Biosaintifika 17 (1) (2025): 80-90 p-ISSN 2085-191X | e-ISSN 2338-7610
Journal of Biology & Biology Education https://journal.unnes.ac.id/nju/index.php/biosaintifika

Microbial Diversity in Hot Spring Soil Microbiome

R. Susanti[1]*, Prasetyo Listiaji[2], Riska Laila Mukaromah[1], Farah Fitrotun Nisa[1], Naina Rizki Kenarni[1], Adi Franata Jaya[1]

[1]Department of Biology, Faculty of Mathematics and Natural Sciences, Universitas Negeri Semarang. Kampus Sekaran, Gunungpati, Semarang 50229, Central Java, Indonesia
[2]Doctoral School of Education, University of Szeged, Hungary

*Corresponding Author: basanatha8@mail.unnes.ac.id

Submitted: 2024-12-04. Revised: 2025-02-02. Accepted: 2025-04-08.

Abstract. Hot springs are natural habitats for thermophilic and hyper thermophilic microorganisms with optimal growth temperatures. This study was conducted using a metagenomic approach to analyze the microbial communities of Nglimut hot spring soils, Mount Ungaran. This study used an exploratory observational method. A total of ten samples of hot spring sediment soil were taken purposively in Nglimut hot spring, Mount Ungaran. A total of 600 grams of hot spring sediment soil samples were used for chemical analysis and 50 grams of samples for metagenomic analysis based on 16S rRNA V3-V4 gene marker regions. The total microbiota sequences analyzed in this study were 103,889 OTUs, consisting of 98,603 Bacteria OTUs and 5,286 Archaea OTUs. At the phylum level, all DNA sequences of soil microbiota bacteria identified 53 phyla, dominated by Proteobacteria (19.42%), Chloroflexi (17.21%), Nitrospirota (13.76%), Bacteroidota (7.67%) and Firmicutes (7.33%). At the order level, 305 bacterial orders were found, dominated by Nitrospirales (12.35%), Burkholderiales (7.99%), Rhizobiales (5.77%), Ignavibacteriales (4.06), and Anaerolineales (3.92%). A total of nine archaea phyla were identified in the hot spring soil, dominated by Crenarchaeota (85.13%), Nanoarchaeota (7.13%) and Halobacterota (5.58%). At the order level, 15 archaea orders were identified, dominated by Nitrosopumilales (64.08%), Nitrososphaerales (14.39%), Woesearchaeales (7.24%), Bathyarchaeia (6.15%), and Methanosarciniales (5.07%). The bacteria that dominate the soil of hot springs are bacteria that can survive at high temperatures (thermophilic) and are able to utilize sulfur. The presence of archaea in hot spring soil helps increase the activity of sulfate-reducing bacteria in sulfur-containing soils.

Keywords: diversity; metagenomic; Mount Ungaran; Nglimut hot spring; soil

How to Cite: Susanti, R., Listiaji, P., Mukaromah, R. L., Nisa, F. F., Kenarni, N. R., & Jaya, A. F. (2025). Microbial Diversity in Hot Spring Soil Microbiome. *Biosaintifika: Journal of Biology & Biology Education, 17(1)*, 80-90.

DOI: http://dx.doi.org/10.15294/biosaintifika.v17i1.20869

INTRODUCTION

Soil microbial diversity refers to the variety and abundance of microorganisms present in soil ecosystems. It includes the various species, genera, and functional groups of bacteria, archaea, fungi, protozoa, and other microorganisms that inhabit the soil (Baliyarsingh *et al*., 2017). Microbial diversity is an important aspect of soil health and plays an important role in various soil processes and functions (Nannipieri *et al*., 2017). Soil is considered one of the most biologically diverse types of ecosystems. Soil ecological functions are largely determined by the activities of soil microorganisms and regulated by relevant interactions between genes and corresponding strains. Microorganisms operate at the center of biological characteristics, biogeochemical processes, and soil ecology (Ma *et al*., 2018).

Research conducted by Odelade and Babalola (2019) revealed that soil microbes, including archaea, bacteria, fungi, and protists are associated with many aspects of soil quality and health. Plant composition and diversity influence the structure and activity of soil microbial communities, suggesting a complex relationship between plants and microbes in ecosystem dynamics (Liu *et al*., 2022). Soil microbes, including pathogenic microbes, are important regulators of plant community dynamics and plant diversity and determine plant abundance. Overall, soil microbes are considered important drivers of plant diversity and productivity in terrestrial ecosystems (van der Heijden *et al*., 2008).

Hot springs are natural habitats for thermophilic and hyper thermophilic microorganisms with optimal growth temperatures of >40 °C and >90 °C, respectively (Lischer *et al*., 2020). Enzymes obtained from thermophilic microorganisms have proven to be very valuable as biocatalysts for industrial and biotechnological purposes. Temperatures in hot springs usually exceed the limits of eukaryotic life (close to 60 °C), which limits the life of bacteria and archaea (Zeldes *et al*., 2015). Each hot spring differs in terms of its temperature, chemical composition, and temperature or light gradient. Hot springs consist of several habitats, such as thermal fluids, microbial mats (horizontally stratified microbial communities), and sediments (Rozanov *et al*., 2017). Thermophilic archaea, including members of the Thermoproteales order, are found in sulfur-rich soils (Satoh *et al*., 2013). Sulfur-oxidizing bacteria such as Sulfobacillus and Acidithiobacillus are known to inhabit hot springs with high sulfur content, obtaining energy from the oxidation of sulfur compounds (Hidayat *et al*., 2017). In addition, sulfate-reducing bacteria such as Desulfurella and Desulfobacter may contribute to sulfur metabolism. Syntrophic interactions between microorganisms are common, with some bacteria performing sulfur oxidation while others utilize byproducts (Plugge *et al*., 2011). A wide variety of microbial strata develop in geothermal hot springs and form unique microbial ecosystems that are physiologically and phylogenetically diverse (Tamazawa *et al*., 2012). By identifying the specific phyla that inhabit hot spring soils, it is possible to better understand the functional roles of these microorganisms and their contribution to the overall ecosystem dynamics of hot spring soils (Baldrian, 2017).

In the environment, microbial communities play an important role in ecosystem function and are involved in many important processes such as nutrient cycling and biodegradation (Gupta *et al*., 2016). As the most active element of the soil ecosystem, microbes can quickly respond to anthropogenic stresses, making them possible indicators of soil quality and health (Rahman *et al*., 2021). This study was conducted using a metagenomic approach to analyze the microbial communities of Nglimut hot spring soils, Mount Ungaran. The data generated in this study can be used as a marker of microbiota stability in hot spring soil ecosystems.

