

GENOTYPIC AND FOREST MANAGEMENT EFFECTS ON SIZE-DENSITY  
FRACTIONATION OF SOIL CARBON IN A FORESTED SPodosol

By

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by

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This work is dedicated to my grandfather and to Dr. George Washington Carver, who taught me the worth and fun of working with plants and soil.

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## LIST OF ABBREVIATIONS

- SOC: soil organic carbon
- OM: organic matter
- PPINES: Pine Productivity Interactions Experimental Study
- DRIFTS: Diffuse Reflectance Infrared Fourier Transform Spectroscopy
- AOM: aggregate organic matter
- POM: particulate organic matter
- SEM: scanning electron microscope
- SOM: soil organic matter

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Soil C accounts for 75 to 85% of terrestrial C, making soil C sequestration an important ecosystem service. This study was undertaken to characterize the soil organic carbon (SOC) pools in a sandy Spodosol of north Florida; to study the aggregate C pool in soils that are considered to have weak aggregation; and to investigate the influence of intensive forest management on SOC pools. A loblolly pine (*Pinus taeda* L.) plantation under two levels of forest management (fertilization and understory control) was evaluated. Dry and wet sieving methods were compared for their applicability in size fractionation. Dry sieving was found to be satisfactory for these soils, as it preserved more structure and the water-soluble SOC components such as esters and amides. The use of organic matter (OM) release associated with aggregate breakdown by sonication allowed simultaneous measurement of aggregate strength and amount of aggregate OM. Aggregate C was found to be an important C pool in these soils, accounting for nearly half of total SOC. Diffuse Reflectance Infrared Fourier Transform Spectroscopy (DRIFTS) spectra showed presence of recently added OM in the 2000 to 250  $\mu\text{m}$  fraction, and more decomposed OM in the  $<53$   $\mu\text{m}$  fraction. The spectra showed clear separation between aggregate and particulate OM. Aggregate OM was characterized by higher esters, amides and

polysaccharides than particulate OM, indicating its susceptibility to decomposition in case of aggregate destruction. The 2000 to 250  $\mu\text{m}$  fraction, especially its light and medium density components, was found to be the C pool most responsive to short-term management-related changes. Intensive management reduced the  $>53 \mu\text{m}$  fraction C as well as aggregate C, probably due to the reduced input of understory roots. The best family (chosen a priori based on growth) added more decomposable C as shown by the higher C content in the light density fraction, and higher N concentration in the medium density under intensive management. The medium family encouraged aggregation as shown by the higher aggregate OM values. These methods and the sensitivity of these SOC pools to the short-term management-related changes offer a promise for better understanding SOC dynamics in sandy Spodosols of Florida.

## CHAPTER 1 INTRODUCTION

What of thee, O earth, (I) dig out,

let that quickly grow over

let me not hit thy vitals,

nor thy heart....

O cleansing one!

Atharva Veda (Whitney, 1905)

The need for conserving natural resources had been recognized by civilizations for over 5000 years. However, the management of these resources for sustainable production is still a challenge. With the rapidly increasing world population, demand for forest products is increasing, while large tracts of forestland are being converted to other uses or being degraded (Forest Resources Assessment [FRA], 2005). The alarming rate of deforestation, especially in the developing countries, demands greater concern for the existing forests. So worldwide, emphasis is being given to the preservation of natural forests and their non-commodity functions (FRA, 2005).

Timber harvest has been restricted in many of the world's natural forests and consequently the onus of providing the world's increasing demand for wood and fiber is now on plantations managed especially for timber and pulp production. Therefore, it is important to increase the productivity of these areas. Concentrating timber production on the best sites will allow the world's wood and fiber demands to be met on fewer acres. It will also allow large areas of native forests to be conserved or preserved (FRA, 2005).

## **Pine Plantations in the Southeastern United States**

Pine plantations are important commercial ecosystems, covering more than 12 million ha in the southern United States (Wear and Greis 2002). Apart from their economic significance, these ecosystems are important for their ecosystem services, such as maintenance of ground water and air quality. Above and below ground C storage in standing biomass and roots and their contribution to soil organic carbon (SOC) is another service that will likely become an economic commodity (e.g., C credits) in the near future.

In the last few decades, increased management intensity has resulted in an unprecedented increase in the yield of woody biomass and litterfall (Dalla-Tea and Jokela, 1991, McCrady and Jokela, 1998). This suggests an even higher potential for C sequestration. However, the long-term impacts of these management changes on SOC pools and on the soil's C sequestration potential are not well documented. There are four important elements of intensive management that have the potential to change nutrient cycling and SOC sequestration: fertilization, chemical understory control, deployment of improved families and site preparation. The first three of those elements are a part of this dissertation. Fertilization has been shown to increase N and P mineralization rates (Polglase et al., 1992b), while understory control has been shown to reduce the SOC (Echeverria et al., 2004). However, no work has addressed genotypic effects of family or their interaction with fertilization and weed control on the distribution and characteristics of SOC.

### **Need for Carbon-wise Management**

Soil organic matter is a key constituent of the complex below-ground ecosystem, affecting a multitude of physical, chemical and biological soil properties. As an important binding agent for soil aggregates, SOC plays a role in soil aeration, water holding capacity and permeability through its effect on soil structure and water holding characteristics. Soil organic matter is

important for soil fertility. Humus is a buffer that influences soil pH. It also has high cation exchange capacity, surpassed only by 2:1 expanding clay minerals that have a higher CEC per unit volume. In most soils, humus accounts for 20 to 90% of the soil CEC (Brady, 2000). SOC is also an important storehouse for N, P, S, and micronutrients. It protects nutrients like N from being leached and protects P and micronutrients from fixation so that they remain available for plant uptake for a longer period. Chelating functional groups in SOC, improve the availability of nutrient cations while reducing the toxicity of others. In sandy soils with low inherent fertility and little clay to perform some of these functions, the significance of SOC in sustaining the productivity of these ecosystems and the need for understanding the C dynamics are even greater.

However, total SOC has been found to be an insensitive indicator of management related changes in sandy soils (Harding and Jokela, 1994). Measurement of changes in C is difficult against the large amount of recalcitrant or inert material already present in the soil. This is especially true for detecting the changes in nutrient supply characteristics of SOC in which the actively cycling fractions of SOC play a major role (Brady, 2000). Physical fractionation techniques based on differences in size and density may provide greater insight into this question by separating the active and inert C pools. Measurement of these pools can also give insight into the mechanisms through which management activities affect total SOC. For example, an increase in light density C suggests that trees may be adding more decomposable organic matter since this fraction is an active form of C (Swanston et al., 2005).

### **Challenges and Opportunities in Sandy Soils**

Sandy soils are generally found to be more resilient to compaction by tillage, but they are probably more susceptible to losses of SOC as there is very little clay to protect it (Shan et al., 2001). Carbon is sequestered when it is protected from decomposition. Protection is generally

provided by sorption onto clay, by incorporation into aggregates, or as a decomposition product that is resistant to further microbial attack. The low clay content of sandy surface soils dictates against the first option, leaving the latter two protection mechanisms as possibilities. Even though the potential for aggregation through the formation of clay-cation-OM complexes (Edwards and Bremner, 1967) is small in these soils, aggregation through the action of fungal hyphae and roots (Tisdall and Oades, 1982) is possible. This suggests a lower potential for C sequestration in the sandy soils, but offers the opportunity to study physical protection mechanisms without the confounding influence of clay. Unfortunately, very little is documented about the biochemical protection of SOC in soils that support southern pine ecosystems. Soil aggregation studies have been conducted in soils with higher clay contents. Therefore, the aggregation in these sandy soils deserves investigation.

This study was conducted with the following main objectives:

- To examine soil C forms in the surface horizon of a sandy Flatwoods Spodosol,
- To study aggregation in this soil; and
- To determine the management-related changes in the different soil C pools.

Three laboratory methods, viz. size fractionation, density fractionation and sonication, were used to study C pools and to determine the effects of forest management on these pools. The results of this study are provided in three chapters. Chapter 2 focuses on two different size fractionation methods, dry and wet sieving, and the C and N characteristics of the size fractions. The hypotheses were that: 1) dry sieving would be a suitable method of fractionation for studying extremely sandy soils with inherently weak structure; 2) the 2000 to 250  $\mu\text{m}$  fraction would be the most important C fraction due to the input of roots and litter; 3) intensive management that uses fertilization and understory competition control to increase aboveground biomass would reduce the C content in the 2000 to 250  $\mu\text{m}$  size fraction due to reduction in

understory root inputs; and 4) fertilizer N added through forest management would be reflected in higher N content in the size fractions.

Chapter 3 focuses on the aggregate morphology, C content, and stability in the surface horizons of sandy Spodosols. Sonication (breakdown of aggregates using ultrasonic energy) was used to measure aggregate stability and the pattern of C release from aggregates of varying stability. The hypotheses tested were that: 1) Surface horizons in a north Florida Spodosol would have more aggregation than has been described through soil mapping due to the high input of above ground litter and roots and higher activity of biological agents of aggregation such as fungal hyphae; 2) aggregate stability would increase with decreasing aggregate size due to increasing surface area of mineral matter available to the action of organic binding agents; 3) particulate organic matter would be the dominant C form over aggregate organic matter because of low clay content to support aggregation; and 4) intensive management would reduce aggregation in the short-term due to the reduction in the input of understory fine roots.

Chapter 4 focuses on the effects of full sib loblolly pine families (families with both parents known, chosen a priori based on their growth performance) and their interactions with management intensity on size-density fractions of SOC. The hypotheses tested in this study were that: 1) the best growing family would exhibit the highest SOC, especially under intensive management, due to high litterfall inputs and the responsiveness of this family to intensive management; 2) the light and medium density fractions would be the main reservoirs of C, since the mineral matter in these soils is predominantly quartz sand with little or no C adsorption capacity; and 3) the light and medium density of the 2000 to 250  $\mu\text{m}$  fraction would be the most responsive pool for detecting family effects because earlier results showed that the 2000 to 250  $\mu\text{m}$  fraction SOC was the most responsive size fraction and because the light and medium

density fractions were most likely to show the effects of recently added organic matter (Romkens et al., 1999).

Chapter 5 summarizes the most important findings of this study. It identifies opportunities for future research and suggests a possible course of action for understanding the impact of forest management on SOC forms and functions.

CHAPTER 2  
EFFECTS OF FOREST MANAGEMENT ON SOIL CARBON AND NITROGEN IN A  
NORTH FLORIDA SANDY SPODOSOL

**Introduction**

The soil C density of Spodosols in Florida ( $20 \text{ kg m}^{-2}$ ; Stone et al., 1993) is higher than the soil C density in a majority of the life zones studied (Post et al., 1982), yet little research has been conducted to study the C profile of these soils. Many Florida Spodosols support southern pine plantations (Adegbidi et al., 2002), which have the potential for significant above ground storage of C (Richter et al., 1995). During the last few decades, intensive management of these plantations has resulted in an unprecedented increase in litterfall and yield of woody biomass (Dalla-Tea and Jokela, 1991), suggesting the potential to further increase above ground C storage. However, the short and long-term impacts of forest management on soil organic carbon (SOC) pools and the C sequestration potential of these soils are poorly documented.

Fertilization and chemical understory control are common silvicultural practices used to increase yields when managing southern pine stands in the southeastern U.S. Chemical understory competition control has been shown to reduce total SOC (Shan et al., 2001; Echeverria et al., 2004) and the mineralization of C and N in both whole soil and soil density fractions (Polglase et al., 1992a, b; Echeverria et al., 2004). Fertilization has been reported to increase mineralization rates, especially for P (Polglase et al., 1992a, b). Yet, previous studies have shown no significant effect of fertilization on total SOC (Harding and Jokela, 1994; Shan et al., 2001), probably because the increased organic matter inputs associated with large growth responses compensated the losses by increased mineralization rates.

Physical fractionation of soil into size and density fractions has been an effective technique for studying the forms and cycling of soil C (Christensen, 1992; Ellert and Gregorich, 1995). Organic matter (OM) changes in the sand fraction have been useful as early indicators of

management-related C changes. For example, two years of barley (*Hordeum vulgare* L.) and alfalfa (*Medicago sativa* L.) cultivation, compared to a bare soil, increased sand fraction C (24 to 60%), N (36 to 45%) and carbohydrates (46 to 83%; Angers and Mehuys, 1990). Likewise, OM (>150  $\mu\text{m}$  fraction) was depleted under continuous maize cultivation, yet recovered rapidly when the land use was returned to pasture (Romkens et al., 1999). In the extremely sandy Spodosols of the southeastern U.S., which often contain less than 5% silt + clay, the sand fraction C is likely to equal total C since the clay fraction of these soils contains mainly quartz and kaolinite with very low C sorption capacity (Harris and Carlisle, 1987). It follows that further fractionation of the sand size C would be required to detect the short-term management related changes in the soil C pools. Fractionation of the SOC into meaningful pools also helps in understanding the processes underlying any possible changes. For example, identifying the pools responsible for different functions such as short-term nutrient turnover (e.g., sand size fraction) and long-term C storage (e.g., fine silt fraction; Liu et al., 2003) may help in the development of more accurate C dynamics models.

Wet sieving is the standard technique for soil size fractionation (Yoder, 1936; Marx et al., 2005). Even though it disrupts macroaggregates (Angers and Giroux, 1996), wet sieving is necessary when working with high clay soils to break the strong aggregates that are >2 mm in diameter. In contrast, the sandy nature of many Coastal Plain soils hinders the formation of large stable macroaggregates. Under these conditions, dry sieving may offer a viable alternative to the more time consuming wet sieving method.

This study was conducted to define the distribution of soil C and N in the surface horizon of a representative sandy Coastal Plain Spodosol and to evaluate the short-term impacts of forest management activities on these soil characteristics. The first study objective compared wet

sieving and dry sieving as alternative methods for investigating C and N distribution among soil size fractions. The hypothesis was that, given the low clay content and weak, single grain to crumb aggregate structure, dry sieving would be a suitable and rapid technique for size fractionation. The second objective was to establish the C and N distribution, as well as the chemical fingerprint of OM in the various size fractions. The assumption was that short-term management impacts would be more identifiable within a size fraction than in the whole soil. We expected the results to show that the majority of the C and N would be in the 2000 to 250  $\mu\text{m}$  fraction, assuming that this fraction received the greatest inputs from roots and aboveground litter. We also expected this soil to contrast with those having considerably more clay and larger C pools in the  $<53 \mu\text{m}$  fraction. The third objective was, through the use of data generated by the first two objectives, to provide an evaluation of the suitability of these methods for determining the short-term impacts of two contrasting forest management intensities. Intensive management, especially with sustained understory competition control, would be expected to reduce SOC content in the 2000 to 250  $\mu\text{m}$  fraction due to the reduction in root C inputs. We anticipated that there would be enhanced N incorporated into all size fractions because of the fertilizer N inputs.

## **Materials and Methods**

### **Experimental Site**

The study site was a loblolly pine (*Pinus taeda* L.) plantation in north Florida (30°24'N lat; 82°33'W long) managed by the Forest Biology Research Cooperative at the University of Florida as part of the Pine Productivity Interactions Experimental Study (PPINES). This long-term study aims at understanding the family x environment interactions in full-sib families of loblolly and slash pine (*P. elliotii* Engelm. var. *elliotii*). The climate is warm, humid subtropical, with 1,394-mm average annual rainfall, 27°C average annual maximum temperature, and 13°C average annual minimum temperature (NOAA, 2002). The soil is classified as a Leon

series (sandy, siliceous, thermic Aeric Alaquod), with <5% silt + clay, and <10 cmol<sub>c</sub> kg<sup>-1</sup> of cation exchange capacity.

Trees were planted in January 2000 in four replicates using a randomized complete block, split plot design. Prior to planting, the entire study was double bedded and treated with the herbicides Arsenal (imazapyr – 1.02 L ha<sup>-1</sup>) and Garlon (triclopyr – 7.02 L ha<sup>-1</sup>) to remove the understory vegetation and to reduce competition. The experimental design was a 2 x 2 x 8 factorial, which included two planting densities (close spacing at 1.3 x 3 m and wide spacing at 3 x 3 m), two management regimes (high and low inputs) and six elite loblolly pine full-sib families, a mix of these elite families, and one poor growing family. We chose treatments that maximized the difference in C inputs to the soil in order to evaluate the capacity for short-term SOC changes, based on biomass production. The high intensity treatment included the most productive family and consisted of sustained understory competition control using herbicides, and annual fertilization using a complete fertilizer. In the high intensity treatment, Arsenal (0.28 L ha<sup>-1</sup>) and Oust (0.14 L ha<sup>-1</sup>) were also applied as necessary to provide sustained understory competition control. The low intensity treatment, planted with the poorest growing family, was chosen for comparison. The families were designated *a priori* based on their growth performance in long-term genetic experiments. This management regime consisted of a one-time fertilizer application and the aforementioned time of planting understory competition control treatment. At age four, when sampling was conducted, the fertilizer added to the high intensity treatment totaled 368 kg ha<sup>-1</sup> N and 128 kg ha<sup>-1</sup> P plus most other essential nutrients (i.e., 121 kg ha<sup>-1</sup> K, 45 kg ha<sup>-1</sup> Mg, 45 kg ha<sup>-1</sup> Ca, 35 kg ha<sup>-1</sup> S, 0.89 kg ha<sup>-1</sup> B, 3 kg ha<sup>-1</sup> Zn, 2 kg ha<sup>-1</sup> Mn, 16 kg ha<sup>-1</sup> Fe, 4 kg ha<sup>-1</sup> Cu, 0.01 kg ha<sup>-1</sup> Mo), while the low intensity treatment had 45 kg ha<sup>-1</sup> N and 50.6 kg ha<sup>-1</sup> P applied as diammonium phosphate. Both treatments were planted at

the 1.3 x 3 m spacing (close spacing - 2,900 trees ha<sup>-1</sup>) and each treatment plot was 480 m<sup>2</sup> in size. The entire study was treated when necessary with insecticides (Dimilin, Pounce or Mimic) for tip moth (*Rhyacionia* spp.) control during the first growing season. Further details of the study site, including the stem volume and above ground biomass are discussed by Roth et al. (2006).

Soil samples were collected from the A horizon at depth increments of 0 to 5 and 5 to 10 cm from each treatment plot in three replicate blocks in September 2003. One combined soil sample for each depth of each plot (treatment within a block) came from four individual soil samples. The four individual soil samples were collected from alternate rows (interbed position), while within an interbed the sample locations were chosen randomly.

### **Laboratory Methods**

Since the soil moisture content is highly variable and “air-dry” conditions are possible in these surface soils under field conditions, soil samples were air-dried and passed through 8000 µm and 2000 µm sieves. The <2000 µm fraction was further size-fractionated in order to contrast dry and wet sieving methods into four size fractions, a macroaggregate fraction 2000 to 250 µm, two microaggregate fractions 250 to 150 µm and 150 to 53 µm, as well as a <53 µm fraction. For dry sieving, samples were sieved in a mechanical shaker for 5 minutes. The purpose was to accomplish size fractionation with minimum destruction of soil structure. A previous study determined that there was no significant difference in weight distribution among the four size classes after sieving for 5 minutes (unpublished data). Therefore, this time frame was used for all dry sieving. Wet sieving followed the procedure of Cambardella and Elliott (1993) without pre-wetting because large aggregates were not common. For each sample, 100 g of soil were added to the coarsest sieve (250 µm) in a tray so that there was standing water 2 cm above the sieve screen. The sample was allowed to stand for 5 minutes and then the sieve was moved up and

down 50 times (for approximately 2 minutes) taking care that the sieve screen broke the water surface every time. The soil suspension in the water tray was then transferred to the next size sieve and the procedure was repeated for each successive sieve (150 and 53  $\mu\text{m}$ ). Each sieve, when removed from the water tray, was allowed to dry for 24 hours. The < 53  $\mu\text{m}$  fraction was collected by sedimentation for 48 hours followed by oven drying. The size fractions were analyzed for total C and N concentrations with a Carlo-Erba CN Analyzer (CE Instruments, model NCS-2500). The >8000  $\mu\text{m}$  and 8000 to 2000  $\mu\text{m}$  fractions were also ground and analyzed for total C and N concentrations and used for calculating C and N contents.

Since these soils have low clay content and no carbonates, loss on ignition (LOI) was used to measure OM content (at 550<sup>0</sup>C for 6h). The ratio of analyzer C to LOI was used to estimate the C content and C:OM ratio in each size fraction. Thermal Gravimetric Analysis (Omnitherm 951 TGA; Dupont Co., Wilmington, DE) confirmed complete combustion of C during LOI.

