Effect of vertical flow exchange on microbial community distributions in hyporheic zones

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The effect of the vertical flow direction of hyporheic flux on the bacterial community is examined. Vertical velocity change of the hyporheic zone was examined by installing a piezometer on the site, and a total of 20,242 reads were analyzed using a pyrosequencing assay to investigate the diversity of bacterial communities. Proteobacteria (55.1%) were dominant in the hyporheic zone, and Bacteroidetes (16.5%), Actinobacteria (7.1%) and other bacteria phylum (Firmicutes, Cyanobacteria, Chloroflexi, Planctomycetes and unclassified phylum OD1) were identified. Also, the hyporheic zone was divided into 3 points – down welling point, mixed welling point and up welling point – through vertical hydraulic gradient, and the bacterial communities were compared and analyzed. When the species diversity index was additionally analyzed based on the pyrosequencing data, richness and diversity of species were observed to be highest in the order of mixed welling point > down welling point > up welling point. Hence, quantitative changes and characteristics of the hyporheic zone were analyzed to have an effect on the resident bacterial communities.

Introduction

The hyporheic zone (Kim et al., 2018) is a part of the groundwater interface in streams where a mixture of surface water and groundwater can be found. The word of hyporheic derived from Greek roots for flow (rheo) and under (hypo). This area is saturated sediments in which surface water and groundwater mix. There are studies of this region as a dynamic and distinguishable interface between surface water and groundwater. (Findlay, 1995; Boulton et al., 1998; Smith et al., 2008; Vogt et al., 2010; Kim et al., 2014a). Hydrologists and ecologists have approached this region with their particular perspectives. There are various literatures to identify and quantify hyporheic exchange flow (Kasahara and Wondzell, 2003; Storey et al., 2003; Cardenas et al., 2004; Westhoff et al., 2011). This water exchange is important to the hyporheic zone health because of its connection with living organisms in the zone (Boulton et al., 2010; Griebler and Avramov, 2015). The advance of hydrodynamic modeling has improved research of hydrological exchange processes at the hyporheic zone (Cardenas and Wilson, 2007; Fleckenstein et al., 2010; Endreny et al., 2011). Also, this zone has plentiful micro-organisms. The hyporheic zone constituents a dynamic hotspot (ecotone) where groundwater and surface water mix (Smith et al., 2008).

This area constitutes a flow path along which surface water down wells into the streamed sediment and groundwater up wells in the stream, travels for some distance before eventually mixing with groundwater returns to the stream channel (Hassan et al., 2015). Surface water enters the hyporheic zone when the vertical hydraulic head of surface water is greater than the groundwater (down welling). On the other hand, surface water with groundwater influence emerges from hyporheic zone where upward direction pressure of subsurface water is greater than that of the stream channel (up welling) (Kim et al., 2011). The zone is composed of permeable gravels, sands and silts and allows significant mixing of surface water with groundwater at the zone. The area is a complicated region whose boundaries are not always easy to define. The exchange of stream water and groundwater in the hyporheic zone is dynamic in space (Kim et al., 2014a) and creates a unique community of the endemic bacteria. The exchange and mixing of groundwater and stream water plays an important role in determining the structure of bacteria diversity by influencing hydraulic and chemical condition (Zimmer and Lautz, 2015). However, there have been only a few data researches reporting on the influence of hydrologic exchange on composition and spatial distribution of the hyporheos (Jones et al., 2015). This study examines the influence of hyporheic exchange on biological characteristics of the hyporheic zone.

Study site

The study area, Haean basin is located between longitude 128°5'-128°11’E and latitude 38°15'-38°20’N with a range in altitude from ca. 339 m to 1,320 m (Fig. 1(a)). These streams leave the basin at the eastern border of the study area, where it eventually converges with Soyang River. The drainage system specifically shows a dendritic pattern. The total length of streams is about 63 km and the streams flow down to the depression of the basin but only one stream flows out in the east of the basin. Therefore, the hydrographic system of the area is relatively
Figure 1. Location of the study area and hyporheic water sampling points.
simple when it compares with other areas (Lee, 2009; Jeong et al., 2012; Lee et al., 2013). Geology of the study area is characterized by Jurassic igneous rocks intruding into the composite metamorphic rocks (Lee, 2009; Kim et al., 2018). The extreme region is mainly made up of alternating meta-sedimentary rocks of mica schist, biotite-feldspar gneiss and quartzite. It has peculiar and interesting topographic feature like a bowl. The features of the area have been formed through prolonged differential erosion and the depressed bottom is made up of granite only (Lee, 2009). The geological section and profile of the basin are shown in Fig. 1(b).

The climate is characterized by distinct wet and dry seasons (Kettering et al., 2012; Kim et al., 2015). From 2005 to 2014, the average annual precipitation is estimated at 1,278.5 mm with 50% falling during the summer monsoon (KMA, 2015). The maximum and minimum of annual precipitations were 1,557 mm in 2011 and 809.5 mm in 2014, respectively (Fig. 2). Based on the precipitations for the decade (from 2005 to 2014), the wet and dry seasons were considered to be from July to September and from October to February, respectively.

Materials and Methods

**Vertical hydraulic Gradient of the Hyporheic zone**

The vertical hydraulic gradient (direction and its magnitude) between groundwater and stream water was evaluated using a piezometer transect. The ambient vertical hydraulic gradient between groundwater and stream water was recorded using piezometer. The direction and magnitude of hydrologic interaction were evaluated using piezometer transects. Each piezometer was made up of 2 inch (internal diameter) PVC (polyvinyl chloride) pipe (Hyun et al., 2011; Kim et al., 2014b). At each transect, a total of 20 piezometers were inserted at 0.1 m depth (Kim et al., 2014a) beneath the streambed adjacent to each seepage meter installation point with a regular interval (see, Fig. 1(c)). The head was measured relative to the stream water surface and vertical hydraulic gradient was determined by dividing by the piezometer insertion depth.

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VHG = \frac{\Delta h}{\Delta l}
\]

where \( \Delta h \) is the difference in water levels of the piezometers and stream water, \( \Delta l \) is the difference in depths between streambed and the point where the piezometer is installed. However, head differences were often very small in the coarse-grained streambeds and thus, three to five measurements were averaged. The positive and negative vertical hydraulic gradients indicate up welling and down welling conditions of the hyporheic zone, respectively (Bartsch et al., 2014).

Hyporheic zone, which is the mixed zone of surface water and groundwater, is where quantitative changes occur dynamically. It was divided into 3 points for analysis through vertical hydraulic gradient in the hyporheic zone: Up welling point where the effect of groundwater is greater, down welling point where the effect of surface water is greater, and mixed welling point where quantitative changes of the groundwater and surface water were evenly observed. Fig. 3(a) shows the installed piezometer and Fig. 3(b) shows the hyporheic water sampling.

