

Highlights of Selected Representative Publications

(As of 11/24/2019)

Numerous papers have been published with total citations of ~ 30,000, H-index, **91** based on Web of Science; Google Scholar Citations >40,000, H-index, **106** based on Google Scholar; (a) 2018, 2019 Global highly cited researcher in Cross-field based on numbers of top 1% highly cited publications by Web of Science. <https://hcr.clarivate.com/>; (b) World's most cited researcher (>99.8% percentile) of ~7 millions scientists across all science and engineering fields (176 subfields of 22 fields) by a composite metric based on the Scopus database from Elsevier (<https://doi.org/10.1371/journal.pbio.3000384>). (c) Most highly cited researcher (H-index > 100) according to their Google Scholar Citations A total of **133** papers published in Nature indexed journals, such as *Science* (4), *Nature Climate Change* (3), *PNAS* (10), *Nature Communication* (4), *Ecology Letters* (2), *The ISME Journal* by Nature Publishing Group (43) (The top one journal in microbial ecology), *Environmental Science & Technology* (22), *Water Research* (10), and *Geochimica et Cosmochimica Acta* (2), and other prestigious journals, e.g., *Nature Ecology and Evolution* (2), *Nature Microbiology* (1), *Nature Review in Microbiology* (2), *Nature Plants* (1), *Microbiology and Molecular Biology Reviews* (1), *mBio* (16), *Ecology* (1), and *Global Change Biology* (9).

The publications below represent the depth and breadth of research led by Dr. Zhou.

A. Sixteen most significant and representative publications

1. Ning, D. Y. Deng, J. M. Tiedje, and **J.-Z. Zhou**. 2019. A General Framework for Quantitatively Assessing Ecological Stochasticity. *Proc. Nat. Acad. Sci.*, 116: 16893-16898.

An ecological community is a dynamic complex system with a myriad of interacting species, which are controlled by various scale-dependent deterministic and stochastic forces. Although with rapid advances in genomic technologies, categorizing biological diversity, particularly microbial diversity, become relatively easy, the great challenge is to quantify the relative importance of deterministic and stochastic processes in controlling community assembly. In this study, a general mathematical framework was developed to quantify ecological stochasticity under different situations in which deterministic factors drive the communities more similar or dissimilar than null expectation. A new index, normalized stochasticity ratio (*NST*), was developed with 50% as the boundary point between more deterministic (<50%) and more stochastic (>50%) assembly. This general null-model-based framework provides an effective and robust tool to ecologists for quantitatively assessing ecological stochasticity. By highlighting the caveats such as model selection, similarity metrics, and spatial scales, this study provides general guidance for appropriate use of null model-based approaches for examining community assembly processes. Although this framework was tested with microbial data, it should also be applicable to plant and animal ecology.

2. Wu, L. *, D. Ning*, B. Zhang*, Y. Li, P. Zhang, X. Shan, Q. Zhang, M. Brown, Z. Li, J. D. Van Nostrand, F. Ling, N. Xiao, Y. Zhang, J. Vierheilig, G. F. Wells, Y. Yang, Y. Deng, Q. Tu, A. Wang, Global Water Microbiome Consortium†, Tong Zhang, Z. He, J. Keller, P. H. Nielsen,

P. J. J. Alvarez, C. S. Criddle, M. Wagner, J. M. Tiedje, Q. He, T. P. Curtis, D. A. Stahl, L. Alvarez-Cohen, B. E. Rittmann, X. Wen, and **J.-Z. Zhou**. 2019. *Global diversity and biogeography of bacterial communities in wastewater treatment plants*. **Nature Microbiology**, in press.

Microorganisms in wastewater treatment plants (WWTPs) are essential for water purification to protect public and environmental health. However, their diversity and the underlying mechanisms are poorly understood. To this end, a global water microbiome consortium (GWMC) was established across more than 70 research groups from 23 countries. Using a systematic global-sampling effort, the 16S rRNA gene sequences from ~1200 activated sludge samples from 269 WWTPs in 23 countries on 6 continents were analyzed. Various theoretical questions were addressed, including species abundance distributions, extent of global microbial diversity, global core bacterial community, latitudinal diversity pattern, scale-dependent distance-decay patterns, structure-functions relationships, and community assembly mechanisms. This is the first comprehensive global scale study to address various theoretical questions in engineered systems. The findings of this study highlight how little we know of the world's microbiome, even in one of the most common and well-controlled systems in the built environment, and have important implications for microbial ecology and wastewater treatment processes.

3. Guo, X.*, X. Zhou*, L. Hale*, M. Yuan, D. Ning, J. Feng, Z. Shi, Z. Li, B. Feng, Q. Gao, L. Wu, W. Shi, A. Zhou, Y. Fu, L. Wu, Z. He, J. D. Van Nostrand, G. Qiu, X. Liu, Y. Luo, J. M. Tiedje, Y. Yang, and **J.-Z. Zhou**. 2019. *Climate warming accelerates temporal scaling of grassland soil microbial biodiversity*. **Nature Ecol & Evol.**, in press

Determining temporal scaling of biodiversity, typically described as species time relationships (STRs), in the face of global climate change is a central issue in ecology, because it is fundamental to biodiversity preservation and ecosystem management. However, whether and how climate change affects microbial STRs remains unclear, mainly due to the scarcity of long-term experimental data. This study examined examined STRs and phylogenetic time relationships (PTRs) of soil bacteria and fungi in a long-term multifactor global change experiment with warming, half precipitation, double precipitation and clipping (annual plant biomass removal). By examining the temporal scaling of soil bacteria and fungi across different treatments, this study provides explicit evidence that STRs exist for bacteria and fungi, and their lineages. This is the first study showing that soil microbes exhibit strong PTRs, with the overall rates significantly lower than STRs. This is also the first time to demonstrate that warming played a predominant role in accelerating both taxonomic and phylogenetic temporal scaling rates of soil microbial communities. The results on warming-enhanced temporal scaling of microbial biodiversity imply that the strategies of soil biodiversity preservation and ecosystem managements may require adjustments in a warmer world.

4. Guo, X., J. Feng, Z. Shi, X. Zhou, M. Yuan, X. Tao, L. Hale, T. Yuan, J. Wang, Y. Qin, A. Zhou, Y. Fu, L. Wu, Z. He, J. D. V. Nostrand, D. Ning, X. Liu, Y. Luo, J. M. Tiedje, Y. Yang, and **J.-Z. Zhou**. 2018. *Climate Warming Leads to Divergent Succession of Grassland Microbial Communities*. **Nature Climate Change**. 8:813-818, 10.1038/s41558-018-0254-2

Understanding the succession, adaptations and mechanisms of biological communities in response to environmental changes, especially anthropogenic changes, is a central issue in ecology and for society. However, very little is known about whether and how climate warming affects the succession of ecological communities, and their underlying mechanisms. This study is the **first** to demonstrate that warming played an important role in accelerating temporal succession towards higher divergence, stochastic processes played dominant roles in controlling the responses of soil microbial communities to climate warming, and experimental warming, acting as a filtering factor, reduced stochasticity as time proceeds. Our results imply that, although successional trajectories of microbial communities are difficult to predict under future climate change scenarios, their composition and structure are projected to be less variable due to warming-driven selection.

