Carbon Sinks to Carbon Sources:

A Soil Analysis of the Colorado Mountain Pine Beetle Epidemic

(Photo taken by student)

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Environmental Systems

Word Count: 3993

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Research Abstract

As the Mountain Pine Beetle (*Dendroctonus ponderosae*) infestation progresses eastward across the continental divide in the Colorado Rocky Mountains, it threatens Ponderosa Pine trees on the eastern side of the divide.[1] This study aims to gauge and understand the effect the impending infestation could have in the future on a soil-carbon level.

The analysis compared soils from Lodgepole Pine Trees in Grand County, CO and Ponderosa Pines in Boulder County, CO in their composition, relative abundances of organic compounds, and CO_2 respiration rates. In doing so, remote sending was used to find the sites for the collection. Then the soils were prepared and packed for GC-MS (Gas Chromatography-Mass Spectrometry), carbon (C) and nitrogen (N) analysis, and a 41-day incubation to measure daily CO_2 respiration rates.

The most significant data found showed an average 2.73 times higher rate of carbon respiration among Boulder County soils, suggesting the microbes in these soils process carbon faster and therefore respire more in a shorter period of time. In analyzing the data, the bulk densities and C/N data were compiled to find respiration per gram C, which indicated the trend of soil-carbon respiration.

[1] Negrón, J. F and Popp, J. B. *Probability of ponderosa pine infestation by mountain pine beetle in the Colorado Front Range.* Elsevier Science B.V.

The far-reaching applications of this study suggest that in addition to the loss of pine tree forests in Colorado, the Mountain Pine Beetle poses the threat of heightened CO_2 emissions by causing an increased rate of respiration in soils where pine trees have decomposed on the eastern side of the continental divide. Though any decomposing forest would increase the microbial respiration of the soil beneath it, this study aimed to gauge what an increase may translate into for specific soils from the eastern and western continental divide in Colorado.

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1: Introduction

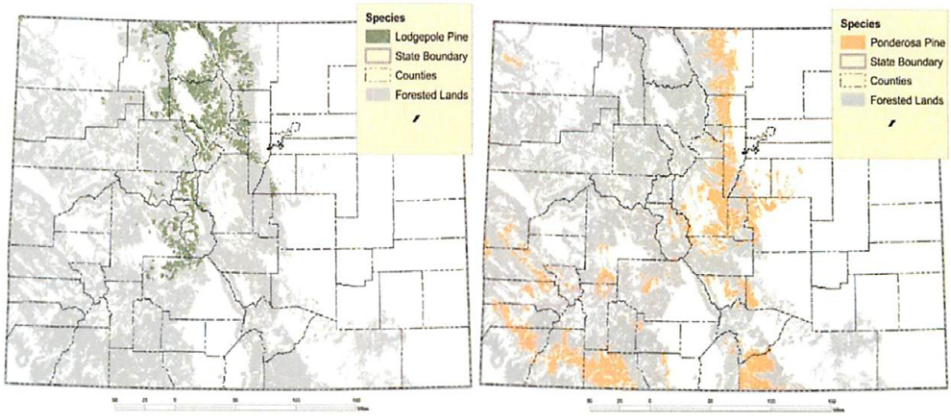
The mountain pine beetle epidemic in the Rocky Mountains has been primarily studied from a perspective of prevention and severity. Almost all research on the rapidly growing infestation has addressed ways to control the number of future outbreaks, and methods of gauging the effect (in number of trees attacked) of *Dendroctonus ponderosae* on forests in the Rocky Mountains. One study, from the University of Colorado, suggests that at least 60 to 70 percent of the forests will die from the epidemic, and Colorado could lose as much as 90 percent of its pine trees[1]. Taking this statistic to be true, the effect of the dying trees on the environment, rather than the effect of the beetles on the trees, may indeed be a more important realm to study. This study addresses the soil under pine beetle infested trees with the overall goal of gauging the differences in soil-carbon microbial respiration rates between the eastern and western sides of the continental divide. The difference between the sides of the divide highlights the difference in the stages of the epidemic, as the western side has had significantly more outbreaks of pine beetle infestations, and the eastern side is predicted to have an equally severe infestation in the near future[2]. The difference in pine tree species also splits across the continental divide, as lodgepole pines are predominantly located on the western side of the divide, while ponderosa pines are on the east:

[1] Neff, T. (10 August 2006). Pine beetle threat looms over county . *Daily Camera* .

[2] Lipsher, S. (5 September 2006). Pine-beetle battle turns desperate. *Denver Post* .

