Supplement method:

Total cell enumeration in groundwater.

One hundred microlitres of groundwater was diluted 1:10 and fixed with 1% (wt:vol) paraformaldehyde solution at 4 ^{o}C. Subsequently, cells were stained with a 1 in 10,000 dilution of SYBR Green I stock solution (Molecular Probes Co. USA) at room temperature in the dark for 2 hours. Subsequently, triplicate cell concentrations were determined for each sample using pre- and post weighed aspirations and side scatter versus green fluorescence detection on a FACSCalibur flow cytometer (Becton Dickinson Inc., USA). To account for machine drift, 0.47 µm Flowcheck microspheres (Polysciences Inc, USA) were added to each sample as an internal fluorescence calibration.

Isolation of naphthalene degraders and conjugation experiments

Naphthalene degraders were isolated by spreading 100 µl of groundwater onto minimal medium agar plates (3) with naphthalene crystals on the lid, which served as the sole carbon source. The plates were incubated at 14 ^{o}C for 1 week. As bacteria grew and formed colonies, each colony was taken and transferred to a new plate. Each purified bacterial colony was identified by sequencing PCR-amplified 16S rDNA using primers 63F and 1387R (Table 2).

The conjugative matings were performed with a plasmid-free recipient *P. putida* UWC1 and each of the Pseudomonad isolates obtained. *P. putida* UWC1 (Rif^{r}, a gift from Dr Andrew Lilly, CEH-Oxford) harbours rifampicin resistant gene within its chromosome, but cannot utilize naphthalene as a sole source of carbon. Conversely, the Pseudomonad isolates were capable of growth on minimal medium with

naphthalene as a sole carbon source, but were not resistant to 100 µg/ml rifampicin. *P. putida* NCIB 9816 (1, 2) (a gift from Peter Williams group at University of Wales, Bangor), that harbours pDTG1 (pWW60), was used as a positive control for subsequent plasmid conjugation experiments. In the conjugation experiments, *P. putida* UWC1 was used as a plasmid recipient and the Pseudomonad isolates as well as *P. putida* NCIB 9816 were used as plasmid donor. Overnight broth cultures of 3 naphthalene degradation isolates, the positive control *P. putida* NCIB9816 and plasmid-free recipient *P. putida* UWC1 were washed three times in PBS and each donor mixed with the *P. putida* UWC1 recipient (200 µl each). Each mating mixture was spotted onto the surface of a cellulose nitrate membrane filter (25 mm diameter, 0.2 µm pore size, Millipore Inc.), and the filters with bacteria side up, were mounted on Luria-Bertani agar (LBA) plate and incubated at 28 ^{o}C for 24 h. Subsequently, the mating mixtures were re-suspended from the filters by vortex-mixing in 10 ml of PBS, diluted and plated onto minimal medium agar plates, supplemented with 100 µg/ml rifampicin and naphthalene as a sole carbon source. Plates were incubated at 28 ^{o}C for 48 hrs and *P. putida* UWC1 conjugants were selected due to acquired naphthalene degradation phenotype and rifampicin resistance.

Plasmid extraction and characterisation

Naphthalene degrading plasmids transferred to recipient *P. putida* UWC1 through mating experiments with natural Pseudomonad isolates were extracted with Nucleoplex BAC DNA kit (Tepnel Co., Manchester, UK) employing a bench-top Nucleoplex BAC Automated DNA Purification System (Nucleoplex, T1000, Tepnel Co., Manchester, UK). For genotypic fingerprinting and plasmid homology

assessment, the extracted plasmids were initially digested with EcoRI and run on a 1% agarose gel.

Plasmids originating from *P. putida* WH1 and WH3 were used as templates to amplify DNA fragments encoding of salicylate hydroxylase (*nah*G) and transcriptional regulator (*nah*R). The primers employed for amplification of *nah*G were NahG1_F and NahG1_R, and for amplification of *nah*R were NahR1_for and NahR_end_R (Table 2), respectively. A proteomics approach was used to identify functional protein and genes of plasmids in *P. fluorescens* WH2 and it is reported in another paper (5).

