A Rapid and Cost Effective Soil Carbon Mineralization Method For Static Incubations

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INTRODUCTION

- Soils can be a net carbon sink if C input is greater than C output (Organic C mineralized to CO_2).
- Soil C mineralization by the biological oxidation of organic C in soil is one of the main means C is returned to the atmosphere.
- Management and climate have a direct and relatively quick impact on biologically active C pools.
- Soil incubations with subsequent determination of CO_2 are becoming common soil assays used to determine active soil organic C (SOC) pools.
- C mineralization rates and soil microbial biomass are also used to evaluate soil quality.
- Traditional methods to determine C mineralized in a static incubation are costly in both labor and equipment.
- A rapid and cost effective method for determining C mineralization rates would allow more routine evaluation of this dynamic pool in both research and in routine soil testing labs.

OBJECTIVE

Objectives:

i) Evaluate if the single cell infrared gas analyzer (IRGA), gas chromatograpy (GC) and NaOH basetrap titration methods of CO_2 determination obtain the same concentrations under static soil incubations over 1, 3, 10 and 21 days.

ii) Evaluate how well these methods correlate to each other across incubation times.

iii) Estimate the limit of quantitation (LOQ).

iv) Estimate the concentration of CO_2 and O_2 gases in the incubation chamber headspace that suppress respiration.

METHODS

- Surface soil samples were collected from a catena sequence from 4 cropping systems across 3 slopes and 2 field replications to obtain a range of labile organic matter (n=24).
- Soils were air dried and ground to pass a 2-mm sieve.
- Soils were weighted out to 30-g with 4 duplications for the 3 detection methods of IRGA, GC, and alkali absorption followed by titration (NaOH) and brought to 50% water filled pore space (n=96).

- **Part 1:**
- IRGA soils were incubated in 500-mL Wheaton serum bottles sealed with a rubber septum and aluminum ring.
- GC and Alkali absorption (NaOH) soils were incubated in 1-L mason canning jars with soils weighted into 60-mL snap jars. Alkali absorption also had a 20 mL vial of 1N NaOH within the canning jar. The lids of the canning jars used for GC analysis were modified by inserting a septa port.
- Bulk density value of 1.0 was artificially obtained by tapping soils into a known volume within incubation bottles. Duplicates were averaged before statistical analysis to normalize the sub-sampling variability (n=24).
- Base traps were switched out on 1, 3, 10 and 21 day increments and titrated to a pH endpoint of 8.3.
- IRGA and GC headspace gases were sampled using a pressure-lok gas syringe sequentially over 1, 3, 10, and 21 day period. The IRGA bottles were vented after the 10 day measurement to mitigate the potential for respiration suppression as the incubation chamber volume was ½ the size of GC and NaOH.

Part 2:

Determination of headspace CO_2 and O_2 gas levels that show suppression of respiration was done over a 30 day period for each 15-g and 30-g sample size in 500-mL Wheaton serum bottles incubated at 30˚C with the average of 24 soils calculated over each time period.

Vented soils were returned to ambient CO_2 and O_2 levels between readings by inserting hypodermic needles and sparging with compressed air.

Oxygen was monitored concurrently with CO_2 by linking the output port of the IRGA analyzer to the input port of a O_2 analyzer.

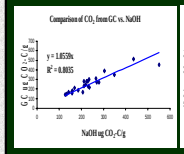
Incubations that had a 30-g sample size were continued up to 50 days with CO_2 and O_2 levels analyzed. On day 35 a 100-mL injection of 100% O_2 was injected to see if CO_2 concentration increased.

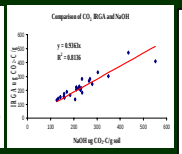
RESULTS – PART 1

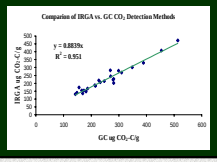
Method Interaction With Time:

	Day 1	Day 3	Day 10	Day 21
	---------- µg CO_2-C/g ----------			
GC	115 a	256 a	618 a	935 a
NaOH	97 a	237 a	541 a	866 a
IRGA	117 a	229 a	410 b	771 a
P-value	0.2012	0.538	0.002	0.1188

- Soils incubated in the 500-mL bottles showed a lower level of respired C at day 10 than the 1-L mason jars.
- After the 10 day reading IRGA soils were vented and did regain values similar to GC and NaOH at day 21.

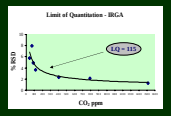


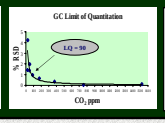


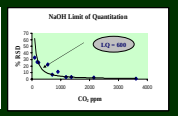


- All methods were strongly correlated with r^2 values of 0.80 and above. The strongest slope (1.05) was found between the regression of NaOH vs. GC, both of which had the larger 1-L incubation chamber.

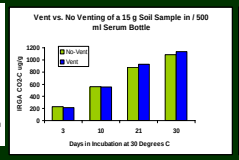
RESULTS – PART 2

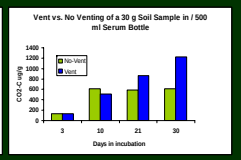




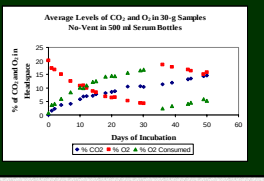


- Limit of Quantitation is defined as the lowest level at which a analytical measurement becomes meaningful in quantifying a result. The empirical value for LOQ is found by examining the inflection point in the above curves of relative standard deviation vs. increasing analyte concentration. The lowest LOQ was found with the GC method of 90 µl CO_2/L followed by 115 for IRGA. The NaOH method had the highest LOQ of 600 µl CO_2/L.





- Mean of 24 soils over 3, 10, 21, and 30 days of 15-g vs. 30-g sample size confirmed that respiration was being suppressed at about day 10 in the 500-mL serum bottles measured with the IRGA.
- Day 10 is, of course, sample dependent, therefore the headspace gas concentration that shows suppression of respiration is required to estimate when the incubation bottles need to be vented back to ambient levels of CO_2 and O_2.



- The graph above shows the average of 24 soils % CO_2 and O_2 in the headspace above a 30-g sub-sample within a 500-mL serum bottle sealed with septa and Al sealing ring. The level of CO_2 and O_2 that showed a slope change and thus suppression where found to be 9.1% and 10.9 % respectively.
- At day 35, 100 mL of 100% O_2 gas was injected into the incubation bottles. This restored ambient O_2 levels but not CO_2 levels. This would indicate that the suppression was due to limited O_2 levels not toxic CO_2 levels as CO_2 levels in the headspace started climbing after day 35.

CONCLUSIONS

- Long-term incubation are achieved by venting the 500 mL serum bottles between readings and/or monitoring the headspace gases to keep CO_2 levels below 10% and O_2 levels above 10%.
- Reduction of the incubation vessel volume by half (IRGA only) showed that venting the headspace is needed in longer incubations. Therefore results at 21 days showed a single-cell IRGA is sufficient technology to assay respired CO_2 from static incubations as results are not statistically different than the gold standard of GC analysis and the traditional alkali absorption followed by titration.
- The sample run time for the IRGA method using out of the box software is 90 or more per hour vs. 26 for the GC and under 10 per hour for base-trap titration (NaOH).
- Equipment cost favor the IRGA method. A single cell IRGA is approximately 1/10th the cost of a GC or automated titrator.
- Equipment costs, simplicity and run times make the single cell IRGA method suitable for research and routine soil analysis.