water

MDPI

Article

Composition of Groundwater Bacterial Communities before and after Air Surging in a Groundwater Heat Pump System According to a Pyrosequencing Assay

Heejung Kim [1], Jong-Koo Mok [2], Youngyun Park [3], Dugin Kaown [1] and Kang-Kun Lee [1,*]

[1] School of Earth and Environmental Sciences, Seoul National University, Seoul 08826, Korea; re503@snu.ac.kr (H.K.); dugin1@snu.ac.kr (D.K.); kklee@snu.ac.kr (K.-K.L.)
[2] Geo3eco Co., Ltd, Kangwon National University, Chuncheon 200-701, Korea; mokjk09@hanmail.net
[3] Department of Geology and Geophysics, Kangwon National University, Chuncheon 200-701, Korea; young-yun@nate.com
* Correspondence: kklee@snu.ac.kr; Tel./Fax: +82-2-873-3647

Received: 29 September 2017; Accepted: 13 November 2017; Published: 15 November 2017

Abstract: The geothermal energy of groundwater has aroused increasing interest as a solution to climate change. The groundwater heat pumps (GWHP) system using groundwater is the most environmentally friendly system to date and has been examined in several studies. However, biological clogging by microorganisms negatively affects the thermal efficiency of the GWHP system. In this study, we employed air surging, the most popular among well management methods, and pyrosequencing to analyze the genetic diversity in bacteria before and after air surging in a geothermal well. Furthermore, the diversity of dominant bacterial genera and those related to clogging were evaluated. The bacterial diversity of the groundwater well increased after air surging. Nevertheless, the proportion of bacterial genera thought to be related to microbiological clogging decreased. In cooling and heating systems based on the geothermal energy of groundwater, the wells should be maintained regularly by air surging to reduce efficiency problems caused by microbiological clogging and to prevent secondary damage to human health, e.g., pneumonia due to human pathogenic bacteria including *Pseudomonas aeruginosa* and *Acinetobacter*.

Keywords: groundwater heat pumps; GWHP; bacterial community composition; efficiency; clogging; pyrosequencing; air surging

1. Introduction

Interest in environmentally friendly energy sources has recently increased particularly because of climate change [1]. Geothermal systems that use geothermal energy—using groundwater for heating and cooling buildings—have been attracting increasing attention [2–4]. This system is the most environmentally friendly approach available and numerous studies have evaluated its performance [5], design guidelines for a standing column well [6], and characteristics [7]. Nonetheless, studies have pointed to limitations of the groundwater heat pump (GWHP) system related to its physicochemical [8] and microbiological thermal efficiency [9–13]. Microorganisms affect the thermal efficiency of a geothermal system; for example, they cause biological clogging [9,12–15]. Some studies evaluated the genetic diversity of bacterial communities in groundwater heat pumps [14], however, there have been no studies on well management. Wells in continuous use over a long period become clogged because of chemical processes such as oxidation and erosion in water pumps, mechanical processes such as mineral sedimentation caused by water–rock interactions, and biological processes related to the accumulation of sediments, which is influenced by bacteria. These factors decrease well efficiency. These factors also reduce the amount of collectable water and may reduce water quality [16]. The National Groundwater Association (NGWA) in the U.S. has been conducting studies on the importance of follow-up groundwater well management for many years, and some researchers concluded that the cause of clogging is the inflow of sand into the well and formation of a thin iron oxide film [16]. In the Netherlands, research suggests that the cause of the aging and clogging of groundwater wells is the clogging of well inlets by fine particles originating from the aquifer [17]. Various countries recognize the challenges in the identification of the causes of clogging and in the development of necessary technologies, but very few studies have addressed follow-up management such as the cleansing of groundwater wells [9,12–19]. There are two main methods, mechanical and chemical, used to maintain or recover well efficiency. Air surging is one of the most widely applied methods for physical recovery of well efficiency; this approach may be combined with the explosion method, jetting, shooting, ultrasonic cleansing, and hydraulic fracturing. In air surging, highly compressed air (25 kg/cm^2) is injected into a groundwater well to cleanse the inner walls of the well or inner casing. This procedure is simple and improves and regenerates groundwater wells by reducing groundwater pump clogging at a low cost with high efficiency [20]. Clogging of a GWHP system well may affect its efficiency, and thus continued and regular cleansing is necessary.

Once the process of extraction of groundwater becomes well established, it is critical to maintain the supply of high-quality groundwater. However, several issues exist in well stewardship. Specifically, aesthetic and health-related aspects make these wells vulnerable [21]. With stricter regulations regarding certain pollutants in place, it becomes the site owner's responsibility to test and manage pollutant levels [22]. Statistical analysis was performed using the SPSS software (SPSS Inc., Chicago, IL, USA); specifically, the statistical tool, factor analysis was employed [23]. Various indicators, such as, NO_3-N, pesticides, and bacteria were used to evaluate the quality of groundwater. To ensure quality, adequate preliminary research, such as a hygiene survey, needs to be conducted before the installation of water well. There is a need for the optimal management of all related activities, including individual behaviors [24,25].

Groundwater heat pumps (GWHPs) is a central heating and cooling system that transfers heat to or from the groundwater by means of cooling in summer and heating in winter. This implies that the groundwater temperature changes inevitably during the circulation in the pump system. Further, the microbial growth in groundwater is closely related to temperature. Focusing on the microbial aspects related to clogging in a groundwater well, this study presents a review of the microbial community in drinking water wells based on the method of pyrosequencing. Flavobacterium and Pseudomonas were the most dominant species, and their growth was shown to have a positive relationship with temperature [26]. In addition, by means of statistical analysis, it was revealed that the higher the ratio of sulfate reducing bacteria (SRB), the more clogging occurred [27]. Therefore, for the proper management of GWHPs, it becomes important to examine the microbial community changes in the face of continuous temperature variations. Air surging is a widely used method for groundwater management and efficient functioning of GWHPs. However, scarce literature exists pertaining to changes in the structure of microbial communities over the course of the application of this method.

