

Final Technical Report to DOE for the Award DE-SC0004601
(Institute for Environmental Genomics, the University of Oklahoma)

Project Title: From Community Structure to Functions: Metagenomics-Enabled Predictive Understanding of Temperature Sensitivity of Soil Carbon Decomposition to Climate Warming

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Abstract

Understanding the responses, adaptations and feedback mechanisms of biological communities to climate change is critical to project future state of earth and climate systems. Although significant amount of knowledge is available on the feedback responses of aboveground communities to climate change, little is known about the responses of belowground microbial communities due to the challenges in analyzing soil microbial community structure. Thus the goal overall goal of this study is to provide system-level, predictive mechanistic understanding of the temperature sensitivity of soil carbon (C) decomposition to climate warming by using cutting-edge integrated metagenomic technologies. Towards this goal, the following four objectives will be pursued: **(i)** To determine phylogenetic composition and metabolic diversity of microbial communities in the temperate grassland and tundra ecosystems; **(ii)** To delineate the responses of microbial community structure, functions and activities to climate change in the temperate grassland and tundra ecosystems; **(iii)** To determine the temperature sensitivity of microbial respiration in soils with different mixtures of labile versus recalcitrant C, and the underlying microbiological basis for temperature sensitivity of these pools; and **(iv)** To synthesize all experimental data for revealing microbial control of ecosystem carbon processes in responses to climate change.

We have achieved our goals for all four proposed objectives. First, we determined the phylogenetic composition and metabolic diversity of microbial communities in the temperate grassland and tundra ecosystems. For this objective, we have developed a novel phasing amplicon sequencing (PAS) approach for MiSeq sequencing of amplicons. This approach has been used for sequencing various phylogenetic and functional genes related to ecosystem functioning. A comprehensive functional gene array (e.g., GeoChip 5.0) has also been developed and used for soil microbial community analysis in this study. In addition, shot-gun metagenome sequencing along with the above approaches have been used to understand the phylogenetic and functional diversity, composition, and structure of soil microbial communities in both temperature grassland and tundra ecosystems. Second, we determined the response of soil microbial communities to climate warming in both temperate grassland and tundra ecosystems using various methods. Our major findings are: (i) Microorganisms are very rapid to respond to

climate warming in the tundra ecosystem, AK, which is vulnerable, too. (ii) Climate warming also significantly shifted the metabolic diversity, composition and structure of microbial communities, and key metabolic pathways related to carbon turnover, such as cellulose degradation (~13%) and CO₂ production (~10%), and to nitrogen cycling, including denitrification (~12%) were enriched by warming. (iii) Warming also altered the expression patterns of microbial functional genes important to ecosystem functioning and stability through GeoChip and metatranscriptomic analysis of soil microbial communities at the OK site. Third, we analyzed temperature sensitivity of C decomposition to climate warming for both AK and OK soils through laboratory incubations. Key results include: (i) Alaska tundra soils showed that after one year of incubation, C_T in the top 15 cm could be as high as 25% and 15% of the initial soil C content at 25°C and 15°C incubations, respectively. (ii) analysis of 456 incubated soil samples with 16S rRNA gene, ITS and GeoChip hybridization showed that warming shifted the phylogenetic and functional diversity, composition, structure and metabolic potential of soil microbial communities, and at different stages of incubation, key populations and functional genes significantly changed along with soil substrate changes. Functional gene diversity and functional genes for degrading labile C components decrease along incubation when labile C components are exhausting, but the genes related to degrading recalcitrant C increase. These molecular data will be directly used for modeling. Fourth, we have developed novel approaches to integrate and model experimental data to understand microbial control of ecosystem C processes in response to climate change. We compared different methods to calculate Q10 for estimating temperature sensitivity, and new approaches for Q10 calculation and molecular ecological network analysis were also developed. Using those newly developed approaches, our result indicated that Q10s increased with the recalcitrance of C pools, suggesting that longer incubation studies are needed in order to assess the temperature sensitivity of slower C pools, especially at low temperature regimes.

This project has been very productive, resulting in 42 papers published or in press, 4 submitted, and 13 in preparation.

Highlights of major results

A. Phylogenetic composition and metabolic diversity of microbial communities in the temperate grassland and tundra ecosystems

For this objective, we have developed a novel phasing amplicon sequencing (PAS) approach for MiSeq sequencing of amplicons. This approach has been used for sequencing various phylogenetic and functional genes related to ecosystem functioning. A comprehensive functional gene array (e.g., GeoChip 5.0) has also been developed and used for soil microbial community analysis in this study. Those tools and shot-gun metagenome sequencing have been used to understand the phylogenetic and functional diversity, composition, and structure of soil microbial communities in both temperate grassland and tundra ecosystems.

