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Respiration and Carbon Dioxide Accumulation in Soil Microcosms

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The thawing of permafrost leads to liberation of soil organic matter and microbial component resulting in significant effect on the carbon cycle. Changes in permafrost soils upon the global climate warming would significantly impact microbial soil activity. Currently, not a great deal is known about what happens to the microbial community after the permafrost thaws. The activity of microbial community was determined in microcosms experiment using respirometry. To assess the CO\textsubscript{2} fluctuations during permafrost thawing a titration experiment was performed. Based on calculations using the respirometric equation it was found that CO\textsubscript{2} production increases with temperature, core depth, ion amendments and duration of time.

Introduction

Regardless that some 25\% of Earth’s terrestrial surface is underlain by permafrost, knowledge of the diversity of microbial life in this extreme habitat is limited (Graham et al. 2012). Cold temperatures play a critical role in the selection and survival of the microorganisms inhabiting these environments as well as the environmental parameters (Bakermans et al. 2012). These environments include high mountains, boreal forests, frozen peat lands, Pleistocene ice complexes, and permafrost sediments up to several hundred meters deep. The total mass of perennially frozen sediments (or permafrost) contains a large amount of buried sequestered ancient organic carbon and these sediments vary widely in soil composition, soil organic matter quality, hydrology, and thermal regimes complicating predictions of carbon flux (Graham et al. 2012). Permafrost is degrading rapidly in response to global climate change, and this is leading to a deeper, seasonally thawed, metabolically active soil layer (Graham et al. 2012). This active soil layer contains a high amount of microorganisms which actively participate in cycling of organic carbon influencing CO\textsubscript{2} and CH\textsubscript{4} flux.

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It is well known that the top layer of soil contains a total estimated number of $2.6 \times 10^{29}$ cells (Whitman et al. 1998). Another poorly studied region of the soil has an estimated of $2.5 \times 10^{29-30}$ cells and is located 8 m below the surface (Whitman et al. 1998). Microorganisms are a vital component in the environment and have been recovered from sediments collected hundreds of meters below the Earth’s surface. Soil microorganisms play many important functions in terrestrial ecosystems, such as in nutrient cycles and decomposition processes. They are also crucial in maintaining cycling of organic matter in soils (Slonczewski et al. 2009).

The thawing of permafrost soil is an important factor in the carbon cycle. As the global climate changes, the permafrost soil thaws, altering soil activity. Little is known about what happens to the microbial community after its liberation from permafrost soils upon thawing. Many factors can change, such as respiration rates, greenhouse gases emissions, and microbial enzymatic activity. The emission of greenhouse gases, including CO$_2$, could increase. To answer some relevant questions about microbial respiration, its rate, and the resulting carbon mass balance, the rates and mechanisms of microbial respiration and their impact on the carbon mass balance was investigated during this study by measuring CO$_2$ fluxes.

Measurement of microbial activity in anaerobic conditions is more challenging than in aerobic conditions, since in permafrost the number of anaerobic bacteria is significantly lower in comparison to aerobic (Vorobyova, Soina et al. 1997). The fermentation products as well as CH$_4$ which are produced in anaerobic microsites can diffuse to aerobic areas with subsequent oxidation to CO$_2$ and H$_2$O. Microbial activity can be determined by measuring CO$_2$ release and/or O$_2$ uptake. CO$_2$ measurement allows an assessment of the balance between the C used in growth relative to substrate decomposition and microbial byproduct formation (Paul and Clark 1996). There are a variety of methods for measuring CO$_2$. A traditional method involves the aeration of the sample in a gas-tight growth chamber (i.e., biometer flask) where a NaOH trap is utilized to collect the evolved CO$_2$ as Na$_2$CO$_3$. BaCl$_2$ can then be added to the NaOH to precipitate the trapped CO$_3^{2-}$ as BaCO$_3$. Residual NaOH is titrated with HCl to determine the CO$_2$ concentration (Paul and Clark 1996, Stotzky 1997). Using a gas chromatograph with a thermal conductivity detector is another method for determining CO$_2$ production from a gas tight growth chamber (i.e., serum bottle with rubber stopper) (Anderson 1982).

