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Isolating the effect of mineral-organic interactions on the decomposition of recalcitrant organic soil carbon

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Isolating the effect of mineral-organic interactions on the decomposition of recalcitrant organic soil carbon

by

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Abstract

Isolating the effect of mineral-organic interactions on the decomposition of recalcitrant organic soil carbon

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The University of Texas at Austin, 2012

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Recalcitrant soil carbon is a poorly understood component of total soil organic carbon (SOC). Although the turnover rate of the recalcitrant fraction is slow, warming temperatures are expected to speed the decomposition of recalcitrant SOC resulting in an increase of atmospheric CO_2 in the future. Several studies show that the oldest SOC is associated with the smallest mineral particles (clays), making direct spectroscopic analysis of old carbon difficult. To overcome the difficulty of analyzing natural samples, we created synthetic soils to examine the association between clay surfaces and specific biomolecules based on the hypothesis that clays with higher surface charge will more strongly bond organic molecules, and also that certain molecules will be better stabilized by clay. We used kaolinite, montmorillonite, or quartz (sand) as a synthetic soil inside 12 mL septum-capped vials, added either dissolved glucose or vanillic acid to each mineral, inoculated with soil microbes, and then purged the vials with a CO_2-free atmosphere. We incubated them and measured the concentration and $\delta^{13}C$ of CO_2 that accumulated in the vials. Respiration rates were significantly higher in experiments containing vanillic acid

than in those containing glucose. Respiration rates were lowest in experiments containing montmorillonite. We repeated the experiment using dilute H_2O_2 as an oxidant, and adding vanillic acid, glucose, or glycine. Vials with montmorillonite showed lower rates of CO_2 accumulation than kaolinite, and both glycine- and glucose-containing experiments had less CO_2 than vanillic acid-experiments. We conclude that the montmorillonite protected the organic matter from oxidation better than sand or kaolinite. Both clays protected organic matter better than sand. In all experiments with clay, the respired CO_2 had lower $\delta^{13}C$ values than bulk substrate. This carbon isotope fractionation is likely due to preferential desorption, followed by oxidation, of ^{12}C- as opposed to ^{13}C- bearing organic molecules. The mineral-organic interaction is a strong bond that explains the old age of labile organic compounds in soils. These results indicate that the clay fraction of soils must be considered for accurate prediction of future land-atmosphere carbon fluxes.

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Chapter 1: Introduction

Soils are one of the largest reservoirs of carbon, with estimates of containing greater than 3300 Pg of carbon worldwide, far more than the amount of carbon stored in terrestrial plant biomass (Tarnocai 2009). Soils contribute a significant amount of CO_2 to the atmosphere (Raich and Schlesinger 1992). In our changing climate, understanding soil organic carbon (SOC) is becoming increasingly important for both potential mitigation strategies and projections of future atmospheric CO_2 concentrations. A majority of soil organic carbon is presumed to have a long mean residence time (von Lutzow and Kogel-Knabner, 2009) which mineralizes to inorganic forms very slowly, over centuries and millennia (Schimel 1994). It is not well understood how this carbon, commonly referred to as recalcitrant for its slow cycling, will respond to temperature changes and this uncertainty in land carbon cycle fluxes translates into about ±200 ppm worth of uncertainties in future predictions of CO_2 in the atmosphere (Friedlingstein et al. 2006). Recalcitrant carbon has a long mean residence time, which we know from radiocarbon dating (Trumbore 1997), but we lack a qualitative understanding of the oldest fraction of SOC and how it is stabilized for such long periods. Various mechanisms suggested to be responsible for the stabilization of recalcitrant carbon include selective decomposition by microbes, physical protection through aggregation or hydrophobicity, and interactions with mineral surfaces. However, the protection by mineral-organic interactions is proving to be the most important factor for SOC in the long-term (von Lutzow et al, 2006). In this report I studied the mineral-organic influence on respiration in simple, synthetic soils in order to isolate the stabilization mechanism of soil minerals apart from other mechanisms that play a minor role in stabilization, but complicate analyses of natural soil systems. I hypothesized that clay minerals with higher

surface area and/or higher cation exchange capacity would bond organic matter more strongly, and result in lower respiration, and that the chemical stability of a particular compound would not have a large effect on respiration rates, except in how easily that compound would bond with the mineral surface.

Chapter 2: Background

Recalcitrant carbon has many different definitions in the literature, but is commonly defined as carbon that is chemically resistant to decomposition (Sollins et al, 1996). Conventional thought is that the bulk of old soil carbon becomes chemically recalcitrant through the humification process, which theoretically creates more stable molecules by degradation of labile plant inputs to soil. However, analysis of the humification process has so far resulted in little evidence that chemically recalcitrant compounds are actually being created from labile starting molecules (Burdon 2001, Kelleher and Simpson 2006, Lehmann 2008). This historical view of how SOC becomes recalcitrant is increasingly questioned, with a growing body of literature suggesting other mechanisms of stabilization at work that are not yet well understood (Hedges and Oades 1997, Mayer and Xing 2001, Czimczik and Masiello 2007, Trumbore 2009, Kleber et al 2011).

The theoretical intrinsic lability or stability of organic compounds is based primarily on the activation energy required for oxidation. Thus carbohydrates, which have a low activation energy to oxidation, are assumed to be labile, while aromatic rings, with a high activation energy, are assumed to be stable (Davidson and Janssens 2006). The activation energy required for oxidation of an organic compound is decreased when energy in the form of heat is added to the system. Using this definition for recalcitrant SOC, current soil carbon models predict a high sensitivity of recalcitrant soil carbon to increasing temperatures (Davidson and Janssens 2006, Bosatta and Agren 1999). But this understanding assumes that the limiting factor in SOC oxidation is temperature, and does not account for microbial preferences or mineral protections. While this higher

temperature sensitivity is corroborated by controlled laboratory soil incubations, field studies show a more minor temperature sensitivity of recalcitrant carbon (Wixon 2009). A mechanistic understanding of this recalcitrant carbon that can explain field observations and laboratory soil incubations is vital to the accuracy of models predicting future soil carbon fluxes. The aim of this report was to isolate the mineral-organic interaction and its effect on respiration by creating simplistic, synthetic soils so that other decomposition-controlling mechanisms could be minimized.

Another issue with the theory of inherently chemically stable molecules is the characteristic increase in $\delta^{13}C$ values of SOC with soil depth and SOC age (e.g. Beckerheidmann and Scharpenseel 1989, Krull 2003, Wynn 2006). Lipids and lignins, which are more thermodynamically stable, have relatively low $\delta^{13}C$ values compared to bulk plant material while cellulose and other carbohydrates which are more degradable have relatively high $\delta^{13}C$ values (Benner 1987, Krull 2003). The increase of $\delta^{13}C$ values of SOC with depth then could potentially be explained by a dominance of lipids and lignins at the soil surface, and of carbohydrates at depth (Huang 1996, Kramer 2003). However, when continuing to look at the soil depth profile, the radiocarbon age of soil organic carbon also increases with depth (Trumbore 1997), and these two trends together have been suggested as evidence that as the age of SOC increases, the relative contribution ratio of labile/stable compounds increases, contradicting the theory that recalcitrant SOC is more stable because of inherent chemical stability of the carbon compounds (Sollins 2006).

