

Research Achievements

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Dr. Jizhong Zhou is a George Lynn Cross Research Professor in the Department of Microbiology and Plant Biology and the School of Civil Engineering and Environmental Sciences and is the Director of the Institute for Environmental Genomics, at the University of Oklahoma (OU). He is a Visiting Senior Scientist in the Earth and Environmental Sciences Area of the Lawrence Berkeley National Laboratory, and an Adjunct Professor in the School of Environment at Tsinghua University, Beijing, China. Dr. Zhou has distinguished himself as an international leader in **genomics-enabled environmental sciences**, especially in areas related to: (i) genomic technologies, (ii) computational technologies, (iii) environmental remediation, (iv) climate change biology, and (v) theoretical ecology.

Dr. Zhou has made several pioneering advances in the area of **genomic technologies**, particularly in the development of various innovative technologies for microbial analyses, which were crucial for the early development of this emerging field. First, his GeoChip¹ is a revolutionary, high throughput technology for addressing a central goal in microbial ecology - linking community structure to function. The most recent generation of GeoChip (Version 5.0) contains 180K probes targeting ~370K functional genes in ~1,500 gene categories important to different microbial processes, including carbon, nitrogen, phosphorus, and sulfur cycling, energy processing, metal resistance and reduction, organic contaminant degradation, stress responses, antibiotic resistance, pathogenesis, viral-host interactions from broad, functionally divergent taxonomic groups, such as bacteria, archaea, fungi, protists, and viruses (mainly phages)². The GeoChip 5 is highly specific, sensitive, and quantitative based on both computational and experimental assays. The GeoChip technology was highlighted in the NRC's report on the *New Science of Metagenomics* (2007), and in the roadmap for NSF's National Ecological Observatory Network (NEON, 2009), and won a R&D 100 Award in 2009 as one of the 100 most innovative scientific and technological breakthroughs that year. Using the GeoChip technology, he has addressed various fundamental questions in ecological theory, climate change, environmental remediation, and human health, as evident by numerous publications (>100) in Nature indexed journals, including *Science*³, *Nature Climate Change*^{4,5}, *PNAS*⁶⁻¹⁰, *Nature Communications*¹¹, *Ecology Letters*¹², *The ISME Journal*^{13,14}, *Environmental Science & Technology*^{15,16}, and *Water Research*^{17,18}, and other prestigious journals, e.g., *Nature Ecology & Evolution*¹⁹, *mBio*^{20,21}, and *Global Change Biology*^{22,23}. In brief, GeoChip has become a powerful technology enabling comprehensive visualization of the functional structure of microbial communities important to energy, global change, ecosystem management and allows microbiologists to address research questions which could not otherwise be undertaken. Second, his methods for effectively extracting high quality, high molecular weight DNA from environmental samples have been very influential and widely used by other scientists. The paper²⁴ describing these methods has been cited >3,200 times and is among the 20 most cited papers in *Applied and Environmental Microbiology* (a leading microbiology journal) since 2008. These methods, because they are capable of recovering high molecular weight DNA from environmental samples, have provided a **foundation** for contemporary metagenomics and molecular microbial ecology. Third, he was the first to discern the problems of heteroduplexes and PCR-induced mutations in 16S rRNA gene-based cloning and developed approaches to minimize such artifacts²⁵. The cloning-based method was very important in early molecular microbial ecology studies in the 1990s. Fourth, he was the first to discern the importance of random sampling in comparing community diversity across different samples^{26,27} and developed mathematical approaches to predict and minimize the artifacts associated with random sampling²⁸, for which the paper describing these approaches was selected as an Editor's Pick. As an mBio's Editor commented, this study would make a fundamental contribution for designing and interpreting microbiome studies. Since most ecological studies involve random sampling, these results and guidance have had a wide impact. His recent perspective on metagenomics technologies for ecological applications²⁹ has also been influential in the field of microbiology. All these studies have been **instrumental** in shaping molecular microbial ecology during its great expansion over the last two decades. In addition, he developed a novel

Cas9 nickase-based tool for editing bacterial genomes³⁰. This study was highlighted by AEM as an Editor's Pick, and the tool has since been used by many other microbiologists for their studies.