METHODS

This study was conducted using an exploratory observational method to identify the composition of microbial communities and the diversity of hot spring sediment soil. Sediment soil samples were collected from Nglimut hot spring, Mount Ungaran, which is located in Gonoharjo Village, Limbangan Subdistrict, Kendal Regency, Central Java, Indonesia (S07°09'30.8"E110°19' 59.4"). Soil samples were taken during the dry season.

Sampling of hot spring soil sediment was carried out at 10 points with a distance between points of 1 m starting from the source of the hot spring towards the water flow. At each point, 500 grams were taken at a depth of 1-10 cm from the ground surface. Sediment soil samples of hot spring that have been taken from the location are carried out physics and chemicals analysis in the form of measuring temperature, humidity, color, and soil texture.

Sedimentary soil samples from hot springs were taken to the laboratory and stored in a refrigerator at -20°C. Then the next day, samples from each sampling point were put into one container. The soil samples were then thoroughly combined (polled) until homogeneous. After being mixed, 50 grams of samples were put into a 250 ml test tube for metagenomic analysis. Then, 600 grams of samples were taken for chemical compound analysis. Soil chemical analysis includes the content of C-organic matter, N-total, K-available, P_2O_5, Ca-dd, Mg-dd, Na-dd, Fe, and S with standard methods.

DNA Isolation and Next Generation Sequencing (NGS)

Microbial genomes were extracted from production field soil and hot spring soil samples using the QIAamp ZymoBIOMICS™ DNA Mini Kit according to the manufacturer's manual. Extracted DNA was stored in a -20°C freezer. Soil microbial diversity and abundance were analyzed based on 16S rRNA V3-V4 gene marker regions. DNA amplification used the Illumina HiSeq 2500 platform for 20 cycles. The primers used were forward primer 338F (5'-GGACTACHVGGGT WTCTAAT-3') and reverse primer 806R (5'-GGACTACHVGGG TWTCTAAT-3') (Holm *et al*., 2019) that bind to the barcode, which is a sequence of eight specific bases in each sample.

Sequencing Data Analysis

Metagenomic analysis of the 16s rRNA soil microbial was performed using QIIME2 (Ver. 2019.4) (Caporaso *et al*., 2010). The paired-end files were demultiplexed using the demux plugin. Quality control was then performed on each sample using the Dada2 plugin (Callahan *et al*., 2016). The abundance and diversity index values were analyzed with 5 diversity indices namely

Shannon (Shannon & Weaver, 1949), Simpson (Simpson, 1949), Chao1 (Chao, 1984), ACE, and Observed OTUs (DeSantis *et al*., 2006). Furthermore, taxonomic preparation was carried out based on the Greengenes 13_8 99% OTU database (Bokulich *et al*., 2018). Heatmap preparation using the heatmap plugin and taxa barplot preparation using Microsoft Excel 2010 (Susanti *et al*., 2024).

Data analysis

Data obtained from each test were analyzed descriptively. Analysis of microbial abundance and diversity of each microbiome was associated with soil chemical and physical parameters.

RESULTS AND DISCUSSION

The microclimate conditions of the Nglimut hot spring have a neutral pH (7.04), 57% humidity and relatively high temperature (61ºC) (Table 1). The physical condition of the hot spring soil is gravelly clay textured and watery because it is located in a river flow, and is brownish yellow in color. The characteristics of the soil texture cause the source of substrate and nutrients for bacterial growth to be not as much as in production land. The microbial population in the rhizosphere soil layer is influenced by the organic matter content of the soil. Soil conditions that lack organic matter cause the microbial population to tend to be low (Wahyuni *et al*., 2016). The vegetation found in the hot spring soil is dominated by moss and ferns.

Based on the results of chemical analysis, the sulfur component was detected quite high (7.86%) (Table 1). The high sulfur content of this study is in line with the results of previous studies. The results of Hamzah *et al*.'s (2013) study showed that sulfur levels in several hot springs in Malaysia ranged from 6.25 to 12.86 mg/L. Sulfur levels in hot springs at Foot of the Mountain in Sedoa Village, North Lore District, Poso Regency ranged from 4.5-9 mg/L (Pogoa & Tahril, 2021). Sulfur components are needed in soil but in ideal amounts. Sulfur acts as a component of essential amino acids (cystine, cysteine, methionine) that play a role in chlorophyll formation and protein synthesis. Most of the sulfur in the soil comes from decomposed organic matter and elemental sulfur (sulfur powder/stone) from volcanic activity. Water-soluble sulfur will be immediately absorbed by plants because this element is needed especially in young plants (Narayan *et al*., 2023).

The P_2O_5 content in the Nglimut hot spring soil of Mount Ungaran was 323 ppm (Table 1), lower than that in the production soil of Mount Ungaran (410 ppm) (Susanti *et al*., 2024). The presence of phosphate in hot spring soil is greatly influenced by the presence of phosphate-solubilizing microorganisms. Bacteria dominate about 50% as phosphate solubilizers, and most phosphate-solubilizing microorganisms are found in the rhizosphere (Kalayu, 2019). The rhizospere microorganisms include the order Rhizobiales. In line with the P_2O_5 content, the relative number of the Rhizospere order in this study was 5.77% (Figure 2A), less than in the production soil of Mount Ungaran (13.17%) (Susanti *et al*., 2024). The amount of Ca-dd in hot spring soil was 16.36% (Table 1), higher than the production soil of Mount Ungaran (10.07%) (Susanti *et al*., 2024). The amount of Ca has a high correlation with the abundance of the Firmicutes phylum (Najar *et al*., 2022). The genus Anoxybacillus which is included in the Firmicutes phylum is able to utilize various heavy metals such as Cu, Mn, Ni, Pb, Zn and Ca (Rawat & Joshi, 2019).