In addition to an increase of SOC age with depth, mineral-associated SOC tends to be older than bulk SOC (Wattel-Koekkek 2003, Kleber 2005, Sollins 2006, Kleber 2011). Analyses of various alumino-silicate mineral surfaces have a relatively old radiocarbon age (Rasmussen 2005, Sollins 2006, Kleber 2011) but also consist of

relatively more labile compounds (Rasmussen 2005, Buurman 2007, Grasset 2009, Kleber 2011) suggesting that the mineral association is probably the most significant source of protection from oxidation. In relation to bulk SOC, carbon associated with mineral surfaces has been found to be both depleted in chemically stable aromatic structures such as lignin and phenols (Guggenberger 1994, Kiem and Kogel-Knabner 2003, Grandy and Neff 2008, Kogel-Knabner 2008, Marschner 2008, Kleber 2011) and enriched in chemically labile O-alkyl and carboxyl structures (Baldock 1992, Mahieu 1999, Spielvogel 2008, Kleber 2011). However the mechanism by which minerals protect organic matter is not well understood and organic matter that is associated with mineral surfaces is difficult to characterize (Flessa 2008), making the study of old organic matter and how it will react to changing temperatures difficult.

Bruun et al. (2010) found that clay content of soils had no correlation with carbon stabilization, but mineralogy did have a significant effect, so clay type is likely an important way to predict recalcitrant carbon stabilization. High Fe and Al contents were found to be highly correlated with stable soil organic carbon (Hobbie 2007, Bruun 2010) as well as with a microbial biomass decrease (Hobbie 2007) which could indicate that minerals containing these elements are the stabilizing mechanism of clays or that the cations drive down pH, reducing microbial activity at those organic matter sites and rendering the C effectively recalcitrant. However, a different study found an inverse relationship between iron content and organic carbon and instead showed a high correlation between surface area and cation exchange capacity with organic carbon coatings, which were discontinuous on the mineral surface (Kahle 2003, Kahle 2004). This discontinuous coverage of organic matter on the mineral surface indicates a high amount of surface specificity for specific biomolecules rather than random sorption (Kahle 2004, Lehmann 2010). In a study which added increasing amounts of organic

carbon to soils, specific surface area decreased with added organic carbon, yet at the highest loadings still had about 59-85% of its available surface area free, (Kaiser and Guggenburger 2003) indicating highly specific site preference of organic matter sorption.

These pieces of evidence gave way to the 'onion layer' theory (Kleber 2007), which is a favored explanation for how organic molecules become so strongly bonded to clay surfaces. The onion layer theory postulates that particular organic molecules bond strongly to the mineral surface, and these molecules in turn form the anchor for successive layers of organic material (Kleber 2007). Analysis of the clay fraction in some natural soils has shown the relatively quick incorporation of new organic matter through both a 14C bomb tracer analysis (Swanston 2005) and a 15N labeled tracer (Strickland 1992) despite the otherwise old radiocarbon dates of mineral-associated carbon, which seems to fit with the onion layer theory as the upper layers of the organo-mineral complex would cycle faster than the more strongly bonded, better protected bottom layer.

The difficulty in qualitative observations of mineral associated SOC alongside the apparent coordination between old carbon, chemically labile carbon, and mineral-associated carbon provides the basis of this study, which led us to examine the respiration of recalcitrant SOC by synthesizing organo-mineral complexes in the laboratory and then oxidizing the organic carbon, rather than attempting to measure in situ mineral-organic interactions. Plant and soil processes result in such a wide array of biomolecules and molecular interactions that observations of these systems are widely differing. This complexity means that isolating stabilization mechanisms in field samples is incredibly difficult, if not impossible, as the huge volume of literature with differing conclusions studying this phenomenon can attest to. Simplifying the mineral-organic complexes will help to shed light on what is happening at the mineral surface with organic compounds.

Chapter 3: Methods

In order to isolate the mineral-organic interaction, I made simple synthetic mineral-organic complexes and incubated them under controlled conditions. I used a kaolinite and a montmorillonite (Ward's Scientific), and a quartz sand sieved to 174 μm in order to have a wide range of surface area and charge values for study (the sieved quartz acts as a control as minimal surface bonding and therefore minimum protection is expected). Approximate values for cation exchange capacity and surface area are listed in Table 1. The starting materials (clays and quartz sand) were found to have negligible amounts of calcium carbonate and organic carbon. I used a 37% HCl solution added to the clays under microscope to watch for reaction with calcium carbonate and was not able to detect anything during the reaction of kaolinite, montmorillonite or sand. To test

Table 1	Chemical Composition	Average External Surface Area (m^2/g)	Cation Exchange Capacity ($cmol_c/kg$)
Quartz Sand	SiO_2	0.1	N/A
Kaolinite	$Al_2Si_2O_5(OH)_4$	5-30	3-15
Montmorillonite	$(Na,Ca)_{0.33}(Al,Mg)_2(Si_4O_{10})(OH)_2$	80-150, *550-650	70-120

Table 1. Approximate characterization values for the minerals used in this report. (Brady and Weil 2002, Bergaya et al 2006). *Internal surface area.

organic carbon content, I weighed untreated clays into previously baked glass tubes, added 250mg of pre-baked copper oxide, sealed the tubes under vacuum and combusted for 24 hours at 500°C. Then I analyzed the gas inside the tubes for CO_2 concentration and carbon isotope composition using a GasBench II coupled to a Thermo Electron 253

operating in continuous flow mode (Fry et al 1996). The amount of organic carbon removed from the surface of the clays via combustion is negligible.

I weighed 1.0 g of quartz, 0.5g of kaolinite or 0.1g of montmorillonite into 12 mL glass exetainer® vials – the different masses are used to minimize the differences in surface area. To sorb specific organic carbon compounds to the mineral surfaces, I dissolved either vanillic acid, glycine, or glucose in 18 M ohm DI water, resulting in a solution approximately 6.2×10^{-5} moles carbon per 1mL of water. These substrates were chosen to distinguish chemical recalcitrance of the molecule from stabilization by the mineral. Vanillic acid can be presumed to be the most chemically stable of these three compounds, due to its aromatic ring. It is a commonly occurring organic acid and can be thought of as an analog for lignin (a lignin monomer). Glycine was chosen to represent proteins and glucose to represent carbohydrates, both of which are presumably more labile than lignin. Proteins may be a particularly important part of the mineral stabilization of organic compounds, binding to the mineral surface and in turn providing a binding site to other organic compounds (Kleber 2007). Although glycine is simple and not a complete protein, it is a good choice as an analog for proteins because the amine and carboxyl groups are likely the key players in surface bonding, and the simple side chain on glycine removes any potentially complicating interactions from the more complex side chains on other amino acids. 10mL of vanillic acid, glycine, or glucose solution were added to each mineral-containing vial, allowed to equilibrate overnight, and then centrifuged and decanted. Since quartz sand in non-absorptive, rather than adding an organic solution, I added 6.2×10^{-4} moles dry organic carbon (1.10 mg vanillic acid, 1.57 mg glucose, and 1.96 mg glycine) to each vial and then wetted each vial with 18 M ohm DI water.

