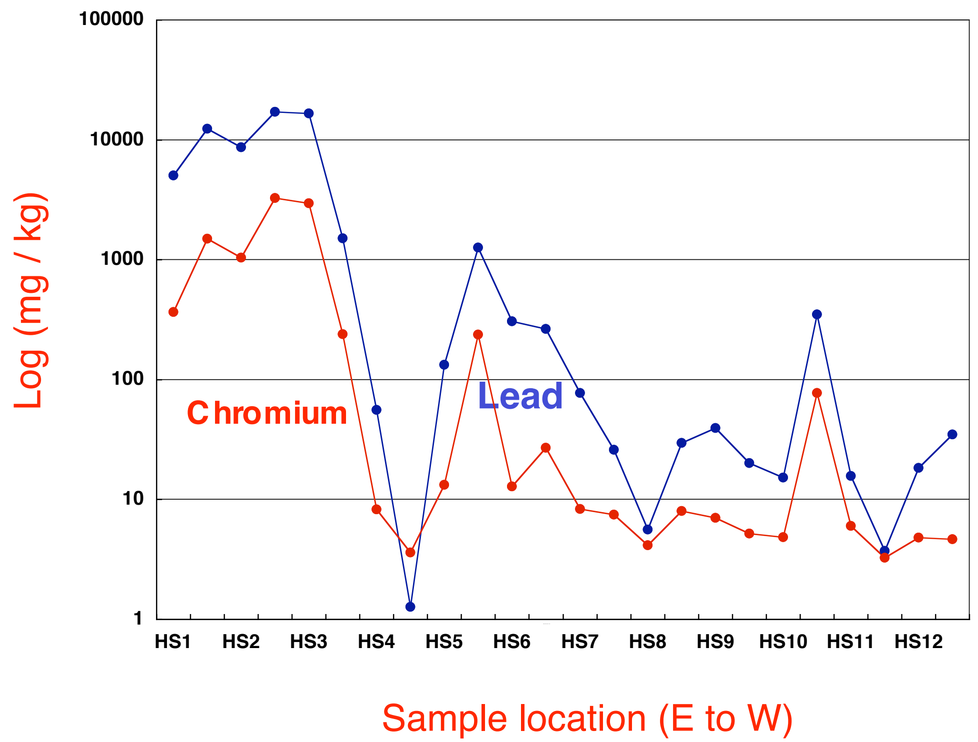


Factors that impact community diversity

- Spatial scale of environmental heterogeneity
- Organic C and Cr(VI) as selective forces
- “Microdiversity” of *Arthrobacter*

Seymour IN Site characteristics

- Site contaminated with Pb, Cr, and hydrocarbons (toluene) in 1960s
- No vegetation cover – little input of natural organic C
- On scale of 20 m, Pb and Cr concentrations change 2-3 orders of magnitude



Arthrobacter FB24 – metal resistance

Metal	Media	MIC
Cr(VI)	1/10 Nutrient Broth	300mM
As (III)	MXBM*, pH=8.0	5mM
As (V)	MXBM*, pH=8.0	250mM
Ni^{+2}	MXBM*, pH=6.0	750 μM
Cd^{+2}	MXBM*, pH=6.0	500 μM
Zn^{+2}	MXBM*, pH=6.0	500 μM
Pb^{+2}	MXBM*, pH=6.0	200 μM
Cu^{+2}	MXBM*, pH=6.0	1mM
Mn^{+2}	MXBM*, pH=6.0	100mM

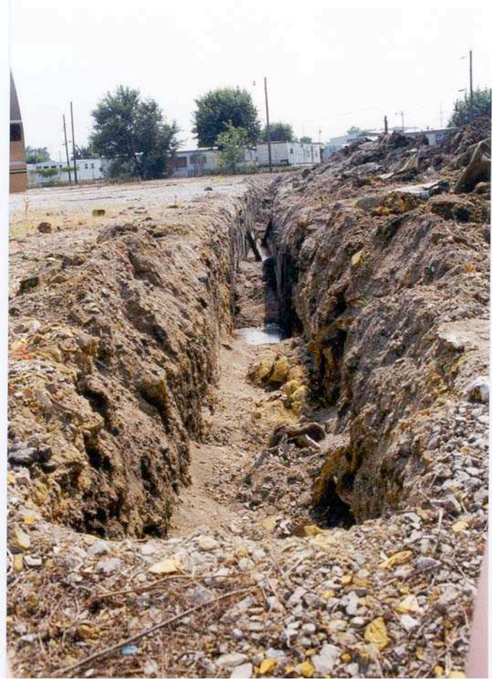
MXBM*: Modified XBM, glycerophosphate (10mM) as phosphate source and glucose (1.7mM) as carbon source

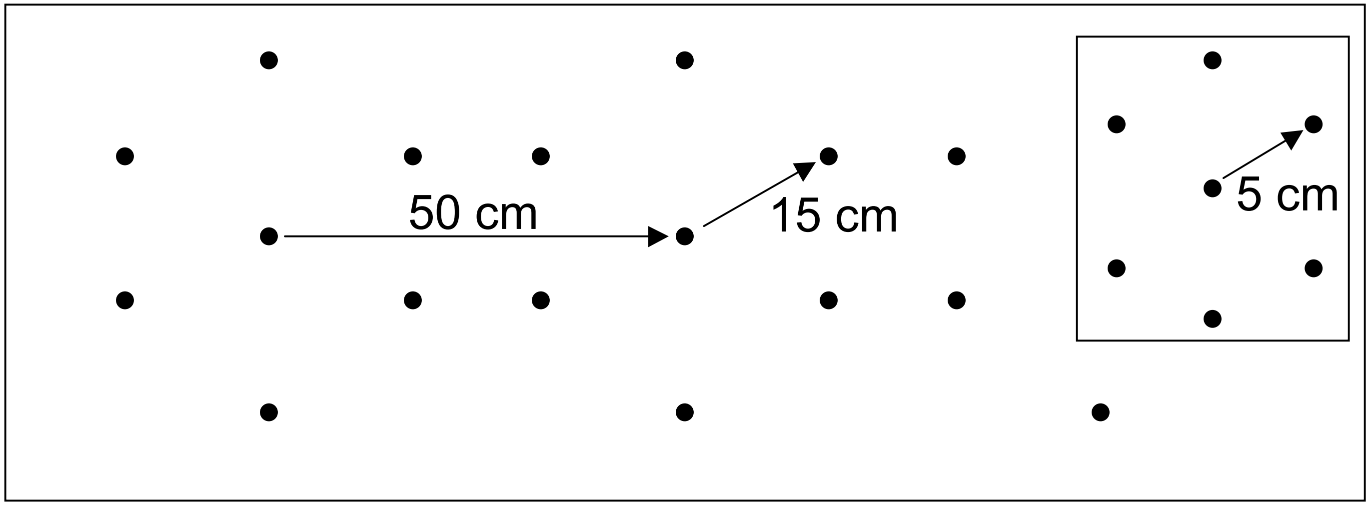
What is the spatial scale of heterogeneity?
A geostatistical analysis

Sampling strategy from face of trench:

- Five arrays (centered 50 cm from each other)
- Each array contains 6 sub-arrays (15 cm from center)
- Each sub-array contains 6 loci (5 cm from sub-array center)
- Three samples (< 1 cm distant) from each sampling locus.

Total: 635 samples





Sequential sample analyses

Performed on triplicate samples from spatial arrays

Soil sample (150 mg)

Microbial activity
Add 0.25 μCi ^{14}C-glucose
Incubate 4 h

Measure $^{14}CO_2$

Community structure
Extract DNA with QBIOGene Fast DNA Spin Kit

DGGE of 16S rDNA PCR amplicons.