Next-generation sequencing methods such as pyrosequencing can characterize bacterial communities in the environment [28,29]. The accuracy of community analysis has recently increased with the increased variety of platforms available. In this study, we analyzed the genetic diversity of bacteria by pyrosequencing before and after air the surging of a geothermal well, and we identified the dominant bacterial genera and those related to clogging.

2. Materials and Methods

2.1. Study Area

The study area was located in Janghak-li, Dong-myeon, Chuncheon city, Gangwon Province. Regarding the geological features of the study area, Mesozoic Chuncheon granite makes up the bedrock, and quaternary alluvium covers the upper layer (Figure 1) [30].

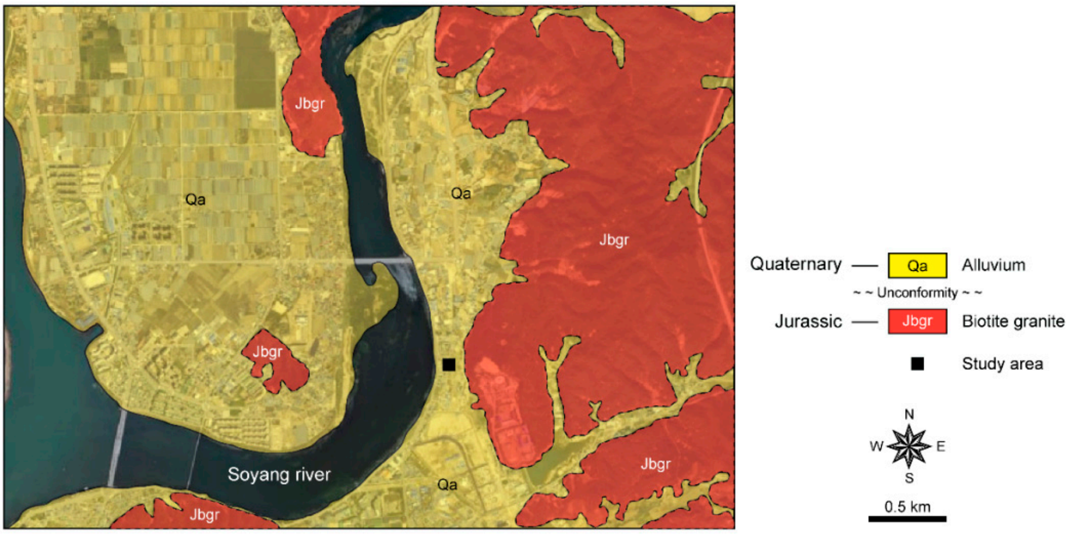

Figure 1. The geologic map of the study area.

The groundwater well installed for this study had an inner diameter of 200 mm and depth of 250 m (Figure 2). The results of boring showed that alluvium was distributed from the ground down to a depth of 9 m, weathered rock was present between 10 and 15 m, soft rock between 16 and 26 m, and moderate rock was found at 26 m and deeper (Figure 1).

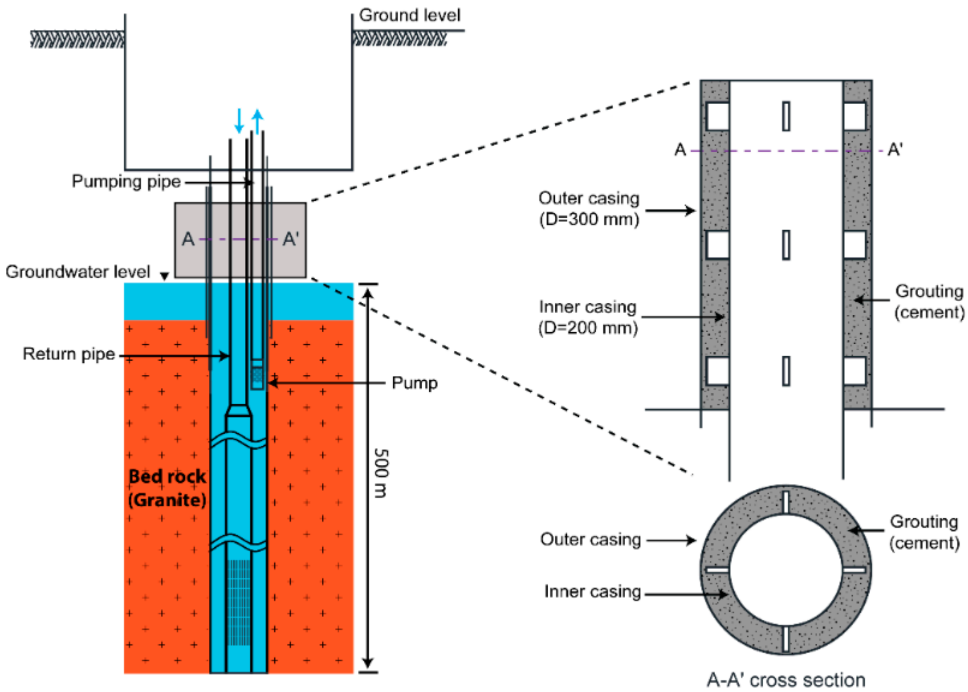

Figure 2. The schematic diagram of the GWHPs well.

Hydraulic conductivity of the study area was 1.92×10^{-4} cm/day and transmissivity was 0.10 m^2/day. A step-drawdown test was conducted to attain an optimal yield and well efficiency. The optimal yield of the study site was 240 m^3/day, and the well efficiency was 66.0%. The permeability of the testbed was 6.0×10^{-12} m^2, and the coefficient of transmissivity was 17.50 m^2/day. Thermal properties such as thermal conductivity, well flow rate, and the thermal power of the GWHP circulation water were 3.23 W/mK, 432 m^3/day, and 98.2 kW, respectively. The mean water temperature in circulation in the GWHP was 16.1 °C, and the highest groundwater temperature was 23.8 °C whereas the lowest was 8.0 °C. The system operated from 10 p.m. to 7:30 a.m. for 9 h and 30 min. The coefficient of performance (COP) of the system was 3.1 and 3.2 for cooling and heating, respectively (Figure 3).