Table 1. The physics and chemical data of Nglimut hot spring, Mount Ungaran, Indonesia

Physics Parameter	Value	Chemical Parameter	Value
pH	7.01	C-organic (%)	1.46
Moisture (%)	57	N-total (%)	0.12
Temperature (°C)	61	K-available (ppm)	132
Texture	Gravelly clay	P_2O_5 (ppm)	323
Color	Yellowish-brown	Ca-dd (cmol/kg)	16.36
		Mg-dd (cmol/kg)	4.44
		Na-dd (cmol/kg)	0.38
		Fe (ppm)	1.98
		S (%)	7.86

Metagenomic Analysis Results

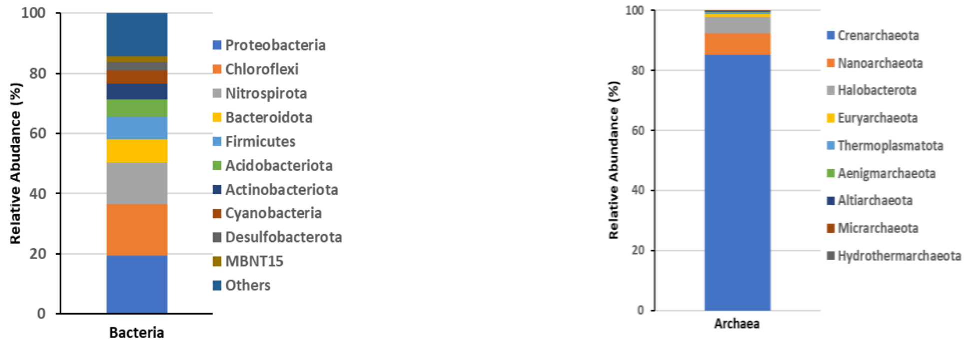
The total microbiota sequences analyzed in this study were 103,889 OTUs (Operational Taxonomic Unit) (Table 2), consisting of 98,603 Bacteria OTUs and 5,286 archaea OTUs. The number of OTUs in the hot spring microbiota is greater than in the productive land of Mount Ungaran, which is 66,124 OTUs (Susanti *et al*., 2024). The findings show that the Bacteria domain is more dominant than the Archaea domain in hot spring soils. This is consistent with research that the Bacteria domain has the highest relative abundance in soil ecosystems. Despite their relatively small numbers, Archaea also play an important role in nutrient cycling and the soil ecosystem. Some Archaea can be involved in nitrogen, methane, and sulfur cycles, and have the ability to survive in extreme environmental conditions (Jung *et al*., 2020). The presence of archaebacteria is as important as eubacteria. According to Jaffe *et al*. (2023), archaebacteria play an important role in environments that have minimal oxygen content. Under these conditions, the increased metabolism of archaebacteria will lead to increased decomposition and turnover of organic matter thus releasing some carbon into the environment (Wang *et al*., 2014). According to Naitam and Kaushik (2021), archaebacteria are also involved in the biogeochemical cycle in the rhizosphere of rice plants through organic matter decomposition and nutrient formation.

The abundance-based coverage estimator (ACE) and Chao1 index values showed that the microbiome in the hot spring soil was highly abundant. The diversity value of hot spring soil is relatively high, as shown by the Shannon index and Simpson index values in Table 2. The high diversity of microbiota in hot spring soil shows that extreme soil conditions still allow the life of various microorganisms that are able to adapt to these conditions. Hot springs have higher sulfur content and temperature (Table 1). Certain types of bacteria are able to survive in the extreme conditions of hot spring soil because they are able to utilize sulfur content and survive at high temperatures, so there are more diverse species (Zhou *et al*., 2016). Certain types of bacteria can survive the extreme conditions of hot spring soils because they are able to utilize sulfur content and survive at high temperatures. They have special adaptations to survive and thrive in these extreme environmental conditions (Kochhar *et al*., 2022).

Table 2. Diversity index

Sequencing Results and Diversity Indices	Value
Operational taxonomic unit (OTUs)	103889
Observed-species	3338
Shannon Index	9187
Simpson Index	0.994
Chao1 Index	3836532
ACE (abundance-based coverage estimator)	3788722
Good’s coverage	0.990

At the phylum level, all DNA sequences of soil microbiota bacteria identified 53 phyla. The five microbial phyla that dominated the hot spring soil were Proteobacteria (19.42%), Chloroflexi (17.21%), Nitrospirota (13.76%), Bacteroidota (7.67%) and Firmicutes (7.33%) (Figure 1A). Proteobacteria are a very diverse microbial phylum and can be found in various environments including hot spring soils. The phylum Proteobacteria also dominates and plays a role in soil fertility in Rubber-Canna (*Hevea brasiliensis-Canna indica*) agroforestry soils (Sasaerila *et al*., 2024). Some members of Proteobacteria can break down sulfur compounds and help in sulfur cycling which is important for soil ecosystems (Wang *et al*., 2021). The Chloroflexi phylum has a dominance of 17.21% in hot spring soil. Chloroflexi has an important role in oxidizing and metabolizing sulfur compounds in hot spring soils. Some species of the phylum Chloroflexi such as *Chloroflexus aurantiacus* and *Oscillochloris trichoides* can oxidize sulfur compounds and contribute to the sulfur cycle (George *et al*., 2020). In addition, some microbes in the phylum Chloroflexi can produce the enzyme sulfur dioxide hydrogenase which plays a role in the breakdown of sulfur dioxide compounds (Wasmund *et al*., 2017). Because of its ability to utilize sulfur, the Chloroflexi phylum is abundant in hot spring soils.



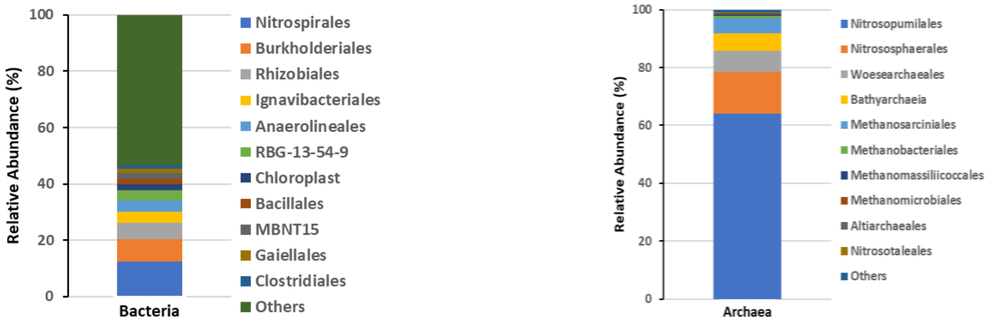
A B

Figure 1. Relative abundance (%) of bacteria (A) and archaea (B) phylum from Nglimut hot spring, Mount Ungaran, Central Java, Indonesia

The abundance of Nitrospirota in hot spring soil in this study reached 13.76% (Figure 1A). Nitrospirota has the ability to perform complete ammonia oxidation (comammox) in an environment with mesothermal conditions (Koch *et al*., 2019). The thermophilic comammox Nitrospira can survive and thrive at relatively high temperatures ranging from 36 to 80°C (Zhang *et al*., 2023). Comammox Nitrospira grows ideally at 50-60°C (Koch *et al*., 2019). This is in accordance with the temperature data on hot spring soil (61ºC) which has an ideal temperature for Nitrospirota growth so that the bacteria are abundant in hot spring soil. Its ability to metabolize nitrogen, thermal adaptation, and oxidative stress in extreme places confirm its ability to survive in the hot spring (Bayer *et al*., 2021).

