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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 constitutes 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 streambed 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, Haeon 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.

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

where Δh is the difference in water levels of the piezometers and stream water, Δl 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 μ m 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

Figure 2. Annual precipitation in the study area from 2005 to 2014.

Figure 3. (a) Installed piezometers and (b) hyporheic water samplings.

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 and compared with other known sequences that were available 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	10.75 (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
OP11†	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.

Figure 4. Regional bacterial diversity in the hyporheic zone (phylum-level).

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, NPS Shannon 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
NPS Shannon	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*, *Anaerosporebacter*, *Anaerostipes*, *Butyricicoccus*, *Carnobacterium*, *Coproccoccus*, *Dendrosporobacter*, *Dialister*, *Fervidicella*, *Lactovum*, *Megamonas*, *Megasphaera*, *Oscillibacter*, *Phascolarctobacterium*, *Proteiniclasticum*, *Proteocatella*, *Pseudoflavonifractor*, *Ruminococcus*, *Saccharibacillus*, *Saccharofermentans*, *Sedimentibacter* and *Succinispira* genus were observed that were not present in the down welling point or up welling point. Additionally, *Bacillus*, *Virgibacillus*, *Alicyclobacillus*, *Tumebacillus*, *Anaerovorax*, *Paenisporosarcina*, *Dethiobacter*, *Sporosarcina*, *Dorea*, *Turicibacter*, *Planifilum* and *Coproccoccus* genus were observed in the down welling point that were not present in the mixed welling point, and only *Arcobacter*, *Sulfurospirillum* and *Sulfuricurvum* 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 *Limnococcus* (46 reads), *Chamaesiphon* (16 reads), *Pleurocapsa* (13 reads), *Snowella* (2 reads) and *Vampirovibrio* genus (1 read) that 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%), *Sulfuricurvum* genus (2 reads, 1.06%) and unidentified ϵ -Proteobacteria (Campylobacterales order; 2 reads, 1.06%) (Supplementary Table S7).

Detailed Analysis of Dominant Bacteria in the up Well-ing 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 Burkholderiales 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 Burkholderiales 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 (16.5%), 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.

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, Chao1, 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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References