The chemical fingerprints of the size fractions were investigated using Diffuse Reflectance Infrared Fourier Transform Spectroscopy (DRIFTS). Samples were scanned before and after ashing (at 550<sup>0</sup>C for 6h) in the mid-infrared on a Varian Digilab FTS-7000 Fourier Transform Mid-infrared Spectrometer (Walnut Creek, CA). Samples were scanned from 4000 to 400  $\text{cm}^{-1}$  at 4  $\text{cm}^{-1}$  resolution using a KBr beamsplitter and DTGS detector and a Pike Autodiff autosampler (Pike Technologies, Madison, WI) using ground, non-KBr diluted samples.

### **Statistical Analysis**

The statistical significance of treatments was analyzed using PROC GLM (SAS, 1996), with forest management intensity, soil depth, soil size fraction, and sieving method as fixed effects and with block as a random effect. The differences were considered significant at  $p < 0.05$ . Since the initial analyses showed a significant depth x fraction interaction, further analyses were

carried out for the individual size fractions. A multiple comparison procedure with a Tukey-Cramer adjustment was used for the post-hoc mean separation.

For the DRIFTS spectra, spectral subtraction of ashed samples from non-ashed samples was used to accentuate OM characterization using GRAMS/AI software Ver. 7.02 (Thermo Galactic, Salem, NH). Discriminant analysis was conducted using SAS (SAS Institute, Cary, NC) partial least squares (PLS) using a modified version of a custom made SAS program for spectral pre-treatments including gap derivatives, scatter correction, and spectral data point averaging (Reeves and Delwiche, 2003). This modified version allowed discriminant analysis to be carried out using SAS PLS while the original program was developed only for quantitative regression analysis. The higher the  $R^2$  of the discriminant analysis and the lower the Root Mean Square Deviation, the better was the separation between main effects as indicated by DRIFTS spectra.

## **Results**

### **Dry vs. Wet Sieving**

Wet sieving reduced the C concentration by 8% (% of fraction, Fig. 2.1) and C content by 30% (% of whole soil; Table 2.1) in the 150 to 53  $\mu\text{m}$  fraction. There was also a significant reduction in the weight of this fraction due to wet sieving (16% of whole soil in dry sieving vs. 14% in wet sieving). In the <53  $\mu\text{m}$  fraction, wet sieving increased the C content by 26% (Table 2.1), but reduced the C concentration by 13% (Fig. 2.1) and C:OM ratio by 12% (Fig. 2.2). Wet sieving reduced the C concentration and C:OM ratio in the 250 to 150  $\mu\text{m}$  fraction, although its C content was not statistically affected by sieving method. Similarly, the 2000 to 250  $\mu\text{m}$  fraction was not affected by the sieving method. The total recovery of C as shown by mass

balance for both methods was not statistically different. Nitrogen concentration or content (Table 2.2) was not affected by the sieving method in any of the size fractions examined.

The effect of sieving method was evident on the chemical fingerprint of the size fractions as measured by DRIFTS (ash-subtracted spectra  $R^2 = 0.94$ ; Table 2.3). Wet sieving reduced C in all four size fractions across the entire spectra as indicated by spectral peak heights (Fig. 2.3). Differences in the 2000 to 53  $\mu\text{m}$  fractions were more pronounced for esters ( $1730\text{ cm}^{-1}$ ), amides ( $1650\text{ cm}^{-1}$ ) and aromatic compounds ( $1530\text{ cm}^{-1}$ ). On the other hand, in the  $<53\text{ }\mu\text{m}$  fraction, the difference between sieving methods was more pronounced in the aliphatic C-H ( $2870, 2930\text{ cm}^{-1}$ ), and polysaccharide ( $1160\text{ cm}^{-1}$ ) peaks.

### **Characterization of Size Fractions**

Carbon concentration (Fig. 2.1) was highest in the  $<53\text{ }\mu\text{m}$  fraction (7.8 to 8.8%) and lowest in the 250 to 150  $\mu\text{m}$  fraction (0.6 to 0.7%). Carbon content (Table 2.1) on the other hand, was highest in the 2000 to 250  $\mu\text{m}$  fraction and lowest in the  $<53\text{ }\mu\text{m}$  fraction. More than 65% of the total C (Table 2.1) in these soils was found in the 2000 to 53  $\mu\text{m}$  fractions, of which about 39% was in the 2000 to 250  $\mu\text{m}$  fraction. N content (Table 2.2) followed a trend similar to C. The C:OM ratios for the 2000 to 250  $\mu\text{m}$  and 150 to 53  $\mu\text{m}$  fractions were 8 to 27% higher than the remaining two fractions (Fig. 2.2), which had ratios equal to the standard Van Bemmelen factor (0.58). The C:N ratios of the fractions were not affected by any of the factors and therefore, are not reported.

The DRIFTS spectra (Fig. 2.4) confirmed chemical fingerprint differences among size fractions (ash-subtracted spectra  $R^2 = 0.94$  to  $0.98$ ; Table 2.3). However, the differences were mainly in the peak heights corresponding to the amount of C in each fraction. The 2000 to 250  $\mu\text{m}$  fraction exhibited the highest aliphatic C-H peaks ( $2870, 2930\text{ cm}^{-1}$ ), while esters ( $1730\text{ cm}^{-1}$ ), amides ( $1650\text{ cm}^{-1}$ ) and polysaccharides ( $1160\text{ cm}^{-1}$ ) were high in both 2000 to 250 and  $< 53$

$\mu\text{m}$  fractions. The peak for aromatic rings at  $1530\text{ cm}^{-1}$  was similar in these latter two fractions, but the peak at  $1580\text{ cm}^{-1}$  was absent in the 2000 to  $250\text{ }\mu\text{m}$  fraction. The ester and amide peaks were absent in the 250 to  $150\text{ }\mu\text{m}$  fraction.

### **Effect of Management Intensity and Soil Depth**

In the 5 to 10 cm depth, low intensity management had 12 and 5% higher C contents in the 2000 to 250 and 250 to  $150\text{ }\mu\text{m}$  fractions, respectively, than under the intensive management regime (Fig. 2.5). Low intensity management also had a 5% higher C content (Fig. 2.5) and 7% higher N content (Fig. 2.6) than intensive management for the 0 to 5 cm depth in the 150 to  $53\text{ }\mu\text{m}$  fraction. In contrast, the high intensity management showed 2% higher C content and 5% higher N content in the  $< 53\text{ }\mu\text{m}$  fraction at the 5 to 10 cm depth. The  $> 2000\text{ }\mu\text{m}$  fraction exhibited 22 to 48% higher C content at 0 to 5 cm depth compared to the 5 to 10 cm depth, but C content in this fraction was not significantly influenced by management intensity.

Soils under the low intensity management regime were higher in OM as shown by consistently higher peak heights of the DRIFTS spectra (Fig. 2.7; ash-subtracted spectra  $R^2 = 0.71$ ; Table 2.3). At the 0 to 5 cm depth, differences were observed for all the four size fractions, while at the 5 to 10 cm depth, a difference was observed only in the 2000 to  $53\text{ }\mu\text{m}$  fractions. This result was observed across the entire spectrum of OM in the size fractions.

### **Discussion**

This study was undertaken with the objective of understanding the distribution of C across the size fractions in a forested Spodosol, with the assumption that the size fractions approximated distinct C pools; and to determine the impact of forest management on these C pools. Southern pine plantations in the southeastern U.S. represent important regional sinks for C (Richter et al., 1995) and understanding the soil C dynamics is an essential step towards sustainable management of these ecosystems.

## **Dry vs. Wet Sieving**

The reduction in C content in the 150 to 53  $\mu\text{m}$  fraction and the increase in C content in the <53  $\mu\text{m}$  fraction (Table 2.1) indicated a significant transfer of C with wet sieving. This was due to breakdown of water-dispersible aggregates in the 150 to 53  $\mu\text{m}$  fraction, where the C and a significant amount of soil mass was washed into the finest fraction. This finding was consistent with previous work (Carter 1992; Angers and Giroux 1996). The reduction in C concentration (Fig. 2.1) and C:OM ratio (Fig. 2.2) of the <53  $\mu\text{m}$  fraction indicated that C was lost even from this fraction as water-soluble C. This interpretation was supported by the DRIFTS data, where esters, amides and aromatic compounds were lost from the 150 to 53  $\mu\text{m}$  fraction and some polysaccharides and aliphatic  $-\text{CH}$  compounds were lost from the <53  $\mu\text{m}$  fraction (Fig. 2.3). However, the statistically equivalent C recovery of the two sieving methods showed that the loss of water-soluble C was small, with amounts being within experimental error. The N concentration and content, on the other hand, was not affected by sieving method, indicating that N in these soils was not likely in a significant water-soluble or water-dispersible (aggregate) form. Therefore, our hypothesis of equivalence between dry and wet sieving was accepted for N distribution and rejected for C distribution, as wet sieving resulted in a redistribution of C. Dry sieving, which is an easier and quicker method than wet sieving, was considered superior to wet sieving for the extremely sandy soils examined in this study, as it preserved the water-dispersible aggregates and water-soluble C.

## **Distribution of C and N among Size Fractions**

More than 65% of the C and N was found in the 2000 to 53  $\mu\text{m}$  fractions (Table 2.1, 2.2), which supported the hypothesis of the importance of these fractions for C content. The <53  $\mu\text{m}$  accounted for less than 10% of the total C. This is unlike soils having higher clay contents, where the C contents tend to be highest in the silt + clay size fractions. Hassink et al. (1997)

found that more than 50% of the soil C was in the < 20  $\mu\text{m}$  fraction (65% clay). Feller (1993) and Bronick and Lal (2005) reported similarly high C contents in the silt + clay fraction. Though the C contents reported for extremely sandy soils vary, there is evidence that forested sandy soils in other parts of the world have similarly high C content within the sand size fractions. For example, forested Spodosols in France (<10% silt + clay) were shown to have 50% of the total C within the sand size fraction (Jolivet et al., 2003). Quideau et al. (1998) reported that, under hardwood forests, 45 to 55% of the total C was associated with the sand size fraction (58% sand). In contrast, when under maize cultivation, a Spodosol (<10% silt + clay) had only 17% of its total C in the sand size fraction (Quenea et al., 2006).

The nature of OM, as evidenced by C:OM ratios (Fig. 2.2), differed among size fractions. The DRIFTS data confirmed this result (Fig. 2.4). The high peaks of esters, amides and aliphatic C in spectra of the 2000 to 250  $\mu\text{m}$  fraction indicated recently added undecomposed OM and the high C:OM ratio of this fraction indicated presence of C rich organic matter. On the other hand, the high aromatic C peak at 1580  $\text{cm}^{-1}$  in the < 53  $\mu\text{m}$  fraction indicated more decomposed OM in this fraction, but also showed high peaks of esters and amides, both of which are easily decomposable C forms.

The C content of organic matter, as measured by the C:OM ratio, is known to change according to soil type and profile depth (Nelson and Sommers, 1982). However, this study indicated that it also changes with fraction size and sieving method used (Fig. 2.2). A 6% underestimation of C would have resulted if the C content of the 2000 to 250  $\mu\text{m}$  fraction was estimated from the amount of OM using the Van Bemmelen factor (0.58). From a methodological perspective, these results indicate that one should consider using fraction-specific conversion ratios when loss on ignition is used to estimate soil fraction C.

## **Impact of Management Intensity**

The higher soil C content associated with the low intensity management regime for the three  $>53 \mu\text{m}$  fractions indicated that intensive forest management reduced the soil C content in as little as 4 years (Fig 2.5). The DRIFTS spectra showed C loss across the entire spectrum of organic matter (Fig. 2.7). A possible cause of this decline would be the reduction of root input caused by the understory control practice. Shan et al. (2001) and Echeverria et al. (2004), working on similar soils in Georgia, have also reported a decrease in SOC due to intensive management, especially due to chemical understory control. Therefore, the hypothesis of C reduction under the high intensity management regime was accepted. However, it is uncertain whether this C reduction would be compensated by the increase in litterfall under the intensive management regime in the longer-term. The hypothesis of higher N content in all fractions was not accepted, since N content showed only a small increase (5%) in the finest fraction (Fig. 2.6). The fate of fertilizer N applied in the intensive management regime is also uncertain. Immobilization of fertilizer N in standing biomass, litter layer, or stored in the subsoil is probably responsible for this. This interpretation is supported by the work of Will et al. (2006), who reported that 68% of applied fertilizer N was stored in the aboveground biomass and forest floor, while only 21% was in the surface soil in loblolly pine stands growing in Georgia.

Size fractionation proved to be a more sensitive method than total SOC measurement for investigating changes in SOC. Management induced change in the 2000 to  $250 \mu\text{m}$  fraction was 23% of the fraction C concentration (Fig. 2.5), which represented greater than 12% change in total SOC, illustrating the sensitivity of this size fraction for assessing impacts of forest management. It is also noteworthy that these differences were observed just four years after the treatments were imposed, supporting the hypothesis that size fractionation enhances the detection of the short-term management induced changes in SOC.

Given the presence of large palmetto roots (*Serenoa repens* (B.) Small.) and other understory plants under the low intensity management regime (Roth et al., 2006), we expected that the fractions  $>2000 \mu\text{m}$  would decrease significantly with increased management intensity. The lack of a statistical C response to management in these fractions, the reduced C content in the 2000 to 250  $\mu\text{m}$  fraction in response to chemical weed control, and the “fresher” nature of the OM as suggested by DRIFTS imply that the large sand fraction accepts the greatest detrital inputs. This interpretation is consistent with the work of Van Rees and Comerford (1986) and Escamilla et al. (1991), who have reported palmetto and other understory root biomass in the 2000 to 250  $\mu\text{m}$  size class under southern pine plantations.

### **Conclusions**

Dry sieving was found to be a useful method for size fractionation for sandy Spodosols when compared to wet sieving, as it preserved more structure and the water-soluble components such as esters and amides. The size fractions were significantly different in all the properties studied. The 2000 to 250  $\mu\text{m}$  fraction was the most important fraction in these soils, as it contained nearly half of the total SOC and was sensitive to the management related changes. The DRIFTS spectra were useful for describing the changes in SOM chemical composition and indicated presence of recently added organic matter in the large sand fraction. Intensive forest management reduced soil C in the  $>53 \mu\text{m}$  fractions, and particularly in the 2000 to 250  $\mu\text{m}$  fraction, in just 4 years, probably due to the reduction in understory roots.

Table 2.1. The effect of wet and dry sieving on C content (g C in size fraction per 100g of whole soil) in soil size fractions for a sandy Spodosol in north Florida.

Fraction	>8000 $\mu\text{m}$	8000 to 2000 $\mu\text{m}$	2000 to 250 $\mu\text{m}$	250 to 150 $\mu\text{m}$	150 to 53 $\mu\text{m}$	< 53 $\mu\text{m}$	Total
Dry	0.31 <sup>†</sup> (0.02) <sup>‡</sup>	0.47 (0.02)	1.1 a <sup>¶</sup> (0.1)	0.36 a (0.02)	0.39 b (0.02)	0.15 a (0.01)	2.8 (0.3)
Wet	0.31 <sup>§</sup> (0.02)	0.47 (0.02)	1.3 a (0.1)	0.32 a (0.02)	0.30 a (0.01)	0.19 b (0.01)	2.9 (0.3)
% of Total	11	17	40	13	14	5	100
	10	16	46	11	10	7	100

<sup>†</sup>Each value is a mean of 36 observations averaged across treatment intensity and soil depth.

<sup>‡</sup> Values in parentheses represent standard error.

<sup>§</sup> The >8000 and 8000 to 2000  $\mu\text{m}$  fractions, though not wet sieved, are necessary for total C content calculations.

<sup>¶</sup> Within a size fraction, the means followed by different letters are statistically different at  $p < 0.05$  showing effect of both depth and sieving method.

Table 2.2. The distribution of N concentration and content among the soil size fractions for a sandy Spodosol in north Florida.

Property	>8000 $\mu\text{m}$	8000 to 2000 $\mu\text{m}$	2000 to 250 $\mu\text{m}$	250 to 150 $\mu\text{m}$	150 to 53 $\mu\text{m}$	< 53 $\mu\text{m}$	Whole soil
Concentration (% of fraction)	0.370 <sup>†</sup> e (0.018)	0.386 e (0.015) <sup>‡</sup>	0.116 c <sup>§</sup> (0.005)	0.030 a (0.002)	0.088 b (0.003)	0.290 d (0.004)	0.082 (0.004)
Content (% of whole soil)	7 a	12 b	39 d	19 c	16 c	7 a	100

<sup>†</sup> Each value is a mean of 72 observations averaged across sieving methods, treatment intensity and soil depth.

<sup>‡</sup> Values in parentheses represent standard error.

<sup>§</sup> The means followed by different letters are statistically different at  $p < 0.05$

Table 2.3 Discriminant analysis of the DRIFTS spectra for a sandy Spodosol in north Florida

Effect	R <sup>2</sup>	Root Mean Square Deviation	Extent of separation between spectra
Block†	0.25 to 0.55	0.29 to 0.38	Very little separation
Sieving method‡	0.94	0.12	Excellent separation
Management intensity§	0.71	0.27	Some separation
Depth¶	0.37	0.40	No separation
Fraction#	0.93 to 0.98	0.06 to 0.12	Excellent separation

† Four blocks (replications across space)

‡ Dry and wet sieving methods

§ High and low management intensities

¶ Depths 0 to 5 cm and 5 to 10 cm

# Fractions 2000 to 250, 250 to 150, 150 to 53 and <53 µm

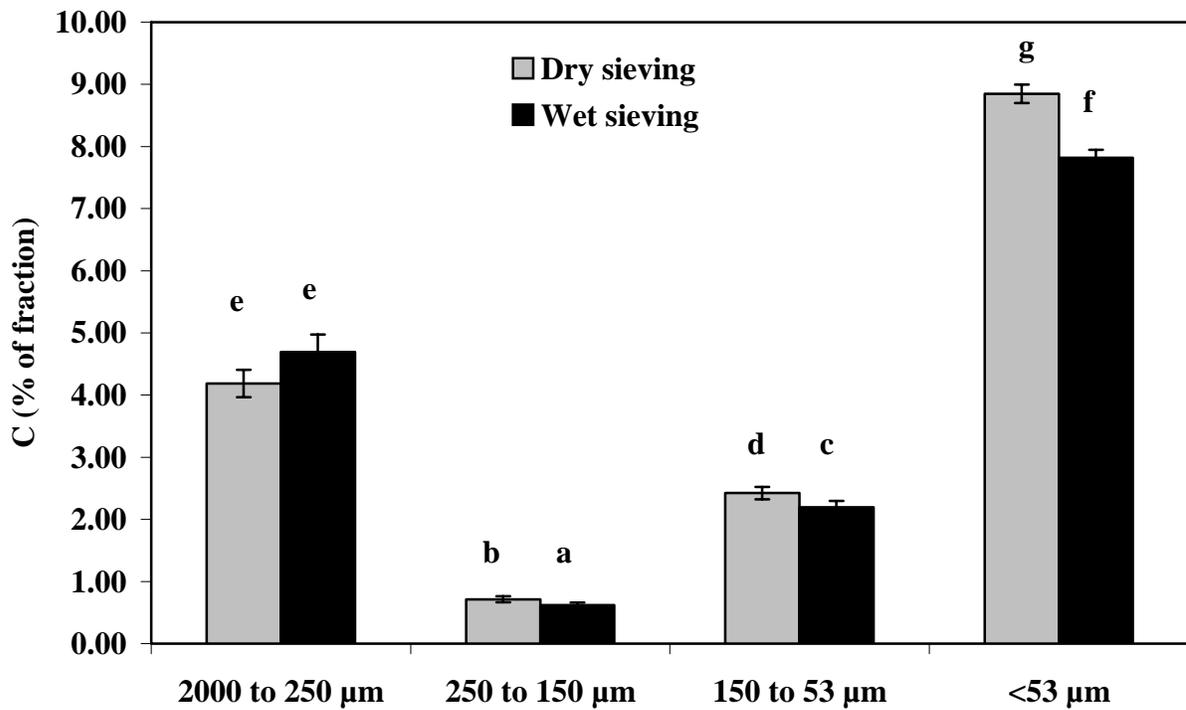


Figure 2.1. Carbon concentrations (% of fraction) of size fractions as affected by the dry and wet sieving for a sandy Spodosol in north Florida. Means followed by different letters are statistically different at  $p < 0.05$ . The error bars represent standard error. Each value is a mean of 36 observations averaged across treatment intensity and soil depth.