Denaturing gradient gel electrophoresis (DGGE) and sequencing

The GC-clamped PCR products were loaded onto a 10% (wt/vol) polyacrylamide gels, with a 35 to 70% urea/formamide denaturing gradient, as previously described (4). Denaturing gradient gels were cast and run using the Ingeny PhorU2 system at 60°C and 100 V for 16 h. Gels were stained with SYBR gold nucleic acid gel stain (Molecular Probes) and visualized by a Versa-Doc Imaging System (Model 3000, BioRad Laboratories, Herts., UK). The bands of interest were cut from gels and eluted in water at 4 ^{o}C overnight. The recovered DNA was re-amplified with primers 338F and 530R, as previously described (Table 2). The re-amplified products were cloned into pGEM-T as per manufacturer's instructions (Promega Co, USA) and transferred to competent cells of *E. Coli* JM109. To verify recovery of the bands of interest, sixteen colonies were randomly selected for PCR with primers GC338F/ 530R, and compared to expected bands by DGGE.

Monitoring naphthalene biodegradation.

HPLC analysis. HPLC was used to measure naphthalene and DMF (solvent) concentrations, at different time points, using a method previously described (3). The cells were removed by passing through 0.22 µm Minisart Syringe End Filters (Sartorius Ltd, UK). Cell-free samples were analyzed on a Dionex liquid chromatography (Dionex Ltd, Camberley, UK), equipped with a photo diode array detector, with a Phenomenex C18 column (250 mm × 3.25 mm, particle diameter 5 mm). Appropriate standards for naphthalene and DMF-specific calibration were prepared in acetonitrile and quantified at 220 nm. An isocratic program was applied with a mobile phase containing 75% acetonitrile, yielding a retention time of 2.75 and 7.12 for DMF and naphthalene, respectively.

Biosensor analysis. A salicylic acid (SA) biosensor was used to monitor the SA concentration during naphthalene biodegradation process, as previously described (3),. Briefly, 100 µl of sample was removed from controls and naphthalene amended treatments at each time point (0, 19, 21, 23, 29, 36, 44, 46, 48, 53, 64, 66 and 69 hrs), and was added to 100 µl overnight culture of *Acinetobacter sp*. ADPWH_lux SA biosensor (3). The SA level was determined by comparing bioluminescence expression from each sample (3).

Reference

1. **Cane, P. A., and P. A. Williams.** 1982. The Plasmid-Coded Metabolism of Naphthalene and 2- Methylnaphthalene in Pseudomonas Strains - Phenotypic Changes Correlated With Structural Modification of the Plasmid Pww60-1. Journal of General Microbiology **128:**2281-2290.
2. **Cane, P. A., and P. A. Williams.** 1986. A Restriction Map of Naphthalene Catabolic Plasmid Pww60-1 and the Location of Some of Its Catabolic Genes. Journal of General Microbiology **132:**2919-2929.
3. **Huang, W. E., H. Wang, L. F. Huang, H. J. Zheng, A. C. Singer, I. P. Thompson, and A. S. Whiteley.** 2005. Chromosomally Located Gene Fusions Constructed in Acinetobacter sp. ADP1 for the Environmental Detection of Salicylate. Environmental Microbiology **7:**1339-1348.
4. **Manefield, M., A. S. Whiteley, R. I. Griffiths, and M. J. Bailey.** 2002. RNA stable isotope probing, a novel means of linking microbial community function to Phylogeny. Applied and Environmental Microbiology **68:**5367-5373.
5. **Yu, X. H., W. E. Huang, and A. S. Whiteley.** 2008. Investigation of naphthalene biodegradation pathway in Pseudomonas fluorescens using a proteomic approach. Environmental Mcirobiology **in preparation**.