A1. Development of phasing amplicon sequencing (PAS) approach for MiSeq sequencing of amplicons of phylogenetic and functional genes

Although high-throughput sequencing, such as Illumina-based technologies (e.g., MiSeq), has revolutionized microbial ecology, adaptation of amplicon sequencing for microbial community analysis is challenging due to the problem of low base diversity. A novel phasing amplicon sequencing approach (PAS) was developed by shifting sequencing phases among different community samples from both directions via adding various numbers of bases (0-7) as spacers to both forward and reverse primers. Our results first indicated that the PAS method substantially ameliorated the problem of unbalanced base composition. Second, the PAS method substantially improved the sequence read base quality (an average of 10% higher of bases above Q30). Third, the PAS method effectively increased raw sequence throughput (~15% more raw reads). In addition, the PAS method significantly increased effective reads (9-47%) and the effective read sequence length (16-96 more bases) after quality trim at Q30 with window 5. In addition, the PAS method reduced half of the sequencing errors (0.54-1.1% less). Finally, two-step PCR amplification of the PAS method effectively ameliorated the amplification biases introduced by the long barcoded PCR primers. Although this new strategy was developed and tested on the 16S rRNA gene, a similar strategy could also be used for sequencing other genes. A paper for this study is submitted for review. We have also developed similar strategy for sequencing fungi ITS, and a variety of functional genes such as *nifH*, *amoA*, *nirK* and *nosZ*, which greatly facilitates the analysis of functional genes in microbial communities. We have used the developed strategy to examine the responses of microbial communities to climate warming. Several papers have been published and/or under review.

A2. Development of GeoChip 5.0 technology for analyzing microbial community functional potential and activity

We have developed a comprehensive functional gene array (GeoChip 5.0), a new platform from Agilent Technologies. GeoChip 5.0 has 167,044 probes designed under strict criteria, covering 395,894 coding sequences from 1,593 functional gene families. GeoChip 5.0 has been designed to targeted genes important to carbon (C), nitrogen (N), sulfur (S), and phosphorus (P) cycling, electron transport, metal homeostasis, organic remediation, stress responses, secondary metabolism, and virus and virulence activity. Meanwhile, GeoChip 5.0 has also been shown to possess incredibly high specificity, sensitivity, quantitations and reproducibility. As the most comprehensive functional gene array, GeoChip 5.0 is expected to be powerful and efficient for molecular level study of microbial communities and their interactions with environmental factors and ecosystem functions.

A3. Development of amplicon-sequencing approaches for uncovering phylogenetic and functional gene diversity and composition

Eco-functional genes involved in nitrogen and carbon cycling were targeted for 454 pyrosequencing and Illumina sequencing in order to resolve changes in microbial functional community structure underlying process changes. For nitrogen cycling, the functional genes *nifH* for N₂ fixation, *nirK* and *nosZ* for denitrification, *amoA* for nitrification by bacteria and archaea were initially targeted. Bacterial laccases, which catalyze the oxidation of various substituted phenolic groups, were also sequenced. The FunGene Pipeline and Repository at the RDP (Michigan State University) has been enhanced to provide the full capability of processing/analyzing sequences for these eco-functional genes. In addition, primers are

continuously developed for other C and N cycling genes, such as *ligE* for C degradation, *pmoA* for methane oxidation, and *nirS* for denitrification. In addition, for phylogenetic diversity, 16S rRNA gene for bacteria and archaea, and ITS and 28S rRNA genes for fungi were developed.

A4. Determining the phylogenetic composition and metabolic diversity of microbial communities in the temperate grassland and tundra ecosystems

We have used MiSeq sequencing of phylogenetic and functional genes, shot-gun metagenome sequencing and GeoChip technologies to analyze soil samples from both warmed and control plots in both ecosystems. We aimed to understand the phylogenetic and metabolic diversity of soil microbial communities, and their responses to warming as well as their relationships with environmental factors. Details were described below.

B. Responses of soil microbial communities to climate warming in the temperate grassland and tundra ecosystems

Various methods were used to understand the response of soil microbial communities to warming in both temperate grassland and tundra ecosystems, and link the community structure with environmental factors.

B1. Microbial feedbacks and vulnerability of permafrost carbon to climate warming in AK

Microbial decomposition of permafrost carbon (C) is one of the most likely potential positive feedbacks from terrestrial ecosystems to atmosphere in a warmer world, and thus understanding microbial mechanisms controlling permafrost C decomposition is critical to project feedbacks to future global climate warming. However, microbial responses (speed, direction and magnitude) in permafrost soils to climate warming remain elusive. Using integrated metagenomic technologies, we demonstrated the rapid feedbacks of microbial communities to climate warming in a tundra warming experiment in Alaska. Consistent with the changes in soil temperature, moisture and ecosystem respiration (Reco), microbial functional community structure shifted dramatically just after one and half year of warming, indicating rapid response and high sensitivity of tundra ecosystem to climate warming. Also, warming stimulated not only functional genes involved in aerobic respiration for degrading both labile and recalcitrant C, contributing to an observed 24% increase in 2010 growing season Reco and 56% increase of decomposition of a standard substrate, but also genes for anaerobic decomposition via denitrification, sulfate reduction and methanogenesis. Nitrous oxide and methane emissions from anaerobic processes are likely to further amplify positive C feedbacks to warming even in this upland tundra ecosystem. In addition, warming greatly enhanced nutrient cycling processes such as nitrogen (N) mineralization, N fixation and phosphorus utilization, which may have contributed to observed increases in plant growth and 30% increase in gross primary productivity (GPP). As a result of simultaneously increased Reco and GPP, growing season net ecosystem exchange did not significantly change after one year of warming; however, there were significant increases in net CO₂ loss from the ecosystem on an annual basis. Together, our results demonstrate the vulnerability of permafrost C to climate warming and the importance of microbial feedbacks in mediating such vulnerability.