Figure 1 – Map

Pine Tree Distribution in Colorado



(Maps Available from Colorado State Forest Service)

In understanding the carbon respiration rates of these soils, the effect of the pine beetle infestation on the global carbon cycle can possibly be gauged. This study aimed to investigate and predict how the effect on carbon dioxide in the atmosphere will be different from decomposing pine beetle infested trees on the eastern versus the western sides of the divide in Colorado. In doing so, as mentioned earlier, the soil microbial respiration rates from soils collected in Grand County, Colorado (Western divide) and Boulder County, Colorado (Eastern divide) were measured. These soils came from samples ten centimeters beneath the duff layer of soils taken from within a three foot radius from the tree's trunk under the tree canopy (mineral layers). The duff layers (biodegrading needles, mostly) were collected and some samples were analyzed, but the rest remain for future analysis.

1.1: Research Question

How are microbial behavior and soil composition different in areas of Mountain Pine Beetle (MPB) infestation on the eastern and western sides of the continental divide, and by year of infestation? Specifically, how do CO_2 respiration rates, Carbon (C) content percentages, Nitrogen (N) content percentages and C:N ratios differ among Lodgepole Pines from Grand County, CO and Ponderosa Pines from Boulder County, CO?

1.2: Hypothesis

It is crucial to consider the effects of the higher annual nitrogen deposition on the eastern side of the continental divide as compared to the western side (2.57 kg/ha/yr in Boulder vs. 2.39 kg/ha/yr in Granby, data from Forest service). Naturally, as a tree dies and begins to decay, organic material will enter the soil and/or be released to the atmosphere.[3] Therefore, organic carbon input and nitrogen deposition will dictate microbial activity and respiration. Since bacteria breaks down organic matter and converts it to CO2 in the presence of oxygen, available nitrogen will be the limiting factor in respiration because the nitrogen-fixing bacteria will respire according to the nitrogen available in the soil.[4]

If the above explanations hold true, then the Boulder County soils will respire at a higher rate and will have a lower C to N ratios, and the older soils (by year of attack) will respire at a higher rate and have lower C to N ratios.

[3] Deacon, J. "The Microbial World: The Nitrogen cycle and Nitrogen fixation" Online Article.

[4] Yoshitake, S et al. (March 2007) Carbon and nitrogen limitation of soil microbial respiration in a High Arctic successional glacier foreland near Ny-Ålesund, Svalbard.

2: Methods

Part I: Determining Soil Collection Sources through GIS

To begin, the Mountain Pine Beetle (MPB) infestation aerial survey record (from forest service: http://www.fs.fed.us/r2/resources/fhm/aerialsurvey/) was downloaded in GIS (shapefile) format for the 1994-2007 infestation data. A new ArcGIS file was created to incorporate the National Forest Service data (from above) and the GIS mapping of Colorado. In the ArcGIS program, the files were filtered for HOST1 108, 113, 115, 122 (lodgepole, limber, spruce, and ponderosa pines), for DCA1 11006 (*dendroctonus ponderosae*), for limits of Colorado, and for overlap between years (removing from most recent year of overlap). Each year of MPB infestation (ArcGIS layer) with county and highway was overlayed onto the existing file. GIS data for soil in infested Colorado counties (from Soil Data Mart) was downloaded. This data would enable soil type to be controlled when determining locations for sampling. Nitrogen deposition GIS data for Colorado was downloaded, allowing Nitrogen deposition values to be taken into account at the sites, so Nitrogen deposition could be ruled out as a source of error in final findings. Geology GIS data for Colorado was downloaded from the USGS, in order to keep geology of locations the same for all sampling sites. Likewise, elevation GIS data for Colorado was downloaded to keep the elevation constant at the sampled sites. To fine the sites, the data was overlaid to find areas of similar characteristics of all above mentioned layers (MPB infestation, soil, nitrogen, geology, elevation [aspect]). The ArcGIS file was then filtered for Grand, Boulder, and Summit Counties, which were in regions where sampling was a reasonable possibility given location constraints. The ArcGIS file was saved as a .kmz file for use with Google Earth, which was used to locate potential sampling sites which held constant to the above-listed factors.

Part II: Sampling at Potential Sites

Using a GPS to locate the picked sites, the locations were driven to for sampling. At each site, a tree was found using the following criteria: it must have needles, but also must be clearly dying due to MPB infestation (reddish needles, dropping branches, unstable trunk, etc.). The circumference of tree, GPS coordinates of tree, geology, year of infestation (both already known from GIS analysis), and any additional comments were recorded in a lab notebook. Two pictures were taken of each tree for future reference: one of the bark, one of the whole tree (record save file on camera). Using a 10cm circumference cylindrical sampling tool, three roughly equidistant samples from around the tree were taken and stored in large (freezer-sized) Ziploc bag. Duff layer (top soil layer) samples were taken from these equidistant sampling sites to fill one medium-sized Ziploc bag per tree. The digging sites were filled in so as to have the least environmental impact from sampling. Steps 2-7 were repeated three times (one for each of 3 trees) per sampling location.

