# ARCFAC V: Project Proposal

## 0 Project Title:

Impact of local geochemistry on the diversity and abundance of methane-oxidizing bacteria in Ny-Ålesund soils.

## 1 Project Summary:

Microbial communities are the prime recyclers of carbon (C) and nitrogen (N) in the terrestrial environment and are central to the flux of soil gases, mobilization of nutrients and metals, and the dissolution of minerals. Unfortunately, patterns of gas release from soils, especially those in the Arctic, are changing due to increasing temperatures in the northern hemisphere. As such, there is an urgent need to better understand microbial mineralization and gas release processes in such soils, especially methane ($CH_4$), the second most important greenhouse gas. As background, methane-oxidising bacteria (methanotrophs) are the main mechanism for reducing atmospheric $CH_4$ and have already been studied in Arctic ecosystems. However, previous work on methanotrophs in permafrost zones has suggested that methanotroph abundances and activity are not consistent with $CH_4$ supply rate; i.e., methanotroph numbers are inexplicitly low relative to $CH_4$ supply in such environs. Low numbers could result from low temperatures and-or short growing seasons; however, they might also result from sampling in the "wrong" places (i.e., the organisms are there, but are being missed) due to inadequate consideration of novel habitat needs of these unusual organisms. Our goal here is to sample sites around Ny-Ålesund to test this basic hypothesis, using a sampling strategy based on new knowledge on mineral conditions that might affect *in situ* methanotroph activity and ecology, specifically the level of Cu and its speciation in conjunction with oxygen and moisture, N, and $CH_4$ levels relative to the permafrost zone. Specifically, we will use structural mapping and stratigraphic data of Brøggerhalvøya to select sites *a priori* that might have characteristically different mineral conditions, and then sample both horizontally and with depth above permafrost across differing oxygen, $CH_{4,}$ and N gradients. Further, we will use state-of-the-art analytical and molecular techniques to characterize the soils, soil gases, and methanotroph distribution and diversity, and then sort the data according to resource ratio theory (RRT), a fundamental ecological framework for relating resource supply and community structure. The ultimate goal will to determine whether Cu-geochemistry vs. sampling issues explain better inconsistency between $CH_4$ supply and methanotroph abundance in Arctic soils, and then determine, Cu conditions are important, whether mineralogy might help provide a framework for predicting methanotroph ecology on a landscape level based geologic data.

## 2 Principal Objective and sub-goals:

**Objective:** To identify and sample sites in the environs of Ny-Ålesund to: (1) determine whether the abundance and speciation of Cu, N, and oxygen in polar soils influences methanotroph abundance, diversity, and activity; (2) correlate methanotroph selection with $CH_4$ flux rates; and (3) examine selection patterns within the context of RRT, providing a possible framework for predicting $CH_4$ flux rates on a landscape level based geologic mapping data.

**Sub-goals:** To do this we will: (i) assemble an expert team in Ny-Ålesund to undertake the current plan; (ii) select, visit, and record sampling sites; (iii) collect, process, dispatch, and analyze pertinent samples; (iv) remove evidence of field activity; (v) statistically compare abundances, distributions, and activity of key microbial functional guilds within the context of RRT; (vi) recommend research directions and stewardship goals aimed at minimizing methane release from polar soils, and; (vii) develop a collaboration strategy for future work.

## 3 Scientific Introduction:

### *3.1 Climate Change, Methane, and the Polar Region*

One of the most prominent signatures of our changing climate is progressively increasing levels of atmospheric methane ($CH_4$). Although atmospheric carbon dioxide ($CO_2$) levels are higher than $CH_4$, $CH_4$ has proportionally greater impact on heat retention within the atmosphere. Therefore, understanding what regulates and, more importantly, suppresses $CH_4$ levels in the environment are of global significance. This is particularly pertinent to the Arctic because as atmospheric warming continues, the rate of permafrost melting is increasing, resulting in the increased physical release of trapped $CH_4$ from "old" ice. Understanding better $CH_4$ suppression mechanisms in the Arctic is critical to our climate, which in central to the proposed work here.

### *3.2 Methanotroph and Methane Oxidation in Arctic Ecosystems*

Methane-oxidizing bacteria (methanotrophs) are ubiquitous in nature and are nature's primary biological mechanism for reducing levels of atmospheric methane (Petit et al., 1999). Previous work has shown that these critical organisms are common in Arctic environs (Wartiainen et al., 2003; Metje and Frenzel, 2007; Svenning et al., in preparation); however, methanotroph abundances appear to be lower than would be expected, particularly given increasing levels of $CH_4$ released from the ice below. Some speculation has been made about why the numbers are so low, although no good answer has been provided. For example, it is possible that no clear point of methanotroph enrichment exists in Arctic soils above permafrost, which suggests that methanotrophs might be distributed equally along the soil profile rather than focused at the interface, which is commonly observed (Hanson and Hanson, 1996). Alternately, Arctic soils may simply not provide appropriate nutrient conditions for methanotrophs, although there is no obvious evidence of this. Finally, cold temperatures might not allow methanotrophs to process all the $CH_4$ in their vicinity (i.e., if $CH_4$ is provided primarily by melting, methanotroph activity might be too low to counterbalance physical process). This project will test these hypotheses through a sampling programme of carefully chosen sites to relate local methanotroph diversity and abundance, and possible factors that might explain where methanotrophs reside and why. Specifically, new theories related to Cu geochemistry will be assessed (see below), in conjunction with previously studied, to potentially explain whether methanotroph abundances are truly low or the organisms reside in different places than previously believed.