B2. Responses of soil microbial communities to climate warming using shotgun metagenome sequencing

We have sequenced 12 soil samples from warming and control using Illumina HiSeq technologies. Our analyses revealed that the heated communities significantly shifted in composition and metabolic potential, which appeared to be at the community-wide. Key metabolic pathways related to carbon turnover, such as cellulose degradation (~13%) and CO₂ production (~10%), and to nitrogen cycling, including denitrification (~12%), were enriched under warming, which was consistent with independent physicochemical measurements. These community shifts were interlinked, in part, with higher primary productivity of the aboveground plant communities stimulated by warming, revealing that most of the additional, plant-derived soil carbon was likely respired by microbial activity. Warming also enriched sporulation related genes and genomes with higher G+C content. Collectively, our results indicate that microbial communities of temperate grassland soils play important roles in mediating feedback responses to climate change and advance the understanding of the molecular mechanisms of community adaptation to environmental perturbations. This study drew a lot of attention by the scientific community, and was highlighted in the editor's spotlight when it was published in *Applied and Environmental Microbiology* (AEM) in March 2013.

We further expanded our efforts and performed similar warming experiments in Alaskan tundra permafrost soils (AK) and compared the results to the Oklahoma temperate grassland soils (OK) to obtain a more complete picture of the effects of warming on belowground microbial communities of terrestrial ecosystems. These warming experiments were performed in parallel at each site for one year at the time of sampling (contrasting with the 10 years of warming at OK site described in AEM). Our analyses of well-replicated 16S rRNA gene amplicon and whole-community shotgun metagenome datasets from Year 1 samples showed that the specific microbial populations and gene/pathways enriched by warming differed between the two locations. Greater taxonomic composition differences were observed at the OK site relative to AK, presumably resulting from longer generation times due to the less optimal conditions for growth at AK. Analysis of fragments of rRNA genes recovered in the shotgun metagenome data revealed no significant shifts in fungal communities at both sites, but that the ratio of fungi to bacteria decreased with warming, indicating that the warming treatment is more favorable for bacteria, at least in the short term. The most pronounced bacterial taxon shifts observed at OK site, which were somewhat also observed at the AK site, were an increase in abundance of *Actinobacteria* and decrease in *Planctomycetes*, both representing major phyla in soils, particularly in regards to C cycling. In terms of functions, the communities of AK warmed plots were enriched in metabolic pathways related to labile carbon mobilization and oxidation whereas fewer of these patterns were observed in the OK communities, indicating that soil C is more vulnerable to microbial respiration at AK. The OK communities were instead enriched in genes involved in heat shock response and cellular surface structures, particularly, trans-membrane transporters for glucosides and ferrous iron. These results, which were consistent with independent physicochemical measurements and process rates determined *in-situ*, were linked with higher primary productivity of the aboveground plant communities stimulated by warming.

B3. Expressions of microbial functional genes important to ecosystem functioning

Soil microbial communities play critical roles in ecosystem functioning and are likely altered by climate warming. However, so far, little is known about warming effects on microbial functional gene activities. Here, we applied GeoChip 3.0 to analyze cDNA reversed transcribed from total RNA to assess expressed functional genes in active soil microbial communities after nine years of experimental warming in a tallgrass prairies. Our results showed that warming significantly altered the community wide gene expressions. Specifically, expressed genes for degrading relatively more recalcitrant carbon were stimulated by warming, likely caused by the plant community shift towards more C₄ species and may decrease the long-term soil carbon stability. In addition, warming changed expressed genes in labile C degradation and N cycling in different directions, possibly reflecting the dynamics of labile C and available N pools when sampling. However, the average abundances of expressed genes in phosphorus and sulfur cycling were all increased by warming, implying a stable trend of accelerated P and S processes which may be a mechanism to sustain higher plant growth. Overall, elucidating functional gene activities enhances our understanding on ecosystem responses to warming through discovering the importance of soil microbial community in regulating ecosystem feedback mechanisms.

More recently, we obtained metatranscriptomics data for 8 samples under warming and control at OK. We are currently trying to integrate those data with the metagenomic data and biochemical measurements toward a system level view of soil microbial communities and their adaptations upon warming. Our immediate future plans, include integration of metatranscriptomics with metagenomics and biochemical measurements for a systems view of microbial communities and assessing whether the gene and pathway differences we saw between warming and control treatments are due to evolutionary adaptations of pre-existing populations (e.g., horizontal gene transfer) or newly emerging populations that are favored by warming

C. Temperature sensitivity of C decomposition to climate warming for both AK and OK soils

We conducted soil incubations of soils from different depths at two constant temperatures (15°C & 25°C). These soils came from a warming experiment in a tundra ecosystem in Alaska and a warming experiment in a grassland in Oklahoma. Three different layers were incubated from Alaska: two surface soils (0 - 15 cm & 15 - 25 cm with high carbon content) and a horizon deeper (>50 cm) within the surface permafrost. From the Oklahoma site, we used soils (0 - 25 cm) from control and warmed plots combined with a root exclusion treatment that had kept new root inputs out over the 8 years of the experiment.