5. Xue, K., M. M. Yuan, Z. J. Shi, Y. Qin, Y. Deng, L. Cheng, L. Wu, Z. He, J. D. Van Nostrand, R. Bracho, S. Natali, E. A. G. Schuur, C. Luo, K. T. Konstantinidis, Q. Wang, J.R. Cole, J. M. Tiedje, Y. Luo, and **J.-Z. Zhou**. 2016. Tundra soil carbon is vulnerable to rapid microbial decomposition under climate warming. *Nature Climate Change*, 6: 595-600, doi:10.1038/nclimate2940.

The northern permafrost stores more than 30% of the global soil organic C, which is a wildcard that could alter the future trajectory of climate change. How microorganisms respond to warming (speed, direction, magnitudes) is critical to determine the fates of permafrost C and the feedbacks of the permafrost ecosystems to climate warming. Although a laboratory study indicated that microbial communities can respond to temperature increase rapidly, evidence from **field experiments** remains scarce. Using integrated metagenomic technologies, particularly GeoChip, this study reported a rapid response of the tundra microbial communities to experimental warming after only 1.5 years of warming. This is the **first** comprehensive field study demonstrating the *in situ* **vulnerability** of tundra soil C to climate warming and the importance of microbial communities in mediating such vulnerability. The results presented in this study have important implications for future climate projection. This study was highlighted as a breakthrough in tundra ecosystem studies by *The Washington Post* (<http://wpo.st/O4GE1>), and it was also specifically highlighted by ASM in its *MICROBES* publication, and DOE OBER. This paper is ranked as the top 1% highly cited based on Web of Science.

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

Climate warming is increasingly leading to dramatic changes in plant and animal biodiversity, but it remains unclear how temperature rising affects microbial biodiversity, particularly in terrestrial soils. In this study, we showed that taxonomic and phylogenetic diversity of soil bacteria, fungi, and nitrogen fixing bacteria all are better predicted by variation in environmental temperature rather than pH, which is consistent with metabolic theory of ecology (MTE). But, the rates of diversity turnover across global temperature gradients are substantially lower than that recorded for trees. These results indicate that the diversity of plant,

and animal, and soil microbial communities will show differential responses to climate change. This study clearly demonstrate that the diversity of different microbial groups has significantly lower rates of turnover across temperature gradients than other major taxa, which has important implications for assessing the effects of human-caused changes in climate, land use, and other factors. This is the **first demonstration** that temperature plays a primary role in shaping microbial diversity in the forest soils, but it could operate in different ways between plants and microorganisms, and that MTE is applicable to microbial communities.

7. Xu, T., Y. Li, Z. Shi, C. L. Hemme, Y. Li, Y. Zhu, J. D Van Nostrand, Z. He, and **J.-Z. Zhou**. 2015. Efficient genome editing in *Clostridium cellulolyticum* via CRISPR-Cas9 nickase. *Appl. Environ. Microbiol.*, 81: 4423-4431, doi: 10.1128/AEM.00873-15 (**Picked up as AEM Spotlight**)

Molecular studies and genetic engineering in many microbes have been hindered by the lack of efficient genetic tools for targeted genome editing and transcriptional control. The CRISPR-Cas9 system, which has been demonstrated to be a revolutionary genome-editing tool for eukaryotic genomes, has the potential to overcome the challenges of manipulating bacterial genomes. This study developed an efficient Cas9 nickase-based genome editing tool to engineer the genome of *Clostridium cellulolyticum*, an important model microorganism for bioenergy research. The developed tool has several main advantages over traditional methods. First, it enables one-step genome editing (e.g., DNA deletion and gene integration) using a single plasmid so it is much faster and more convenient. Also, it has a nearly 100% editing efficiency and an extraordinary precision which allows for targeted editing at similar sites. In addition, it has the capability of multiplex editing to modify two or more different genomic loci simultaneously. With this tool, unprecedented genome editing in *C. cellulolyticum* towards the improvement of cellulose degradation via promoter/gene integration has been conducted. This study was spotted by AEM as an Editor Pick. Since the publication of this study several months ago, more than 50 institutes around the world have requested the genetic system for their studies. It is expected that in the near future Cas9 technology will spark a revolution in microbial genetic studies.

8. **Zhou, J.-Z.**, Y. Deng, P. Zhang, K. Xue, Y. Liang, J. D. Van Nostrand, Y. Yang, Z. He, L. Wu, D. A. Stahl, T. C. Hazen, J. M Tiedje, and A. P. Arkin. 2014. Stochasticity, Succession and Environmental Perturbations in a Fluidic Ecosystem. *Proc. Nat. Acad. Sci.*, 111: E836-E845

Unraveling the rules that govern assembly and succession of biological communities is a central topic, but poorly understood, in ecology. It is generally believed that both deterministic and stochastic processes play important roles in shaping community composition and structure, but their relative importance remains elusive. To understand their relative importance in mediating microbial community assembly and succession, a novel framework comprised of four theoretical models was developed and tested with a fluidic groundwater ecosystem after nutrient addition. This study showed that community assembly and succession were driven by a dynamic, time-dependent interaction of stochastic and deterministic processes, with stochastic forces dominating. This is the **first** time to demonstrate that community succession responding to nutrient amendment is primarily controlled by stochastic processes, rather than deterministic processes as traditional ecological succession theory predicted. This is also the

first demonstration showing that the drivers controlling biodiversity and community succession are dynamic rather than static. By identifying the mechanisms controlling microbial community assembly and succession, this study makes seminal contribution to the mechanistic understanding essential for a predictive microbial ecology of natural and managed ecosystems. The importance of this study is also evidenced as Editor's Pick in PNAS and Genomeweb. This study was also highlighted by OBER (<http://genomicscience.energy.gov/program/berhighlights.cfm>) as OBER Research Highlights. It is also listed as a highly cited publication (top 1%) in Web of Science.

9. **Zhou, J.-Z.**, Y.H. Jiang, Y. Deng, Z. Shi, B.-Y. Zhou, K. Xue, L. Wu, Z. He, and Y. Yang. 2013. *Random Sampling Process Leads to Overestimation of β -Diversity of Microbial Communities.* **mBio 4:** e00324-13 (doi:10.1128/mBio.00324-13).

Although large-scale metagenome sequencing technology such as PCR-based-amplicon sequencing has revolutionized the studies of microbial communities, it suffers from several inherent drawbacks such as high sequencing errors, biases, poor quantitation, and very high technical variations, which could greatly overestimate microbial biodiversity. Based on general sampling theory, in this study, mathematical approaches were developed to predict and minimize the artifacts associated with random sampling processes. Along with a previous study in ISME Journal (ISME J, 5:1303, top 1% highly cited), our study was the **first** to document the low reproducibility problem of high throughput sequencing-based detection technology, discern the phenomenon of random sampling artifacts in comparing community diversity across different samples, and provide the first **explicit** evidence to demonstrate the importance of random sampling processes in estimating microbial β -diversity, which has not been adequately recognized and addressed in microbial ecology. As indicated by a mBio Editor as **Editor's Pick**, this study would make a fundamental contribution for designing and interpreting microbiome research because high throughput sequencing technologies have been widely used in microbial ecology. Since most ecological studies are involved in random sampling, the conclusions learned from this study should also be applicable to other ecological studies in general.