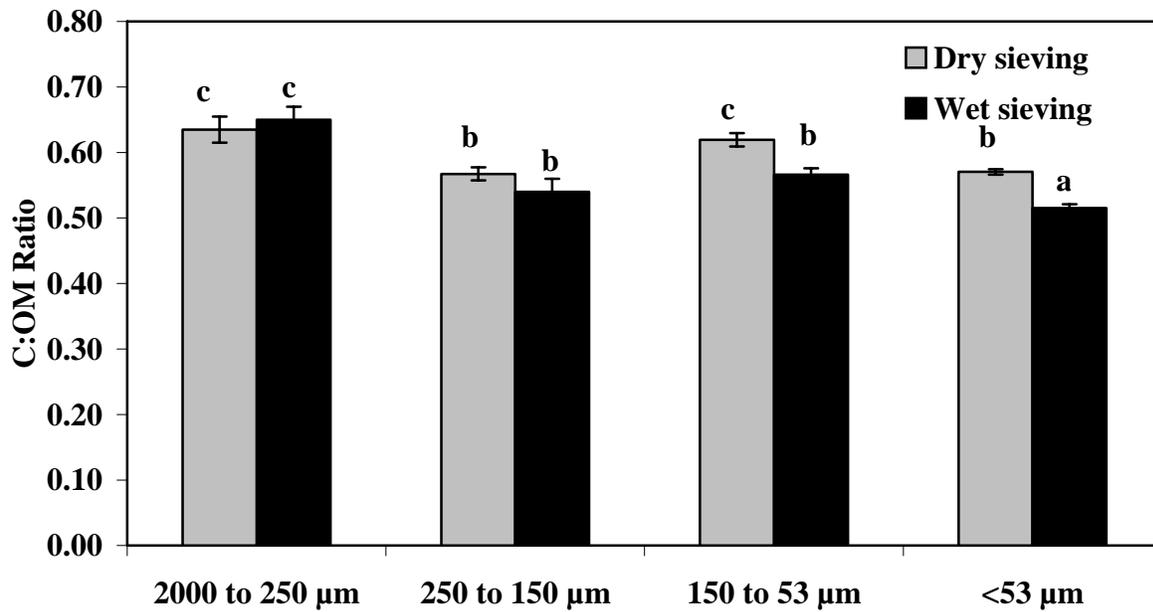


Figure 2.2. The interaction between fraction size and sieving method on the ratio of C to organic matter (C:OM) for a sandy Spodosol in north Florida. The means followed by different letters are statistically different at  $p < 0.05$ . The error bars represent standard errors. Each value is a mean of 36 observations averaged across management intensity and soil depth.

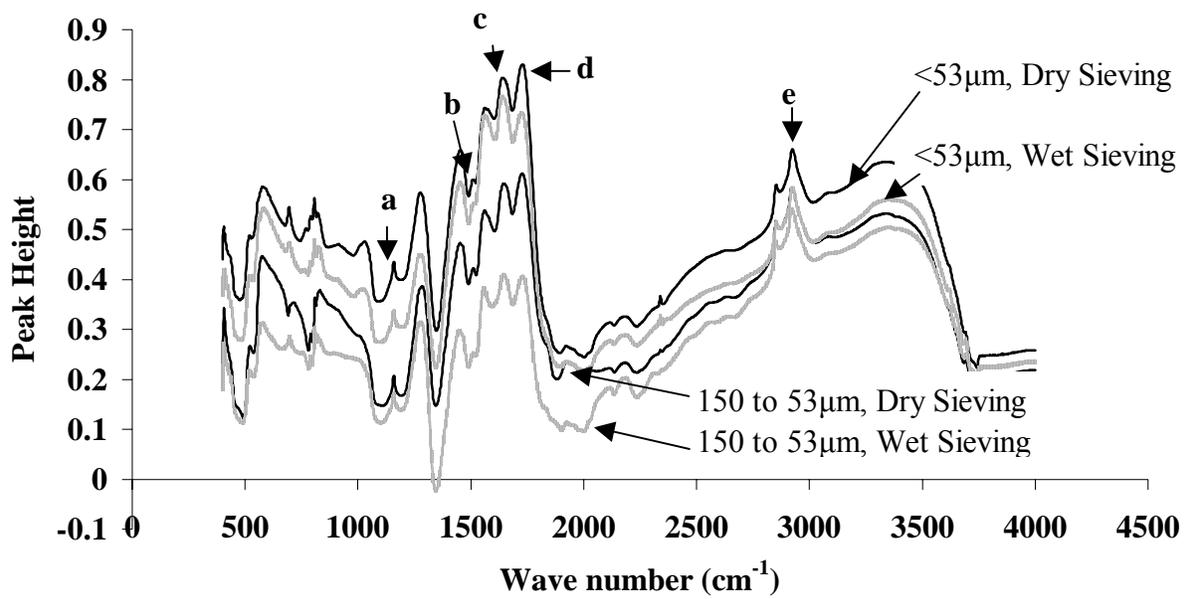


Figure 2.3. Diffuse Reflectance Infrared Fourier Transform Spectroscopy spectra showing effect of sieving method (150 to 53 and <53  $\mu\text{m}$  fractions, low intensity, 5 to 10 cm depth) for a sandy Spodosol in north Florida. a. polysaccharides, b. aromatic compounds, c. amides, d. esters, e. aliphatic  $-\text{CH}$ .

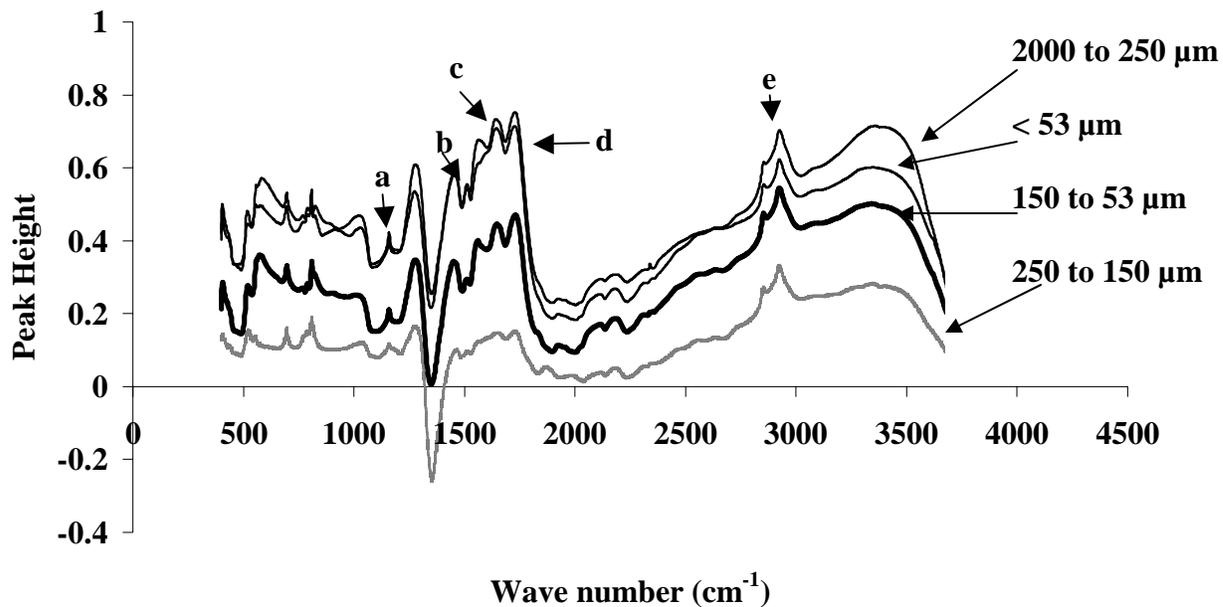


Figure 2.4. Diffuse Reflectance Infrared Fourier Transform Spectroscopy spectra showing effect of fraction size (dry sieving, high intensity, 0 to 5 cm depth) for a sandy Spodosol in north Florida. a. polysaccharides, b. aromatic compounds, c. amides, d. esters, e. aliphatic -CH.

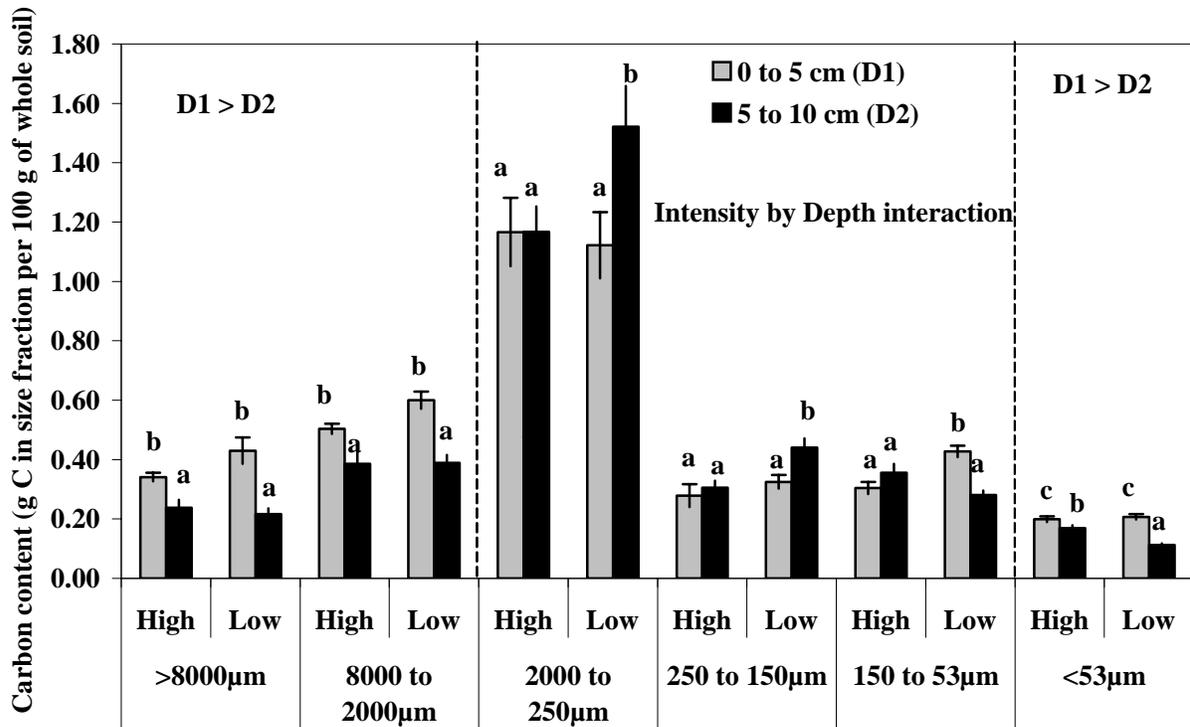


Figure 2.5. Effect of management intensity and soil depth on the C content of the soil size fractions for a sandy Spodosol in north Florida. Within a size fraction, the means followed by different letters are statistically different at  $p < 0.05$ , showing the effect of both management intensity and depth. Each value is a mean of 18 observations averaged across sieving methods.

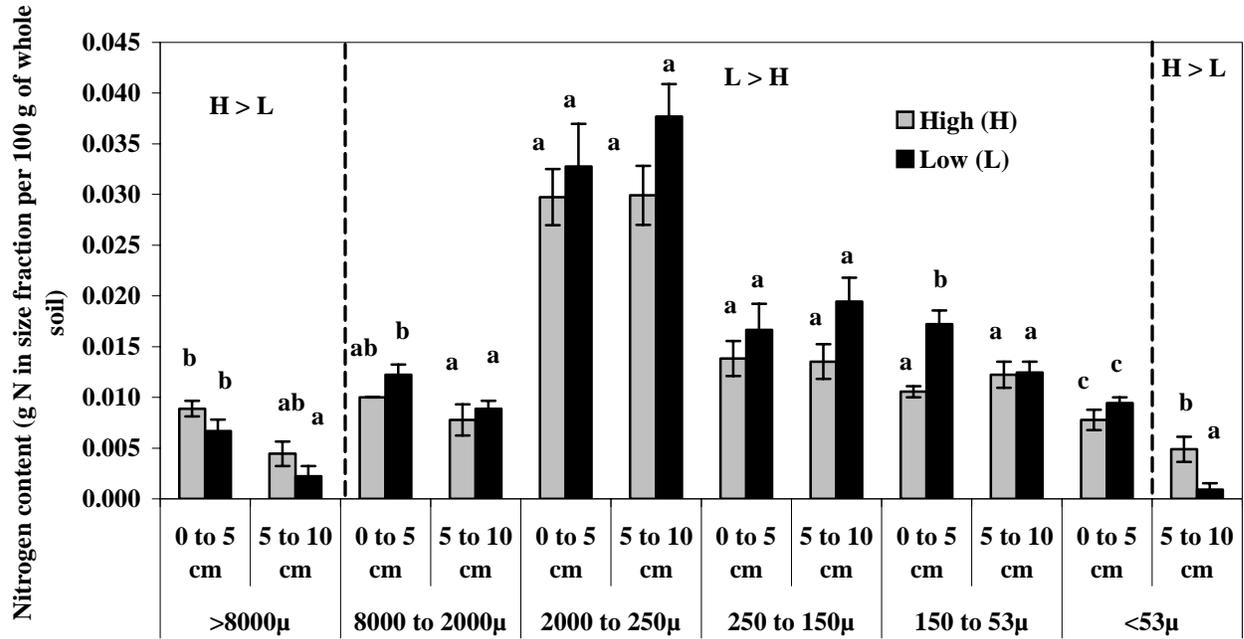


Figure 2.6. Effect of management intensity and soil depth on N content (g N in a soil fraction per 100g of whole soil) in soil size fractions for a sandy Spodosol in north Florida. Within a size fraction, the means followed by different letters are statistically different at  $p < 0.05$ , showing the effect of both management intensity and depth. Each value is a mean of 18 observations averaged across sieving methods.

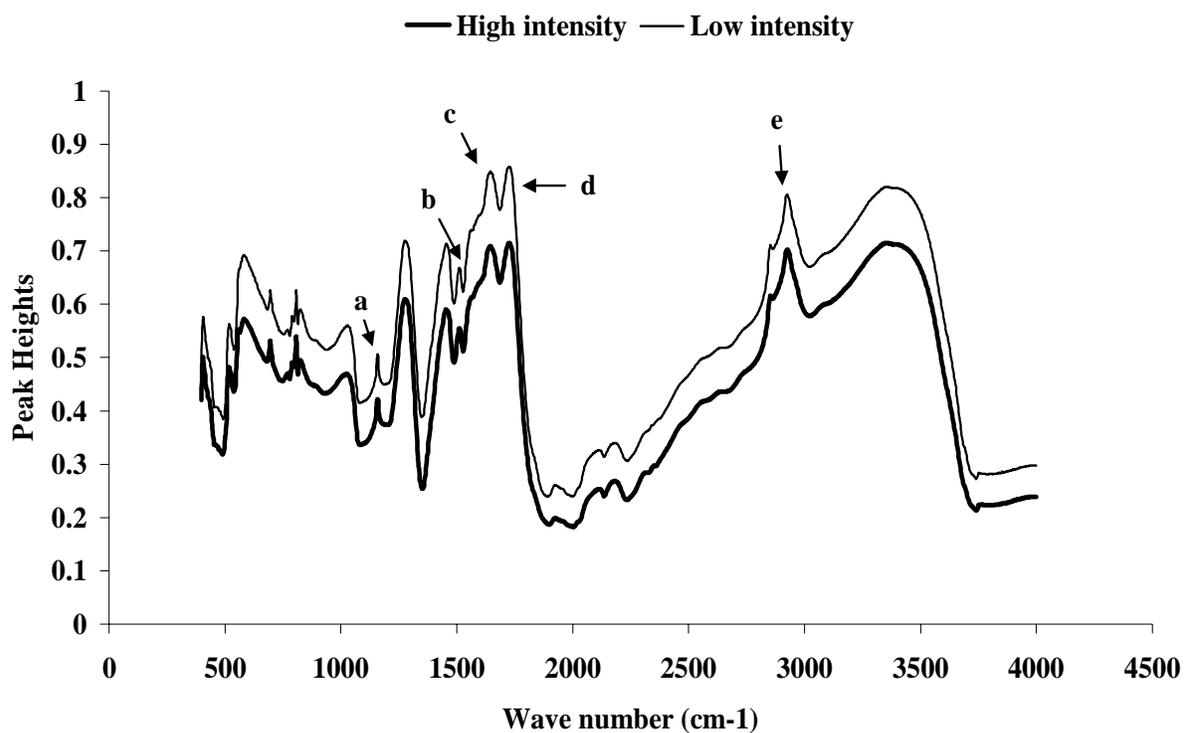


Figure 2.7. Diffuse Reflectance Infrared Fourier Transform Spectroscopy spectra showing effect of intensity (2000 to 250  $\mu\text{m}$  fraction, dry sieving, 0 to 5 cm depth) for a sandy Spodosol in north Florida. a. polysaccharides, b. aromatic compounds, c. amides, d. esters, e. aliphatic  $-\text{CH}$ .

CHAPTER 3  
SOIL AGGREGATION AND AGGREGATE CARBON IN A FORESTED SOUTHEASTERN  
COASTAL PLAIN SPodosol

**Introduction**

Carbon-wise soil management requires an understanding of the processes by which soil C is sequestered. Yet, understanding of these processes for forested soils of the southeastern USA is limited. Secondary forests in the southeastern USA accumulate C at a rate greater than 70 MT  $y^{-1}$  (Richter et al., 1995), which implicates them as important regional C sinks. Southern pine plantations are important components of these forests, covering more than 12 million ha (Wear and Greis, 2002). Many of these plantations are underlain by sandy Spodosols (Adegbidi et al., 2002), which represent the dominant soil order in Florida, covering 27% of the state (Stone et al., 1993). Many Spodosols are exceptionally sandy with less than 5% silt + clay and less than 10  $cmol_c kg^{-1}$  of cation exchange capacity (Carlisle et al., 1981, 1988, 1989; Sodek et al., 1990).

Forest fertilization and chemical weed control are two management inputs that have increased productivity of southern pines in these landscapes. However, fertilization has not promoted an increase in soil C (Harding and Jokela, 1994; Shan et al., 2001); while chemical weed control, presumably by reducing detrital inputs of understory plants, has reduced the soil C content (Shan et al., 2001; Echeverria et al., 2004). The effects of these practices on the development of aggregates and the soil C contained within them have yet to be considered.

Soil organic carbon (SOC) can be protected from decomposition through four mechanisms: sorption onto clay particles (chemical protection), incorporation into aggregates (physical protection), movement to subsoils (translocation), and biochemical transformation into products that are resistant to microbial attack (biochemical protection; Six et al., 2002; Blanco-Canqui and Lal, 2004; Jiménez and Lal, 2006). The soil structure of Florida's Spodosols is described as weak crumb to granular or single grain (Carlisle et al., 1981, 1988, 1989; Sodek et al., 1990),

suggesting poor soil aggregation. In these soils, the low cation content limits aggregate formation through clay-polyvalent cation-organic matter complexes (Edwards and Bremner, 1967), though aggregates can form under the influence of microbial products, fungal hyphae, and roots (Tisdall and Oades, 1982; Blanco-Canqui and Lal, 2004). The potential for chemical protection of soil C is also limited by the low clay content. Given these factors, the interest in aggregation in sandy Spodosols in the southeastern U.S. has been low as evidenced by the few studies addressing this topic.

In Russian Spodosols, aggregation did respond to agricultural management. Water-stable aggregation and total soil C content increased after just two years under the influence of a grass-clover mixture, but decreased when followed by spring wheat (Buchkina and Balashov, 2001). In Florida, soil aggregation was found to be influential in P dynamics. Higher water-extractable P and heavy metals, along with slower rates of release, were found in the 500 to 250  $\mu\text{m}$  and 250 to 125  $\mu\text{m}$  aggregates compared to the smaller size fractions (Zhang et al., 2003). Sandy Spodosols in Florida represent a unique soil condition and it was deemed necessary to better understand aggregation under these conditions.