Part III: Preparation for Samples for Analysis

All mineral samples were placed in tin foil containers and masses were recorded. They were then dried in a drying oven at 35 degrees Celsius. A new (dry) mass was recorded for the samples. This process was repeated for the duff layers samples. The dry mass divided by three times the volume of the cylindrical shovel (three times for the three equidistant samples taken) used for sampling provided the bulk density, which was important in Part IV for calculating porosity. Then, each sample was split and sieved with a 2mm sieve. In between each sample, the sieve was cleaned with water and methanol and was dried in the drying oven at 100 degree Celsius to decrease contamination between the soils through careful sterilization. Each sample, after it was dried, split, sieved and the masses were recorded, was placed into a small sample bag with its label and a <2mm marking to distinguish it from other soil fractions. For the duff layer samples, coffee grinders were used to grind the duff layers to approximately

850 micrometers. The duff layers were stored in small bags and also labeled with the same system as the mineral layer samples. The coffee grinders, too, were sterilized with methanol between samples.

Part IV: Incubation of Mineral soils

Roughly 8g of split/sieved soil was taken and placed in a jar for each sample. The mass was recorded (of the soil). The porosity percentage was determined for each sample using the following equation: Porosity = (1/(Bulk Density/Sample Density))*100. Then, 60% of the porosity was taken as the value for the amount of water to be initially added to each jar to maintain constant moisture (to maintain controlled respiration rates among all samples). Then, the samples were wet accordingly and capped in air-tight jars which contained the jars which they were already in. For the actual measurement, two air tight jars willed solely with air from the room were capped as "blank" samples to calculate ambient CO_2. These jars with the samples were uncapped one day prior to measurement and re-capped three hours prior to measurement. Using designated syringe to gather air samples from each jar, measurements were taken from each jars using the following steps:

a. Press CO_2 analysis on computer, then auto-generate cells making 20 cells, then calibrate the TOC machine, turn on O_2 flow and press start
b. Press set when ready and when the start button is lit up, proceed
c. Press the green button on TOC analyzer, then pump air in to top of syringe, release at steady flow taking 15 seconds
d. Record the first blank samples and use repeat function to average them into one cell
e. Record data for each sample repeating every other one to ensure precision

TOC (total organic carbon) measurements were taken on days 1, 2, 5, 8, and every week after through day 41, when a trend was apparent and established[5].

Part VI: Carbon and Nitrogen Analysis

Roughly 1g of the mineral layer and duff layer soils were measured and placed into jars. The soils were acidified with just enough 0.1M HCl that the soil began to "fizz" and bubbles appeared

[5] Shumacher, B. *Methods for the determination of Total Organic Carbon (TOC) in Soils and Sediments.* Las Vegas, NV: Environmental Protection Agency.

accompanied by a hissing noise. This process is necessary to remove carbonate which will offset data if included in the mass spectroscopy. The soils were left overnight to ensure that they had properly dried. The following day, the soils were ground with a mortar and pestle and then packed into labeled capsules. These capsules were taken and 20mg of each soil was packed into tin foil packets designated for the mass spectrometer. Using pliers and a high-accuracy scale with an enclosed measurement area, the tins were placed into a tray and their masses were recorded. The tray was inserted into the Carbon/Nitrogen Analyzer (specific mass spectrometer). The data from the C/N Analyzer gave a percentage of carbon and nitrogen in each sample using the recorded masses. The process was repeated for the duff layer soils although that data has not yet been analyzed, since the duff layer addition is pending analysis as a whole.

Part VII: GCMS (Gas Chromatography-Mass Spectrometry)

Samples were prepared for Mass Spectrometry by taking each soil from each site and averaging them by mass so as to have a 33:33:33 ratio for each soil (amount of soil by mass) in a site (i.e. samples G1A, G1B, G1C become G1, and are composed of proportionate masses of each sample). This was done because the organic composition of each site was much more pertinent than the organic composition of each sample, which would not differ from the sampling location as a whole. The samples were acidified using 15% HCl and were split, grided, and packed into small capsules for GCMS analysis. Each sample was injected into the GCMS analyzer one at a time. The provided computer application for the collection of data was used and the data was exported to MatLab7 for conversion, then to AMDIS, where it was saved as a text file (.txt). The text file was then opened with Microsoft Excel and all "types" and "sources" (the data titles differentiating the organic composition of the soils) were sorted into different columns for percent-based composition among the sampling sites. Then, using partial sums, each organic compound or type of compound (aliphatic, aromatic, or fatty-acid methyl ester) was

sorted and a percent-composition of each was found. The data was graphed to show a comparison among the sites.