- Bartsch, S., Frei, S., Ruidisch, M., Shope, C.L., Peiffer, S., Kim, B., and Fleckenstein, J.H., 2014, River-aquifer exchange fluxes under monsoonal climate conditions. *Journal of Hydrology*, v. 509, pp. 601–614.
- Boulton, A.J., Findlay, S., Marmonier, P., Stanley, E.H., and Valett, H.M., 1998, The functional significance of the hyporheic zone in streams and rivers. *Annual Review of Ecology and Systematics*, v. 29, 59–81.
- Boulton, A.J., Detry, T., Kasahara, T., Mutz, M., and Stanford, J.A., 2010, Ecology and management of the hyporheic zone: stream-groundwater interactions of running waters and their floodplains. *Journal of the North American Benthological Society*, v. 29, pp. 26–40.
- Cardenas, M.B., Wilson, J.L., and Zlotnik, V.A., 2004, Impact of heterogeneity, bed forms, and stream curvature on subchannel hyporheic exchange. *Water Resources Research*, v. 40, pp. 1–13.
- Cardenas, M.B. and Wilson, J.L., 2007, Hydrodynamics of coupled flow above and below a sediment–water interface with triangular bedforms. *Advances in water resources*, v. 30, pp. 301–313.
- Endreny, T., Lautz, L., and Siegel, D.I., 2011, Hyporheic flow path response to hydraulic jumps at river steps: Flume and hydrodynamic models. *Water Resources Research*, v.47, pp. 1–10.
- Findlay, S., 1995, Importance of surface-subsurface exchange in stream ecosystems: The hyporheic zone. *Limnology and oceanography*, v. 40, pp. 159–164.
- Fleckenstein, J.H., Krause, S., Hannah, D.M., and Boano, F., 2010, Groundwater-surface water interactions: New methods and models to improve understanding of processes and dynamics. *Advances in Water Resources*, v. 33, pp. 1291–1295.
- Griebler, C. and Avramov, M., 2015, Groundwater ecosystem services: a review. *Freshwater Science*, v. 34, pp. 355–367.
- Hassan, M.A., Tonina, D., Beckie, R.D., and Kinnear, M., 2015, The effects of discharge and slope on hyporheic flow in step-pool morphologies. *Hydrological Processes*, v. 29, pp. 419–433.
- Hyun, Y., Kim, H., Lee, S.S., and Lee, K.K., 2011, Characterizing streambed water fluxes using temperature and head data on multiple spatial scales in Munsan stream, South Korea. *Journal of Hydrology*, v.402, pp. 377–387.
- Jeong, J.J., Bartsch, S., Fleckenstein, J.H., Matzner, E., Tenhunen, J.D., Lee, S.D., and Park, J.H., 2012, Differential storm responses of dissolved and particulate organic carbon in a mountainous headwater stream, investigated by high-frequency, in situ optical measurements. *Journal of Geophysical Research: Biogeosciences*, v. 117, pp. G03013.
- Jones, I., Growns, I., Arnold, A., McCall, S., and Bowes, M., 2015, The effects of increased flow and fine sediment on hyporheic invertebrates and nutrients in stream mesocosms. *Freshwater Biology*, v. 60, pp. 813–826.
- Kasahara, T. and Wondzell, S.M., 2003, Geomorphic controls on hyporheic exchange flow in mountain streams. *Water Resources Research*, v. 39, pp. SBH–3.
- Kettering, J., Park, J.H., Lindner, S., Lee, B., Tenhunen, J., and Kuzyakov, Y., 2012, N fluxes in an agricultural catchment under monsoon climate: a budget approach at different scales. *Agriculture, ecosystems & environment*, v.161, pp.101–111.
- Kim, H., Hyun, Y., and Lee, K.K., 2011, Hydro-ecological characterization in groundwater dependent ecosystem. *Journal of Korean Wetland Society*, v.11, pp. 1–8 (in Korean with English abstract).
- Kim, H., Lee, K.K., and Lee, J.Y., 2014a, Numerical verification of hyporheic zone depth estimation using streambed temperature. *Journal of Hydrology*, v. 511, pp. 861–869.
- Kim, H., Lee, J.Y., Park, Y., Hyun, Y., and Lee, K.K., 2014b, Groundwater and stream water exchange revealed by water chemistry in a hyporheic zone of agricultural area. *Paddy and Water Environment*, v. 12, pp. 89–101.
- Kim, H., Lee, J.Y., and Lee, K.K., 2014c, Thermal Characteristics and Bacterial Diversity of Forest Soil in the Haeen Basin of Korea. *The Scientific World Journal*, v. 2014, pp. ID247401.
- Kim, H., Kaown, D., Mayer, B., Lee, J.Y., Hyun, Y., and Lee, K.K., 2015, Identifying the sources of nitrate contamination of groundwater in an agricultural area (Haeen basin, Korea) using isotope and microbial community analyses. *Science of the Total Environment*, v. 533, pp. 566–575.
- Kim, H., Kaown, D., Mayer, B., Lee, J.Y., and Lee, K.K., 2018, Combining pyrosequencing and isotopic approaches to assess denitrification in a hyporheic zone. *Science of the Total Environment*, v. 631, pp. 755–764.
- KMA(Korean Meteorological Administration), 2015, Homepage of Korean Meteorological Administration. <http://www.kma.go.kr> [accessed on 30th October 2018].
- Lee, J.Y., 2009, Importance of hydrogeological and hydrologic studies for Haeen basin in Yanggu. *Journal of Geological Society of Korea* v. 45, pp. 405–414 (in Korean with English abstract).
- Lee, J.Y., Lee, K.S., Park, Y., Choi, H.M., and Jo, Y.J., 2013, Chemical and isotopic compositions of groundwater and stream water in a heavy agricultural basin of Korea. *Journal of the Geological Society of India*, v. 82, pp. 169–180.
- Smith, J.W.N., Bonell, M., Gibert, J., McDowell, W.H., Sudicky, E.A., Turner, J.V., and Harris, R.C., 2008, Groundwater–surface water interactions, nutrient fluxes and ecological response in river corridors:

translating science into effective environmental management. *Hydrological Processes*, v. 22, pp. 151–157.

Storey, R.G., Howard, K.W., and Williams, D.D., 2003, Factors controlling riffle-scale hyporheic exchange flows and their seasonal changes in a gaining stream: A three-dimensional groundwater flow model. *Water Resources Research*, v. 39, pp. 1–17.

Vogt, T., Schneider, P., Hahn-Woernle, L., and Cirpka, O.A., 2010, Estimation of seepage rates in a losing stream by means of fiber-optic high-resolution vertical temperature profiling. *Journal of Hydrology*, v. 380, pp. 154–164.

Westhoff, M.C., Gooseff, M.N., Bogaard, T.A., and Savenije, H.H.G., 2011, Quantifying hyporheic exchange at high spatial resolution using natural temperature variations along a first-order stream. *Water Resources Research*, v. 47, pp. 1–13.

