The Long-Term Effects of Climate on the Respiration Rates of Recalcitrant Soil Microbes

Simon Wong

Abstract Changes in global climate have been linked to rising seawater levels and the extinction of many endemic and endangered species. However, one link that has not been thoroughly explored is the effect of long-term temperature increases on the microbes that inhabit recalcitrant carbon pools. I attempted to answer this question by extracting soil samples from two sites in Northern California and having the samples undergo a warm and cold treatment. The soil samples were placed in manually adjusted incubators to simulate global warming scenarios. During the nine month-long incubations, changes in Q10, which represented the rate of CO_2 respiration per ten degree increase and acclimation patterns, were noted. In the end, it was seen that long-term incubations do significantly change the Q_{10} value from the accepted value of two. For the Blodgett samples the trends in Q_{10} values were highly variable. However, complications in testing methodology may have contributed to the variability seen. The Q_{10} values for Tonzi samples were more consistent, as the warm samples showed signs of acclimation and had significant deviations in $Q_{10.}$ From the ANOVA test on neither temperature nor location were a significant factors enough to contribute to the Q_{10} values seen.

Introduction

The carbon dioxide reservoir from the organic soil pool is one of the largest potential sources of carbon dioxide. According to estimates, the world's soil contained about 2,400 gigatons of organic carbon that is around two meters deep (Batjes *et al* 2006). This large pool of carbon has important implications for the future effects of global warming. According to the IPCC Special Report on Carbon Dioxide Capture and Storage (IPCC 2007), even if future emissions were to stop within the coming years, it is expected that the atmospheric concentration of CO_2 would still approach 450-750 parts per million. This will result in the saturation of the CO_2 terrestrial storage with an additional 220-2200 $GtCO_{2,\ if}$ current global warming scenarios continue as predicted.

Likewise, if current global temperatures continued to accelerate this could result in higher rates of decomposition of organic matter by microbes. The decomposition can lead to a more progressive release of $CO_{2,}$ which can trap more infrared radiation thus leading to higher temperatures (Kirschbaum 1995). The higher temperatures induces more decomposition of soil organic matter (SOM), therefore leading to a positive feedback cycle (Cox *et al.* 2000).

The most worrisome factor is the decomposition of the recalcitrant soil. Recalcitrant soils are deep pockets of SOM that usually adhere to cation metals and are generally inaccessible to decomposition (Lloyd & Taylor 1994). The organic carbon content of the recalcitrant pool is estimated to be 5-7 times larger than the labile pool. With higher temperatures, more surplus energy is available for the soil microbes to use as activation energy for their enzymes to break down recalcitrant soil. (Bosatta & Agren 1996).

To quantify such changes in CO_2 respiration, the Q_{10} mathematic model was used for explaining and quantifying global warming trends. The Q_{10} temperature coefficient equation used by soil climate researchers to predict global warming feedback loops and cycles (Davidson et al 2006) is written as

$$Q_{10} = \left(\frac{R_2}{R_1}\right)^{\left(\frac{10}{T_2 - T_1}\right)}$$

In this model, R_2 presents the new respiration rate of the soil sample at temperature = T_2, while R_1 represents the base respiration rate. T_1 is equal to the baseline temperature (usually 10°C). T_2 is equal to T_1 - 10. The equation stipulated that for every 10 °C increase in soil temperature, respiration rates should double (Davidson & Janssesns *et al* 2006).

For most researchers, Q_{10} values should be around two (Davidson *at el* 2006). For example, if the temperature increased from 10 to 20 °C, that should result in a doubling of microbial decomposition of organic matter. The variance of this figure is usually ±.2 (Yuste unpublished). However, if current climate scenarios continue the accepted value may not stay around two and this may result in changes to microbial community structure. The major concern in this equation is that it does not account for the layer of recalcitrant soil that would be available for degradation by increasing global temperatures.

Current research conducted in this field of the organic soil pool breakdown is inadequate because it does not take into account how long-term changes affect the soil community structure. The most recent studies (Fang *et al.* 2005) have focused primarily on simulating soil recalcitrant pools at high temperatures on shorter time scales. Even with these results it is still uncertain how higher temperatures may affect Q_{10}. It is traditionally accepted that warmer temperatures lead to higher Q_{10}'s. According to the most recent studies conducted (Davidson 2006) Q_{10} appeared to be relatively stable around 2.01 to 2.3 in warm conditions. However, since there are so many uncertainties ,additional studies with more varied climate models over longer time frames should be conducted (Kirschbaum 2000).