Part VIII: Data Analysis

The data were organized using Microsoft Excel. The following steps were taken in the spreadsheet to manipulate data:

- Determine soil-carbon mass from carbon percentages (see Part VI) multiplied by initial soil masses for each sample
- Make columns for time capped, time injected, time elapsed, blank CO2, measured CO2, and respiration rate (ug-C/g-C-soil/day/cm3)
- Repeat above for each day and soil sample, using [(((Measured CO2-Blank CO2)/Time Elapsed)/(1000000))*(0.01952)*44*60*24*1000*1000)/Carbon mass of soil] as the equation to determine the respiration rate

The C:N ratios and respiration rates were examined for consistency: If consistent then the incubation trends had a basis (in data) to be relied upon for further analysis. If not, the samples were examined to determine the causes for this discrepancy. The respiration rate trends were graphed over time for the locations (Boulder, CO, Granby, CO), by year of first-reported infestation, by percent change in respiration, by carbon, nitrogen, and C:N ratios. Using AMDIS data from the GC Mass Spectroscopy (see part VII), the soil composition was mapped out by location and year (of first-reported infestation) for the samples). Using Statistica (PC application), an ANOVA analysis was run to ensure the reliability (statistically) of the data.

Safety Procedures (Hazardous Chemicals, Item #4)

- Chemicals: 15%HCl, Methanol
- Precautions: Wear gloves, goggles, lab coat
- In case of spill: Use H_2O and acid neutralizer
- Disposal of chemicals: Hazardous waste is put in appropriate storage containers, labeled, and stored in a satellite accumulation area by waste type

3: Data

Figure 2: Graph

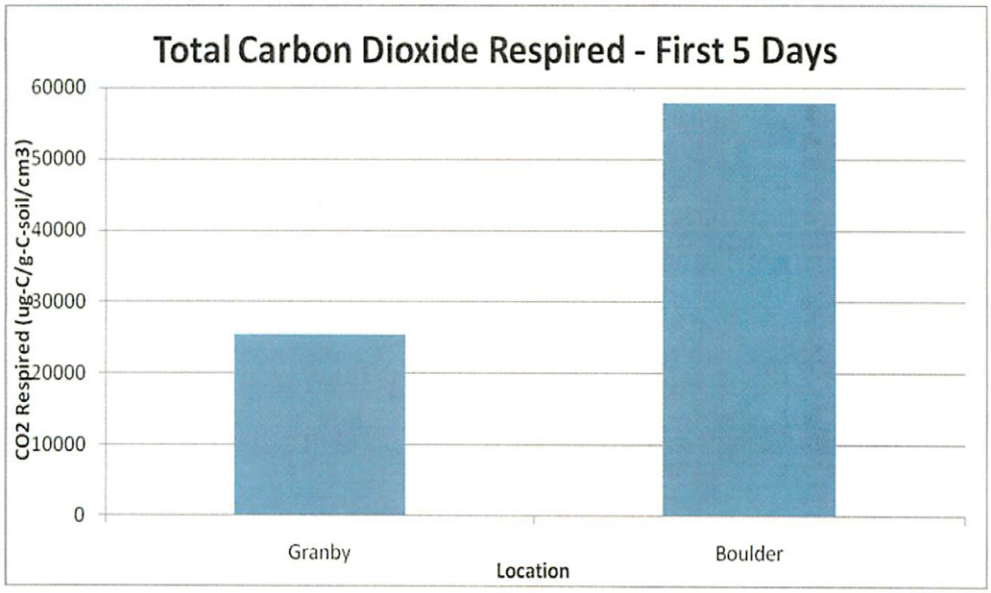


Figure 2: The first 5 days indicate how the soils respire as they would naturally. Without time to normalize carbon and nitrogen levels, this time period gives us the best indication of what a typical respiration rate would be.

Figure 3: Graph

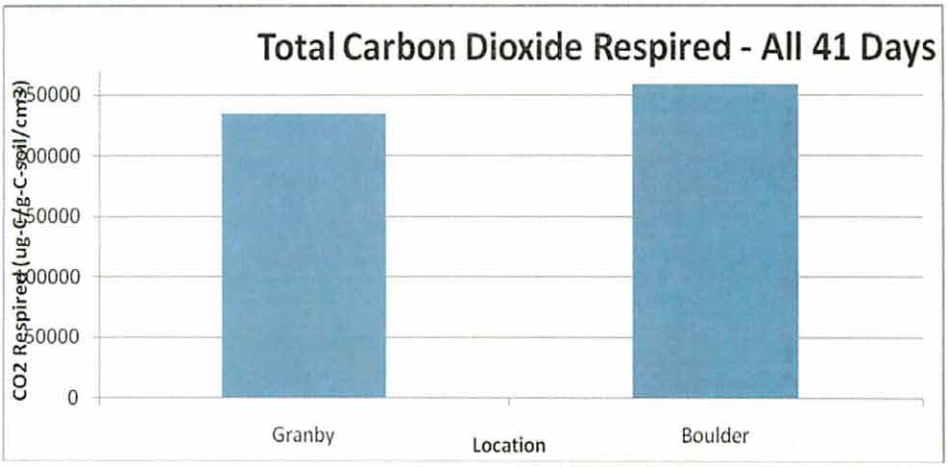


Figure 3: The first 41 days encompass the entire measurement period. The total respired CO_2 is extremely similar between the two sites. This is because after a month-and-a-half long period, the carbon and nitrogen levels have stabilized with the same periods of rewetting and aeration for all the soils.