At the order level, the bacterial community in the hot spring soil in this study found 305 orders. The six highest orders were Nitrospirales (12.35%), Burkholderiales (7.99%), Rhizobiales (5.77%), Ignavibacteriales (4.06), Anaerolineales (3.92%), RBG-13-54-9 (3.68%) and Cloroplast (2.16%). Other orders such as Baciallales, MBNT15, Gaiellales, and Clostridiales were each found at less than 2% (Figure 2A). Nitrospirales, including the genus Nitrospira, are chemolithoautotrophic nitrite-oxidizing bacteria (NOB) that are widely distributed in the environment, including hot springs (Daims *et al*., 2016). These microorganisms obtain energy through nitrite oxidation and CO_2 fixation, and can perform alternative energy metabolism and complete ammonia oxidation (comammox Nitrospira) (Mueler *et al*., 2023).

The Burkholderiales order in Korean hot springs was found to have 128 OTUs (Panda *et al*., 2016), while in this study 7791 OTUs were found (relative abundance of 7.99%) (Figure 2A). Meanwhile, in hot spring water from Bantang (China), the relative abundance of the Burkholderiales order was identified as reaching 10.58% (Fang & Yan, 2022). Bacteria in the order Burkholderiales have been reported to be involved in pathogen suppression, and may play a role in soil nitrogen fixation (Aguirre-von-Wobeser *et al*., 2018). In addition, Burkholderiales also act as a degrader of organic compounds, such as pentachlorophenol and dichlorophenoxyacetate as pesticide chemicals. Pentachlorophenol is widely found in fungicides, herbicides, and biocides, so it is classified as the most toxic chlorophenolic pollutant in the environment (Tong *et al*., 2015).



A B

Figure 2. Relative abundance (%) of bacteria (A)and archaea (B) order from Nglimut hot spring Mount Ungaran, Central Java, Indonesia

In soil, the Rhizobiales order (5.77%; Figure 2A) plays a role in nitrogen fixation (Jones, 2015). The Rhizobiales order is widely categorized as a group of P-solubilizing and N-fixing bacteria. The phosphate-solubilizing bacteria group plays a role in increasing the availability of P in the soil so that it is easily absorbed by plants while N-fixing bacteria use N as a source of growth (Timofeeva *et al.*, 2023). Bacteria dominate about 50% as phosphate solubilizers, while fungi only contribute 0.5%. And most phosphate-solubilizing microorganisms are found in the rhizosphere (Kalayu, 2019).

Order Anaerolineales (3.92%, Figure 2A) is a thermophilic microbe (Moreno *et al*., 2023), because it can survive in hot spring ecosystems. Thermophiles are divided into three, namely moderate thermophiles (optimum growth temperature 50-60°C), extreme thermophiles (optimum growth temperature 60-80°C), and hyperthermophiles (optimum growth temperature 80-110°C) (Gupta *et al*., 2014). According to Lee et al. (2022) thermophilic bacteria can produce thermostable enzymes (proteolytic, lipolytic and amylolytic) that allow bacteria to survive at high temperatures. In the hot springs in this study, the temperature reached 61°C (Table 1), so it still supports the growth of Anaerolineales. In production land, Anaerolineales can act as a decomposer of organic matter (Wright & Lima, 2021). As reported by Ji *et al*. (2018), the number of Anaerolineales is very abundant in the process of straw decomposition.

Order Gaiellales (<2%; Figure 2A) can cause damage to the surrounding ecosystem through biological processes such as the chlorination effect on pentachlorophenol (Li *et al.*, 2019). The results of the study by Liang *et al.* (2021) showed that Gaiellales are abundant in environments with acidic conditions. Hot spring sediment soil in this research has a pH value of 7.01 which is included in neutral pH (Table 1), causing the abundance of Gaiellales to be not too high at 1.72% (Figure 2A).

A total of nine archaea phyla were identified in the hot spring soil, dominated by Crenarchaeota (85.13%), Nanoarchaeota (7.13%) and Halobacterota (5.58%). Other phyla such as Euryarchaeota, Thermoplasmatota, Aenigmarchaeota, Altiarchaeota, Micrarchaeota, and Hydrothermarchaeota were each found at less than 1% (Figure 1B). Some Crenarchaeota species can assist in the degradation of organic compounds such as amino acids and carbohydrates in hot spring soils (Song *et al.*, 2010). The presence of Crenarchaeota in hot spring soils (85.13%; Figure 1B), can affect the composition and activity of other bacteria and fungi in the soil (Köhler *et al*., 2023). The presence of Crenarchaeota can increase the activity of sulfate-reducing bacteria in sulfuric soils (Balk *et al*., 2015). Nanoarchaeota (7.13%; Figure 1B) are small cells that depend on other archaeal cells for their growth and replication. Nanoarchaeota were originally discovered in marine hydrothermal environments and hot springs. Species in the order Nanoarchaeota are obligate ectobionts, with different host species (Munson-McGee *et al*., 2020).

At the order level, the archaebacteria community in the hot spring soil microbiota in this study found 15 orders, dominated by Nitrosopumilales (64.08%), Nitrososphaerales (14.39%), Woesearchaeales (7.24%), Bathyarchaeia (6.15%) and Methanosarciniales (5.07%). Other orders were each found at less than 1% (Figure 2B). Nitrosopumilales (64.08%; Figure 2B) are thermophilic archaea (Jia *et al.*, 2014). The water and soil temperatures at the hot springs in this study were 61°C (Table 1), thus supporting the growth of thermophilic bacteria. Nitrosopumilales is mesophilic to moderately thermophilic, capable of aerobically oxidizing ammonia, and some of them are able to use urea as a substrate. The Nitrosopumilales is also autotrophic, fixing CO_2 via the 3-hydroxy-propionate/4-hydroxybutyrate pathway (Qin *et al*., 2016). The relative abundance of the Nitroso-sphaerales in this study was 14.39% (Figure 2B). Nitrososphaerales is mesophilic (Araujo *et al*., 2018), shows chemolithoautotrophic metabolism through ammonia oxidation and CO_2 fixation (Kerou *et al*., 2015), and is able to oxidize ammonia to obtain energy (Mutschlechner *et al*., 2020). Ammonia oxidizing bacteria play a role in the nitrification process, releasing NO_3 and NO_2 which can be used by plants (Hu *et al*., 2014).