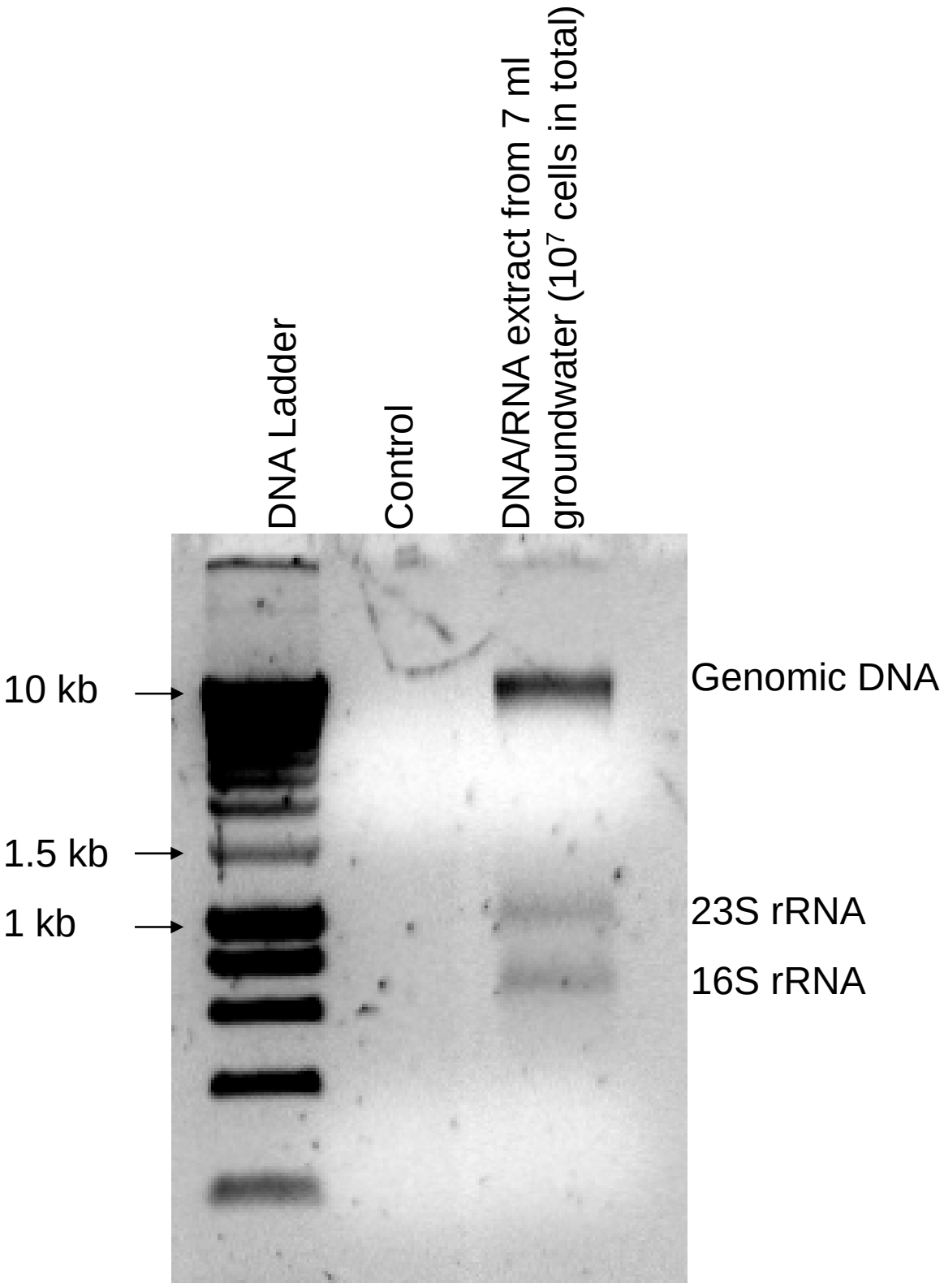

Figure S1. DNA/RNA extraction from groundwater. Both genomic DNA and 16S rRNA from 7 ml groundwater (1.5×10^6 cells/ml) was efficiently extracted.