### *3.3 Methanobactin and Cu-Geochemistry: Controls of Methane Flux in Nature?*

Methanotrophs are ubiquitous in nature; however, habitat factors that influence methanotroph ecology, and implicitly, *in situ* $CH_4$ flux, are poorly understood, despite extensive recent studies (Bodelier et al., 2000; Mohanty et al. 2006). Moisture content and pH, as well as local $CH_4$, oxygen, and N levels have been conditionally shown to influence methanotrophic selection and activity (Hanson and Hanson 1996), but none of these factors provide a consistent explanation for the distribution of methane-oxidizing organisms in the environment. Interestingly, copper (Cu), which is central to metabolism in methanotrophic bacteria (Murrell et al., 2000), has not been studied in field systems, which is surprising since Cu is an essential component of particulate methane monooxygenase (pMMO), nature's most efficient enzyme at $CH_4$ catalysis. Furthermore, Cu regulates MMO expression in methanotrophs that express both pMMO and soluble MMO (sMMO; the alternate $CH_4$ oxidizing enzyme), and also affects the selection of type I (tend to only express pMMO) versus type II (express pMMO and sMMO) strains based upon laboratory experiments (Graham et al., 1993).

We recently showed that *in situ* methanotrophic ecology may be controlled by the relative ability of some methanotrophs to mobilize and acquire Cu from mineral phases to support pMMO expression (Knapp et al., 2007). Specifically, a small, fluorescent chromopeptide, called methanobactin (mb) (Kim et al., 2004), was purified that mediates Cu acquisition and promotes pMMO expression. We hypothesized that Cu sequestration by mb from natural Cu sources, especially in geochemical habitats, is the rate limiting step in *in situ* pMMO expression and, as such, mb:Cu interactions might explain where and when pMMO is expressed, methanotroph ecology, and $CH_4$ oxidation patterns in nature. We recently tested this hypothesis using a set of RT-PCR expression assays to quantify pMMO, soluble MMO, and 16S-rRNA gene transcript levels under progressively more stringent Cu supply conditions. Figure 1 show that as Cu bioavailability for pMMO transcription reduces from $CuCl_2$ to Cu-oxide to Cu-silicates, implying that Cu-geochemistry and mb-mediation might impact MMO expression in nature, and may provide an explanation for why $CH_4$ oxidation rates might be locally lower than expected, including in Arctic systems. This question will be central to sampling and studies in this project; however, work will done within the context of other previously identified habitat factors, including $CH_4$, oxygen, and N supply conditions (satisfying objectives 1 and 2).

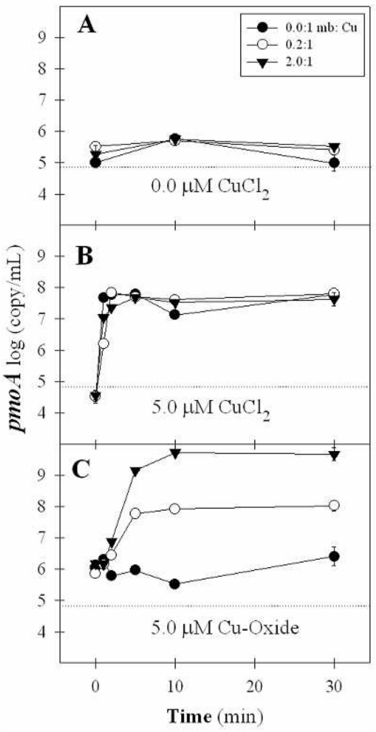

**Figure 1 – mb alters pMMO expression with different Cu sources**

*3.4 Resource-Ratio Theory: Framework for Design of Sampling Regime*

Resource-ratio theory (RRT) was formulated to provide a logical framework to examine how resource competition impacts species composition and diversity in natural communities (Tilman 1982). Although it was developed to explain plant biodiversity, it has been used to describe phytoplankton species succession in lakes, hydrocarbon biodegradation, and other phenomena (Smith et al, 1998; Graham and Curtis 2003), and is ideally suited to guiding studies on the relationship between resources supply level and community structure for methanotrophs. Specifically, RRT predicts that the "equilibrium" biomass, composition, and functional activity of a community are a function of resource supply rates and ratios in the system (i.e., available Cu, N, $CH_4$, and oxygen). RRT is particularly useful here because it is quantitative, can be calibrated (which we propose to do through our sampling program), and deals explicitly with competition in multiple resource systems, which describes well selection and activity of methanotrophs. Specifically, we propose to sample an array of sites (carefully chosen based on local Cu geology, oxygen and methane profiles, moisture conditions etc.), characterize resource conditions in detail for each sample, and then compare methanotroph selection (both abundance and diversity) against parameters, such as total Cu, "bioavailable" Cu (through Cu speciation determination on sample soils), oxygen, methane, and nitrogen. Ideally, at least 15 to 20 samples will be assessed to calibrate the theory (satisfying objective 3).