**Analysis of Pyrosequencing Reads**

The samples were filtered through 0.2 µm filter and returned to the laboratory and stored in a -70°C refrigerator until DNA extraction and pyrosequencing of 16S rRNA analysis. The DNA of sampled was extracted using a FastDNA Spin Kit (Qbiogene, USA) as specified by the manufacturer. The quality of extracted DNA was checked by standard agarose gel electrophoresis and stored at -20°C. The DNA concentration was determined using a UV-VIS Spectrophotometer (Mechasys Co. Ltd., Korea). 16S rRNA genes were amplified using forward and inverse primers to distinguish each sample prior to sequencing at Chun Laboratory (Kim et al., 2015). Amplification condition for PCR was i) an initial denaturation step of 94°C or 5 min, ii) 30 cycles of
denaturation, and annealing (94°C for 30 sec followed by 55°C for 45 sec), and iii) an extension step at 72°C for 90 sec. Pyrosequencing was conducted using 454 Genome Sequence FLX Titanium Junior (Roche, NJ, USA) by Chun Laboratory (Seoul, South Korea). Distinct sequences have been deposited in the Sequence Read Archive (Chun Lab). The full sequences were analysed in the NCBI (National Center for Biotechnology Information) database. The NCBI web site can help to access to genomic information. The tools in the NCBI allow users to perform a BLAST (Basic Local Alignment Search Tool) search for similar sequences in the GenBank. A search for sequence similarities with known genes was performed using a BLAST analysis. Identification of the conserved region and protein translations and analysis of amino acids were performed using BioEdit Sequence Alignment Editor (Ibis Biosciences, USA).

Bacterial communities of down welling point (PDHS4, groundwater < surface water), mixed welling point (PMHS8, groundwater ≈ surface water) and up welling point (PUHS20, groundwater > surface water) in the hyporheic zone were analyzed using a pyrosequencing assay. Total of 37,511 reads were analyzed in the three regions based on bacterial 16S rRNA partial gene (approximately 490 bp). Excluding reads where primer mismatch, non-target, low quality, short length, chimera, matching or similarity of 80% or below with eukaryotes were observed, total of 20,242 reads (PMHS8 6,065 reads, PDHS4 7,603 reads and PUHS20 6,574 reads) were analyzed (Table 1).

**Total Bacterial Communities**

When the bacterial communities inhabiting the hyporheic zone were analyzed at the phylum-level, Proteobacteria phylum was dominant with 11,067 reads (55.1%), and Bacteroidetes phylum had 3,238 reads (16.5%), Actinobacteria phylum had 1,516 reads (7.1%), Acidobacteria phylum had 885 reads (3.9%), Firmicutes phylum had 685 reads (3.5%), Cyanobacteria phylum had 490 reads (2.3%), Chloroflexi phylum had 464 reads (2.2%), Planctomycetes phylum had 435 reads (2.1%), and unclassified phylum OD1 had 240 reads (1.2%). Other phylum [Gemmatimonadetes, Armatimonadetes, Verrucomicrobia, Nitrospirae, Elusimicrobia, Chlorobi, Fibrobacteres, Lentisphaerae, Fusobacteria, Deinococcus-Thermus and unclassified (TM7, GN02, TM6, OP11, OP3, MATCR, WS3, WS5, AD3 and SR1)] were identified to be lower than 1–0.05% (data below 0.05% not shown) (see, Table 1).

**Results and Discussion**

**Down Welling Point (PDHS4)**

In the down welling point which is largely affected by the surface water among the hyporheic zone, Proteobacteria phylum was dominant with 44.38%, followed by Actinobacteria (14.14%), Acidobacteria (10.75%), Chloroflexi (6.04%), Bacteroidetes (5.95%), Planctomycetes (5.55%), Firmicutes (1.93%), Gemmatimonadetes (1.82%), unclassified TM7 (1.33%), Armatimonadetes (1.18%) and Nitrospirae (1.09%). α-Proteobacteria class, δ-Proteobacteria class, Actinobacteria phylum and Acidobacteria phylum were observed to be relatively high in the down welling point distinctively compared to mixed welling point and up welling point (Table 1 and Fig. 4).

**Mixed Welling Point (PMHS8)**

In the mixed welling point which is equally affected by groundwater and surface water, Proteobacteria phylum was dominant with 54.11%, followed by Bacteroidetes (16.72%), Bacteroidetes (6.56%), Firmicutes (6.29%), Cyanobacteria (5.85%), unclassified OD1 (2.18%), unclassified TM7 (1.2%) and others (7.09%). Mixed welling point showed dominance and percentage that are between those of down welling and up welling points, but Firmicutes phylum, Cyanobacteria phylum, and ε-Proteobacteria class were observed at relatively higher percentage compared to down welling and up welling points (See, Table 1 and Fig. 4).

**Up Welling Point (PUHS20)**

In the up welling point, Proteobacteria phylum was dominant with 66.81%, followed by Bacteroidetes (26.86%), Firmicutes (2.34%) and others (3.99%). Sum of Proteobacteria and Bacteroidetes phylum accounted for 93.67% of the total in the up welling point, showing a characteristic of increasing dominance compared to down welling point and mixed welling point while percentages of other phyla were
Table 1. Analysis of bacterial diversity in the hyporheic zone (phylum-level)

