Assessing soil microbial populations responding to crude-oil amendment at different temperatures using phylogenetic, functional gene \((alkB)\) and physiological analyses

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The following is the list of additional supporting materials for this article.

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**Supplemental Methods Details.**

**Supplemental Figure S1.** Depletion of \(n\)-alkane components of crude-oil added to soil and incubated at 25, 10 and 4°C.

**Supplemental Figure S2.** Detection of alkane hydroxylase genes in crude-oil contaminated soil incubated at 4°C (Day 19).
Methods Details:

Nutrient solution. Final concentrations: \((\text{NH}_4)_2\text{SO}_4\) (1.1 mM), \(\text{NH}_4\text{NO}_3\) (5.9 mM), \(\text{KH}_2\text{PO}_4\) (0.7 mM), \(2\text{K}_2\text{HPO}_4\) (0.7 mM), \(\text{KOH}\) (0.28 mM), \(\text{H}_2\text{SO}_4\) (0.28 mM), \(\text{MgCl}_2\) (1.6 mM), \(\text{CaCl}_2\) (3.2 mM), and \(\text{FeCl}_2\) (0.02 mM)\) (1).

GC-MS Analysis. 1 g (dry wt) subsamples were removed from replicate soil vessels at several time points, solvent-extracted, and analyzed as described previously (2). Loss of total \(n\)-alkane fractions from control treatments with autoclaved soils was 15.0±8.6% after a 50-day incubation, likely due to enhanced volatilization in air-purged reaction vessels. Thus, the % of \(n\)-alkane depletion in non-sterile treatments was normalized to the amount of each \(n\)-alkane fraction recovered from control treatments at corresponding time points.

Unexpected product in alkB PCR amplification. A faint ~980 bp-PCR product was obtained with R4 primers and \(P.\ putida\) GPo1 DNA as a template, although this non-specific product was never observed with soil DNAs as templates. The sequence of this larger, non-specific product did not show high similarity to known sequences in GenBank, thus could be ignored when the expected size products for R4 primers (242-bp) were obtained.

Isolation of alkane degrading bacteria. Alkane-degrading bacteria were isolated as described previously (3) by plating serially diluted soil slurry on minimal medium (Xm) plates (4) with \(n\)-hexadecane supplied as the sole carbon source through the vapor phase. The plates were incubated at 25°C. A total of 24 colonies were randomly selected from plates inoculated with \(10^{-4}\) to \(10^{-5}\) dilutions of crude-oil amended soil incubated for 48 days at 25°C.

References.


Figure S1. Depletion of \textit{n}-alkane components of crude-oil added to soil and incubated at 25, 10 and 4°C. Each bar represents the mean from duplicate samples; the error bars represent the standard error.
Figure S2. Detection of alkane hydroxylase genes in crude-oil contaminated soil incubated at 4 °C (Day 19). Agarose gel image showing PCR amplification products with group-specific alkB primers, defined in Figure 3. The sizes (base pairs) of the standard fragments in lane M (repeated on each side of the gel) are shown on the left.