10. **Zhou, J.-Z.**, K. Xue, J.-P. Xie, Y. Deng, L.Y. Wu, X.L. Cheng, S.F. Fei, S.P. Deng, Z.-H. He, J.D. van Nostrand, and Y.Q. Luo. 2012. *Microbial Mediation of Carbon Cycle Feedbacks to Climate Warming.* **Nature Climate Change**, 2:106-110.

Although understanding the responses and adaptations of biological communities to environmental changes is critical in ecology, how microbial communities respond to environmental changes is poorly understood and controversial. Using integrated genomics technologies with isotope and ecosystem process analysis, this study discovered that microorganisms play crucial roles in regulating soil carbon dynamics through three primary feedback mechanisms: shifting microbial community composition, differentially stimulating genes for degrading labile but not recalcitrant carbon, and enhancing nutrient-cycling processes. These results have fundamental implications for projecting future climate change. Currently, global climate models have ignored the importance of microbial communities in regulating carbon dynamics. However, this study indicated that ecological models must consider incorporating the dynamics of microbial communities for more accurate and reliable

predictions. Furthermore, identification of coherent fingerprints to signature climate warming is of a great challenge. The results from this study also provide key signatures to identify positive or negative feedbacks of microbial communities to climate warming, which could then be incorporated for modeling prediction. In a word, these results represent ground-breaking discoveries in global change biology, and ranked by Faculty 1000 as the top 2% of the published articles in biology and medicine, and as a highly cited publication (top 1%) in Web of Science.

11. **Zhou, J.-Z.**, Y. Deng, F. Luo, Z. He, and Y.F. Yang. 2011. *Phylogenetic Molecular Ecological Network of Soil Microbial Communities in Response to Elevated CO₂*. *mBio*, 2(4): doi:10.1128/mBio.00122-11.

In an ecosystem, various microorganisms interact with each other to form complicated networks. Elucidating such network interactions and their responses to environmental changes is an important topic in ecology, but is of a grand challenge in microbial ecology. Although high-throughput metagenomic technologies can rapidly produce a massive amount of data, one of the greatest difficulties is how to extract, analyze, synthesize, and transform such a vast amount of information into biological knowledge. This study described a novel random matrix theory (RMT)-based conceptual framework to analyze metagenomics data for **unambiguously** identifying interaction networks in microbial communities, and presents unique strategies to discern **keystone** populations, which is a formidable task in microbial ecology. Also, this study, along with a previous study (mBio, 1: e00169-10), is the **first** to document that the network interactions among different phylogenetic and functional populations in soil microbial communities were substantially changed by a global change factor such as an elevated CO₂ level, which could have important implications in assessing the responses of ecosystems to climate change. The novel framework developed in this study will allow microbiologists to address research questions unapproachable previously by focusing on network interactions (i.e. beyond the parts list).

12. Hazen, T. C., E. Dubinsky, T. DeSantis, G. Andersen, Y. Piceno, N. Singh, J. Jansson, A. Probst, S. Borglin, J. Fortney, W. Stringfellow, M. Bill, M. Conrad, L. Tom, K. Chavarria, T. Alusi, R. Lamendella, D. Joyner, C. Spier, J. Baelum, M. Auer, M. Zemla, R. Chakraborty, E. Sonnenthal, P. D'haeseleer, H.-Y. Holman, S. Osman, Z.M. Lu, J. van Nostrand, Y. Deng, **J.-Z. Zhou**, and O. U. Mason. 2010. *Deep-sea oil plume enriches Indigenous oil-degrading bacteria*. *Science*, 330: 204-208.

Due to the vast diversity and as-yet uncultivated status, detection, characterization and quantification of microorganisms in natural settings are very challenging, and linking microbial biodiversity to ecosystem processes and functions is even more difficult. To meet these challenges, comprehensive functional gene arrays (e.g. GeoChip series) for dissecting functional structure of microbial communities were developed and used to analyze microbial communities from a variety of environments. As an example, in this study, GeoChip 4.0 was used as the **primary** tool for assessing the shifts of microbial **functional** composition, structure and activities in response to oil spill of the deep-sea in the Gulf of Mexico --- which was described by President Barack Obama in a prime-time address as "the worst environmental disaster America has ever faced". GeoChip analysis revealed that a variety of

hydrocarbon-degrading genes/populations exist in the deep sea environment, and there were strong linkages between particular hydrocarbon-degrading genes and the hydrocarbon concentrations in the Deep Horizon oil. Primarily based on GeoChip results, along with other integrated analyses, this study convincingly concluded that the potential exists for *intrinsic* bioremediation of the oil plume in the deep-sea, which has been confirmed by many other studies. A subsequent publication with GeoChip data alone in ISME J (6: 451-460) was particularly highlighted by *Science for Environment Policy* of European Commission (http://ec.europa.eu/environment/integration/research/research_alert_en.htm). This and other studies clearly demonstrated that GeoChip is an excellent, unique high throughput technology for enabling comprehensive visualization of the functional structure of microbial communities and for establishing the relationships between biodiversity and ecosystem functioning - a central goal in microbial ecology. More importantly, this study provides a shining example on how GeoChip technology is used in an integrated synergistic fashion to address complex emergent environmental problems, which is a cornerstone of basic science.

13. **Zhou, J.-Z.**, Sanghoon Kang, Christopher W. Schadt, and Charles T. Garten, Jr. 2008. *Spatial Scaling of Functional Gene Diversity across Various Microbial Taxa*. **Proc Nat. Acad. Sci.** 105: 7768-7773.

Determining the spatial scaling of organisms and the underlying mechanisms shaping biotic communities is a central goal in community ecology. One of the most well documented spatial patterns in plant and animal communities is taxa-area relationship (TAR), which is one of the principal generalizations in ecology. TAR is fundamental to our understanding of the distribution of global biodiversity, but remains elusive in microbial communities. Using GeoChip-based metagenomic technologies, this is the **first** study to demonstrate that the forest soil microbial communities exhibit TARs at **whole** community level across various functional and phylogenetic groups, all with z values < 0.1 . This study also revealed that the spatial turnover rates of microbial communities (z values, < 0.1) are generally lower than those observed in plants and animals (with typical z values of 0.25), which are most likely due to the unique biology of microorganisms. More importantly, this study provides strong evidence to support the general claim that TAR is a universal law in biology. The results presented in these studies have important implications for biodiversity conservation, ecosystem restoration, environmental management, and climate change. The conclusions presented in this study are supported by several of our more recent studies (Deng et al. 2018. *Environ. Microbiol.* 20:3504-3513; Liang et al. 2015. *mBio*, 6: e00240-15).

14. **Zhou, J.-Z.**, B. C. Xia, D. S. Treves, T. L. Marsh, R. V. O'Neill, L.-Y. Wu, A. V. Palumbo and J. M. Tiedje. 2002. *Spatial and resource factors influencing high soil microbial diversity*. **Appl. Environ. Microbiol.** 68: 326-334.

This study reports **unusual** diversity patterns in microbial communities in surface soils, which are not typical in plant and animal communities. Further **mathematical** simulation indicated that spatial isolation was the key mechanism shaping microbial community structure. This was the **first** study to focus on the mechanisms that control microbial diversity, and help explain explained why microbial diversity is so high in soils. Further studies are focused on the

mechanisms controlling community assembly, and factors (stochastic vs deterministic) shaping microbial community diversity and succession.