| Phylum            | % (number of reads) | Total reads | Ratio (%) |
|-------------------|---------------------|-------------|-----------|
|                   | PDHS4              | PMHS8       | PUHS20    |           |
| Proteobacteria*   |                     |             |           |           |
| α-Proteobacteria  | 20.79 (1,581)      | 7.75 (470)  | 1.17 (77) | 2,128     | 10.51     |
| β-Proteobacteria  | 13.80 (1,049)      | 28.54 (1,731)| 50.27 (3,305)| 6,085 | 30.06     |
| γ-Proteobacteria  | 6.62 (503)         | 12.91 (783) | 14.25 (937) | 2,223 | 10.98     |
| δ-Proteobacteria  | 3.09 (235)         | 2.11 (128)  | 0.53 (35)  | 398 | 1.97      |
| ε-Proteobacteria  | 0.08 (6)           | 3.12 (189)  | 0.58 (38)  | 233 | 1.15      |
| Bacteroidetes     | 5.95 (452)         | 16.72 (1,020)| 26.86 (1,766)| 3,238 | 16.00     |
| Actinobacteria    | 14.14 (1,075)      | 6.56 (400)  | 0.62 (41)  | 1,516 | 7.49      |
| Acidobacteria     | 10.73 (817)        | 0.69 (42)   | 0.40 (26)  | 885  | 4.37      |
| Firmicutes        | 1.93 (147)         | 6.29 (384)  | 2.34 (154) | 685  | 3.38      |
| Chloroflexi       | 6.04 (459)         | 0.36 (22)   | 0.14 (9)   | 490  | 2.42      |
| Planctomycetes    | 5.55 (422)         | 0.49 (30)   | 0.18 (12)  | 464  | 2.29      |
| Cyanobacteria     | 0.83 (63)          | 5.85 (357)  | 0.23 (15)  | 435  | 2.15      |
| OD1†              | 0.83 (63)          | 2.18 (133)  | 0.67 (44)  | 240  | 1.19      |
| TM7†              | 1.33 (101)         | 1.20 (73)   | 0.30 (20)  | 194  | 0.96      |
| Gemmatimonadetes  | 1.82 (138)         | 0.15 (9)    | 0.08 (5)   | 152  | 0.75      |
| Armatimonadetes   | 1.18 (90)          | 0.34 (21)   | 0.06 (4)   | 115  | 0.57      |
| Nitrospirae       | 1.09 (83)          | 0.21 (13)   | 0.03 (2)   | 98   | 0.48      |
| Verrucomicrobia   | 0.70 (53)          | 0.54 (33)   | 0.14 (9)   | 95   | 0.47      |
| GN02†             | 0.33 (25)          | 0.49 (30)   | 0.26 (17)  | 72   | 0.36      |
| TM6†              | 0.66 (50)          | 0.21 (13)   | 0.02 (1)   | 64   | 0.32      |
| OP1†              | 0.54 (41)          | 0.16 (10)   | 0.05 (3)   | 54   | 0.27      |
| OP3†              | 0.17 (13)          | 0.36 (22)   | 0.09 (6)   | 41   | 0.20      |
| Elusimicrobia     | 0.11 (8)           | 0.38 (23)   | 0.18 (12)  | 43   | 0.21      |
| Chlorobi          | 0.32 (24)          | 0.21 (13)   | 0.08 (5)   | 42   | 0.21      |
| MATCR†            | 0.16 (12)          | 0.28 (17)   | 0.05 (3)   | 32   | 0.16      |
| WS3†              | 0.34 (26)          | 0.03 (2)    | 0.00 (0)   | 28   | 0.14      |
| Fibrobacteres     | 0.05 (4)           | 0.25 (15)   | 0.09 (6)   | 25   | 0.12      |
| WS5†              | 0.08 (6)           | 0.18 (11)   | 0.06 (4)   | 21   | 0.10      |
| Lentisphaerae     | 0.05 (6)           | 0.20 (12)   | 0.03 (2)   | 18   | 0.09      |
| Fusobacteria      | 0.00 (0)           | 0.21 (13)   | 0.05 (3)   | 16   | 0.08      |
| Deinococcus-Thermus| 0.05 (4)          | 0.11 (7)    | 0.03 (2)   | 13   | 0.06      |
| AD3†              | 0.16 (12)          | 0.00 (0)    | 0.00 (0)   | 12   | 0.06      |
| SR1†              | 0.00 (0)           | 0.10 (6)    | 0.06 (4)   | 10   | 0.05      |
| Synergistetes     | 0.00 (0)           | 0.11 (7)    | 0.00 (0)   | 7    | 0.03      |
| Tenericutes       | 0.00 (0)           | 0.07 (4)    | 0.03 (2)   | 6    | 0.03      |
| NKB19†            | 0.04 (3)           | 0.02 (1)    | 0.02 (1)   | 5    | 0.02      |
| TDNP†             | 0.00 (0)           | 0.03 (2)    | 0.03 (2)   | 4    | 0.02      |
| BRC1†             | 0.04 (3)           | 0.00 (0)    | 0.00 (0)   | 3    | 0.01      |
| WS1†              | 0.03 (2)           | 0.00 (0)    | 0.00 (0)   | 2    | 0.01      |
| GN04†             | 0.01 (1)           | 0.00 (0)    | 0.02 (1)   | 2    | 0.01      |
| Spirochaetes      | 0.03 (2)           | 0.00 (0)    | 0.00 (0)   | 2    | 0.01      |
| OMAN†             | 0.01 (1)           | 0.00 (0)    | 0.00 (0)   | 1    | <0.01     |
| 10BAV†            | 0.01 (1)           | 0.00 (0)    | 0.00 (0)   | 1    | <0.01     |
| Unidentified      | 0.31 (24)          | 0.31 (19)   | 0.02 (1)   | 44   | 0.22      |
| Total             | 7,603              | 6,065       | 6,574      | 20,242 | 100.00 |

*Total 11,067 reads (55.10%) were analyzed. Details are as follows; PDHS4 (44.38%, 3,374 reads), PMHS8 (54.11%, 3,301 reads) and PUHS20 (66.81%, 4,392 reads).

†Unclassified phylum.
low. Especially, Proteobacteria phylum (specifically β-Proteobacteria class) and Bacteroidetes phylum were high at 22.43% and 20.91%, respectively, compared to the down welling point while percentages of Actinobacteria, Acidobacteria, Chloroflexi, Planctomycetes, Gemmatimonadetes, Armatimonadetes, Verrucomicrobia, Nitrospirae, Chlorobi and unidentified phylum (TM7, TM6, OP11 and WS3) were low (see, Table 1 and Fig. 4).

**Analysis of Bacterial Species Diversity Index**

In order to analyze bacterial species diversity in the down welling, mixed welling and up welling points, operational taxonomic unit (OTUs) and biotic diversity index (ACE, Chao1, JackKnife, NPShannon and Shannon) were analyzed (Kim et al., 2014c). In the mixed welling point, only JackKnife index was low and the rest of the diversity indices were high. In the up welling point, all indices including OTUs were low. Thereby, its species richness and diversity were evaluated as low compared to the mixed welling and down welling points. Therefore, bacterial species richness and diversity of the three regions were the highest in the order of mixed welling point > down welling point > up welling point (Table 2).

**Detailed Analysis of Dominant Bacteria in the Down Welling Point**

Actinobacteria phylum, Acidobacteria phylum, α-Proteobacteria class and δ-Proteobacteria class, which showed high percentage in the down welling point compared to up welling point were subjected to detailed analysis. Actinobacteria phylum was identified with higher percentage in the down welling point (1,075 reads, 14.14%) than the up welling point (41 reads, 0.62%). Detailed analysis of the down welling point (1,075 reads) showed that Arthrobacter genus was dominant with 367 reads (34.14%), and high percentage of Gaiella genus, Streptomyces genus and Cryobacterium genus were observed in the down welling point compared to the up welling point (Supplementary Table S1). Acidobacteria phylum was identified with relatively high percentage in the down welling point (817 reads, 10.75%) compared to the up welling point (26 reads, 0.40%). Detailed analysis of the down welling point (817 reads) showed that Blastocatella genus (132 reads) and Koribacter genus (86 reads) were dominant, and its difference with the up welling point was analyzed (Supplementary Table S2). Also, α-Proteobacteria class had 1151 reads accounting for 46.86% among the Proteobacteria phylum in the down welling point, showing difference from 14.24% and 1.75% in the mixed welling point and up welling point, respectively. Among them, Rhizobiales order had 1006 reads showing dominance among α-Proteobacteria class, which was different from other regions (Supplementary Table S3). Detailed analysis of δ-Proteobacteria class showed 235 reads, which was relatively higher than those of the mixed welling point (128 reads) and up welling point (35 reads). Among them, Myxococcales order was dominant with 70 reads, showing difference with other regions (Supplementary Table S4).

**Table 2. Diversity index of regional bacterial diversity in the hyporheic zone**

| Diversity index | PDHS4 | PMHS8 | PUHS20 |
|-----------------|-------|-------|--------|
| OTUs*           | 3018  | 3126  | 2307   |
| ACE             | 7938.0| 8491.8| 5676.6 |
| Chao1           | 5886.3| 5991.2| 4151.7 |
| JackKnife       | 9431.4| 8771.2| 5385.6 |
| NPShannon       | 7.08  | 7.18  | 6.69   |
| Shannon         | 6.901 | 7.019 | 6.524  |

*OTUs were defined as groups of sequences sharing 00%.
**Detailed Analysis of Dominant Bacteria in the Mixed Welling Point**

Relatively high bacterial diversity was observed in the mixed welling point compared to the up welling and down welling points (see, Table 2). Analysis of the bacteria phylum (or class)-level in the mixed welling points showed high percentages of Firmicutes, Cyanobacteria, ε-Proteobacteria, Elusimicrobia, Lentisphaerae, Fusobacteria, Deinococcus-Thermus, Synergistetes, Tenericutes and unclassified (OD1, GN02, OP3, MATCR and WS5) compared to the up welling point and down welling point (see, Table 1). Particularly, Firmicutes (6.29%), Cyanobacteria (5.85%), ε-Proteobacteria (3.12%) and unclassified OD1 (2.18%) showed higher percentages than the means of the down welling point and up welling point, which were 2.34%, 0.23%, 0.58% and 0.75%, respectively. Rest of the phyla showed percentages lower than 0.5% in the mixed welling point, but they were still relatively higher than those of the down welling or up welling point (see, Table 1).