3.1 WET SORPTION

To ensure that all of the carbon in solution was in fact adsorbed onto the surface of the clays (or else encased in clay colloids) I used two methods. After centrifuging each clay-organic vial and decanting the liquid, I used a UV spectrophotometer to measure the amount of solute still in solution after reaction with clay, using the assumption that lower light absorbance in the supernatant would equate to greater adsorption by the clay of the dissolved substrate. Because this method was difficult to use for clay-glucose and clay-glycine supernatants, and might have been complicated by clay particles that stayed suspended in solution in clay-vanillic acid supernatants, I used a second method to double check my assumption. I decanted the supernatant then filled Thermo Electron 3x5 mm pressed silver capsules with the liquid and evaporated the water, precipitating any organic matter still in solution. I then placed each silver capsule inside individual pre-baked glass tubes, added 250mg copper oxide wire, and sealed each tube under vacuum. These tubes are baked for 24 hours at 500°C, and the combusted organic matter is then analyzed as CO_2 for concentration and isotopic carbon composition via a GasBench II coupled to a Thermo Electron 253 operating in continuous flow mode (Fry et al 1996). These two methods together ensured that mineral sorption of the organic substrates was occurring during the overnight reaction of minerals and organic molecule solutions, and by analyzing the CO_2 produced from the organic substrate in the solution I was able to determine the $\delta^{13}C$ value of the organic carbon still in solution, and then calculate the $\delta^{13}C$ value of the organic carbon sorbed to the clay in each vial before incubation.

3.2 RESPIRATION

I then capped each vial of mineral-organic mixture with a septum cap and performed two different sets of incubation experiments. Headspace CO_2 produced during incubation was transferred by syringe to UHP He-flushed exetainer® vials. The

concentration and stable carbon isotope composition of the CO_2 were measured using a GasBench II coupled to a Thermo Electron 253 operating in continuous flow mode.

The first incubation experiments were designed to investigate the protection of OM from microbial respiration. Each vial was purged with CO_2 free air, and the clay-organic complexes were then inoculated with 0.05 mL of soil-equilibrated water in order to introduce soil microbes. To obtain soil-equilibrated water, I took a small sample of soil and mixed in water at a 1:5 ratio. Then after 4 hours the mineral soil settled out of solution and the water remaining was used to inoculate the vials. Each vial was then incubated at 26˚C with small aliquots of the headspace gas being sampled periodically for changes in CO_2 levels. I experimented with several different incubation periods, incubating for up to 16 days with gas samples taken at 4, 6 and 16 days, and over the shorter range of 24 hours with samples taken approximately every four hours. In these experiments, only glucose and vanillic acid compounds were added to the long term experiments, while vanillic acid, glucose and glycine are used in the short-term experiment, as described above.

3.3 OXIDATION

The second experiment was designed to abiotically oxidize organic matter on the clay surface, which avoids potential complications of changes in microbial community during incubation, i.e. selective pressures in the synthetic environment such as pH effects, absence of nutrients, microbial metal toxicity, etc. In these experiments, the headspace in each prepared (as above) mineral-organic vial was purged with UHP He and 0.1 mL of 10% H_2O_2 (fewer moles of H_2O_2 than would be needed to oxidize all of the carbon adsorbed to the clay surface) was injected into each vial. H_2O_2 is intended as an analog for oxidative enzymes secreted by microorganisms. The vials were then mixed

continuously using a Scientific Equipment Products Tube Rotator for at least 2 days before sampling headspace gas. In these experiments glucose, vanillic acid, and glycine are used as substrates, as described above. The headspace gas in each vial was transferred by syringe to UHP He-flushed exetainer® vials. The concentration and stable carbon isotope composition of the CO_2 were measured using a GasBench II coupled to a Thermo Electron 253 operating in continuous flow mode.

To account for respired CO_2 that was dissolved in water inside the vials, each value is corrected based on the pH and volume of water inside the vial. pH was measured using a AR25 Dual Channel pH/Ion Meter. CO_2 contents used in this report are a reflection of headspace CO_2 as well as dissolved inorganic species of carbon, giving total organic carbon oxidized during the incubation. The correction is substantial for montmorillonite experiments and is small for other experiments. The $\delta^{13}C$ values of respired CO_2 are also corrected based on reported fractionation factors (Deines et al 1974, Mook et al 1974, Clark and Fritz 1997). Results are reported as a ratio of moles CO_2 respired to grams of carbon in the vial initially.

3.4 DRY SORPTION

Another complication that I considered was the adsorption of CO_2 to clay surfaces after it had been produced. To test for this, and whether it was a significant problem for my analysis, I filled 12 mL glass exetainer® vials with 1.0 g of dry quartz, 0.5g of dry kaolinite or 0.1g of dry montmorillonite and closed with septum caps. For this experiment, I also had vials with no mineral as controls. I then purged the vials with UHP He, baked them in the oven at 60°C for 6 hours, and purged with UHP He again. Then vials were injected with 5 mL of 5000 ppm CO_2–in-air. Headspace gas was sampled after 24 hours and the concentration and stable carbon isotope composition of the CO_2 were

measured using a GasBench II coupled to a Thermo Electron 253 operating in continuous flow mode.

Chapter 4: Results and Discussion

4.1 Wet Sorption

Incomplete sorption of the organic substrate in solution onto the clay minerals resulted in an isotope fractionation, depicted in Figure 1. The glucose left in solution after reaction with clay is lighter than the starting value, meaning that the glucose sorbed by clay must have had a higher $\delta^{13}C$ value of approximately -6.5‰ on kaolinite-glucose and -7.5‰ on montmorillonite-glucose. This helps to explain the results shown below, in which the respired/oxidized CO_2 is has higher $\delta^{13}C$ values than the starting substrate. Although I do not have exact values for vanillic acid and glycine sorption, I assume that similar fractionations will occur resulting in a higher $\delta^{13}C$ value for the beginning substrate in the vial and continue the discussion of the results from that perspective.

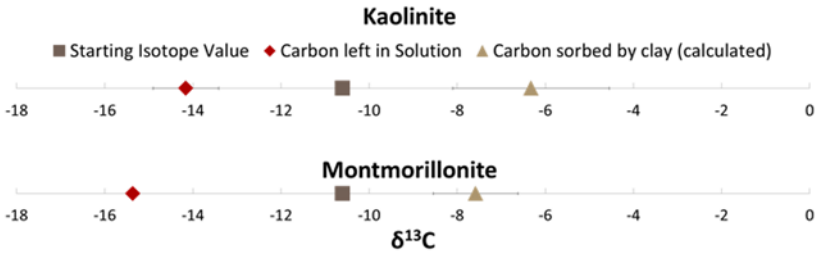

Figure 1. Wet sorption of glucose solution. The square is the starting $\delta^{13}C$ value of glucose, while the diamond is the measured value of DOC left in solution after reaction with clay. The triangle is the calculated value of glucose that must have been sorbed by the clay.