### *3.5 Integrated Sampling and Measurement Methods*

Central to the work proposed here is methanotroph community characterization, which will rely on both quantitative and qualitative molecular biological techniques, combined with specific Cu speciation and other analysis on soil samples. Qualitative molecular methods will be employed to describe community diversity and trends in methanotroph prevalence, whereas quantitative methods will be used to determine abundances for comparison among resource supply conditions, mineral conditions, and relative of abundances. RRT will be used to relate the prevalence of type vs. type II methanotrophs and prevalence of pMMO vs. sMMO expression vs. resource conditions.

## **4 Work Content and implementation plan:**

### *4.1 Sampling Site Selection*

Our general objective will be to identify specific sites around Ny-Ålesund that provide a matrix of differing habitat conditions, chosen to i) best inform RRT for analysis, and ii) examine relationships between resource supply conditions and methanotroph selection and ecology. Specifically, we will choose sites that range from Cu-rich to Cu-poor conditions, differing depth to permafrost, the presence or absence of clear oxic-anoxic interfaces in the soil profile, and differing N supply levels within the practical context of sites that are likely accessible by foot. Special effort will be placed on finding sites with different Cu geochemistry (e.g., organics, oxides, silicates, carbonates etc.) to assess the possible role of Cu bioavailability on methanotroph selection and abundance and also sites with clear and less clear oxic-anoxic interfaces to assess the role of "interface" on local distribution of methanotrophs along the soil profile.

Strategic decisions on sites for possible sampling will be made prior to arrival at Ny Ålesund, largely based on existing geologic information (Bergh et al., 2000). Although rock beds that are explicitly sulphide- or Cu-rich are not known in Ny Ålesund, bed rock composition and geochemistry does vary considerably in the Brøggerhalvøya area (e.g. between the Mesozoic-Cenozoic sedimentary strata and the metamorphic basement rocks, the two dominant components of the area). Therefore, the potential for Cu enrichment and variation will be highest within the Tertiary shales and coal deposits near Ny Ålesund, and the adjacent quartz-magnetite schists in local basement rocks. Further, habitat and surface soils underlain by carbonate rocks, evaporates (gypsum), and oxide-rich silicified bed rocks, might also reflect contrasting Cu-geochemistry, which will be considered in site selection. Discussions will be held among team members to choose areas for sampling prior to arrival in the field; however, precise locations will not be selected until on-site evidence are available, such as oxygen profiles, permafrost depths, and $CH_4$ levels.

### *4.2 Site Logistics*

Some of the potential sites at Ny-Ålesund are in remote areas, and may be difficult to reach. Therefore, logistical support from the Norsk Polarinstitutt would be extremely useful. It is planned that a research team of 6 scientists will arrive in Ny-Ålesund and will stay for between 5 and 7 days, depending upon individual schedules (see Table). During this period, board and lodging facilities will be needed at Kings Bay AS.

The first day will include safety training, establishing a small working laboratory for sample processing and soil analyses, and for setting up our analytical equipment, including at gas chromatograph (GC) for $CH_4$ analysis. Although we will not bring a microscope, access to a microscope would be helpful and office space for 2-3 persons would be very advantageous.

Concurrent to laboratory set-up, discussions about site selection will occur, and an initial field reconnaissance will be performed to better plan the sampling regime. Specifically, the geologists (JR & SB) will examine various on-site site sampling options across the region, including sites on the Brøgger peninsula and proximal areas (ideally, with a variety of habitat based on assumed or detected Cu, N, and permafrost conditions). After initial investigations, the team will visit each possible site to collect and process samples for ultimate molecular microbial characterization, soil metal and nutrient analysis, and oxygen and $CH_4$ profile determinations.

### *4.3 Site Sampling and Sample Processing*

Each site visit will be initiated by preliminary coring to determine characteristics of each soil profile, including the collection of samples for gas analysis. Physical samples will then be collected in the field (using local composite samples, but also samples collected with depth at some sites) and then transported to the laboratory for processing. Samples will be processed as soon as possible after sampling by imbedding biomass into agarose plugs that will later be used for cell lysis and preparation for molecular biological analysis of harvested DNA. In concert with this sampling, core samples will be sub-sampled for Cu and N speciation analysis (by JR in her home laboratory). Sub-samples mineral analysis will be preserved accordingly in parallel to samples collected for molecular characterization to place observed microbial data into resource-based framework. The majority of the samples will be transported intact to the University of Tromsø and then sub-divided to distribute to the different collaborators for specific molecular studies, including DNA /RNA isolations, and filtration for membrane isolation. A small set of sub-samples will be processed *in situ* (e.g., the addition of specific preservatives for mRNA of MMO gene transcripts) that require immediate preservation.

