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Aerobic Methane Production from a Red Oak Tree Leaf

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ABSTRACT

Recent studies have shown that there is more methane in the atmosphere than previously thought. After discovering large amounts of methane emission above tropical rainforest area, Frank Keppler and colleagues performed research concerning aerobic methane emission from plants. Keppler concluded that some plants may be capable of aerobic methane production. Methane emissions are an important area of research since methane is one of the most prominent greenhouse gases being released into the atmosphere. Furthermore, for my research I chose to examine the methane emissions from a plant from the ecosystem of the United States, the red oak tree leaf, *Quercus rubra*, using gas chromatography with a flame-ionizing detector. I collected red oak tree leaves and cut them into 0.2g samples using aseptic techniques and incubated 20 samples in the dark at 30°C and 20 samples in the presence of 300 µmol/s?m² of light at 30°C. At the times T=0 hours and T=24 hours, the concentration of methane was measured by gas chromatography with a flame-ionizing detector. The paired t-test comparing the difference of methane produced by samples incubated in the dark at 30°C found that there was a statistically significant amount of methane produce in the time interval from T=0 hours to T=24 hours. Also, the paired t-test comparing the difference in methane production for the samples incubated the presence of 300 µmol/s?m² of light at 30°C showed that there was a statistically significant amount of methane produced from time T=0 hours to T=24 hours. Both of the paired t-tests had a p value < 0.001 showing that the change in production of aerobic methane was not due to chance. Next, a t-test was performed comparing the amount of methane produce from the samples incubate in the dark at 30°C to the amount of methane produced from the samples incubated in the presence of 300 µmol/s?m² of light at 30°C. The results of this t-test found a p value of 0.715 which showed that there was not a statistical significance between the amounts of aerobic methane produced in the samples incubated in the dark when compared with the sample incubated in the presence of 300 µmol/s?m² of light indicating that the amount of methane produced was not more active when incubated in the dark or when incubated in the light. The results of this research imply that methane is being produced aerobically and that the aerobic production of methane is not dependent on the presence of light or dark.

Keywords: *methane, greenhouse gas, anthropogenic, methanogenesis, aerobic*

INTRODUCTION

When considering greenhouse gases that are emitted into the atmosphere, carbon dioxide and methane are the two most important. When comparing the warming of the earth by one kilogram of methane and one kilogram of carbon dioxide, methane warms 23 times more than carbon dioxide (Keppler 2007). Over the past 150 years, the total methane concentration in the atmosphere has almost tripled with close to 600 million metric tons being produced each year from natural and anthropogenic sources (Keppler 2007). Anthropogenic sources constitute 20% of the methane concentration while biogenic sources constitute 80% of the methane concentration (Potter 2006). Scientists believed that methane was produced in locations with reduced levels of oxygen where decomposition occurred, such as that of agricultural rice paddies and swamp lands (Kruglinski, 2006). Most all of the sources of methane production were thought to be known until recently when Frankenberg et al (2005) studied atmospheric levels of methane using a SCIAMACHY instrument which is a scanning imaging absorption spectrometer for atmospheric chartography. The SCIAMACHY measurements indicated higher than expected methane concentrations during the dry season in a tropical region as well as higher than expected methane concentrations surrounding evergreen forests (Frankenberg, 2005). In 2006, while working at the Max-Planck Institute for Nuclear Physics in Heidelberg Germany, Keppler et al published a study in which they found that plants including leaves of ash (*Faxinus excelsior*), leaves of beech (*Fagus sylvatica*), sweet vernal grass (*Anthoxanthum* odoratum), maize (*Zea mays*), and wheat (*Triticum aestivum*) used the process of aerobic methanogenesis (Keppler, 2006). Until Keppler's publication, it was thought that methanogenesis was only an anaerobic bacterial process. Keppler and colleagues found that sunlight and increased temperature had an influence on the amount of methane produced from their experimental plants (Keppler, 2006). In 2007, Rodriguez studied aerobic methane production by a banana plant (Musa acuminate) following the techniques used in Keppler's experiment. Rodriguez's results did correlate with Keppler's and his colleagues. I performed an experiment similar to Rodriguez, the only difference being that I studied the aerobic methane emission from red oak tree leaves, a plant common in the United States.

MATERIALS AND METHODS

Standard Curve

To create my standard curve, I used a 15ppm concentration of CH_4 in air from Scott Specialty Gas in Plumsteadville, Pennsylvania. For my standard curve, I used injections starting at 20 µL followed by two 10 µL increases followed by four 20 µL increases up to 120 µL injections. I had an R^2 value of 0.9929 and a standard curve equation of y(ng) = (5.3764E-5) x(mV*s) - 0.5597.

Procedure

I used aseptic techniques similar to Rodriguez while collecting and preparing the red oak tree leaves. I cut the leaves into 40 sample pieces all weighing approximately 0.2 grams. I then sterilized the leaf matter with a 5% bleach solution for 30 seconds followed by de-ionized water for 30 seconds. I then used aseptic techniques to place the sample pieces into appropriately labeled 22 mL glass vials and capped the vials with gastight screw tops with Teflon lined rubber septa. I took a 50 µL injection using a gastight syringe and measured the concentration of methane from the sample by injecting the sample into the Claurs 500 gas chromatography with flame-ionizing detector (FID). I placed the first 20 samples into a dark incubator with a constant temperature of 30 °C. I placed the last 20 sample into an incubator with a constant temperature of 30°C and 300 µmol/s?m² of light. I incubated the samples for 24 hours. At T=24 I measured the methane concentration using the same technique used to measure the samples at T=0.