4.2 Respiration

Results of the soil microbe respiration experiments are shown in Figure 2. After four days sand-vanillic acid and kaolinite-vanillic acid vials had the greatest build-up of

CO_2, with less in the montmorillonite-vanillic acid vials (Figure 2C). These results agreed with the stated hypothesis that minerals with greater surface charge provide stronger

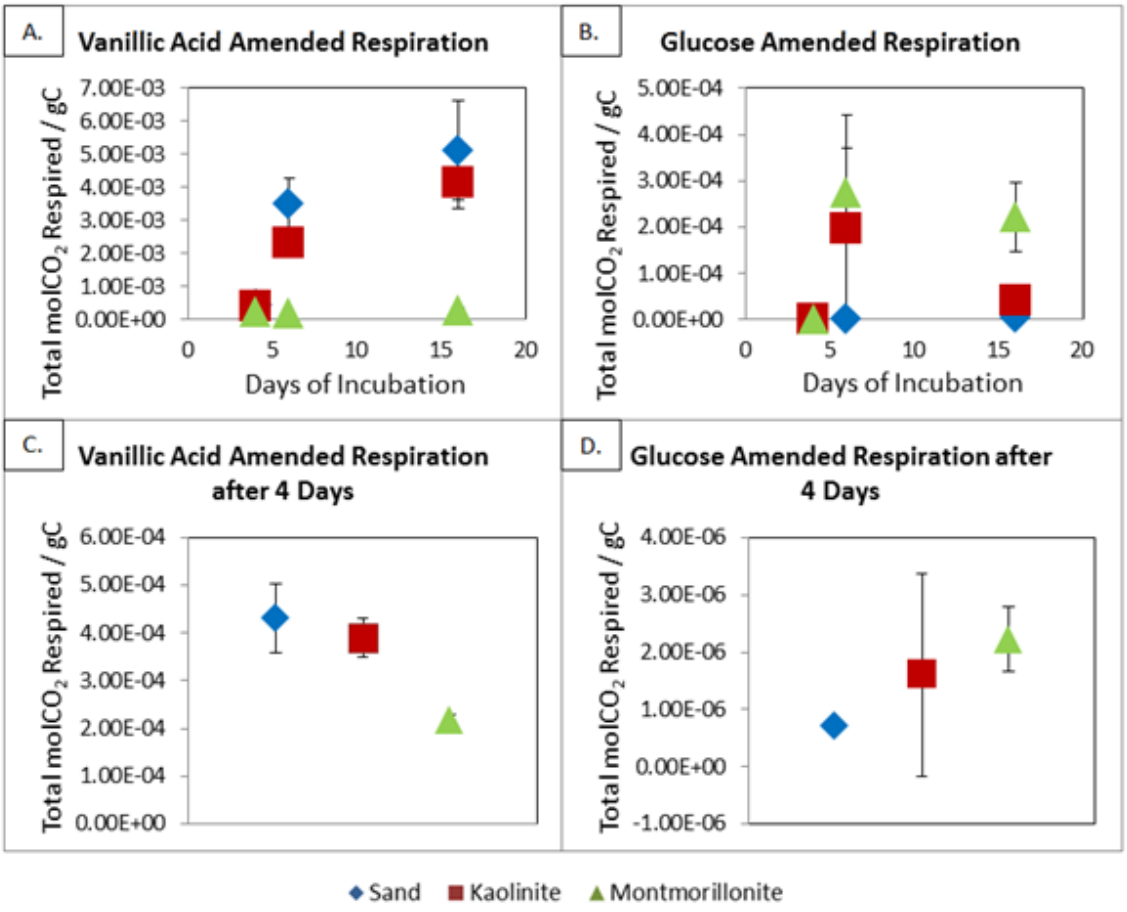

Figure 2. A-D show the results from the long term incubation experiments with soil microbe inoculations. A and B are incubations that were sampled over a period of 16 days, with headspace gas samplings taken at 4, 6 and 16 days. Total respired CO_2 increased with time for both the vanillic acid and glucose, but the relative rates among different minerals were opposite for vanillic acid and glucose.

protection, and suggest that montmorillonite offered the greatest protection of vanillic acid through sorption properties. However, in the glucose amended incubations (Figure 2D) the trend was opposite, with montmorillonite-glucose vials producing the greatest

amounts of CO_2 while sand-glucose vials had a more depressed respiration during the first four days (kaolinite-glucose was not statistically different from either sand-glucose or montmorillonite-glucose, but sand-glucose versus montmorillonite-glucose had a p=.001 value). As the length of incubation increased, the trends for CO_2 in both vanillic acid and glucose amended vials continued, with montmorillonite-glucose vials showing greater respiration than sand-glucose vials. The sand-vanillic acid vials showed a much greater accumulation of CO_2 in the headspace than montmorillonite-vanillic acid vials (Figure 2A,B). At all sampling points, respiration rates were significantly higher ($p<0.001$), by at least an order of magnitude in vials with vanillic acid than vials with glucose (except in the case of montmorillonite-organic vials at 6 and 16 days at which CO_2 respiration gC^{-1} was not statistically different between vanillic acid or glucose amendements).

The unexpected results from glucose-amended trials, which displayed montmorillonite as being less protective than sand, could have a variety of explanations. First, the glucose is being sorbed by the clay, resulting in stabilization (respiration is lower for glucose-amended experiments than for vanillic acid-amended experiments) but the quartz sand is inhibiting the microbial metabolism in some other way, perhaps because of the lower surface area which could provide less area for microbial interactions, or the way in which quartz sand vials had to be treated differently than clay i.e., adding solid organic substrate rather than dissolved organic substrate. The glucose is made of sizeable crystals while vanillic acid is a very fine powder, so the glucose may not have dissolved sufficiently in sand vials to become completely accessible to microbes. CO_2 respiration gC^{-1} in kaolinite-glucose and montmorillonite-glucose vials, where the glucose was dissolved and then sorbed, was not statistically different at any point during the glucose experiments, but was statistically smaller than kaolinite-vanillic

acid and montmorillonite-vanillic acid vials. So while sand-glucose vials did not have accessible glucose and so had inaccurate results, altogether the results suggest that vanillic acid was less protected than glucose, and that clay minerals protected organic matter from respiration better than sand, as seen in the vanillic acid experiments.

A second possible explanation for the low respiration in sand-glucose vials is that as the microbial population starts consuming organic matter, eventually it does begin to die and recycle itself. Since the only nourishment provided to the microbes is carbon compounds, any other nutrients, such as nitrogen and potassium, may become limiting and have to come from either the microbial population itself as it recycles, or from the crystal structure of the clays and quartz. The clays could be providing nitrogen from the crystal structure if microbes are able to degrade it, while sand would have no nutrients, being composed of SiO_2. Thus clay minerals could have a minimized nutrient limitation in the respiration experiments, while microbes in vials with sand would be severely nutrient limited. This explanation is not favored because clay-vanillic acid and sand-vanillic acid treatments did not show trends of CO_2 respired gC^{-1} similar to the trends observed in clay-glucose and sand-glucose treatments.

Vanillic acid is a more stable compound than glucose, yet in these incubation trials soil microbes consistently produced more CO_2 from vanillic acid than from glucose, calling into question the assumption that the aromatic structure of vanillic acid slows its degradability. However, a third problem with these results is that the short life-span of some microbes, particularly in a limiting environment, means that at some point microbes start dying and recycling their own bodies, resulting in CO_2 production that is not solely from the carbon compounds I added to the vials. This complication led me to run the experiment over a shorter time-scale to attempt to limit this influence on the respiration data.

