

overtone region at 1080 and 800 to 780  $\text{cm}^{-1}$ , the 1050  $\text{cm}^{-1}$  polysaccharide band, and the CH band at 1370  $\text{cm}^{-1}$ .

### Comparison of Decalcified Soils with Untreated Soils

The PCA analysis showed spectral differences between the untreated and decalcified samples for all four sites. In KBS, Lamberton, and Hoytville, the separation was clear with decalcification, while in the Wooster soils the separation occurred only when examining each depth individually (not shown). In the Lamberton samples the difference between decalcified and untreated spectra was more pronounced for deeper layers (Fig. 8).

In all sites, component loadings showed that the decalcification increased absorption at the CH bending region between 1450 and 1370  $\text{cm}^{-1}$  characteristic of surface soils. Acidification decreased absorption at the clay OH stretching band near 3600  $\text{cm}^{-1}$ . It is possible that the acidification and washing process removed sesquioxide clays from the sample, explaining the drop in absorbance at 3600  $\text{cm}^{-1}$ . The acidification may have also removed portions of the SOM. For example, in the Hoytville and Lamberton samples, acidification also reduced absorption at the 1223  $\text{cm}^{-1}$  aromatic C–H band.

The Lamberton soils are the only ones that actually show a decrease in the calcite/dolomite band near 2517  $\text{cm}^{-1}$  (data not shown). Lamberton, like the Hoytville soil, formed on calcareous parent material and have B horizons containing calcium carbonate. The Lamberton soil, however, has more mineral C below 50 cm, with 5.7  $\text{g kg}^{-1}$  compared with 1.8  $\text{g kg}^{-1}$  for Hoytville (Paul et al., 2001). The KBS soils in turn, has little mineral C at all depths (Paul et al., 2001), and as expected there was no effect on the calcite/dolomite bands with acidification on the KBS soils.

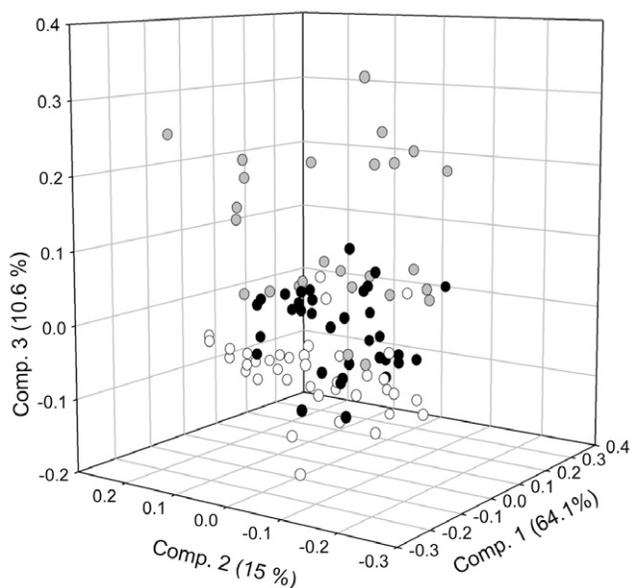


Fig. 7. Principal components analysis of the neat Mid-infrared spectra of unincubated and unfractionated soil. All samples from the KBS, Wooster, Hoytville, and Lamberton included. White is 0 to 20 cm, black is 25 to 50 cm, gray is 50+ cm. The percentage of the spectral variance accounted for each component is in parenthesis.

### Changes of Soil Fraction Spectra during Incubation

The PCA results show that the clay and light fraction spectra changed during laboratory incubation (Fig. 9 and 10). The spectral properties of the POM and silt fractions were little affected by incubation (data not shown). For the LF and clay, including all sites still showed good separation between incubation times, indicating that the type of spectral changes during incubation were observed across sites (Fig. 9 and 10).