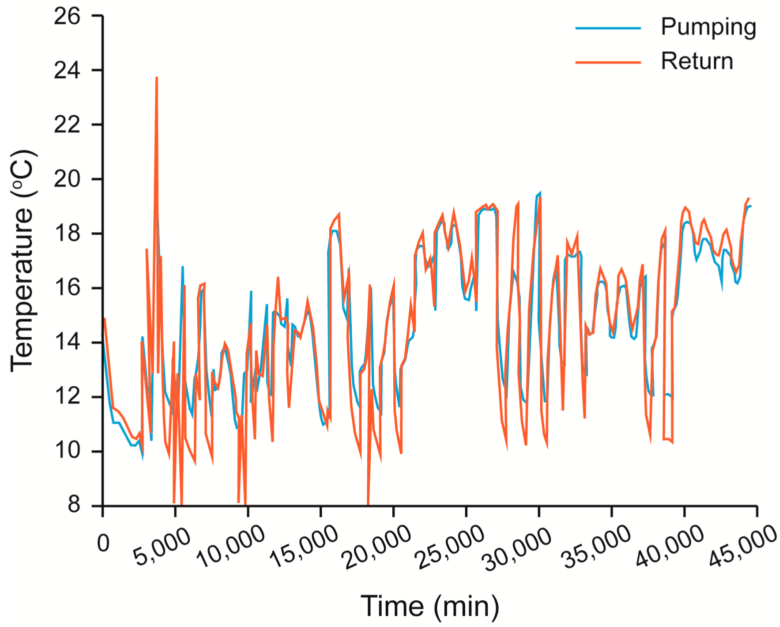

Figure 3. GWHPs Operation schedule and temperature of groundwater.

For constant heat production by a GWHP, it is necessary to consider the surrounding geological characteristics. Figure 4 shows the results of RQD (rock quality designation) and TCR (total core recovery), which were determined for the safe and economical design of geothermal heat production. The characteristics of the site revealed that the underground part of the groundwater well as well-developed insulation and a dense structure. The geothermal well was installed in August 2014 and was monitored until November 2016. Air surging was conducted in August 2015 to improve the efficiency of the geothermal well and to facilitate its maintenance.

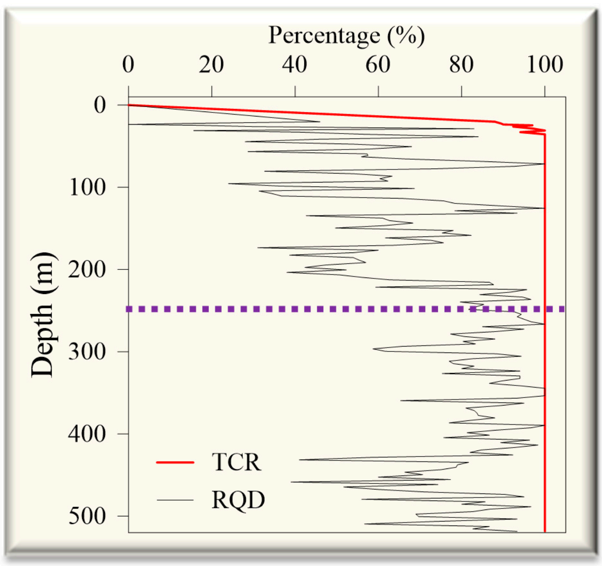

Figure 4. Rock quality designation (RQD) and total core recovery (TCR) data from the study site.

2.2. Water Sampling and Chemical Analysis

Groundwater before and after cleansing was sampled on 25 August 2015 in order to assess water quality before and after air surging. The onsite water quality (temperature, pH, electrical conductivity (EC), oxidation reduction potential (ORP), and dissolved oxygen) was evaluated in the field, and the water quality analysis (Ca^{2+}, Na^{+}, Mg^{2+}, K^{+}, Zn^{2+}, Fe^{2+}, Al^{3+}, NO_3^{-}, Cl^{-}, SO_4^{2-}, F^{-}, and SiO_2) was carried out in the laboratory. The onsite water quality parameters were measured with a portable meter, YSI556 (YSI, Yellow Springs, OH, USA). For indoor water quality analysis, water was pumped until there was no difference in the water temperature, and a water sample was collected when the water quality stabilized. The collected sample was passed through 0.45 µm filter paper to remove impurities. Nitric acid at the concentration 0.05 N was used for acid treatment, and the anion and cation samples were collected into 100 mL sterile water collection bottles, frozen below 4 °C, and transported to the laboratory. Laboratory analysis was performed by the Natural Science Research Support Center of Sangji University.

2.3. Air Surging

Air surging was conducted in August 2015. The air surging procedure involved pressurized air at ~2000 psi (150 atm), which was injected into the screen of the casing or near well walls in the aquifer of study site groundwater well. Figure 5a shows a photo of interior of the groundwater well before air surging and (b) a view inside the groundwater well after air surging.

