Correspondence on discrimination of shifts in soil microbial communities using nonequilibrium thermal dissociation and gel pad array technology.

Alex E. Pozhitkov[1] and Peter A. Noble[2]*

1, Gulf Coast Research Laboratory,
University of Southern Mississippi
703 East Beach Dr
Oceans Springs MS 39564
Email: alexander.pozhitkov@usm.edu

2, *Corresponding author.
201 More Hall,
Civil and Environmental Engineering,
University of Washington,
Seattle, WA 98195
Email: panoble@washington.edu

Content

Figure S-1

Table S-1

Table S-2

Table S-3

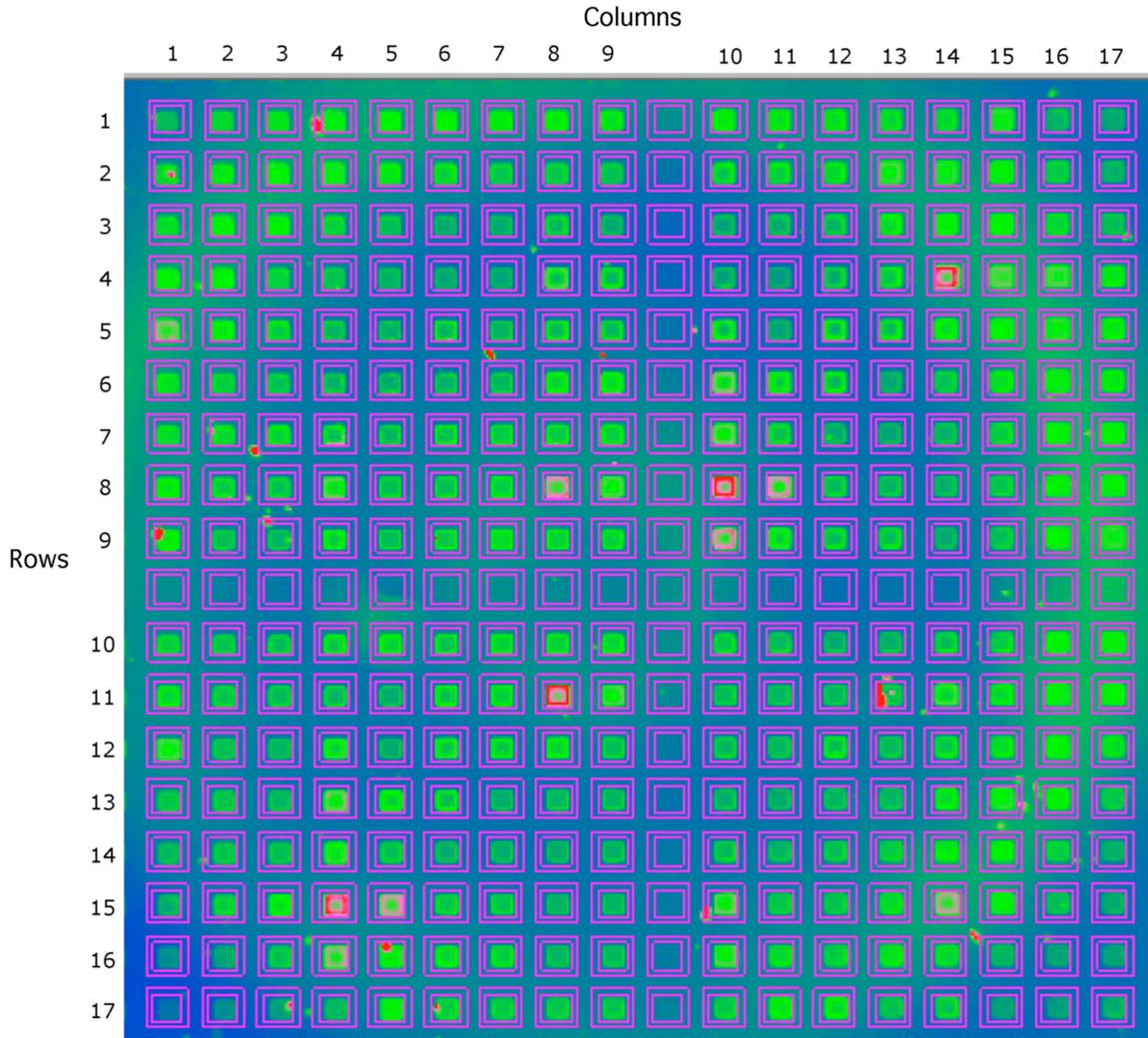Literature Cited

Total number of pages: 6

Figure S-1. Color-enhanced image of a gel pad array showing the inner and outer grids framing the gel pads used by Fotin et al. (4) image processing software. Gel pads, green; frames, pink; background, blue.