Component loadings show that the LF loses absorbance during incubation at the 3400  $\text{cm}^{-1}$  OH/NH band, the 2920 to 2860  $\text{cm}^{-1}$  CH band, the region between 900 and 1200  $\text{cm}^{-1}$  that includes the aromatic C–H band, and a band near 480  $\text{cm}^{-1}$  (Fig. 11). The losses of absorbance in the OH area, and in the area between 900 and 1200  $\text{cm}^{-1}$  (due to Si–O inversion, but not in the LF) are consistent with carbohydrate materials being lost over time. The results confirm observations by others that the 3400  $\text{cm}^{-1}$  band marks decomposable plant material (Haberhauer and Gerzabek, 1999; Janik et al., 2007). Likewise, Spaccini et al. (2001) found that the aliphatic bands in the 2920 to 2860  $\text{cm}^{-1}$  regions can be used to study mineralization rates of corn residues in soil. During LF incubation, bands for clay OH, carboxylate/aromatics at 1630  $\text{cm}^{-1}$ , and protein/amide (1650 and 1570  $\text{cm}^{-1}$ ) increase. It is possible that the spectral signatures for mineral bands and recalcitrant C become prominent during decomposition as the labile organic material is mineralized and lost from the sample. The increase in protein could be explained by the growth of the microbial biomass and production of microbial proteins during incubation. Calderón et al. (2006) found that infrared bands associated with proteins increased during manure incubation. Besides the production of microbial proteins, the spectral data suggests that there is an enrichment in highly aromatic C. Increase in the aromatic band

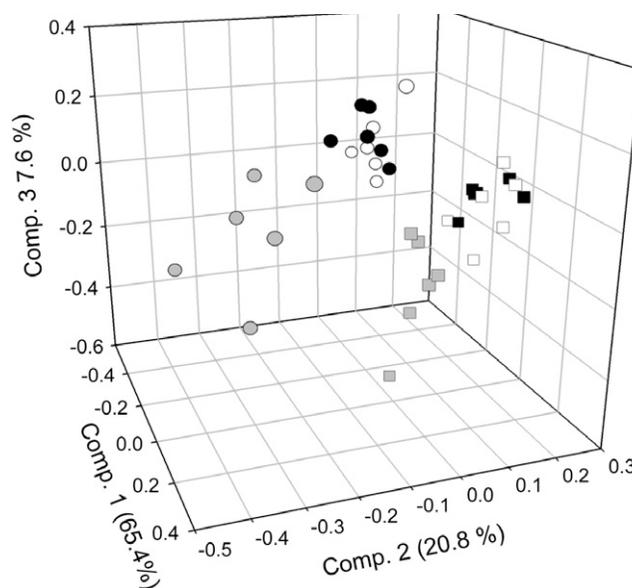


Fig. 8. Principal components analysis of the Lamberton time zero samples. Square samples are decalcified, circles are untreated. Gray are 50+ cm depth, black are 25- to 50-cm depth, and white are the 0- to 20-cm depth. The percentage of the spectral variance accounted for each component is in parenthesis.

suggest that humic acids are left over after the easily degraded C is removed. Artz et al. (2006) used the ratio of 1600/1030 (carboxylate/polysaccharide) as an index of decomposition in peat, suggesting that the band near 1630 represents a relatively recalcitrant form of organic C, probably a combination of lignin and humics. The previous study with this set of soils, as exemplified by the data in Table 2, showed that the LF lost more C than the other size fractions, confirming that the LF contains labile plant and microbial material, and can be responsible for short-term soil fertility (Haile-Mariam et al., 2008). The LF C decreased 65% on average during the 800-d incubation, and some of the LF C was transferred to other fractions.

Our spectral analysis showed that the LF fraction contained some clay. We can use previous data from this experiment (Haile-Mariam et al. (2008) to calculate the contribution of the per kilogram clay-associated C3-C to the  $^{13}\text{C}$  value of  $-13.9\text{‰}$  in the LF fraction (Table 2). The LF of this soil had  $360\text{ g C kg}^{-1}$  relative to  $420\text{ g C kg}^{-1}$  in the added corn stover (data not shown). The clay contained  $20\text{ g C kg}^{-1}$ . Thus  $<1\%$  of the C of the LF can be attributed to clay-associated C. This indicates that although LF is shown by MidIR to contain some clay, it also contained other forms of stabilized C not associated with clay. One example could be charcoal. Additional studies are needed to elucidate these observations.