The abundance of Woesearchaeales in the hot spring in this study was 7.24% (Figure 2B). Woesearchaeales can live in various environments such as groundwater (Castelle *et al*., 2015), surface water (Ortiz-Alvarez & Casamayor, 2016), activated sludge (Xu *et al*., 2017), and wetlands (Long *et al*., 2016). Woesearchaeales have the ability to activate carbon and hydrogen metabolism in low oxygen conditions (Castelle *et al*., 2015). According to Liu *et al*. (2018), oxygen availability is one of the determining factors in the abundance of Woesearchaeales. The Methanosarcinales order includes anaerobic methanogens, so it is found in large numbers in low-oxygen environments (Crevecoeur *et al*., 2016). Oxygen in the environment is limited because the soil is always flooded, greatly supporting the growth of anaerobic microbes (Hu *et al*., 2013). The anaerobic decomposition process of organic matter by Methanosarcinales produces methane gas (Buan, 2018). Synthesizing methane is a necessity for methanogenic microbes, carried out to save energy using the Wolfe Cycle (Thauer, 2012).

The important findings of this study are the characteristics of the microbial community in the Ngimut hot spring of Mount Ungaran and its role in the ecosystem. The microbial community can be further used to understand the dynamics and adaptation of microbes, the reciprocal relationship between microbes and the environment, and to find microbial enzymes that can be utilized for industry, agriculture and the environment.

CONCLUSION

The hot spring soil microbiota is more dominated by the Bacteria domain than the Archaea domain. The bacteria that dominate the soil hot springs are bacteria that can survive at high temperatures (thermophilic) and are able to utilize sulfur. The presence of archaea in hot spring soil helps increase the activity of sulfate-reducing bacteria in sulfur-containing soils. It is necessary to further explore the thermophilic enzymes produced by microbes in hot springs for the development of modern biotechnology.

REFERENCES

Aguirre-von-Wobeser, E., Rocha-Estrada, J., Shapiro, L.R., & de la Torre, M. (2018). Enrichment of Verrucomicrobia, Actinobacteria and Burkholderiales drives selection of bacterial community from soil by maize roots in a traditional milpa agroecosystem. *PloS ONE, 13*(12), e0208852. https://doi.org/10.1371/journal.pone.0208852

Araujo, A.S.F., Mendes, L.W., Bezerra, W.M., Nunes, L.A.P.L., Lyra, M.D.C.C.P., Figueiredo, M.D.V.B., & Melo, V.M.M. (2018). Archaea diversity in vegetation gradients from the Brazilian Cerrado. *Brazilian Journal of Microbiology, 49*, 522-528. https://doi.org/10.1016/j.bjm.2017.08.010

Baldrian, P. (2017). Forest microbiome: diversity, complexity and dynamics. *FEMS Microbiology Reviews, 41*(2), 109-130. https://doi.org/ 10.1093/femsre/fuw040

Baliyarsingh, B., Nayak, S.K., & Mishra, B.B. (2017). Soil microbial diversity: an ecophysiological study and role in plant productivity. In: S.K. Nayak & B.B. Mishra, *Advances in Soil Microbiology: Recent Trends and Future Prospects* (pp. 1-17). https://doi.org/10.1007/978-981-10-7380-9_1

Balk, M., Keuskamp, J.A., & Laanbroek, H.J. (2015). Potential activity, size, and structure of sulfate-reducing microbial communities in an exposed, grazed and a sheltered, non-grazed mangrove stand at the red sea coast. *Frontiers in Microbiology, 6*, 1-13. https://doi.org/10.3389/fmicb.2015.01478

Bayer, B., Saito, M.A., McIlvin, M.R., Lücker, S., Moran, D.M., Lankiewicz, T.S., Dupont, C.L., & Santoro, A.E. (2021). Metabolic versatility of the nitrite-oxidizing bacterium *Nitrospira marina* and its proteomic response to oxygen-limited conditions. *ISME Journal, 15*(4), 1025-1039. https://doi.org/10.1038/s41396-020-00828-3

Bokulich, N.A., Kaehler, B.D., Rideout, J.R., Dillon, M., Bolyen, E., Knight, R., Huttley, G.A., & Caporaso, J.G. (2018). Optimizing taxonomic classification of marker-gene amplicon sequences with QIIME 2's Q2-feature-classifier plugin. *Microbiome, 6*(1), 1-17. https://doi.org/10.1186/s40168-018-0470-z

Buan, N.R. (2018). Methanogens: pushing the boundaries of biology. *Emerging Topics in Life Sciences, 2*(4), 629-646. 581-583. https://doi.org/10.1042/ETLS20180031

Callahan, B.J., McMurdie, P.J., Rosen, M.J., Han, A. W., Johnson, A.J.A., & Holmes, S.P. (2016). DADA2: high-resolution sample inference from illumina amplicon data. *Nature Methods, 13*(7), 581-583. https://doi.org/10.1038/nmeth.3869

Caporaso, J.G., Kuczynski, J., Stombaugh, J., Bittinger, K., Bushman, F.D., Costello, E.K., Fierer, N., Peña, A.G., Goodrich, J.K., Gordon, J.I., Huttley, G.A., Kelley, S.T., Knights, D., Koenig, J.E., Ley, R.E., Lozupone, C.A., McDonald, D., Muegge, B.D., Pirrung, M., Reeder, J., Sevinsky, J.R., Turnbaugh, P.J., Walters, W.A., Widmann, J., Yatsunenko, T., Zaneveld, J., & Knight, R. (2010). Correspondence QIIME allows analysis of high-throughput community sequencing data intensity normalization improves color calling in solid sequencing. *Nature, 7*(5), 335-336. https://doi.org/10.1038/nmeth0510-335

Castelle, C.J., Wrighton, K.C., Thomas, B.C., Hug, L.A., Brown, C.T., Wilkins, M.J., Frischkorn, K.R., Tringe, S.G., Singh, A., & Markillie, L. M. (2015). Genomic expansion of domain archaea highlights roles for organisms from new phyla in anaerobic carbon cycling. *Current Biology, 25*(6), 690-701. https://doi.org/10.1016/j.cub.2015.01.014.

Chao, A. 1984. Nonparametric estimation of the number of classes in a population. *Scandinavian Journal of Statistics,11* (4), 265-270.