The purpose of this research was to study aggregation and its relation to SOC in a representative forested Spodosol of northern Florida. The first objective was to observe the aggregation present in these soils. We hypothesized that aggregation, albeit weak, was present in the  $< 2$  mm fraction in these extremely sandy soils because of the high input from root turnover and aboveground litter. The second objective was to determine the strength of aggregates in the  $< 2$  mm fraction, and quantify the amount of aggregate C. The hypotheses related to this objective were that: (1) aggregate strength, as measured by an aggregate's resistance to dispersion, would increase with decreasing aggregate size, and (2) that particulate C would be the dominant pool of

SOC. The first hypothesis grew from the concept that smaller particles have greater surface area available for binding; hence, the aggregate's strength/stability would be greater. The second hypothesis recognized that SOC could be found as either particulate organic matter (POM) or aggregate organic matter (AOM). Given the weak structure often described for Coastal Plain Spodosols, the dominance of POM would be expected.

The third objective of this research was to provide preliminary information on the short-term influence of two contrasting management intensities on the amount and distribution of AOM and POM. The hypothesis was that intensive management (more fertilization and chemical weed control) would equate to decreases in aggregation. It was expected that reduced root turnover of understory plants, resulting from sustained chemical control, would cause reduced aggregation and short-term reductions in soil C.

## **Materials and Methods**

### **Experimental Site**

A loblolly pine (*Pinus taeda* L.) plantation in north Florida (30°24'N lat; 82°33'W long) was the study site and it was managed by the Forest Biology Research Cooperative at the University of Florida as part of the Pine Productivity Interactions Experimental Study (PPINES; Roth et al., 2006). This long-term study aims at understanding the family x environment interactions in full-sib loblolly and slash pine (*P. elliottii* Engelm. var. *elliottii*) families. The climate is warm, humid subtropical, with 1,394 mm average annual rainfall, 27°C average annual maximum temperature, and 13°C average annual minimum temperature (NOAA, 2002). The soil is classified as a Leon series (sandy, siliceous, thermic Aeric Alaquod), with < 5% silt + clay, and < 10 cmol<sub>c</sub> kg<sup>-1</sup> of cation exchange capacity.

The trees were planted in January 2000 in four replicates using a randomized complete block, split plot design. Prior to planting, the entire study was double bedded and treated with the

herbicides Arsenal (imazapyr, 1.02 L ha<sup>-1</sup>) and Garlon (triclopyr, 7.02 L ha<sup>-1</sup>) to remove the understory vegetation and to provide a competition free environment. The experimental design was a 2 x 2 x 8 factorial, which included two planting densities (close spacing at 1.3 x 3 m and wide spacing at 3 x 3 m), two management regimes (high and low inputs), and six elite loblolly pine full-sib families, a mix of these elite families, and one poor growing family. The families were designated *a priori* based on their growth performance in long-term genetic experiments. Two treatment combinations representing the maximum differences in biomass production were selected in order to evaluate the capacity for short-term SOC changes, with the idea that the differences in input would be reflected in the differences in SOC pools. The high intensity treatment included the most productive family under sustained understory competition control using herbicides and annual fertilization with a complete fertilizer. The low intensity treatment was planted with the poorest performing family and managed with a one-time fertilizer application and understory competition control at planting. The fertilizer added to the high intensity treatment totaled 368 kg ha<sup>-1</sup> N and 128 kg ha<sup>-1</sup> P plus nearly all other essential nutrients (i.e., 121 kg ha<sup>-1</sup> K, 45 kg ha<sup>-1</sup> Mg, 45 kg ha<sup>-1</sup> Ca, 35 kg ha<sup>-1</sup> S, 0.89 kg ha<sup>-1</sup> B, 3 kg ha<sup>-1</sup> Zn, 2 kg ha<sup>-1</sup> Mn, 16 kg ha<sup>-1</sup> Fe, 4 kg ha<sup>-1</sup> Cu, 0.01 kg ha<sup>-1</sup> Mo); while the low intensity treatment included 45 kg ha<sup>-1</sup> N and 50.6 kg ha<sup>-1</sup> P applied as diammonium phosphate. In the high intensity treatment only, Arsenal (0.28 L ha<sup>-1</sup>) and Oust (0.14 L ha<sup>-1</sup>) provided sustained understory competition control. Both treatments were planted at the 1.3m x 3 m spacing (close spacing - 2,900 trees ha<sup>-1</sup>) and each treatment plot was 480 m<sup>2</sup> in size. The entire study was treated when necessary with insecticides (Dimilin, Pounce or Mimic) for tip moth (*Rhyacionia* spp.) control during the first growing season.

Soil samples were collected from the A horizon at depth increments of 0 to 5 cm and 5 to 10 cm from each treatment plot in three replicate blocks in September 2003. One combined soil sample from each depth of each plot (treatment within a block) came from four individual soil samples. The four individual soil samples were collected from alternate interbed rows, while within an interbed, sample locations were chosen randomly.

### **Laboratory Methods**

Soil samples were air-dried and passed through a 2 mm sieve. They were then dry sieved to separate the aggregates into four size classes with minimum disruption of aggregation (a macroaggregate fraction 2000 to 250  $\mu\text{m}$ , two microaggregate fractions 250 to 150  $\mu\text{m}$  and 150 to 53  $\mu\text{m}$  as well as a <53  $\mu\text{m}$ ). A preliminary study determined that there was no significant difference in weight distribution among the four size classes after shaking the sieves for 5 minutes. Therefore, this time frame was used for all dry sieving.

The first part of the investigation included a microscopic examination of aggregate morphology in the dry sieved size fractions. The intent was to determine if there was identifiable aggregation and a positive result would justify examining other objectives. Aggregate samples were examined and photographed using a dissecting light microscope (Carl Zeiss 475003-9902) with a mounted digital camera (Sony MVC FD90). A scanning electron microscope (SEM) (JEOL JSM 6400) equipped with an energy-dispersive x-ray fluorescence elemental microanalysis (EDX) system was used for obtaining images and silica dot maps within aggregates. Samples were prepared for SEM by mounting on C stubs and coating with C.

Upon finding aggregates and noting that they were a significant component of the soil matrix, the next step was to examine aggregate strength and to determine the quantity of C contained in stable aggregates. This analysis was performed on the three >53  $\mu\text{m}$  fractions using sonication to input energy into a water-soil system. Sonication has been used for aggregate

disruption (North, 1976; Six et al., 2001; Swanston et al., 2005) because, unlike chemical dispersion techniques, it avoids chemical modification of the organic matter. It also allows measurement of aggregate strength on an energy basis, which allows for a quantitative comparison of samples. Energy inputs to the size fractions ranged from 0 to 27,000 J and were achieved with a Sonic Dismembrator (Fisher Scientific, model 500) by using a range of amplitude (20 to 60%) and time (1 to 7 min) combinations. The energy output was given by the sonicator and was calculated by internal software using voltmeter readings recorded every 10 seconds (Fisher Scientific, personal communication, 2006). The energy output thus calculated was replicable (coefficient of variation < 10%). The pulse method (60 sec ON and 30 sec OFF) was used to avoid an excessive rise in temperature.

Each size fraction was sonicated at incremental energy levels. This was accomplished by using one sub-sample for each energy level until complete aggregate breakdown was achieved. Microscopic observation and release of soil organic matter (SOM) was used to ensure that all the aggregates were disrupted. This analysis was repeated on the >53  $\mu\text{m}$  fractions of 12 soil samples representing three replications of both management intensities and soil depths. For each sample, sonication was done at 9 to 11 energy levels (one sub-sample for each energy level). For each sub-sample, 2 g soil was weighed into a 250 mL beaker to which 100 mL water was added. The suspension was sonicated at the desired energy level. Depth of immersion of the sonicator probe was kept constant at 10 mm, as this variable is known to influence the degree of disruption (North, 1976). The suspension was then passed through the same size sieve used to obtain the size fraction (e.g., 250  $\mu\text{m}$  sieve for the 2000 to 250  $\mu\text{m}$  fraction). The SOM remaining on the sieve and the SOM passing through the sieve were measured by loss on ignition.

Organic matter passing through the sieve after sonication was termed aggregate organic matter (AOM), as it contained finer organic matter held inside the aggregates that was released after aggregate disruption. It was expressed as percent of total organic matter in each sample. Organic matter remaining on the sieve after sonication was termed particulate organic matter (POM) which, after complete aggregate dispersion, contained SOM of the same size. Energy input was plotted against AOM to obtain the response curve for each sample. The AOM lost at 0 J represented the organic matter associated with water-dispersible aggregates. The POM data are not reported because the focus of this paper is aggregation and also because POM can be derived from the AOM data ( $POM = 100 - AOM$ ).

Upon finding that AOM was a significant component of total organic matter, the chemical nature of AOM and POM was investigated through the use of Diffuse Reflectance Infrared Fourier Transform Spectroscopy (DRIFTS). Samples were scanned before and after ashing (at 550°C for 6 h) in the mid-infrared on a Digilab FTS-7000 Fourier Transform Mid-infrared Spectrometer (Varian Instruments; Walnut Creek, CA). Samples were scanned from 4000 to 400  $cm^{-1}$  at 4  $cm^{-1}$  resolution using a KBr beamsplitter and DTGS detector and a Pike Autodiff autosampler (Pike Technologies, Madison, WI) using ground, non-KBr diluted samples. Spectral subtraction of ashed samples from non-ashed samples was performed to accentuate differences in organic matter characterization using GRAMS/AI software Ver. 7.02 (Thermo Galactic, Salem, NH).

### **Statistical Analysis**

The equation,  $y = (a*x) (b + x)^{-1}$  was used to fit the energy vs. AOM release data for each size fraction; where,  $a$  = maximum AOM lost for the size fraction,  $b$  = energy level at  $0.5*a$ ,  $x$  = sonicator energy output (J) and  $y$  = AOM. The energy output for each individual run was used for this analysis.

PROC MIXED (SAS, 1996) was used to contrast the effects of management intensity and depth based on a completely randomized design, with energy level (i.e., each unique combination of amplitude and time), size fraction, management intensity and soil depth as fixed effects and block as a random effect. The differences were considered significant at  $p < 0.05$ . There was a significant energy level x management intensity x size fraction interaction. Therefore, further statistical analyses were run separately for each size fraction. A multiple post-hoc comparison procedure with the Tukey-Cramer adjustment was used.

## **Results**

### **Aggregate Morphology**

Microscopic observation identified two qualitative categories of aggregation. Irregular shaped aggregates (Fig. 3.1.1 – 3.1.4) had mineral matter, organic debris and fungal hyphae and/or fine roots enmeshed together. Spherical aggregates exhibited mineral matter encrusted on organic debris or plant remains (Fig. 3.1.5) with or without fungal hyphae and/or fine roots (Fig. 3.1.6). The internal structure of the aggregates, as shown by the silicon dot maps (Fig. 3.1.1 – 3.1.4), indicated mineral matter embedded in the organic matter. Images suggested a role of fungal hyphae and fine roots in aggregate formation, either through mechanical enmeshing (Fig. 3.1.1 - 3.1.5) or through encrustation of mineral matter on plant remains (Fig. 3.1.7).

### **Quantifying Organic C in Aggregates**

On average, 46% of the total soil C (Table 3.1) was contained in the soil aggregates. As the energy input to the soil sample increased, aggregates were destroyed, increasing the amount of AOM removed from that size class of soil material (Fig. 3.2). Eventually a plateau was reached, indicating that all the organic matter that could be removed from the aggregates was removed (Fig. 3.3). This finding was supported by microscopic observations (Fig. 3.1.8, 3.1.9), which indicated that only POM was present when the plateau was reached. The energy level at which

this plateau was reached exceeded the strength of the most stable aggregates in the size fraction studied. The energy required for the complete breakdown of aggregates (Fig. 3.3) increased in the order 250 to 150  $\mu\text{m}$  (8500 J) < 2000 to 250  $\mu\text{m}$  (13300 J) < 150 to 53  $\mu\text{m}$  (16422 J).

The shapes of the size fraction response curves were different (Fig. 3.3) among the three size fractions, which indicated diversity in the nature of aggregates. The aggregates in the 250 to 150  $\mu\text{m}$  fraction were the least stable among the three fractions, losing the highest proportion of its organic matter (y) at all energy levels (x). The 150 to 53- $\mu\text{m}$  fraction had the most stable aggregates (Fig. 3.3, Table 3.2). The 2000 to 250  $\mu\text{m}$  and 250 to 150  $\mu\text{m}$  fractions (Fig. 3.3) exhibited a step-wise loss of organic matter, with steps at 2500 J and 6000 J (Fig. 3.3). The 150 to 53  $\mu\text{m}$  fraction (Fig. 3.2, 3.3) exhibited a continuous spectrum of organic matter loss with increasing energy input. The variability (Table 3.2) of organic matter release in AOM decreased with decreasing fraction size, while within a fraction, the variability decreased with increasing energy output. The highest variability was observed at 0 J (i.e., in the water-dispersible aggregates). All three size fractions were susceptible to organic matter loss by wetting, with values ranging from 5 to 17% (Table 3.2).

The selected equation proved satisfactory to model the response of AOM to sonication energy output for 250 to 150  $\mu\text{m}$  ( $R^2 = 0.93$ ) and 150 to 53  $\mu\text{m}$  ( $R^2 = 0.96$ ) fractions (Table 3.2). For the 2000 to 250  $\mu\text{m}$  fraction, however, the equation did not work as well ( $R^2 = 0.84$ ) due to the well-defined steps in the loss of organic matter.

The DRIFTS spectra separated the POM and AOM fractions (Fig. 3.4). The AOM fraction exhibited higher quantities of polysaccharides ( $1160\text{ cm}^{-1}$ ), aromatic rings ( $1500\text{ cm}^{-1}$ ), esters ( $1730\text{ cm}^{-1}$ ) and amides ( $1650\text{ cm}^{-1}$ ) than the POM fraction. The negative peak at  $1350\text{ cm}^{-1}$  on the POM spectra was due to distortions caused by the spectral subtraction and should be ignored.

The peaks at  $2880\text{ cm}^{-1}$  and  $2950\text{ cm}^{-1}$  were due to aliphatic -CH groups. Peak heights indicated higher content of these materials in the AOM component within the size fractions.

### **Effect of Management Intensity**

The effects of energy input and size fractions on AOM in the size fractions were significant at  $p < 0.01$  (Table 3.3). There was also a significant energy level x management intensity x size fraction interaction in the AOM data. The effect of management intensity on AOM was statistically significant in the 2000 to  $250\text{ }\mu\text{m}$  fraction, with an energy level x management intensity interaction present (Fig. 3.5). At lower energy levels, treatment differences were small. However, beyond about 6,000 J the low intensity treatment had higher AOM. The energy level x management intensity interaction was also significant for the 250 to  $150\text{ }\mu\text{m}$  fraction, but the absolute difference between the management intensities was less than 4%. The 150 to  $53\text{ }\mu\text{m}$  fraction was not affected by the intensity of management.

### **Discussion**

Forested Spodosols of southeastern U.S. are important regional C sinks (Richter et al., 1995) and understanding the C sequestration mechanisms in these soils can help in maintaining and improving the C storage potential of these forests. Physical protection of C by incorporation into aggregates is an important mechanism of C sequestration and this study was conducted with an objective of understanding the different aspects of aggregation in these soils.

### **Aggregate Morphology, Stability and OM Content**

The microscopic observations confirmed the presence of aggregates and a range of aggregate forms (Fig. 3.1). Thus, the hypothesis regarding the presence of aggregates was accepted. The images further implicated the role of fungal hyphae and fine roots in aggregation (Fig. 3.1.1-3.1.5), while the DRIFTS spectra suggested polysaccharides as an aggregate binding agent (Fig. 3.4). Oades (1993) discussed the importance of biological agents of aggregation in

sandy soils, since the abiotic mechanisms of aggregation are most important in soils with clay contents greater than 15% (Horn, 1990). The binding action of polysaccharides, secreted by fungi or bacteria, has also been previously reported (Tisdall, 1994; Caesar-Tonthat, 2002; Blanco-Canqui and Lal, 2004). Our observations provide justification to further study the significance of roots, fungi and polysaccharides as biological agents for aggregation in these sandy soils, while questioning their role in sequestering C.

Sonication facilitated making quantitative estimates of aggregate stability and aggregate C. The hypothesis (second objective) suggesting an inverse relationship between aggregate size and stability was rejected because the 250 to 150  $\mu\text{m}$  fraction was less stable than both the 2000 to 250  $\mu\text{m}$  and 150 to 53  $\mu\text{m}$  fractions (Fig. 3.3). The higher aggregate strength of the 2000 to 150  $\mu\text{m}$  fraction, which was counter to our hypothesis, appeared to be a function of a microaggregate/macroaggregate hierarchical structure (see discussion below; Oades and Waters, 1991).

Aggregates, through physical occlusion, protect organic matter from destructive agents such as physical breakdown by tillage, removal of finer particles by erosion, or decomposition by soil organisms of different sizes. Not all aggregates offer protection from all of these agents. The extent of protection depends on the size of pores within the aggregates and the strength of the aggregates, which in turn depends on the binding agents and the size of the primary particles in the aggregate. The aggregate strength should indicate the extent of mechanical protection (e.g., from breakdown by tillage), but the extent of protection from the soil microbes is not certain. In the absence of sufficient clay, it is possible that one form of organic matter may be protected by another form. For example, a kind of biochemically inert material such as aromatic compounds may discourage the entry of microbes inside the aggregates and protect the labile organic matter

inside. Sonication, as proposed in this study, combined with chemical analyses of the C lost from aggregates of differential stability, offers the opportunity to advance these studies.

The hypothesis suggesting the dominance of POM was only partially supported, since this fraction accounted for just over half of the total organic matter. On the other hand, the possibility of partial breakdown of POM during sonication does suggest that a greater proportion of total SOM may be in this pool. Though AOM is an important pool of sequestered C, POM may be a more important in determining the short-term turnover of essential nutrients (Haynes, 2005).

### **Effect of Management Intensity**

The AOM in the 2000 to 250  $\mu\text{m}$  fraction was reduced by intensive management (Fig 3.5). Therefore, the hypothesis of a short-term reduction in aggregation by intensive management was accepted. The effect of management intensity could be partly attributed to changes in fine root biomass. Intensive management, especially chemical control of understory plants, has been reported to decrease the fine root biomass and length (Escamilla et al., 1991; Shan et al., 2001). Higher decomposability due to fertilization (Polglase et al., 1992a) may also contribute to reductions in AOM. However, it is unclear whether these differences between management intensities will be sustained over time. The higher levels of productivity and C inputs reported under intensive management (Dalla Tea and Jokela, 1991; Jokela and Martin, 2000) could result in longer-term opportunities for higher aggregation; especially after canopy closure, when nutrient deficiencies reduce site productivity levels under the low intensity management regime (Jokela et al., 2004).

### **Methodological Considerations**

The response of AOM in the 2000 to 250  $\mu\text{m}$  size fraction to management intensity (Table 3.1, Fig 3.5) illustrated the sensitivity of the sonication technique to soil organic matter changes in as few as four years after treatment. It also used operationally defined C fractions that could be

related to meaningful C pools. The two pools separated by this procedure within a size fraction, POM and AOM, showed a marked difference in their chemical composition as indicated by the DRIFTS spectra (Fig. 3.4). The AOM showed higher content of polysaccharides, phenols, esters and amides; of which polysaccharides have already been shown to function as binding agents (Tisdall, 1994). The higher aromatic C content also suggested the presence of more biochemically inert organic matter. Higher amounts of esters and amides, on the other hand, suggested that this fraction is susceptible to decomposition if the aggregates were destroyed, since esters and amides are highly reactive C forms. The differences in chemical composition indicated that this method should be useful in separating soil C into more functional pools and lead to better conceptualization of the cycling of SOC when used in conjunction with chemical decomposition/mineralization studies.

North (1976) used a similar method of aggregate strength measurement, which has been reproduced by others (Schmidt et al., 1999; Roscoe et al., 2000). However, North (1976) used the amount of clay lost as an indicator of aggregate destruction, which made it unsuitable for the highly sandy soils examined in this study. Clay can become saturated with organic matter (Hassink et al., 1997) and so it is not necessarily an appropriate measure for protection of C in soils with low clay content. This indicates the suitability of organic matter release instead of clay release in the sonication technique. The method described here can also be used to study aggregates of varying stability by sonicating the soil at different energy levels and analyzing the remaining aggregates for properties such as age or mineralizability.