Detailed analysis of Firmicutes phylum (384 reads) in the mixed welling point showed dominance of Firmicutes phylum with 160 reads (41.67%) of *Trichococcus* genus, and 66 reads (17.19%) of *Clostridium* genus. *Clostridium* genus had 21 reads in the down welling point, but they were not present in the up welling point. In the mixed welling point, *Acetivibrio*, *Acidaminobacter*, *Anaeromusa*, *Anaerosinus*, *Anaerospobacter*, *Anaerotipes*, *Butyrivibrio*, *Carnobacterium*, *Coprococcus*, *Dendrosporobacter*, *Diaester*, *Fervidicella*, *Lactovum*, *Megamonas*, *Megasphaera*, *Oscillibacter*, *Phascolarctobacterium*, *Proteiniclasticum*, *Proteocatella*, *Pseudoflavonifractor*, *Ruminococcus*, *Saccharibacillus*, *Saccharoflavus*, *Sedimentibacter* and *Succininspira* genus were observed that were not present in the down welling point or up welling point. Additionally, *Bacillus*, *Virgibacillus*, *Alicyclobacillus*, *Tumebacillus*, *Anaerovorax*, *Paenisporosarcina*, *Deltobacter*, *Sporosarcina*, *Dorea*, *Turbicector*, *Planifilum* and *Coprococcus* genus were observed in the down welling point that were not present in the mixed welling point, and only *Arcobacter*, *Sulfurospirillum* and *Sulfurivorans* genus were observed in the up welling point among Firmicutes phylum, showing differences with the mixed welling point (Supplementary Table S5). Detailed analysis of Cyanobacteria phylum (357 reads) revealed 242 reads (67.79%) of *Prochlorococcus* genus, and 46 reads (12.97%) of *Limnococcus* genus. *Chamaesiphon* genus (128 reads, 67.72%) and unidentified genus (11 reads, 7.15%) were not observed in the down welling point and up welling point (Supplementary Table S6). Detailed analysis of ε-Proteobacteria class (189 reads) revealed *Arcobacter* genus (128 reads, 67.72%), *Sulfurospirillum* genus (57 reads, 30.16%), *Sulfurivorans* genus (2 reads, 1.06%) and unidentified ε-Proteobacteria (Campylobacteroidales order; 2 reads, 1.06%) (Supplementary Table S7).

**Detailed Analysis of Dominant Bacteria in the up Welling Point**

Proteobacteria and Bacteroidetes phylum that exist at high percentage in the up welling point were analyzed in detail. Analysis of Proteobacteria phylum at the class-level revealed that α-Proteobacteria existed in the down welling point at 20.79%, which showed 19.62% difference from 1.17% in the up welling point, and β-Proteobacteria existed in the down welling point at 13.80% and in the up welling point at 50.27%, showing 30.47% difference. Additionally, 7.63% difference was observed with γ-Proteobacteria in the down welling point (6.62%) and up welling point (14.25%), and 2.56% difference was observed with δ-Proteobacteria between the down welling point (3.09%) and up welling point (0.53%). These results showed differences in the bacterial diversity in the down welling point and up welling point within the hyporheic zone (Table 1). Detailed analysis of 3,305 reads of β-Proteobacteria that are dominant in the up welling point revealed 3,118 reads (94.34%) of Burkholderiaceae order (Supplementary Table S8). Among them, *Comamonas* genus was dramatically higher in the up welling point at 60.46% than in the down welling point (0.77% among Burkholderiaceae order), and the difference between *Malikia* genus [Down welling point (2.19%) and Up welling point (25.53%)] and *Acidovorax* genus [Down welling point (0.90%) and Up welling point (3.08%)] was also observed (Supplementary Table S9).

Detailed analysis of Bacteroidetes phylum revealed the difference between the up welling point and down welling point in Flavobacteria class and Sphingobacteria class. Flavobacteria class was dominant in the up welling point at 1,645 reads among 1,766 reads of Bacteroidetes phylum, accounting for 93.15%, but it showed 52 reads (11.50%) in the down welling point among 425 reads of Bacteroidetes phylum, showing difference with the up welling point (Supplementary Table S3). Detailed analysis of 1,645 reads of Flavobacteria class showed the dominance of *Flavobacterium* genus at 1,456 reads (88.51%). Sphingobacteria class accounted for 372 reads (82.30%) among Bacteroidetes phylum in the down welling point but had only 56 reads in the up welling point, showing difference in the bacterial communities between the down welling and up welling points (Supplementary Table S10).

**Conclusion**

Quantitative changes of the hyporheic zone were examined by installing a piezometer on the site, and a total of 20,242 reads were analyzed using a pyrosequencing assay to investigate the diversity of bacterial communities. Proteobacteria (55.1%) were overall dominant in the hyporheic zone, and Bacteroidetes (16.3%), Actinobacteria (7.1%) and other bacteria phylum (Firmicutes, Cyanobacteria, Chlororflexi, Planctomycetes and unclassified phylum OD1) were identified. Also, the hyporheic zone was divided into 3 points – down welling point, mixed welling point and up welling point – through vertical hydraulic gradient, and the bacterial communities were compared and analyzed. In the down welling point, α-Proteobacteria class, δ-Proteobacteria class, Actinobacteria phylum and Acidobacteria phylum were relatively high compared to mixed welling point and up welling point. *Arthrobacter* genus in the Actinobacteria phylum, *Blastocatella* genus in the Acidobacteria class, *Rhizobiales* order in the α-Proteobacteria class, and *Myxococcales* order in the δ-Proteobacteria class were dominant, and the difference between mixed welling point and up welling point were observed. In the mixed welling point, Firmicutes phylum, *Cyanobacteria* phylum and ε-Proteobacteria class were identified with higher percentage compared to the down welling point and up welling point. *Trichococcus* genus in the Firmicutes phylum, *Prochlorococcus* genus in the Cyanobacteria phylum and *Arcobacter* genus in the ε-Proteobacteria class were dominant, and the difference between
down welling point and up welling point was observed. In the mixed welling point, various species were identified that were not present in the up and down welling points.

In the up welling point, Proteobacteria and Bacteroidetes phylum were dominant, and the sum of two phyla was 93.67% indicating high dominance. Especially in β-Proteobacteria of Proteobacteria and Flavobacteria class of Bacteroidetes, which showed far higher dominance compared to the down welling point and mixed welling point, Comamonas genus and Flavobacterium genus were identified with high percentage, respectively, showing differences between the down welling point and mixed welling point. When the species diversity index was additionally analyzed based on the pyrosequencing data, richness and diversity of species were observed to be highest in the order of mixed welling point > down welling point > up welling point. Hence, quantitative changes and characteristics of the hyporheic zone were analyzed to have an effect on the resident bacterial communities.

The composition of bacterial communities was influenced by the direction of the vertical component of hyporheic flux. The indexes of bacterial diversity such as OUT, ACE, Chol, NP Shannon and Shannon were low in the region where surface water or ground water were dominant, respectively. However, the indexes were the highest for the sections influenced by both surface water and groundwater.