My experiment focused on simulating long-term increases global temperatures on soil core samples and calculating the $Q_{10.}$ By calculating the Q_{10}·s through the different climate scenarios I can indirectly measure CO_2 respiration. Secondly, my experiment focused on how Q_{10} can be related to acclimation. Acclimation is the physiological changes that microbes can undertake to better cope with changing environments. For example, when microbes are exposed to the cold condition, the microbes can switch to a different set of enzymes that allow it to better cope with the lower temperature and degrade organic carbon substrates more efficiently therefore leading to higher Q_{10} values .

This resulted in a higher CO_2 flux than one would have expected in the cold environment. As for samples in the warm condition, there should be a net reduction in CO_2 flux as time progressed in order to conserve soil organic matter. So in the warm samples, acclimation by the microbes should produce Q_{10} values that gradually decrease. It is important to test for acclimation effects because recent studies (Luo *et al* 2001) have shown that even increases in temperature of 2°C induced Great Plain prairie glasses to acclimates and burn off less CO_2. The overall acclimation effects allow the microbes to change carbon substrate usage rate to better suit the environment.

Methods

Study Sites The two test sites were Blodgett, which is near the Sierra Nevada Mountains, and Tonzi Ranch, which is located just a few miles outside of Sacramento. The geographical location of the two sites can be seen in Figure 1. These two test sites were chosen because they represented two of the most common soil types in California. Blodgett as one can see from the Figure 1 is dominated by mostly ponderosa pine (*Pinus ponderosa L*) and rainfall occurs mostly from September to March. The Blodgett soil is very sandy and more dependent on the availability of soil moisture. The second site, Tonzi ranch, is mainly an oak savanna field site. The woodland is mainly composed of blue oak trees (*Quercus douglasii*) and grey pine trees (*Pinus sabiniana*), (submitted Yuste et al. 2006) The soil is characterized as more clay-like, dry and temperature-dependent. In both locations, the climate is Mediterranean-like with warm dry summers and cold wet winters.

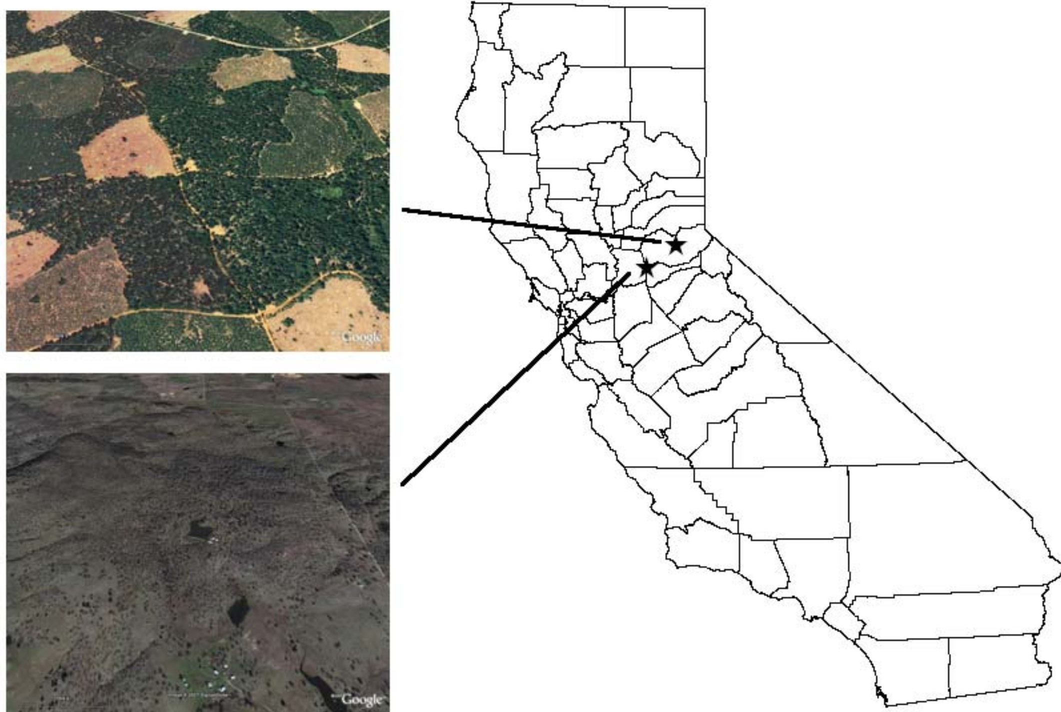

Figure 1: A map displaying the two test field sites. The top panel belongs to Blodgett; it has a relatively high density of the Ponderosa pine woods. An interesting detail to note is the relative shading that Blodgett test site provided. The bottom panel is the Tonzi test site, where the terrain is much more barren and varied when compared to Blodgett. The vegetation is composed mainly of short scrubs and other non-deciduous trees.