### **Aggregate Structure in Coastal Plain Spodosols – Additional Considerations**

The structure in Spodosols has been described as weak (Carlisle et al., 1981, 1988, 1989; Sodek et al., 1990). Consequently, aggregation in these soils has received little, if any, attention. Oades and Waters (1991) studied the patterns of aggregate breakdown in Mollisols, Alfisols and

Oxisols. In Oxisols, the aggregates broke down to release primary particles. In Mollisols and Alfisols, the authors reported a hierarchical structure; where the larger, weaker aggregates broke down to release smaller, stronger aggregates, before breaking down into primary particles. Results from this study indicated that the surface horizon of sandy Coastal Plain Spodosols also had an aggregate hierarchy, as exhibited by the step-wise breakdown of aggregates in the 2000 to 250  $\mu\text{m}$  and 250 to 150  $\mu\text{m}$  fractions (Fig. 3.3). In the 2000 to 250  $\mu\text{m}$  fraction, the least stable aggregates were disrupted at energies  $\leq 2500$  J. At energy levels greater than 2500 J, a plateau in percent OM lost (AOM) indicated that there was no further aggregate destruction until 3700 J. We interpret this energy level as the threshold for aggregates of second order, which were destroyed between 3700 J to 6000 J. A second plateau was observed between 6000 J to 7500 J. This was the threshold for the third order of aggregates, which started breaking down at 7500 J and the loss went on until 13300 J when all the aggregates in this fraction were destroyed. In the 2000 to 250  $\mu\text{m}$  fraction, the well-defined steps indicated a well-developed structure. In the 250 to 150  $\mu\text{m}$  fraction, although the first two steps were observed at the same energy levels, they were less distinct and all the aggregates were destroyed at 8500 J, indicating a poorly developed structure. However, the 150 to 53  $\mu\text{m}$  fraction exhibited a continuous spectrum, suggesting that this fraction was simply a continuum of aggregates of different stabilities. This behavior appears unique for these Spodosols and requires further study, especially since this C pool remained unaffected by the intensive management regime.

Edwards and Bremner (1967) defined microaggregates as the water stable aggregates bound by strong clay-polyvalent metal-organic matter complexes. The authors used 250  $\mu\text{m}$  as the separation point between macro and microaggregates. Tisdall and Oades (1982) also used 250  $\mu\text{m}$  as the separation point between micro and macroaggregates. They described

microaggregates as those stabilized by organo-mineral complexes and resistant to disruption by wetting, cultivation or other disturbances. In contrast, macroaggregates were described as those stabilized by roots and fungal hyphae, having varying stability depending upon management and other factors. Apparently, the separation point at 250  $\mu\text{m}$  was chosen based on the increase in aggregate strength and difference in binding agents. However, the similar behavior of the 2000 to 250 and 250 to 150  $\mu\text{m}$  fractions, as well as the low stability and presence of roots and fungal hyphae in the 250 to 150  $\mu\text{m}$  fraction, suggests that 150  $\mu\text{m}$  is a more appropriate separation point for our soils. The 150 to 53  $\mu\text{m}$  fraction, although stable and unaffected by management, was susceptible to loss by wetting (Table 3.2). This indicates that size alone is insufficient to define aggregates types in these sandy textured soils. A quantitative measure of aggregate strength such as the energy required to achieve complete aggregate disruption can be used to improve the definition. It should be noted that sonication was used to measure mechanical strength. Chemical modifications may break aggregates before the threshold for mechanical failure is reached. These factors, in addition to the limited potential for clay or cation binding, indicate the necessity of a different approach for studying aggregation in sandy Spodosols.

### **Conclusions**

This study found that aggregates form in sandy Spodosols and they have a hierarchal structure in the large soil size fractions. The use of organic matter release instead of clay release after aggregate breakdown by sonication was useful for studying aggregate properties. It allowed simultaneous measurement of aggregate strength and amount of aggregate organic matter. Aggregate C was an important pool in these soils and the intensive management regime used to enhance pine plantation productivity reduced this C pool significantly in the short-term. Results from this study highlight the necessity of using a different approach for aggregate classification in sandy soils as well as for quantification of aggregate characteristics like stability. Finally,

results from this study highlight the need to assess the long-term, management-related changes using quantifiable C pools for assessing the metrics of sustainability.

Table 3.1. Amount of organic C held in soil aggregates for a sandy Spodosol in north Florida.

Size fraction ( $\mu\text{m}$ )	C content in size fraction <sup>†</sup> (g C in size fraction $100 \text{ g}^{-1}$ soil)	C content in soil aggregates (g C in fraction $100 \text{ g}^{-1}$ soil)
2000 to 250	1.1	0.69 (63%) <sup>‡</sup>
250 to 150	0.36	0.30 (84%)
150 to 53	0.39	0.28 (72%)
< 53	0.15	NA <sup>§</sup>
Total	2.8	1.28 (46%)

<sup>†</sup> The C content was measured by loss on ignition and the standard Van Bemmelen factor (0.58) was used for conversion of organic matter into C content.

<sup>‡</sup> The values in parentheses represent the average proportion of organic matter lost from the size fraction after sonication at the highest energy level. The percent loss remained the same for organic matter and C. See Table 3.2 for the statistical analysis.

<sup>§</sup> NA: Not Applicable, since this fraction was not analyzed for aggregation.

Table 3.2. Energy output of the sonicator probe and the amount of organic matter lost from each soil size fraction as aggregate organic matter (2000 to 250; 250 to 150 and 150 to 53  $\mu\text{m}$ ) for each energy level for a sandy Spodosol in north Florida.

2000 to 250 $\mu\text{m}$		250 to 150 $\mu\text{m}$		150 to 53 $\mu\text{m}$	
Energy (J)	Aggregate OM (%†)	Energy (J)	Aggregate OM (%)	Energy (J)	Aggregate OM (%)
0 (0)	5‡ a§ (1¶)	0 (0)	17 a (2)	0 (0)	6 a (1)
945 (6)	14 b (1)	936 (11)	39 b (1)	2813 (34)	23 b (1)
2729 (64)	22 c (2)	2468 (55)	54 c (1)	5997 (55)	45 c (2)
3793 (21)	23 c (2)	3805 (19)	60 c (1)	9445 (57)	59 d (1)
5973 (35)	45 d (2)	5969 (44)	76 d (1)	12186 (84)	65 e (1)
7446 (47)	47 de (3)	7450 (52)	78 d (1)	13513 (270)	67 ef (1)
8481 (42)	55 ef (2)	8467 (54)	82 d (1)	16422 (362)	70 f (1)
9489 (67)	58 fg (3)	9467 (65)	83 d (1)	20447 (536)	71 f (1)
10505 (55)	60 fg (2)	10516 (44)	84 d (1)	22297 (208)	72 f (1)
13367 (55)	60 fg (3)	NA††	NA	NA	NA
19691 (312)	63 g (2)	NA	NA	NA	NA
$y = (95.7 * x) / (7508.3 + x)$ # $R^2 = 0.84$		$y = (97.1 * x) / (1773.2 + x)$ $R^2 = 0.93$		$y = (98.4 * x) / (7038.2 + x)$ $R^2 = 0.96$	

† The aggregate OM or AOM is expressed as a percent of the total OM in the size fraction.

‡ Each value is a mean of 12 samples.

§ Within a size fraction, the means followed by different letters are statistically different at  $p < 0.05$  and show the effect of sonication energy level. The Tukey-Cramer adjustment for mean separation was used.

¶ Values in parentheses represent standard error.

# The equations are in the form  $y = (a * x) / (b + x)$ ; where, a = maximum AOM, b = energy level at  $0.5 * a$ , x = sonicator energy output (J) and y = AOM.

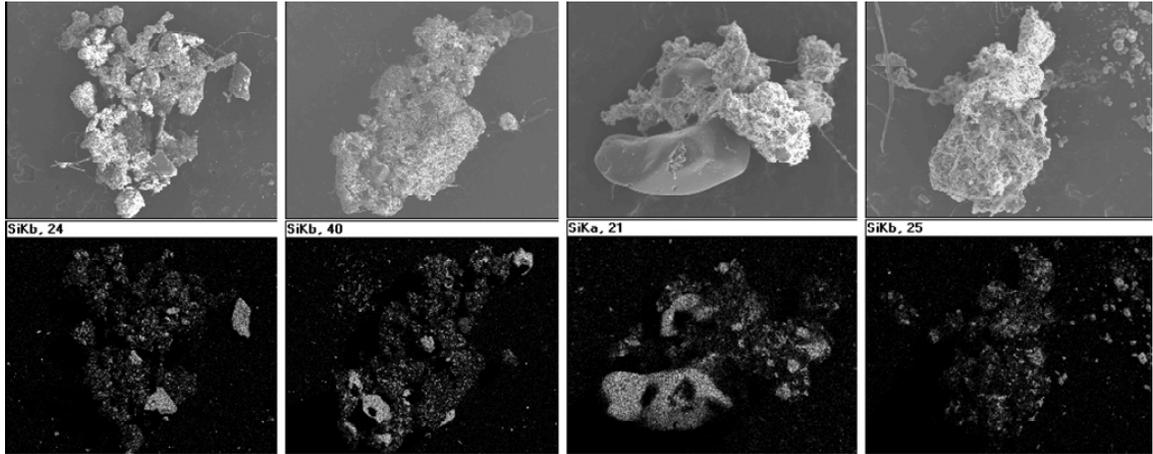
†† NA = Not applicable.

Table 3.3. Effects of forest management intensity and soil depth on aggregate organic matter in the 2000 to 250, 250 to 150 and 150 to 53  $\mu\text{m}$  fractions for a sandy Spodosol in north Florida.

Main Effects / size fraction	2000 to 250 $\mu\text{m}$	250 to 150 $\mu\text{m}$	150 to 53 $\mu\text{m}$
Management intensity	p = 0.03	NS	NS
Soil depth	NS†	NS	NS
Intensity*depth	NS	NS	NS
Energy level	p < 0.01		
Fraction size	p < 0.01		
Energy Level x Intensity x Fraction	p = 0.02		

†NS = Not significant (p>0.05)

Figure 3.1. Observations of soil aggregation in a sandy surface horizon of a Coastal Plain Spodosol. Figures 3.1.1 to 3.1.4 are irregularly shaped aggregates showing fungal hyphae, organic debris and mineral matter enmeshed together. Magnification: 1.1 = 87X, 1.2=55X, 1.3 = 295X, 1.4 = 217X. Figures 3.1.5 to 3.1.7 show spherical aggregates, which are encrustations of mineral matter on organic debris combined with fungal hyphae/fine roots. Figures 3.1.8 and 3.1.9 are photographs of the 250 to 150  $\mu\text{m}$  fraction. Figure 3.1.8 shows the soil after dry sieving, while Figure 3.1.9 is after sonication and shows clean sand grains and particulate organic matter but no aggregates.

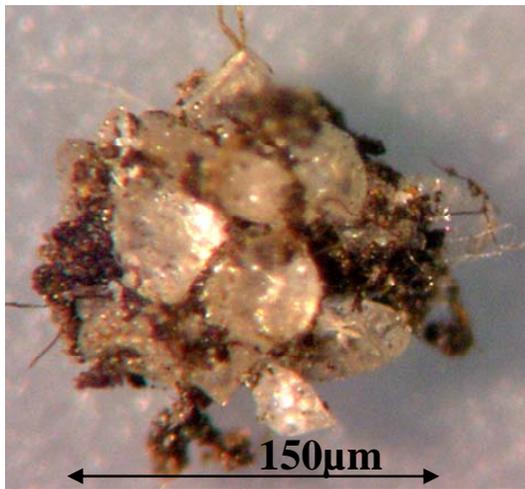


(1)

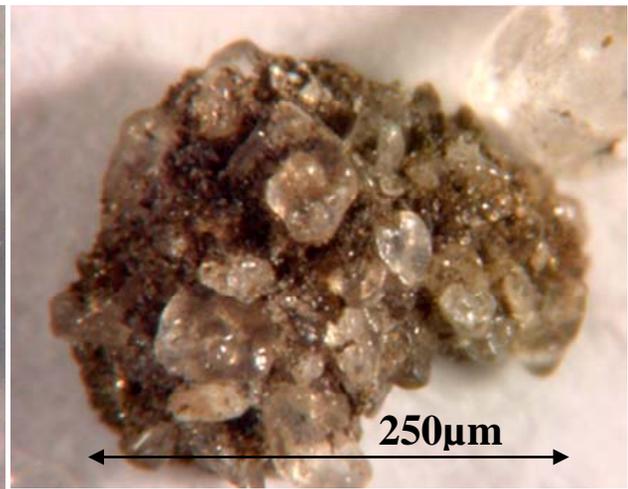
(2)

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(5)



(6)



(7)

(8)

(9)

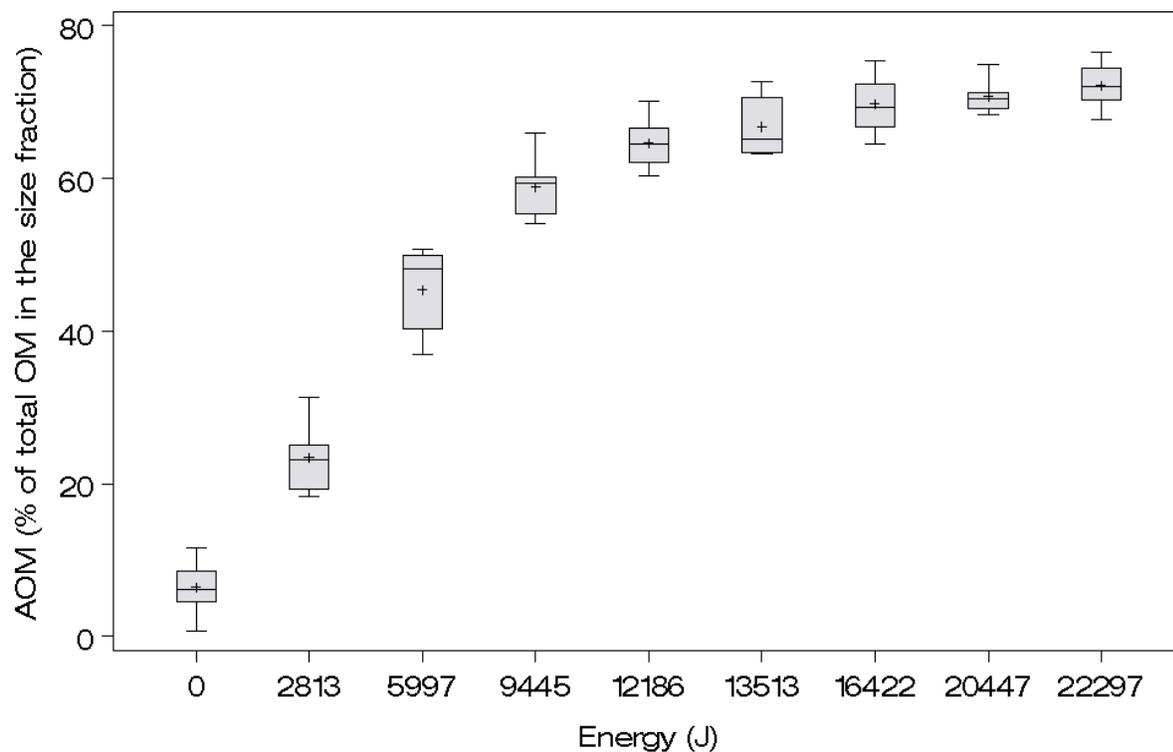


Figure 3.2. Effect of sonication energy input on the loss of aggregate organic matter (AOM, % of total OM in size fraction) after sonication of the 150 to 53  $\mu\text{m}$  fraction. The error bars represent the range of values ( $n = 12$  samples), while the box represents interquartile range (upper quartile = 75<sup>th</sup> percentile, lower quartile = 25<sup>th</sup> percentile). The plus sign in the box represents the mean and the line in the box represents the median.

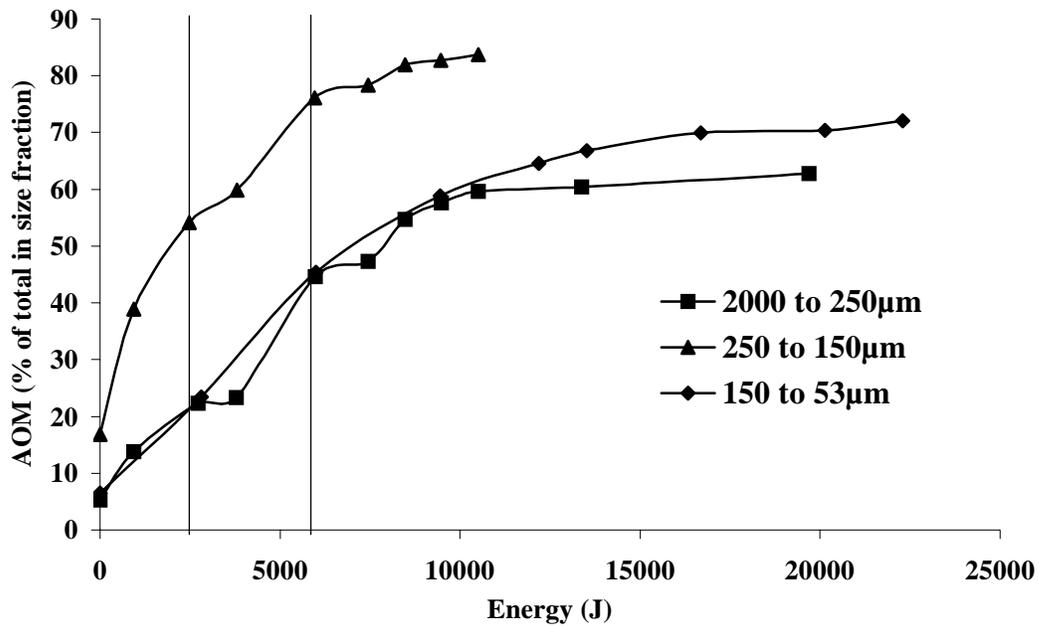


Figure 3.3. Loss of aggregate organic matter (AOM, % of total OM in size fraction) with increasing energy for the various soil size fractions. The vertical lines indicate the steps in the continuity of organic matter lost from aggregates for the 2000 to 250 and 250 to 150  $\mu\text{m}$  fractions. Each data point represents the mean of 12 samples.

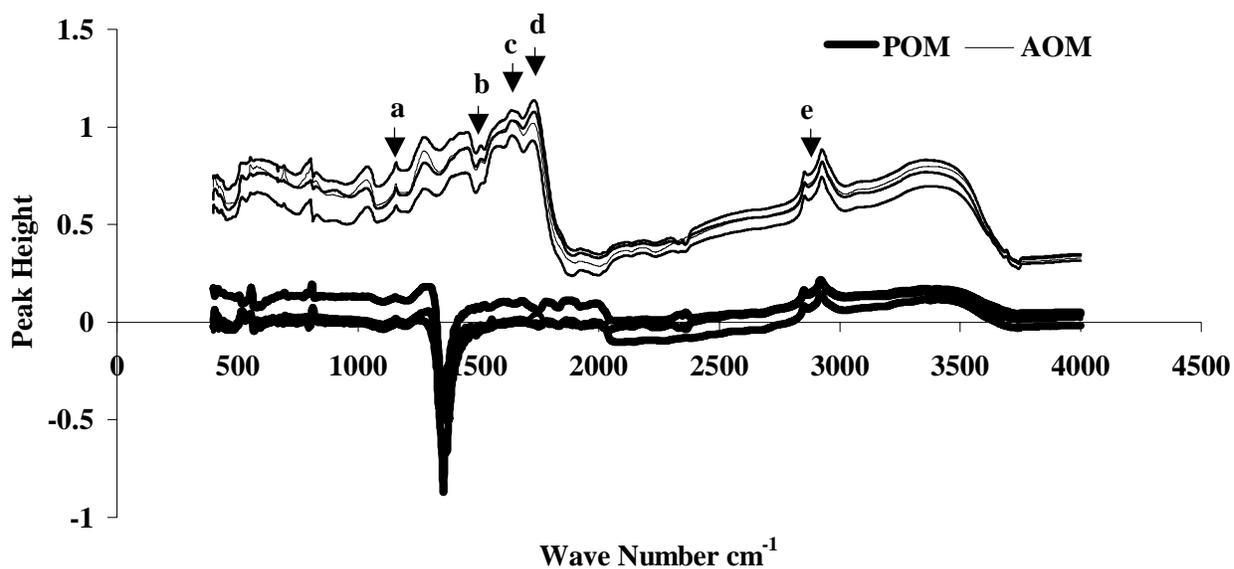


Figure 3.4. Diffusive Reflectance Infra-red Fourier Transformed Spectra (DRIFTS) showing characteristics of particulate organic matter (POM) and aggregate organic matter (AOM) of the 250 to 150  $\mu\text{m}$  fraction for a sandy Spodosol in north Florida. The AOM spectra show peaks are: (a) polysaccharides (b) aromatics (c) esters (d) amides (e) C-H bonds.