The clay fraction accounts for the largest portion of total soil C in these Corn Belt soils, so changes in clay C quality during laboratory incubation could have important implications for large-scale soil C dynamics. There was a change in SOC distribution during the incubation with a drop in both the LF and clay fractions in spite of the fact that the clay has the oldest MRTs for both the corn-derived C4 and original forest-derived C3 components (Table 2). Component 2 of the clay fraction PCA shows

that during incubation, the clay fraction loses absorbance at the quartz overtone bands between  $1800$  and  $2000\text{ cm}^{-1}$ , and the CH deformation band between  $1270$  and  $1460\text{ cm}^{-1}$  (Fig. 12). The  $1460$  to  $1270\text{ cm}^{-1}$  region is high in the Lamberton clay fraction (Fig. 1), but is shown to contain some mineral bands in the whole soil spectra (Fig. 2). Absorbance losses at  $1730\text{ cm}^{-1}$  suggest losses of carbonyls in esters, which should be relatively easy to split during decomposition. The clay increases in absorbance at a prominent band near  $1205\text{ cm}^{-1}$ , and at the  $3400\text{ cm}^{-1}$  OH/NH band. Spectral changes of the clays are mostly the reverse of the patterns obtained during the LF incubation throughout most of the MidIR spectral range, demonstrating that LF decomposition follows a very different chemistry than the transformations of the clay fraction. Specifically the following spectral changes in the clays during incubation are opposite to the spectral changes in the LF: (i) increase in the OH/NH band at  $3400\text{ cm}^{-1}$ , (ii) decrease in the carbohydrate band at  $2200$  to  $2000\text{ cm}^{-1}$ , (iii) decrease throughout a large spectral region between  $1730$  and  $1250\text{ cm}^{-1}$  that includes bands for aromatics, aliphatics, amides, proteins, phenolics, and (iv) decrease in absorbance in the region between  $650$  and  $100\text{ cm}^{-1}$  and the peak at  $479\text{ cm}^{-1}$ . Haile-Mariam et al. (2008) showed that the clay C concentration did not change as much as the LF during the incubation, although there was a small decline in the Hoytville soil. Table 2 shows that clay has the oldest C for both corn and noncorn derived SOC, suggesting that the clay fraction can be considered very stable and highly processed compared with the rest of the size fractions. We hypothesize that the spectroscopic changes in the clay fraction are explained mostly by a change in the quality of the organic material, instead of the amount of C lost.

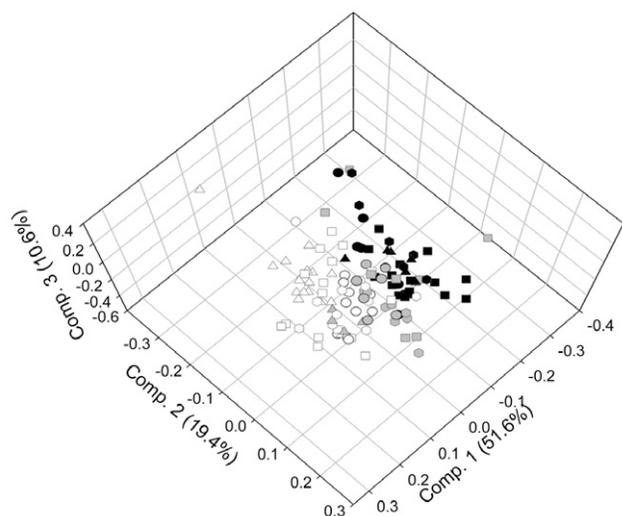


Fig. 9. Principal components analysis of the light fraction samples. All four sites are included. W.K. Kellogg Biological Station samples are squares, Lamberton samples are triangles, Hoytville samples are circles, and Wooster samples are hexagons. Time zero is white, samples incubated for 440 d are gray, and 800-d incubation are black. The percentage of the spectral variance accounted for each component is in parenthesis.