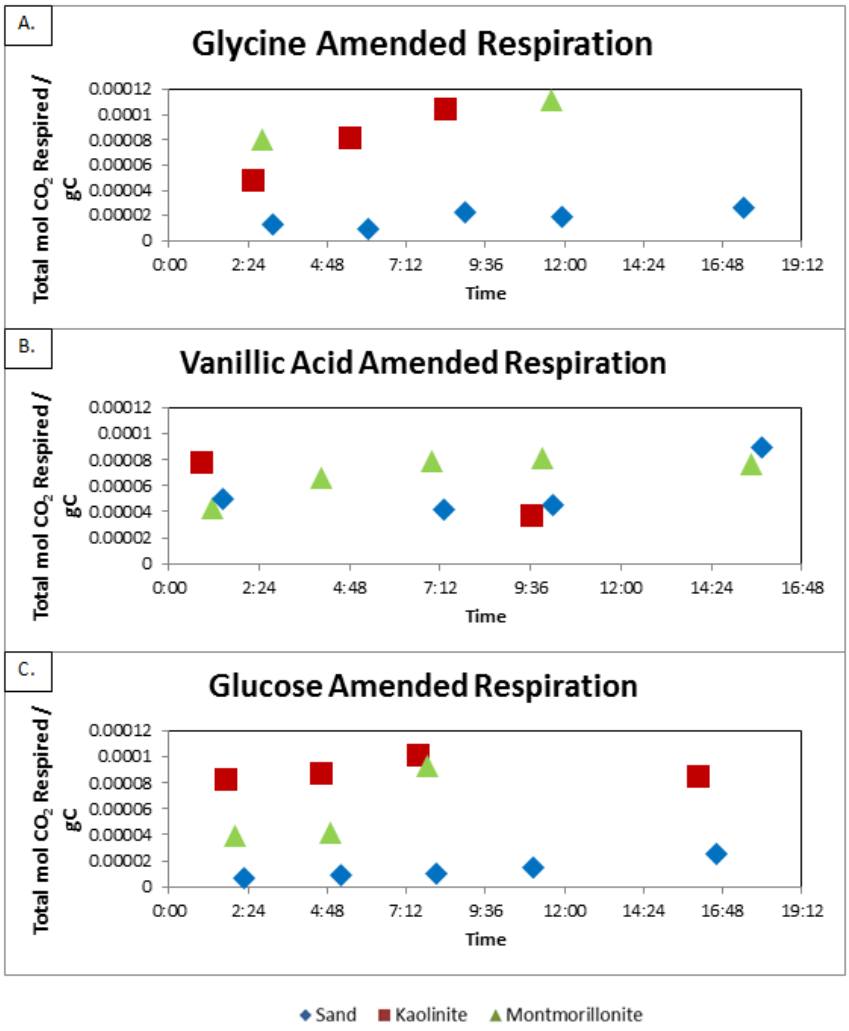

Figure 3. A-C are CO_2 measurements taken over the first 24 hours of incubation. Several samplings were unexpectedly off-scale and each time measurement only has one data point, but overall there is a consistent increase in CO_2 over the 24 hour period in each treatment.

Figure 3 has the results from incubations carried out over a 24 hour period. During the first 24 hours there is a steady build-up of CO_2 in the headspace and, contrary to the original hypothesis, those vials with quartz sand as the mineral had the least

amount of CO_2 for each organic substrate (Figure 3). Glycine and glucose amended vials had very similar trends of CO_2 respired gC^{-1}. The sand-glycine vial has the lowest CO_2 respiration gC^{-1} with a very slight increase over time, then the montmorillonite-glycine vial starts at about double the CO_2 respiration gC^{-1} at the first data point while kaolinite-glycine has about double the CO_2 respiration gC^{-1} in the montmorillonite-glycine vial. The sand-glucose vials also have the lowest CO_2 respiration gC^{-1}, then montmorillonite-glucose has approximately double, followed by kaolinite-glucose with the highest moles CO_2 respired gC^{-1}. The results from vials amended with vanillic acid don't show a clear trend, and the moles CO_2 respired gC^{-1} are very similar among the mineral treatments. However, looking at respiration values between substrates, vanillic acid amended vials do show higher CO_2 respiration gC^{-1} than in glucose amended vials at most data points, and higher CO_2 respiration gC^{-1} than in sand-glycine vials as well as at least the first data point from the kaolinite-glycine vial. These results support the conclusion drawn from the longer incubations that chemical composition of OM is less important for stabilization than previously thought. However, in both glucose and glycine amended incubations, sand vials had the lowest CO_2 respiration gC^{-1} which is contrary to the hypothesis, but this trend is also seen in the longer incubations with glucose, so the same explanations as above may apply. It is important to note though that although there are not enough data points from clay vials amended with either glucose or glycine to draw firm conclusions, it does seem as if kaolinite may have slightly higher CO_2 respiration gC^{-1} than montmorillonite when amended with either glucose or glycine, supporting the hypothesis that clays with higher surface charge protect OM better.

Table 2	$\delta^{13}C$ (‰ vs VPDB)
Glucose	-11
Glycine	-40
Vanillic Acid	-28

Table 2. $\delta^{13}C$ values for the substrates used in this report.

The isotope results from the incubations are less clear. The starting $\delta^{13}C$ value of the carbon in the glucose used is approximately -11‰ whereas the value of the carbon in vanillic acid is -28‰ (Table 2). However, as discussed in section 4.1, incomplete sorption resulted in clays preferentially sorbing ^{13}C-glucose, so the starting glucose isotopic value is heavier, closer to -6.5‰ for kaolinite-glucose and -7.5‰ for montmorillonite-glucose. Although I do not have exact values for vanillic acid or glycine fractionations, I assume that fractionations would also occur during sorption of these substrates, resulting in heavier starting $\delta^{13}C$ substrate values than reported in Table 2, and the rest of the discussion will be considered in light of this assumption. Previous incubation experiments have shown that microbes respire CO_2 with lower $\delta^{13}C$ values than bulk substrate (e.g. Blair et al 1985). Although in each vial with vanillic acid the CO_2 measured is heavier than the starting isotopic value of the bulk vanillic acid, it is most likely lighter than actual sorbed vanillic acid in the vials before incubation. The trend does hold that respired CO_2 in montmorillonite incubations had the lowest $\delta^{13}C$ value and respired CO_2 in sand incubations had the highest $\delta^{13}C$ value (Figure 4). Alternatively, these unexpected results could suggest some weird isotope effects as they indicate that heavy isotopes of carbon are more readily desorbed from the clay surface

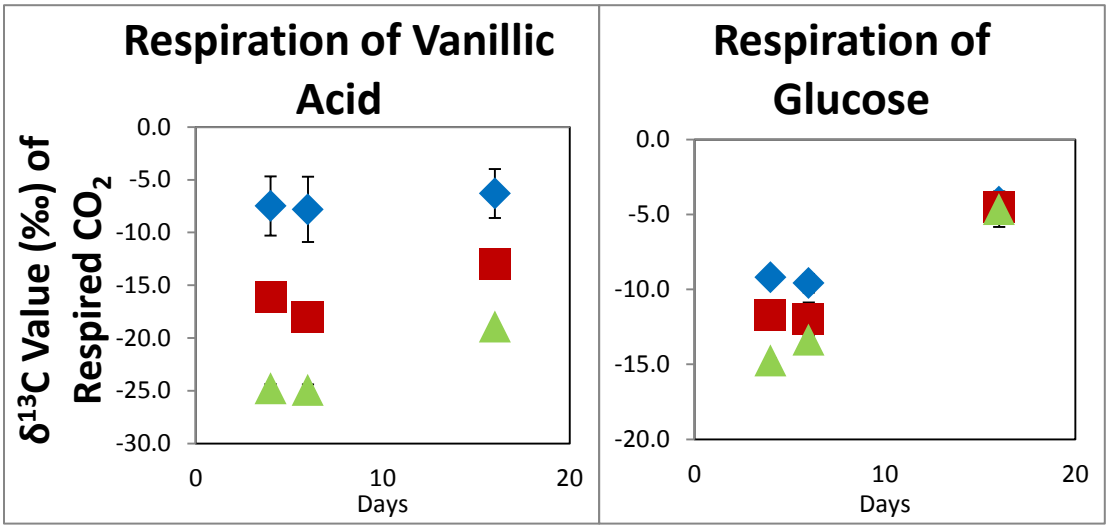

Figure 4. Isotopic values of measured CO_2 in long-term respiration incubations.