Figure 4: Graph

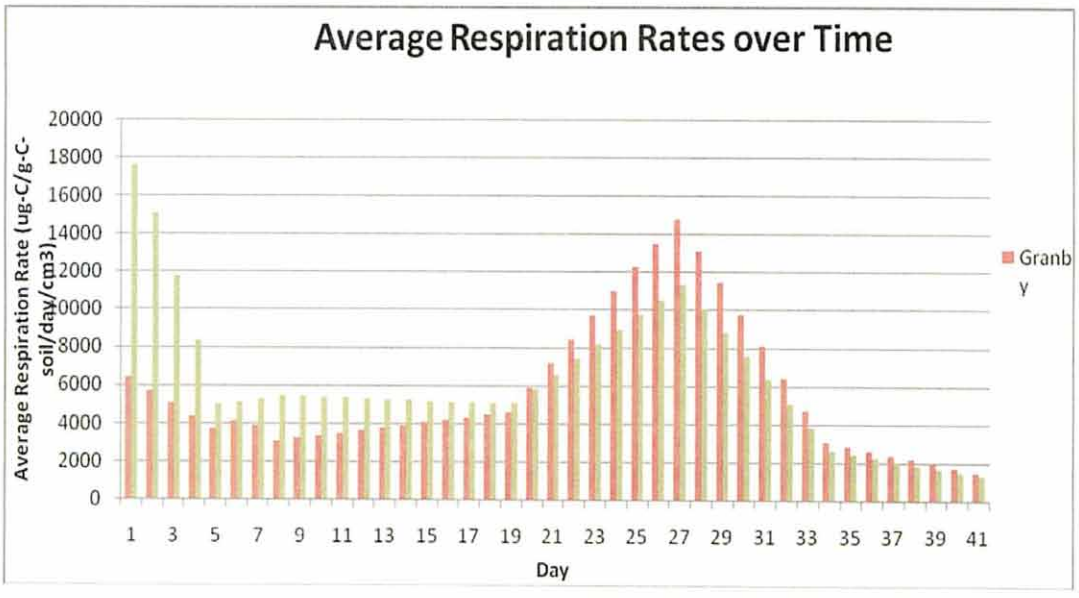
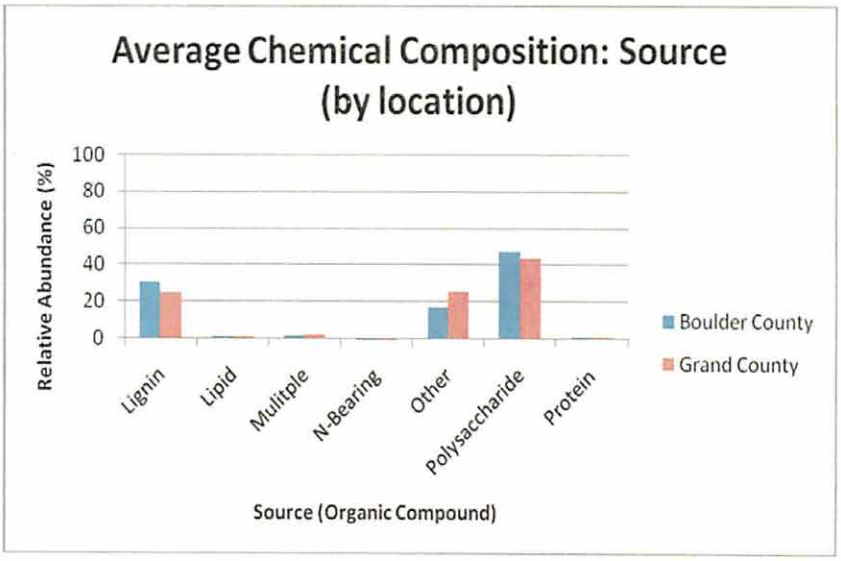
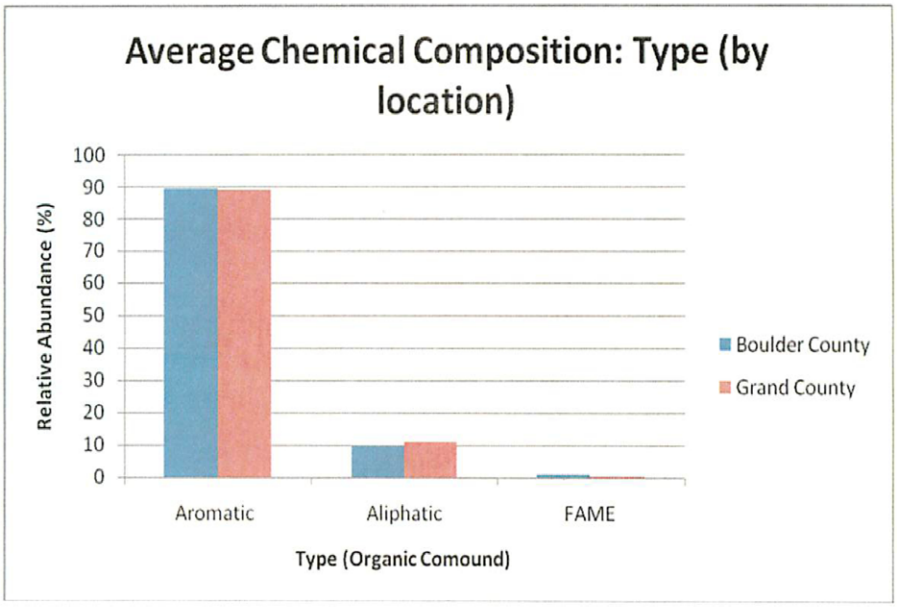