### *4.4 Sample Characterization*

Soil samples will be characterized for selected nutrients (JR & DG), soil physical properties (JR & SB), gases (PF), and molecular microbial community characteristics (SMM, DG, & PF). Nutrient, soil, and gas analysis will be performed according to standard procedures, whereas various molecular biological methods will be used for community characterization. For example, molecular fingerprint analyses by 16S rDNA DGGE (Denaturing Gradient Gel Electrophoresis) will be used to determine general specie dominance in soils, whereas diagnostic *pmoA* microarray analyses (Bodrossy et al. 2003) will used to survey MMO prevalence and contrast observed methanotroph communities against previous communities stored within the *pmoA* microarray probe database. Quantitative methods will focus on real-time PCR and RT PCR for quantifying genes and gene transcripts, respectively, on samples, especially related to MMO gene targets and signature 16S targets that are characteristic of different methanotroph groups. As noted, samples will be collected, specially preserved as needed, and segregated for analysis in the different home laboratories. DG will be primarily responsible for quantitative molecular characterization, JR will lead nutrient and geochemical analysis, and SMM will characterize community diversity and also ancillary microbial groups (e.g., methanogens) that might impact observed methanotrophic community conditions.

| Project/campaign period and Milestones/Activities | From Date: | To Date: |
|---|---|---|
| **In Ny-Ålesund** | | |
| Organize field equipment/establish sampling areas | 02.08.08 | 02.06.08 |
| Set-up field laboratory | 03.08.08 | 03.08.08 |
| Preliminary reconnaissance | 03.08.08 | 03.08.08 |
| Collect and process samples | 03.08.08 | 08.08.08 |
| Organize samples and equipment for departure | 08.08.08 | 09.08.08 |
| **In Home Laboratories** | | |
| Sub-sample field samples and distribute | 10.08.08 | 17.08.08 |
| Nutrient analysis – Cu speciation | 17.08.08 | 15.09.08 |
| Microbial diversity characterization | 17.08.08 | 15.10.08 |
| Abundance quantification of selected samples | 15.09.08 | 15.11.08 |
| Examine resource supply vs. abundances within RRT | 15:10.08 | 01.12.08 |
| Journal article 1 | 01.12.08 | 01.02.09 |
| Journal article 2 | 01.02.09 | 01.04.09 |

## 5 Integration, Collaboration and Networking:

This is a small effort compared with many of the other projects in the region. However, we feel it addresses more than one major theme within the mandate of the ARCFAC V programme. We hope to discover fundamental explanation(s) for why methanotroph abundances in permafrost regions are inconsistent with the ample and increasing supply of methane from melting ice. Specifically, we will integrate forefront knowledge of methanotroph biochemistry into a field programme that simultaneously quantifies methanotroph diversity and abundance, and soil geochemistry to better under understand factors that links methane supply and methane oxidation in Arctic systems. These data will then be placed into the framework of RRT to allow the systematic extrapolation local observations to a more global scale, hopefully identifying geological markers that might be linked to biological activity. An added benefit of this endeavour is that our team is using similar methods to ask the same question in sub-Arctic, temperate, and tropical environs, so that observations in the Arctic will integrated into more global data on methane flux from other ecosystems.

This is a new network and that combines researchers both from the biological (DWG, SMM & PF) and physical sciences (JR & SB), although sub-groups with the team have been very successful in related work (e.g., publications in *Science, Nature, PNAS,* and other forefront journals). Such a synthesis is essential for long term progress in the studying of *in situ* methane ecology and activity, which will cross disciplines and have broad dissemination. Further, the team includes researchers with previous Arctic experience (SMM, SB & PF), which is critical for research success in difficult polar environs, although the core of the new work will be driven by fundamental work that has never been considered before in the Arctic methanotroph studies (DG & JR). Finally, the work complements existing funding and research objectives, which ensures meaningful analysis of samples collected in the programme. As two examples, DG is leading ECOSERV an EU FY6 multi-national research effort studying applications of ecological theory to biological systems; JR leads a team studying the relationship between trace metal mineralogy on biogeochemical activity (funded by the US NSF); and PF and SM are cooperating in METHECO, a nine-group European consortium studying methane-oxidizing bacteria under different habitat conditions.

## 6 Publication & Outreach plans:

- The project will benefit education and training efforts about the Arctic tundra, terrestrial microbial ecology, soil-air gas interchanges, geochemistry, and climate models for students, especially at University of Tromso, Newcastle University, and University of Kansas, primarily through small well-defined sub-tasks on collected samples performed by master students at each university.
- The research team is highly published in internationally prestigious journals and it is expected that manuscripts of equivalent impact will result from the proposed research. In fact, field studies relating trace metal geochemistry, nutrient bioavailability, and methanotroph ecology have never been performed before, especially in Polar Regions, therefore it is reasonable to expect seminal results from the proposed work.
- Furthermore, such publications will almost certainly be of great interest to the larger scientific community because results will likely influence global gas flux models and have significant implications to more general climate change studies.
- Finally, we expect that publications aimed at the popular press will result from the work (our past work on this topic has), although the nature of the articles will depend on discoveries. For example, our past work related to climate change gained public attention that resulted in international press attention to results (e.g., our recent *PNAS* paper was chosen as the "cover article" for that issue).