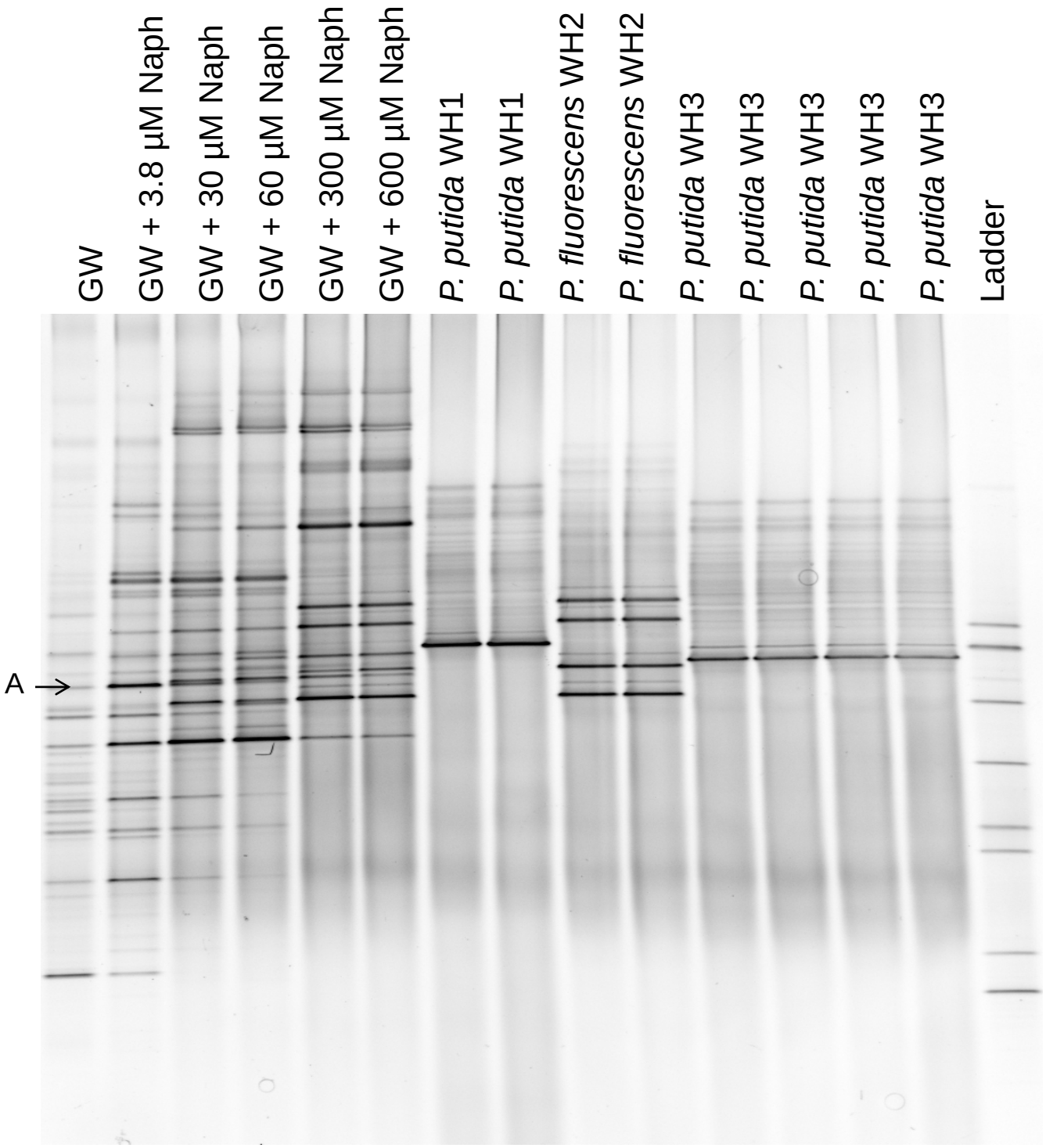

Figure S2. Naphthalene concentrations affected the groundwater microbial communities. With increasing naphthalene concentrations, microbial biodiversity decreased and *Pseudomonas fluorescens* WH2 gradually became dominant species in the communities. Band A was identified as *Acidovorax sp..*