15. Liu, S., **J.-Z. Zhou**, C.-L. Zhang, D. R. Cole and T. J. Phelps. 1997. *Thermophilic Fe(III)-reducing bacteria from the deep subsurface: The evolutionary implications. Science* 277: 1106-1109.

Reports a unique group of metal-reducing bacterial cultures that are the oldest (~200 million years) viable cultured bacteria available from the deepest subsurface (2,000 meters underground). The bacteria obtained from this study belong to *Thermotoga* *ethanolicus*. Subsequent studies showed that the isolates from the deep subsurface are able to efficiently produce ethanol using both glucose and xylose.

16. **Zhou, J.-Z.**, M. A. Bruns, and J. M. Tiedje. 1996. *DNA recovery from soils of diverse composition. Appl. Environ. Microbiol.* 62: 316-322.

This pioneering study presented a robust and efficient method to isolate high quality of DNA from a variety of soils. It has been highly influential and widely used by other scientists in molecular ecology as evidenced by citations of > **3200** times based on Google Scholar. It is among the **12** most cited papers in Applied and Environmental Microbiology (2008-2018 statistics). Because it recovers high molecular weight pure DNA from soils with high yields and purity, this method laid a **foundation** for the widely used metagenomics (by Jo Handelsman) and other molecular microbial ecology studies.

B. Additional 10 most significant publications

1. Cheng, L., N. Zhang, M. Yuan, J. Xiao, Y. Qin, Y. Deng, Q. Tu, K. Xue, J. D Van Nostrand, L. Wu, Z. He, X. Zhou, M. B. Leigh, K. T Konstantinidis, E. A.G. Schuur, Y. Luo, J. M Tiedje, and **J.-Z. Zhou**. 2017. *Warming enhances old organic carbon decomposition through altering functional microbial communities. ISME J*, 11: 1825-1835, doi:10.1038/ismej.2017.48.

Global soil organic matter (SOM) contains nearly three times as much carbon (C) as the atmosphere and changes in soil C stocks may have a major impact on future atmospheric carbon dioxide concentrations and climate. Over the past two decades, much research has been devoted to examining the influence of warming on SOM decomposition in topsoil. Most SOM, however, is old and stored in subsoil. The fate of subsoil SOM under future warming remains highly uncertain. In this study, we showed that warming of approximately 2 °C in a tallgrass prairie significantly increased SOM decomposition in the subsoil. We also found that warming-induced decomposition of SOM was millennia-old and this effect was largely associated with shifts in functional gene structure of microbial communities. By coupling stable isotope probing with metagenomics, we showed that microbial communities in warmed soils possessed a higher relative abundance of key functional genes involved in the degradation of organic materials with varying recalcitrance than those in control soils. These findings provide new insights into the stability of the vast pool of old SOM in soil under future climate change scenarios.

2. Hemme, C.L., S. J. Green, L. Rishishwar, O. Prakash, A. Pettenato, R. Chakraborty, A. M. Deutchbauer, J. D. Van Nostrand, L. Wu, Z. He, I. K. Jordan, T. C. Hazen, A. P. Arkin, J. E. Kostka, and **J.-Z. Zhou**. 2016. Lateral Gene Transfer in a Heavy Metal-Contaminated Groundwater Microbial Community. *mBio*, 7(2):e02234-15. doi:10.1128/mBio.02234-15.

Lateral gene transfer (LGT), along with positive selection and gene duplication, are the three main mechanisms that drive adaptive evolution of microbial genomes and communities, but their relative importance is unclear. Some recent studies suggested that LGT is a major adaptive mechanism for microbial populations in response to changing environments, and hence, it could also be critical in shaping microbial community structure. However, direct evidence of LGT and its rates in extant natural microbial communities in response to changing environments is still lacking. Our results presented in this study provide explicit evidence that LGT played a crucial role in driving the evolution of a groundwater microbial community in response to extreme heavy metal contamination. It appears that acquisition of genes critical for survival, growth, and reproduction via LGT is the most rapid and effective way to enable microorganisms and associated microbial communities to quickly adapt to abrupt harsh environmental stresses.

3. **Zhou, J.-Z.**, W. Liu, Y. Deng, Y. Jiang, K. Xue, Z. He, J. D. Van Nostrand, L. Wu, Y. Yang, and A. Wang. 2013. Stochastic assembly leads to alternative communities with distinct functions. *mBio*, 4: e00584-12

Using many well-controlled replicate bioreactors and GeoChip technologies, this study demonstrated that stochastic assembly leads to alternative communities with distinct ecosystem functions. This provides the **first** empirical support for the critical role of community assembly in influencing ecosystem functioning, which is a fundamental issue in ecological and environmental science.

4. He, Z.H., M. Xu, D. Ye, S. Kang, L. Kellogg, L.Y. Wu, J. D. Van Nostrand, S. E. Hobbie, P. B. Reich, and **J.-Z. Zhou**. 2010. Metagenomic analysis reveals a marked divergence in the structure of belowground microbial communities at elevated CO₂. *Ecol. Lett*, 13: 564-575.

This study used GeoChip and other metagenomic technologies to demonstrate that 10 years of field exposure of a grassland ecosystem to elevated CO₂ dramatically altered the composition and structure of the belowground microbial community. While genes involved in decomposing recalcitrant C remained unchanged, those involved in labile C degradation and C and N fixation were increased under elevated CO₂. This was the **first** comprehensive study at the whole community level to document the responses of soil microbial communities to anthropogenic changes. This type of study has important implications for understanding the feedback responses of terrestrial ecosystems to climate change (e.g., elevated CO₂, warming, nitrogen deposition, precipitation), and for improving climate modeling. The results of this study were highlighted by *Science* (Naeem et al. 2012. *Science*: 336: 1401-1406) as a convincing demonstration of the frontier of integrative biodiversity studies.

5. Wang, F., H. Zhou, J. Meng, X.T. Peng, L. Jiang, P. Sun, C. Zhang, J. D. Van Nostrand, Y. Deng, Z. He, L. Wu, **J.-Z. Zhou**, X. Xiao. 2009. GeoChip-based Analysis of Metabolic

Diversity of Microbial Communities at the Juan de Fuca Ridge Hydrothermal Vent. Proc Nat. Acad. Sci, 106: 4840-4845.

First study in using high throughput genomics technologies (GeoChip 3.0) to describe the metabolic diversity of hydrothermal vent systems and demonstrate that the hydrothermal microbial communities are metabolically and physiologically highly diverse, and the communities undergo rapid dynamic succession and adaptation in response to the steep temperature and chemical gradients across the chimney.

6. He, Z., T. J. Gentry, C. W. Schadt, L. Wu, J. Liebich, S. C. Chong, W.M. Wu, B. Gu, P. Jardine, C. Criddle, and **J.-Z. Zhou**. 2007. GeoChip: A comprehensive microarray for investigating biogeochemical, ecological, and environmental processes. *The ISME Journal*, 1: 67-77 (News Release by Nature Press Office: (http://www.nature.com/ismej/press_releases/index.html) (Among the 5 top-cited articles since the journal first published in 2007; http://sci.scientific-direct.net/view_online.asp?1443164&e5885b4707419d59&18) (Among the 5 top-cited articles since the journal first published in 2007).