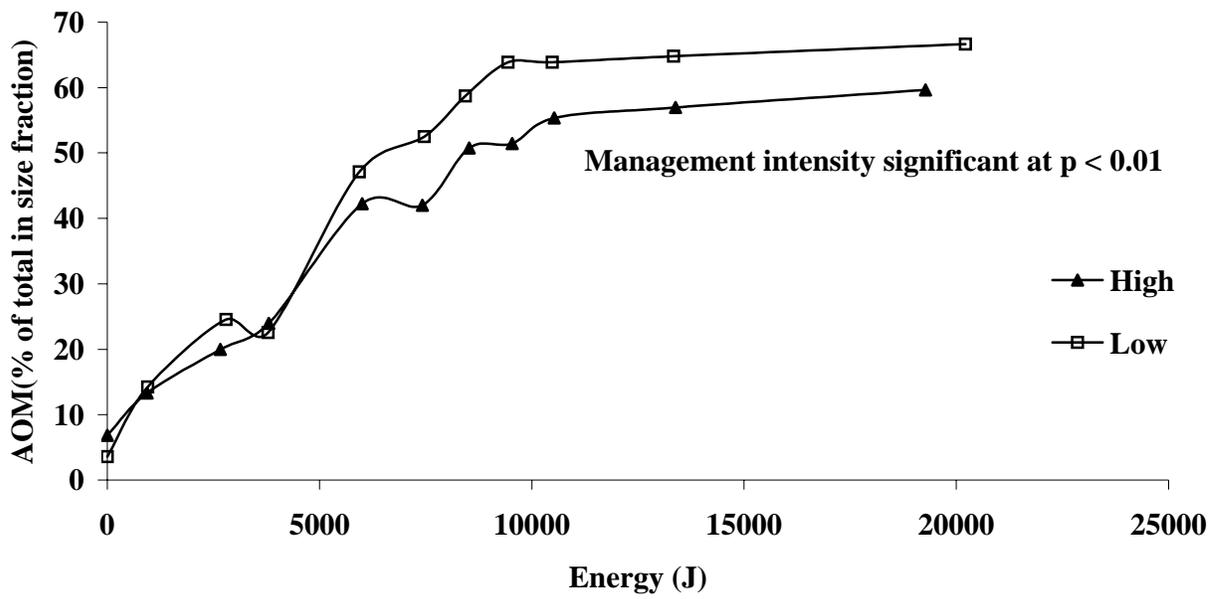


Figure 3.5. Effect of forest management intensity on the amount of aggregate organic matter (AOM, % of total OM in size fraction) for the 2000 to 250  $\mu\text{m}$  fraction for a sandy Spodosol in north Florida. Each data point represents the mean of 6 samples.

CHAPTER 4  
GENOTYPIC AND FOREST MANAGEMENT EFFECTS ON SIZE-DENSITY  
FRACTIONATION OF SOIL CARBON IN A FORESTED SPodosol

**Introduction**

Genetic deployment of fast growing and disease resistant families is a key factor for enhancing forest plantation productivity (McKeand et al., 2003). Contrasting tree species are also known to differentially influence aspects of soil C accumulation and cycling. For example, acidic soil condition beneath spruce (*Picea* spp.) canopies has reportedly lowered microbial biomass and produced lower rates of microbial CO<sub>2</sub> than the soils beneath beech (*Fagus* spp.) or oak (*Quercus* spp.) forests (Anderson and Domsch, 1993). Scrub oak (*Q. dumosa* Nutt) also has been shown to contain more soil organic carbon (SOC) in all soil size fractions compared to soils under coulter pine (*Pinus coulteri* B. Don.); while litter C was higher beneath the pine forests (Quideau et al., 2000). The chemical character of SOC can also be influenced by species. In the last example, O-alkyl C decreased from the litter to the macro organic matter (water-floatable) and fine silt size fractions for both species, but the decrease was more pronounced under pine, indicating greater decomposition of cellulose and hemicellulose in pine litter.

Families within a single species may also differ in their adaptative capabilities and biomass accumulation. For example, loblolly pine (*Pinus taeda* L.) is an important plantation species in the United States, Brazil and Argentina, among other countries (Schultz, 1999). Families of loblolly pine vary in commercially and ecologically important qualities, such as biomass production, light use efficiency, and nutrient use efficiency (Pope, 1979; Crawford et al., 1991; McCrady and Jokela, 1998) as well as fusiform rust resistance (Schmidt, 2003) and response to tropospheric ozone (Taylor, 1994). However, it is not well-known if and how families affect the accumulation of soil C. The differential growth response of families to intensive forest management argues that they may be a factor influencing amount and quality of

SOC. Since genetically improved families of loblolly pine are an important element of intensive management, a better understanding of the influence of genetics, and their interaction with forest management practices on SOC is warranted.

One of the major mechanisms involved in management-related changes in SOC is the change in quality and quantity of organic matter inputs. Density fractionation has been used by many researchers to understand these changes (Romkens et al., 1999; Echeverria et al., 2004), as this technique separates the organic matter from the mineral matter and accentuates the differences in organic matter. The light and medium density pools are reported to be actively recycling fractions with higher C and N concentrations and faster turnover rates. In contrast, the heavy density fraction is reported to be passive, characterized by low C and N concentrations and slow turnover rates (Swanston et al., 2005). As families can be expected to differentially impact SOC through litter and root C inputs, density fractionation may offer sufficient sensitivity to detect these differences.

This study was undertaken with the overall objective of closing this gap in knowledge. The first objective was to study the short-term effects of family and family x management interactions on the surface soil C pools. The hypothesis related to this objective was that the best family (family designations chosen *a priori* based on growth performance in long-term genetic experiments) would promote the greatest increase in SOC, and the effect would be most pronounced under intensive management. This hypothesis was based on the observation that the best family used in this study was highly responsive to fertilization, and produced the most litterfall under intensive management (E.J. Jokela, unpublished data).

The second objective was to determine the profile of C and N in a typical forested Spodosol and identify the size-density fractions most responsive to the varying levels of forest

management. The first hypothesis related to this objective was that the medium and light density fractions would be the main reservoirs of C and N in these soils. This was based on the premise that the mineral fraction in these soils is predominantly quartz sand, with little or no sorption capacity for SOC. The second hypothesis was that the light and medium density fractions in the 2000 to 250 $\mu$ m fraction would be the responsive pools for detecting genotypic differences in SOC as influenced by varying management intensity. This hypothesis was formulated on the observation that in preliminary studies (Chapter 2, 3); this fraction responded most to management intensity. It was expected that the effects on organic matter would be accentuated by separating the density fractions.

## **Materials and Methods**

### **Experimental Site**

A loblolly pine plantation in north Florida (30°24'N lat; 82°33'W long) was the study site. It is managed by the Forest Biology Research Cooperative at the University of Florida, as part of the Pine Productivity Interactions Experimental Study (PPINES). This long-term study aims at understanding the family x environment interactions in full-sib families of loblolly and slash pine (*P. elliotii* Engelm. var. *elliotii*). The climate is warm, humid subtropical, with 1,394 mm average annual rainfall, 27°C average annual maximum temperature and 13°C average annual minimum temperature (NOAA, 2002). The soil is classified as a Leon series (sandy, siliceous, thermic Aeric Alaquod), with <5% silt + clay and <10 cmol<sub>c</sub> kg<sup>-1</sup> of cation exchange capacity.

The trees were planted in January 2000 in four replicates using a randomized complete block, split plot design. Prior to planting, the entire study was double bedded and treated with the herbicides Arsenal (imazapyr – 1.02 L ha<sup>-1</sup>) and Garlon (triclopyr – 7.02 L ha<sup>-1</sup>) to remove the understory vegetation and to reduce competition. The experimental design was a 2 x 2 x 8 factorial including two planting densities (close spacing at 3 x 1.3 m and wide spacing at 3 x 3

m), two management regimes (high and low input) and six elite loblolly pine full-sib families, a mix of these elite families and one poor growing family. Out of these, six treatment combinations (1 x 2 x 3) representing the close spacing, both management regimes and three families were chosen. The full-sib families were chosen *a priori* based on their above-ground growth performance in long-term genetic experiments and included the best grower, a medium grower and the poorest grower (above ground biomass 45.2, 42.6 and 40.6 Mg ha<sup>-1</sup> respectively at age 5; Roth et al., 2006).

The high intensity management regime consisted of sustained understory competition control and annual fertilization using a complete fertilizer. The low intensity management regime consisted of a one-time fertilization and understory competition control at planting. At age 6, when the soil was sampled, the fertilizer added to the high intensity treatment totaled 368 kg ha<sup>-1</sup> N and 128 kg ha<sup>-1</sup> P plus nearly all other essential nutrients (i.e., 121 kg ha<sup>-1</sup> K, 45 kg ha<sup>-1</sup> Mg, 45 kg ha<sup>-1</sup> Ca, 35 kg ha<sup>-1</sup> S, 0.89 kg ha<sup>-1</sup> B, 3 kg ha<sup>-1</sup> Zn, 2 kg ha<sup>-1</sup> Mn, 16 kg ha<sup>-1</sup> Fe, 4 kg ha<sup>-1</sup> Cu, 0.01 kg ha<sup>-1</sup> Mo). The low intensity treatment had 45 kg ha<sup>-1</sup> N and 50.6 kg ha<sup>-1</sup> P applied as diammonium phosphate. In the high intensity treatment, the herbicides Velpar or Oust and Glyphosate applied at labeled rates provided sustained understory competition control. The entire study was treated when necessary with insecticides (Dimilin, Pounce or Mimic) for tip moth (*Rhyacionia* spp.) control during the first growing season. Each treatment plot was 480 m<sup>2</sup> in size.

Soil samples were collected from the A horizon of interbeds at soil depth increments of 0 to 5 and 5 to 10 cm from each treatment plot in three of the four replicate blocks in March 2005. Two composite samples for each depth of each plot (treatment within a block) came from about six individual soil samples. The individual soil samples for each composite sample were

collected from alternate rows (interbed position), in two lines diagonally across the plot. The core method was used to measure bulk density (0 to 5 cm depth) based on two samples per treatment plot.

## **Laboratory Methods**

### **Size fractionation**

Soil samples were air-dried and passed through a 2000  $\mu\text{m}$  sieve. The  $<2000 \mu\text{m}$  fraction was further size fractionated using dry sieving (see Chapter 2). Four size fractions, a macroaggregate fraction 2000 to 250  $\mu\text{m}$ , two microaggregate fractions (250 to 150  $\mu\text{m}$  and 150 to 53  $\mu\text{m}$ ), as well as a  $<53 \mu\text{m}$  fraction were obtained. The size fractions, including the  $> 2 \text{ mm}$  size fraction, were ground to a fine powder and analyzed for total C and N concentrations on a Carlo-Erba Analyzer (CE Instruments, model NCS-2500). The three 2000 to 53  $\mu\text{m}$  fractions were also analyzed for organic matter content using loss on ignition.

### **Density fractionation**

The most widely used liquids for density separation are water, sodium polytungstate and Ludox, an inert silica suspension (Christensen, 1992; Cambardella and Elliott, 1993; Meijboom et al., 1995). Sodium polytungstate is expensive, toxic and is reported to hinder mineralization (Sollins et al., 1984). Ludox was tested initially in this study but appeared to dissolve some C from the size fractions, as shown by the considerably darkened supernatant. Therefore, a modified density separation procedure based on Meijboom et al. (1995) was used, with water as a separating liquid.

For each size fraction, 10 g soil was added to a 50 mL beaker. Twenty-five mL water was added to this beaker and the organic matter was separated by swirling and decanting into a 600mL beaker. The process was repeated until no more organic matter could be visually separated. The light fraction floating on top of the water in the 600 mL beaker was periodically

transferred to another beaker to avoid co-precipitation of the light density fraction (settling down under the weight of medium density fraction). Organic matter, which could not be separated from mineral material, was termed the heavy fraction. The suspension in the 600 mL beaker was added to a 250 mL funnel with the suspension of light density material added on top. Organic matter, which settled in water, was termed the medium density fraction, while organic matter, which floated on water, was termed the light density fraction. The 2000 to 250  $\mu\text{m}$  fraction needed 24 hrs to separate light and medium densities, while the 250 to 150  $\mu\text{m}$  fraction needed 12 hrs to achieve a clear separation. The separated fractions were passed through the same size sieve used for obtaining the size fraction (e.g., 250  $\mu\text{m}$  sieve for the 2000 to 250  $\mu\text{m}$  fraction) in order to separate the water-dispersible aggregate fraction. The water-dispersible fraction was defined as the organic matter passing through the sieve after the size fractions were density-fractionated with water. The samples were then air-dried and used for further analysis.

Preliminary studies indicated that in the 150 to 53  $\mu\text{m}$  fraction, more than 90% of the SOC was in the medium density fraction. Therefore, this fraction was not density fractionated.

### **Sonication**

The medium density and heavy density fractions of the 2000 to 250 and 250 to 150  $\mu\text{m}$  fractions, as well as the whole 150 to 53  $\mu\text{m}$  fraction, were further fractionated into aggregate organic matter (AOM) and particulate organic matter (POM) using sonication. In this analysis, a 5 g sample was used for the heavy density fractions, a 1 g sample for the medium density fractions and a 2 g sample for the 150 to 53  $\mu\text{m}$  whole fractions. The energy level for sonication was chosen based on the preliminary analysis (Chapter 3). The 2000 to 250  $\mu\text{m}$  and 150 to 53  $\mu\text{m}$  fractions were sonicated at 20000J for (60% amplitude, 5 minutes; pulse method 60 sec ON and 30 sec OFF), while the 250 to 150  $\mu\text{m}$  fraction was sonicated at 10000J (40% amplitude, 5

minutes; pulse method 60 sec ON and 30 sec OFF). Loss on ignition was used to measure the AOM and POM separated by sonication.

### **Statistical Analysis**

The statistical significance was analyzed using 'PROC MIXED' (SAS, 1996), with management intensity, depth, size fraction, and family as fixed effects and block and replication (nested within block) as random effects. Differences were considered significant at  $p < 0.05$ . As the initial analyses showed that the size and density fractions were significantly different from each other with interactions between depth, intensity and family, further analyses were carried out for the individual fractions. To analyze the significant interactions within each size-density fraction, the contrast procedure was used for post-hoc comparisons to ascertain differences among least square means.

## **Results**

### **Management Intensity and Family Effects**

Family and forest management intensity did not affect SOC and N among the size fractions, except for the  $>2000 \mu\text{m}$  size fraction. In this fraction, the high intensity management regime reduced the C:N ratio by 17% (60 in high intensity vs. 73 in low intensity). However, the density fractions of both the 2000 to 250  $\mu\text{m}$  and 250 to 150  $\mu\text{m}$  size fractions exhibited significant effects of management intensity and family. In the 2000 to 250  $\mu\text{m}$  light density fraction, the best family produced higher C content than the poor family (Fig 4.1). On the other hand, the medium family exhibited higher AOM in the 250 to 150  $\mu\text{m}$  medium density fraction (Fig 4.2). There was also an intensity x family interaction for N concentration in the 2000 to 250  $\mu\text{m}$  medium density (Fig 4.3) and AOM in the heavy density fractions (Fig 4.4). The medium family had significantly higher soil N concentration when grown under the low intensity management regime (Fig 4.3). The best family trended toward higher N concentrations under the

high intensity management regime, with the difference being statistically significant at  $p = 0.06$ . In the 2000 to 250  $\mu\text{m}$  heavy density fraction (Fig 4.4), the medium family showed higher AOM under the high intensity management regime (best family 68% vs. medium family 80%).

### **Distribution of C and N in the Size-density Fractions**

Among the size fractions, C concentration (% of fraction) was highest in the  $>2000 \mu\text{m}$  fraction (Table 4.1), while C content (% of whole soil), was highest in the 2000 to 250  $\mu\text{m}$  fraction (Fig 4.5). Among the density fractions, C content was highest in the medium density (47-85%; Fig 4.6) in both the 2000 to 250 and 250 to 150  $\mu\text{m}$  fractions. The C concentration was highest in the light density 2000 to 250  $\mu\text{m}$  fraction (Table 4.2). The light density material of the 250 to 150  $\mu\text{m}$  fraction accounted for  $<1\%$  of the fraction's weight and C content. However, it accounted for 6 to 10% of the C in the 2000 to 250  $\mu\text{m}$  fraction. The water-dispersible fraction accounted for 7 to 16% of the total C in the size fractions. The medium density of the 250 to 150  $\mu\text{m}$  fraction showed the highest AOM (81 to 82%; Table 4.3), while the medium density of the 2000 to 250  $\mu\text{m}$  fraction showed the lowest amounts (65 to 66%).

Nitrogen concentrations in the size fractions followed the same trend as C (Table 4.1), but the N content was highest in the 250 to 150  $\mu\text{m}$  fraction (Fig 4.5). The C: N ratio was highest in the  $>2000 \mu\text{m}$  fraction (55-73; Table 4.1) and lowest in the 250 to 150  $\mu\text{m}$  fraction (9-16). Among the density fractions, N content was highest in the heavy density, especially in the 250 to 150  $\mu\text{m}$  fraction (84 to 92% of total N in the fraction; Fig 4.7). The heavy density also exhibited extremely low C: N ratios (2.2-3, Table 4.2). The C: N ratio of the light density in the 2000 to 250  $\mu\text{m}$  fraction was highest (56 to 58, Table 4.2) among the density fractions and was similar to the  $>2000 \mu\text{m}$  fraction (55 to 73, Table 4.1).

## **Effect of Depth**

The 0 to 5 cm depth had higher C and N concentrations in all size fractions except the >2000  $\mu\text{m}$  fraction, which had its higher C concentration at the 5 to 10 cm depth (Table 4.1). However, the effect was more prominent for C than for N. There was also a significant depth x fraction interaction in C and N contents of the soil fractions (Fig 4.5). The 2000 to 250  $\mu\text{m}$  fraction exhibited higher content at the 0 to 5 cm depth, while all other fractions exhibited higher content at the 5 to 10 cm depth. The 250 to 150  $\mu\text{m}$  fraction in particular exhibited significantly higher N content (42 vs 28% of total soil N) at the 5 to 10 cm depth.

There was also a depth x density fraction interaction. In both size fractions, the medium density had higher C concentration at the 0 to 5 cm depth, while the other three density fractions had higher C concentration at the 5 to 10 cm depth (Table 4.2). The 0 to 5 cm depth also showed higher AOM in the 2000 to 250  $\mu\text{m}$  heavy density fraction, while the 5 to 10 cm depth showed higher AOM in the 150 to 53  $\mu\text{m}$  fraction and higher POM in the 2000 to 250  $\mu\text{m}$  heavy density fraction (Table 4.3).

## **Discussion**

This study was undertaken with the objective of understanding how forest management activities and genetic deployment affect the C in forested Spodosols of the southeastern U.S. Utilization of genetically improved seedlings for growth and disease resistance, coupled with management practices that include understory competition control and fertilizer applications, are common approaches used for increasing the productivity of managed plantation forests in the southeastern U.S. Yet, the impacts of management practices and genetics on soil related processes are still poorly documented.

## Effects of Family

The best performing family added more decomposable organic matter to the soil as shown by the higher C content in the light density fraction (Fig 4.1). The light density fraction has been reported to be highly mineralizable due to its high C and N concentration and lack of mineral protection (Romkens et al., 1999; Swanston et al., 2005). The best family also showed a trend toward higher soil N concentrations under the high intensity management regime (Fig 4.3). This family was characterized by much higher yield under intensive management. It had 31% higher stem volume and 11% more aboveground biomass as compared to the poor performing family at age 5 (Roth et al., 2006). It also exhibited higher N concentrations in the foliage (Jokela, E.J., unpublished data). These factors suggest that a combination of the best family and the intensive management regime may be favorable for faster C turnover rates. Although this is a positive factor for availability of nutrients such N, P and S in poor fertility soils, the consequences for long-term C sequestration are uncertain. These data document early trends through age 6 and it will be necessary to determine whether these effects are sustained over time.