Sampling equipment: A stainless steel core soil sampler, which is a heavy iron cased battering device, was used to obtain the soil samples. At the end of the device is a container, that collected the soil sample into a stainless steel core approximately 80 cm^3 (4.4 x4.4 x 5 cm). To measure the soil CO_2 flux, I used a closed flow system created by my graduate supervisor, Jorge Yuste. The measurement technique follows methodology set forth by Fan *et al* 2005 and Yuste *et al* 2006. The system included a Li-Cor 6262 infrared gas analyzer. The pump worked by drawing air out of each container and running it through two valve acyclic flow meters that slowed the air down to be analyzed. Next, the infrared analyzer screened the samples. The data were stored in a Campbell Scientific data logger which stores data on CO_2 flux, water vapor, pressure and temperature.

Sampling procedure Soil samples in Tonzi were collected at the end of April 2006 and Blodgett samples were collected at the end of August 2006. The temporal difference allowed the soil to be exposed to a wide temperature range so that seasonal effects are controlled. The selection of the sampling sites was based on stratified random sampling. In the end, five understory sites were chosen along with five open sites. From each of the open or understory sites, three samples were extracted. A total of ten sites were chosen with three experimental types for each site for a total of 30 samples. The three samples per site were approximately separated from each other by .5 meters.

Once extracted, the samples were covered in plastic wrap and stored in a glass jar (7x 5x 5cm). The samples were labeled according to three criteria; by test site (Tonzi or Blodgett), test locations (understory or open area) and treatment (cold, warm, control). The samples were then taken back to the Baldocchi Biometeology lab UC Berkeley, where they were weighed and dried in the laboratory oven for two days at 40 ° C. The moisture content was then calculated from subtracting the dry weight from initial weight. Afterwards, the 30 samples were stored in jars with water saturated sponges. The sponges gradually kept the soil saturated by releasing water in a stepwise-capillary action. The samples were watered every five days to ensure ample water concentration. The warm condition samples were then placed in an incubator and set to 25˚C. The cold condition samples were placed in another incubator and set to 5˚C. The control samples were sampled for carbon and nitrogen isotope testing. Each of the sites was separated from each other by at least 20 meters. This allowed us to ignore most spatially – linked factors such as nutrient dispersion by roots and carbon pool mixing factors.

In this experiment, there were controls for many outside artifacts such as root biomass and litter inputs. To control for litter decomposition, I removed all the grass clippings and organic litter from the surface of soil. For the root respiration, when the roots are detached from the overall root system the roots died off and do not produce CO_2. Finally, soil bulk density is controlled for by having metallic soil containers that restricted the volume change of the soil.

A typical respiration measurement started by removing the sample jar from the incubator. Next, the top of the jar lid was unscrewed and the CO_2 respiration measuring

cup was placed into the jar headspace. The exact starting and ending time of sample incubation was recorded. In all cases, data from the first few seconds of incubations were discarded as disturbances to soil caused to CO_2 decomposition to be irregular (Fang and Moncrieff 2001). The average time per measurement was around thirty seconds. After taking the measurements of all thirty samples, the samples were placed back into incubator and the incubator was set to the next temperature.

The respiration measurements generally followed the pattern of first having the cold and warm treatments measured simultaneous for CO_2 respiration. Next, both cold and warm treatment were placed in cold incubator and set to 10°C. Next, the samples were placed back in the incubator and set to the next temperature; this continued until 40°C was reached. Then the samples were incubated from 40° to 10°C in 10° intervals. After the last measurement, the cold samples were placed back into the cold incubator and set to 5°C and the warm samples were placed back into warm incubator at 25°C.

