8-10-2012

Measurement of the Respiratory Quotient of Peat

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8/10/2012
BIOL 5800 Undergraduate Research
Summer 2010

Introduction

Respiratory quotient (RQ) is the ratio of CO_2 produced to O_2 consumed by an organism. Complete respiration of glucose will give an RQ of 1 as described by the formula $C_nH_{2n}O_n+nO_2 \rightarrow nCO_2+nH_2O$. The respiration of molecules with lower oxygen content, such as lipids, give RQ values of less than one, whereas in cases of anaerobic metabolism, an increase in biomass or the respiration of substances such as humic, oxalic and citric acids the respiratory quotient can be greater than one. In complex systems such as soil, Dilly (2003) found that the RQ varied dramatically, and changed within the same soil under varying conditions. Similarly, Hollender et al. (2003) found RQ was informative in determining the underlying metabolic mechanisms, such as nitrification processes. Dilly (2004), studied the effects of various organic compounds on RQ, and found that beech forest soils amended with cellulose or humic acid maintained RQ values greater than one for more than 20 days after application.

Measurement of RQ involves a simultaneous detection of the changes in CO_2 and O_2. The objective of this study was to demonstrate a technique to measure the RQ in a closed static system.

Materials and Methods

Preparation of peat

To produce uniformity, commercial Canadian peat moss was well mixed and loosely packed into a 3 gallon planting pot. The pot was then watered with a dilute nutrient solution and set to rest in a greenhouse. This initial process allowed for microbial populations to stabilize and reduces the effect of an initial growth spike induced by soil disruption and nutrient addition. After a rest period of 7 days, 20 grams of uniformly moistened peat was place in 1 liter Mason jars for 1 day to further allow equilibration of microbial populations. Each jar lid was fitted with a septum to allow for gas extraction via syringe (for CO_2 sampling) and a small hole to allow the oxygen sensors to be installed. The lids were also fitted with 1 meter of 3.18 mm (1/8th inch) diameter polyethylene tubing to allow pressure equilibration and prevent leaks caused by the expansion and contraction due to pressure. This allowed for a 2 ml air buffer against the expansion and contraction of air due to temperature and pressure changes. A pH of 4.8

was determined by suspending a small amount of peat in deionized water. This low pH reduced bicarbonate interactions with CO_2 solubility in the moisturized peat.

Figure 1: 1 liter Mason jars fitted with septa and oxygen sensors for gas measurements.

The far right jar is an empty control.

CO_2 Measurement

A LI-COR LI-6251 IRGA (Infrared Gas Analyzer) was used in conjunction with a Campbell Scientific CR10T datalogger to measure CO_2 concentration. Each measurement consisted of a 5 ml sample being injected into a CO_2 free air stream. The induced change in CO_2 concentration of this air stream was detected by the IRGA and output as a voltage to the datalogger, where software ascertained the peak of this signal which was then converted to ppm CO_2 and recoded. The system was calibrated each day it was used by injecting a sample of reference gas, from which a correction multiplier was calculated that accounted for current pressure and temperature conditions.

Oxygen measurement

The measurement of O_2 was monitored continuously using an oxygen probe in a method similar to that described by Blonquist et al. Four Oxygen sensors (Apogee Instruments Model-SO) were mounted to read the oxygen concentration of the 1 L Mason jar. The data, along with measurements from a thermocouple and a pressure sensor (Apogee Instruments SB-100), were acquired using a Campbell Scientific CR1000 datalogger. The pressure

equilibration of the system allowed for pressure corrections to be applied based on atmospheric pressure. The sensors output was corrected to remove the effects of temperature and pressure.

Results

Each jar, other than the control, showed an increase in CO_2 and decrease in O_2, as seen in Figure 2. Each data set was regressed against time to obtain relative change in gas concentration per day; a scatterplot and the corresponding linear regression are plotted in Figure 3. The respiration rate was calculated using the equation $\frac{\Delta\mu mol\ gas}{mol\ air * day} * 0.034\ mol\ air = \frac{\Delta\mu mol\ gas}{day}$. These rates, along with the RQ values for each replicate jar, are reported in Table 1. The coefficient of variation for the 3 reps was 6.9% and 22% for CO_2 and O_2 respectively.