The medium performing family had higher levels of aggregation as shown by the higher AOM values in the 250 to 150  $\mu\text{m}$  medium density fraction (Fig 4.2). A possible explanation for this effect is the difference in root biomass and root architecture of these families, since fine roots are important aggregating agents (Tisdall and Oades, 1982). This family also exhibited higher N under the low intensity management regime (Fig 4.3), which was reported to have greater aggregation (Chapter 3). This suggests a role of aggregates in N storage. In the 2000 to 250  $\mu\text{m}$  heavy density fraction, this family showed higher aggregation under the high intensity management regime (Fig 4.4), suggesting that the positive impact of this family on aggregation was independent of management intensity. It is possible that the genotypic effects were better expressed under intensive management as it has been reported to reduce the environmental

variation (Lopez-Upton et al., 1999). This result also offers a possibility for improving the C sequestration potential of these ecosystems without sacrificing yield through choices of family deployment.

### **Fraction Characteristics and Effect of Depth**

The high C:N ratios of the medium and light density in the 2000 to 250  $\mu\text{m}$  fraction (Table 4.2), which were similar to those of the  $> 2000 \mu\text{m}$  fraction (Table 4.1), indicated that OM in these fractions mainly came from recently added litter and root biomass. Similar high C:N ratios, especially for the light density fraction have been reported by many authors (Swanston et al., 2004; Gregorich et al., 2006; Liao et al., 2006) This interpretation was supported by the higher C content in the 2000 to 250  $\mu\text{m}$  size fraction (Table 4.1), as well as in the medium density of this fraction at the 0 to 5 cm depth (Table 4.2), which can be expected to receive higher root and litter inputs. This was in accordance with the conclusion in Chapter 2 that the 2000 to 250  $\mu\text{m}$  fraction received fresh organic matter. The high C:N ratio of the 250 to 150  $\mu\text{m}$  medium density fraction (Table 4.2) indicated that this fraction also received fresh organic matter inputs.

The high N content of the 250 to 150  $\mu\text{m}$  fraction (42% of total N at 5 to 10 cm depth; Table 4.1) indicated that it may be responsible for the storage of N. It also exhibited the highest proportion of aggregate C as shown by the high AOM values (Table 4.2), which supports the importance of aggregates for the N storage in these soils. Within this size fraction, the heavy density showed the highest N content (84 to 92% of the N; Fig 4.7) and lowest C: N ratios (2.2 to 3, Table 4.3). The heavy density 2000 to 250  $\mu\text{m}$  fraction also had low C:N ratios. This finding highlights the importance of heavy density soil material in the N storage of these soils. Zhong and Makeschin (2006) also reported that the heavy density fraction contained more labile N than the light density fraction in temperate forest soils. Stable sorption of nitrogenous compounds like amides on the mineral surfaces (Sollins et al., 2006) can be one of the mechanisms responsible

for the high N content and low C/N ratios of the heavy density fractions. Presence of microbial N protected by the aggregates may represent another mechanism. Foster (1988) reported that aggregates offered prime sites for microbes due to the protection from sudden changes in moisture and from predation by the protozoa and other large predators. Therefore, it is likely that the N stored in the aggregates is of microbial origin. Although these ratios are unusually low, Tscherko et al. (2003) reported similar values for microbial biomass (lowest value reported 1.4). A comparison of the N content in AOM and mineral fraction or the measurement of microbial C/N ratios by fumigation extraction would be necessary to provide further insight into this phenomenon.

### **Methodological Considerations**

The modified density fractionation procedure offered many advantages. There was minimal chance of chemical alteration of the organic matter since only water was used during the procedure. This also made the procedure inexpensive and easy to use in any laboratory. It allowed fraction-wise estimation of the water-dispersible aggregates, for which there is no other method available at present. In agricultural soils, only the water-stable aggregates are usually measured, since these soils are continually exposed to splash and wind erosion. However, the forested soils are protected from the erosive forces by a thick litter layer. Therefore, the water-dispersible aggregates are likely to be important for C dynamics as well as the short-term cycling of N, P and S in these soils. The medium and light density fractions separated by this procedure showed effects of family and management intensity on the SOC pools as early as six years after the treatments were imposed. This indicated the applicability of this method for detecting management related changes. Other researchers, using different procedures, have also reported the sensitivity of light and medium density fractions to the short and long-term management related changes (Romkens et al., 1999; Echeverria et al., 2004).

## Conclusions

The 2000 to 250  $\mu\text{m}$  fraction contained the most C, while the 250 to 150  $\mu\text{m}$  fraction was most important for N storage. Of the density fractions studied, the medium density contained the most C, while the heavy density contained the most N. The best growing family added more decomposable C as indicated by higher C in the light density 2000 to 250  $\mu\text{m}$  fraction, while the medium family encouraged better aggregation, as shown by higher AOM. These findings indicate that understanding the short-term as well as long-term genotypic effects on soil C would be necessary for carbon-wise management of the forested Spodosols in north Florida. Water-dispersible aggregates were an important component of these soils, as they accounted for 7 to 16% of the C in the two largest size fractions. The modified density fractionation procedure used in this study offered an inexpensive and effective way for separating soil C pools and for detecting management related changes in them.

Table 4.1. Characteristics of the size fractions for a sandy Spodosol in north Florida

Variable	Depth	>2000 $\mu\text{m}$	2000 to 250 $\mu\text{m}$	250 to 150 $\mu\text{m}$	150 to 53 $\mu\text{m}$	<53 $\mu\text{m}$
C concentration (% of fraction)	0 to 5 cm	18.8 <sup>†</sup> h (1.0)	2.21 e (0.13) <sup>‡</sup>	0.43 b (0.02)	1.48 d (0.06)	9.99 g (0.36)
	5 to 10 cm	23.6 i (1.1)	0.89 c (0.08)	0.17 a (0.02)	0.66 c (0.04)	5.83 f (0.26)
N concentration (% of fraction)	0 to 5 cm	0.35 de (0.02)	0.071 c (0.004)	0.029 ab (0.002)	0.063 c (0.003)	0.365 e (0.016)
	5 to 10 cm	0.33 de (0.01)	0.030 ab (0.002)	0.024 a (0.002)	0.037 b (0.002)	0.228 d (0.009)
C:N Ratio	0 to 5 cm	55 d (1)	31 c (1)	16 b (1)	24 c (1)	28 c (2)
	5 to 10 cm	73 e (2)	31 c (1)	9 a (1)	18 b (2)	27 c (2)

<sup>†</sup>Each value is a mean of 36 observations averaged across treatment intensity and families.

<sup>‡</sup> Values in parentheses represent the standard error.

<sup>§</sup> Within a variable, the means followed by different letters are statistically different at  $p < 0.05$  showing the effects of both depths and size fraction.

Table 4.2. Characteristics of the density fractions for a sandy Spodosol in north Florida

Variable	Fraction	Depth	Heavy	Medium	Light
C concentration (% of fraction)	2000 to 250 $\mu\text{m}$	0 to 5 cm	0.12 <sup>†</sup> a <sup>§</sup> (0.01) <sup>‡</sup>	29.7 b (0.7)	35.5 c (0.9)
		5 to 10 cm	0.10 a (0.01)	30.7 bc (0.7)	34.5 bc (0.9)
	250 to 150 $\mu\text{m}$	0 to 5 cm	0.08 a (0.02)	22.4 b (0.5)	20.6 ¶ (4.5)
		5 to 10 cm	0.07 a (0.01)	19.9 b (0.9)	17.6 NA
N concentration (% of fraction)	2000 to 250 $\mu\text{m}$	0 to 5 cm	0.04 a (0.001)	0.75 c (0.01)	0.66 bc (0.04)
		5 to 10 cm	0.04 a (0.001)	0.64 b (0.02)	0.61 b (0.04)
	250 to 150 $\mu\text{m}$	0 to 5 cm	0.041 b (0.001)	0.55 c (0.02)	1.05 (0.12)
		5 to 10 cm	0.036 a (0.001)	0.48 c (0.03)	0.82 NA
C:N Ratio	2000 to 250 $\mu\text{m}$	0 to 5 cm	3.0 a (0.3)	40.3 c (0.8)	55.8 d (2.9)
		5 to 10 cm	2.6 a (0.4)	50.8 b (1.7)	58.5 d (2.5)
	250 to 150 $\mu\text{m}$	0 to 5 cm	2.2 a (0.6)	42.2 b (1.6)	18.7 (2.6)
		5 to 10 cm	2.3 a (0.4)	45.4 b (2.0)	21.5 NA

<sup>†</sup> Each value is a mean of 36 observations averaged across treatment intensity and families.

<sup>‡</sup> Values in parentheses represent standard error.

<sup>§</sup> Within a variable, the means followed by different letters are statistically different at  $p < 0.05$  showing effect of both depth and density, within a size fraction.

<sup>¶</sup> The light density in 250 to 150  $\mu\text{m}$  was less than  $<0.01\%$  of the fraction weight and only 5 samples had enough weight for analysis. Therefore, no statistical analysis was carried out for this fraction.

<sup>#</sup> The weight and C content of the water-dispersible fraction was measured by difference and not analyzed directly. Therefore, the characteristics of this fraction were not reported.

Table 4.3. Distribution of aggregate organic matter (AOM) and particulate organic matter (POM) in the different size-density fractions for a sandy Spodosol in north Florida

Variable	Depth	2000 to 250 $\mu$ m		250 to 150 $\mu$ m		150 to 53 $\mu$ m
		Medium	Heavy	Medium	Heavy	Whole
AOM (% of total OM in size fraction)	0 to 5 cm	65 <sup>†</sup> a (2) <sup>‡</sup>	77 b (1)	82 a (1)	73 a (2)	79.5 a (0.5)
	5 to 10 cm	66 a (1)	70 a (1)	81 a (1)	63 a (3)	81.3 b (0.8)
POM (% of total OM in size fraction)	0 to 5 cm	35 a (2)	23 a (1)	18 a (1)	27 a (2)	20.5 a (0.5)
	5 to 10 cm	34 a (1)	30 b (1)	19 a (1)	37 a (3)	18.7 a (0.8)

<sup>†</sup>Each value is a mean of 34-36 observations averaged across treatment intensity and families.

<sup>‡</sup> Values in parentheses represent standard error.

<sup>§</sup> Within a size-density fraction, the means followed by different letters are statistically different at  $p < 0.05$  showing the effect of depth.

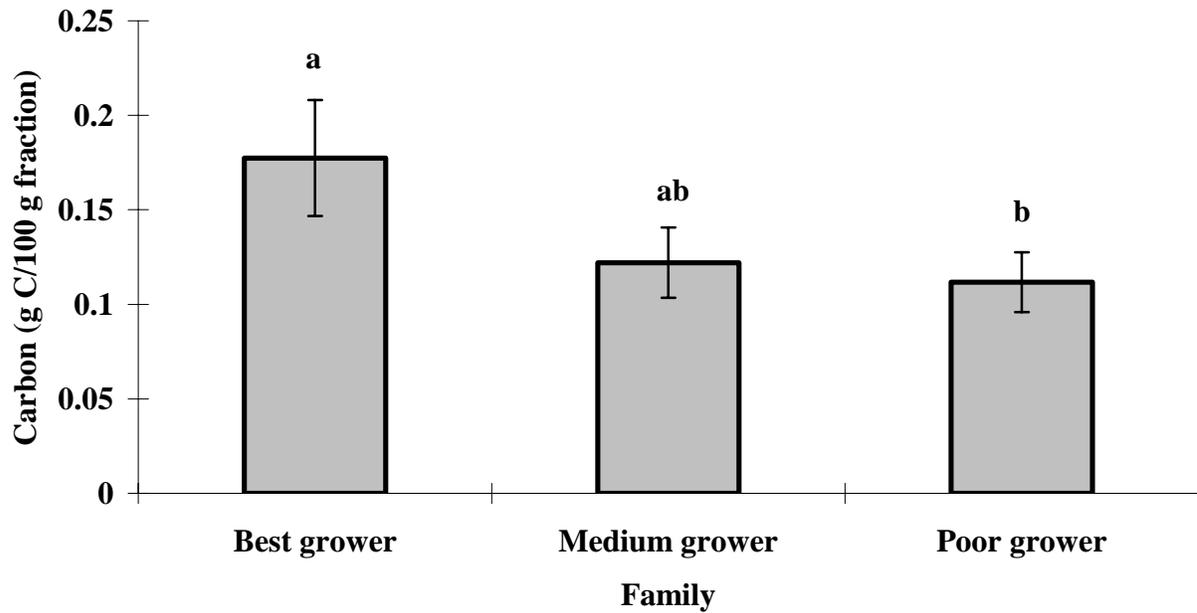


Figure 4.1. Effects of family on the C content of the 2000 to 250  $\mu\text{m}$  light density fraction for a sandy Spodosol in north Florida. The means followed by different letters are statistically different at  $p < 0.05$ . The error bars represent the standard errors. Each value represents the mean of 24 observations averaged across management intensity and soil depth.

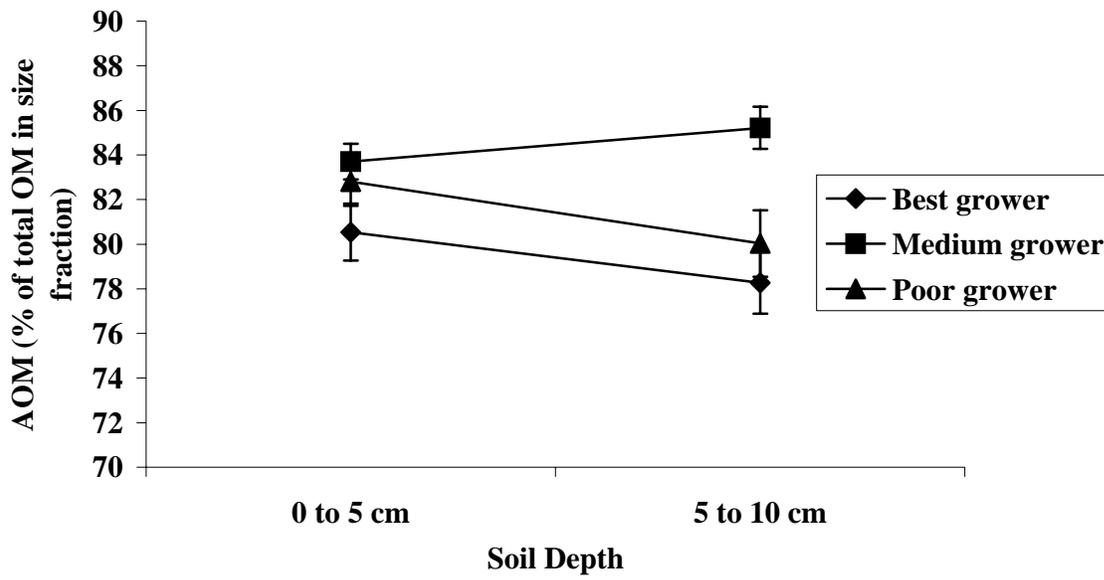


Figure 4.2. Family x Depth interaction in aggregate organic matter (AOM) of the medium density, 250 to 150 μm fraction for a sandy Spodosol in north Florida. The error bars represent the standard errors. Each value represents the mean of 12 observations averaged across management intensities.

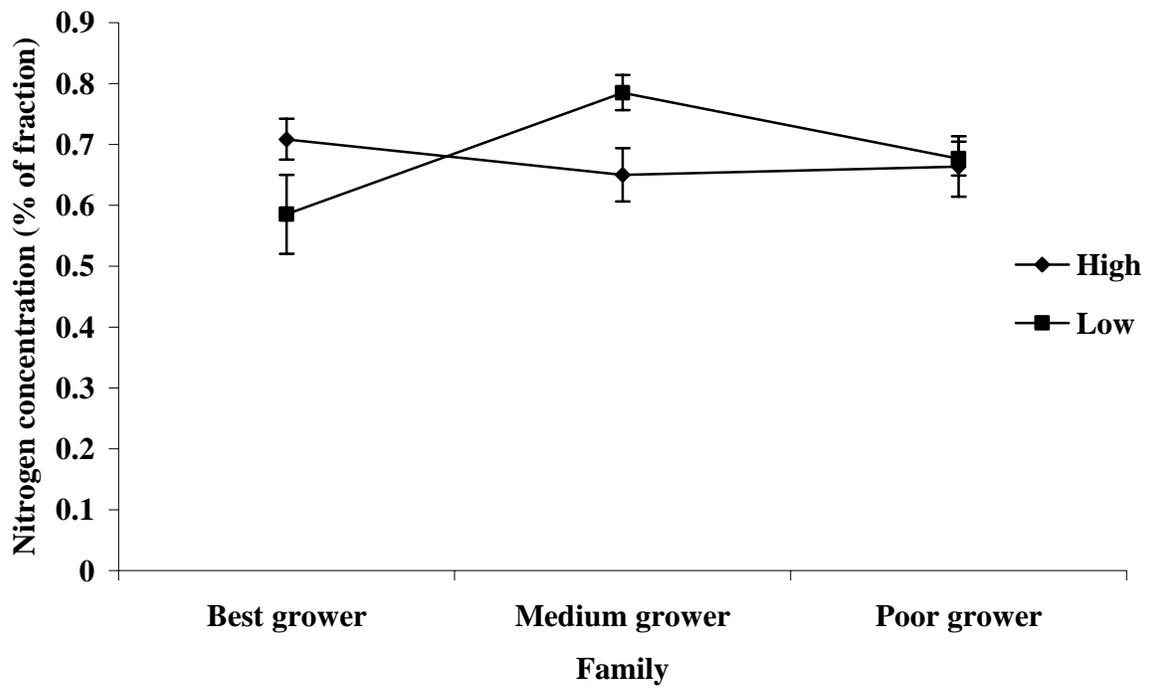


Figure 4.3. Effects of management intensity and family on N concentrations in the 2000 to 250  $\mu\text{m}$  medium density fraction for a sandy Spodosol in north Florida. The error bars represent the standard errors. Each value represents the mean of 12 observations averaged across management intensity and soil depth.

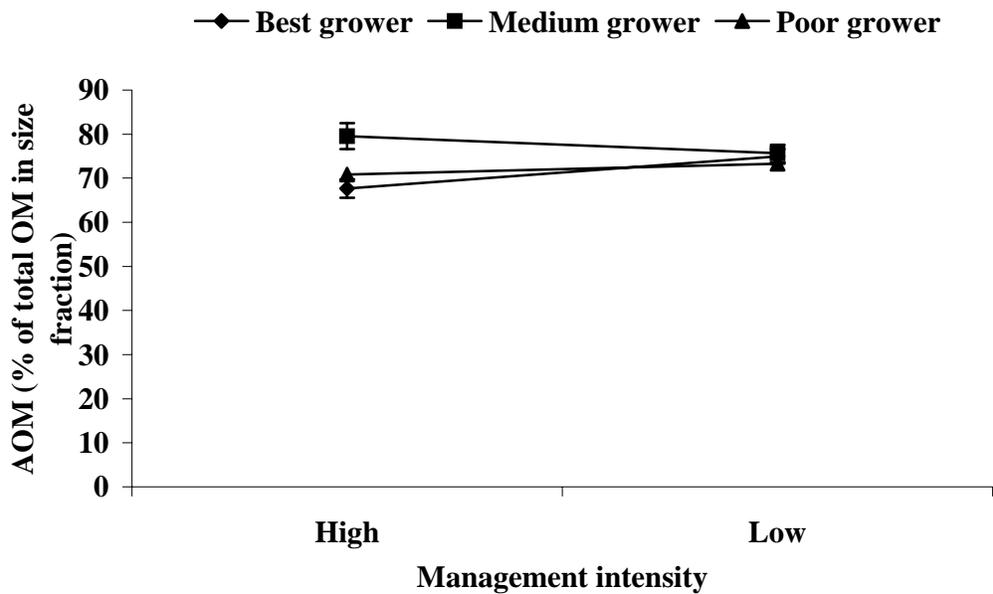


Figure 4.4. Family x management intensity interaction in aggregate organic matter (AOM) of the heavy density, 2000 to 250  $\mu\text{m}$  fraction for a sandy Spodosol in north Florida. The error bars represent the standard errors. Each value represents the mean of 12 observations averaged across soil depths.