## 8 Consortium and Participants

David Graham (DG) is Professor of Civil Engineering and is Team Leader of ECOSERV, a Marie Curie Excellence Project based at Newcastle University applying ecological theory, mathematics, and molecular biology to understanding engineered biological systems. He expert in methanotroph biochemistry and ecology and has led projects on relationships between nutrient speciation and bioavailability in many microbial systems, including pioneering work on Cu acquisition in methanotrophs. Further, he was the first to apply RRT theory in terrestrial microbiology, which laid the groundwork for the use of this framework in microbial ecology. Finally, he is led many projects in applied microbiology and engineering (funded by EU, US, and Canadian sources), and has field experience in the Canadian Arctic studying the impact of mining on arctic habitat. Jennifer Roberts (JR) is an Associate Professor of Geology at the University of Kansas with over 10 years of expertise straddling geology and microbiology, especially related to the affect of trace metals embedded in minerals on *in situ* methanotroph and methanogen selection in terrestrial systems. She is expert in both laboratory and field assessments in biogeochemistry. Peter Frenzal (PF) is Professor of Biogeochemistry at the Max-Planck-Institute for Terrestrial Microbiology and is a world-leading expert in the biogeochemistry and microbiology of ricefields, wetlands, and other systems that have soil-plant-water interfaces. PF is the project leader of METHECO, which is multi-national collaboration studying microbial diversity, process dynamics, and stability in global methane consumption. Mette M. Svenning (MMS) is a Professor of Biology at the University of Tromsø and has extensive experience in methanotroph molecular ecology in Arctic habitats, including work in the Ny-Ålesund, Longyearbyen and Pyramiden regions. She has led projects at Svalbard for over 10 years, including both laboratory and field work leading to PhD and Masters Dissertations and is collaborator in METHECO with PF. Steffen Bergh (SB) is a Professor of Geology at the University of Tromso and is the leading expert in the geology of Spitsbergen, having extensive field experience an knowledge of the region.

| Project Team Members | From: | To: | Number of Days (days) | New/Last Visit |
|---|---|---|---|---|
| Leader - David W Graham | 02.08.08 | 09.08.08 | 7 | Yes |
| Jennifer A Roberts | 02.08.08 | 09.08.08 | 7 | Yes |
| Peter Frenzel | 02.08.08 | 09.08.08 | 7 | Yes |
| Mette M Svenning | 02.08.08 | 09.08.08 | 7 | No/2007 |
| Steffen Bergh | 02.08.08 | 07.08.08 | 5 | No/2007 |
| Christiane Graef (student) | 02.08.08 | 0.9.08.08 | 7 | Yes |

## 9. References

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Kulczycki, E., D.A. Fowle, C.W. Knapp, D.W. Graham, & J.A. Roberts. (2007) Methanobactin-promoted dissolution of Cu-substituted borosilicate glass. *Geobiol.* 5:251-263.

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Petit J.R. et al (1999) Climate and atmospheric history of the past 420,000 years from the Vostok ice core, Antarctica. *Nature* **399**:429-436.

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## 10 Attachments

CV for David Graham
CV for Jennifer Roberts
CV for Peter Frenzel
CV for Mette Svenning
CV for Steffen Bergh

## *Short CURRICULUM VITAE*, David W Graham

Born 04.16.54; Received PhD in environmental engineering in 1992 (Univ. of Arizona). Visiting Professor, School of Civil Engineering and Geosciences, Newcastle University from 2006; Professor of Environmental Engineering, University of Kansas from 1992. Team Leader of ECOSERV, a FP6 Marie Curie Excellence Grant based at Newcastle University.

### Research interests

(1) Biochemistry and ecology of methane-oxidation bacteria in aquatic and terrestrial ecosystems. (2) Molecular basis of the production, chemical properties, and ecological implications of small extracellular copper-binding compounds. (3) The application of chaos, resource-ratio, and other basic ecological theories to the study biological processes. (4) Environmental quality in remote locales, ranging from the tropics to the arctic. (5) Interactions human activity, eutrophication, the fate of organic aquatic contaminants, and land management practices. (6) The application of molecular biological and molecular chemical techniques to the study of contaminant fate and attenuation in “natural” systems. (7) Linkage and calibration of toxicological end-points (e.g., gene expression biomarkers) to local water chemistry and microbiology in ecosystems.

### Selected relevant publications (from over 60)

•Knapp, C.W., D. A. Fowle, E. Kulczycki, J.A. Roberts, & **D.W. Graham**. (2007) Regulation of copper acquisition and MMO gene expression by methanobactin in methane-oxidizing bacteria in the natural environment. *Proc. Nat. Acad. Sci - USA.* **104**:12040-12045.
•Kulczycki, E., D.A. Fowle, C.W. Knapp, **D.W. Graham**, & J.A. Roberts. (2007) Methanobactin-promoted dissolution of Cu-substituted borosilicate glass. *Geobiol.* **5**:251-263**.**
•**Graham, D.W**., C.W. Knapp, E.S. Van Vleck, K. Bloor, T.B. Lane, & C.E. Graham. (2007) Experimental demonstration of chaotic behavior in biological nitrification. *ISME J.*. 1:385-393.
•Kim, H.J., N. Galeva, C.K. Larive, M. Alterman, & **D.W. Graham.** (2005) Purification and physiochemical properties of methanobactin: A chalkophore from *Methylosinus trichosporium* OB3b. *Biochem.*, **44**:5140-5148.
•Kim, H.J., **D.W. Graham**, A.A. DiSpirito, M.A. Alterman, N. Galeva, C.K. Larive, D. Asunskis, & P.M.A. Sherwood. (2004) Methanobactin, a copper-acquisition compound from methane-oxidizing bacteria. *Science*, 305:1612-1615.
•Knapp, C.W. & **D.W. Graham.** (2004) Development of alternate ssu-rRNA probing strategies for characterizing aquatic microbial communities. *J. Microb. Methods*, 56:323-330.
•Curtis, T.C., I.M. Head, & **D.W. Graham**. (2003) Theoretical ecology in engineering biology. *Environ. Sci. Technol.*, 37:64A-70A.
•Kim, H.J. & **D.W. Graham**. (2003) Effects of oxygen and nitrogen conditions on the transformation kinetics of 1,2-Dichloroethenes by *Methylosinus trichosporium* OB3b and its $sMMO^C$ mutant. *Biodegradation*, 14:407-414.
•**Graham, D.W.**, A.S. Lindner, & H.J. Kim. (2002) Methanotrophic Bacteria. In: *Encyclopedia of Environmental Microbiology*, Ed. G. Bitton., Wiley Publishing, New York, N.Y.
•Kim, H.J. & **D.W. Graham**. (2001) Effect of oxygen level on simultaneous nitrogenase and sMMO expression and activity in *Methylosinus Trichosporium* Ob3b and its $sMMO^c$ mutant, PP319: Aerotolerant $N_2$ fixation in PP319. *FEMS Microbiol. Lett*, 201:133-138.
•Smith, V.H., **D.W. Graham**, & D. Cleland. (1998). Application of resource ratio theory to hydrocarbon biodegradation. *Environ. Sci. Technol.*, 32:3386-3395.