Figure 4: Graph of the average daily respiration rates by site. The large (and decreasing) difference between the two sites is expressed in the above two graphs. The increases and decreases correspond to days in which the soils were rewetted. While the soils were measured 8 times over the 41-day period, the other days of days were calculated based on averages.

Figures 5,6: Graphs





Figures 5 and 6: These graphs show the chemical similarities the Grand County and Boulder County soils share, which are necessary for a valid comparison. Using the data shown in these Figures 5 and 6, the site "G3" was removed from the study because of its compositional dissimilarity. Note that the sites were selected to be constant through GIS remote sensing data so the composition is not surprising. To view the relative abundances by site (i.e. G1, G2, etc.), graphs are provided in the accompanying binder.

Figure 7 – Table

Average Daily Carbon Respiration Rates – Mineral Soils (**all values in µg-C/g-C-soil/day/cm3**)

Day	Site - G1	Site – G2	Site - G4	Site - B1	Site - B2
1	6334.874	6810.574	6181.426	21324.72	13898.39
2	5781.57	6947.376	4502.202	21317.63	8909.68
5	3833.742	4419.799	2906.686	7071.432	2956.542
8	2845.009	3530.66	2972.121	7267.138	3670.383
19	5170.305	4799.339	3907.443	6323.746	3801.079
27	15098.53	24105.69	5182.598	12062.61	10570.35
34	3443.917	3143.342	2666.274	2884.313	2350.27
41	1930.068	1410.581	1268.903	1701.545	978.1046

Figure 6 represents the mean carbon respiration rates recorded through TOC analysis (see Part IV of methods) for the mineral soils. The table shows the averaged values, with blank (ambient) CO_2 taken into account, as well as carbon percentage, and the rate was computed from measured CO_2 in each enclosed soil jar. The data in Figure 6 correspond to the graph in Figure 3. The data were used to calculate the total carbon respired over the incubation period (see Figures 1 and 2).

Figure 8 – Table

Carbon/Nitrogen Relative Abundances, C:N Ratio

Sample ID	Mass (mg)	N (mg)	C (mg)	N %	C %	C:N Ratio
G1A	9.370	0.015	0.597	0.158	6.375	40.3481
G1B	9.996	0.008	0.262	0.085	2.625	30.88235
G1C	10.538	0.01	0.322	0.099	3.052	30.82828
G2A	10.862	0.008	0.169	0.073	1.557	21.32877
G2B	9.754	0.014	0.351	0.148	3.596	24.2973
G2C	9.594	0.005	0.135	0.054	1.412	26.14815
G3A	10.804	0.011	0.275	0.103	2.548	24.73786
G3B	10.908	0.01	0.307	0.087	2.816	32.36782
G3C	10.194	0.017	0.458	0.166	4.493	27.06627
G4A	9.960	0.012	0.465	0.117	4.672	39.93162
G4B	9.374	0.02	0.598	0.218	6.383	29.27982
G4C	9.748	0.015	0.392	0.155	4.024	25.96129
B1A	10.182	0.013	0.32	0.124	3.143	25.34677
B1B	10.660	0.006	0.142	0.059	1.33	22.54237
B1C	10.918	0.006	0.142	0.058	1.301	22.43103
B2A	10.242	0.018	0.325	0.174	3.175	18.24713
B2B	10.450	0.014	0.245	0.132	2.343	17.75

Figure 7 shows the carbon and nitrogen relative abundances for each sample run through the specialized mass spectrometer (see Part VI of methods). The data were incorporated to normalize respiration per gram of carbon for Part VI of the methods.