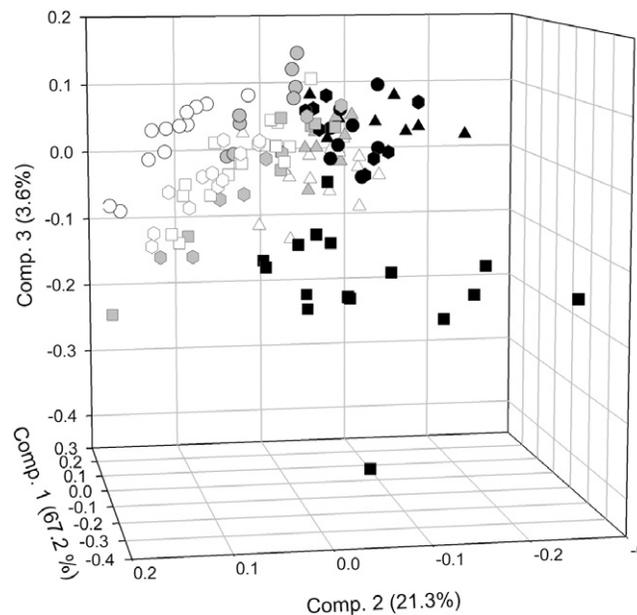


Fig. 10. Principal components analysis of the clay-sized fraction samples. All four sites are included. W.K. Kellogg Biological Station samples are squares, Lamberton samples are triangles, Hoytville samples are circles, and Wooster samples are hexagons. Time zero is white, samples incubated for 440 d are gray, and 800-d incubation are black. The percentage of the spectral variance accounted for each component is in parenthesis.

## CONCLUSIONS

This study used MidIR to measure the functional groups and absorbing bonds of both OM and soil mineral constituents

of soils that had been previously characterized by both  $^{14}\text{C}$  (Paul et al., 2001),  $^{13}\text{C}$  (Collins et al., 1999), and long-term incubation and fractionation (Haile-Mariam et al., 2008). It provided valuable molecular structural information to help interpret the SOM dynamics studies conducted previously on these soils. The analysis demonstrates that the spectral differences between the LF, POM silt, and clay fractions are greater than those attributable to soil type and location. Depth effects that relate to the very significant differences in previously noted MRTs for these horizons were also observed. The loss, during incubation, of spectral bands in the LF fraction associated with those from plant residues confirms the previously measured changes in C/N ratio and the  $^{13}\text{C}$  tracer data indicating that recent corn-derived C is the most labile, but also moves fairly rapidly into the older fractions. The extensive differences noted between the LF and the related POM fraction requires further study. The soil mineral absorption associated with the POM and its close relation to the silt fraction will have to be further delineated.

The fact that the spectral characteristics of clay-associated SOM, shown by tracers to be the oldest fraction, changed extensively during incubation indicates multiple interactions in what is thought to be a resistant fraction. Also puzzling are the extensive changes brought about by the delimiting with weak acid. Organic constituents were affected by this treatment in addition to those expected by the loss of carbonate-spectral absorption.

The clays were separated clearly from all the other constituents by the PCA analysis of the spectra and the signals for the prairie, grassland-derived, Wisconsin samples were further separated from those of the forest-derived soils. Ashing and mathematical subtraction of the mineral signals from those of the whole soil showed that this was in part attributable to the quality of the SOM in this fraction but there may also be mineralogical differences. This is in agreement with the observations of Collins et al. (2000) that the dynamics of the originally, prairie-derived SOM are different from those of forest-derived constituents. The clay fraction showed aromatic absorption that has in the past been associated with charcoal and humification. But, both these peaks and those associated with the higher N content of the clays, even after correcting for fixed ammonia in the clays, require further investigation.