First comprehensive functional gene arrays (i.e., GeoChip 2.0) for geochemical, ecological and environmental studies, which are important tools for providing direct linkages of microbial genes/populations to ecosystem processes and functions. This study was highlighted by Nature Press Office, many newspapers, NSF National Ecological Observatories Network (NEON) Roadmap and the Metagenomics Report by National Academy of Sciences. The GeoChip technology has changed the way scientists conduct research on many aspects of biogeochemistry, microbial ecology, environmental sciences, and systems biology related to global changes, energy production, environmental protection, agriculture, and industry, as well as human health. This GeoChip and subsequent new versions have been extensively used to address research questions in many ecosystems, including soils, marine sediments and water columns, river sediment, hydrothermal vents, ocean crust, groundwater, waste water treatment plants, bioreactors, microbial fuel cells, oil pipelines, bioleaching, and human microbiomes. Many novel insights were obtained.

7. Liu, Yongqing*, **J.-Z. Zhou***, Marina Omelchenko, Alex Beliaev, Amudhan Venkateswaran, Julia Stair, Liyou Wu, Dorothea K. Thompson, Dong Xu, Igor B. Rogozin, Elena K. Gaidamakova, Min Zhai, Kira S. Makarova, Eugene V. Koonin, and Michael J. Daly. 2003. Transcriptome dynamics of *Deinococcus radiodurans* recovering from ionizing radiation. *Proc. Nat. Acad. Sci*, 100: 4191-4196. *equal contribution. (Reviewed by Jeremy S. Edwards and John R. Battista. 2003. *TRENDS in Biotechnology*: 21: 381-382).

Reports whole genome wide regulations of the responses of *Deinococcus* to the lethal effects of ionizing radiation. Results suggest that *Deinococcus* can efficiently recover from the damage by complex networks and metabolic pathway switching.

8. Shi, S., E. E. Nuccio, S. J. Zhou, Z. He, **J.-Z. Zhou**, and M. K. Firestone. 2016. The interconnected rhizosphere: High network complexity dominates rhizosphere assemblages. *Ecol. Lett.*, 19: 926-936.

In many environments, microorganisms coexist in complex arrays in which interactions among members are essential for community assembly and ecosystem function. However, very few Rhizosphere networks were substantially more complex than those in surrounding soils, indicating the rhizosphere has a greater potential for interactions and niche-sharing. Using random matrix theory (RMT)-based network approach, this study showed that network complexity increased as plants grew, even as diversity decreased, highlighting that interactions and niches are dimensions of community organization overlooked by univariate diversity. Covariations were predominantly positive (>80%), suggesting that extensive mutualistic interactions may occur among rhizosphere bacteria. This study also revealed quorum-based signaling as one potential strategy. Putative keystone taxa often had low relative abundances, suggesting low-abundance taxa may significantly contribute to rhizosphere function. This study identifies a previously undocumented dimension of the rhizosphere, and offers insight into fundamental properties of these soil habitats.

9. Xue, K., M. M. Yuan, J. Xie, D. Li, Y. Qin, L. E. Hale, L. Wu, Y. Deng, Z. He, J. D. Van Nostrand, Y. Luo, J. M. Tiedje, and **J.-Z. Zhou**. 2016. Annual Removal of Aboveground Plant Biomass Alters Soil Microbial Responses to Warming. *mBio*, 7: e00976-16; doi: 10.1128/mBio.00976-16.

Global change involves simultaneous alterations in multiple aspects, including climate warming and land management practice (e.g. clipping). Clipping (i.e., harvesting aboveground plant biomass) is a common practice in agriculture and for bioenergy production. However, microbial responses to clipping in the context of climate warming are poorly understood. However, the interactive effects between warming and clipping on ecosystems remain elusive, particularly in microbial ecology. This study found that clipping alters microbial responses to warming and demonstrated the antagonistic interactions between clipping and warming on microbial functional genes. Clipping alone or combined with warming enriched genes for degrading relatively recalcitrant carbon, likely reflecting the decreased quantity of soil carbon input from litter, which could weaken long-term soil C stability and trigger a positive warming feedback. All these results illustrate the complexity of microbial responses to more realistic scenarios with multiple environmental changes, and have important implications in assessing and predicting the consequences of global climate change.

10. Deng, Y. Z. He, J. Xiong, H. Yu, M. Xu, S. E. Hobbie, P. B. Reich, C. W. Schadt, A. Kent, E. Pendall, M. Wallenstein, and **J.-Z. Zhou**. 2016. Elevated carbon dioxide accelerates the spatial turnover of soil microbial communities. *Glob. Chang. Biol.*, doi: 10.1111/gcb.13098.

Several studies indicated that elevated CO₂ (eCO₂) significantly affects the diversity, composition, structure, function, interaction and dynamics of soil microbial communities. However, little is known about its impacts on the geographic distribution. Based on GeoChip data (GeoChip 3.0), this study examined the effects of eCO₂ on microbial geographic distributions by focusing on distance-decay relationships of soil microorganisms. This is the first study showing that the spatial turnover rate of soil microbial communities was accelerated

under eCO₂ among disparate ecosystems. The results from this study provides valuable insights into in predicting microbial community dynamics and the underlying assembly mechanisms under the future climate change scenarios.

C. Other significant representative publications

11. Liang, Y., Y. Jiang, F. Wang, C. Wen, K. Xue, Y. Qin, Y. Yang, L. Wu, **J.-Z. Zhou**, and B. Sun. 2016. Long-term soil transplant simulating climate change with latitude significantly alters microbial temporal turnover. *ISME J*, 2561-2572. doi: 10.1038/ismej.2015.78.

The temporal turnover and stability of microbial communities in response to climate change is a critical, but poorly understood, issue in microbial ecology. To understand soil microbial community temporal turnover, a long-term soil transplant experiment was conducted in three agricultural experimental stations over large transects from a warm temperate zone to a subtropical zone, and to a cold temperate zone. The experimental results indicated that temperature increase leads to a faster succession rate of microbial communities as well as lower species richness, and compositional changes compared to *in situ* and temperature cooling. This study provides important insights into the impacts of climate change factors on the fundamental temporal scaling of microbial biodiversity.

12. Deng, Y., P. Zhang, Y. Qin, Q. Tu, Y. Yang, Z. He, C.W. Schadt, and **J.-Z. Zhou**. 2016. Network succession reveals the importance of competition in response to emulsified vegetable oil amendment for uranium bioremediation. *Environ. Microbiol.*, in press, doi:10.1111/1462-2920.12981.

Discerning network interactions among different species/populations in microbial communities has recently received great attention in microbial ecology, but little information is available about temporal dynamics of microbial network interactions in response to environmental perturbations. In this study, based on GeoChip data (GeoChip 3.), a new random matrix theory (RMT)-based network approach was developed to discern network succession in groundwater microbial communities in response to emulsified vegetable oil (EVO) amendment for uranium bioremediation. The experimental results showed that negative interactions predominated in time-series functional gene networks, suggesting strong competition among different microbial species in the groundwater systems. These results provide mechanistic explanations of the decreased phylogenetic diversity during environmental perturbations.