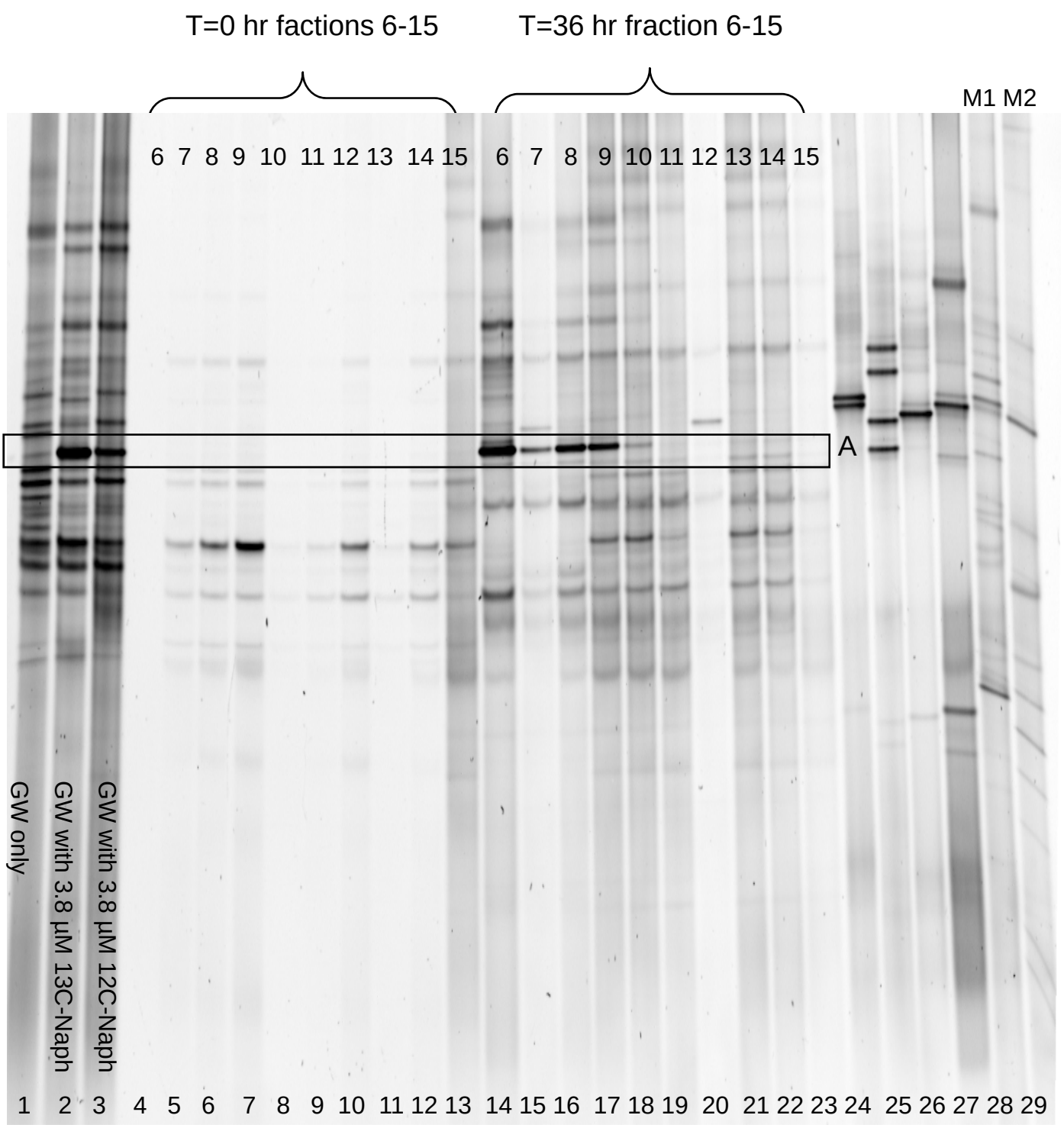

Figure S3. Microbial community profile relating to degradation species. Lane 1-3 represents groundwater only, groundwater pulsed with 3.8 µM 12C- and 13C-naphthalene at 36 hrs. Lane 4-13 and lane 14-23 respectively represents RNA fractions 6-15 from equilibrium density gradients at 0 hr and 36 hrs after 13C-naphthalene pulse. Lane 24-26 represents 3 pure cultures isolated from groundwater. Lane 27-29 are unrelated DGGE markers. It suggests that the *in-situ* naphthalene degrader was not species which were isolated from groundwater. Band A was identified as *Acidovorax sp..*