and available for respiration whereas the light isotope of carbon is held stable. This is unexpected since heavy carbon should make a surface bond with a higher activation energy. As the $\delta^{13}C$ value of the CO_2 decreases with increasing surface area and charge, these results suggest a greater amount of protection in montmorillonite than in sand, as expected. In the glucose trials the contrast between clay and sand is less stark but consistent, showing a greater protection of heavy isotopes of carbon on the clay surface. The idea of a carbon isotope fractionation during sorption of organic matter has not previously been tested (to my knowledge) but preliminary results (discussed in section 4.1) show that preferential sorption of heavy isotopes by clay minerals does occur.

The $\delta^{13}C$ values of the CO_2 in the 24 hour experiment are shown in Figure 5. The biggest fractionation between substrate and CO_2 occurs in the mineral-glycine experiments – the glycine used has an approximate $\delta^{13}C$ value of -40‰ and the lightest

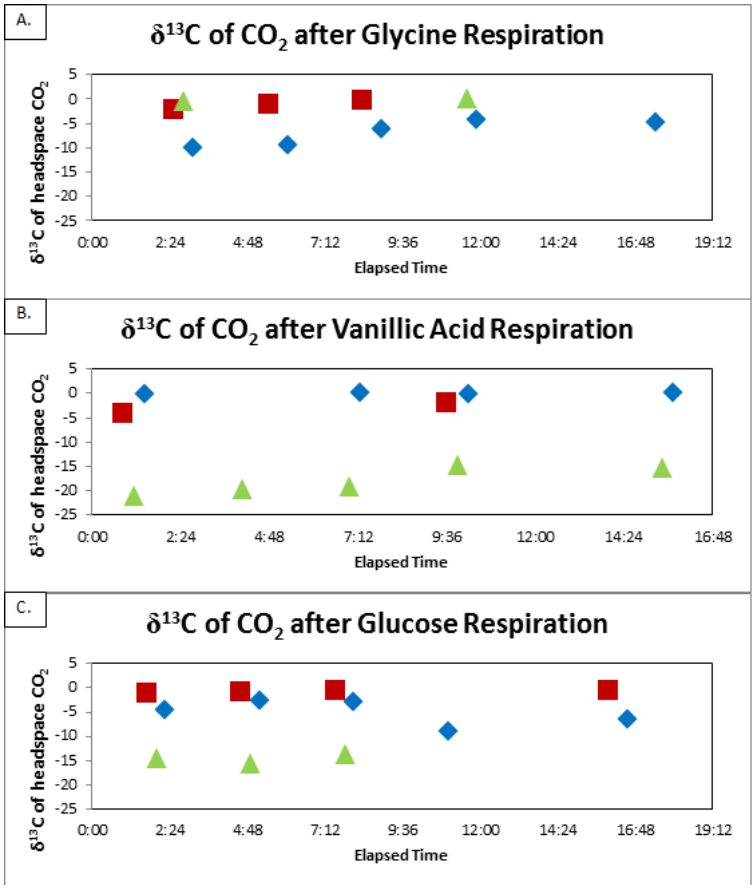

Figure 5. Stable carbon isotope ratios of headspace CO_2 in short-term respiration incubations. The green triangles represent vials with montmorillonite, the red squares represent vials with kaolinite, and the blue diamonds represent vials with quartz sand. Each point represents a single measurement so statistical significance could not be tested, but trends within data sets over time show a fairly consistent increase in the $\delta^{13}C$ value of CO_2. Because of this, I think it is reasonable to report these observations that show increasingly heavier CO_2 across all minerals and substrates used.

CO_2 measured is approximately -9‰ for sand-glycine vials. However, again I am making the assumption that a fractionation would occur during clay sorption of glycine, as occurred during sorption of glucose, resulting in a heavier starting glycine $\delta^{13}C$ value than bulk glycine reported in Table 2. CO_2 produced in sand-glycine vials has the lowest $\delta^{13}C$ values, although increasing over time, while CO_2 in kaolinite-glycine and

montmorillonite-glycine vials has larger, very similar $\delta^{13}C$ values. All of the plots have a general trend of increasingly heavier CO_2 over the period of incubation, with the exception of mineral-glucose vials which had fairly consistent values throughout the measurement period of approximately -15‰ for CO_2 in montmorillonite-glucose vials, -9‰ for CO_2 in sand-glucose vials, and -2‰ for CO_2 in kaolinite-glucose vials. Montmorillonite-glucose vials are the only ones over the range of samples taken during the 24 hour period that produced CO_2 that was lighter than the starting substrate, which is what is expected if microbial processes are controlling decomposition. Both montmorillonite-glucose and montmorillonite-vanillic acid vials have lighter CO_2 than either sand- and kaolinite-glucose or sand- and kaolinite-vanillic acid, although again both sand- and kaonlinite- treatments have CO_2 with indistinguishable $\delta^{13}C$ values from each other. Even though these values are still heavier than the beginning substrates, as discussed above this may potentially be explained by fractionations that are happening during the original creation of the mineral-organic complexes, and so the fact that the most sorptive clay is comparatively accumulating more $^{12}CO_2$ in its headspace indicates preferential protection of heavy carbon.

To test whether microbial respiration of glucose, glycine, and vanillic acid was responsible for the CO_2 accumulation found, vials with no substrate were incubated after inoculating with soil water as in the amended vials, and sterile treatments with no substrate and with no inoculation were also incubated. The results are shown in Figure 6. The respirations rates in these control experiments are several orders of magnitude lower than the respirations in glucose and vanillic acid amended incubations, indicating that the source of CO_2 in amended trials (Figures 2-5) is the organic substrate that was added.