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### Table S1. Detailed analysis of Actinobacteria genus in hyporheic zones

| Genus          | Number of reads | PDHS4 | PMHS8 | PUHS20 |
|----------------|-----------------|-------|-------|--------|
| 4P003540_g     | 2               | 0     | 0     | 0      |
| AB021325_g     | 4               | 3     | 0     | 0      |
| AB240334_g     | 3               | 0     | 0     | 0      |
| AB245397_g     | 2               | 0     | 0     | 0      |
| AB630582_g     | 17              | 0     | 0     | 0      |
| Acl_B_g        | 0               | 1     | 0     | 0      |
| Aciditerrimonas| 1               | 0     | 0     | 0      |
| Actinoallomurus| 2               | 0     | 0     | 0      |
| Actinomadura   | 1               | 0     | 0     | 0      |
| Actinoplanes   | 4               | 6     | 0     | 0      |
| Aeromicrobium  | 1               | 0     | 0     | 0      |
| AF408985_g     | 1               | 0     | 0     | 0      |
| AF498716_g     | 2               | 0     | 0     | 0      |
| AJ507468_g     | 0               | 1     | 0     | 0      |
| AJ863193_g     | 1               | 0     | 0     | 0      |
| Alpinimonas    | 6               | 8     | 0     | 0      |
| AM277063_g     | 1               | 1     | 0     | 0      |
| AM991247_g     | 110             | 1     | 1     | 1      |
| Amnibacterium  | 1               | 0     | 0     | 0      |
| Angustibacter  | 2               | 0     | 0     | 0      |
| Aquiluna       | 2               | 29    | 2     | 0      |
| Arthrobacter   | 367             | 15    | 4     | 0      |
| Asanoa         | 1               | 0     | 0     | 0      |
| AY234624_g     | 0               | 0     | 1     | 0      |
| Brooklawnia    | 2               | 0     | 0     | 0      |
| Cellulomonas   | 0               | 1     | 0     | 0      |
| Chryseobacterium| 0              | 1     | 0     | 0      |
| Collinsella    | 0               | 0     | 2     | 0      |
| Conexibacter   | 7               | 0     | 0     | 0      |
| Corynebacterium| 0               | 1     | 0     | 0      |
| Cryobacterium  | 25              | 0     | 0     | 0      |
| Demequina      | 0               | 0     | 1     | 0      |
| DQ125551_g     | 2               | 0     | 1     | 0      |
| EF016795_g     | 1               | 0     | 0     | 0      |
| EF018137_g     | 0               | 1     | 0     | 0      |
| EF516392_g     | 1               | 1     | 0     | 0      |
| EF516411_g     | 1               | 0     | 0     | 0      |

### Table S1. (continued)

| Genus          | Number of reads | PDHS4 | PMHS8 | PUHS20 |
|----------------|-----------------|-------|-------|--------|
| EF516773_g     | 1               | 0     | 1     | 0      |
| EF520360_g     | 2               | 0     | 0     | 0      |
| EF584530_g     | 0               | 0     | 1     | 0      |
| EF632905_g     | 10              | 0     | 0     | 0      |
| EU289467_g     | 1               | 0     | 0     | 0      |
| EU335168_g     | 1               | 0     | 0     | 0      |
| EU374107_g     | 0               | 1     | 0     | 0      |
| EU801210_g     | 2               | 5     | 0     | 0      |
| EU861899_g     | 14              | 0     | 0     | 0      |
| EU861937_g     | 3               | 0     | 0     | 0      |
| EU980607_g     | 2               | 0     | 0     | 0      |
| FJ478790_g     | 1               | 0     | 0     | 0      |
| FJ478799_g     | 25              | 0     | 2     | 0      |
| FM209069_g     | 3               | 0     | 1     | 0      |
| FM886842_g     | 1               | 0     | 1     | 0      |
| FN436189_g     | 1               | 0     | 0     | 0      |
| FN554394_g     | 5               | 0     | 0     | 0      |
| FN687458_g     | 6               | 0     | 0     | 0      |
| FN811204_g     | 10              | 0     | 0     | 0      |
| Friedmanniella  | 1               | 0     | 0     | 0      |
| Frigoribacterium| 0              | 1     | 0     | 0      |
| Gaiella         | 95              | 5     | 1     | 0      |
| Gordonibacter   | 0               | 2     | 0     | 0      |
| GQ387490_g     | 34              | 26    | 0     | 0      |
| GQ396807_g     | 4               | 1     | 0     | 0      |
| GQ396959_g     | 2               | 0     | 1     | 0      |
| GQ487899_g     | 3               | 0     | 0     | 0      |
| GU305765_g     | 13              | 0     | 1     | 0      |
| Herbiconiux     | 0               | 1     | 0     | 0      |
| HM445971_g     | 0               | 4     | 0     | 0      |
| HM748665_g     | 6               | 0     | 0     | 0      |
| HQ674860_g     | 1               | 1     | 0     | 0      |
| HQ864103_g     | 0               | 1     | 0     | 0      |
| HQ910322_g     | 1               | 0     | 0     | 0      |
| Humibacillus    | 1               | 0     | 0     | 0      |
| Ilumatobacter   | 6               | 0     | 0     | 0      |
| Jatrophihabitans| 8               | 0     | 0     | 0      |
### Table S1. (continued)

| Genus              | Number of reads | PDHS4 | PMHS8 | PUHS20 |
|--------------------|-----------------|-------|-------|--------|
| JN588609_g         | 2               | 0     | 0     |        |
| Kitasatospora      | 11              | 1     | 0     |        |
| Knoellia           | 1               | 0     | 0     |        |
| Leifsonia          | 0               | 0     | 2     |        |
| Leucobacter        | 0               | 1     | 0     |        |
| Longispora         | 1               | 0     | 0     |        |
| Lysinimonas        | 1               | 0     | 0     |        |
| Marmoricola        | 1               | 0     | 0     |        |
| Microbacterium     | 9               | 5     | 0     |        |
| Micrococcus        | 0               | 1     | 0     |        |
| Microlunatus       | 2               | 0     | 0     |        |
| Micromonospora     | 2               | 1     | 0     |        |
| Microterricola     | 3               | 1     | 0     |        |
| Mycobacterium      | 21              | 0     | 4     |        |
| Nakamuraella       | 9               | 0     | 0     |        |
| Nocardia           | 2               | 0     | 0     |        |
| Nocardioides       | 24              | 5     | 0     |        |
| Nocardiosis        | 1               | 0     | 0     |        |
| Nonomuraea         | 2               | 0     | 0     |        |
| Oryzihumus         | 27              | 1     | 0     |        |
| Phycicoccus        | 20              | 0     | 0     |        |
| Planktoluna        | 2               | 6     | 0     |        |
| Planktophila       | 12              | 208   | 7     |        |
| Propioniciclava    | 2               | 2     | 2     |        |
| Propionicimonas    | 3               | 1     | 2     |        |
| Rhodococcus        | 6               | 1     | 0     |        |
| Rhodoluta          | 9               | 43    | 2     |        |
| Saccharomonospora  | 1               | 0     | 0     |        |
| Salinibacter       | 2               | 1     | 0     |        |
| Sanguibacter       | 0               | 2     | 0     |        |
| Sinomomas          | 1               | 0     | 0     |        |
| Streptomyces       | 44              | 0     | 0     |        |
| Streptosporangium  | 1               | 0     | 0     |        |
| Terrabacter        | 14              | 0     | 0     |        |
| Tetraspera         | 5               | 2     | 1     |        |
| Tropheryma         | 1               | 0     | 0     |        |
| **Total**          | 1,075           | 400   | 41    |        |