13. **Zhou, J., Z. He**, Y. Yang, Y. Deng, S.G. Tringe and L. Alvarez-Cohen 2015. High-Throughput Metagenomic Technologies for Complex Microbial Community Analysis: Open and Closed Formats. *mBio* 6(1):e02288-14. doi:10.1128/mBio.02288-14.

This paper provides a critical review and synthesis of the most commonly applied “open-format” and “closed-format” detection technologies. The characteristics, advantages, and disadvantages of various metagenomic technologies were discussed within the context of environmental applications and focus on analysis of complex microbial systems, such as those in soils. In addition, crucial issues and considerations were discussed to apply complementary

high-throughput molecular technologies to address important ecological questions.

14. Liang, Y., L. Wu, I. M. Clark, K. Xue, Y. Yang, J. D. Van Nostrand, Y. Deng, Z. He, S. McGrath, J. Storkey, P. R. Hirsch, B. Sun, and **J.-Z. Zhou**. 2015. Over 150 years long-term fertilization alters spatial scaling of microbial biodiversity. **mBio** 6(2):e00240-15. doi:10.1128/mBio.00240-15

Spatial scaling is a critical issue in ecology, but how anthropogenic activities such as fertilization affect spatial scaling is poorly understood, especially for microbial communities. Most studies to date are based on different sites that may not be truly comparable or on short-term perturbations. This study examined spatial patterns of microbial communities at the 150 year-old Park Grass Experiment, the oldest continuous grassland experiment in the world. This study is the first showing that long-term fertilization has dramatic impacts on spatial scaling of microbial communities and biodiversity. By identifying the spatial patterns and their underlying mechanisms in response to long-term fertilization, this study makes fundamental contributions to predictive understanding of microbial biogeography.

15. Zhou, A., K. L. Hillesland, Z. He, W. Schackwitz, Q. Tu, G.M. Zane, Q. Ma, Y. Qu, D. A. Stahl, J. D. Wall, P. Adam, T. C. Hazen, M. Fields, A. P. Arkin, and **J.-Z. Zhou**. 2015. Rapid selective sweep of pre-existing polymorphisms and slow fixation of new mutations in experimental evolution of *Desulfovibrio vulgaris*. **ISME J**, 9:2360-2372, doi:10.1038/ismej.2015.45

Linking phenotypes to genotypes is a central issue in evolutionary biology. To understand the genetic basis of microbial evolutionary adaptation to salt (NaCl) stress, the bacterial cells were evolved under salt stress or non-stress conditions for 1,200 generations. Whole genome sequencing analysis showed that mutations in a few genes contributed substantially to the high salt tolerance. Rapid selective sweep of pre-existing mutations was followed by slow fixation of new mutations. The mutations in both essential genes and non-essential genes could lead to loss- or gain-of-function of the genes, beneficial for salt tolerance or general growth advantages under high-salt conditions. The experimental results revealed that a few beneficial mutations could dramatically improve salt tolerance.

16. Zhang, P., J. D. Van Nostrand, Z. He, R. Chakraborty, Y. Deng, D. Curtis, M. W. Fields, T.C. Hazen, A. P. Arkin, and **J.-Z. Zhou**. 2015. A slow-release substrate stimulates groundwater microbial communities for long-term in-situ Cr(VI) reduction. **Environ Sci & Technol.**, 49:12922-12931., DOI: 10.1021/acs.est.5b00024

Cr(VI) is a widespread environmental contaminant that is highly toxic and soluble, and microbes-based bioremediation is a potential effective approach for Cr immobilization. This study identified some key functional populations/groups involved in microbial processes important to long-term Cr(VI) reduction after a long-term (>3.5 yrs) field bioremediation experiment by injecting complex hydrogen-release compound. The community dynamics was analyzed with GeoChip (GeoChip 4.0). This is the first field study on the diversity, composition, structure, and dynamics of groundwater microbial communities in response to substrate amendment for Cr(VI) bioremediation, and it provides great mechanistic insights into our understanding of *in-situ* potential of Cr(VI) bioremediation and significant linkages

between microbial communities and long-term Cr(VI) reduction. Such knowledge will help us in future designing strategies for long-term bioremediation of various contaminated groundwater (e.g., reduction of other metals, nitrate removal, and dechlorination of solvents).

17. Wang, C., X. Wang, D. Liu, H. Wu, X. Lu, Y. Fang, W. Cheng, W. Luo, P. Jiang, J. Shi, H. Yin, **J.-Z. Zhou**, X. Han and E. Bai. 2014. Aridity threshold in controlling ecosystem nitrogen cycling in arid and semi-arid grasslands. *Nature Comm.* DOI: 10.1038/ncomms5799

Arid and semi-arid biomes cover >40% of the global terrestrial area and provide crucial ecosystem services. Although higher aridity and more extreme rainfall events in drylands are predicted under climate change, it is unclear how changing precipitation regimes may affect nitrogen (N) cycling, especially in areas with extremely high aridity. Using isotope and recent GeoChip technology (GeoChip 5.0), this study showed that the increase of gaseous N losses was higher than the increase of net plant N accumulation with increasing aridity index (AI) below AI=0.32, while the opposite was favored above this threshold. The findings of this study highlight the importance of changing precipitation regimes on nutrient cycling, biodiversity conservation, and ecosystem services of arid and semi-arid areas.

18. Luo, C., L. M. Rodriguez-R, E. R. Johnston, L. Wu, L. Cheng, K. Xue, Q. Tu, Y. Deng, Z. He, J. Z. Shi, M. M. Yuan, S. A. Rebecca, D. Li, Y. Luo, E. A.G. Schuur, P. Chain, J. M. Tiedje, **J.-Z. Zhou**, and K. T. Konstantinidis. 2014. Soil microbial community responses to a decade of warming as revealed by comparative metagenomics. *Appl. Environ. Microbiol.* 80: 1777-1786. (*Picked up as AEM Spotlight*).

How soil microbial communities will respond to climate change and how these responses will affect the carbon balance in soils remain important unresolved issues, limiting our understanding of the roles of soil biota in mediating climate change. Using shotgun sequencing approach, this study compared well-replicated metagenomes from temperate grassland soils that underwent a decade of experimental warming by 2°C to unheated controls. Significant changes were observed in the composition and functional gene content of microbial communities, thereby mediating the respiration of the additional, plant-derived carbon fixed as an effect of warming. Altogether the experimental results indicate that the microbial communities of the temperate grassland soils play important roles in regulating the feedback responses to climate change. This study was highlighted by AEM as a Spotlight.

19. Hillesland, K. L., S. Lim, J. Flowers, N. Pinel, N.s Elliott, G. Zane, Y. Qin, L. Wu, S. Turkarlan, N. Baliga, **J.-Z. Zhou**, J. Wall, and D. Stahl. 2014. Erosion of functional independence early in the evolution of a microbial mutualism. *Proc. Nat. Acad. Sci.*, 111: 14822–14827, doi: 10.1073/pnas.1407986111.

Although many species have evolved to function as specialized mutualists, often to the detriment of their ability to survive independently, there are few, if any, well controlled observations of the evolutionary processes underlying the genesis of new mutualisms. Using experimental evolution, mutagenesis and whole genome sequencing, this study showed the loss of sulfate respiration was a consequence of mutations in one or more of three key genes in the pathway for sulfate respiration. The experimental results suggest a tradeoff between

performance in the mutualistic environment and maintaining the flexibility to survive alone, and explain why sulfate reducers share a common ancestor with many species specialized for cooperation with methanogens.