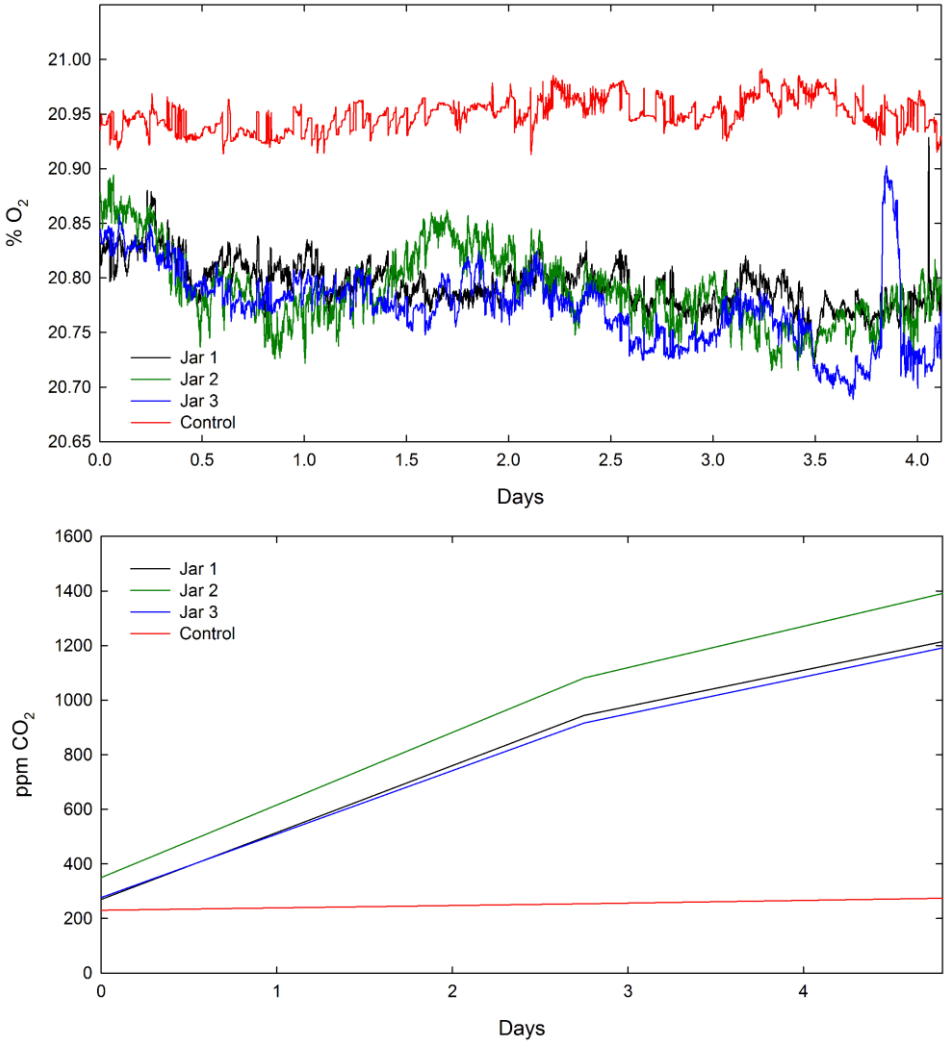

Figure 2: The change in CO_2 and O_2 in a 1 L jar.