### Table S2. Detailed analysis of Acidobacteria genus in hyporheic zones

| Genus            | Number of reads | PDHS4 | PMHS8 | PUHS20 |
|------------------|-----------------|-------|-------|--------|
| AB179509_g       | 2               | 0     | 0     |        |
| AB240310_g       | 5               | 0     | 0     |        |
| AB252946_g       | 5               | 0     | 0     |        |
| AB355055_g       | 9               | 0     | 0     |        |
| Acidobacterium   | 4               | 0     | 0     |        |
| AF234701_g       | 3               | 0     | 0     |        |
| AM180888_g       | 7               | 0     | 0     |        |
| AY212581_g       | 1               | 0     | 0     |        |
| AY281358_g       | 52              | 0     | 0     |        |
| AY921986_g       | 1               | 0     | 0     |        |
| Blastocatella    | 132             | 6     | 0     |        |
| Bryobacter       | 2               | 0     | 0     |        |
| DQ083303_g       | 0               | 1     | 0     |        |
| DQ139454_g       | 1               | 0     | 0     |        |
| DQ451510_g       | 5               | 0     | 0     |        |
| DQ453805_g       | 15              | 0     | 0     |        |
| DQ648914_g       | 8               | 0     | 0     |        |
| DQ829648_g       | 4               | 0     | 0     |        |
| DQ833485_g       | 4               | 0     | 0     |        |
| DQ906882_g       | 1               | 0     | 0     |        |
| Edaphobacter     | 2               | 0     | 0     |        |
| EF492943_g       | 1               | 0     | 0     |        |
| EF999370_g       | 0               | 1     | 0     |        |
| EU192989_g       | 4               | 0     | 0     |        |
| EU335275_g       | 15              | 1     | 0     |        |
| EU373937_g       | 1               | 0     | 0     |        |
| EU445199_g       | 11              | 0     | 0     |        |
| EU652507_g       | 4               | 0     | 0     |        |
| EU676413_g       | 1               | 0     | 0     |        |
| EU686607_g       | 16              | 0     | 0     |        |
| EU861837_g       | 1               | 0     | 0     |        |
| EU881271_g       | 1               | 0     | 0     |        |
| FJ416115_g       | 2               | 0     | 0     |        |
| FJ478829_g       | 5               | 0     | 0     |        |
| FJ478953_g       | 7               | 0     | 0     |        |
| FJ479026_g       | 8               | 0     | 0     |        |
| FJ479597_g       | 2               | 0     | 0     |        |
| GQ214092_g       | 1               | 0     | 0     |        |
| GQ302594_g       | 2               | 0     | 1     |        |
Table S2. Detailed analysis of α-Proteobacteria order in hyporheic zones

| Order                | % (number of reads) | PDHS4 | PMHS8 | PUHS20 |
|----------------------|---------------------|-------|-------|--------|
| Caulobacterales      | 2.97 (133)          | 17.03 (80) | 27.28 (21) |
| Rhodobacterales      | 8.41 (166)          | 10.64 (50) | 6.49 (5) |
| Rhodospirillales     | 1.58 (25)           | 12.77 (60) | 9.09 (7) |
| Sphingomonadales     | 12.27 (194)         | 30 (141) | 20.77 (16) |
| Total                | 1,581               | 470 | 77 |

Table S3. Detailed analysis of β-Proteobacteria order in hyporheic zones

| Order                | % (number of reads) | PDHS4 | PMHS8 | PUHS20 |
|----------------------|---------------------|-------|-------|--------|
| Deferrisoma_o        | 0.42 (1)            | 0.00 (0) | 0.00 (0) |
| Desulfobacca_o       | 0.00 (0)            | 0.00 (0) | 2.85 (1) |
| Desulfobacterales    | 0.85 (2)            | 0.78 (1) | 0.00 (0) |
| Desulfobulbaceae_o   | 0.00 (0)            | 0.78 (1) | 2.85 (1) |
| Desulfubacterales    | 0.00 (0)            | 3.91 (5) | 2.85 (1) |
| Desulfuromonadales   | 5.11 (12)           | 3.91 (5) | 2.85 (1) |
| EF574244_o           | 0.85 (2)            | 0.78 (1) | 0.00 (0) |
| EU335163_o           | 0.42 (1)            | 0.00 (0) | 0.00 (0) |
| EU617842_o           | 0.85 (2)            | 0.00 (0) | 0.00 (0) |
| EU861868_o           | 1.28 (3)            | 0.78 (1) | 0.00 (0) |
| FM253572_o           | 23.84 (56)          | 2.34 (3) | 0.00 (0) |
| GUS67808_o           | 0.42 (1)            | 2.34 (3) | 0.00 (0) |
| HM243977_o           | 0.42 (1)            | 0.78 (1) | 0.00 (0) |
| Myxococcales         | 29.79 (70)          | 21.88 (28) | 8.57 (3) |
| OM27                 | 0.42 (1)            | 0.78 (1) | 2.85 (1) |
| SAR324               | 0.00 (0)            | 0.78 (1) | 0.00 (0) |
| Spirobacillus_o      | 8.09 (19)           | 17.19 (22) | 14.3 (5) |
| Syntrophobacterales  | 0.42 (1)            | 0.00 (0) | 0.00 (0) |
| Syntrophohabdabaceae_o | 0.42 (1)      | 0.00 (0) | 0.00 (0) |
| Total                | 235                 | 128 | 35 |
| Genus         | Number of reads | PDHS4 | PMHS8 | PUHS20 |
|--------------|-----------------|-------|-------|--------|
| AB237727_g   |                 | 0     | 1     | 0      |
| Acetivibrio  |                 | 0     | 1     | 0      |
| Acidaminobacter |             | 0     | 3     | 0      |
| AF304435_g   |                 | 0     | 2     | 0      |
| AM183112_g   |                 | 0     | 1     | 0      |
| AM406061_g   |                 | 0     | 2     | 0      |
| AM500748_g   |                 | 0     | 3     | 0      |
| Anaeromusa   |                 | 0     | 1     | 0      |
| Anaerobius   |                 | 0     | 1     | 0      |
| Anaerospirillum |             | 0     | 2     | 0      |
| Anaerostipes |                 | 0     | 3     | 0      |
| AY532583_g   |                 | 0     | 1     | 0      |
| Blautia      |                 | 3     | 2     | 0      |
| Butyriccoccus|                 | 0     | 1     | 0      |
| Carnobacterium|               | 0     | 2     | 0      |
| Clostridium  | 11   | 53    | 0      |
| Clostridium g22 |         | 0     | 3     | 0      |
| Clostridium g25 |         | 0     | 2     | 0      |
| Clostridium g4 |             | 10    | 6     | 0      |
| Clostridium g7 |             | 0     | 1     | 0      |
| Clostridium g9 |             | 0     | 1     | 0      |
| Coprococcus g2 |             | 0     | 1     | 0      |
| D16279_g     |                 | 0     | 1     | 0      |
| Dendrospirillum |             | 0     | 1     | 0      |
| Dialister   |                 | 0     | 1     | 0      |
| DQ071484_g   |                 | 1     | 3     | 0      |
| DQ206415_g   |                 | 0     | 1     | 0      |
| DQ777915_g   |                 | 0     | 1     | 0      |
| EF403870_g   |                 | 1     | 1     | 0      |
| EF404752_g   |                 | 0     | 1     | 0      |
| EU753611_g   |                 | 0     | 1     | 0      |
| EU845632_g   |                 | 0     | 4     | 0      |
| Eubacterium g2 |             | 2     | 2     | 0      |
| Exiguobacterium|           | 5     | 4     | 0      |
| Faecalibacterium |        | 1     | 4     | 0      |
| Fervidicella |                 | 0     | 2     | 0      |
| FJ799146_g   |                 | 0     | 1     | 0      |
| FJ880290_g   |                 | 0     | 2     | 0      |
| GQ406188_g   |                 | 0     | 3     | 0      |
| GQ871709_g   |                 | 1     | 1     | 0      |
| GU112184_g   |                 | 0     | 2     | 0      |
| GU324404_g   |                 | 1     | 1     | 0      |
| GU454868_g   |                 | 0     | 1     | 0      |
| HM123979_g   |                 | 0     | 1     | 0      |
| HQ132444_g   |                 | 0     | 1     | 0      |
| HQ904156_g   |                 | 0     | 2     | 0      |
| Lactobacillus|                 | 2     | 6     | 0      |
| Lactococcus  |                 | 3     | 12    | 0      |
| Lactovum     |                 | 0     | 1     | 0      |
| Leuconostoc  |                 | 1     | 7     | 0      |
| Megamonas    |                 | 0     | 7     | 0      |