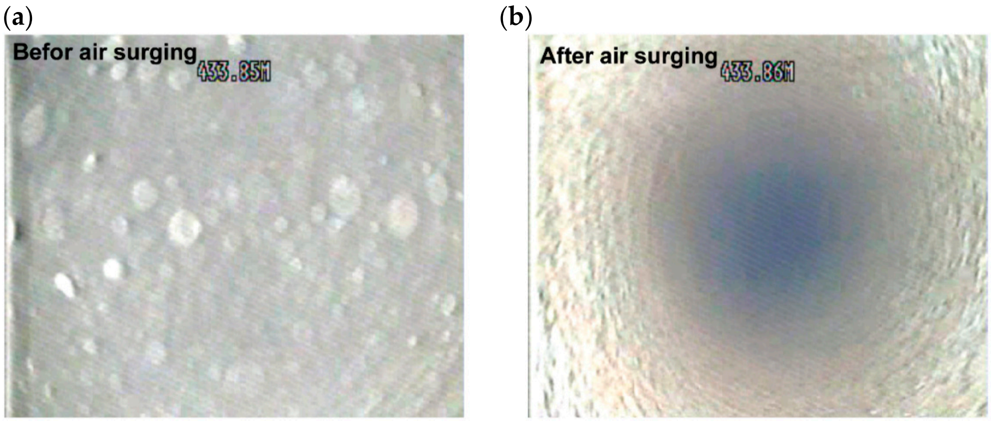

Figure 5. (**a**) A photo of the interior of the groundwater well before air surging, (**b**) a photo of the interior of the groundwater well after air surging.

2.4. Microbial Analysis

A 3000 mL sample of groundwater was passed through a 0.2 µm pore size filter (Millipore, Billerica, MA, USA). Total DNA was extracted according to the manufacturer's instructions after filtering by means of the i-Genomic Soil DNA Extraction Mini Kit (iNtRON Biotechnology, Kyungki-Do, Korea) and the extraction was confirmed using a Qubit fluorometer (Invitrogen, Carlsbad, CA, USA). The 8-27F and 1492-1510R primers were used to amplify a fragment of the bacterial 16S ribosomal RNA (rRNA) gene. The procedures, including reagent composition, reaction conditions, and electrophoresis for polymerase chain reaction (PCR) amplification, were the same as those described by Lee et al. [28]. An amplicon band of approximately 1.5 kb was observed (data not shown). Pyrosequencing analysis of the PCR product was conducted by Macrogen Co., Ltd. (Seoul, Korea). The platform used for the analysis was the MiSeq v4 hyper variable, and the results were analyzed after special processing, including the exclusion of nonspecific nucleic acid sequences.

3. Results and Discussion

3.1. Physicochemical Data

The water temperature ranged between 11.7 and 22.5 °C. Seasonal changes were clearly detectable, with the highest temperatures in the summer (21.6 °C) and lowest in winter (11.7 °C); pH showed a wide range, between 4.4 and 8.3, but the typical range was between 6.0 and 8.3, except for the value measured in July 2016. There was no seasonal change in pH. Dissolved oxygen concentration ranged from 1.42 to 5.88 mg/L and was relatively high in the summer (4.10 mg/L) during rainfall, whereas in other seasons, it showed general average of DO values in a groundwater (2.23 mg/L). Electrical conductivity ranged from 186 to 350 μS/cm and was the lowest in May and highest in August 2016. Except for these two periods, electrical conductivity was 235 μS/cm, showing stable values (Figure 6).

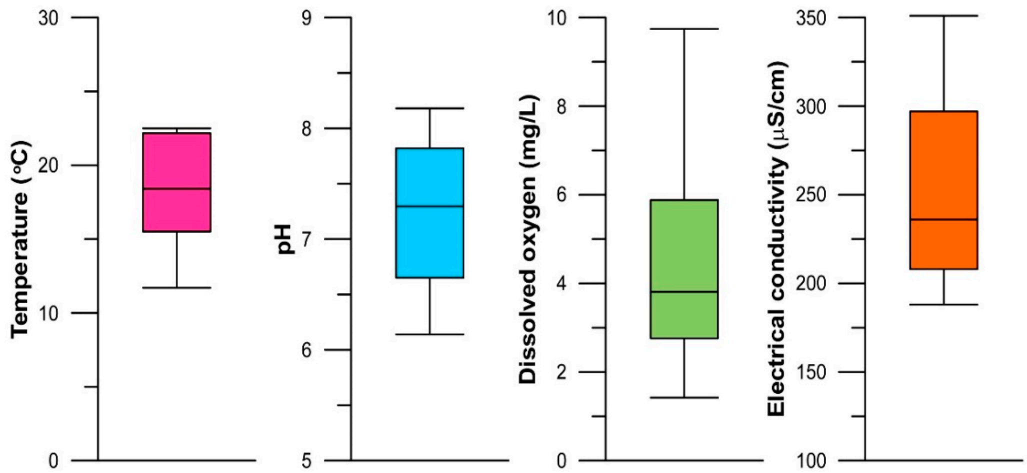

Figure 6. The range of measured water temperatures, pH, dissolved oxygen concentrations, and electrical conductivity in the well being monitored.

Groundwater was compared before and after air surging. The water temperature, pH, ORP, and EC before air surging were 19.2 °C, 6.7, −8.9 mV, and 220 μS/cm, respectively. The water temperature was dropped steadily after the air surging procedure, whereas pH decreased after air surging and then tended to increase. ORP and EC increased and then decreased (Figure 7). Changes in temperature of the groundwater seemed to be influenced by the atmosphere, and the fluctuations of parameters such as EC, ORP and pH varied greatly after air surge but recovered over time. Air surging does not seem to have a significant effect on the physicochemical properties of the groundwater wells.