Extractable Metal
Nitric acid digestion

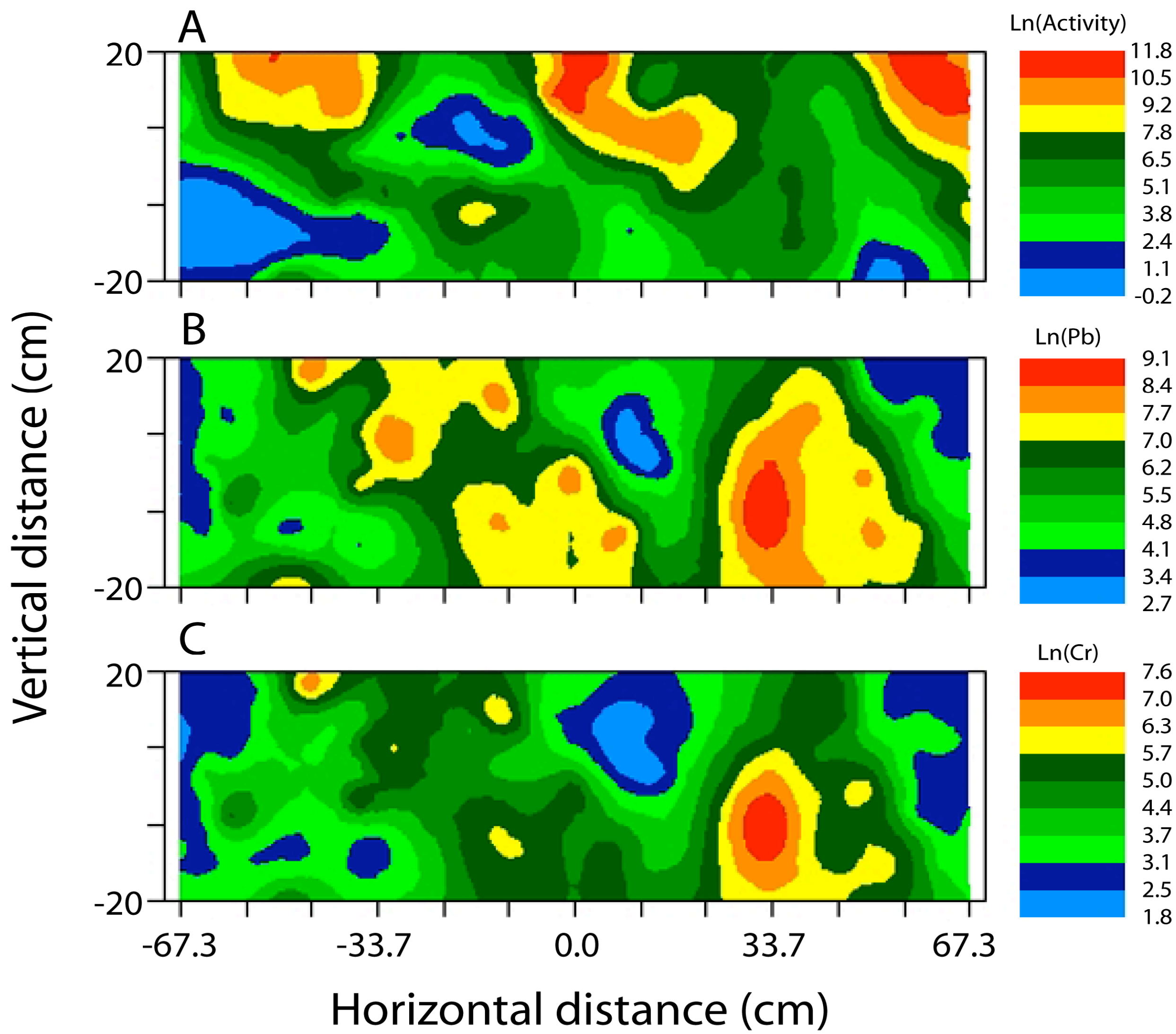
ICP-AES analysis of Pb and Cr

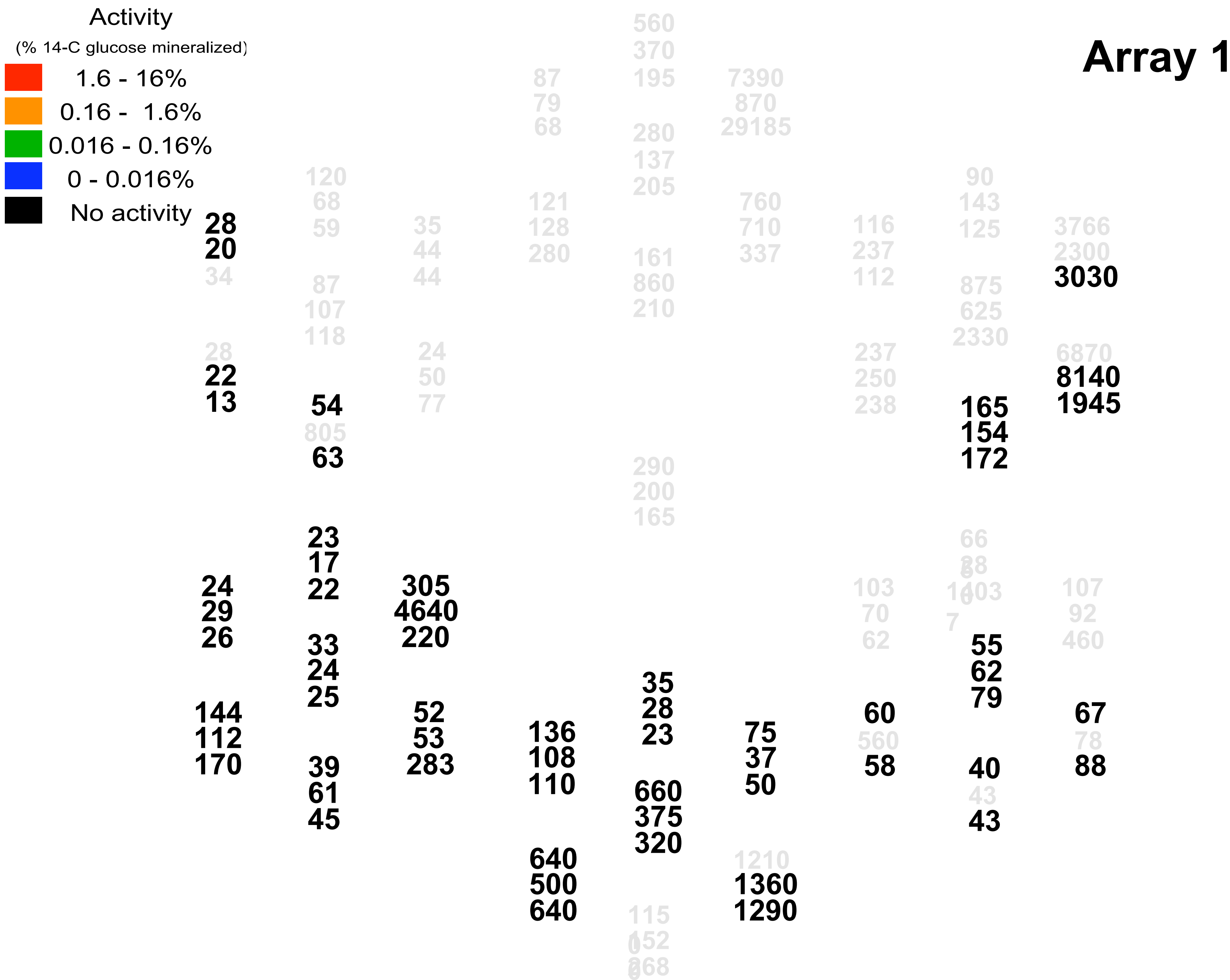
Geostatistical analysis – Block Kriging

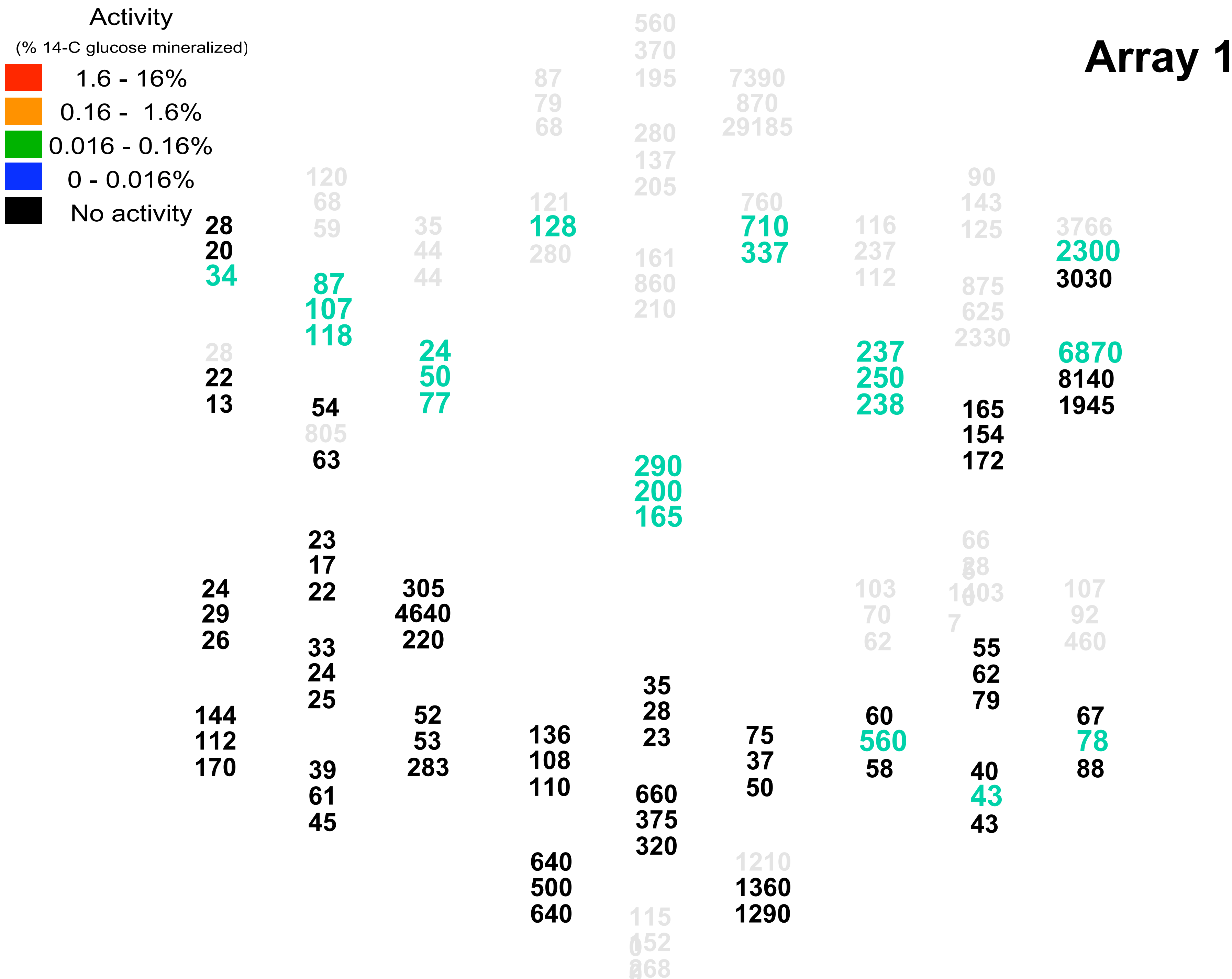
Metabolic Activity

Lead

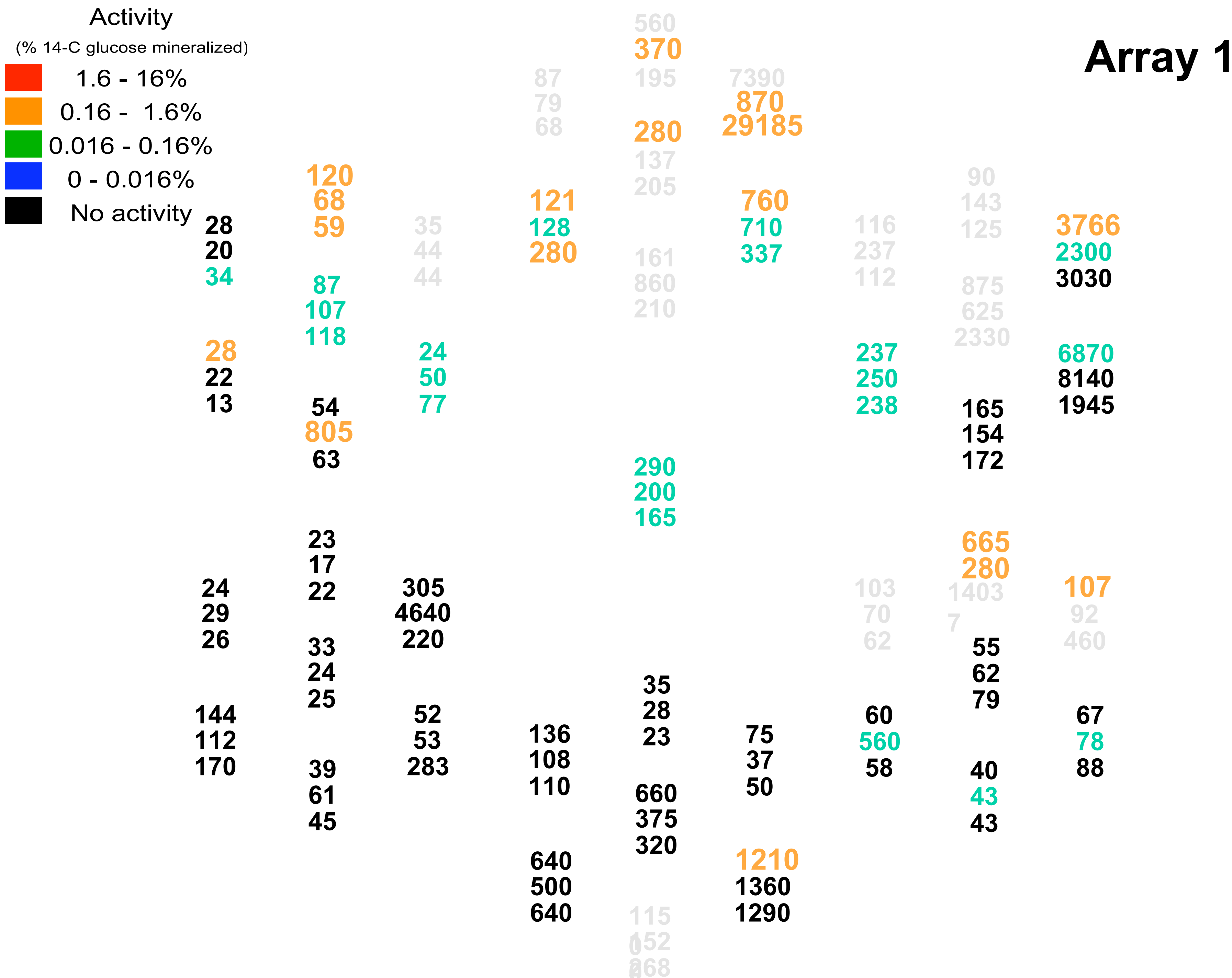
Chromium

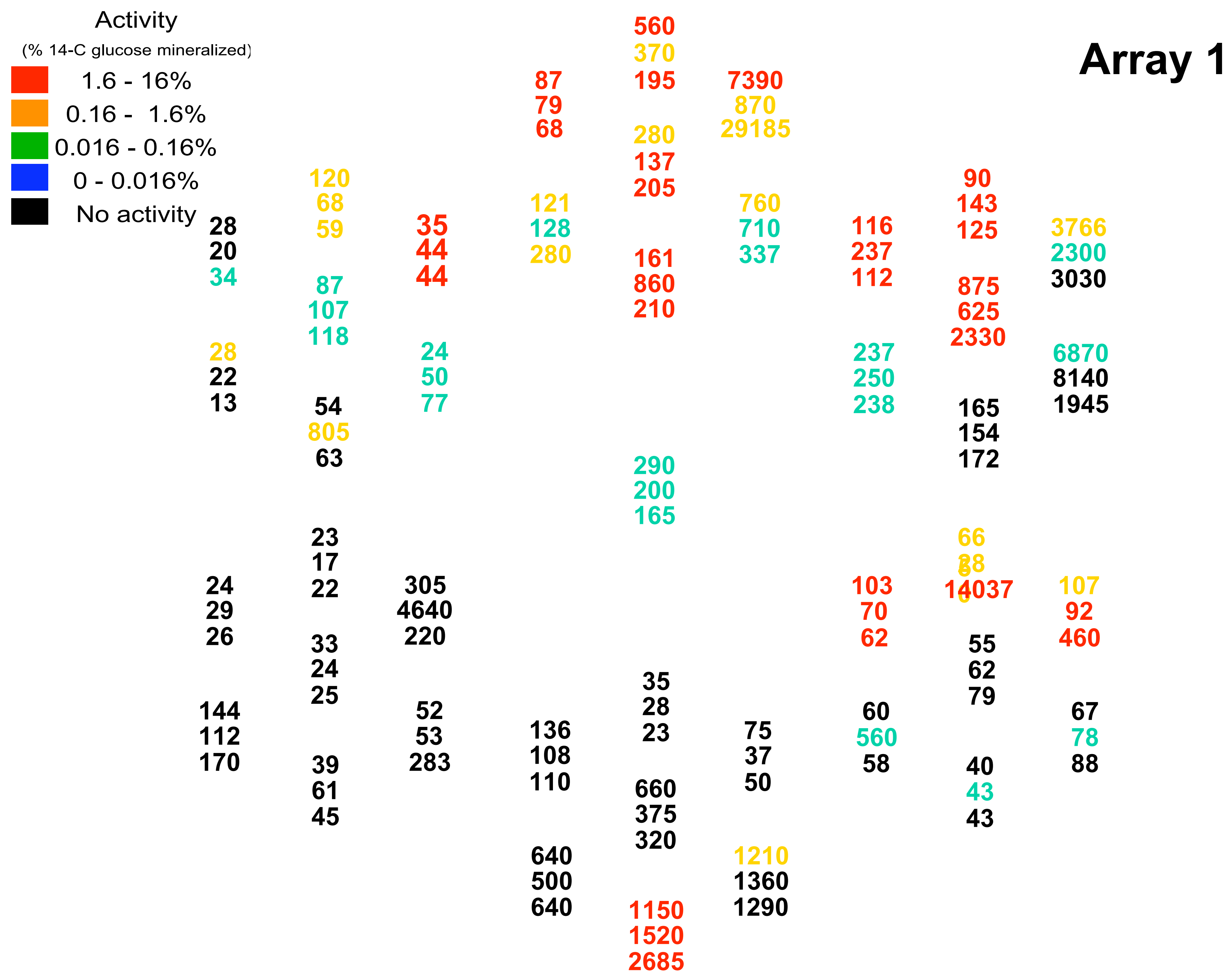



Activity
(% 14-C glucose mineralized)
1.6 - 16%
0.16 - 1.6%
0.016 - 0.16%
0 - 0.016%
No activity
Array 1
28 20 34
28 22 13
24 29 26
144 112 170
120 68 59
87 107 118
54 805 63
23 17 22
33 24 25
39 61 45
35 44 44
24 50 77
305 4640 220
52 53 283
87 79 68
121 128 280
136 108 110
640 500 640
560 370 195
280 137 205
161 860 210
290 200 165
35 28 23
660 375 320
115
7390 870 29185
760 710 337
75 37 50
1210 1360 1290
116 237 112
237 250 238
103 70 62
60 560 58
90 143 125
875 625 2330
165 154 172
66
55 62 79
40 43 43
3766 2300 3030
6870 8140 1945
107 92 460
67 78 88