A similar microcosm experiment to the one conducted here was performed previously at Princeton University (Princeton, NJ) between July 2010 and October 2010. A series of anaerobic microcosm experiments were conducted on active-layer and permafrost soil in order to refine sampling and analysis strategies for the thawing experiments. These experiments were performed at 4°C and 22°C under four different growth conditions: 80:20 H$_2$/CO$_2$, 30 mM acetate, 30 mM methanol, and no amendment. Their results showed that both CH$_4$ and CO$_2$ production were highest at 22°C on the CO$_2$/H$_2$ substrate. The permafrost layer with methanol amendment that was incubated at 22°C had the highest CH$_4$ production (Onstott 2010).

Based on the results of these experiments, it could be hypothesized that if bacterial respiration is temperature-sensitive, warmer temperatures will result in a corresponding increase in bacterial respiration. Based on high proportion of readily reversible to metabolic activity cells in the permafrost (Vorobyova, Soina et al. 1997), we hypothesized that the greater respiratory activity will be in permafrost layer than in the more shallow active layers and CO$_2$ will increase with increasing of temperature. The results of the Princeton
experiments also suggest that microbial populations differ between soil type (i.e., active layer versus permafrost), among nutritional amendments, and along a temperature gradient.

**Methods**

Using an anaerobic Coy chamber (Coy Laboratory Products Inc., Grass Lake, MI), anaerobic microcosms were established with each microcosm consisting of 10 g soil and 10 ml water in a 50 ml serum vial sealed with a butyl rubber stopper and aluminum crimp seal. Three layers of cores were used in this experiment: D1-upper active (sticky, muddy, with a few plant roots), D2-lower active (rocky, sandy, no roots), and D3-permafrost (fast-thawing, “smoothie like” mud, no roots). Four types of nutrient amendments were used as individual treatments: 1) 1 mM sodium nitrate, 2) 1 mM sodium sulfate, 3) 1 mM glucose or 4) no amendment. All microcosms were incubated at 0, 6 and 15°C for 8 months. Every month, CO$_2$ and methane production was measured by gas chromatography (Agilent Technologies 6890N Network GC System, Santa Clara, CA).

The microcosms with low activity under anaerobic conditions were selected to investigate if the change in redox conditions from anaerobic to aerobic would increase microbial respiration. The serum vials were opened, and the contents were transferred into biometer flasks. The serum vials were rinsed with 2 ml of phosphate buffered saline (PBS) and the buffer transferred to the flasks in order to ensure all contents were transferred from the original serum vial. Glucose (10% stock solution) was added at final concentration of 5 mM to all flasks. Sodium hydroxide solution (7 ml of 2.5 M) was added to the side arm and the flask openings were secured with rubber stoppers. The side arm stopper contained a needle recovery port. These flasks were then incubated in the dark at different temperatures (0°C, 6°C, 15°C). To measure CO$_2$ production, 7 ml of NaOH were recovered from the side arm every 24 hours for 2 weeks. Fresh NaOH solution was added back into the side arm after each sampling. The CO$_2$ concentration in the collected NaOH was measured by reacting the solution with 10 mL of 1 N (or 0.5 M) BaCl$_2$ solution. This reaction created a cloudy precipitate and the samples were centrifuged (Dynac® Centrifuge: Becton Dickinson & Company, Parsippany, NJ) at 3500 X g for 15 minutes (room temperature) to obtain a pellet. If a firm pellet was not formed, the sample was re-centrifuged for an additional five minutes. The supernatant fraction was retrieved and 10 drops of phenolphthalein indicator were added to the flask until the mixture turned pink. HCl was then added to the flask using the titration method. The acid reacted with the amount of NaOH remaining. The amount of NaOH available to titrate was inversely proportional to the amount of CO$_2$ it contained. At the endpoint of the titration, the amount of HCl used was determined. This allowed for an estimation of how much CO$_2$ was produced over a specific period of time and, consequently, the net rate of respiration of soil organisms within the soil samples.

**Results**

A comparison of CO$_2$ accumulation over time within a selected experimental parameter of temperature, nutrient amendment or soil layer was made (Figs. 1, 2, and 3). For water amendment of D2 active layer CO$_2$ accumulation was higher at 6°C, followed by CO$_2$ accumulation at 0° and 15°C (Fig. 1), however CO$_2$ accumulation did not vary at nitrate (Fig. 2) and sulfate (not shown) amendments. The accumulation of CO$_2$ was higher for water
amendment of permafrost D3 layer (Fig. 3). Changing from anaerobic to aerobic redox conditions enhanced CO$_2$ production regardless of treatment and temperature. Over time, the amount of CO$_2$ increased in all aerobic microcosms, but minor differences in respiration rate were seen with temperature, treatment, or core depth. It was observed that samples at 6°C generally showed higher levels of respiratory activity (Fig. 4), and samples with the amendment of water without an electron acceptor (i.e., nitrate or sulfate) had higher levels of activity (Fig. 5). Samples from permafrost (D3) soil had higher levels of activity than upper (D1) and lower (D2) active layers (Fig. 6).
Discussion