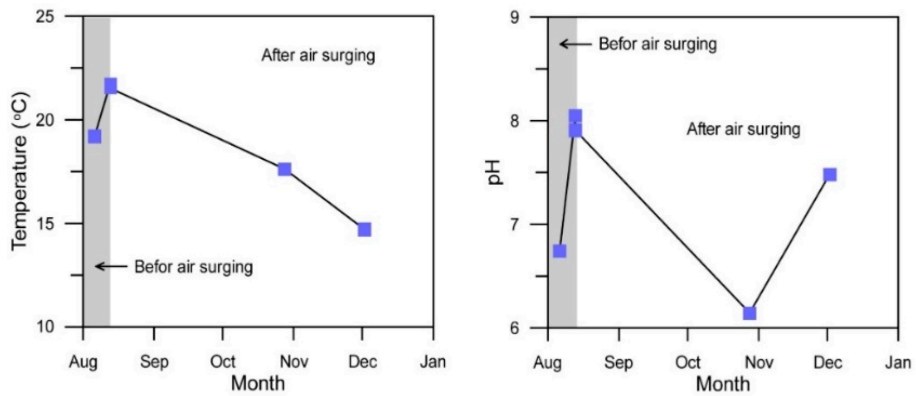

Figure 7. *Cont.*

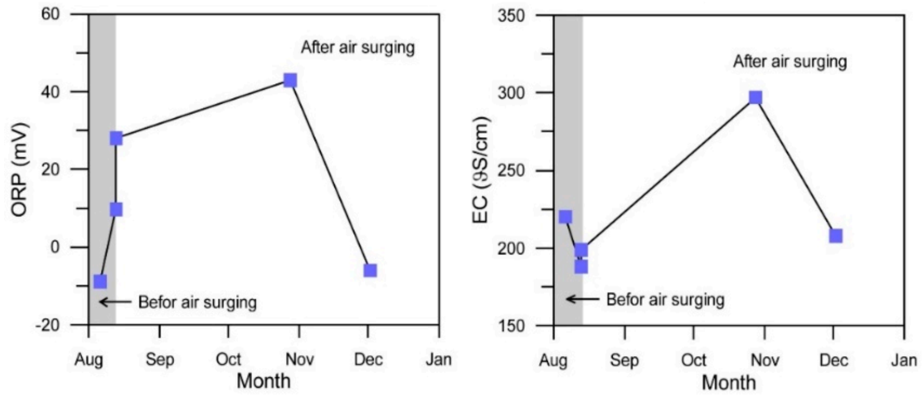

Figure 7. Temporal variation of water temperature, pH, dissolved oxygen, and electrical conductivity before and after air surging.

3.2. Microbial Data

Pyrosequencing analysis indicated that the sample analyzed by means of a rarefaction curve could explain the metagenomic findings (data not shown). In this analysis, 709,386 and 668,033 reads were obtained before and after air surging, respectively. Biodiversity increased from 5.0 before air surging up to 6.5 after (Table 1), and the dominance showed a minimal difference with values of 0.93 and 0.97 (Table 1), respectively. Particularly, 519 operational taxonomic units (OTUs) were detected before air surging and 775 after, showing an increase of 256 OTUs. The number of OTUs with a 0.5% prevalence rate or higher increased from 25 to 41, whereas the number of OTUs with prevalence 0.1–0.4% increased from 48 to 89. Thus, bacterial communities became more diverse after air surging (Table 1).

Table 1. A comparison of operational taxonomic units (OTUs) and diversity indices before and after air surging. The percentages indicate a prevalence rate.

Air Surging	Number of OTUs			Diversity Index	
	Total	**>0.5%**	**0.1–0.4%**	**Shannon**	**Simpson**
Before	519	25	48	5.00	0.93
After	775	41	89	6.50	0.97

Phylum level analysis showed that the phylum Proteobacteria was dominant with prevalence 83.6%, and that Bacteroidetes was the second most dominant phylum, accounting for 6.7% of OTUs in the geothermal well. The pattern changed after the air surging. The prevalence of Proteobacteria decreased to 46.6% after air surging and additional phyla were detected, such as Actinobacteria, Bacteroidetes, Acidobacteria, and Chloroflexi (Figure 8). Particularly, the proportion of the class Gammaproteobacteria in Proteobacteria decreased from 39.4% to 1.8% (decrease of 37.6%) and that of the class Betaproteobacteria decreased by 8.4%. The prevalence of classes belonging to Proteobacteria changed too; for example, the prevalence of class Deltaproteobacteria increased from 0.4% to 7.8% (increase of 7.4%; Supplementary Materials Table S1).

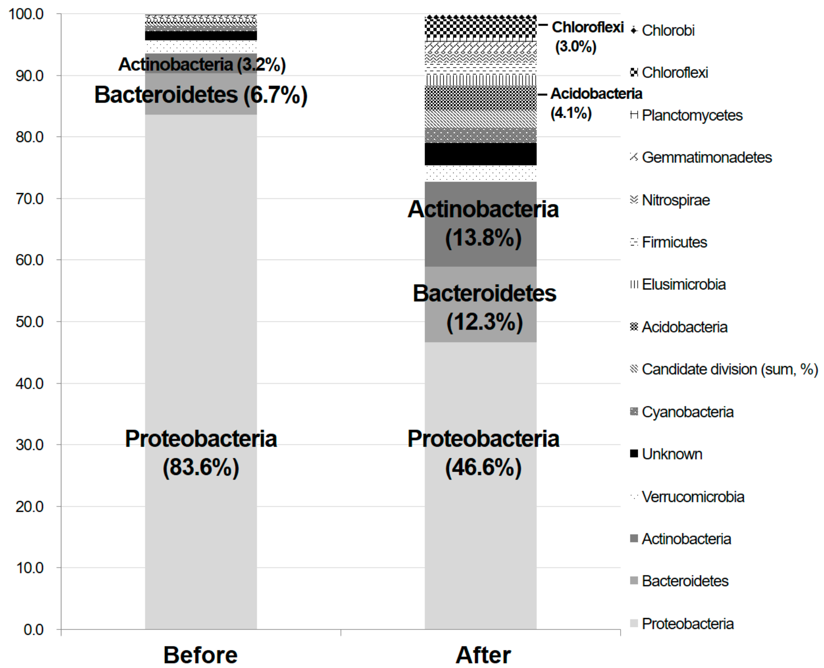

Figure 8. Comparison of phylum level distributions before and after air surging.