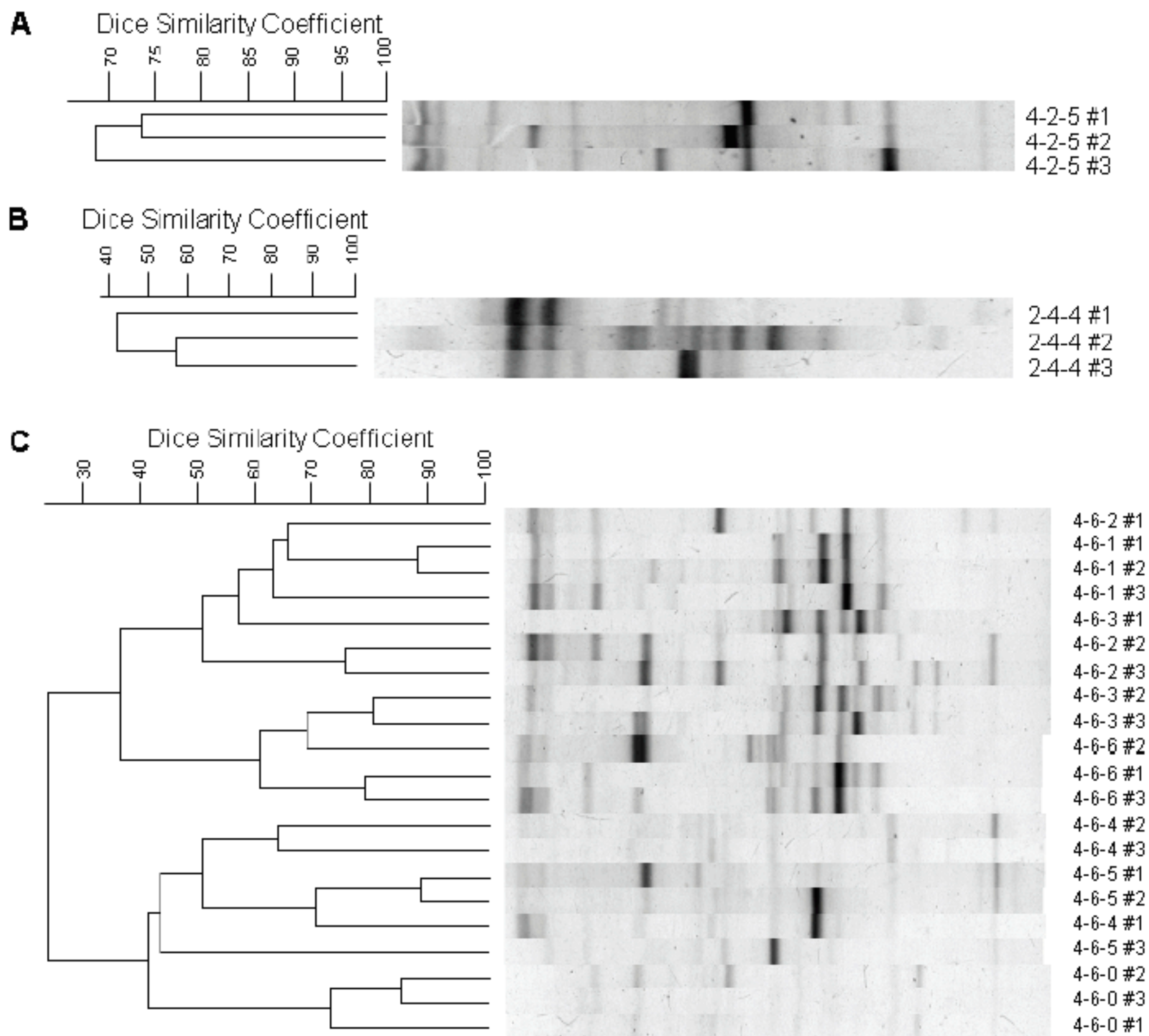
Activity
(% 14-C glucose mineralized)
1.6 - 16%
0.16 - 1.6%
0.016 - 0.16%
0 - 0.016%
No activity
Array 1
28 20 34
120 68 59
87 107 118
35 44 44
24 50 77
28 22 13
54 805 63
87 79 68
121 128 280
560 370 195
280 137 205
161 860 210
290 200 165
7390 870 29185
760 710 337
116 237 112
237 250 238
90 143 125
875 625 2330
165 154 172
3766 2300 3030
6870 8140 1945
24 29 26
23 17 22
33 24 25
305 4640 220
144 112 170
39 61 45
52 53 283
136 108 110
640 500 640
35 28 23
660 375 320
115
75 37 50
1210 1360 1290
103 70 62
60 560 58
66
55 62 79
40 43 43
107 92 460
67 78 88


Activity
(% 14-C glucose mineralized)
1.6 - 16%
0.16 - 1.6%
0.016 - 0.16%
0 - 0.016%
No activity
Array 1
28
20
34
28
22
13
24
29
26
144
112
170
120
68
59
87
107
118
54
805
63
23
17
22
33
24
25
39
61
45
35
44
44
24
50
77
305
4640
220
52
53
283
87
79
68
121
128
280
136
108
110
640
500
640
560
370
195
280
137
205
161
860
210
290
200
165
35
28
23
660
375
320
115
152
868
7390
870
29185
760
710
337
75
37
50
1210
1360
1290
116
237
112
237
250
238
103
70
62
60
560
58
90
143
125
875
625
2330
165
154
172
665
280
1403
7
55
62
79
40
43
43
3766
2300
3030
6870
8140
1945
107
92
460
67
78
88

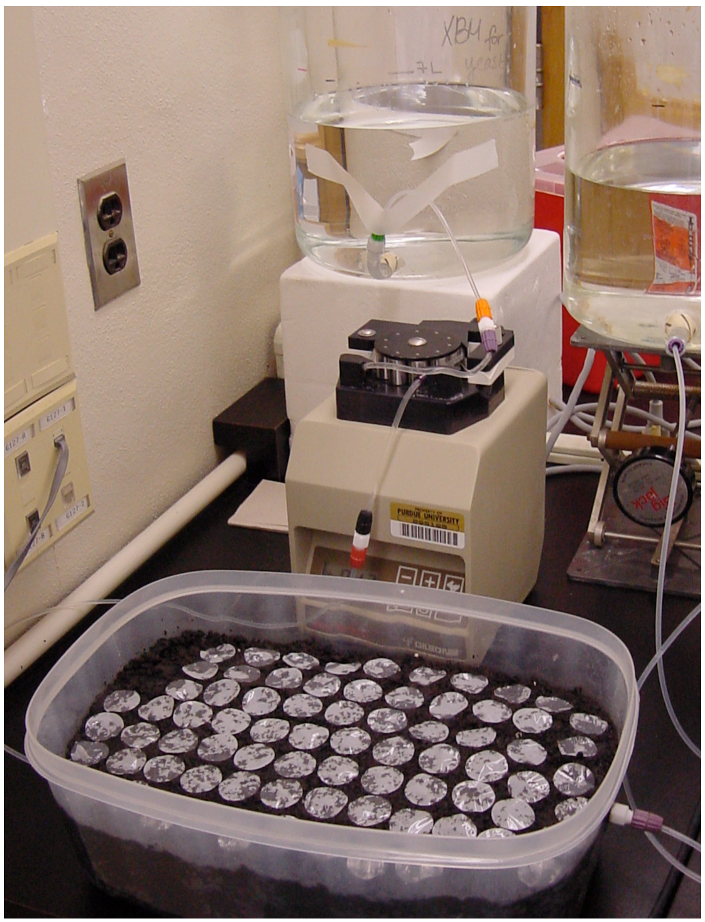


Array 1
Activity
(% 14-C glucose mineralized)
1.6 - 16%
0.16 - 1.6%
0.016 - 0.16%
0 - 0.016%
No activity
28 20 34
28 22 13
24 29 26
144 112 170
120 68 59
87 107 118
54 805 63
23 17 22
33 24 25
39 61 45
35 44 44
24 50 77
305 4640 220
52 53 283
87 79 68
121 128 280
136 108 110
640 500 640
560 370 195
280 137 205
161 860 210
290 200 165
35 28 23
660 375 320
1150 1520 2685
7390 870 29185
760 710 337
75 37 50
1210 1360 1290
116 237 112
237 250 238
103 70 62
60 560 58
90 143 125
875 625 2330
165 154 172
66 88 14037
55 62 79
40 43 43
3766 2300 3030
6870 8140 1945
107 92 460
67 78 88

Community composition at < 1 cm intervals



Loci with high proportions of Cr-resistant microbes?