***Short CURRICULUM VITAE*, Jennifer A. Roberts**

Born 30/09-1972; received PhD in geology in 2000 (The University of Texas at Austin). She was a National Research Council postdoctoral fellow 2000-2001 in microbial transport (U.S. Geological Survey). Assistant Professor of Geomicrobiology from 2001-2007 in the Department of Geology at the University of Kansas (KU). Currently, Associate Professor, Department of Geology, KU.

**Past and ongoing research interests:**
General aspects of microbe:mineral interactions and the geological and ecological implications of these interactions in subsurface environments including: (1) Role of silicate mineralogy and trace element content in dictating abundance and speciation of attached microbial communities, (2) Microbial acquisition of nutrient and terminal electron acceptors from primary silicates and rates of BTEX degradation in aquifers, (3) Preferential microbial weathering of primary silicates containing limiting trace elements, (4) The role of methanobactin in the dissolution of Cu-bearing silicates and methane-oxidation rates in methanotrophs, (5) Low-temperature dolomite precipitation mediated by methanogenic microorganisms, (6) Microbial precipitation of carbonate minerals and their influence on mineralogy and morphology.

**Selected relevant publications (please note J.R. Rogers is J.A. Roberts):**

•Knapp, C., Fowle, D.A., Kulczycki, E., **Roberts, J.A**., & Graham, D.W., Methane monooxygenase gene expression mediated by methanobactin in the presence of copper sources, *PNAS*, **104**:12040-12045.

•Crowe, S.A., O'Neill, A.H., Kulczycki, E., Weisener, C.G., **Roberts, J.A**., & Fowle, D.A. (2007) Reductive release of trace metals from sediments: the importance of manganese. *Geomicrobiol. J*, **24**:157-165.

•Mauck, B.S. & **Roberts, J.A.** (2007) Mineralogic control on diversity of surface-adherent microbial communities in a petroleum-contaminated aquifer. *Gemicrobiol. J* **24**:167-177.

•Kulczycki, E., Fowle, D.A., Knapp, C., Graham, D.W. & **Roberts, J.A**. (2007) Methanobactin-promoted dissolution kinetics of synthetic glass. *Geobiol.* **5**:251-263.

**•Roberts, J.A**., Fowle, D.A., Hughes, B.T., & Kulczycki, E. (2006) Attachment behavior of Shewanella putrefaciens to magnetite under aerobic and anaerobic conditions. *Geomicrob. J* **23**:631-640.

•Bennett, P.C., Engel, A.S., & **Roberts, J.A.** (2006) Counting and imaging bacteria on mineral surfaces: In Methods of Investigating Microbial-Mineral Interactions, CMS Workshop Lectures, Vol. 14, J. P.A. Maurice and L.A. Warren eds., The Clay Mineral Society, Chantilly, VA, 37-78.

**•Roberts, J.A.** (2004) Inhibition and enhancement of microbial surface colonization: the role of silicate composition. *Chem. Geol.* **212**:313-327.

**•Rogers, J.R.** & Bennett, P.C. (2004) Mineral stimulation of subsurface microorganisms: release of limiting nutrients from silicates. *Chem. Geol*. **203**:91-108.

**•Roberts, J.A**., Bennett, P.C., Macpherson, G.L., González, L.A., & Milliken, K.L. (2004) Microbial precipitation of dolomite in groundwater: Field and laboratory experiments. *Geology* **32**:277-280.

•Bennett, P.C., **Rogers, J.R**., Hiebert, F.K, & Choi, W.J. (2001) Mineralogy, mineral weathering, and microbial ecology. *Geomicrobiol J* **18**:3-19.

**•Rogers, J.R**., Bennett, P.C., and Choi, W.J. (1998) Feldspars as a source of nutrients for microorganisms. *American Mineralogist*, **83**:1532-1540.

***Short CURRICULUM VITAE*, Peter Frenzel**

Received Dr. rer. nat. from University of Heidelberg, Germany and Dr. rer. nat. habil. (venia legend in Ecology) from University of Marburg, Germany. Currently Professor at the University of Marburg (since 2001) and based at the Max Plank Institute. Chair of the EuroDIVERSITY Scientific Committee. Project leader of METHECO, an EU consortium cooperating on methane oxidizing bacteria and their function under different of environmental conditions and habitats.