Data Analysis After each incubation experiment, the data set were exported to Microsoft Word where the relevant incubation was separated and sorted in Microsoft Excel. Data kept included the CO_2 concentration measured in terms of parts of CO_2 per million parts of air, the concentration of H_2O in the jar headspace, the volume that a mole of air occupied in the container. To account for changes the ideal gas law of nRT =PV was applied to give the correct volume of the gas occupied in the sample soil container. From these data sets the CO_2 flux mmol per m^3 was calculated.

Next, all data of the slopes was arranged into eight groupings based on location and treatment. The SigmaPlot program was then used to calculate the CO_2 respiration slope and Q_{10} values. The SigmaPlot equation for the Q_{10} model came in the form of S*b ^ (t-t_b/10). The variable S, represented the average initial respiration slope value of the soil sample at 10° C. The coefficient T_b represents the baseline temperature, while t represented the temperature of the incubation cycle. The variable b, is the Q_{10} value. Finally, the influence of temperature and test site location on carbon respiration was analyzed by a two-way ANOVA with five replicates per test site and temperature treatment.

Results

To analyze the results of the incubation data the replicates of each sample location and treatment were grouped and the average Q_{10} values were calculated. From the raw data of the calculated Q_{10} values, it appeared that the Q_{10} values varied considerably from the accepted value of two. The deviation from the accepted value of two was less in the Tonzi samples Figure 2 when compared to the Blodgett samples in Figure 3. Overall, the Blodgett samples showed greater variations in Q_{10} values ranging from as low as .4087 to as high as 3.654. The most drastic change occurred in the Blodgett warm treatment samples. Where the initially sampled the Q_{10} values were 3.206 and 3.623, while the next samplings produced values that plunged to .3572 and 1.4599. The reason for this drastic drop in output will be discussed in the discussion.

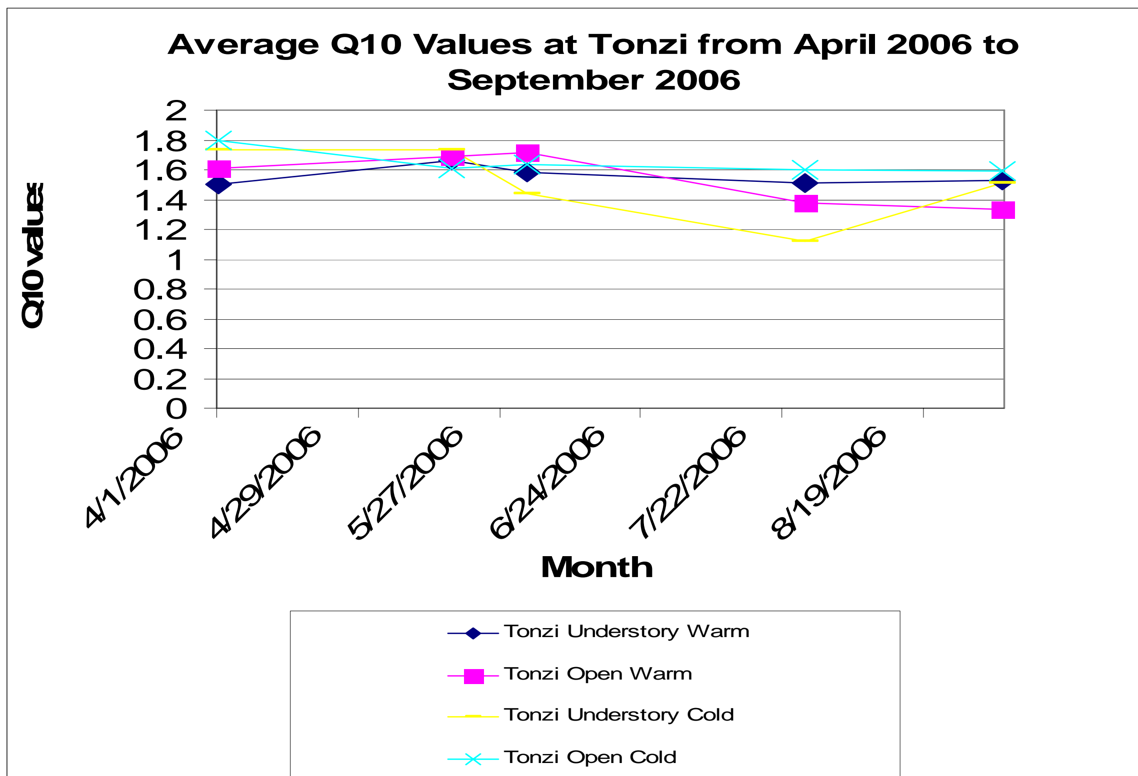

Figure 2: Average Q10 values of Tonzi Samples from April 2006 to September 2007. Note that the two cold treatment samples did not show signs of acclimation as their slopes decreased over the course of the trials. While the two warm treatments showed signs of acclimation as the slope decreased as temperature and time ranges increased.