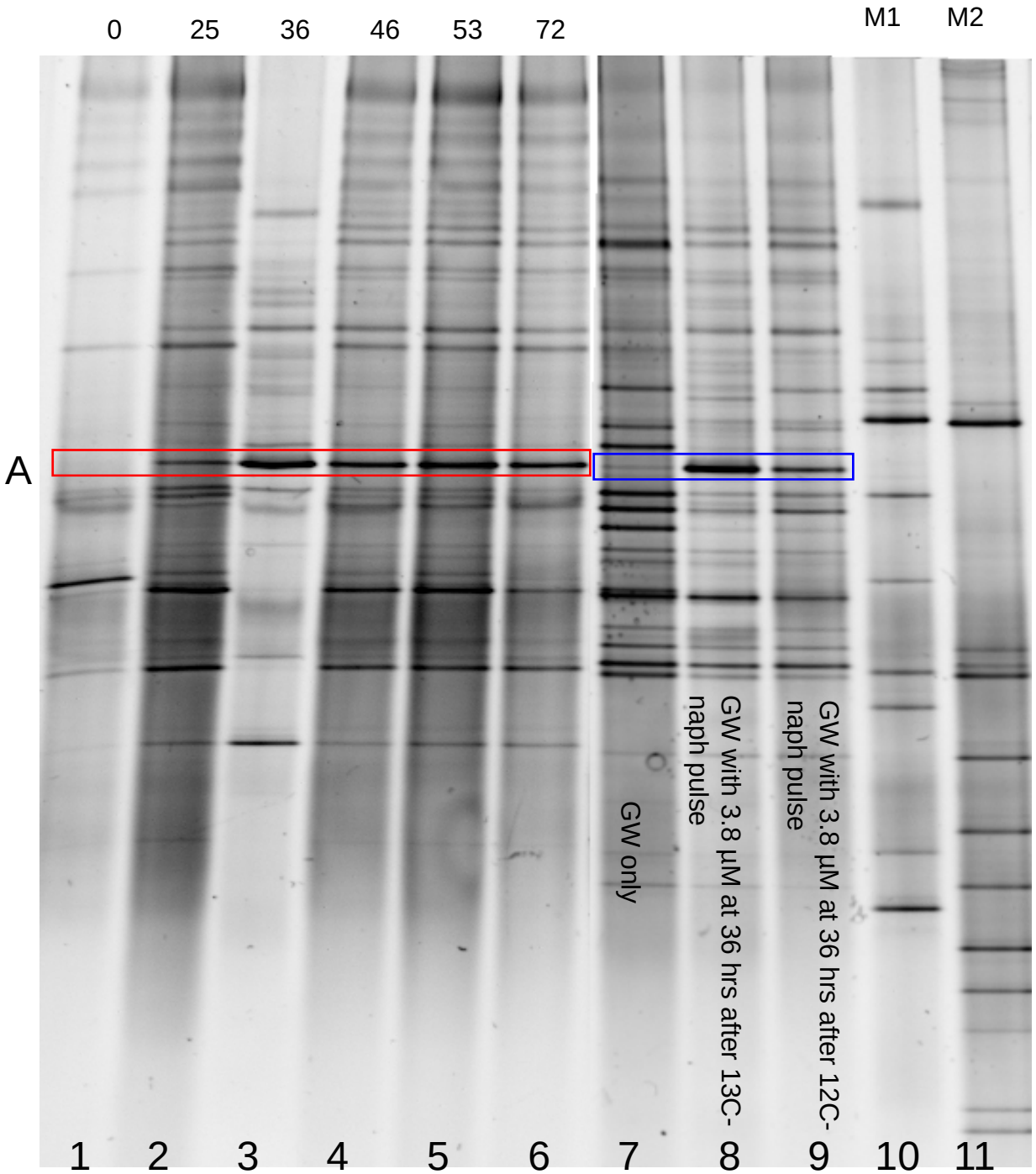

Figure S4. DGGE profile of microbial communities present in fractions 6 (^{13}C-RNA) from equilibrium density gradients at 0-72 hrs after the ^{13}C-naphthalene pulse. Lane 1-6 represents 0, 25, 36, 48, 53 and 72 hrs respectively, lane 7-9 represents groundwater only, groundwater pulsed with 3.8 µM ^{12}C- and ^{13}C-naphthalene at 36 hrs. Lane 10-11 represents unrelated DGGE marker1 and marker2. The profile suggested that a particular community member (highlighted bands), appeared in 13C-RNA fraction, was a primer naphthalene degrader *in-situ*. Cut-band A sequence indicated that it was *Acidovorax sp*..