20. Chan, Y., J. D. Van Nostrand, **J.-Z. Zhou**, S. B. Pointing, and R. L. Farrell. 2013. *Functional ecology of an Antarctic Dry Valleys landscape*. **Proc. Nat. Acad. Sci**, 110: 8990-8995. doi: 10.1073/pnas.1300643110.

Using GeoChip-based metagenomics technology (GeoChip 4.0), this study identified the functional traits of microorganisms that drive community assembly and interactions at the McMurdo Dry Valleys, which are among the most threatened environments from climate change due to their polar location and unique ecology. A suite of previously unappreciated Antarctic microbial stress response pathways, thermal, osmotic, and nutrient limitation responses were discerned, which offer tangible clues to the mechanisms behind the enduring success of microorganisms in this seemingly inhospitable terrain. The insights also expand our knowledge of how such communities tolerate extreme environmental stress and their capacity to respond to future changes, including climate and human impacts.

21. Hemme, C. L., Y. Deng, T. J. Gentry, M. W. Fields, L. Wu, Z. Fang, S. Barua, K. Barry, S. Green-Tringe, D. B. Watson, Z. He, T. C. Hazen, J. M. Tiedje, E. M. Rubin and **J.-Z Zhou**. 2010. *Metagenomic insights into evolution of a uranium-contaminated groundwater microbial community*. **The ISME J**, 5: 660-672. (**Highlighted by The Scientist**, <http://www.the-scientist.com/blog/display/57342/>)

First community level metagenomic sequencing analysis to document the impacts of anthropogenic change (environmental contaminants) on microbial communities, and to demonstrate the importance of lateral gene transfer in the adaptation of a microbial community to environmental change.

22. **Zhou, J.-Z.**, Qiang He, C. L. Hemme, A. Mukhopadhyay, K. Hillesland, A. Zhou, Z-H. He, J. D. Van Nostrand, T. C. Hazen, D. A. Stahl, J. D. Wall, and A. P. Arkin. 2011. *How sulfate reducing microorganisms cope with stress: lessons from systems biology*. **Nat Rev. Microbiol.**, 9: 452-466. (**Specially invited**)

This invited review provides a comprehensive synthesis on crucial new insights obtained using systems biology tools to study the stress responses of sulfate reducing microorganisms, particularly *Desulfovibrio* spp., at the cell, population, community and ecosystem levels. Microbes of this clade are important in bioremediation of DOE's legacy wastes.

23. **Zhou, J.-Z.** *, L.-Y. Wu*, Y. Deng, X.-Y. Zhi, Y.-H. Jiang, Q.-C. Tu, J.-P. Xie, J. D. Van Nostrand, Z.-H. He, and Y.-F. Yang. 2011. *Reproducibility and Quantitation of Amplicon Sequencing-Based Detection*. **ISME J**, 5:1303-1313

First report on testing the reproducibility and quantitation of amplicon-based sequencing approach and highlight the importance of random sampling processes in estimating beta diversity of microbial community structure. This study has important implications on

experimental design, statistical analysis and data interpretation of high throughput metagenomics sequencing data.

24. **Zhou, J.-Z.**, Y. Deng, F. Luo, Z.-H. He, Q. Tu, and X.Y. Zhi. 2010. *Functional Molecular Ecological Networks*. **mBio** 1(4):e00169-10. doi:10.1128/mBio.00169-10.

Provides a novel conceptual framework to construct interaction networks in microbial communities based on high throughput functional gene array hybridization and sequencing data. It also first documents that elevated carbon dioxide in the atmosphere dramatically altered the network interactions in the soil microbial communities, which could have important implications in assessing the responses of ecosystems to climate change. This study provides a novel framework to allow microbiologists to address research questions unapproachable previously by focusing on network interactions, and hence it could and a new research direction in microbial ecology.

25. Lin, L., H. Song, Q. Tu, Y. Qin, A. Zhou, W. Liu, Z. He, **J.-Z. Zhou**, and J. Xu. 2011. *Thermoanaerobic Glycobiome Reveals Mechanisms of Pentose and Hexose Co-utilization in Bacteria*. **PLoS Genetics**, 7(10): e1002318. doi:10.1371/journal.pgen.1002318

First thermoanaerobic glycobiome to unravel novel genetic features in carbon catabolism that might be of immediate industrial implication. This study also provides novel strategies and targets in fermentation- and genome-engineering.

26. Zhou, A.-F, Z.H. He, A. M. Redding, A. Mukhopadhyay, C. L. Hemme, M. P. Joachimiak, K. S. Bender, J. D. Keasling, D. A. Stahl, M. W Fields, T. C. Hazen, A. P. Arkin, J. D. Wall, and **J.-Z. Zhou**. 2010. *Hydrogen peroxide-induced oxidative stress responses in Desulfovibrio vulgaris Hildenborough*. **Environ Microbiol**, 12: 2645-2657.

With metabolic activity assays, temporal transcriptional and translational profiling analyses provided a comprehensive picture of the direct and indirect effects of H₂O₂ on the oxidative stresses. Examination of the stress response of deletion mutants of fur and perR indicated that PerR and Fur may be coordinately involved in the regulation of oxidative stress response in D. vulgaris. This study provides a complicated molecular mechanism to defend against the H₂O₂-induced stresses

27. He, Z., Y. Deng, J. D. Van Nostrand, Q. Tu, M. Xu, C. Hemme, X. Li, L. Wu, T. J. Gentry, Y.F. Yin, J. Liebich, T. C. Hazen, and **J.-Z. Zhou**. 2010. *GeoChip 3.0 as a high throughput tool for analyzing microbial community structure, composition, and functional activity*. **ISME J.**, 4: 1167-1179.

An updated GeoChip 3.0 with details for selected gene families as well as its application for analysis of effects of plant diversity on the functional diversity and structure of soil microbial communities. The GeoChip technology won a 2009 R&D 100 Award.

28. Gao, H., Z.K Yang, S. Barua, S. B. Reed, M. F. Romine, K. H. Nealson, J. K. Fredrickson, J. M. Tiedje, and **J.-Z. Zhou**. 2009. *Reduction of nitrate in Shewanella oneidensis depends on*

atypical NAP and NRF systems with NapB as a preferred electron transport protein from CymA to NapA. The ISME J., 1-11.

Using mutagenesis to define the functions of the genes involved in nitrate reduction, and novel insights on gene functions were obtained. A novel conceptual model for dissimilatory nitrate reduction to ammonium is proposed.

29. Gao, H. Zamin K. Yang, Terry J. Gentry, Liyou, Wu, Christopher W. Schadt, and **J.-Z. Zhou**. 2007. Microarray-based Analysis of Microbial Community RNAs by Whole Community RNA Amplification (WCRA). *Appl. Environ. Microbiol.* 73: 563-571. **(The 8th of the top 20 papers most requested in 2007 (January to March) by AEM).**

First demonstration of representative, quantitative and sensitive amplification of RNAs at the whole community genome level, which makes it possible to use microarrays to detect the functional activities of microbial communities from real environmental samples in a high-throughput fashion.