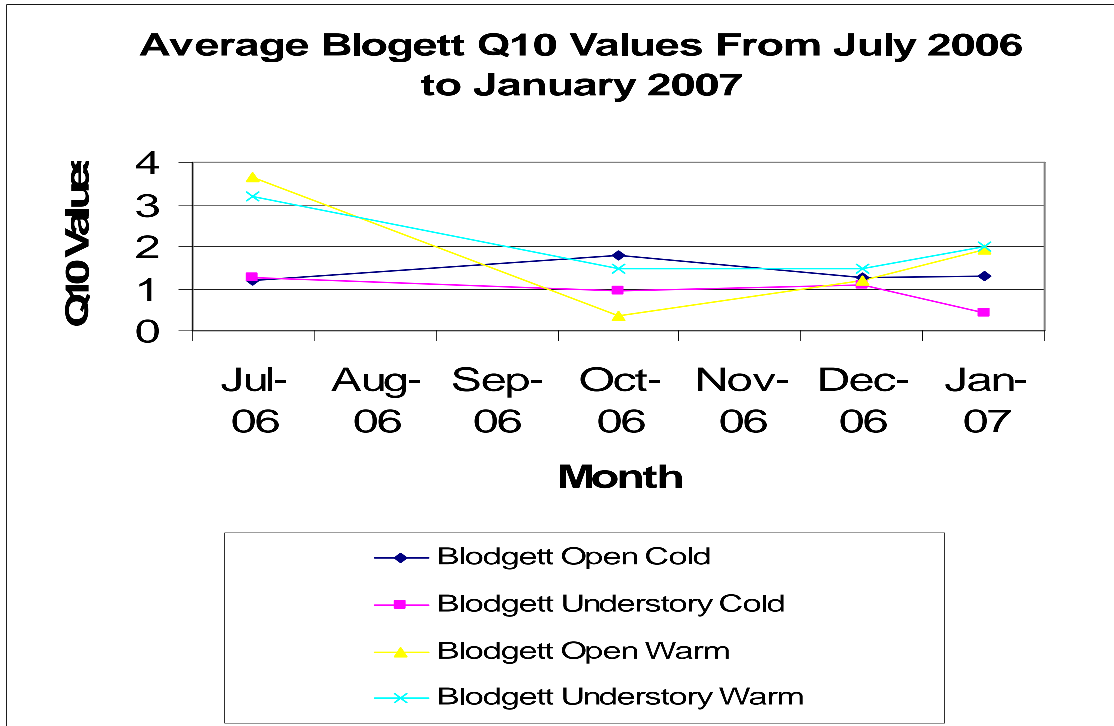

Figure 3: Average Q10 values of Blodgett Samples from July 2006 to January 2007. The warm samples showed a steady progression to a lower Q10 value. While the samples in the cold treatment did not show the expected increase in Q10 values as incubation continued.

Overall, the Tonzi warm treatment soil samples exhibited signs of acclimation as the initially high Q_{10} values declined when the temperature ranges shifted to the range of 30-40°C. The increase in the duration of incubations also produced signs of acclimation. Both the Tonzi open and understory warm samples displayed a steady dip in Q_{10} values as temperatures increased. The Tonzi understory soil types, regardless of temperature condition, decreased to the around the same Q_{10} value of (~1.6). As the Q_{10} values for the cold treatments only increased in the Tonzi open samples. For the other treatments there was only a decline in Q_{10} values. The most extreme case came from the Blodgett understory cold samples as Q_{10} plunged from 1.25 to .4. The precipitous drop is contradictory to what acclimation should have produced. Of all the four cold treatments only in the Blodgett Open cold treatment samples was there evidence of acclimation.