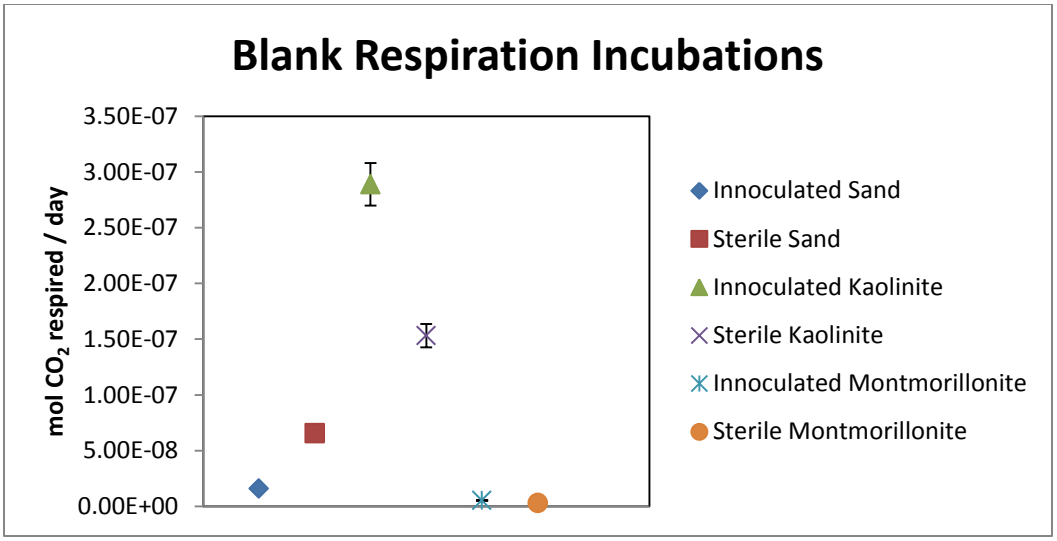

Figure 6. CO_2 accumulation measured in vials with no added organic substrate that were either inoculated or kept sterile. The headspace was purged with helium and then the vial incubated and measured for CO_2 after 4 days.

4.2 Oxidation

The CO_2 respiration gC^{-1} results from experiments with hydrogen peroxide as an oxidant are shown in Figure 7. When comparing vials amended with similar amounts of vanillic acid and H_2O_2, more CO_2 was produced per gram of carbon added to each vial in experiments with kaolinite-vanillic acid than experiments with montmorillonite-vanillic acid, suggesting that montmorillonite is better able to protect vanillic acid, whether through inner layer or surface adsorption. The bottom panel of Figure 7 shows a comparison of the accumulated CO_2 in vials with montmorillonite-vanillic acid, montmorillonite-glycine, or montmorillonite-glucose. Montmorillonite-vanillic acid vials had the highest accumulation of CO_2 while montmorillonite-glycine had the lowest accumulation of CO_2. This indicates that even when using a chemical oxidant, the same bonding principles are controlling oxidation as in the incubation experiments. Vanillic acid is not as well protected as glycine or glucose.

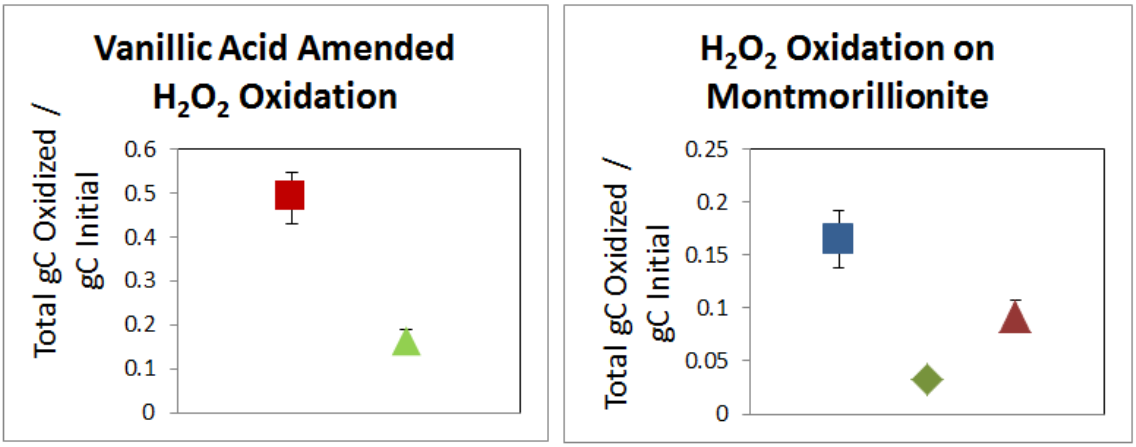

Figure 7. The left graph is a comparison of the oxidation potential of vanillic acid on kaolinite (red square) and montmorillonite (green triangle). The right graph is a comparison of the oxidation potential on montmorillonite of different substrates used: vanillic acid (blue square), glycine (green diamond), and glucose (red triangle).

The carbon isotope results obtained in this experiment (Figure 8) are slightly more confusing. Vanillic acid amended vials show accumulated CO_2 that is lighter than the bulk vanillic acid $\delta^{13}C$ value, and the headspace of montmorillonite-vanillic acid has light CO_2 when compared to CO_2 in kaolinite-vanillic acid vials. CO_2 from glycine amended vials had the most dramatic isotope results, with kaolinite-glycine vials producing heavier CO_2 while the CO_2 in montmorillonite-glycine vials is about 20‰ lighter than the beginning substrate. As explained above, the kaolinite glycine results can potentially be explained by a fractionation that occurs during initial sorption by the clay, and overall these results suggest that montmorillonite is preferentially protecting organic compounds with ^{13}C, explained by stronger sorption bonds between the surface and heavy carbon. CO_2 in kaolinite-glucose and montmorillonite-glucose vials showed very little difference, but both kaolinite-glucose and montmorillonite-glucose vials contained CO_2 that was lighter isotopically than the bulk glucose. Because of the drastic differences between

minerals that were glycine amended, I think it's likely that a carbon fractionation is occurring during sorption, and that this preferential sorption of heavy isotopes of carbon may explain part of the increasing $\delta^{13}C$ with depth commonly found in soil profiles. Again this method was tested without added substrate (Figure 9).