Figure 9 - Table

Details of Soil Relative Abundances of Organic Compounds by Source and Type (See Figures 5, 6):

	Boulder Soils (% Abundance)	Granby Soils (% Abundance)
Lignin	30.3389371	25.013012
Lipid	1.65504187	1.69620301
Mulitple	1.78712571	2.41361056
N-Bearing	0.23970654	0.1985616
Other	16.8835414	25.503452
Polysaccharide	47.4652342	43.6693508
Protein	1.63041319	1.50581005
Aromatic	89.2166406	88.8383249
Aliphatic	9.58859388	10.8438288
FAME	1.19476557	0.31784632

Note: FAME = Fatty Acid Methyl Esther in Figure 9

4: Discussion

Mineral Incubation Results:

Analysis by location of attacked trees (**Main Study**)–

Finding: The average Boulder county respiration rate was 2.73 times the average Grand County rate initially, and remained higher until day 19 of measurement

Brief Explanation: At the beginning of an incubation cycle there are vastly different amounts of available nutrients for microbial respiration. Since the Boulder county soils have a higher annual nitrogen deposition and lower C:N ratios (more nitrogen relatively), N is not initially a limiting component to microbial respiration and the Boulder County soils respire at a significantly higher rate.

Finding: On day one, there was a 173.37% difference between Grand County and Boulder average respiration rates, on day 41, there was a 12.80% difference.

Brief Explanation: The simple reasoning for this result is the same as above. With no added inputs to the microbial system, over time it balances out as no new Nitrogen is added to the system. Therefore, over time (after about 19 days), the level of Nitrogen is similar in all the soils and the respiration rates are limited accordingly.

Analysis by year of first-reported infestation of attacked trees (**Secondary analysis**)–

Finding: No distinct trend could be found (in neither Statistica nor Excel)

Brief Explanation: While the data proved to be inconclusive, it does not mean the year of infestation is insignificant as it relates to CO_2 respiration. Naturally, a tree takes many years to decompose. While the needles may fall of an create a small spike in carbon respiration within a few years, the carbon and nitrogen levels should (and likely do) rebalance as no large new carbon input is added to the system. Therefore, over a short (5-10 year) period of time, the age of the infestation will not affect microbial respiration rates.

Carbon/Nitrogen Analysis Results:

(Note: the main purpose of this analysis was to find the % carbon so it could be used to normalize respiration rates by gram of carbon)

Analysis by location of attacked trees (**Main Study**)–

Finding: The average Carbon to Nitrogen ratio of the Boulder County and Grand County soils on average was found to be 27.8% lower in Boulder Co. (29.4:1 in Grand Co. vs. 21.3:1 in Boulder Co.)

Brief Explanation: Since Boulder County has a higher annual Nitrogen Deposition, it follows that there would be more nitrogen in the soil (i.e. higher % nitrogen) and a lower percentage of carbon. So, naturally, the Carbon to Nitrogen ration would be lower with less Carbon and more Nitrogen.

Analysis by year of first-reported infestation of attacked trees (**Secondary analysis**)–

Finding: The 2008 Boulder County soils had an average 18.0 C:N ratio, while the 1998 Boulder County soils had an average 23.4 C:N ratio (23.2% difference), while there was no distinct trend by year among Grand County soils.

Brief Explanation: Over time, dead trees obviously begin to decompose. At a microbial level, this means a large input of carbon. Therefore, the older the infestation of the tree, the more carbon is in the soil (from the decomposed input) and the less Nitrogen there is (used in the respiration process by the Nitrogen cycle). It is therefore logical that an older infested tree's soil would have a higher C:N ratio than a newer infestation's soil[6].

Gas Chromatography-Mass Spectrometry Results:

(Note: This analysis was done solely for the purpose of providing a background for comparison – chemical similarity – with which to effectively relate the soils)

Analysis by location of attacked trees (**Main Study**)–

Finding: Boulder County and Grand County soils are chemically comparable and there is a compositional basis for comparison.

[6] Madritch, M and Hunter, M. *Intraspecific litter diversity and nitrogen deposition affect nutrient dynamics and soil respiration*. Oecologia Journal, February 2004.

Brief Explanation:

Explained by details of soil relative abundances of organic compounds by source and type (See Figures 5, 6) and actual data (See Figure 9).