The overall results of CO_2 respiration rates across Tonzi samples were relatively consistent. At the end of the incubation study, all samples had a slight decrease in Q_{10}

when compared to the first measurement taken. The Tonzi open area warm treatment showed the greatest overall decrease from 1.6083 to 1.3319. While the least variation came from the Tonzi understory warm samples, which initially had a Q_{10} of 1.50 and increased to a final Q_{10} value of 1.53. As for the Blodgett warm samples displayed the greatest variance at 1.80. The Blodgett open cold and understory cold both had relatively low standard deviation values of .27 and .36. The sample in which no consistent trend was established occurred with the Blodgett open cold samples.

Table 1: Summary of the ANOVA results of the importance of either location, temperature or a combination of the temperature and location on soil respiration rates.

ANOVA

Source of Variation	*SS*	*df*	*F*	*P-value*	*F crit*
Location	0.917682	3	1.395962	0.349079	2.699393
Temperature	0.664193	1	1.728284	0.191762	3.940163
Interaction	0.881207	3	0.764325	0.516798	2.699393

The results of the ANOVA test for the influence of temperature on Q_{10} came to be (26 samples, p =.19, F critical 3.940) as for location it was (26 samples, p=.34, F critical 2.69). This result suggested that temperature is a larger factor on carbon respiration than the location of the site but neither factors had a significant p level that was lower than .05. So in the end, neither temperature nor location on microbial CO_2 respiration was significant enough of a factor.

Discussion

From the study, the evidence supported the idea that long term increases in global temperatures does impact the carbon respiration rates of microbes. The Tonzi data suggested of a link between increasing global temperatures and massive carbon dioxide flux changes. For the cold treatment samples, there was a steady decline in Q_{10} values as the temperatures increased. Correspondingly, when warm treatment samples underwent higher temperatures the CO_2 respiration rates went down slightly indicating that less SOM was utilized.

In general, Tonzi sampling turned out to give much more normative results, while Blodgett gave incubation respiration readings that were more extreme. Overall, it was expected that Blodgett gave more extreme readings because the soils contained much more organic SOM. Therefore, it would be more inclined to be burn off more recalcitrant soil and release a higher CO_2 reading. The results from the Blodgett samples in the warm treatment were important because it meant that the prolonged temperatures might have induced the microbes to switch enzyme systems to reduce even higher rates of decomposition. This can have important implications since higher temperatures may slow the CO_2 respiration thus helping to conserve SOM for future usage.

Taken as a whole, the results were inconclusive in delineating an exact pattern of how global climate changes may induce changes in global soil respiration rates. Despite, some fluxuations in samples from Blodgett the exhibited Q_{10} values that were within reasonable limits. According to the ANOVA test results and qualitative trends from Figures 2 and 3, the temperature had much more of an impact in determining Q_{10} values then test location. Initially, I believed that the location could have confounded the results of the analysis since different geology and geographical factors could have modified the soil. However, my ANOVA test results disproved that.

For the results of the incubations in Blodgett, one fundamental error that might have confounded the results was the damage to soil microbes caused by a lacking of watering the samples that occurred during October 20th to October 30th 2006. It was during my third incubation round for Blodgett that the CO_2 respiration readout confirmed the severity of the damage, as there was no active CO_2 respiration for the samples above 40°C. When the normal watering schedule was established again much of the Blodgett warm samples had cracks on the surface of the soil core and some even had white fungi mycelium growing on the top surface layer. These errors may have influenced the Q_{10} value and is reflected by the lower average Q_{10} values.

One interesting modification to this experiment would have been the usage of thermocouples. The thermocouples are used to track variations in temperature across the vertical soil core. Since heat takes times to uniformly spread from top to bottom, it is important to keep track of the variations to know when the recalcitrant soil is actually warmed (Davidson 2006) and for what duration. Most of the recalcitrant pockets of soils

are locked up in carbon substrate so it is helpful to know the average exposure time of the soil samples to each temperature range. Another interesting component to include in future studies is the usage of carbohydrate-based polymers or nutrients (nitrogen fertilizers and carbohydrates). Would the extra nutrients serve lead to an overall increase in CO2 respiration or would it lead to an inhibition of the degradation of recalcitrant soil organic matter?

The implications of these results should serve as a warning to most scientists, politicians and citizens of the Earth because it showed that global warming does have an impact in changing the CO_2 respiration of soil microbes. It is still unclear which factor either a change in the soil microbial composition or microbial enzyme usage played a larger factor. In the future, this area still needs to be explored in greater details with more biochemical analysis. Biochemical tests like the analysis of lipoproteins and enzyme detection methods should be used to help determine how the exact mechanism of change occurred. These tests need to be conducted for a more prolonged period of time to see the eventually fate of more remote pockets of recalcitrant soil.

