1 S1 SUPPORTING INFORMATION

2 Divergent microbial communities in groundwater 3 and overlying soils exhibit functional redundancy for 4 plant-polysaccharide degradation

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15 Running title: Plant polysaccharide degradation in groundwater and soil

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Materials and Methods

Monitoring of physicochemical parameters in microcosms during SIP incubation

During incubation, the concentration of total inorganic carbon (TIC) as well as pressure and pH were monitored every 1 to 2 weeks. Quantification of TIC was based on CO_2 partial pressure in the headspace of the incubations determined by GC measurements on a Hewlett Packard 5890 instrument (Hewlett Packard, Palo Alto, CA, USA) with a thermal conductivity detector, equipped with a Chromosorb 102 column (2 m x 1/8'', Alltech, Unterhaching, Germany). The injector temperature was set to 150 °C and the detector temperature to 175 °C. A volume of 100 µl from the headspace of the incubations was injected and separated with Helium as carrier gas at a pressure of 22 psi and 50 °C, CO_2 was detected after 1:35 min. Pressure in the incubations was measured with a portable digital pressure meter GMH 3111 (Greisinger electronic, Regenstauf, Germany) before taking samples for GC measurements, and pH was measured with a WTW Sentix Mic probe attached to a WTW pH 330 pH-meter (WTW electronic, Graz, Austria). Pressure and pH were used to calculate the concentrations of dissolved hydrogen carbonate for determination of TIC as previously described (1).

Bacterial 16S rRNA gene amplicon sequencing by LGC

Illumina MiSeq amplicon sequencing targeting bacterial 16S rRNA genes was performed on all samples using the primer combinations Bakt_785R/Bakt_341F (2). Illumina MiSeq amplicon sequencing was carried out by LGC Genomics GmbH (Berlin, Germany). The PCRs included about 5 ng of DNA extract, 15 pmol of each forward primer 341F 5'-NNNNNNNNNNTCCTACGGGNGGCWGCAG and reverse primer 785R 5'-

41 NNNNNNNNNNTGACTACHVGGGTATCTAAKCC in 20 uL volume of 1 x MyTaq buffer
42 containing 1.5 units MyTaq DNA polymerase (Bioline) and 2 µl of BioStabII PCR Enhancer
43 (Sigma). For each sample, the forward and reverse primers had the same 10-nt barcode
44 sequence, resulting in an amplicon size of approximately 470 bp, including the V3 and V4
45 region of the bacterial 16S rRNA gene. PCRs were carried out for 30 cycles using the
46 following parameters: 2 min 96 °C pre-denaturation; 96 °C for 15 s, 50 °C for 30 s, 70 °C for
47 90 s. DNA concentration of amplicons of interest was determined by gel electrophosesis .
48 About 20 ng amplicon DNA of each sample were pooled for up to 48 samples carrying
49 different barcodes. If needed PCRs showing low yields were further amplified for 5 cycles.
50 The amplicon pools were purified with one volume AMPure XP beads (Agencourt) to remove
51 primer dimer and other small mispriming products, followed by an additional purification on
52 MinElute columns (Qiagen). About 100 ng of each purified amplicon pool DNA was used to
53 construct Illumina libraries using the Ovation Rapid DR Multiplex System 1-96 (NuGEN).
54 Illumina libraries were pooled and size selected by preparative gel electrophoresis.
55 Sequencing was done on an Illumina MiSeq using V3 Chemistry (Illumina).

56 References

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