C1. Long-term laboratory incubation

To determine the temperature sensitivity of microbial respiration (Q_{10}) in soils with different mixtures of labile versus recalcitrant C, we are conducting aerobic incubations of soils from a warming experiment in Alaska (AK) and Oklahoma (OK). Six field biological replicates under warming and non-warming conditions have been established at both AK and OK. From the AK site, soils at three depths were collected, while at the OK site soils from deep collar (no plants) and outside deep collar (with plants) were collected, providing soil samples with a wide range of C quality. These soil samples are incubating at two constant temperatures (15°C & 25°C) in laboratory. Altogether, 120 soil jars (microcosms) were set up and each microcosm contains eight 10-g subsoil vials. In the second phase of this project, these long-term incubation experiments are being continued and now the incubations are approaching 700 days. Carbon

fluxes have been measured periodically over the course of the incubation, and each set of soils will have two years of incubation by February 2014. Total amount of carbon respired at any measurement time was calculated by interpolating between measurement days assuming a linear trend in carbon fluxes; total carbon (C_T) respired through time was fitted with a two-pool C model.

Results from the Alaska tundra soils showed that after one year of incubation, C_T in the top 15cm could be as high as 25% and 15% of the initial soil C content at 25°C and 15°C incubations respectively. The fast decomposing C pool (C_F) accounted for up to 5% of the initial C content in the top 15 cm soils. Both, C_T and C_F decreased with depth but no field warming effect was detected. Overall average turnover time for C_F was of around 60 days at these laboratory conditions. Turnover time for C_S varied from 10 years in top soils to around 60 years in soils near the permafrost at 15°C incubation and decreased by half at the higher incubation temperature. Total C respired (C_T) in soils from Oklahoma accounted for 5% at 15°C and 10% of the initial soil C content at 25°C after one year of incubation. Fast decomposing C reached up to 4% of the initial soil C content at 25°C with an average turnover time of up to 100 days.

Various pilot experiments have been also carried out to determine the optimal conditions for performing priming experiments. New series of priming experiments will be established once the optimal conditions are figured out. This work will be continued for the new project (Award No. DE-SC0010715) currently also funded by DOE.

C2. The dynamics of soil microbial communities during a long-term incubation

To determine the temperature sensitivity of soil microbial communities important to recalcitrant carbon (C) and their underlying molecular basis, we analyzed microbial communities of 456 incubated soil samples based on 16S gene, ITS and GeoChip hybridization. At OK warming site, dissimilarity analysis of the 16S rRNA amplicons showed that the phylogenetic structure of soil microbial communities significantly differed between the root exclusion and with root ($p = 0.03$), between the incubation temperatures at 15 and 25°C ($p = 0.001$), and among the incubation time for 14, 100, and 280 days ($p = 0.03$) although no significant warming effects were observed ($p = 0.8$). The deep collar soils incubated at 15°C for 14 days showed distinct warming effects on the microbial communities with increased abundances for *Planctomycetes*, *Bacteroidetes*, *Chlamydiae*, and *Verrucomicrobiawere*, but decreased abundances for *Acidobacteria*, *Actinobacteria*, *Gemmatimonadetes*, *Nitrospira*, *Firmicutes*, *Armatimonadetes*, *Chloroflexi*, and *Proteobacteria*. However, after 280 days, such differences disappeared. Also, some warming effects were observed in deep collar soils when incubated at 25°C for 14 days, but such warming effects were not seen after three-or nine-month incubation. In addition, GeoChip results showed a significant difference in the functional diversity. When incubated at 15°C, most labile degradation genes had lower abundances in the root exclusion under warming after 14 days, but higher abundances after 280 days. Changes of C degradation gene for Soils from Long-term warming had strong co-relationship with degradation of Fast and Slow C.

In AK site, a significant warming effect was observed in the soils from 0-15 cm when incubated at 25°C for two weeks. In those samples, *Proteobacteria*, *Actinobacteria*, *Acidobacteria*, *Chloroflexi*, *Firmicutes*, and *Gemmatimonadetes* had higher abundances in the warmed soils, while *Planctomycetes*, *Chlamydiae*, *Armatimonadetes*, *Verrucomicrobia*, *Bacteroidetes* had lower abundances. Significant differences in microbial population abundances

between warmed and control soils from 0-15 cm were also observed when incubated at 15°C after three months, and the soils from >35 cm after 9 months. From GeoChip analyses, differences were observed among soil depths, between treatments, incubation temperatures, and incubation time. For the incubations at 25°C, consistent differences in carbon degradation genes between warmed and un-warmed soils from 15-25 cm were observed along the incubation time points.

These results indicate that warming effects were significant impact on the phylogenetic and functional diversity and structure of soil microbial communities in these ecosystems. Microbial communities at different stages of incubation change along with soil substrate changes. Functional gene diversity and functional genes for degrading labile C components decrease along incubation when labile C components are exhausting, but the genes related to degrading recalcitrant C increase. These molecular data will be directly used for modeling.

Based on laboratory incubation data, various pilot experiments have been also carried out to determine the optimal conditions for single cell analysis and stable isotope analysis. Also, a proposal is prepared to JGI for deep single cell and metagenomic analyses of these incubated samples.

D. Integration of experimental data and modeling approaches for understanding microbial control of ecosystem C processes in response to climate change

We have compared different methods to calculate Q_{10} for estimating temperature sensitivity. Also, new approaches for Q_{10} calculation and molecular ecological network analysis were developed. In addition, we will incorporate all tools developed in this study into KBase as the newly funded project (Award No. DE-SC0010715) is going on.