Zimmer, M.A. and Lautz, L.K., 2015, Pre-and postrestoration assessment of stream water–groundwater interactions: effects on hydrological and chemical heterogeneity in the hyporheic zone. *Freshwater Science*, v. 34, 287–300.

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Supplementary

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

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

Table S1. (continued)

Genus	Number of reads		
	PDHS4	PMHS8	PUHS20
EF516773_g	1	0	1
EF520360_g	2	0	0
EF584530_g	0	0	1
EF632905_g	10	0	0
EU289467_g	1	0	0
EU335168_g	1	0	0
EU374107_g	0	1	0
EU801210_g	2	5	0
EU861899_g	14	0	0
EU861937_g	3	0	0
EU980607_g	2	0	0
FJ478790_g	1	0	0
FJ478799_g	25	0	2
FM209069_g	3	0	1
FM886842_g	1	0	1
FN436189_g	1	0	0
FN554394_g	5	0	0
FN687458_g	6	0	0
FN811204_g	10	0	0
Friedmanniella	1	0	0
Frigoribacterium	0	1	0
Gaiella	95	5	1
Gordonibacter	0	2	0
GQ387490_g	34	26	0
GQ396807_g	4	1	0
GQ396959_g	2	0	1
GQ487899_g	3	0	0
GU305765_g	13	0	1
Herbiconiux	0	1	0
HM445971_g	0	4	0
HM748665_g	6	0	0
HQ674860_g	1	1	0
HQ864103_g	0	1	0
HQ910322_g	1	0	0
Humibacillus	1	0	0
Ilumatobacter	6	0	0
Jatrophihabitans	8	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
Nakamurella	9	0	0
Nocardia	2	0	0
Nocardioides	24	5	0
Nocardiosis	1	0	0
Nonomuraea	2	0	0
Oryzihumus	27	1	0
Phycococcus	20	0	0
Planktoluna	2	6	0
Planktophila	12	208	7
Propioniciclava	2	2	2
Propionicismonas	3	1	2
Rhodococcus	6	1	0
Rhodoluna	9	43	2
Saccharomonospora	1	0	0
Salinibacterium	2	1	0
Sanguibacter	0	2	0
Sinomonas	1	0	0
Streptomyces	44	0	0
Streptosporangium	1	0	0
Terrabacter	14	0	0
Tetrasphaera	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. (continued)

Genus	Number of reads		
	PDHS4	PMHS8	PUHS20
GQ472805_g	7	0	1
Granulicella	3	0	0
GU127807_g	1	0	0
GU187031_g	64	3	3
GU187034_g	1	0	0
GU260705_g	79	5	2
GU727715_g	1	0	0
HM061812_g	8	1	0
HM131972_g	3	0	0
HM243779_g	2	0	0
HM243842_g	2	0	0
HM445331_g	29	3	0
HM748715_g	1	0	0
Holophaga	6	6	7
HQ010775_g	3	0	0
HQ166644_g	1	0	0
HQ190378_g	2	0	0
HQ190410_g	19	5	2
HQ190481_g	6	0	0
HQ445629_g	2	0	0
HQ445649_g	3	0	0
HQ445680_g	7	4	1
HQ445759_g	0	1	1
HQ645210_g	9	0	0
HQ864177_g	8	0	0
JF429092_g	1	0	0
JF718667_g	8	0	0
Koribacter	86	0	3
Solibacter	28	0	0
Telmatobacter	2	0	0
Z95718_g	6	0	0
Z95727_g	73	5	5
Z95729_g	1	0	0
Total	817	42	26

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

Order	% (number of reads)		
	PDHS4	PMHS8	PUHS20
AY957891_o	0.13 (2)	0.42 (2)	0 (0)
Caulobacteriales	2.97 (47)	4.68 (22)	3.9 (3)
EF573150_o	0.32 (5)	0.42 (2)	0 (0)
FJ612212_o	0 (0)	0.21 (1)	0 (0)
Kordiimonadales	0 (0)	0.21 (1)	1.3 (1)
Micavibrio_o	0.13 (2)	2.13 (10)	1.3 (1)
Parvularculales	0.06 (1)	0 (0)	0 (0)
Rhizobiales	63.63 (1006)	21.49 (101)	29.87 (23)
Rhodobacteriales	8.41 (133)	17.03 (80)	27.28 (21)
Rhodospirillales	10.50 (166)	10.64 (50)	6.49 (5)
Rickettsiales	1.58 (25)	12.77 (60)	9.09 (7)
Sphingomonadales	12.27 (194)	30 (141)	20.77 (16)
Total	1,581	470	77