**Past and ongoing research interests:**
The ecology, biogeochemistry, and microbiology of methanotrophic and methanogenic bacterial processes in rice fields, wetlands, and other soil-water systems, including the impacts permanent or temporal flooding, local vegetation (adapted to water-saturated soils), and soil geochemistry. The study of physical and biological phenomena at oxic-anoxic interfaces that develop after flooding events. Formation of oxic-anoxic interfaces resulting from local plant root activity and microbial interactions in such zones. Recent focus has been on the plant interface in rice fields, and on methanogenic pathways in both rice fields and natural wetlands.

**Selected relevant publications:**
- Metje, M. & **Frenzel, P**. (2007). Methanogenesis and methanogenic pathways in a peat from subarctic permafrost. *Environ Microbiol* **9**:954-964.
- Bodelier, P.L.E., Sorrell, B.K., Drake, H.L., Küsel, K., Hurek, T., Reinhold-Hurek, B. et al. (2006). Ecological aspects of microbes and microbial communities inhabiting the rhizosphere of wetland plants. In *Wetlands and Natural Resource Management*. Verhoeven,J.T.A., Beltman,B., Bobbink,R., & Whigham,D.F. (eds). Springer, pp. 205-238.
- Krüger, M., **Frenzel, P.**, Kemnitz, D., & Conrad, R. (2005). Activity, structure and dynamics of the methanogenic archaeal community in a flooded Italian rice field. FEMS Microbiol Ecol 51: 323-331.
- Metje,M. & **Frenzel, P**. (2005). The effect of temperature on anaerobic ethanol oxidation and methanogenesis in an acidic peat from a northern wetland. Appl Environ Microbiol 71: 8191-8200.
- Purvaja, R., Ramesh, R., & **Frenzel, P**. (2004). Plant-mediated methane emission from an Indian mangrove. **Global Change Biol** *10*:1825-1834.
- Krüger, M. & **Frenzel, P.** (2003). Effects of N-fertilisation on $CH_4$ oxidation and production, and consequences for $CH_4$ emissions from microcosms and rice fields. *Global Change Biol* **9**:773-784.
- Krüger, M., Eller, G., Conrad, R., & **Frenzel, P.** (2002). Seasonal variation in pathways of $CH_4$ production and in $CH_4$ oxidation in rice fields determined by stable carbon isotopes and specific inhibitors. *Global Change Biol* **8**:265-280.
- Bosse, U. & Frenzel, P. (2001). $CH_4$ emission from a West Siberian mire. *Suo* **52**:99-114.
- Bodelier, P.L.E., Roslev, P., Henckel, T., and Frenzel, P. (2000). Stimulation by ammonium-based fertilizers of methane oxidation in soil around rice roots. *Nature* **403**:421-424.
- Lehmann-Richter, S., Großkopf, R., Liesack, W., **Frenzel, P**., and Conrad, R. (1999). Methanogenic archaea and CO2-dependent methanogenesis on washed rice roots. *Environ Microbiol* **1**:159-166.

## *Short CURRICULUM VITAE*, Mette M Svenning

Born 02.01.55; Received PhD in biology in 1991 (Univ. of Tromsø). Professor Department of Biology, University of Tromsø from 2002. Head of Department of Biology 2002-2009. Member of Board for Science, Research Council of Norway (2003-2007). Steering Committee Member; Nordic Centre of Excellence Pilot Programme (2003-2007) and European Science Foundation Life, Earth and Environmental Sciences (2007-2009).

### Research interests

(1) Genetic diversity and function of indigenous soil populations of rhizobia, symbiotic cyanobacteria and methanotrophs. (2) Interactions between agricultural important legumes under various climatic conditions. (3) Symbiotic nitrogen fixing bacteria are used as «model organisms» for comparative studies of differences in structural and functional diversity in experimental laboratory studies and their performance and persistence are followed in sub arctic and arctic field studies. (4) The presence and activity of methane oxidising bacteria-methanotrophs in northern high organic ecosystems are studied both through isolation of organisms and *in situ*. (5) A culture collection from different latitudes is used for physiological and taxonomic studies. (6) A new novel method has been developed for isolation of methanotrophs from soil. (7) Arctic field studies, including ongoing projects at Pyramiden, Adventdalen and Solvatn, Ny-Ålesund.

### Recent publications

•Duodu S., Georg Carlsson G., Huss-Danell K. and **Svenning M.M**. (2007). Large genotypic variation but small variation in $N_2$ fixation among rhizobia nodulating red clover in soils of northern Scandinavia. *J Appl Microbiol*. **102**:1625-1635.

•Wartiainen I., Hestnes A.G., McDonald Ian R. and **Svenning M.M**. (2006). *Methylobacter tundripaludum* sp. nov., a methane-oxidising bacterium from arctic wetland soil on the Svalbard islands, Norway (78°N). *Int. J Syst Evol Microbiol* **56**:109-113.

•Wartiainen I., Hestnes A.G., McDonald Ian R. and **Svenning M.M.** (2006). *Methylocystis rosea* sp. nov., a novel methanotrophic bacterium from Arctic wetland soil, Svalbard, Norway (78° N). *Int. J Syst Evol Microbiol* **56**:541-547.