7: Conclusion

Essentially, the results of this study suggested three main conclusions: Boulder County soils had a higher average soil microbial carbon respiration rate than Grand County soils, Boulder County soils had a lower average C:N ratio than Grand County soils, and no distinct trend could be found for the year of the soils as it relates to microbial respiration.

Within the study, there were several possible sources of error which could have affected the results. The first, and likely most consequential, source of error was labile Carbon. The results of the GCMS analysis showed that the Boulder County soils had a higher average relative abundance of Lignin and Polysaccharides, which are among the easiest organic compounds to be broken down by microbes. Though the differences between the Boulder and Granby percentages of these organic compounds was only 5% at most, the discrepancy could have influenced the data. While it is unlikely that labile carbon could have changed the results significantly, the only way to know would be to analyze the results again, differentiating by Lignin and Polysaccharide relative abundances. Another source of error could be chemical contaminants from road spills or other sources, but this is unlikely to have any possible effect after the results have been normalized through GCMS analysis. Finally, the likely reason that the year-based analysis did not provide any meaningful data is that the time span of the year of attack of the trees sampled was far too short (10 years) to start to have an impact on the soils, since the trees had not fully decomposed.

The main consequence of the study suggests that the eastern side of the continental divide will undergo more carbon microbial respiration as an effect of the pine beetle infestation. As a

consequence, carbon emissions for the Denver-metro area and other regions east of divide will be higher. This discrepancy between respiration rates by location is best expressed by carbon sequestration, where the lower the sequestration (C stored in soil), the higher the respiration rate.[7]

In continuing this study, a C/N analysis on the duff layers (before and after incubation) and a C/N analysis on the mineral layers (after incubation) will be run. The purpose of the first C/N analysis on the duff layers will be to normalize respiration data. The other two analyses will provide insight into whether or not the theory that uncapping the soils changed the ultimate respiration rates has merit. As stated earlier, a statistical differentiation of the data based on labile carbon from the GCMS organic compound relative abundance measurements should be analyzed to rule out labile carbon as a source of error, or to discover its impact on the data.

[7] Sundermeier, A, Randall R and Rattan L. *Soil Carbon Sequestration-Fundamentals*. The Ohio State University Extension Educator.

Works Cited

Deacon, Jim. "The Microbial World: The Nitrogen cycle and Nitrogen fixation". Available from <http://www.biology.ed.ac.uk/research/groups/jdeacon/microbes/nitrogen.htm>

This source provided information on the fundamentals of the nitrogen cycle, which ended up being crucial in the analysis of the observed results.

Lipsher, Steve. "Pine-beetle battle turns desperate." *Denver Post* 6 Sept. 2006

This article talked about the severity of the pine beetle infestation in Colorado on the western side of the divide as well as the migration of beetles to Ponderosa Pines and the eastern side of the divide in Colorado.

Madritch, Michael and Mark Hunter. "Intraspecific litter diversity and nitrogen deposition affect nutrient dynamics and soil respiration". Springer Berlin / Heidelberg. Oecologia Journal, February 2004.

This study helped to explain Nitrogen deposition and how it relates to soil respiration. Since \ N-deposition was cited as a main cause of the results observed, this source was a great starting point in piecing together the data into a coherent conclusion.

Neff, Todd. "Pine beetle threat looms over county ." Daily Camera 10 Aug. 2006

This article was perhaps the most crucial to the project. Since this research has results whose applications are speculative, understanding how many trees are going to die as a result of the infestation allows it to be placed in an understandable, meaningful context.

Negrón , José F. , and John B. Popp. "Probability of ponderosa pine infestation by mountain pine beetle in the Colorado Front Range." *Elsevier Science B.V.* Available online from ScienceDirect.com.

This article from Elsevier Science Journal talked about the likelihood of the progression of the pine beetle infestation in Colorado. It was valuable in providing a contextual basis for the study.

Shumacher, Brian A., Ph.D. "Methods for the determination of Total Organic Carbon (TOC) in Soils and Sediments." Environmental Protection Agency. Las Vegas, NV, 2002.

This study simply provided a model soil-organic-carbon incubation method, which was the main analytical portion of this study.

Sundermeier, Alan, Randall Reeder and Rattan Lal. "Soil Carbon Sequestration-Fundamentals." The Ohio State University Extension Educator.

This study explained Soil Carbon Sequestration, which is the ultimate explanation for differences in soil-carbon respiration rates.

Yoshitake, Shinpei et al. Carbon and nitrogen limitation of soil microbial respiration in a High Arctic successional glacier foreland near Ny-Ålesund, Svalbard. Available from <http://www3.interscience.wiley.com/journal/118495136/abstract?CRETRY=1&SRETRY=0>

This study explained why higher nitrogen levels in soils allowed for more microbial carbon-respiration, which is exactly what was observed in this research experiment.