Table S4. Detailed analysis of δ -Proteobacteria order in hyporheic zones

Order	% (number of reads)		
	PDHS4	PMHS8	PUHS20
AF269002_o	1.28 (3)	0.00 (0)	0.00 (0)
AY921748_o	0.00 (0)	0.78 (1)	0.00 (0)
Bdellovibrionales	2.13 (5)	10.16 (13)	5.71 (2)
CU925466_o	15.33 (36)	6.25 (8)	8.57 (3)
Deferrisoma_o	0.42 (1)	0.00 (0)	0.00 (0)
Desulfobacca_o	0.00 (0)	0.00 (0)	2.85 (1)
Desulfobacteriales	0.85 (2)	0.78 (1)	0.00 (0)
Desulfobulbaceae_o	0.00 (0)	0.78 (1)	0.00 (0)
Desulfovibrionales	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)	5.47 (7)	0.00 (0)
FJ889281_o	7.66 (18)	21.09 (27)	51.45 (18)
FM253572_o	23.84 (56)	2.34 (3)	0.00 (0)
GU567808_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)
Syntrophobacteriales	0.42 (1)	0.00 (0)	0.00 (0)
Syntrophorhabdaceae_o	0.42 (1)	0.00 (0)	0.00 (0)
Total	235	128	35

Table S5. Detailed analysis of Firmicutes genus in hyporheic zones

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
Anaerosinus	0	1	0
Anaerosporebacter	0	2	0
Anaerostipes	0	3	0
AY532583_g	0	1	0
Blautia	3	2	0
Butyricicoccus	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
Dendrosporobacter	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. (continued)

Genus	Number of reads		
	PDHS4	PMHS8	PUHS20
Megasphaera	0	1	0
Oscillibacter	0	2	0
Paenibacillus	12	2	0
Phascolarctobacterium	0	1	0
Propionispira	1	7	0
Proteiniclasticum	0	11	0
Proteocatella	0	7	0
Pseudoflavonifractor	0	1	0
Psychrobacillus	1	5	0
Psychrosinus	2	1	0
Ruminococcus	0	1	0
Ruminococcus_g2	0	3	0
Ruminococcus_g5	0	3	0
Saccharibacillus	0	1	0
Saccharofermentans	0	2	0
Sedimentibacter	0	1	0
Streptococcus	1	3	0
Subdoligranulum	1	1	0
Succinispira	0	2	0
Trichococcus	12	160	0
Ureibacillus	1	1	0
Dethiobacter	1	0	0
EF558947_g	1	0	0
GQ480002_g	1	0	0
HQ660796_g	1	0	0
AF050591_g	1	0	0
Bacillus	27	0	0
EF404788_g	1	0	0
Virgibacillus	1	0	0
Alicyclobacillus	1	0	0
Tumebacillus	3	0	0
AB239481_g	2	0	0
Anaerovorax	1	0	0
Paenisporosarcina	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
Sulfuricurvum	0	0	1
Arcobacter	0	0	34
Sulfurospirillum	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
Limnotherix	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
EU134274_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
JF747790_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)
DQ395705_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)
Ferritrophales	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)
Nitrosomonadales	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
Actinimicrobium	3	3	0
AF236011_g	0	2	2
AF289156_g	0	5	0
AF418942_g	13	33	11
AJ565430_g	0	7	8
AJ575697_g	2	2	1
AJ863277_g	13	7	4
Albidiferax	11	25	28
AM777983_g	55	5	0
Aquabacterium	4	17	17
Aquincola	4	0	1
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	1885
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
Hermiimonas	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	16	1
Kinneretia	1	1	0
Leptothrix	8	24	11
Limnobacter	0	2	0
Limnohabitans	11	108	2
Malikia	17	331	796
Massilia	21	18	8
Methylibium	1	3	0
Mitsuaria	0	0	1
Oxalobacter	0	1	0
Pandoraea	0	1	0
Paucibacter	2	12	8
Pelomonas	0	5	4
Piscinibacter	2	0	1
Polaromonas	38	12	7
Polyangium_g1	1	0	0
Polynucleobacter	9	86	5
Pseudoduganella	0	0	2
Pseudorhodoferrax	2	3	17
Ralstonia	2	5	0
Ramlibacter	7	1	2
Rhizobacter	9	10	2
Rhodoferrax	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	% (number of reads)		
	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