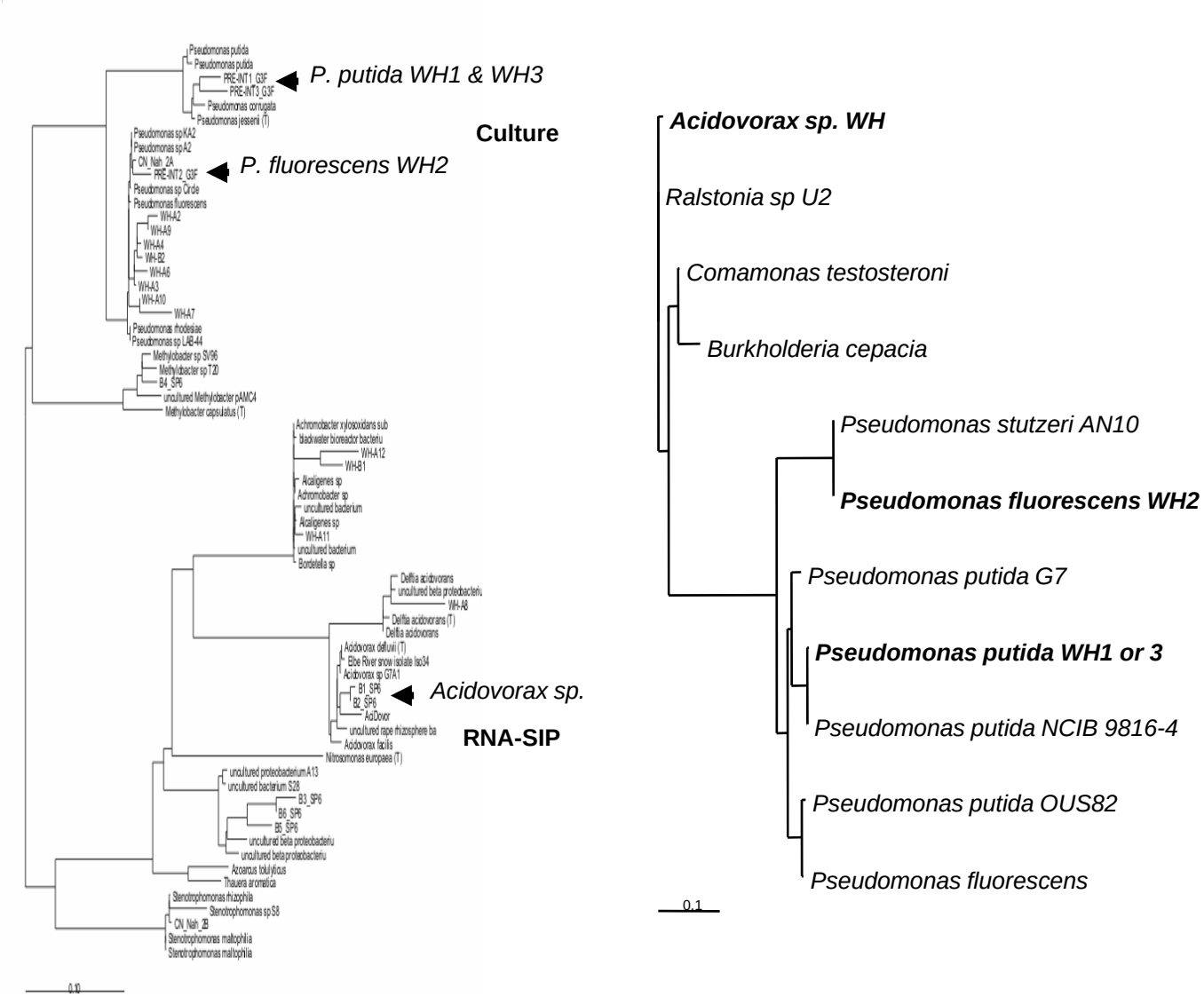

Figure S5. (A) 16S rRNA based sequence diversity of naphthalene degraders isolated from the soil and groundwater of the contaminated site and comparison to those sequences recovered by RNA-SIP. The sequences of 16S rRNA indicates that strains WH1, 2 and 3 were closely related to *Pseudomonas spp.* and sequences recovered from RNA-SIP were related to *Acidovorax sp*.. (B) Phylogenetic analysis of naphthalene dioxygenase gene (NDO) gene sequences recovered from groundwater communities relating to the *Acidovorax* sp. and three Pseudomonad isolates.