D1. Comparison of different models for estimating temperature sensitivity of soil organic matter

Although the temperature sensitivity (Q_{10}) of soil organic matter (SOM) decomposition has been widely studied, the estimations substantially depend on the methods used with specific assumptions. Thus we compared several commonly used methods plus a new and more process-oriented approach for estimating Q_{10} of SOM decomposition based on laboratory incubation data. The methods included one-pool (1P) model, two-discrete-pool (2P) model, three-discrete-pool (3P) model, and time-for-substrate (T4S) Q_{10} method. The process-oriented approach is a three-transfer-pool (3PX) model that resembles the soil part of commonly used Earth system models. Temperature sensitivity and other parameters in the models were estimated from the cumulative CO_2 emission using the Bayesian Markov Chain Monte Carlo (MCMC) technique. Our results indicated that the 1P model is not adequate for Q_{10} estimation because it cannot adequately simulate the dynamics of SOM decomposition. All the multi-pool models fitted the soil incubation data well. The Akaike information criterion (AIC) analysis suggested that the 2P model is the most parsimonious. All models, but not the T4S method, underestimated Q_{10} of bulk soil, which was directly calculated from released CO_2 in the first incubation day. As the incubation progressed, Q_{10} estimated from the 3P model was greater than that from the 3PX models because the continuous C transfers from the slow and passive pools to the active pool were not included in the 3P model. The T4S method estimated Q_{10} of labile SOM accurately, but overestimated that of resistant SOM. The similar structure of 3PX model with soil part of Earth

system models provides a possible approach, via the data assimilation techniques, to incorporate the existing data from a large number of incubation experiments into these large-scale models.

We conducted inverse analyses of data from long-term (385 days) incubation experiments with two types of soil (from plant interspace and underneath plants, respectively) to deconvolute soil carbon (C) efflux into different source pools. We analyzed the two data sets with one-, two- and three-pool models and used probability density functions as a criterion to judge the best model to represent the data sets. Our results indicated that soil C release trajectories over the 385 days of the incubation study were best modeled with a two pool C-model. For both soil types C was released from the labile pool within the first ten days of the incubation study. Decomposition of C in the recalcitrant pool contributes to the total CO₂ efflux by 9 -11% at the beginning of the incubation. At the end of the experiment, 75- 85% of the initial SOC was released over the incubation period. Our analysis also indicated that the labile C pool of the soil type plant was larger but decomposed more slowly than that in the interspace. This deconvolution analysis is based on information contained in incubation data to separate carbon pools. Results from this pool-based analysis can be easily incorporated into ecosystem models.

D2. Development of new data integration approaches

A new model was developed in this study. The main objectives are to calculate Q₁₀ using a data model fusion technique, and to evaluate dynamics of Q₁₀ with C recalcitrance. Q₁₀ and turnover rates for different C pools and fraction of each C pool can be optimized using Bayesian probability inversion and a Markov chain Monte Carlo (MCMC) technique. This approach generates posterior probability density functions of model parameters. A previously published dataset, which consisted of 12 soils from 6 sites along a mean annual temperature (MAT) gradient (2 - 25.6°C), using three incubation temperatures (15, 25 and 35°C) and an incubation period of 588 days was used to test the model. When a 2-pool model was used, parameters related to C pool size and turnover rates could be very well constrained. Q₁₀ for labile pool was well constrained either between 15-25°C or between 25-35°C. But Q₁₀ for slow pool was only constrained well between 25 - 35 C. Over the incubation period (588 days), slow pool accounted for 13.6%, 44.3% and 66.9% of the total CO₂ effluxes, respectively. For the 3-pool model, parameters related C pool size and turnover rates for labile and slow pools were also very well constrained, but turnover rate for the passive pool was not well constrained. Q₁₀ for labile and slow pools was well constrained either between 15 - 25°C or between 25 - 35°C. But Q₁₀ for passive pool was only constrained between 25-35°C. Over the incubation period, the passive pool accounted for 4.5%, 14.5% and 26.9% of the total CO₂ effluxes, respectively. The simulated and observed soil respiration rates fit well ($r^2 > 0.995$) for all the three temperatures either using 2- or 3-pool model. The result also indicated that Q₁₀s increased with the recalcitrance of C pools. Longer incubation studies are needed in order to assess the temperature sensitivity of slower C pools, especially at low temperature regimes.