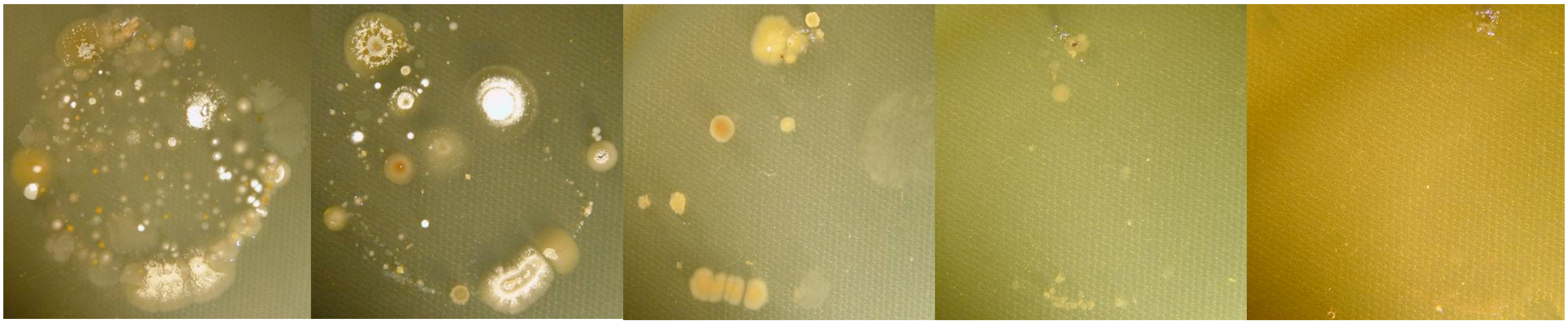


- Fifty soil samples (150 mg each) from
  - Forest soil (Ross Reserve)
  - Pb and Cr contaminated soil (Seymour site)
- Extract cells, deposit ca. 100 on filter
- Incubate in soil incubation chamber
  - Microcolonies form (4-8% of total cells)
- Replicate-plate filters onto nutrient agar with increasing concentrations of Cr(VI).

Comparison of Culturable Cr^R Microbes

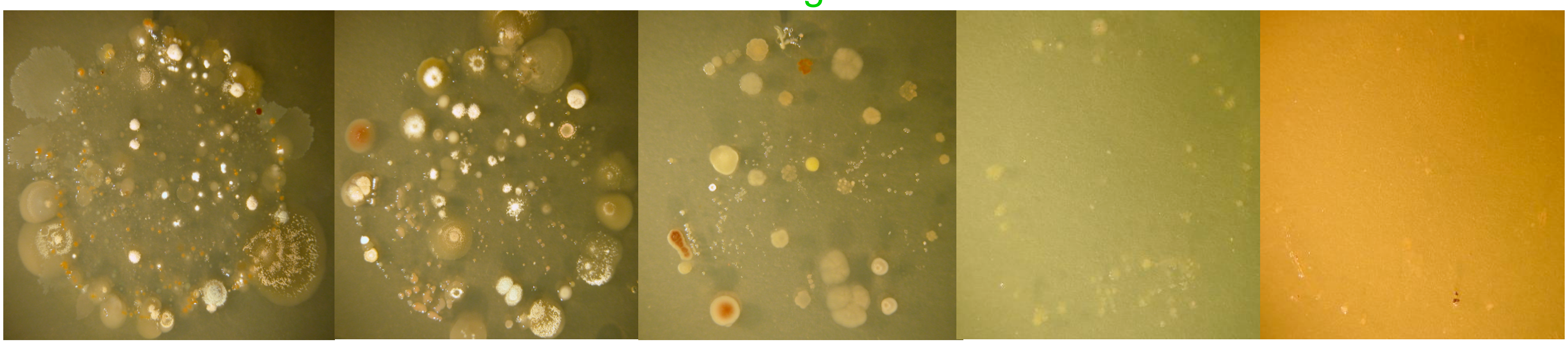
Rapid Decrease in total cfu's with Increasing Cr

Forest soil



Slowed Decrease in total cfu's with Increasing Cr

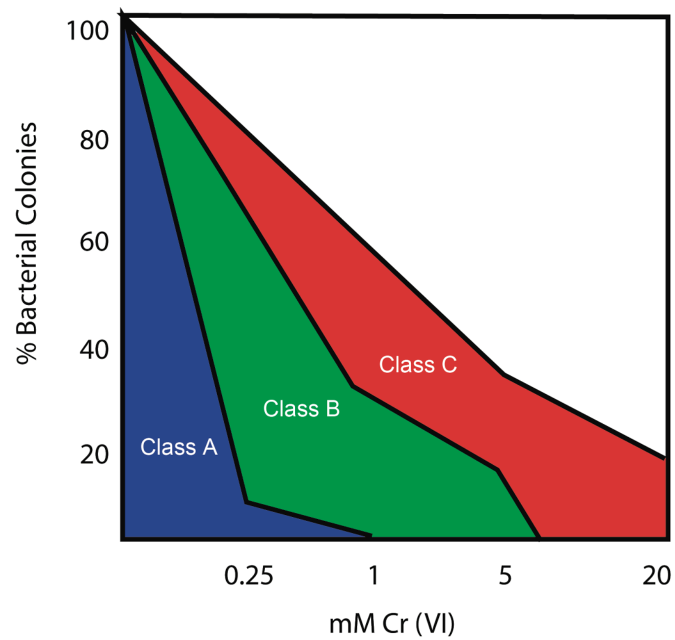
Contaminated site



0 mM Cr | 0.25 mM Cr | 1 mM Cr | 5 mM Cr | 20 mM Cr

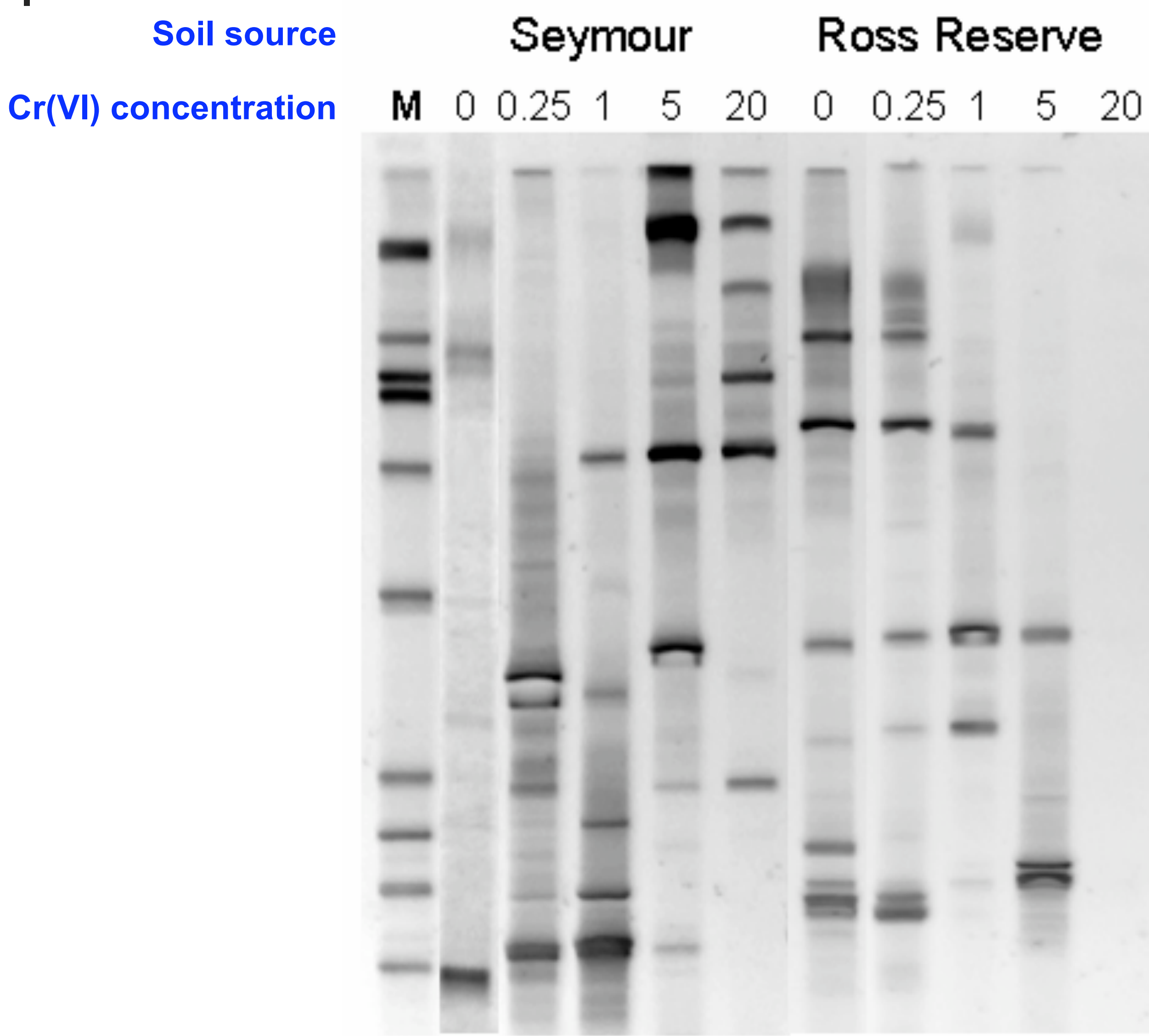
Localized foci of Cr resistant bacteria

Functional responses of communities to Cr (VI):