Dr. Zhou has also made several pioneering advances in the area of **computational technology**. First, based on the random matrix theory (RMT), a relatively young mathematical theory proposed in 1960s, he developed a novel, sensitive, and robust approach with mathematically defined thresholds for predicting network interactions in microbial communities³¹⁻³³, overcoming a great challenge in network biology. The network pipeline³⁴ has been widely used by microbial ecologists with little computational background, as evidenced by the >36,000 networks constructed by >3,000 users. This early pathbreaking work represents paradigm-shifting, transformative research in microbial ecology. Prior to this, most microbial biodiversity studies ignored the interactions and associated functions among different microorganisms. More recently, he has developed a novel approach for inferring direct dependences in association networks, called **iDIRECT** (Inference of Direct and Indirect Relationship with Effective Copula-based Transitivity), by overcoming/ameliorating several mathematical problems (e.g. underdetermination, self-looping and interaction strength overflow) in network ecology. Second, he has developed various novel mathematical approaches to **quantitatively** estimate the relative importance of deterministic vs stochastic processes in governing community assembly – a major bottleneck in ecology. His most recent innovation provides an effective and robust tool for quantitatively assessing ecological stochasticity⁸. Another novel general framework for quantitatively inferring community assembly mechanisms by phylogenetic-bin-based null model analysis (iCAMP) is capable of reliably and accurately estimating the relative roles of selection, dispersal, and “drift”. These new general tools are not only crucial for microbial ecology, but also for macroorganism ecology. In addition, he has pioneered the development of novel mathematical frameworks to incorporate massive genomics information into Earth system models – an urgent issue with the greatest challenge in global change research. He was the first to demonstrate that incorporating microbial functional traits into ecosystem models can substantially reduce model uncertainty and improve modeling prediction in response to long-term experimental warming, and elevated CO₂, and/or nitrogen deposition. These groundbreaking studies have been instrumental in steering microbial ecology in new research directions.

Dr. Zhou has made several pioneering discoveries in the area of **environmental remediation**. First, over the last 20 years, he has pioneered studies on groundwater microbiomes in response to extreme heavy metal contamination by illustrating: (a) the impact of heavy metal contamination on the functional community structure^{35,36}, (b) crucial roles of horizontal gene transfer in driving the evolution of microbial communities in response to contamination³⁷, (c) microbial mechanisms underlying successful in situ bioremediation to reduce high uranium contamination to below drinking water standards^{1,38-43}, (d) community assembly processes governing the succession of microbial communities to substrate amendment^{615,16,44,45}, and (e) artificial intelligence-assisted predictions of groundwater geochemistry, contamination and functions with omics data^{20,46}. Second, using GeoChip and other technologies, he and his colleagues demonstrated a broad capacity and high potential for *intrinsic* bioremediation of the Deep Water Horizon oil plume in the Gulf of Mexico³, which was described by President Barack Obama in a prime-time address as "the worst environmental disaster America has ever faced". A subsequent publication with GeoChip data alone in *The ISME Journal*¹⁴ was specially highlighted by the European Commission's *Science for Environment Policy*. These studies also provide a shining example of how GeoChip technology is used in an integrated synergistic fashion to address complex emergent environmental problems, a cornerstone of basic science. Third, he recently established a Global Water Microbiome Consortium (GWMC). With a systematic global-sampling effort, he and his colleagues examined global diversity and biogeography of microbiomes from ~1200 activated sludge samples collected from 269 wastewater treatments (WWTPs) in 23 countries across 6 continents⁴⁷. 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. In addition, Dr. Zhou is an international leader in studying functional genomics to assess microbial responses to

environmental stresses – a core of microbial ecology - via his central role in the first comprehensive book on Microbial Functional Genomics⁴⁸. He pioneered the elucidation of microbial cellular responses to contaminants and various stressors, energy metabolism and regulatory networks in several environmentally important microorganisms, including *Shewanella*, *Desulfovibrio*, *Rhodanobacter*, as well as *Clostridium* and *Thermoanaerobacter*. Many new insights have been revealed using integrated functional genomics/systems biology approaches, especially with sulfate-reducing microorganisms as demonstrated by his invited synthesis in *Nature Reviews Microbiology*⁴⁹.