**Table S5. Detailed analysis of Firmicutes genus in hyporheic zones (continued)**

| Genus         | Number of reads | PDHS4 | PMHS8 | PUHS20 |
|--------------|-----------------|-------|-------|--------|
| Bacillus     | 27   | 0     | 0      |
| EF404788_g   |                 | 1     | 0     | 0      |
| Virgibacillus|                 | 1     | 0     | 0      |
| Alcycolbacillus|             | 1     | 0     | 0      |
| Tumebacillus |                 | 3     | 0     | 0      |
| AB239481_g   |                 | 2     | 0     | 0      |
| Anaerovorax  |                 | 1     | 0     | 0      |
| Paenisporesarcina |         | 13    | 0     | 0      |
| Eubacterium g5 |             | 2     | 0     | 0      |
| Eubacterium g8 |             | 2     | 0     | 0      |
| Sporosarcina |                 | 1     | 0     | 0      |
| DQ394658_g   |                 | 1     | 0     | 0      |
| Dorea        |                 | 1     | 0     | 0      |
| Turicibacter |                 | 1     | 0     | 0      |
| HQ716403_g   |                 | 1     | 0     | 0      |
| AB298726_g   |                 | 1     | 0     | 0      |
| DQ887956_g   |                 | 1     | 0     | 0      |
| Planifilum   |                 | 1     | 0     | 0      |
| Coprococcus  |                 | 2     | 0     | 0      |
| AM500798_g   |                 | 1     | 0     | 0      |
| AM277340_g   |                 | 2     | 0     | 0      |
| GQ897654_g   |                 | 3     | 0     | 0      |
| Sulphurivorax|                 | 0     | 0     | 1      |
| Arcobacter   |                 | 0     | 0     | 34     |
| Sulphurosprillum |           | 0     | 0     | 3      |
| **Total**    | 147  | 384   | 38     |
Table S6. Detailed analysis of Cyanobacteria genus in hyporheic zones

| Genus         | Number of reads |
|---------------|-----------------|
|               | PDHS4 | PMHS8 | PUHS20 |
| AB240501_g    | 0     | 1     | 1      |
| AB354619_g    | 0     | 1     | 2      |
| AJ536844_g    | 0     | 1     | 0      |
| AJ583204_g    | 0     | 1     | 0      |
| AY375144_g    | 0     | 5     | 3      |
| Chamaesiphon  | 0     | 16    | 0      |
| Chroococcus_g2| 0     | 1     | 0      |
| DQ128639_g    | 1     | 1     | 2      |
| EF032660_g    | 0     | 2     | 0      |
| EF580987_g    | 0     | 1     | 0      |
| EU101276_g    | 0     | 3     | 0      |
| EU491779_g    | 0     | 1     | 0      |
| FJ236035_g    | 0     | 1     | 0      |
| FJ425638_g    | 0     | 1     | 0      |
| FJ625338_g    | 1     | 1     | 0      |
| GQ451200_g    | 0     | 2     | 2      |
| HM124232_g    | 0     | 1     | 0      |
| JF733399_g    | 0     | 2     | 0      |
| Limnococcus   | 0     | 46    | 0      |
| Limnothrix    | 0     | 3     | 1      |
| Merismopedia  | 0     | 3     | 0      |
| Microcystis   | 0     | 3     | 0      |
| PCC7335_g     | 0     | 1     | 0      |
| Phormidium_g6 | 0     | 1     | 0      |
| Pleurocapsa   | 0     | 13    | 0      |
| Prochlorococcus| 28   | 242   | 3      |
| Snowella      | 0     | 2     | 0      |
| Vampirovibrio | 0     | 1     | 0      |
| FJ543055_g    | 1     | 0     | 0      |
| GU444060_g    | 5     | 0     | 0      |
| EU1134274_g   | 1     | 0     | 0      |
| AY493962_g    | 4     | 0     | 0      |
| EU753634_g    | 5     | 0     | 0      |
| AJ544083_g    | 2     | 0     | 0      |
| JF417809_g    | 1     | 0     | 0      |
| Woronichinia  | 2     | 0     | 0      |
| Nostoc_g1     | 1     | 0     | 0      |
| Anabaena_g2   | 1     | 0     | 0      |
| EF018129_g    | 2     | 0     | 0      |
| Nostoc        | 2     | 0     | 0      |
| JF737898_g    | 4     | 0     | 0      |
| Microcoleus   | 1     | 0     | 0      |
| Cylindrospermum| 1    | 0     | 0      |
| FJ810552_g    | 0     | 0     | 1      |
| Total         | 63    | 357   | 15     |

Table S7. Detailed analysis of ε-Proteobacteria genus in hyporheic zones

| Genus         | Number of reads |
|---------------|-----------------|
|               | PDHS4 | PMHS8 | PUHS20 |
| Arcobacter    | 4     | 128   | 34     |
| Sulfurospirillum| 1    | 57    | 3      |
| FJ747790_g    | 0     | 2     | 0      |
| Sulfuricurvum | 1     | 2     | 1      |
| Total         | 6     | 189   | 38     |