	% of Samples in functional class:		
Soil source	A	B	C
Seymour (contaminated)	36	48	16
Ross Reserve (pristine)	76	24	0

DGGE fingerprints from biomass on plates



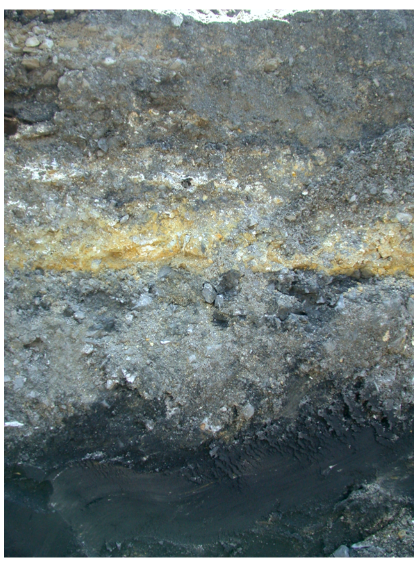
The impact of selective forces

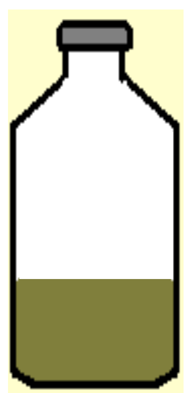
Microcosm experiments

Energy sources: glucose vs. xylene vs. protein

Terminal electron acceptors: O_2 vs. NO^{-3} vs. Fe^{+3}

Cr(VI): Acute inhibition of 50, 75 or 90%



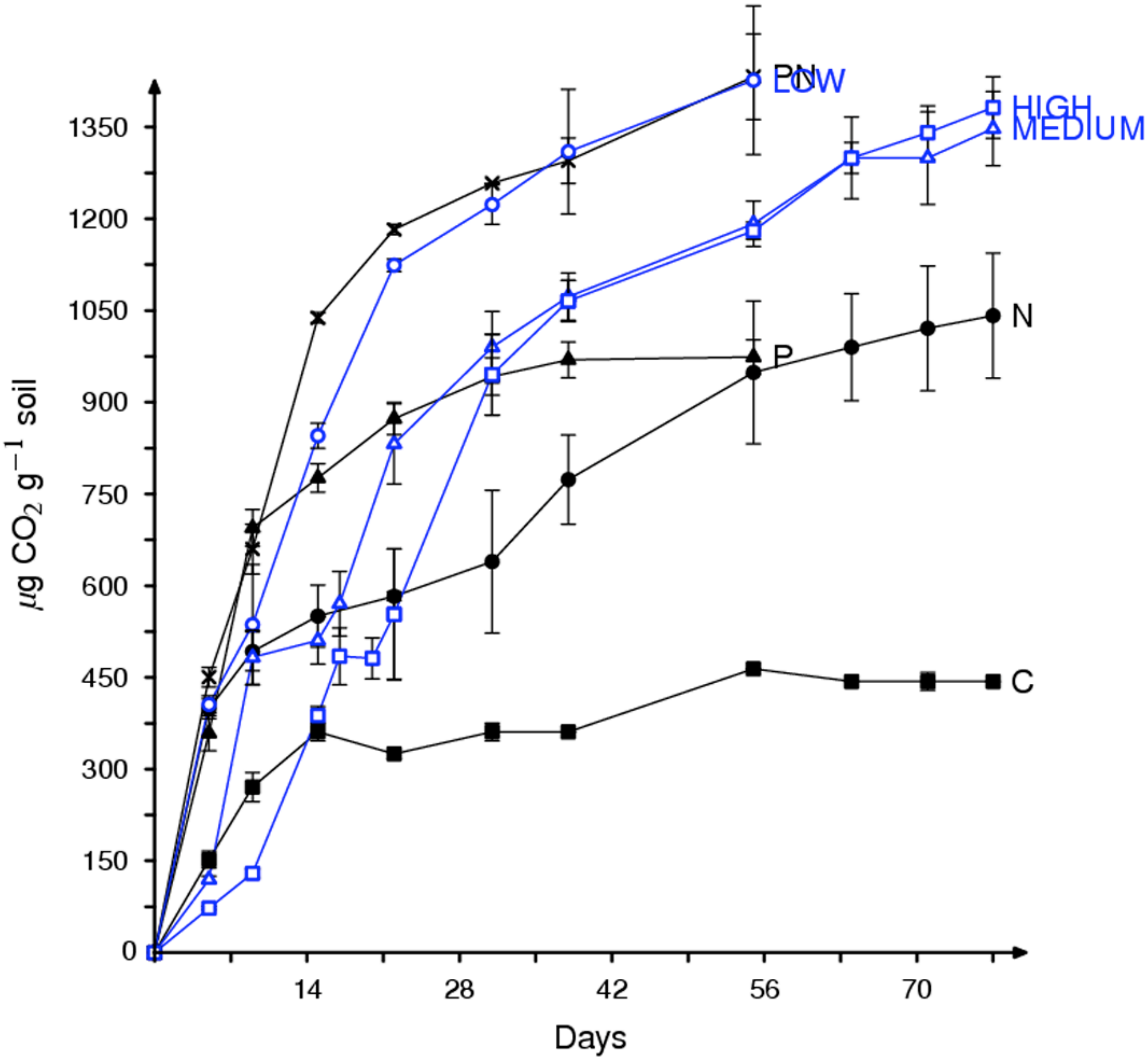


- 10 g soil
- Organic energy source – 30 mg
- Terminal electron acceptor
- Chromium