Products delivered

A. In preparation

1. Rosvel Bracho, Edward A.G. Schuur, Susan Natali, , Elaine Pegoraro, K. Grace Crummer, Jizhong Zhou, Liyou Wu, Huaqun Yin, Kai Xue, Mengting Yuan, Jin Zhang, Ye Deng, Zhili He, Jim Cole, James M. Tiedje, Konstantinos Konstantinidis, Junyi Liang, & Yiqi Luo. Temperature sensitivity of soil organic matter decomposition in permafrost soils under experimental warming.
2. Cheng, Lei Yiqi Luo, Liyou Wu, Joy D. Van Nostrand, Ye Deng, Yujia Qin, Zhili He, Kai Xue, Jin Zhang, Mengting Yuan, Mary Beth Leigh, Edward A.G. Schuur, James M. Tiedje and Jizhong Zhou. Warming facilitates old carbon loss from soil through altering microbial communities, In preparation for PNAS
3. Cheng, Lei, Joy D. Van Nostrand, Yujia Qing, Ye Deng, Liyou Wu, Zhili He, Yiqi Luo, James M. Tiedje, Jizhong Zhou et al. Pyrosequencing analysis of 16S rRNA gene amplicons of soil microbial communities shows a low reproducibility, In preparation for ISME
4. Li D, C Schädel, R Conant, J Zhou, Y Luo, A data-model fusion technique to evaluate the temperature sensitivity of SOM decomposition, in preparation.
5. Wen, Chongqing, Liyou Wu, Joy D Van Nostrand, Yujia Qin, Ye Deng, Feifei Liu, Jizhong Zhou. Reproducibility of Amplicon Sequencing of Microbial phynogenic and functional Community on Illumina MiSeq sequencing platform
6. Wen, Chongqing, Yujia Qin, Liyou Wu, Joy D Van Nostrand, Ye Deng, Feifei Liu, Jizhong Zhou. Analyses of PCR bias and sequence errors of amplicon sequencing of Illumina Miseq sequencing by sequencing a bacteria mock community using varies methods. Wu, Liyou, Yuja Qin, Ye Deng1, Qichao. Tu, Jianping. Xie, YiQi Luo, Joy D. Van Nostrand1, Rebecca Sherry, ZhiLi He, Jizhong. Zhou. Microbial Community Shifts in Grassland Soil under Long-term Warming Conditions Revealed by Multiplex Pyrosequencing. In preparation
7. Xu X, Shi Z, Li D, Rey A, Liang J, Ruan H, Craine JM, Zhou J, Luo Y. Global assessment of relationships between soil carbon decomposition and soil properties using long-term incubation data: a data-assimilation. In preparation.
8. Xue, Kai et al. Interactive effects of Clipping and Warming on Soil Microbial Communities in a Tallgrass Prairie, In preparation
9. Xue, Kai, Jianping Xie, Aifen Zhou, Dejun Li, Liyou Wu, Ye Deng, Zhili He, Joy D. Van Nostrand, Yiqi Luo, Jizhong Zhou. Warming alters expressions of microbial functional genes important to ecosystem functioning, In preparation.
10. Xue, Kai, Jianping Xie, Dejun Li, Liyou Wu, Deng Ye, Zhili He, Joy D. Van Nostrand, Yiqi Luo, James M. Tiedje, Jizhong Zhou. Land Use Practice Altered Microbial Feedback Responses to Climate Warming, In preparation.
11. Yin, Huaqun, Liyou Wu, , Rosvel Bracho, Edward A.G. Schuur, Elaine Pegoraro, , Kai Xue1, Mengting Yuan, Ye Deng, Zhili He, Jim Cole, James M. Tiedje, Konstantinos Konstantinidis, Junyi Liang, Katherine Todd-Brown, Yiqi Luo and Jizhong Zhou. Responses of microbial communities to climate warming: results from long term soil temperature incubations, .
12. Yuan, Mengting, Lei Cheng, Liyou Wu, Ye Deng, Kai Xue, Tong Yuan, Joy D. Van Nostrand, Zhili He, Edward A.G. Schuur, Yiqi Luo, James M. Tiedje, and Jizhong Zhou. Influence of Experimental Warming on Soil Microbial Communities in Two Different Ecosystems. In preparation.

13. Yuan, Mengting, Jin Zhang, Liyou Wu, Kai Xue, Lei Cheng, Ye Deng, Joy D. Van Nostrand, Zhili He, Edward A.G. Schuur, and Jizhong Zhou. Climate warming induced permafrost thaw changes soil microbial communities. In preparation.

B. *Submitted*

1. Xue, Yuan et al. Rapid Microbial Feedbacks Mediates Vulnerability of Permafrost Ecosystem Carbon to Climate Warming, Submitted to Nature Climate Change.
2. Li D, X Xu, J Zhou, Y Luo, Synthesis of temperature sensitivity (Q10) of soil organic carbon decomposition based on laboratory incubation studies. Ready to be submitted to Global Change Biology.
3. Penton CR, Yang C, Tiedje J, Liu F, Ma J, Yuan M, Zhang J, Xue K, Van Nostrand J, Yuan T, Wu L, He X, Schuur EAG, Zhou J. Changes in Nitrogen-Fixing (*nifH*) community structure across an Alaskan permafrost thaw gradient. Appl. Environ. Microbiol. *Under Review*.
4. Wu, Liyou, Chongqing Wen, Yujia Qin, Huaqun Yin, Qichao Tu, Joy D. Van Nostrand, Tong Yuan, Menting Yuan, Ye Deng, and Jizhong Zhou. Phasing amplicon sequencing for robust microbial community analysis. In review, Nucleic Acids Research.

C. *Published/In press*

BOOK CHAPTERS

1. JD Van Nostrand and J Zhou. 2014. “Applications of functional gene microarrays for profiling microbial communities”. In Comprehensive Analytical Chemistry: Applications of Advanced Omics Technologies: From Genes to Metabolites, V Garcia-Canas, A Cifuentes, and C Simo (Eds). Elsevier, Philadelphia, PA, USA. 2014.
2. C. Luo, L. M. Rodriguez-R, and K. T. Konstantinidis. “A user’s guide to quantitative and comparative analysis of metagenomic datasets”. In Methods in Enzymology, “Microbial Metagenomics, Metatranscriptomics, and Metaproteomics”. E. F. DeLong (Ed.). Elsevier, Philadelphia, PA, USA. 2013.