Crevecoeur, S., Vincent, W.F., & Lovejoy, C. (2016). Environmental selection of planktonic methanogens in permafrost thaw ponds. *Scientific Reports, 6*(1), 1-10. https://doi.org/10.1038/srep31312

Daims, H., Lücker, S., & Wagner, M. (2016). A new perspective on microbes formerly known as nitrite-oxidizing bacteria. *Trends Microbiology, 24,* 699-712. https://doi.org/10.1016/j.tim.2016.05.004

DeSantis, T.Z., Hugenholtz, P., Larsen, N., Rojas, M., Brodie, E.L., Keller, K., Huber, T., Dalevi, D., Hu, P., & Andersen, G.L. (2006). Greengenes, a chimera-checked 16S rRNA gene database and workbench compatible with ARB. *Applied and Environmental Microbiology, 72*(2), 5069-5072. https://doi.org/10.1128/AEM.03006-05.

Fang, S., & Yan, J. (2022). Analysis of prokaryotic microbial diversity in hot spring water from Bantang (China) using the targeted amplicon analysis. *All Life, 15*(1), 325-339. https://doi.org/10.1080/26895293.2022.2049899

George, D.M., Vincent, A.S., & Mackey, H.R. (2020). An overview of anoxygenic phototrophic bacteria and their applications in environmental biotechnology for sustainable resource recovery. *Biotechnology Reports, 28,* e00563. https://doi.org/10.1016/j.btre.2020.e00563

Gupta, A., Gupta, R., & Singh, R.L. (2016). Microbes and environment. In: R.L. Singh (Ed). *Principles and Applications of Environ-mental Biotechnology for a Sustainable Future* (pp. 43-84). https://doi.org/ 10.1007/ 978-981-10-1866-4_3

Gupta, G., Srivastava, S., Khare, S.K., & Prakash, V. (2014). Extremophiles: An overview of microorganism from extreme environment. *International Journal of Agriculture Environment Biotechnology, 7*,371-380. https://doi.org/10.5958/2230-732X.2014. 00258.7

Hamzah, Z. , Rani, N.L.A., Saat, A., & Wood, A.K. 2013. Determination of hot springs physicochemical water quality potentially use for balneotherapy. *Malaysian Journal of Analytical Sciences, 17*(3), 436-444.

Hidayat, M.Y., Saud, H.M., & Samsudin, A.A. (2017). Isolation and characterisation of sulphur oxidizing bacteria isolated from hot spring in malaysia for biological deodorisation of hydrogen sulphide in chicken manure. *Media Peternakan, 40*(3), 178-187. https://doi.org/10.5398/medpet.2017.40.3.178

Holm, J.B., Humphrys, M.S., Robinson, C.K., Settles, M.L., Ott, S., Fu, L., Yang, H., Gajer, P., He, X., McComb, E., Gravitt, P.E., Ghanem, K.G., Brotman, R.M., & Ravel, J. (2019). Ultrahigh-throughput multiplexing and sequencing of >500 basepair amplicon regions on the illumina HiSeq 2500 platform. *mSystems, 4*(1), e00029-19. https://doi.org/10.1128/msystems.0002919.

Hu, H.-W., Zhang, L.-M., Yuan, C.-L., & He, J.-Z. (2013). Contrasting euryarchaeota communities between upland and paddy soils exhibited similar pH-impacted biogeographic patterns. *Soil Biology and Biochemistry, 64,* 18-27. https://doi.org/10.1016/j.soilbio.2013.04.003

Hu, H.-W., Xu, Z.-H., & He, J.-Z. (2014). Ammonia-oxidizing archaea play a predominant role in acid soil nitrification. *Advances in Agronomy, 125,* 261-302. https://doi.org/10.1016/B978-0-12-800137-0.00006-6

Jaffe, A.L., Castelle, C.J., & Banfield, J.F. (2023). Habitat transition in the evolution of bacteria and archaea. *Annual Review of Microbiology, 44*(2), 22-29. https://doi.org/10.1146/annurev-micro-041320-032304

Ji, Y., Liu, P., & Conrad, R. (2018). Response of fermenting bacterial and methanogenic archaeal communities in paddy soil to progressing rice straw degradation. *Soil Biology and Biochemistry, 124,* 70-80. https://doi.org/10.1016/j.soilbio.2018.05.029

Jia, C., Zhang, C.L., Xie, W., Wang, J.-X., Li, F., Wang, S., Dong, H., Li, W., & Boyd, E.S. (2014). Differential temperature and pH controls on the abundance and composition of H-GDGTs in terrestrial hot springs. *Organic Geochemistry, 75,* 109-121. https://doi.org/10.1016/j.orggeochem.2014.06.009

Jones, R.T. (2015). A comprehensive survey of soil Rhizobiales diversity using high-throughput DNA sequencing. *Biological Nitrogen Fixation, 2,* 769-776. https://doi.org/10.1002/9781119053095.ch76

Jung, J., Kim, J.S., Taffner, J., Berg, G., & Ryu, C.M. (2020). Archaea, tiny helpers of land plants. *Computational and Structural Biotechnology Journal, 18,* 2494-2500. https://doi.org/10.1016/j.csbj.2020.09.005

Kalayu, G. (2019). Phosphate solubilizing microorganisms: promising approach as biofertilizers. *International Journal of Agronomy, 2019*, 4917256. https://doi.org/10.1155/2019/4917256

Kerou, M., Alves, R.J.E., & Schleper, C. (2015). Nitrososphaerales. *Bergey's Manual of Systematics of Archaea and Bacteria, 4*, 1-4. https://doi.org/10.1002/9781118960608.cbm00055

Koch, H., van Kessel, M.A.H.J., & Lücker, S. (2019). Complete nitrification: insights into the ecophysiology of comammox Nitrospira. *Applied Microbiology and Biotechnology, 103*(1), 177-189. https://doi.org/10.1007/s00253-018-9486-3

Kochhar, N., Kavya, I.K., Shrivastava, S., Ghosh, A., Rawat, V.S., Sodhi, K.K., & Kumar, M. (2022). Perspectives on the microorganism of extreme environments and their applications. *Current Research in Microbial Sciences, 3*, 100134. https://doi.org/10.1016/j.crmicr.2022.100134

Köhler, J.M., Ehrhardt, L., & Günther, P.M. (2023). Archaeal and extremophilic bacteria from different archaeological excavation sites. *International Journal of Molecular Sciences, 24*(6), 5519. https://doi.org/10.3390/ijms 24065519

Lee, Y.J., Ganbat, D., Oh, D., Kim, H., Jeong, G.E., Cha, I.T., Kim, S.B., Nam, G., Jung, Y.J., & Lee, S.J. (2022). Isolation and characterization of thermophilic bacteria from hot springs in Republic of Korea. *Microorganisms, 10*(12), 2375. https://doi.org/10.3390/microorganisms10122375