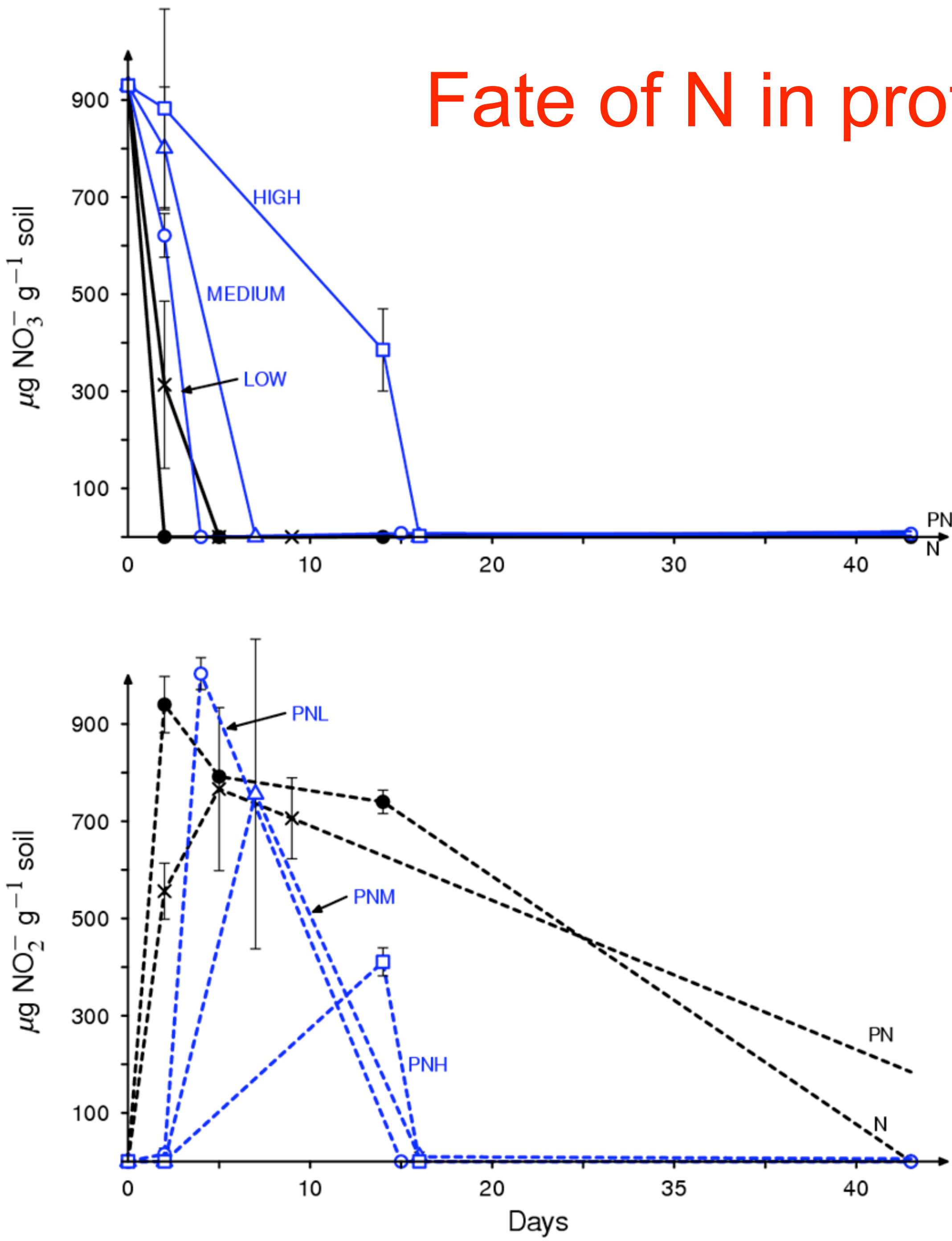
5 ml H_2O

The impact of selective forces – microcosm experiments

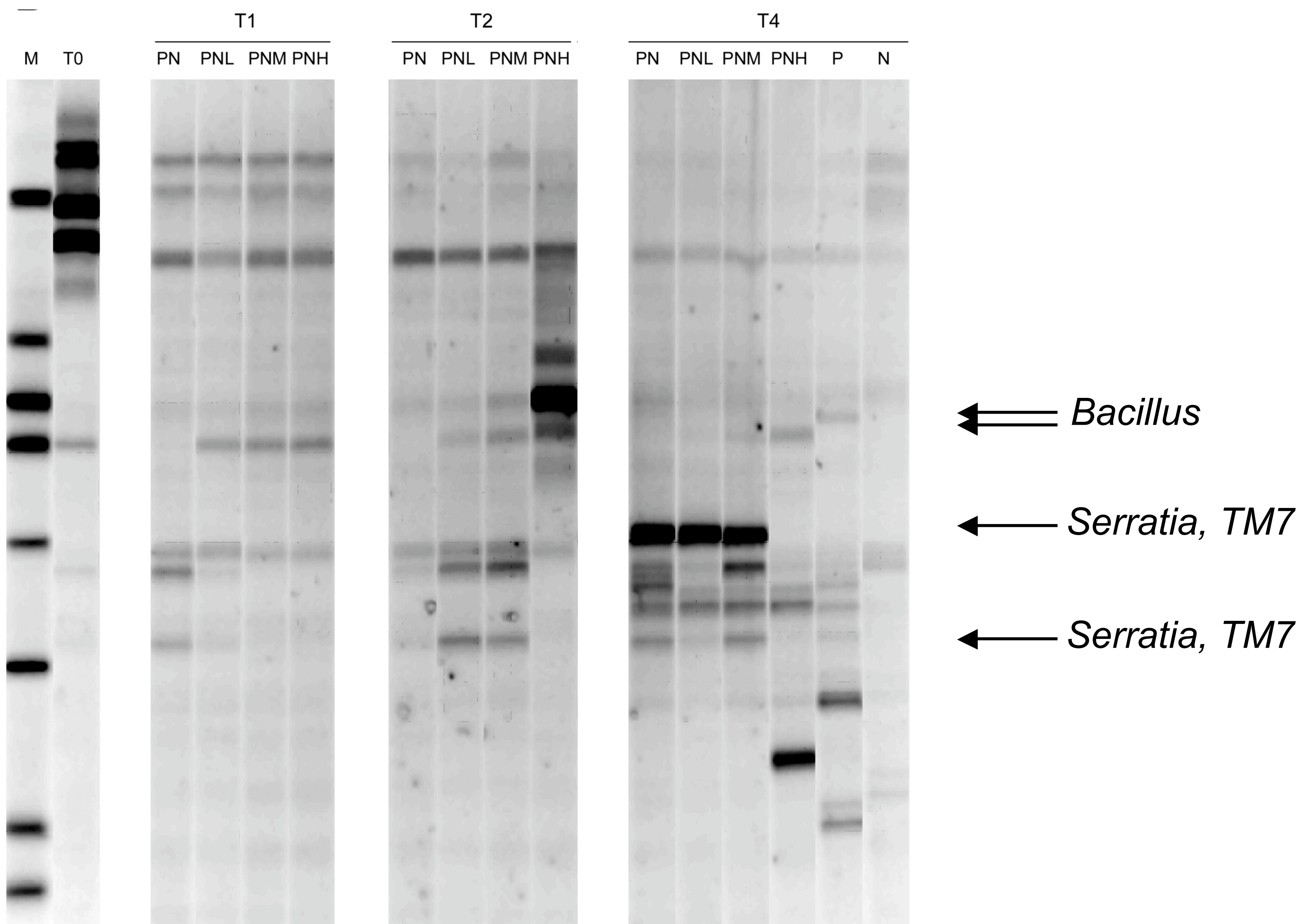
Protein / nitrate / Cr(VI) – CO_2 production



Fate of N in protein microcosms



DGGE profiles - protein



Physiological and genetic “microdiversity”

Cultures were isolated from an aerobic microcosm to which xylene and “high” level of Cr^{6+} was added

<u>Selection</u>:

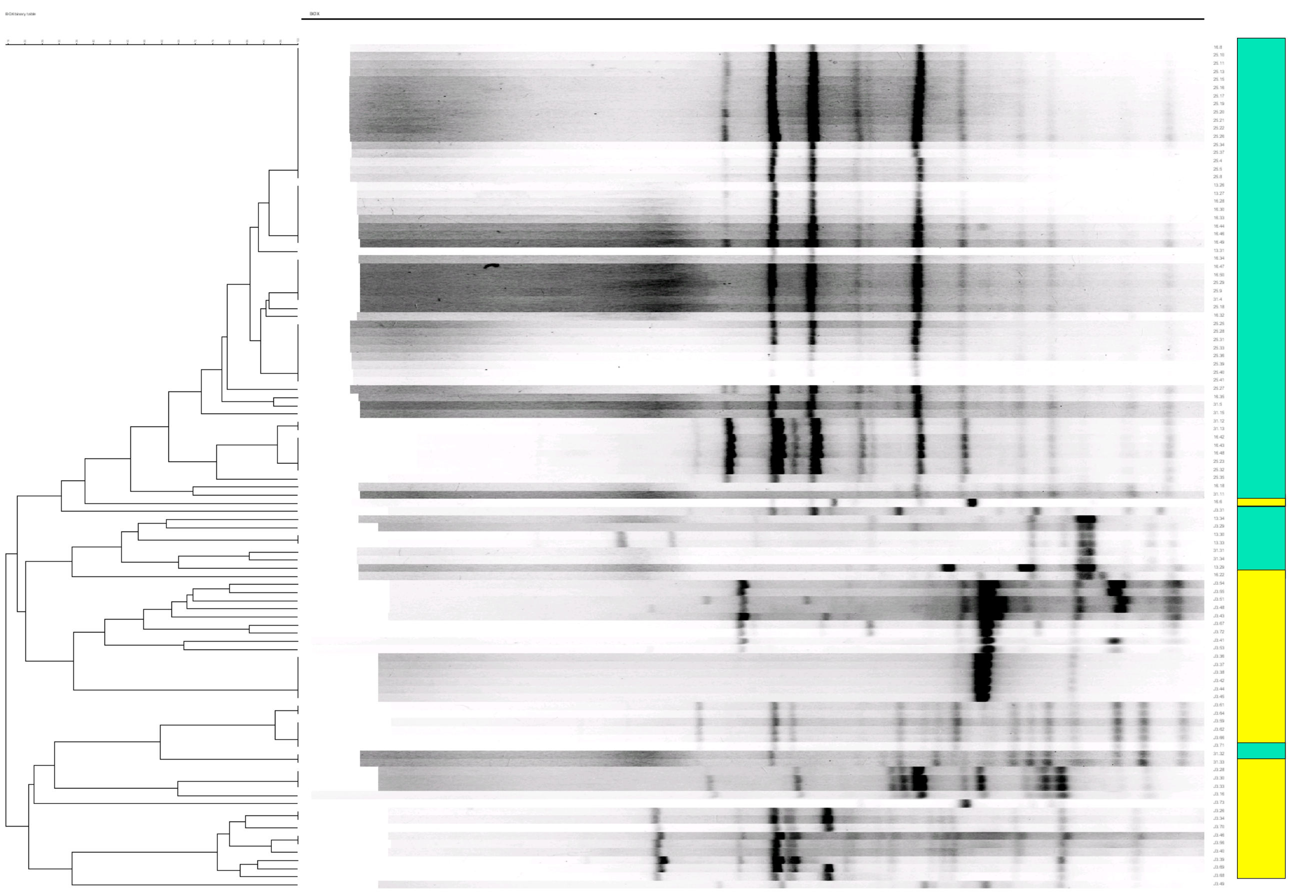
- 5 mM Cr^{6+} on complex media
- Xylene - mineral salts medium

<u>Results</u>:

- 38 Cr^{6+} resistant *Arthrobacter*
- 103 Xylene degraders
  - 66 *Arthrobacter*
  - 23 *Rhodococcus*
  - 14 *Pseudomonas*
- “0” Cr^{6+} resistant/xylene degraders

Physiological and genetic "microdiversity"