RESEARCH ARTICLES

3. Deng, J, Y Gu, J Zhang, K Xue, Y Qin, M Yuan, H Yin, Z He, L Wu, EAG Schuur, JM Tiedje, and J Zhou. Shifts of tundra bacterial and archaeal communities along a permafrost thaw gradient in Alaska. Mol Ecol. DOI: 10.1111/mec.13015.
4. Liang, J., Li, D., Shi, Z., Tiedje, J. M., Zhou, J., Schuur, E. A. G., Konstantinidis, K. T., Luo, Y. 2015. Methods for estimating temperature sensitivity of soil organic matter based on incubation data: A comparative evaluation. Soil Biology and Biochemistry. 80:127-135.
5. Tu, Q., M. Yuan, Z. He, Y. Deng, K. Xue, L. Wu, S. Hobbie, P. Reich, and J.-Z. Zhou. 2015. Fungal communities respond to long-term elevated CO2 by community reassembly. Appl. Environ. Microbiol., doi:10.1128/AEM.04040-14, in press

6. Xu X. R.A. Sherry, S Niu, J. Zhou, Y. Luo. 2012. Long-term experimental warming decreased labile soil organic carbon in a tallgrass prairie, *Plant and Soil*, 361:307-315.
7. S Liu, F Wang, K xue, B Sun, Y Zhang, Z He, JD Van Nostrand, J Zhou, and Y Yang. 2014. The interactive effects of soil transplant into colder regions and cropping on soil microbiology and biogeochemistry. *Enviro Micrbiol*. DOI: 10.1111/1462-2920.12398
8. Luo, L. M. Rodriguez-R, and K. T. Konstantinidis. 2014. MyTaxa: an advanced taxonomic classifier for genomic and metagenomic sequences. *Nucleic Acids Res*. doi: 10.1093/nar/gku169.
9. Luo, 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, R. A. Sherry, D. Li, Y. Luo, E. A. Schuur, P. Chain, J. M. Tiedje, J. Zhou, and K. T. Konstantinidis. 2014. Soil microbial community responses to a decade of warming as revealed by comparative metagenomics. *Appl. Environ. Microbiol*. 80(5):1777-86.
10. FS Paula, JLM Rodrigues, J Zhou, L Wu, RC Mueller, BS Mirza, BJM Bohannan, K Nusslein, Y Deng, JM Tiedje, and VH Pellizari. 2014. Land use change alters functional gene diversity, composition and abundance in Amazon forest soil microbial communities. *Mol Ecol* DOI: 10.1111/mec.12786
11. L. M. Rodriguez-R, and K. T. Konstantinidis. 2014. Estimating coverage in metagenomic datasets and why it matters. *The ISME Journal*. 8:2349-2351.
12. L. M. Rodriguez-R, and K. T. Konstantinidis. 2014. Bypassing cultivation to identify bacterial species. *ASM Microbe Magazine*. March issue.
13. Q Tu, Z He, and J Zhou. 2014. Strain/species identification in metagenomes using genome-specific markers. *Nucl Acids Res*. 42:e67
14. Q Tu, H Yu, Z He, Y Deng, L Wu, JD Van Nostrand, A Zhou, J Voordeckers, YJ Lee, Y Qin, CL Hemme, Z Shi, K Xue, T Yuan, A Wang, and J Zhou. 2014. GeoChip 4: a functional gene-array-based high-throughput environmental technology for microbial community analysis. *Mol Ecol Resources*. DOI: 10.1111/1755-0998.12239
15. J Zhou, Y Deng, P Zhang, K Xue, Y Liang, JD Van Nostrand, Y Yang, Z He, L Wu, DA Stahl, TC Hazen, JM Tiedje, and AP Arkin. 2014. Stochasticity, succession, and environmental perturbations in a fluidic ecosystem. *Proc Nat Acad Sci USA*. 111:E836-E845.
16. Yuki Chan, Joy D. Van Nostrand, Jizhong Zhou, Stephen B. Pointing and Roberta L. Farrell. 2013. Functional ecology of an Antarctic dry valley. *Proc. Nat. Acad. Sci. USA*, 110:8990-8995
17. Z He, J Xiong, AD Kent, Y Deng, K Xue, G Wang, L Wu, JD Van Nostrand and J Zhou. 2013. Distinct responses of soil microbial communities to elevated CO₂ and O₃ in a soybean agro-ecosystem. *ISME J*. 8:714-726.
18. D Li, C Schadel, ML Haddix, EA Paul, R Conant, J Li, J Zhou, and Y Luo. 2013. Differential responses of soil organic carbon fractions to warming: Results from an analysis with data assimilation. *Soil Biol Biochem* 67:24-30.
19. D Li, X Zhou, L Wu, J Zhou, and Y Luo. 2013. Contrasting responses of heterotrophic and autotrophic respiration to experimental warming in a winter annual-dominated prairie. *Glob Change Biol*. 19:3553-3564.
20. Li, X.Y., Y. Deng, Q. Li, C. Lu, J. Wang, H.W. Zhang, J.G Zhu, J.-Z. Zhou, and Zhili He. 2013. Shifts of functional gene representation in rhizosphere microbial communities under elevated ozone. *The ISME J*, 7: 660-71.