•Johansen A., Knudsen I.M.B., Binnerup S.J., Winding A., Johansen J.E., Jensen L.E., Andersen K.S., **Svenning M.M.** and Bonde T.A. (2005). Non-target effects of the microbial control agents *Pseudomonas fluorescens* DR54 and *Clonostachys rosea* IK726 in soils cropped with barley followed by sugar beet: a greenhouse assessment. *Soil Biol Biochem* **37**:2225-2239.

•Binnerup S.J, **Svenning M.M**., Hestnes A.G., Wartiainen I. (2005). Methane oxidising bacteria as environmental indicators. Nordic Council of Ministers, TemaNord 2005:522

•Fagerli I.-L. and **Svenning M.M.** (2005). Arctic and subarctic soil populations of *Rhizobium leguminosarum* biovar *trifolii* nodulating three different clover species, characterised by diversity at chromosomal and symbiosis loci. *Plant Soil* **275**:371-381.

•Wartiainen I., Hestnes A.G. and **Svenning M.M.** (2003). Methanotrophic diversity in high arctic wetland on the islands of Svalbard (Norway) - denaturing gradient gel electrophoresis analysis of soil DNA and enrichment cultures. *Can. J Microbiol*. **49**:602-612.

•**Svenning M.M.**, Wartiainen I., Hestnes A.G. and Binnerup S.J. (2003). Isolation of methane oxidising bacteria from soil by use of a soil substrate membrane system. *FEMS Microbiol. Ecol.* **44**:347-354.

## *Short CURRICULUM VITAE*, Steffen G Bergh

Born 24/7-1954; received PhD in geology in 1987 (Univ. of Tromsø). Professor of Structural Geology and Tectonics at University of Tromsø, Norway since in 1992. Visiting Scientist at University of Durham, UK, University of Wyoming, USA, Northern Arizona University, USA, Nordic Volcanological Institute, Iceland.

**Past and ongoing research interests:**
General aspects of crustal assembly and continental growth, including (1) Onshore-offshore linkage of Meozoic-Tertiary extensional faults in the Mid-Norwegian shelf margin and plate tectonic implications, (2) Post-Caledonian tectonics and structural evolution of Svalbard with special attention to transcurrent and convergent Tertiary tectonics linked to the Barents shelf transform plate boundary, (3) Archaean and Palaeoproterozoic crustal evolution in North Norway, (4) Onshore-offshore tectonic controls on Cenozoic denudation, topography and landscape evolution in western Fennoscandia. (5) Precambrian petrology, geochemistry and crustal evolution of rift sequences and province-bounding, ductile shear zones in North Norway, (6) Caledonian orogen in North Norway, tectono-thermal evolution.

**Selected relevant publications**:

- **Bergh, S.G**., Ohta, Y., Andresen, A., Maher, H.D., Braathen, A. & Dallmann, W.K. 2003: Geological map of Svalbard, 1:100 000 sheet B8G St. Jonsfjorden. Norsk Polarinstitutt Temakart nr. 34.
- **Bergh, S.G.** & Grogan, P. (2003) Tertiary structures of the Sørkapp-Hornsund Region, South Spitsbergen, and implications for the offshore southern extension of the fold-thrust belt. *Norwegian J Geol* **83**:43-60.
- **Bergh, S.G**., Maher, H.D.Jr., & Braathen, A. (2000) Tertiary divergent directions from partitioned transpression, Brøggerhalvøya, Spitsbergen. *Norwegian J Geol* **80**:63-82.
- Braathen, A., **Bergh, S.G**., Maher, H.D., Andresen, A., Hansen, A-I., & Bergvik, A. (1999a). Kinematics of the Isfjorden-Ymerbukta fault; a dextral oblique thrust ramp in the Tertiary fold-thrust belt of Spitsbergen. *Norwegian J Geol* **79**:227-240.
- Braathen, A., **Bergh, S.G**., & Maher, H.D. (1999b). Application of a critical wedge taper model to the Tertiary, transpressional fold-and thrust belt on Spitsbergen. *Geol Soc Am Bull* **111**:1468-1485.
- **Bergh, S.G**., Braathen, A., & Andresen, A. (1997). Interaction of basement-involved and thin-skinned tectonism in the Tertiary fold-and thrust belt of Central Spitsbergen, Svalbard. *Am. Ass Petrol.Geol.Bull*. **81**:637-661
- Maher, H.D., **Bergh, S.G**., Braathen, A., & Ohta, Y. (1997) Svartfjella, Eidembukta, and Daudmannsodden lineament:Tertiary orogen-parallel motion in the crystalline hinterland of Spitsbergen's fold-thrust belt. *Tectonics* **16**:88-106
- Braathen, A. & **Bergh, S.G**. (1995). Kinematics of Tertiary deformation in the basement-involved fold-thrust complex, Western Nordenskiøldland, Svalbard; tectonic implications based on fault-slip data analysis. *Tectonophysics* **249**:1-29
- Maher, H.D., Braathen, A., **Bergh, S.G**., Dallmann, W, & Harland, B.W. (1995). Tertiary or Cretaceous age for Spitsbergens fold-thrust belt on the Barents Shelf? *Tectonics*. **14**:1321-1326.