Dr. Zhou has made several ground-breaking discoveries related to **climate change biology**. First, he discovered that microorganisms play central roles in regulating soil carbon (C) dynamics through **three** primary feedback mechanisms⁵. This was the **first** study revealing functional differences in microbial communities due to warming that may affect projections of climate warming. These results represent critical findings in global change biology, and was ranked by Faculty 1000 as among the top 2% of published articles in biology and medicine. Recently, he discovered that climate warming leads to divergent succession of grassland microbial communities⁵⁰, and reduces microbial diversity, but increases network complexity and stability. Also, using integrated genomics technologies with isotope and process analysis, he demonstrated the *in situ* vulnerability of tundra soil C to climate warming and the importance of microbes in mediating such vulnerability⁴. This study was highlighted as a breakthrough by *The Washington Post*, Department of Energy, and *ASM Microbe* because northern permafrost stores more than 30% of the global soil organic C, and is a wildcard that could alter the future trajectory of global climate change. In addition, he demonstrated that long-term field exposure of a grassland ecosystem to elevated CO₂ dramatically altered the composition and structure of the belowground microbial community¹², which was the **first** comprehensive study at the whole community level to document the responses of soil microbial communities to elevated CO₂. These results were highlighted by *Science* as a convincing demonstration of the frontier of integrative biodiversity studies (Naeem et al. 2012. *Science*: 336: 1401-1406). All of these studies have made conceptual advances in climate change biology, and lead to a paradigm shift by demonstrating the urgent needs of incorporating microbial functional traits into global change modeling because whether microbially mediated feedback is positive or negative depends on which functional guilds are affected by climate changes and to what magnitude.

Dr. Zhou has also made several meritorious contributions to the field of **theoretical ecology**. First, in contrast to the traditional view of microbial assembly, several of his pioneering studies have explicitly demonstrated the importance of stochastic processes underlying microbial community assembly - a central but poorly understood topic in ecology⁵¹. He was the **first** to discern unusual microbial diversity patterns in soils and, through mathematical modeling analysis, revealed that spatial isolation (i.e. dispersal limitation) was the major underlying mechanism⁵², and to discover that divergent microbial communities with different functions can be formed under identical environmental conditions⁵³. He provided the **first** evidence that microbial community **succession** is stochastic rather than deterministic⁶, and that the responses of grassland soil microbial communities to climate change are primarily governed by stochastic processes⁵⁴. He has recently demonstrated that the microbiome assembly in global WWTPs is also more stochastic⁴⁷, in complete contrast to conventional thought because WWTPs are well-controlled engineered ecosystems. Such findings have important implications for studying ecosystem functioning, biodiversity preservation, and ecosystem management. Also, using integrated genomic technologies, his studies convincingly demonstrated that species area relationships (SARs), a universal law in ecology, generally hold for microorganisms, but that microorganisms have substantially lower spatial turnovers (z values, < 0.1) due to their unique biology^{7,55,56}. His recent study on the temporal scaling of soil bacteria and fungi provides explicit evidence for the existence of species-time relationships (STRs), another possible universal law in biology⁵⁴. This study is the **first** to demonstrate that there is phylogenetic-time relationships (PTRs), and that warming accelerates both STRs and PTRs. In addition, contrary to a common belief, he and his colleagues demonstrated that taxonomic and phylogenetic diversity of soil bacteria, fungi, and nitrogen fixing bacteria all are better predicted by temperature than pH⁵⁷. This was the **first** study in microbial

ecology showing that the metabolic theory of ecology (MTE) is a powerful framework for predicting broad large-scale microbial biodiversity, but further theoretical modelling development is needed to account for the unique characteristics of microorganisms.

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