Table S-1. Table corresponding to Figure S-1 indicating if the grid frame was correctly positioned in the center of the pad (1) or not (0). Forty-five of the 289 (15.6%) gel pads were correctly positioned (i.e., not touching the grid frames at the start of the thermal dissociation) in this example. Correct positioning of all frames, at any time, was not possible using the image acquisition software. Moreover, the position of the frames changed during the thermal dissociation.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
5	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
6	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
7	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
8	0	0	0	0	0	0	0	0	0	0	0	1	1	1	1	0	0
9	0	0	0	1	1	1	1	1	1	0	0	1	1	1	1	0	1
10	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	1	1
11	0	0	0	0	0	0	0	0	0	0	1	0	0	1	1	0	0
12	0	0	0	0	0	1	1	1	1	1	0	1	1	1	0	0	0
13	0	0	0	0	0	0	1	1	1	0	0	0	0	0	0	0	0
14	0	0	0	0	0	0	1	1	1	0	0	0	0	0	0	0	0
15	0	0	0	0	0	0	0	1	1	0	0	0	1	1	1	1	0
16	0	0	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0
17	0	0	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0

Table S-2. Comparison of the capability to detect differences in probes between sites for Functional ANOVA and initial signal intensity (SI) methods. These data are based on the results from Figure 2 in Eyers et al. (1). Shown is the number of probes that were significantly different (+) between sites and those that were not (-) based Functional ANOVA results, and the number of probes that were significantly different (+) and those that were not (-) based on initial SI (+) between sites. Two-sided Fisher's exact test revealed no difference between methods.

Method	Number of probes:		Total
	+[a]	-[b]	
Functional ANOVA	11	12	23
Initial SI	16	7	23
	27	19	46

[a], that were significantly different in their analysis shown in Figure 2
[b], that were not significantly different in their analysis shown in Figure 2

Table S-3. Agreement/disagreement of results from Figure 2 in Eyers et al. (1) that were based on Functional ANOVA and initial signal intensity (SI). Shown is the number of probes with significant differences between sites for both methods (+,+), the number of probes yielding significant differences between site for one method but not the other (+,-) and vice versa (-,+), and the number of probes that were not significantly different between sites for both methods (-,-). Two-sided Fisher's exact test revealed no difference between methods.

Functional ANOVA	Initial SI		Total
	+	-	
+	8	3	11
-	8	4	12
Total	16	7	23

Literature Cited

1. Eyers, L.; Smoot, J. C.; Smoot, L. M.; Bugli, C.; Urakawa, H.; McMurry, Z.; Siripong, S.; ElFantrousii, S.; Lambert, P.; Agothos, S. N.; Stahl D. A. Discrimination of shifts in a soil microbial community associated with TNT-contaimination using a Functional ANOVA of 16S rRNA hybridized to oligonucleotide arrays. *Environ. Sci. Technol.* **2006**, *40,* 5867-5873.

2. Pozhitkov, A.; Chernov, B.; Yershov, G.; Noble, P.A. Evaluation of gel pad oligonucleotide array technology using artificial neural networks. *Appl. Environ. Microbiol.* **2005**, *71,* 8663–8676.

3. IstackX, http://staff.washington.edu/pozhit/default.htm, 2005.

4. Fotin, A. V.; Drobyshev, A. L.; Proudnikov, D. Y.; Perov, A. N.; Mirzabekov, A. D. Parallel thermodynamic analysis of duplexes on oligodeoxyribonucleotide microchips. *Nucleic Acids Res.* **1998**, *26,*1515-1521.

5. Brazma, A.; Hingamp, P.; Quackenbush, J.; Sherlock, G.; Spellman, P.; Stoeckert, C.; Aach, J.; Ansorge, W.; Ball, C. A.; Causton, H. C.; Gaasterland, T.; Glenisson, P.; Holstege, F.C.P.; Kim, I. F.; Markowitz, V.; Matese, J. C.; Parkinson, H.; Robinson, A.; Sarkans, U.; Schulze-Kremer, S.; Stewart, J.; Taylor, R.; Vilo, J.; Vingron, M. Minimum information about a array experiment (MIAME)—toward standards for array data. *Nat. Genet.* **2001**, *29,* 365-371.