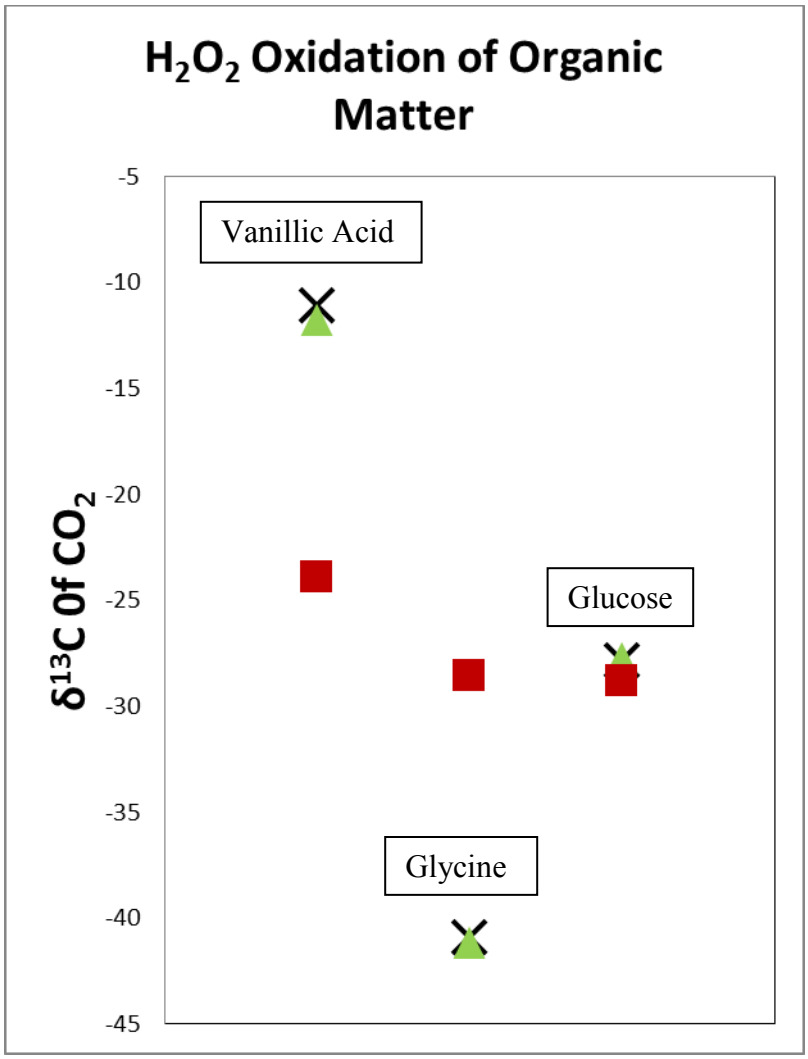

Figure 8. Isotopic values of carbon oxidized by the addition of hydrogen peroxide. Black X marks starting $\delta^{13}C$ values of substrate while green triangles are montmorillonite and red squares are kaolinite (quartz sand is not used in these experiments).

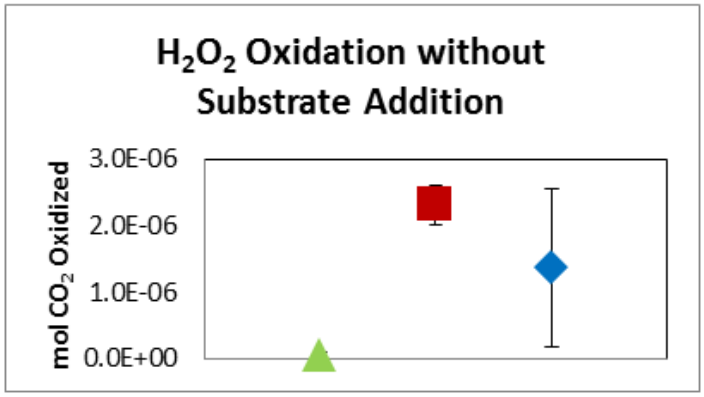

Figure 9. Hydrogen peroxide oxidations of minerals that had no organic matter added to ascertain whether there was a significant background carbon contribution. The green triangles represent vials with montmorillonite, the red squares represent vials with kaolinite, and the blue diamonds represent vials with quartz sand. The values are orders of magnitude smaller than substrate added values, and insignificant.

4.4 Dry CO_2 Adsorption

The weird isotope effects inconsistently observed throughout this experiment led us to the dry batch adsorption experiments in Figure 10. Kaolinite only adsorbs a little bit of CO_2 from the headspace but montmorillonite clay adsorbed approximately 55% of all of the CO_2 injected into the headspace. Concurrent with the increases in adsorption with the different minerals was a $\delta^{13}C$ value of the CO_2 that got increasingly lighter the more CO_2 was adsorbed. Using a mixing equation, an isotopic value for the CO_2 adsorbed onto the clay surface can be calculated. Montmorillonite adsorbed CO_2 with a $\delta^{13}C$ value of -2.11‰, approximately 4‰ heavier than the starting CO_2.

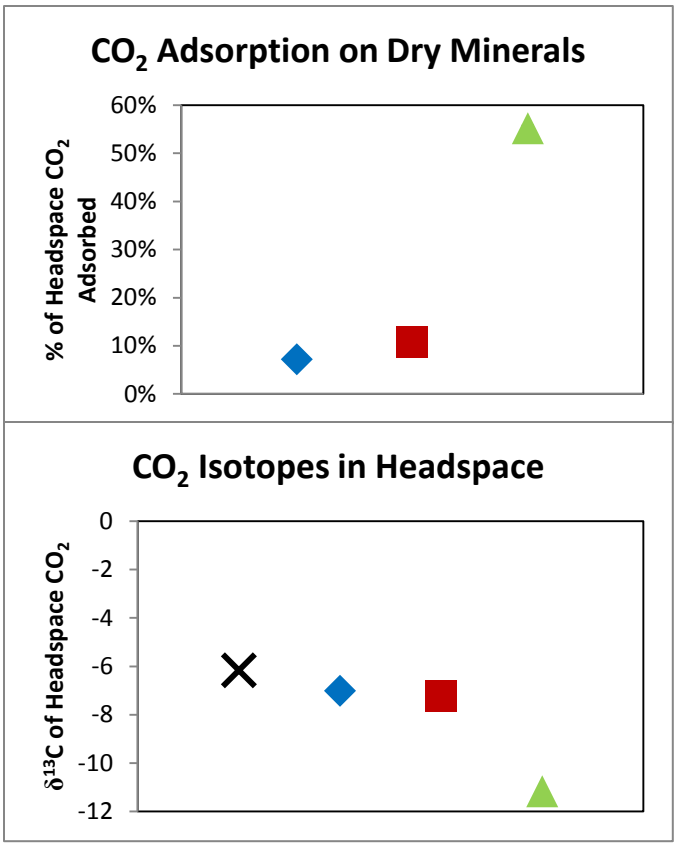

Figure 10. Results from dry CO_2 batch adsorption: the black x marks the starting values of the CO_2 used, the green triangles represent vials with montmorillonite, the red squares represent vials with kaolinite, and the blue diamonds represent vials with quartz sand. Increases in adsorption of CO_2 coincided with a decrease in the $\delta^{13}C$ values of the CO_2 still in the headspace.

The large adsorption of CO_2 by sorptive clays is especially interesting here because it indicates an additional control on respiration. If clays are adsorbing CO_2, then when soil CO_2 production is high as during the summer, observations made at typical soil gas wells will be showing a slower rate of respiration than is actually occurring. Conversely, when pCO_2 of the soil decreases, clays will probably desorb CO_2 and respiration will seem to be greater than it actually is. For soils with a large clay content this could be a significant problem, and render observational data on those soils

inaccurate. In the incubation experiments conducted in this study, this adsorption was not accounted for and may have a significant effect, and resulting in both higher respiration rates than were measured, as well as heavier CO_2 production than observed (since the dry clay preferentially sorbed $^{13}CO_2$, then presumably my CO_2 measurements are underestimating $^{13}CO_2$ in the gas samples). However, I don't think this is likely for two reasons: the first is that these incubations are kept saturated so I would expect CO_2 to be dissolved in the water rather than sorbed onto the clay surface as would happen in natural soils which are not at field capacity. Dissolved inorganic species are accounted for in the calculations. The second reason is that pCO_2 of the incubations is very high, so that surface sorption of CO_2 by clays, even if it is occurring, is most likely negligible in this closed system.

Chapter 5: Conclusion

Soils are complex systems that are vital to understand but difficult to elucidate. The results show definitively that clay bonding of organic carbon is a significant factor when considering respiration in soils. Incubations with clays showed less respiration/oxidation of organic matter. Additionally, clays preferentially sorbed organic molecules with heavy carbon, and then preferentially protected heavy carbon from both oxidative enzymes and H_2O_2. The resistance of organic compounds to oxidation when associated with clays of higher surface area and charge indicates that rather than slow carbon turnover rates being subject to changes in temperature, they are subject to the amounts and quality of clay in a soil.

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