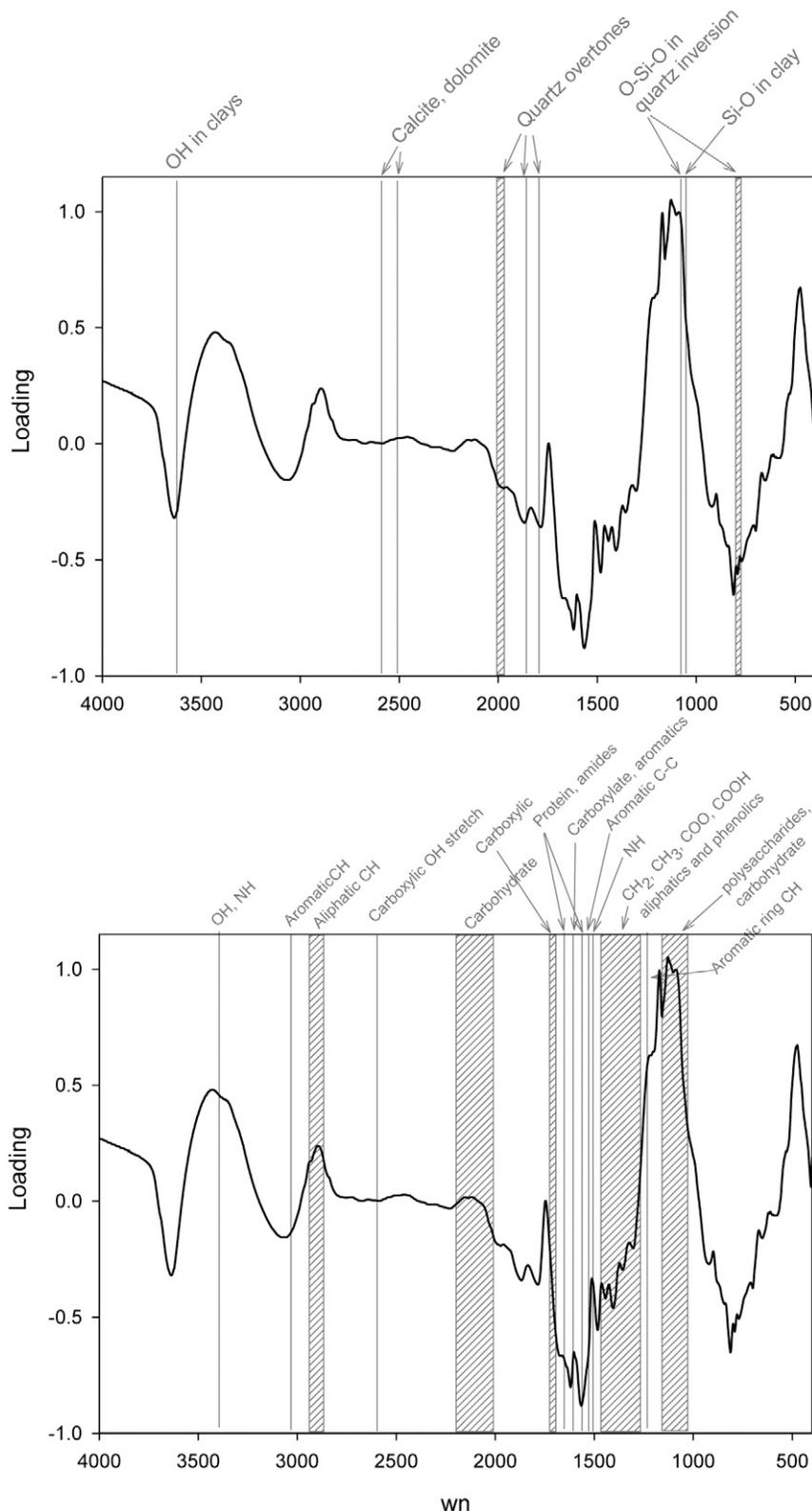


Fig. 11. Component 1 loadings for the principal components analysis shown in the light fraction PCA in Fig. 9; wn, wavenumbers in  $\text{cm}^{-1}$ . Top graph has the mineral bands indicated, bottom graph has the organic bands.

There are a number of approaches for determining the molecular structure of SOM relative to its dynamics. Plante et al. (2009) analyzed soils from the same Hoytville site by using Pyrolysis-molecular beam mass spectrometry (Py-MBMS). Although, they looked at humic acid fractions and whole soils while the present study looked at particle-size fractions and whole soils, some observations can be made regarding these two studies. Both MidIR and Py-MBMS are data-rich and fast, but MidIR is nondestructive. Both techniques require extensive statistical analysis, and both give a good relation to total soil C and clearly separate soils at depth. Pyrolysis-molecular beam mass spectrometry characterizes the molecular weight of heat breakdown products. It gave good resolution of humic acid fractions and cultivated vs. grassland or forest soils. Mid-infrared relies on the vibrational absorbance of functional groups of SOM. This study showed the clear resolution of the SOM signals from particle-size fractions, which differ in the age and chemistry of organic C. One advantage of MidIR over Py-MBMS is that mineral features like clays, silicates, and carbonates can be identified. This however can also be a disadvantage until the different bands have been clearly differentiated.

The techniques are complementary and both could benefit from increased use of internal standards to help quantify the data obtained. The use of multiple techniques, on similar soil samples, that have been characterized relative to their dynamics by tracers helps to look at SOM from different perspectives and also brings out the merits of the different techniques.

Many studies have now established that MidIR with multivariate analysis is a powerful tool to develop accurate calibrations for total soil C. Clearly, infrared spectra contain wealth of information about soil C quality that can be exploited with judicious interpretation. Our results also show that MidIR spectroscopy can be used as a screening tool to quickly distinguish different soil types. Soils with different amounts of calcareous materials can also be resolved by absorbance at  $2517\text{ cm}^{-1}$ .