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Appendixes

Table 2: Table with each individually calculated Q10 value per sample and incubation time. The second number in each cell is the standard error.

	4/26/2006	5/03/2006	5/17/2006	6/15/2006	7/25/2006	4/18/2006	9/04/2006
TU1W	1.7521	1.6519 .01	1.8294 .04	1.4312 .01	1.8265 .12	1.8205 .05	1.0170 .056
TU2	1.5399 ,0.	1.7049	1.6743	1.5706	1.3249	1.527	1.238

W		.0 0	.0 8	.0 7	.0 8	.0725	.044
TU3 W	1.4893,.26 7	1.4591 .0 4	1.6388 .0 6	2.1056 .0 4	1.1411 .0 9	1.559 .0394	1.544 .030
TU4 W	1.6574 .0 1	1.5307 .01	1.7939 .0 5	1.5425 .0 0	1.4630 .0 1	2.927 .0669	1.379 .082
TU5 W	1.6391 .0 1	1.6992 .0 2	1.8966 .0 5		1.7586 .0 8	1.477 .055	3.5380.278
TO1 W	1.7485 .0 3	1.7794 .0 2	1.6308 .0 5	1.6368 .0 5	1.2451 .0 8	1.682 .0341	2.85 .053
TO2 W	1.6836 .0 3	1.7100 .0 1	1.8047 .0 0	1.7335 .0 5	1.6941 0. 1	1.40 .0268	1.589 .0391
TO3 W	1.7711 .0 2	1.5788 .0 2	1.6837 .0 2	1.4852 .0 8		1.582 .0312	1.675 .0391
TO4 W	1.7491 .0 2	1.7620 .0 1	1.7169 .0 3	1.4877 .0 3		1.5039 .0 1	2.403 .0552
TO5 W	1.9166 .055	1.6626 .0 1		1.6824 .0 0	2.0758 .1 2		1.405 .093
TU1C	1.3871 .0 2	1.4278 .0 1	1.5023 .0 1	1.4265 .0 3	2.7949 .2 5	1.6952 0. 0	
TU2C	1.3081 .0 0	1.4032 .0 2		1.4957 .0 2	1.7252 .0 3	1.5885 0. 0	
TU3C	1.2762 .0 1	1.3061 .0 0		1.3856 .0 4	1.0108 .0 3	1.6727 .0 2	
TU4C		1.2970 .0 2		1.5743 .0 1	1.1368 .0 1	1.5767.051 5	
TU5C	1.3875 .0 1	1.6161 .0 4		2.2011 .0 7	1.2580 .0 3		
TO1C	1.3395 .0 1	1.3048 .0 4	1.3740 0. 9	2.0025 0. 1		1.7096 .0 3	
TO2C	1.3343 .0 0	1.3334 .0 1		1.7107 .0 4	1.0508 .0 1	1.6258 0. 1	
TO3C	1.3340 .0 1	1.4943 .0 2		1.2838 .0 3	1.2748 .033	1.5843 .0 1	
TO4C	1.3994 .0 2		1.3436 .0 2	1.5544 .0 3	1.2438 .0 7	1.6173 .0 1	
TO5C	1.2940 .7	1.4373 .0	1.3408 .0		1.2257 .0	1.6520 .0	

	2	1	1		7	1	

Table 3: The calculated average Q10 values per treatment type in Tonzi that was used to construct Figure 2

	4/26/06	5/17/06	6/15/06	7/26/06	9/03/06
Tonz Understory Warm	1.5015	1.6652	1.5803	1.5097	1.5313
Tonzi Open Warm	1.6083	1.6929	1.7170	1.3783	1.3319
Tonzi Understory Cold	1.7333	1.7376	1.4407	1.1164	1.5104
Tonzi Open Cold	1.7986	1.6095	1.6364	1.6032	1.5943

Table 4 : A table of the average Q10 values per incubation cycle in Blodgett that was used to construct Figure 3

	7/26/06	10/25/06	12/20/06	1/17/07
Blodgett Open Cold	1.1816	1.7866	1.2718	1.3064
Blodgett Understory Cold	1.2594	.9387	1.1026	.4087
Blodgett Open Warm	3.6543	.3572	1.1834	1.9307
Blodgett Understory Warm	3.2026	1.4599	1.4817	2.0044