Li, H., Jiang, Y., Chen, L., Chen, Y., Wen, X., & Tao, L. (2019). Carbon sources mediate microbial pentachlorophenol dechlorination in soils. *Journal of Hazardous Materials, 373*, 716-724. https://doi.org/10.1016/j.jhazmat.2019.03.109

Liang, J., Yu, X., Cao, Y., Zhang, J., Yan, N., Xue, L., Cai, X., & Shen, G. (2021). Effect of quicklime on microbial community in strong acidic soil. *Journal of Soil Science and Plant Nutrition, 21*, 1771-1781. https://doi.org/10.1007/s42729-021-00478-0

Lischer, K., Putra, A.B.R.D., Guslianto, B.W., Avilla, F., Sitorus, S.G., Nugraha, Y., & Sarmoko. (2020). The emergence and rise of indigenous thermophilic bacteria exploration from hot springs in Indonesia. *Biodiversitas, 21*(11), 5474-5481. https://doi.org/10.13057/biodiv/d211156

Liu, X., Li, M., Castelle, C.J., Probst, A.J., Zhou, Z., Pan, J., Liu, Y., Banfield, J.F., & Gu, J.-D. (2018). Insights into the ecology, evolution, and metabolism of the widespread Woesearchaeotal lineages. *Microbiome, 6*, 1-16. https://doi.org/10.1186/s40168-018-0488-2

Liu, S., Sun, Y., Shi, F., Liu, Y., Wang, F., Dong, S., & Li, M. (2022). Composition and diversity of soil microbial community associated with land use types in the agro-pastoral area in the upper yellow River Basin. *Frontiers in Plant Science, 13*, 1-14. https://doi.org/10.3389/fpls.2022.819661

Long, Y., Yi, H., Chen, S., Zhang, Z., Cui, K., Bing, Y., Zhuo, Q., Li, B., Xie, S., & Guo, Q. (2016). Influences of plant type on bacterial and archaeal communities in constructed wetland treating polluted river water. *Environmental Science and Pollution Research, 23*, 19570-19579. https://doi.org/10.1007/s11356-016-7166-3

Ma, B., Zhao, K., Lv, X., Su, W., Dai, Z., Gilbert, J. A., Brookes, P.C., Faust, K., & Xu, J. (2018). Genetic correlation network prediction of forest soil microbial functional organization. *The ISME Journal, 12*(10), 2492-2505. https://doi.org/10.1038/s41396-018-0232-8

Moreno, I.J., Brahamsha, B., Donia, M.S., & Palenik, B. (2023). Diverse microbial hot spring mat communities at Black Canyon of the Colorado River. *Microbial Ecology, 86*, 1-18. https://doi.org/10.1007/s00248-023-02186-x

Mueller, A.J., Daebeler, A., Herbold, C.W., Kirkegaard, R.H., & Daimset, H. (2023). Cultivation and genomic characterization of novel and ubiquitous marine nitrite-oxidizing bacteria from the Nitrospirales. *The ISME Journal, 17*, 2123-2133. https://doi.org/10.1038/s41396-023-01518-6.

Munson-McGee, J.H., Rooney, C., & Young, M.J. (2020). An uncultivated virus infecting a Nanoarchaeal parasite in the hot springs of Yellowstone National Park. *Journal of Virology, 94*(3), e01213-19. https://doi.org/10.1128/JVI.01213-19

Mutschlechner, M., Praeg, N., & Illmer, P. (2020). Soil-derived inocula enhance methane production and counteract common process failures during anaerobic digestion. *Frontiers in Microbiology, 11*, 1-18. https://doi.org/ 10.3389/fmicb.2020.572759

Naitam, M.G., & Kaushik, R. (2021). Archaea: an agro-ecological perspective. *Current Microbiology, 78*(7), 2510-2521. https://doi.org/10.1007/s00284-021-02537-2

Najar, I.N., Sharma, P., Das, S., Sherpa, M.T., Kumar, S., & Thakur, N. (2022). Bacterial diversity, physicochemical and geothermometry of South Asian hot springs. *Current Research in Microbial Sciences, 3*, 100125. https://doi.org/ 10.1016/j.crmicr.2022. 100125

Nannipieri, P., Ascher, J., Ceccherini, M.T., Landi, L., Pietramellara, G., & Renella, G. (2017). Microbial diversity and soil functions. *European Journal of Soil Science, 68*(1), 12-26. https://doi.org/10.1111/ejss.4_12398

Narayan, O.P., Kumar, P., Yadav, B., Dua, M., & Johri, A.K. (2023). Sulfur nutrition and its role in plant growth and development. P*lant Signaling & Behavior, 18*(1), 2030082. https://doi.org/10.1080/15592324.2022.2030082

Odelade, K.A., & Babalola, O.O. (2019). Bacteria, fungi and archaea domains in rhizospheric soil and their effects in enhancing agricultural productivity. *International Journal of Environmental Research and Public Health, 16*(20), 3873. https://doi.org/10.3390/ijerph162 03873.

Ortiz, R., & Casamayor, E.O. (2016). High occurrence of Pacearchaeota and Woesearchaeota (*Archaea superphylum* DPANN) in the surface waters of oligotrophic high-altitude lakes. *Environmental Microbiology Reports, 8*(2), 210-217. https://doi.org/10.1111/1758-22 29.12370

Panda, A.K., Bisht, S.S., De Mandal, S., & Kumar, N.S. (2016). Bacterial and archeal community composition in hot springs from Indo-Burma region, North-East India. *AMB Express, 6*, 111 (2016). https://doi.org/10.1186/s13568-016-02 84-y

Plugge, C.M., Zhang, W., Scholten, J.C.M., & Stams, A.J.M. (2011). Metabolic flexibility of sulfate-reducing bacteria. *Frontiers in Microbiology, 2*, 1-8. https://doi.org/10.3389/ fmicb.2011.00081

Pogoa, M., & Tahril. (2021). Analysis of sulfur content in the hot springs at the foot of the mountain in Sedoa Village North Lore District Poso Regency. *Media Eksakta, 17*(2), 98-101. https://doi.org/10.22487/me.v17i2.1289

Qin, W., Martens-Habbena, W., Kobelt, J.N., David, A., & Stahl, D.A. (2016). Nitrosopumilales. *Bergey's Manual of Systematics of Archaea and Bacteria*. https://doi.org/10.1002/9781118960 608.obm00122

Rahman, N.S.N.A., Hamid, N.W.A., & Nadarajah, K. (2021). Effects of abiotic stress on soil microbiome. *International Journal of Molecular Sciences, 22*(16), 9036. https://doi.org/10.3390/ijms22169036

Rawat, N., & Joshi, G.K. (2019). Bacterial community structure analysis of a hot spring soil by next generation sequencing of ribosomal RNA. *Genomics, 111*(5), 1053-1058. https://doi.org/10.1016/j.ygeno.2018.06.008.