In the genus level analysis, the top seven genera accounted for 67.3% of OTUs in the geothermal well before air surging. Among them, the genus *Gallionella* was dominant, accounting for 17.4% of OTUs, and the genus *Acinetobacter* was in second place at 15.8%. *Pseudomonas* accounted for 11.7% of OTUs. The class Betaproteobacteria-OTU25, *Stenotrophomonas*, class Betaproteobacteria-OTU05, and *Enterobacter* had relatively high prevalence rates before air surging, and these rates tended to decrease after the purging. Candidatus *Planktophila*, which had prevalence of 2.4% before air surging, became dominant at 11.2% after air surging, and the prevalence rates of the class Betaproteobacteria-OTU05, phylum Bacteroidetes-OTU44, and class Betaproteobacteria-OTU25 ranged from 5.2% to 6.4% after air surging.

In addition, the genera with prevalence rates lower than 1.0%, or below the detection limit before air surging, such as *Geobacter* (3.5%) and *Sulfuritalea* (2.4%) increased in prevalence after air surging; thus, diversity increased in general (Figure 9 and Supplementary Materials Table S2). Analysis of bacterial genera that were thought to be related to clogging revealed that the dominant OTU was the genus *Gallionella*, which accounted for 17.4% of OTUs before air surging. *Gallionella* is known as an anaerobic iron bacteria. Only a single species, *G. ferruginea*, has been reported previously [31,32], and its role in the environment remains unclear.

Nevertheless, iron reducing bacteria use oxygen to form rust and can create slimy materials that get attached to well pipes, pumps, and plumbing fixtures [33]. Furthermore, OTUs with prevalence rates of 11.7% and 2.2% were identified as *Pseudomonas* and *Flavobacterium*. These genera inhabit a wide variety of environments and play important roles in the environment [33]. Among them, some species are reported to produce exopolysaccharides and are related to biofilms [14,34]. In contrast, *Gallionella* prevalence decreased from 17.4% to 1.7% after air surging, *Pseudomonas* decreased from 11.7% to below 0.1%, and *Flavobacterium* from 2.2% to 0.4%. The genus known to purify organisms, *Geobacter*, increased in prevalence from 0.1% to 3.5% (Figure 9 and Supplementary Materials Table S2).

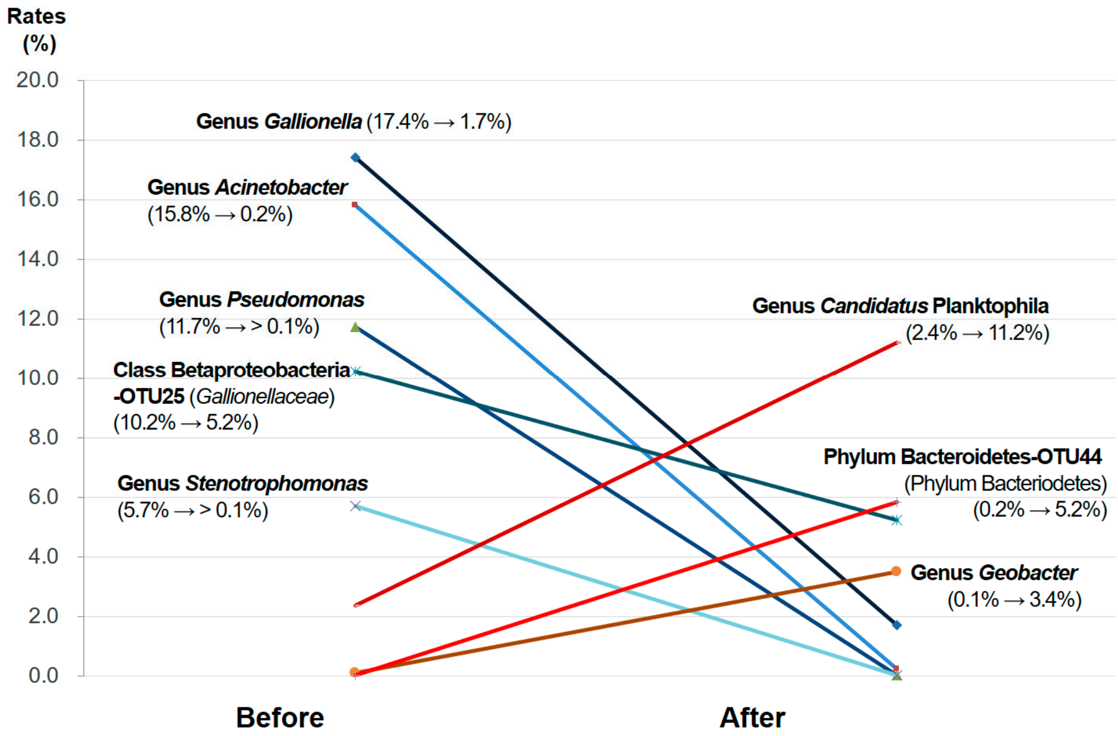

Figure 9. Comparison of major changes in genus or OTU level prevalence rates of taxa before and after air surging.

The genus *Acinetobacter*, which was the second most dominant, accounting for 15.8% of OTUs prior to air surging, inhabits environments such as soil, freshwater, sewage, and wastewater, and 44 species have been reported [35]. This genus is known to perform an important function mainly in soil. However, A. *baumannii* and A. *lwoffi* belonging to the genus *Acinetobacter* are suspected human pathogens that are the source of strains causing pneumonia and may cause various other infections such as the infection of skin wounds, bacteremia, and meningitis [36,37]. The genus *Acinetobacter* decreased in prevalence from 15.8% to 0.2% after air surging. During the practical use of cooling and heating by means of geothermal wells, secondary damage may occur, and thus follow-up monitoring should be conducted in order to eliminate possible human pathogenic bacteria, e.g., *P. aeruginosa* and *Acinetobacter*.