30. Luo*, F., D. Yang*, J. Zhong*, Haichun Gao, Latifur Khan, Dorothea K. Thompson and **J.-Z. Zhou**. 2007. Constructing gene co-expression networks and predicting functions of unknown genes by random matrix theory. *BMC Bioinformatics*, 8:299 (17 pages). *Equal contribution.

A novel, reliable, sensitive, and robust approach for automatically identifying functional modules and predicting gene functions using a mathematically defined threshold predicted by random matrix theory (RMT) was developed and applied to the analysis of transcriptional modular networks in yeast, E. coli and Shewanella oneidensis. This novel network approach is useful in predicting the functions of hypothetical proteins and delineating expression network modules.

31. Wu, L*, X. Liu*, C. W. Schadt, and **J.-Z. Zhou***. 2006. Microarray-based analysis of sub-nanogram quantities of microbial community DNAs using Whole Community Genome Amplification (WCGA). *Appl. Environ. Microbiol.* 72:4931-4941. *equal contribution. **(The 11th of the top 20 papers most requested in 2006 (October to December) by AEM)**

First demonstration of representative, quantitative and sensitive amplification of DNAs at the whole community genome level, which makes it possible to use microarrays to analyze microbial communities from a variety of environments.

32. Li, Xingyuan, Zhili He, and **J.-Z. Zhou**. 2005. Selection of optimal oligonucleotide probes for microarrays using multiple criteria, global alignment and parameter estimation. *Nucl. Acids Res.* 33:6114-6123

New tool with novel algorithms for rapid designing specific probes based on many homologous sequences, one of the best tools for probe design. This is widely used in scientific community.

33. Beliaev, Alexander S., Dawn M. Stanek, Joel A. Klappenbach, Liyou Wu, Daad A. Saffarini, Margie F. Romine, Kenneth H. Nealson, Jim K. Fredrickson, and **J.-Z. Zhou**. 2005. Electron

Acceptor-Induced Shifts in Shewanella oneidensis MR-1 Gene Expression Profiles. J. Bacteriol., 187:7138-7145.

Reveal fundamental differences between metal and non-metal respiratory pathways of *S. oneidensis*, where the coordinate induction of detoxification and stress response genes play a key role in adaptation of this organism under metal-reducing conditions. The results also suggest that the relative paucity of genes involved in electron transfer to metals is likely due to the low-specificity and the opportunistic nature of the metal-reducing electron transport pathways.

34. **Zhou, J.-Z.**, D.K. Thompson, Y. Xu, and James M. Tiedje. 2004. *Microbial Functional Genomics*. 624pp. John Wiley & Sons, Hoboken, New Jersey.

The **first** comprehensive book in microbial functional genomics field.

35. Wan, X.-F. *, N.C. VerBerkmoes*, L. A. McCue, D. Stanek, H. Connelly, L. Wu, X. Liu, T. Yan, A. Leaphart, R. L. Hettich, **J.-Z. Zhou**, and D. K. Thompson. 2004. *Defining the Shewanella oneidensis FUR Regulon: Integration of Genome-Wide Expression Analysis, Proteome Characterization, and Regulatory Motif Discovery*. *Equal contribution. **J. Bacteriol.** 186: 8385-8400.

Using systems biology approaches to define Fur regulons, this study suggested, for the first time, a possible role for 4 operons and 8 ORFs of unknown function in iron metabolism or iron transport-related functions.

36. Rhee, S.K*, X. Liu, L. Wu, S. C. Chong, X. Wan and **J.-Z. Zhou***. 2004. *Detection of genes involved in biodegradation and biotransformation in microbial communities using 50-mer oligonucleotide microarrays*. **Appl. Environ. Microbiol.** 70:4303-4317. *Equal contribution.

First report on constructing the 1st generation of GeoChip (GeoChip 1.0) with 50mer oligonucleotide probes for environmental studies, demonstrating its application to environmental studies as specific, sensitive and quantitative detection tools. This study first demonstrates that 50mer oligonucleotide probes instead of PCR product probe work well for environmental applications, which greatly simplifies the design of comprehensive functional gene arrays for environmental studies.

37. Thompson D.K, A. S. Beliaev, C. S. Giometti, S. L. Tollaksen, T. Khare, D. P. Lies, Kenneth H. Nealson, H. Lim, J. Yates III, C. C. Brandt, J. M. Tiedje, and **J.-Z. Zhou** 2002. *Transcriptional and Proteomic Analysis of a Ferric Uptake Regulator (Fur) Mutant of Shewanella oneidensis: Possible Involvement of Fur in Energy Metabolism, Transcriptional Regulation, and Oxidative Stress*. **Appl. Environ. Microbiol.** 68:881-892.

First demonstration that Fur is a global regulator rather than local regulator based on microarray analysis. Also, it is the first time to reveal that Fur serves as both repressor and activator.

38. Murray, A. E., D. Lies, G. Li, K. Nealson, **J.-Z. Zhou**, J. M. Tiedje. 2001. DNA:DNA hybridization to microarrays reveals gene-specific differences between closely related microbial genomes. *Proc. Nat. Acad. Sci.* 98: 9853-9858.

Reports that microarrays-based genomic hybridization has the ability to understand and display specific differences between closely related organisms providing a window into understanding microheterogeneity, bacterial speciation, and taxonomic relationships.

39. Hurt, R. A., X. Qiu, L. Wu, Y. Roh, A. V. Palumbo, J. M. Tiedje, and **J.-Z. Zhou**. 2001. Simultaneous Extraction of RNA and DNA from Environmental Samples. *Appl. Environ. Microbiol.* 67: 4495-4503.

The first robust, simple, rapid, and effective method for simultaneous recovery of both RNA and DNA from soils of diverse composition, which are critical to examine microbial activities, especially when the biomass is lower.

40. Wu, L.Y., D. Thompson, G.-S. Li, R. Hurt, H. Huang, J. M. Tiedje, and **J.-Z. Zhou**. 2001. Development and Evaluation of Functional Gene Arrays for Detection of Selected Genes in the Environment. *Appl. Environ. Microbiol.* 67: 5780-5790.

First report that microarrays can be potentially used as specific, sensitive and quantitative tools for environmental analysis. This study is well cited with >400 times since its publication.

41. Qiu, X. Y., L.-Y. Wu, H. Huang, P. E. McDonel, A.V. Palumbo, J. M. Tiedje, and **J.-Z. Zhou**. 2001. Evaluation of PCR-generated chimeras, mutations, and heteroduplexes with 16S rRNA gene-based cloning. *Appl. Environ. Microbiol.* 67: 880-887.

First study to discern the problems of heteroduplexes and PCR-induced mutations in the 16S rRNA gene-based cloning approach and to develop approaches to minimize such artifacts. These studies have been instrumental in shaping molecular microbial ecology because the PCR-based cloning approach is the most widely used and powerful approach for analyzing biological samples. This study is well cited with >380 times since its publication.

42. Deysh, S. N., N. S. Panikov, W. Liesack, R. Grobkopf, **J.-Z. Zhou**, and J. M. Tiedje. 1998. Isolation of acidophilic methane-oxidizing bacteria from northern peat wetlands. *Science* 282: 281-284.

First report to document acidophilic methane-oxidizing bacteria in the vast acidic northern wetlands, which are the largest natural source of atmospheric CH₄. These types of bacteria could play a major role in the CH₄ cycle.