Rozanov, A.S., Bryanskaya, A.V., Ivanisenko, T.V., Malup, T.K., & Peltek, S.E. (2017). Biodiversity of the microbial mat of the Garga hot spring. *BMC Evolutionary Biology, 17*(2), 254. https://doi.org/10.1186/s12862-017-1106-9

Sasaerila, H.Y., Effendi, Y., Wijihastuti, R.S., Pambudi, A., & Nicola, F.D. (2024). Studies on the short-and long-term effects of Rubber-Canna agroforestry through soil analysis and a metagenomic approach. *Biosaintifika: Journal of Biology & Biology Education*, 16(1), 73-88. http://dx.doi.org/10.15294/biosaintifika.v15i1. 3514.

Satoh, T., Watanabe, K., Yamamoto, H., Yamamoto, S., & Kurosawa, N. (2013). Archaeal community structures in the solfataric acidic hot springs with different temperatures and elemental compositions. *Archaea, 2013*, 7238 71. https://doi.org/10.1155/2013/723871

Shannon, C.E., & Weaver, W. (1949). The mathematical theory of communication. University of Illinois Press, Urbana.

Simpson, E.H. (1949). Measurement of diversity. *Nature, 163*(1), 688. https://doi.org/10.1038/ 163688a0.

Song, Z.Q., Chen, J.Q., Jiang, H.C., Zhou, E.M., Tang, S.K., Zhi, X.Y., Zhang, L.X., Zhang, C.L., & Li, W.J. (2010). Diversity of Crenarchaeota in terrestrial hot springs in Tengchong, China. *Extremophiles, 14*(3), 287-296. https://doi.org/10.1007/s00792-010-0307-6

Susanti, R., Widiatningrum, T., Jaya, A.F., Nisa', F.F., Kenarni, N.R., & Mukaromah, R.L. (2024). Metagenomic analysis of soil forest microbiota on Mount Ungaran, Central Java, Indonesia. *IOP Conference Series: Earth and Environmental Science 1315*, 012018. https://doi.org/10.1088/1755-1315/1315/1/012 018.

Tamazawa, S., Takasaki, K., Tamaki, H., Kamagata, Y., & Hanada, S. (2012). Metagenomic and biochemical characterizations of sulfur oxidation metabolism in uncultured large sausage-shaped bacterium in hot spring microbial mats. *PLoS ONE, 7*(11), 1-11. https://doi.org/10.1371/journal.pone.0049793

Thauer, R.K. (2012). The wolfe cycle comes full circle. *Proceedings of the National Academy of*

Sciences of the United States of America, 109, 15084-15085. https://doi.org/10.1073/pnas.1213193109

Timofeeva, A.M., Galyamova, M.R., & Sedykh,S.E. (2023). Plant growth-promoting soil bacteria: nitrogen fixation, phosphate solubilization, siderophore production, and other biological activities. *Plants, 12*(24), 4074. https://doi.org/10.3390/plants12244074

Tong, H., Hu, M., Li, F., Chen, M., & Lv, Y. (2015). Burkholderiales participating in pentachlorophenol biodegradation in iron-reducing paddy soil as identified by stable isotope probing. *Environmental Science: Processes & Impacts, 17*(7), 1282-1289.

Van Der Heijden, M.G.A., Bardgett, R.D., & Van Straalen, N.M. (2008). The unseen majority: soil microbes as drivers of plant diversity and productivity in terrestrial ecosystems. *Ecology Letters*, *11*(3), 296-310. https://doi.org/10.1111/j.1461-0248.2007. 01139.x

Wang, F.-P., Zhang, Y., Chen, Y., He, Y., Qi, J., Hinrichs, K.-U., Zhang, X.-X., Xiao, X., & Boon, N. (2014). Methanotrophic archaea possessing diverging methane-oxidizing and electron-transporting pathways. *The ISME Journal, 8*(5), 1069-1078. https://doi.org/10.1038/ismej.2013.212.

Wang, M., Wang, L., Shi, H., Liu, Y., & Chen, S. (2021). Soil bacteria, genes, and metabolites stimulated during sulfur cycling and cadmium mobilization under sodium sulfate stress. *Environmental Research, 201*(12), 111599. https://doi.org/10.1016/j.envres.2021.111599

Wasmund, K., Mußmann, M., & Loy, A. (2017). The life sulfuric: microbial ecology of sulfur cycling in marine sediments. *Environmental Microbiology Reports, 9*(4), 323-344. https://doi.org/10.1111/1758-2229.12538

Wright, M.S., & Lima, I.M. (2021). Identification of microbial populations in blends of worm castings or sugarcane filter mud compost with biochar. *Agronomy, 11*(8), 1671. https://doi.org/10.3390/agronomy11081671

Xu, D., Liu, S., Chen, Q., & Ni, J. (2017). Microbial community compositions in different functional zones of Carrousel oxidation ditch system for domestic wastewater treatment. *AMB Express, 7,* 1-13. https://doi.org/10.1186/s13568-017-0336-y

Zeldes, B.M., Keller, M.W., Loder, A.J., Straub, C. T., Adams, M.W.W., & Kelly, R.M. (2015). Extremely thermophilic microorganisms as metabolic engineering platforms for production of fuels and industrial chemicals. *Frontiers in Microbiology, 6,* 1-17. https://doi.org/10.3389/fmicb.2015.01209

Zhang, Y., Liu, T., Li, M.M., Hua, Z.S., Evans, P., Qu, Y., Tan, S., Zheng, M., Lu, H., Jiao, J-Y., Lücker, S., Daims, H., Li, W-J., & Guo, J. (2023). Hot spring distribution and survival mechanisms of thermophilic comammox Nitrospira. *The ISME Journal, 17*(7), 1-11. https://doi.org/10.1038/s41396-023-01409-w

Zhou, J., Deng, Y., Shen, L., Wen, C., Yan, Q., Ning, D., Qin, Y., Xue, K., Wu, L., He, Z., Voordeckers, J.W., Nostrand, J.D., Buzzard, V., Michaletz, S.T., Enquist, B.J., Weiser, M.D., Kaspari, M., Waide, R., Yang, Y., & Brown, J.H. (2016). Temperature mediates continental-scale diversity of microbes in forest soils. *Nature Communications, 7,* 12083. https://doi.org/10.1038/ncomms12083