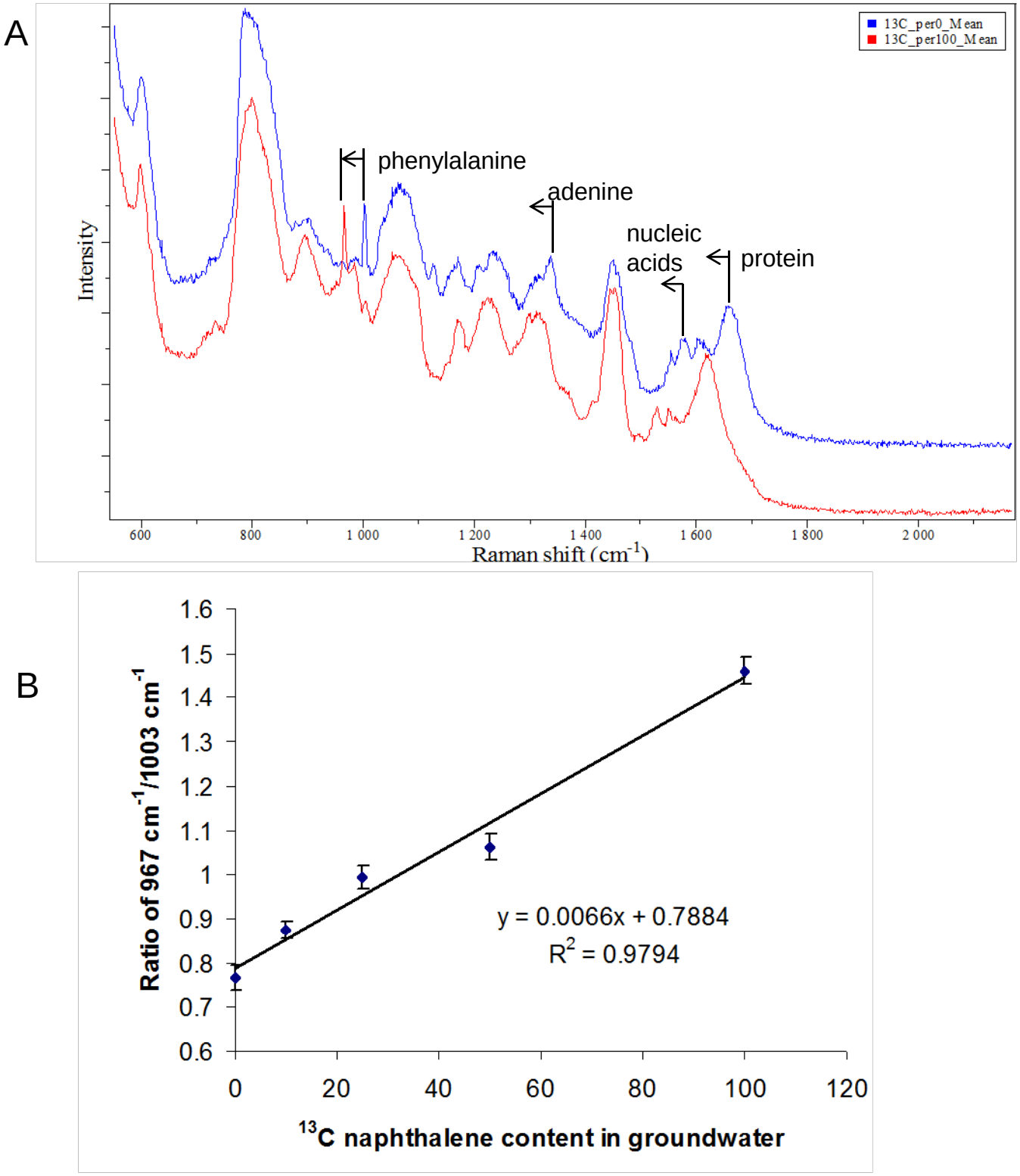

Figure S6. Some bands of Raman spectra of individual bacteria shifted as they integrated 13C-naphthalene into the cells. (A) Raman spectra obtained for living cells of *P. fluorescens* WH2 grown in minimal media containing either 12C or 13C labelled naphthalene as the sole source of carbon. (B) Relationship between 13C-naphthalene content and 'red' shift ratios of individual bacteria (RSR; calculated as intensity at 967 cm^{-1} / intensity at 1003 cm^{-1}) when analyzed under five 13C labeling conditions (n = 5-10 cells for each point).