Rep-PCR of 104 *Arthrobacter* Isolates

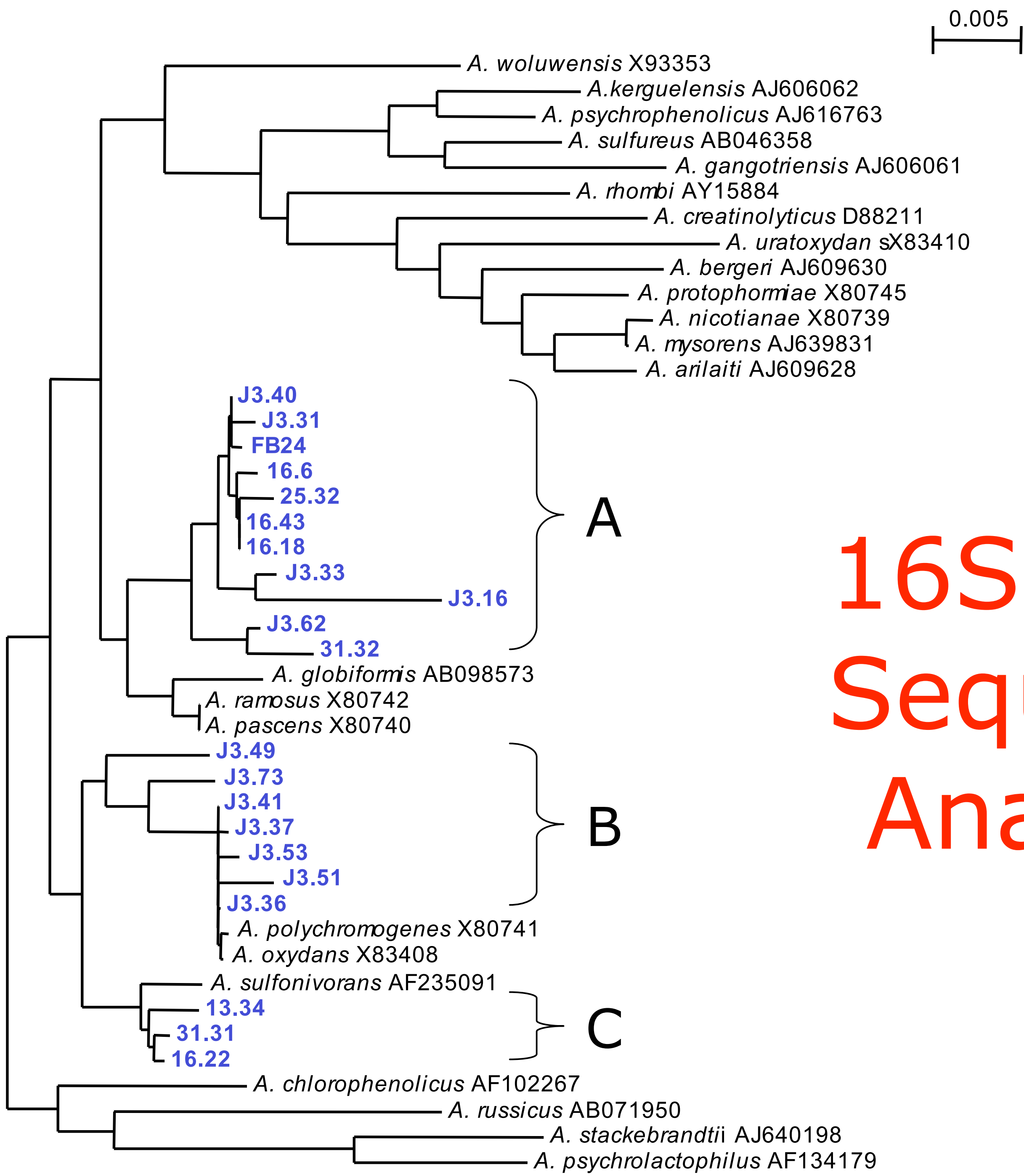


xylene degrader

Cr^{6+} resistant

13 sibling groups – 20 strains examined in phenotypic studies

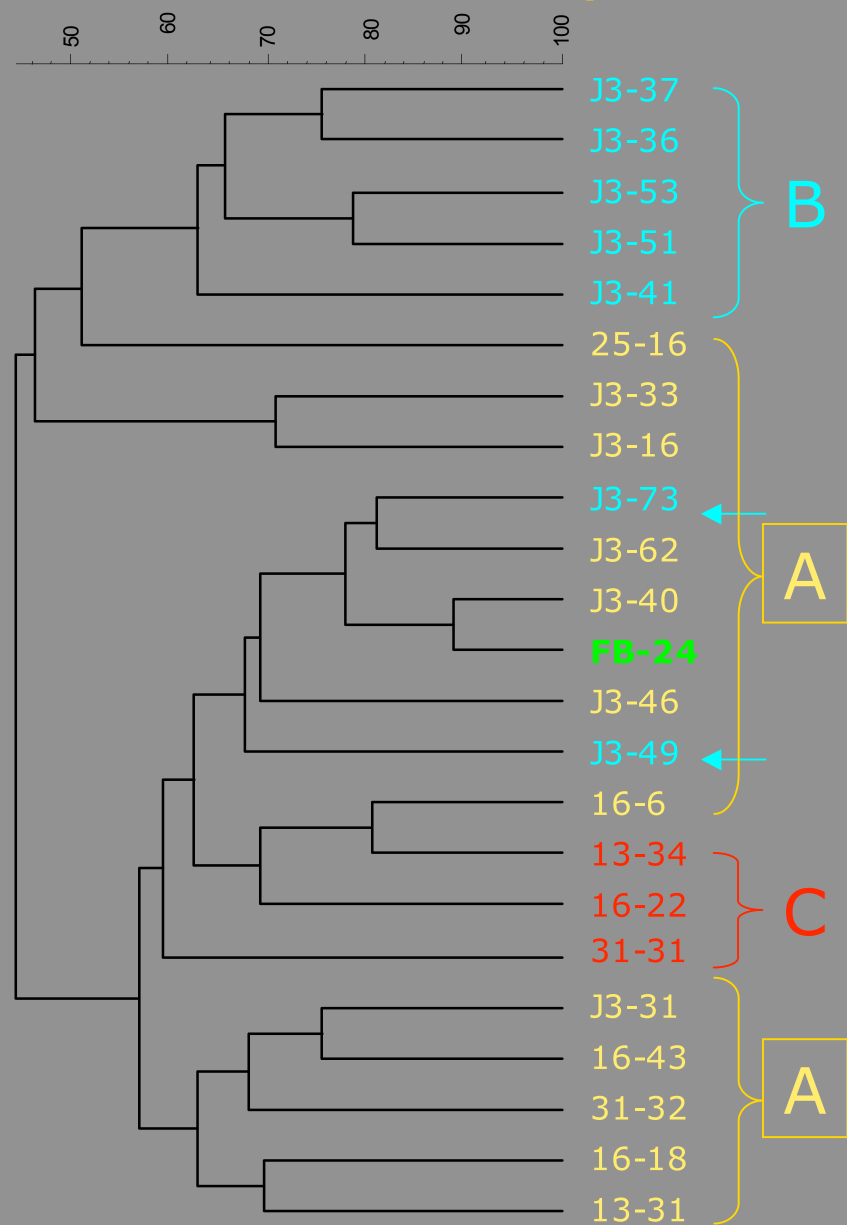
16S rRNA Sequence Analysis



Phenotypic variation among *Arthrobacter* isolates

Strain ID	rRNA group	rep-PCR group	Cr (mM)	Cd (μM)	Ni (μM)	Zn (μM)	Xylene	Toluene	Ethyl-benzene
16-43	A	1	0.25	500	750	750	+	+	+
25-32	A	1	0.25	500	1000	750	+	+	+
16-18	A	2	0.25	500	750	500	+	+	+
16-6	A	3	0.25	500	500	500	+	+	+
J3-31	A	4	50	500	25	500	-	-	-
J3-62	A	11	20	250	250	250	+	+	+
31-32	A	11	0.25	250	250	250	+	+	+
J3-16	A	12	1.0	100	250	100	-	-	-
J3-33	A	12	2.5	100	250	100	-	-	-
J3-40	A	13	20	500	250	500	-	-	-
FB24	A	13	300	500	750	500	-	-	-
J3-41	B	7	150	250	25	250	-	-	-
J3-51	B	7	200	250	25	500	-	-	-
J3-53	B	7	150	250	25	250	-	-	-
J3-36	B	8	150	250	100	250	-	-	-
J3-37	B	8	150	250	100	250	-	-	-
J3-73	B	9	60	250	25	250	-	-	-
J3-49	B	10	0.25	500	1000	500	-	-	-
13-34	C	5	0.25	250	250	500	+	+	+
31-31	C	5	0.25	500	750	750	+	+	+
16-22	C	6	0.25	500	250	750	+	+	+

Carbon source utilization patterns (Biolog GP)



Colleagues

Department of Biological Sciences

<u>Current</u>
Josie Becker
Militza Carrerro
Tina Henne
Kurt Jerke
Dr. Peter Kourtev

<u>Past</u>
Nadia Carmosini
Patrick Curtis
Jennifer Groh
Prof. Wei Shi

Department of Agronomy
Prof. Cindy Nakatsu
Weimin Chen

Prof. Ron Turco
Janet Joynt
Fred Beasley
Marti Vargha
Brett Baldwin
Megi Kourteva

USDA Soil Tilth Lab -- Dr. Tim Parkin
Worcester Polytechnic -- Prof. Jayson Wilbur