21. Penton CR, St. Louis D, Cole JR, Luo Y, Wu L, Schuur EAG, Zhou J, Tiedje JM. 2013. Fungal diversity in permafrost and tallgrass prairie soils under experimental warming conditions. *Applied and Environmental Microbiology* 79, 7063-7072.
22. Penton CR, Johnson T, Quensen J, Tiedje J. 2013. Functional genes to assess nitrogen cycling and aromatic hydrocarbon degradation: primers and processing matter. *Frontiers in Terrestrial Microbiology* 17: Sept 2013 DOI: 10.3389/fmicb.2013.00279
23. Schädel C, Y Luo, S Fei, S Schaeffer, 2013. Separating soil CO₂ efflux into C pool specific decay rates via inverse analysis of soil incubation data, *Oecologia*. 171:721-732.
24. Meiying Xu, Zhili He, Ye Deng, Liyou Wu, Joy D van Nostrand, Sarah E Hobbie, Peter B Reich and Jizhong Zhou. 2013. Elevated CO₂ influences microbial carbon and nitrogen cycling. *BMC Microbiology* 2013, 13:124.
25. J Zhou, YH Jiang, Y Deng, Z Shi, BY Zhou, K Xue, L Wu, Z HE, Y Yang. 2013. Random Sampling Process Leads to Overestimation of β -Diversity of Microbial Communities. *mBio*. 4:e00324-13
26. Aifen Zhou, Zhili He, Yujia Qin, Zhenmei Lu, Ye Deng, Qichao Tu, Christopher L. Hemme, Joy D. Van Nostrand, Liyou Wu, Terry C. Hazen, Adam P. Arkin, and Jizhong Zhou. 2013. StressChip as a High Throughput Tool for Assessing Microbial Community, *Environ Sci Tech* 47:9841-9849
27. Zhou, J., Y. Jiang, Y. Deng, Z. Shi, 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).
28. Deng, Y., Y. Jiang, Y. Yang, Z. He, F. Luo, and J.-Z. Zhou. 2012. Molecular Ecological Network Analyses. *BMC Bioinformatics*, 13:113
29. Deng, Y., Z. He, M. Xu, Y. Qin, J. D. Van Nostrand, L. Wu, B. A. Roe, G. Wiley, S. E. Hobbie, P. B. Reich, and J.-Z. Zhou. 2012. Pyrosequencing of 16S rRNA genes reveals a shift of soil microbial composition and structure under elevated carbon dioxide. *Appl. Environ. Microbiol.*, in press
30. Luo, D. Tsementzi, N. Kyrpides, and K. T. Konstantinidis. 2012. Individual genome assembly from complex community short-read metagenomic datasets. *The ISME J.* Apr; 6(4):898-901.
31. Luo, D. Tsementzi, N. C. Kyrpides, T. Read and K. T. Konstantinidis. 2012. Direct comparisons of Illumina vs. Roche 454 sequencing technologies on the same microbial community DNA sample. *PLoS ONE*. 7(2): e30087.
32. Reith, F., R., J. Brugger, C. M. Zammit, A. L. Gregg, K. C. Goldfarb, G. L. Andersen, T. Z. DeSantis, Y. M Piceno, E. L. Brodie, Z. Lu, Z. He, J.-Z. Zhou, and S. A. Wakelin. 2012. Influence of geogenic factors on microbial communities in metallogenic Australian soils. *The ISME J.* 6: 2107-2118.
33. Wang, H., Z. He, Z.M. Lu, J.-Z. Zhou, J. D. Van Nostrand, X.H. Xu, and Z.J. Zhang. 2012. Genetic linkage of sediment carbon pools and microbial functions in subtropical freshwater wetlands responding to experimental warming. *Appl. Environ. Microbiol.*, 78: 7652-7661
34. Xu, X., Y. Luo, J.-Z. Zhou. 2012. Carbon Quality And The Temperature Sensitivity Of Soil Organic Carbon Decomposition In A Tallgrass Prairie. *Soil Biol. & Biochem.* Doi:10.1016/j.soilbio.2012.03.007.

35. Xu, X., R. A. Sherry, S. Niu, J.-Z. Zhou, Yiqi Luo. 2012. Long-term experimental warming decreased labile soil organic carbon in a tallgrass prairie. *Plant and Soil*, 50: 142-148. DOI: 10.1007/s11104-012-1265-9
36. Xu, X., S. Niu, R. A. Sherry, X. Zhou, J.-Z. Zhou, Y. Luo. 2012. Interannual variability in responses of belowground NPP and NPP partitioning to long-term warming and clipping in a tallgrass prairie. *Global Change Biology*, 18:1648-1656.
37. 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. (Ranked by Faculty 1000 as the top 2% of the published articles in biology and medicine).
38. He, Z., D. J. Van Nostrand, Y. Deng, and Z.-J. Zhou. 2011. Development and applications of functional gene microarrays in the analysis of the functional diversity, composition, and structure of microbial communities. *Front. Environ. Sci. Engin.* 5: 1-20.
39. Wang, J., J. D. Van Nostrand, L. Wu, Z. He, G. Li, and J.-Z. Zhou. 2011. Evaluation of whole-community genome DNA amplification methods with microarrays. *Appl. Environ. Microbiol.* 77: 4241-4245, (doi:10.1128/AEM.01834-10).
40. 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. (*Equal contribution).
41. 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, *Equal distribution).
42. 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, *equal contribution). Zhou, J., et al., Microbial mediation of carbon-cycle feedbacks to climate warming. *Nature Climate Change*, 2012. 2(2): p. 106-110.

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