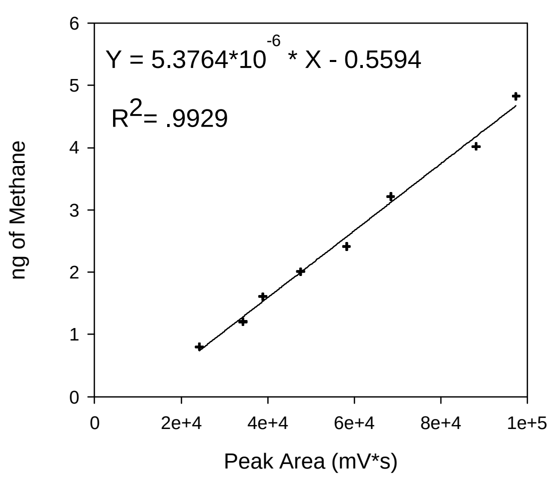

Figure 1. Standard Curve for Sample Methane Concentrations.

RESULTS

The data was analyzed by running a paired t-test in a statistical program called SigmaStat 3.5®. The paired t-test was comparing the amount of methane produced at T=0 hours to T=24 to determine if there was a statistical significance between the amount of methane being produced at the two different times. According to the results of the paired t-test, the red oak tree leaves that were incubated in the dark and in 300 µmol/s?m² of light both showed significant aerobic methane production with p value < 0.001 for each test. To further analyze my data, I used SigmaStat 3.5® to run a t-test to compare the samples when the ng of methane produced per hour and mass of the leaf samples were being accounted for. This test showed a p value of 0.715 which did not show a significant difference of methane emissions between the light and dark samples.

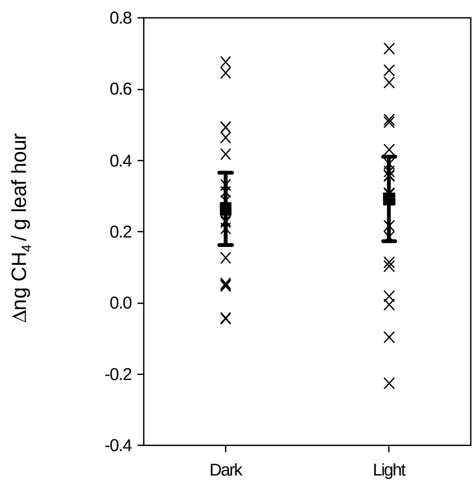

Figure 2. Change of methane concentrations per hour per gram of leaf for samples incubated in dark at 30°C as well as samples incubate in the presence of 300 µmol/s?m² of light at 30°C. The means of the two data sets are indicated by the ¦ 's. The 95% confidence intervals are indicated by the horizontal bars.

DISCUSSION

This experiment was an experiment similar to Rodriguez's experiment testing differences in methane production produced aerobically when plant matter was incubated in the dark and in light at 30°C at the times T=0 and T=24. The difference between this experiment and Rodriguez's experiment was the plants that were used. I used a red oak tree leaf as my sample plant matter, a plant found in the United States ecosystems rather than a banana plant, a plant found in a tropical ecosystem. According to the results of the paired t-test, the red oak tree leaf does produce amounts of methane that show a statistically significant difference for the samples that were incubated in the dark as well as for the samples that were exposed to 300 µmol/s?m² of light. Furthermore, the data analysis showed that there was not a statistical significance between the samples exposed to the light when compared to the samples exposed to the dark. This shows that aerobic methane production is not dependent on light or dark.

There has been a great deal of research regarding the methane production from plants found in tropical ecosystems. More recently there has been published research regarding the methane production from plants found the ecosystem of the United States. It is thought that the amount of methane released into the atmosphere from the ecosystem of the United States differs depending on the location of the plants (Potter, 2006). Furthermore, factors that are variables to the methane production from plants include cyclic temperature, sediments in soil, and the dynamics of water tables (Potter, 2006).

Valuable information would be gained if further studies were performed regarding the amounts of methane produced from varying plants in varying ecosystems. Further studies of aerobic methane production that could be performed include testing the methane production from a variation of plants native to the ecosystem of the United States as well as from a variation of plants native to the ecosystems of other nations. Also, the methane production of varying plants could be tested during different seasons, as well as when the plants are growing in different soils with various known sediments in their growing soil.

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LITERATURE CITED

Evans, J.R., Neill, S., and Wingler, A. Resolving methane fluxes. New Phytologist 175, 1-4 (2007).

Frankenberg, C., Meirink, J.F., van Weele, M., Platt, U. and Wagner, T. Assessing methane emissions form global space-borne observations. Science 308, 1010-1014 (2005).

Harvey, S. How does the greenhouse grow?. Natural History 115, 14 (2006).

Keppler, F., Hamiltion J.T.G., Brass, M., and Rockmann, T. Methane emissions from terrestrial plants under aerobic conditions. Nature 439, 187-191 (2006).

Keppler, F. and Rockmann, T. Methane, plants and climate change. Scientific America 296, 52-57 (2007).

Kruglinski, S. Plants grab control of the global greenhouse. Discover 27, 15 (2006).

Potter, C., Klooster, S., Hiatt, S., Fladeland, M., Genovese, V., and Gross, P. Methane emissions form natural wetlands in the united states: satellite-derived estimations based on ecosystem carbon cycling. Earth Interactions 10, 1 (2006)

Rodriguez, Jamie. Aerobic methane production by banana plant. Cantaurus 15, 21-23 (2007).