The respirometry measurements allowed accurate comparison of microbial activity in a number of microcosms. Changing from anaerobic to aerobic incubations resulted in CO$_2$ production, regardless of treatment or temperature. Over time, the amount of CO$_2$ increased for all aerobic microcosms, while minor differences in respiratory activity were observed with temperature, treatment and soil layer (depth). The variations in CO$_2$ accumulation at time periods 263-306 h (Fig. 1) could have resulted from better utilization of glucose.
at 6°C where the temperature had increased above the freezing point, yet still within the temperature growth range of most psychrophilic organisms. Additionally, the 15°C incubations may have provided a less optimal growth environment for some psychrophiles. Other treatment conditions (Fig. 2) show no variations in CO$_2$ accumulation over time.

Figure 2 shows 6°C having a higher CO$_2$ accumulation average when compared to temperatures 0°C and 15°C; however, there were no statistically significant differences between the three temperatures. The limited variation in CO$_2$ production could be due to the fact that the original microcosm setup was initially performed under anaerobic conditions. The lower redox environment in these microcosms may have altered the microbial community composition and reduced the numbers of aerobic bacteria during the 8 months of anaerobic incubation prior to being exposed to oxygen. Overall, the microbial respiration rate when the active layer and permafrost soils were exposed to oxygen increased to 0.4 mmol CO$_2$ g$^{-1}$ day$^{-1}$.
The results obtained from Princeton University researchers differed from that presented in this study. Their microcosms showed significant CO$_2$ production under anaerobic conditions. This variation was likely due to the lower concentration (1 mM) of nutrients used for amendments in this study. Indeed, the Princeton study utilized a higher nutrient concentration (30 fold higher) which resulted in higher CO$_2$ accumulation. This also demonstrates that as nutrient levels rise, CO$_2$ production increases due to microbes having more bioavailable nutrients to metabolize.

The addition of nutrients such as nitrate or sulfate did not stimulate CO$_2$ production in fact the higher CO$_2$ production resulted from adding just water (Fig. 5). Thus, it could be speculated that the addition of water, which could represent the accumulation of water during thawing, was sufficient to mix available nutrients for increased microbial respiration. When CO$_2$ production was examined by soil depth (upper active (D1), lower active (D2) and permafrost (D3) layers), it was found that the permafrost D3 samples had more total accumulated CO$_2$ compared to either active layer regardless of the incubation temperature or nutrient amendment (Fig. 6). Furthermore, in the water only amendments, the amount of CO$_2$ accumulated over time was higher for the permafrost layer than for the active layers (Fig. 3). These results provide support that increased temperatures that result in thawing of the arctic soils cause increase CO$_2$ production by permafrost microbial communities. It has been concluded that if this microcosm experiment was performed only under aerobic conditions, there may have been a more dramatic increase in CO$_2$ accumulation at temperature increase. Based on the results of this microcosm experiment, it could be predicted that as the global temperature increases and the permafrost thaws, the resulting increase in CO$_2$ production could exacerbate global climate change.

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About the Author

Jasity Murphy is a recent UT graduate with a Bachelor of Science degree in Biological Sciences and a major in Biology. She has been working with microcosms since the summer of 2011 and has enjoyed her experience in undergraduate research. She hopes to continue her educational and scientific pursuits at a university or hospital. She is especially interested in the field of immunology.

About the Advisor

Dr. Tatiana Vishnivetskaya is a Research Associate III at the University of Tennessee, the Center for Environmental Biotechnology. Her work focuses on the use molecular assays to examine the molecular microbial diversity of permafrost and track changes in metabolic activity during environmental changes. She received her Ph.D. in 2003 from Russian Academy of Sciences. She has more than 80 presentations at scientific meetings and published abstracts, 55 publications. She was awarded Significant Event Award for team achievement in Launch of the BESC Cultivation Pipeline (2008) and in Development of New Techniques for Pyrosequencing (2010). Her research interests include the molecular microbial ecology, applied microbiology, microbial genomics, metagenomics and life in extreme environments. Her professional activities include: Review Editor for the Journal of Frontiers in Extreme Microbiology, ad-hoc reviewer for several peer review scientific journals, and proposal reviewer for NSF.