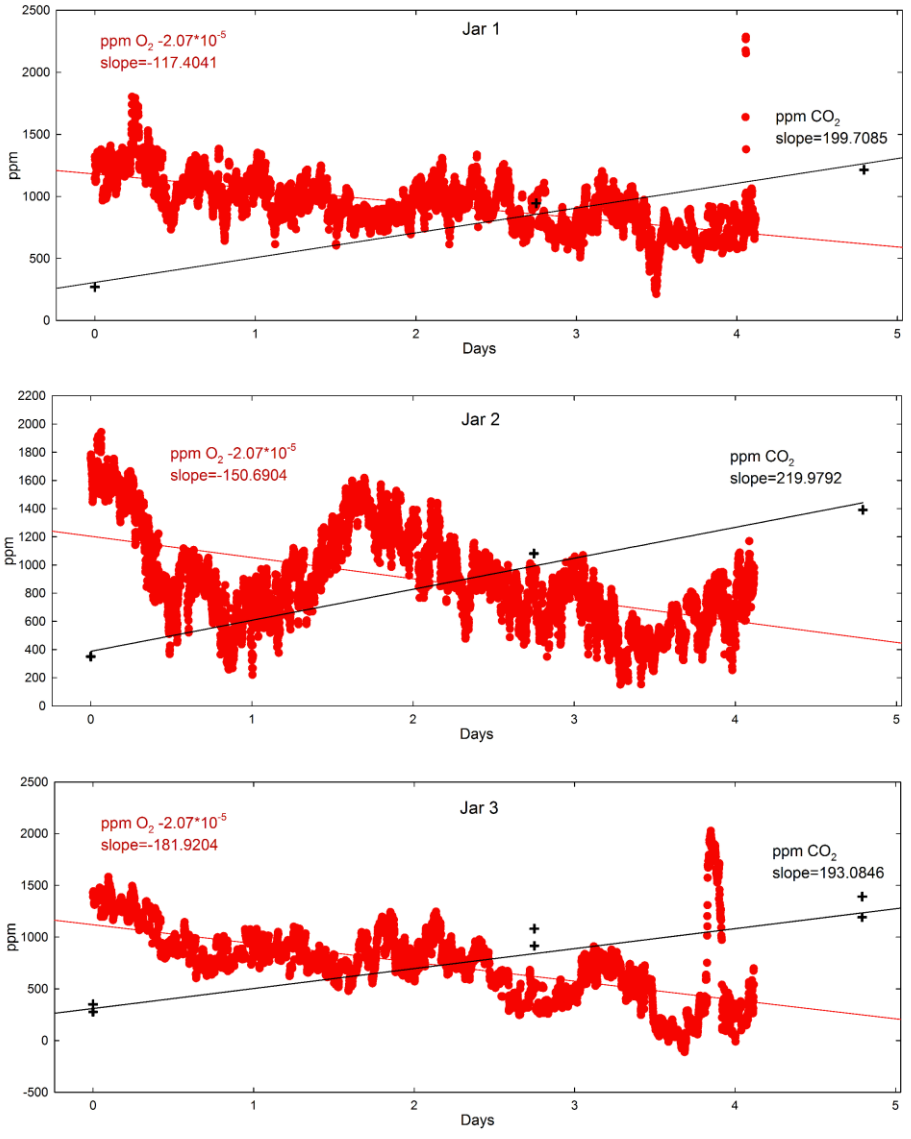

Figure 3: Comparison of respiration rates measured by CO_2 and O_2

Jar #	resp. rate by CO_2 ($\mu mol\ CO_2\ per\ day$)	resp. rate by O_2 ($\mu mol\ O_2\ per\ day$)	Respiratory Quotient ($\frac{CO_2}{O_2}$)
1	6.79	3.99	1.70
2	7.48	5.12	1.46
3	6.56	6.19	1.06

Table 1: Table of respiration rates and RQ for each replicate jar.

Discussion

The RQ values above one indicated either an artifact of respiration or an imperfection of the method. The theoretical calculation of RQ for a carbohydrate such that

$$C_xH_yO_z + \left(x + {}^{y}/_{4} - {}^{z}/_{4}\right)O_2 \rightarrow xCO_2 + ({}^{y}/_{2})H_2O$$

is

$$RQ = {}^{x}\Big/x + {}^{y}/_{4} - {}^{z}/_{2}$$

Under ideal aerobic respiration, to obtain a RQ>1 requires carbohydrates of the type

$${}^{x}\Big/x + {}^{y}/_{4} - {}^{z}/_{2} > 1 \quad => \quad z > {}^{y}/_{2}$$

Such carbohydrates include oxalic ($C_2H_2O_4$), malic ($C_4H_6O_5$) and citric acid ($C_6H_8O_7$).

Dilly (2003) found respiratory quotients above 1 for many soil types days after nutrients had been added, even for compounds that would produce RQ>1 under ideal aerobic respiration, which he attributed to the accumulation of biomass. When carbon intermediates are removed from the citric acid cycle before oxidative decarboxylation via the electron transport chain, CO_2 is released with no consumption of oxygen, thus high RQ values.

The peat was widely dispersed in the jar and pockets of anaerobic respiration were not likely to be present in sufficient amounts to significantly impact the RQ. Therefore, if the RQ values are valid, the carbohydrates in the peat, $C_xH_yO_z$, would have to be of a chemical composition such that $z > {}^{y}/_{2}$ or there would have to be a sufficient accumulation of biomass.

Given that the coefficient of variation for CO_2 is low in comparison to O_2, there is also the possibility that the oxygen sensors could not adequately detect respiration in the conditions of this experiment. The small amount of peat in the comparison to the volume of the jar resulted in very low changes in oxygen concentration in comparison to the ambient O_2 level. These low relative changes are difficult to detect.

Another source of error is the exchange of gas via the pressure equilibration tube. The temperature change throughout the experiment was approximately 2.25° C, which corresponds to a 0.75% change in volume. Such a change would equate to 7.5 ml of air moving either in or out of the tube. Given that the tube volume was approximately 2 ml, and that the temperature fluctuated up and down multiple times during the experiment, a significant amount of gas exchange with the exterior of the jar would have taken place. Since the gases are

changing in opposite directions, lowering the value of RQ. Also, the magnitude of the individual respiration rates would be damped.

Conclusions

While the system does detect the RQ, a more sensitive system is needed to obtain accurate values. The system could be optimized for oxygen detection, such as using a smaller volume jar or a greater amount of peat. Smaller jar volumes would potentially cause the accumulation of CO_2 and cause the measurement to go off scale. Due to the discrepancies in relative gas concentration changes, a more robust method should be utilized to obtain accurate RQ values, with either a more sensitive oxygen measurement or a CO_2 measurement with a wider range.

References

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