Table S8. Detailed analysis of β-Proteobacteria genus in hyporheic zones

| Genus         | % (number of reads) |
|---------------|---------------------|
|               | PDHS4 | PMHS8 | PUHS20 |
| AB294329_o    | 0.09 (1) | 0.00 (0) | 0.00 (0) |
| AB308366_o    | 7.05 (74) | 0.35 (6) | 0.15 (5) |
| Burkholderiales| 74.0 (776) | 83.42 (1,444) | 94.34 (3,118) |
| DQ009366_o    | 1.33 (14) | 0.17 (3) | 0.03 (1) |
| DQ95705_o     | 4.1 (43) | 0.46 (8) | 0.06 (2) |
| DQ469205_o    | 0.00 (0) | 0.06 (1) | 0.03 (1) |
| EU786132_o    | 4.39 (46) | 0.4 (7) | 0.15 (5) |
| Ferritrophicales| 0.00 (0) | 0.06 (1) | 0.00 (0) |
| Gallionellales| 1.53 (16) | 0.29 (5) | 0.00 (0) |
| Methylophilales| 2.76 (29) | 1.33 (23) | 0.06 (2) |
| Neisseriales | 1.04 (11) | 5.03 (87) | 2.23 (74) |
| Nitrosonomadales| 0.57 (6) | 0.00 (0) | 0.00 (0) |
| Rhodocyclales| 1.14 (12) | 4.16 (72) | 1.42 (47) |
| Sterolibacterium_o| 1.71 (18) | 1.5 (26) | 0.94 (31) |
| Thiobacillus_o| 0.00 (0) | 0.06 (1) | 0.00 (0) |
| Thiobacter_o  | 0.09 (1) | 0.12 (2) | 0.00 (0) |
| Zoogloea_o    | 0.19 (2) | 2.59 (45) | 0.57 (19) |
| Total         | 1.049 | 1.731 | 3.305 |
### Table S9. Detailed analysis of Burkholderiales order in hyporheic zones

| Order | Number of reads | PDHS4 | PMHS8 | PUHS20 |
|-------|-----------------|-------|-------|--------|
| 4P000609.g | 1 | 1 | 5 | |
| 4P002413.g | 0 | 2 | 0 | |
| 4P003243.g | 0 | 2 | 0 | |
| AB076847.g | 2 | 1 | 1 | |
| AB240255.g | 49 | 0 | 0 | |
| AB672287.g | 9 | 1 | 0 | |
| Acidovorax | 7 | 49 | 96 | |
| Actimicrobium | 3 | 3 | 0 | |
| AF236011.g | 0 | 2 | 2 | |
| AF289156.g | 0 | 5 | 0 | |
| AF418942.g | 13 | 33 | 11 | |
| AJ565430.g | 2 | 2 | 1 | |
| AJ575697.g | 13 | 7 | 4 | |
| Albidiferax | 11 | 25 | 28 | |
| AM777983.g | 55 | 5 | 0 | |
| Aquabacterium | 4 | 17 | 17 | |
| Aquinculum | 4 | 1 | 0 | |
| AY218568.g | 6 | 7 | 19 | |
| AY234747.g | 182 | 1 | 0 | |
| AY328716.g | 0 | 2 | 0 | |
| Azohydromonas | 1 | 0 | 0 | |
| Bordetella | 0 | 2 | 0 | |
| Brachymonas | 0 | 4 | 0 | |
| Burkholderia | 18 | 0 | 1 | |
| Caenimonas | 10 | 1 | 0 | |
| Collimonas | 0 | 1 | 0 | |
| Comamonas | 6 | 322 | 1985 | |
| Cupriavidus | 4 | 1 | 0 | |
| Curvibacter | 6 | 35 | 9 | |
| Delftia | 0 | 1 | 0 | |
| Derxia | 0 | 0 | 4 | |
| DQ450176_g | 0 | 4 | 0 | |
| DQ469209_g | 0 | 1 | 0 | |
| DQ520167_g | 6 | 7 | 1 | |
| Duganella | 5 | 43 | 5 | |
| EF580985.g | 1 | 1 | 8 | |
| EU104128.g | 0 | 2 | 4 | |
| EU636042.g | 28 | 2 | 1 | |
| EU735703.g | 1 | 1 | 0 | |
| EU800906.g | 0 | 1 | 0 | |
| EU801607.g | 5 | 2 | 0 | |
| EU937973.g | 8 | 3 | 3 | |
| FJ517712.g | 9 | 8 | 4 | |
| FJ624877.g | 2 | 0 | 1 | |
| FJ660572.g | 0 | 4 | 5 | |
| FJ755754.g | 1 | 3 | 0 | |

### Table S9. (continued)

| Order | Number of reads | PDHS4 | PMHS8 | PUHS20 |
|-------|-----------------|-------|-------|--------|
| FM209333_g | 0 | 6 | 0 | |
| FN668029_g | 0 | 4 | 1 | |
| Giesbergeria | 2 | 3 | 8 | |
| GU134931_g | 1 | 11 | 0 | |
| Herbaspirillum | 47 | 6 | 2 | |
| Herminiimonas | 29 | 2 | 0 | |
| HM445266_g | 2 | 0 | 0 | |
| HQ166641_g | 7 | 0 | 0 | |
| HQ178927_g | 6 | 1 | 0 | |
| HQ324854_g | 1 | 2 | 1 | |
| HQ910358_g | 1 | 0 | 0 | |
| Hydrogenophaga | 7 | 19 | 20 | |
| Ideonella | 9 | 23 | 4 | |
| Inhella | 1 | 3 | 0 | |
| Janthinobacterium | 12 | 30 | 25 | |
| JF697503_g | 1 | 3 | 1 | |
| JN679217_g | 4 | 18 | 1 | |
| Kinneretia | 1 | 1 | 0 | |
| Leptothrix | 8 | 24 | 11 | |
| Limnobacter | 0 | 2 | 0 | |
| Limnobahitans | 11 | 108 | 2 | |
| Malibiscia | 17 | 331 | 796 | |
| Massilia | 21 | 18 | 8 | |
| Methylibium | 1 | 3 | 0 | |
| Mitsukuria | 0 | 0 | 1 | |
| Oxalobacter | 0 | 1 | 0 | |
| Pandoraea | 0 | 1 | 0 | |
| Paucibacter | 2 | 12 | 8 | |
| Pelomonas | 0 | 5 | 4 | |
| Piscinibacter | 2 | 0 | 1 | |
| Pseudomonas | 38 | 12 | 7 | |
| Polynucleobacter | 9 | 86 | 5 | |
| Pseudomonas | 0 | 2 | 0 | |
| Pseudorhodoferax | 2 | 3 | 17 | |
| Raslonia | 2 | 5 | 0 | |
| Ramlibacter | 7 | 1 | 2 | |
| Rhizobacter | 9 | 10 | 2 | |
| Rhodoferax | 5 | 10 | 10 | |
| Rivibacter | 1 | 1 | 0 | |
| Simplicispira | 4 | 5 | 2 | |
| Sphaerotilus | 4 | 14 | 2 | |
| Sutterella | 0 | 2 | 0 | |
| Undibacterium | 5 | 24 | 45 | |
| Variovorax | 5 | 1 | 2 | |
| Xenophilus | 19 | 12 | 0 | |
| Z93984_g | 0 | 2 | 4 | |
| Total | 1,049 | 1,731 | 3,305 |
Table S10. Detailed analysis of Bacteroidetes genus in hyporheic zones

| Genus         | PDHS4  | PMHS8  | PUHS20 |
|---------------|--------|--------|--------|
| Bacteroidia   | 1.33 (6) | 31.96 (326) | 3.4 (60) |
| Cytophagia    | 4.87 (22) | 6.18 (63) | 0.28 (5) |
| Flavobacteria | 11.5 (52) | 36.76 (375) | 93.15 (1,645) |
| Sphingobacteria | 82.3 (372) | 25.1 (256) | 3.17 (56) |
| Total         | 452    | 1,020  | 1,766  |