Introduction: The primary objective of this project is to trace the co-production and co-utilization of methanogenic substrates in natural and simulated gases and waters from low thermal maturity coal beds, a slowly biodegraded form of natural organic matter. While some previous studies have inferred that methanogenesis in low thermal maturity coal beds is dominated by hydrogenotrophic methanogenesis, recent studies suggest that simultaneous processes could contribute more than one methane precursor (e.g., acetate, H₂, methanol), linked to more than one mechanism of CH₄ generation. However, steady-state concentrations of methane precursors (including H₂) in coal beds are poorly understood.

Activities to date: To date, PI Vinson’s group has implemented a method for simultaneous gas chromatographic analysis of CH₄, CO₂, and trace levels of H₂; and has set up and maintained batch incubations of simulated coal bed waters and gases, including headspace gas analysis. Due to recruiting difficulties for the fall 2018 semester, student-supported effort on the PRF-funded project began in January 2019. Therefore, the project is modestly behind schedule.

In 2018, Vinson obtained a new reduction gas detector (RGD) for the packed column GC system used in this study. The RGD, obtained using funds other than this award, unlocked new capabilities related to this project, allowing the approach to be refined. The RGD combined with the existing system allows simultaneous analysis of the essential gases in this study, CH₄, CO₂, and H₂, in a single injection.

In spring 2019, Vinson’s group established the H₂ analysis method and began two anaerobic batch incubations, representing in total twelve treatments, each incubated in triplicate. The first incubation is natural methanogenic wetland sediments in freshwater and high-sulfate media buffered to pH 7 with phosphate buffers. By design, the wetland sediment is the sole carbon source. The purpose of the sediment incubations is to validate our H₂ measurements in methanogenic vs. sulfate-reducing conditions and to maintain a mixed fermentative-methanogenic fluid that can be used to transfer anaerobes to other incubations. Results of the wetland sediment incubation indicate that the methanogenic treatment contains headspace H₂ corresponding to the low methanogenic range, while the sulfate-containing treatment exhibits lower headspace H₂ (<5 ppm), likely typical of sulfate reduction (Figure 1). Headspace CH₄ in the methanogenic treatment has leveled off, likely indicating that the initial supply of easily metabolized electron donors has been consumed. This condition is suitable for transferring the fermentative-methanogenic fluid into the coal incubations. The second incubation is low-thermal-maturity Powder River Basin coal as primary carbon source, incubated in sterile media ranging from fresh to 3 M NaCl, prepared with vitamin, nutrient, and mineral additions, and buffered to pH 7 with phosphate buffers. A high-sulfate sterile media incubation provides a sulfate-reducing environment. The coal incubations have been monitored for CH₄, CO₂, and H₂, defining the baseline prior to adding a microbe-bearing fluid, and water samples have been collected for acetate and methanol analysis. It is intriguing that the coal incubations have released H₂ and CO₂ to the saline treatments to a varying degree (Figure 2).

Collaboration: The PRF award has allowed Vinson to strengthen working relationships with collaborators in complementary areas of expertise, contributing to project objectives: (1) Thomas Darrah, Ph.D. of Ohio State University, with expertise in noble and trace gas geochemistry; and (2) Matthew Kirk, Ph.D. of Kansas State University, with expertise in geomicrobiology. In July 2019, Vinson visited Darrah’s lab to learn methods of introducing field-collected gas samples to the GC system for trace H₂ gas analysis without air contamination. Vinson has also interacted with Kirk on anaerobic incubation design.

Students supported to date: To date, the award has supported three graduate students. In spring and summer 2019, one continuing MS student each term was supported by research assistantships.
funded by the award. Vinson trained both students in basic principles of anaerobic incubations and gas chromatographic analysis. In the current fall 2019 semester, a new MS student is being supported by a research assistantship funded by the award. The new student has taken responsibility for the anaerobic incubations.

Overall, to date the PRF award has allowed Vinson to apply new tools and techniques to a longstanding subsurface problem, has enabled Vinson to strengthen collaborations with key investigators, has provided support for Vinson to mentor future geoscience professionals, and has provided Vinson’s lab group with training in new problems and techniques.

Figure 2. Representative preliminary headspace $H_2$ in Powder River Basin coal anaerobic incubations of varied media salinity.