4. Conclusions

This study analyzed the genetic diversity of bacteria, by pyrosequencing, in a geothermal well before and after air surging. The groundwater of the geothermal well was neglected for 24 months before air surging. Before the procedure, the well showed a relatively low bacterial diversity, and the proportion of the bacterial genera believed to be related to microbiological clogging was high. After air surging, diversity increased, whereas clogging-related taxa decreased in prevalence. Further studies of microorganisms that may cause secondary damage are needed. According to our results, it is necessary to perform regular maintenance at least once every 24 months by air surging in order to improve efficiency and to reduce the risk of secondary damage such as the biological clogging of cooling and heating systems that are based on the geothermal energy of groundwater.

Supplementary Materials: The following are available online at www.mdpi.com/2073-4441/9/11/891/s1, Table S1: Class comparison of phylum Proteobacteria before and after air surging, Table S2: Comparison of major bacterial communities before and after air surging.

Acknowledgments: This study was supported by the Korean Ministry of Environment under "GAIA project (2014000530001)".

Author Contributions: Heejung Kim, Jong-Koo Mok and Kang-Kun Lee were responsible for research design. Heejung Kim, Youngyun Park and Dugin Kaown analyzed the data, and prepared figures. Heejung Kim drafted the main text. Jong-Koo Mok and Youngyun Park participated in the sampling. Heejung Kim, Dugin Kaown and Kang-Kun Lee polished the manuscript. All authors participated in discussions and editing.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Park, Y.; Kwon, K.S.; Kim, N.; Lee, J.Y.; Yoon, J.G. Change of geochemical properties of groundwater by use of open loop geothermal cooling and heating system. *J. Geol. Soc. Korea* **2013**, *49*, 289–296.
2. Ahn, C.M.; Han, J.S.; Kim, C.G.; Park, Y.C.; Mok, J.K.; Jang, B.J. Monitoring of geothermal systems wells and surrounding area using molecular biological methods for microbial species. *J. Soil Groundw. Environ.* **2012**, *17*, 23–32. [CrossRef]
3. Lee, J.Y. Current status of ground source heat pumps in Korea. *Renew. Sustain. Energy Rev.* **2009**, *13*, 1560–1568. [CrossRef]
4. Kwon, K.S.; Lee, J.Y.; Mok, J.K. Update of current status on ground source heat pumps in Korea. *J. Geol. Soc. Korea* **2012**, *8*, 193–199. (In Korean)
5. Rees, S.J.; Spitler, J.; Deng, Z.; Orio, C.; Johnson, C. A study of geothermal heat pump and standing column well performance. *ASHRAE Trans.* **2004**, *110*, 3–13.
6. Hahn, J.; Han, H.; Hahn, C.; Kim, H.S.; Jeon, J.S. Design guidelines of geothermal heat pump system using standing column well. *Econ. Environ. Geol.* **2006**, *39*, 607–613. (In Korean)
7. Freedman, V.L.; Waichler, S.R.; Mackley, R.D.; Horner, J.A. Assessing the thermal environmental impacts of an groundwater heat pump in southeastern Washington State. *Geothermics* **2012**, *42*, 65–77. [CrossRef]
8. Park, Y.; Kim, N.; Lee, J.Y. Geochemical properties of groundwater affected by open loop geothermal heat pump systems in Korea. *Geosci. J.* **2015**, *19*, 515. [CrossRef]
9. Jo, Y.J.; Lee, J.Y.; Lim, S.Y.; Hong, G.P. A review on potential effects of installation and operation of ground source heat pumps on soil and groundwater environment. *J. Soil Groundw. Environ.* **2009**, *14*, 22–31.
10. Jo, Y.J.; Lee, J.Y.; Kim, C.G.; Han, J.S. Effects of grouts and temperature change on microorganisms in geothermal heat pump. *J. Soil Groundw. Environ.* **2009**, *14*, 22–31.
11. York, K.P.; Jahangir, J.Z.M.G.; Solomon, T.; Stafford, L. Effects of a large scale geothermal heat pump installation on aquifer microbiota. In Proceedings of the 2nd Stockton International Geothermal Conference, 16–17 March 1998; p. 8. Available online: http://citeseerx.ist.psu.edu/viewdoc/download;jsessionid=B671D9CD2872ACAC6B5FDF3C52262B86?doi=10.1.1.510.1293&rep=rep1&type=pdf (accessed on 9 November 2017).
12. Kwon, M.J.; Sanford, R.A.; Park, J.; Kirk, M.F.; Bethke, C.M. Microbiological response to well pumping. *Ground Water* **2008**, *46*, 286–294. [CrossRef] [PubMed]
13. Abudalo, R.A.; Bogatsu, Y.G.; Ryan, J.N.; Harvey, R.W.; Metge, D.W.; Elimelech, M. Effect of ferric oxyhydroxide grain coatings on the transport of bacteriophage PRD1 and Cryptosporidium parvum oocysts in saturated porous media. *Environ. Sci. Technol.* **2005**, *39*, 6412–6419. [CrossRef] [PubMed]
14. Kim, H.; Lee, S.; Park, J.; Joun, W.T.; Kim, J.; Kim, H.; Lee, K.K. Genetic prokaryotic diversity in boring slime from the development of a groundwater heat pump system. *Microbiol. Biotechnol. Lett.* **2016**, *44*, 550–556. [CrossRef]
15. Sowers, L.S.; Epstein, C.; York, K.P.; Van Guilder, B.; Jahangir, S.; Steinberg, A.; Stafford, L.; Pierce, J.; Brownhill, J.; Solomon, T. Impact of a large scale geothermal project on its surface and subterranean environment. In Proceedings of the 7th International Conference on Thermal Energy Storage MEGASTOCK, Sapporo, Japan, 18–21 June 1997; Volume 1, pp. 85–90.
16. Houben, G.; Treskatis, C. *Water Well Rehabilitation and Reconstruction*; McGraw-Hill: New York, NY, USA, 2007, ISBN 10-0071486518.
17. Rinck-Pfeiffer, S.; Ragusa, S.; Sztajnbok, P.; Vandevelde, T. Interrelationships between biological, chemical, and physical processes as an analog to clogging in aquifer storage and recovery (ASR) wells. *Water Res.* **2000**, *34*, 2110–2118. [CrossRef]