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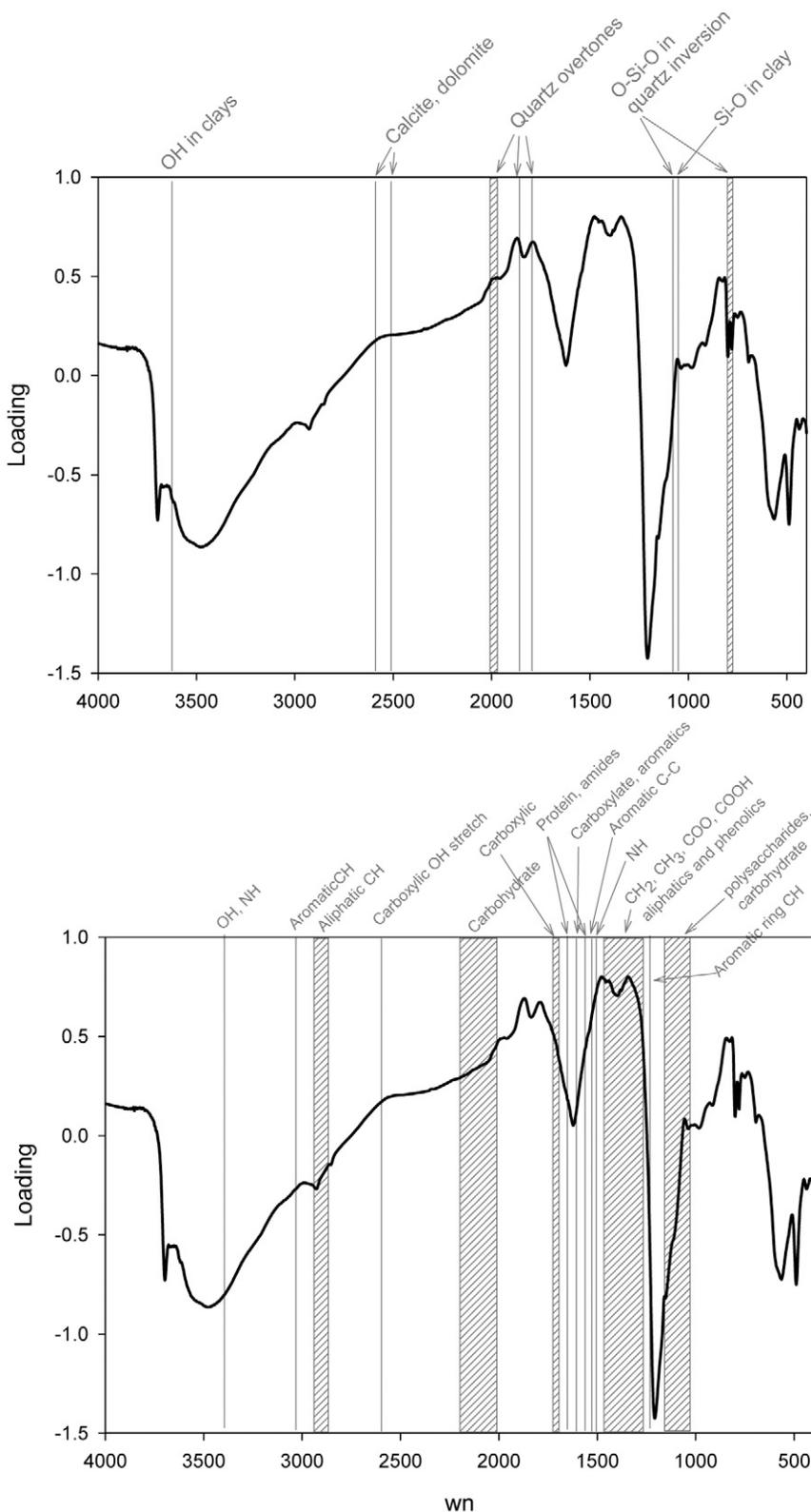


Fig. 12. Component 2 loadings for the principal components analysis shown in the clay fraction PCA in Fig. 10; wn, wavenumbers in  $\text{cm}^{-1}$ . Top graph has the mineral bands indicated, bottom graph has the organic bands.

Minnesota, and the KBS NSF-LTER, Michigan State University, for access to the research plots.

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