18. Gino, E.; Starosvetsky, J.; Kurzbaum, E.; Armon, R. Combined chemical-biological treatment for prevention/rehabilitation of clogged wells by an iron-oxidizing bacterium. *Environ. Sci. Technol.* **2010**, *44*, 3123–3129. [CrossRef] [PubMed]
19. Ralph, D.E.; Stevenson, J.M. The role of bacteria in well clogging. *Water Res.* **1995**, *29*, 365–369. [CrossRef]
20. Kim, G.B.; Kim, B.W.; Kim, S.Y. Improvement of well efficiency through well development in a pumping well. *J. Soil Groundw. Environ.* **2010**, *15*, 39–49.
21. Malecki, K.M.C.; Schultz, A.A.; Severtson, D.J.; Anderson, H.A.; VanDerslice, J.A. Private-well stewardship among a general population based sample of private well-owners. *Sci. Total Environ.* **2017**, *601*, 1533–1543. [CrossRef] [PubMed]
22. Flanagan, S.V.; Marvinney, R.G.; Zheng, Y. Influences on domestic well water testing behavior in a Central Maine area with frequent groundwater arsenic occurrence. *Sci. Total Environ.* **2015**, *505*, 1274–1281. [CrossRef] [PubMed]
23. Kreutzwiser, R.; Loë, R.; Imgrund, K.; Conboy, M.J.; Simpson, H.; Plummer, R. Understanding stewardship behaviour: Factors facilitating and constraining private water well stewardship. *J. Environ. Manag.* **2011**, *92*, 1104–1114. [CrossRef] [PubMed]
24. Gosselin, D.C.; Headrick, J.; Tremblay, R.; Chen, X.H.; Summerside, S. Domestic Well Water Quality in Rural Nebraska: Focus on Nitrate-Nitrogen, Pesticides, and Coliform Bacteria. *Ground Water* **1997**, *17*, 77–87. [CrossRef]
25. Stetzenbach, L.D.; Kelly, L.M.; Sinclair, N.A. Isolation, Identification, and Growth of Well-Water Bacteria. *Ground Water* **1986**, *24*, 6–10. [CrossRef]
26. Navarro-Noya, Y.E.; Suárez-Arriaga, M.C.; Rojas-Valdes, A.; Montoya-Ciriaco, N.M.; Gómez-Acata, S.; Fernández-Luqueño, F.; Dendooven, L. Pyrosequencing Analysis of the Bacterial Community in Drinking Water Wells. *Microb. Ecol.* **2013**, *66*, 19–29. [CrossRef] [PubMed]
27. Van Beek, C.G.E.M.; van der Kooij, D. Sulfate-Reducing Bacteria in Ground Water from Clogging and Nonclogging Shallow Wells in The Netherlands River Region. *Ground Water* **1982**, *20*, 298–302. [CrossRef]
28. Lee, S.; Chung, H.M.; Park, E.R. Characteristics of bacteria in the living room and bathroom of a residential environment using the pyrosequencing method. *Microbiol. Biotechnol. Lett.* **2016**, *44*, 84–88. [CrossRef]
29. Kim, H.; Kaown, D.; Kim, C.S.; Lee, S. Analysis of bacterial diversity in water from the Han River water source protection area via a pyrosequencing assay. *Iran. J. Environ. Health Sci. Eng.* **2016**, *42*, 274–279. [CrossRef]
30. Lee, D.S.; Nam, G.S.; Lee, H.Y.; Yang, S.Y. *Chuncheon Geology Report (1:50,000)*; Korea Institute of Geoscience and Mineral Resources: Daejeon, Korea, 1974; pp. 1–18.
31. Falagas, M.E.; Karveli, E.A.; Kelesidis, I.; Kelesidis, T. Community acquired Acinetobacter infections. *Eur. J. Clin. Microbiol. Infect. Dis.* **2007**, *26*, 857–868. [CrossRef] [PubMed]
32. Jeong, J.M.; Park, Y.C.; Jo, Y.J.; Lee, J.Y. Time series analysis of groundwater level fluctuation data Cheonjeonri, Chuncheon, Gangwon-do. *J. Geol. Soc. Korea* **2010**, *46*, 171–176.
33. List of Prokaryotic Names with Standing in Nomenclature (LPSN). Genus Acinetobacter. 2017. Available online: http://www.bacterio.net/acinetobacter.html (accessed on 23 May 2017).
34. Lee, S.; Weon, H.Y.; Han, K.; Ahn, T.Y. *Flavobacterium dankookense* sp. nov., isolated from a freshwater reservoir, and emended descriptions of *Flavobacterium cheonanense*, *F. chungnamense*, *F. koreense* and *F. aquatile*. *Int. J. Syst. Evol. Microbiol.* **2012**, *62*, 2378–2382. [CrossRef] [PubMed]
35. List of Prokaryotic Names with Standing in Nomenclature (LPSN). Genus Gallionella. 2017. Available online: http://www.bacterio.net/gallionella.html (accessed on 23 May 2017).
36. Ryan, K.J.; Ray, C.G. (Eds.) *Sherris Medical Microbiology*, 4th ed.; McGraw Hill: New York, NY, USA, 2004, ISBN 0-8385-8529-9.
37. Dent, L.L.; Marshall, D.R.; Pratap, S.; Hulette, R.B. Multidrug resistant Acinetobacter baumannii: A descriptive study in a city hospital. *BMC Infect. Dis.* **2010**, *10*, 196. [CrossRef] [PubMed]