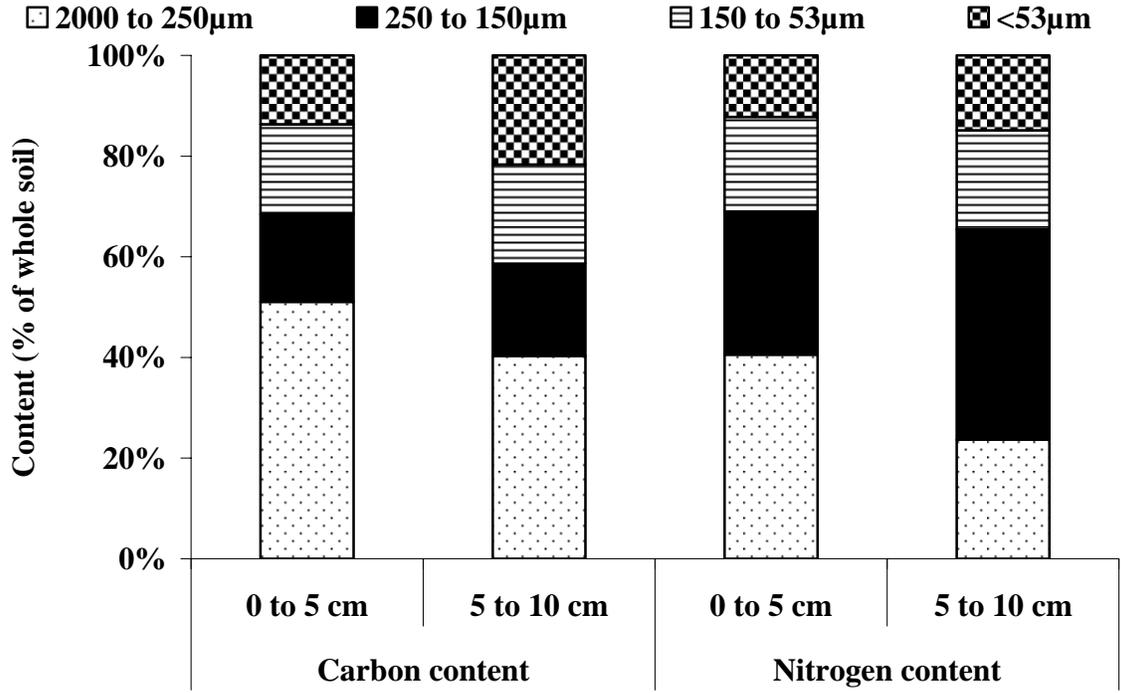


Figure 4.5. Effects of soil depth on C and N contents among the various size fractions for a sandy Spodosol in north Florida.

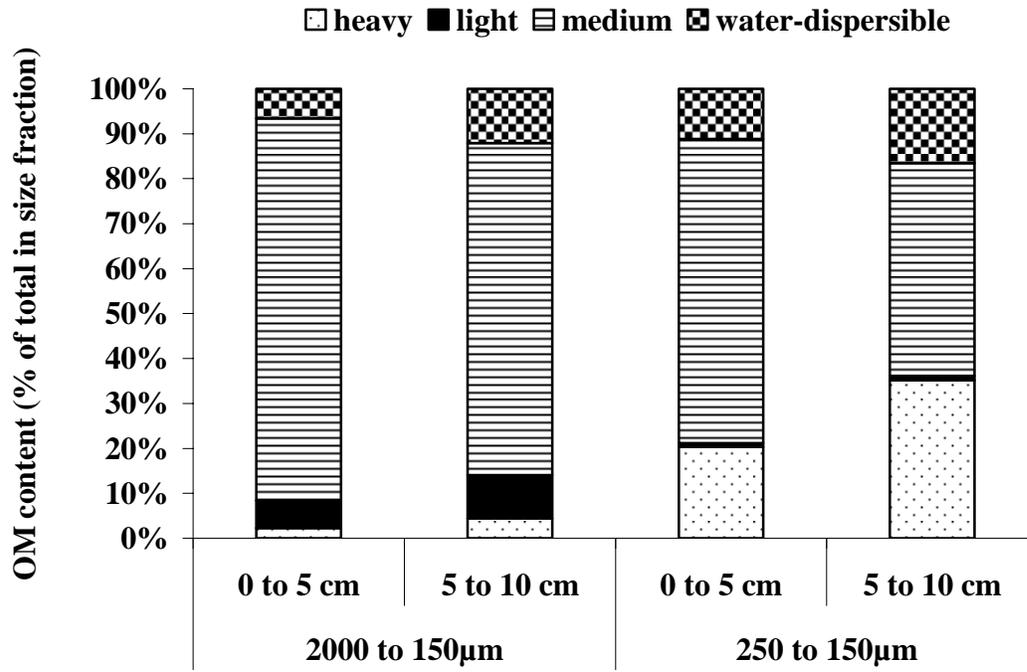


Figure 4.6. Distribution of C among the various density fractions for a sandy Spodosol in north Florida.

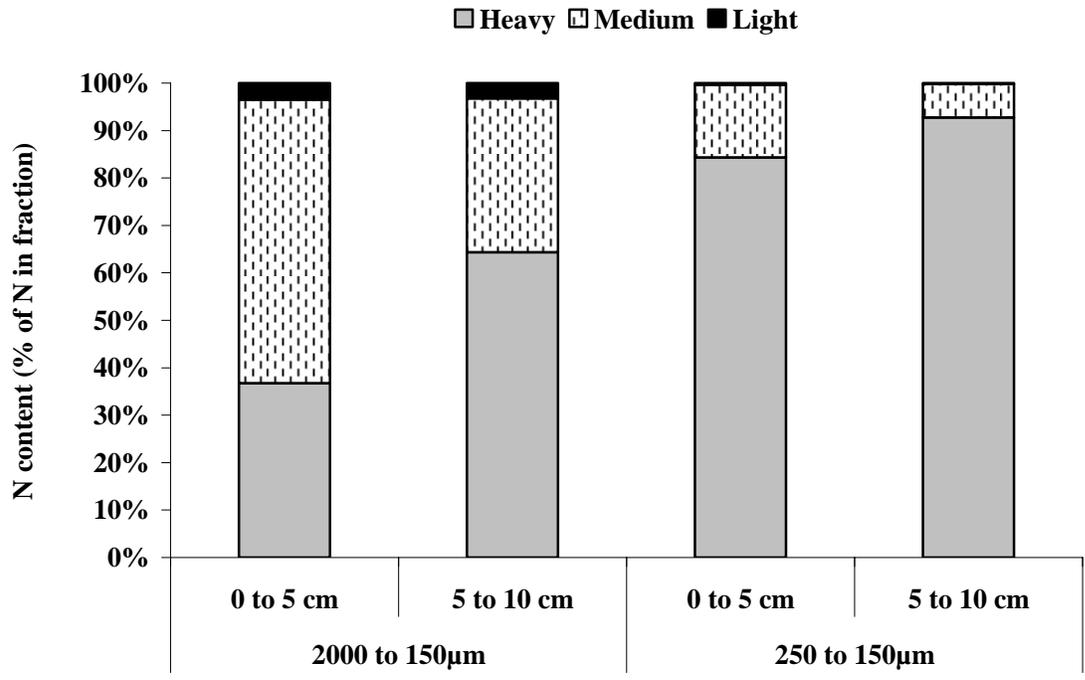


Figure 4.7. Distribution of N among the various density fractions for a sandy Spodosol in north Florida.

## CHAPTER 5 SUMMARY AND CONCLUSIONS

Soil organic carbon (SOC) and its chemical nature in sandy Spodosols under loblolly pine plantations in the southeastern U.S. are poorly described. The objectives of this dissertation were to investigate aggregation in a representative Spodosol in north Florida under pine management, to characterize SOC pools in the A horizon, and to determine the impact of management activities and genetics on the SOC profile. This research required an adaptation of the size-density fractionation methods to suit the sandy nature of the surface soils, since the weak structure required less invasive methods for size and density fractionation. The low clay content also ruled out using clay release after sonication as an indicator of aggregate destruction. Described below is a critical evaluation of the main findings of this dissertation with focus on how this research contributed to understanding soil C dynamics in forest soils.

### **Methodological Contributions**

Dry sieving was shown to function as well as, or better than, the more widely used wet sieving technique. Dry sieving preserved more of the weak structure and the water-soluble components within the soil, such as esters and amides (Chapter 2). As dry sieving is considerably faster than wet sieving, it offers economy of time and effort for sandy textured soils.

When sonication was used to disrupt soil aggregates, the loss of organic matter instead of clay was used as a measure of aggregate stability. The use of organic matter was a superior procedure because (1) there is little clay to measure in these soils and (2) organic matter in aggregates and aggregate stability can be measured together (Chapter 3). This procedure also offers opportunities for understanding the nature of aggregates in these soils as it allows investigation of aggregate hierarchy when using incremental energy input and organic matter

release. This procedure should also be useful in analyzing the chemical nature of organic matter in aggregates of differing strength.

The density fractionation procedure used in this study is an inexpensive and chemically mild technique of organic matter fractionation, since only water was used during the procedure (Chapter 4). The three density fractions; heavy, medium and light, were different from each other in their appearance as well as C and N concentrations. They also differed in their response to changes in forest management. It is clear that this procedure separates three distinct C pools of different chemical characteristics.

Use of DRIFTS spectra, especially the spectral subtraction technique used in this study (Chapter 2), proved useful in identifying differences in organic matter pools and in understanding the changes in chemical composition resulting from sieving method, soil aggregate size, and forest management. This technique enabled identification of major functional compounds that differed among C pools. In particular, it was useful in separating aggregate C and particulate C, showing higher concentration of esters, amides and polysaccharides in aggregate C.

The fractionation method also proved useful in detecting SOC changes that occurred due to forest management practices in as little as 4 to 6 y. Although this study focused on C, these fractionation methods could also be used to study the distribution and dynamics of other soil nutrients and pollutants.

### **Aggregation and Physical Protection**

The structure of the surface soil of Florida's Spodosols has been described as weak. Therefore, aggregation has received little, if any, attention. This was the first study to focus on the soil aggregate C in a Florida Spodosol. Results suggested that nearly half of the C was held by the aggregates (Chapter 3). This study also showed that, in spite of their mechanical stability,

the aggregates were susceptible to management related influences. Therefore, C-wise management will require an understanding of the nature and stability of these aggregates. Further studies on differences in aggregate stability and response to alternative management practices could offer further insights into the soils' potential to protect SOC through aggregates as a physical protection mechanism. Even though this study measured the stability of aggregates and the amount of C held inside the aggregates, quantification of the mineralization potential and C dating of these pools would be necessary before the C sequestration potential of the various C pools could be established.

### **Influence of Management Intensity and Family**

The distribution of C across different fractions and the responsiveness of these fractions to management intensity and families are summarized in Figs 5.1 and 5.2. Intensive management did not reduce SOC throughout all soil C pools, but only in specific size-density fractions; especially the 2000 to 250  $\mu\text{m}$  fraction (Chapter 2, 3, 4). It is reasonable to assume that this change resulted from reduced C input when the understory root turnover was reduced by sustained weed control practices. The response of this fraction to management and family was unexpected in some ways. First, the management effect was most pronounced in the stable aggregates that had strength of 6000 J or higher (Chapter 3). This indicates that measuring the mechanical strength of aggregates would not be sufficient to determine the mechanisms through which intensive management practices impact aggregates. An investigation of the chemical changes in these aggregates due to management practices would be necessary. For example, a comparison of the DRIFTS spectra of the aggregate organic matter would indicate whether intensive management reduces the amount of polysaccharides, which are reported to work as binding agents (Tisdall, 1994).

In the 2000 to 250  $\mu\text{m}$  fraction, the heavy density, which is usually considered a passive fraction (Romkens et al., 1999), also responded to management (Chapter 4). This was probably due to predominance of quartz sand in these soils, which has little or no adsorption capacity to offer chemical protection. When modeling C dynamics in sandy soils, one will need to reconsider the usual definitions of active and passive fractions.

Another surprising result was the effect of management intensity on the chemical character of SOM as evidenced by the DRIFTS spectra (Chapter 2). Management intensity was not expected to affect aromatic C in such a short time frame. Change in the chemical composition of the inputs, either litter or roots, might be responsible for this effect and a spectroscopic analysis of the OM inputs under differing management intensities should help to address this question.

The best growing family added more N at the 0 to 5 cm depth and had greater C content in the 2000 to 250  $\mu\text{m}$  light density fraction than the other two families (Chapter 4). The medium growing family, on the other hand, exhibited higher AOM in the 250 to 150  $\mu\text{m}$  medium density fraction and the 2000 to 250  $\mu\text{m}$  heavy density fractions. This illustrates how families may differentially influence soil properties. The best family is reported to have higher foliar N (Jokela E.J., unpublished data) and above ground biomass production (Roth et al., 2006) in response to N fertilization. This opens an avenue for objective-based deployment of families for a variety of management objectives and ecosystem services (e.g., long-term C sequestration versus improved nutrient turnover). However, family did not significantly affect the total soil C content of the size fractions. As this study assessed treatment and family differences through age 6, a follow-up investigation repeated at age 12 to 15 y may be more definitive in examining changes in soil C due to management activities.

### **Active and Passive C Pools Identified In This Study**

Identifying the most active C pools would help in understanding the mechanisms behind the total SOC changes and aid in efficient resource allocation for future research efforts. The characteristics of the size-density fractions used in this study indicate their functionality, which will help in relating the operationally defined C fractions to meaningful C pools.

The 2000 to 250 $\mu$ m fraction was found to be the C pool most responsive to forest management activities. The C content of the whole fraction, as well as the aggregate C in this fraction, responded to management intensity (Chapter 2, 3). In contrast, the light and medium density fraction in this size fraction responded to family differences (Chapter 4). The chemical nature of SOC, as shown by the peaks for esters, amides and aliphatic C compounds, indicated the presence of recently added, undecomposed organic matter in this fraction (Chapter 2). Since this fraction accounted for nearly half of the total SOC, the changes in this fraction should be useful for documenting expected changes in total SOC.

The 250 to 150  $\mu$ m fraction was also sensitive to the effects of management intensity (Chapter 2) and family (Chapter 4). However, the DRIFTS spectra of this fraction did not offer any explanation for the differences. Even though the C and N concentrations were the lowest in this fraction, it accounted for more than 50% of the soil weight and contained 42% of total soil N (Chapter 4). This highlights the importance of this fraction for SOC and N dynamics. Since this fraction contained the highest proportion of aggregate C (84%; Chapter 2), further study of the aggregates should help in understanding the nature of C in this fraction and the role of aggregation in soil C cycling. For example, measurement of the amount of glomalin in aggregates of different stability or under different management intensities may help in identifying the exact effect of forest management activities on aggregation.

The <53 $\mu\text{m}$  fraction was also responsive to forest management activities, even though the magnitude of change was small (2% change in C content, Chapter 2) and exhibited high peaks of esters and amides. These peaks, combined with the presence of a significant amount of aromatics further indicated the presence of decomposed OM combined with fresh undecomposed OM (Chapter 2). However, this fraction was not studied in detail, as it accounted for less than 10% of the total SOC.

The 150 to 53 $\mu\text{m}$  fraction on the other hand, was probably the most stable C pool, as shown by its lack of response to management through age 6 and its high aggregate stability at age 4 (Chapter 3). This fraction also exhibited a unique behavior in terms of response to sonication energy output because it did not exhibit aggregate hierarchy. However, this fraction had a small (5%) decrease in SOC content under the intensive management regime at age 4 (Chapter 2), indicating the presence of more decomposable C forms. Similar effects were not detected at age 6. A future comparison of the chemical composition of this fraction at the two sampling times should help explain these differences.

Of the three density fractions studied, the medium density fractions were most responsive to both forest management intensity and family in both the 2000 to 250 $\mu\text{m}$  and 250 to 150  $\mu\text{m}$  fractions (Chapter 4). The medium density fraction contained the most C. The light density in the 2000 to 250  $\mu\text{m}$  fraction accounted for 7 to 16% of the fraction C and also responded to family differences. The high C: N ratios of these two fractions suggested the presence of relatively undecomposed organic matter and the importance of these two density fractions in C cycling. In contrast, the heavy density fraction was found to be important for N dynamics, as it contained the highest amount of N. The extremely low C: N ratios (2 to 3) implied that the N in this fraction

was probably microbial N. A study of the fungal and bacterial populations in these density fractions could provide further insights into the C and N dynamics of these soils.

### **Additional Research Needs**

This study could have been improved. The samples were prepared by air drying for two weeks and sieving, which is a standard method of sample preparation. However, the soils might have been passed through the 2 mm sieve when still field moist (with light pressure). While these soils can be near air-dry during dry down periods, they were moist when sampled. It is not known if sieving in a field-moist condition would have given different results. I suspect the results would have been similar; yet it would be worth running a comparative evaluation. Use of the bulk density cores for the measurement of the >2mm fraction would also have been preferable, especially for the volumetric C measurements.

Another possible improvement would have been to collect the water-dispersible C by sedimentation during the density fractionation procedure (Chapter 4) instead of measuring it by difference. This fraction is likely to be important for the short-term recycling of C and nutrients and it may be a worthwhile component in future studies.

### **Modeling C Dynamics**

Even though modeling carbon dynamics was not a part of this study, the results suggested that use of the carbon pools identified in this study could aid carbon-modeling efforts. The general approach is to use conceptual carbon pools in modeling (e.g., Century model, Parton et al., 1987; see Smith et al., 1997 for a comparison of various carbon models). However, in most cases the actual measurement of soil organic carbon is limited to total soil carbon. The physical fractionation techniques, such as size and density fractionation, can improve the accuracy of these models by using operationally defined and hence directly measurable carbon fractions to represent the conceptual carbon pools. For example, the Century model (Parton et al., 1987) used

three soil carbon pools, active, slow and passive, based on turnover rates. The characteristics of different size-density fractions in this study suggested that the carbon content in the light and medium density of the 2000 to 250 and 250 to 150  $\mu\text{m}$  fractions could be used to represent the active pool, whereas the carbon content of the 150 to 53 and  $<53 \mu\text{m}$  fractions could be used to represent the slow or passive pools. Similarly, Hassink and Whitmore (1997) used two pools of carbon, protected and non-protected, for modeling the buildup and decline of organic carbon with and without addition of organic matter. Their model was based on the concept that clay plays an important role in physical protection of organic matter, but the protection capacity of the soil is limited. The carbon content of the  $<53\mu\text{m}$  fraction in this study could represent the clay-protected carbon pool, while the  $>53\mu\text{m}$  fractions can represent the unprotected carbon pool. However, more study of the turnover rates and chemical characteristics of these size-density fractions would be necessary for this purpose.

The following model, though far from adequate, represents an initial outline. It uses the continuity equation as the basis and instead of focusing on the right side of the equation, which incorporates various processes and flows, it focuses on the left side of the equation, which represents the effect of these processes on changes in carbon pools. Thus, the changes in total SOC can be separated into changes in labile C (C associated with the active fractions) and protected C (C associated with passive fractions). The equation can be rewritten as follows:

$$(dC/dt)_x = dC_{\text{labile}} + dC_{\text{protected}}$$

$$(dC/dt)_x = dC_{\text{labile}} + dC_{\text{protected (physical)}} + dC_{\text{protected (chemical)}} + dC_{\text{protected (biochemical)}}$$

where,

$C_{labile} = \text{light fraction} + \text{particulate organic matter in medium and heavy density in both 2000 to 250 } \mu\text{m and 250 to 150 } \mu\text{m fractions.}$

$C_{protected} (\text{physical}) = \text{aggregate organic matter as measured by loss on sonication in medium and heavy density of the 2000 to 250 } \mu\text{m and 250 to 150 } \mu\text{m fractions} + C \text{ in 150 to 53 } \mu\text{m fraction}$

$C_{protected} (\text{chemical}) = \text{negligible}$

$C_{protected} (\text{biochemical}) = f(\text{polyphenol content, lignin/N ratio})$

However, it must be reiterated that even though AOM and the heavy density organic matter are considered passive C pools, the AOM in the medium density of 250 to 150  $\mu\text{m}$  fraction and the AOM in the heavy density of 2000 to 250  $\mu\text{m}$  fraction were influenced by management intensity and family in as few as 6 years. Also, the particulate organic matter, which is considered to be a labile pool, did not respond to any of these treatment effects. Therefore, a study of the mineralization potentials and C dating is required to confirm the C protection potential of these size-density fractions.

In conclusion, the major contributions of this study were 1) development of C fractionation techniques suitable for the sandy soils, 2) establishing the importance of aggregates in these soils, which was a neglected subject until now, 3) establishing the profile of C in terms of content distribution and, 4) identification of the most responsive C pools to the management-related changes as well as the pools that have potential for long-term C sequestration.

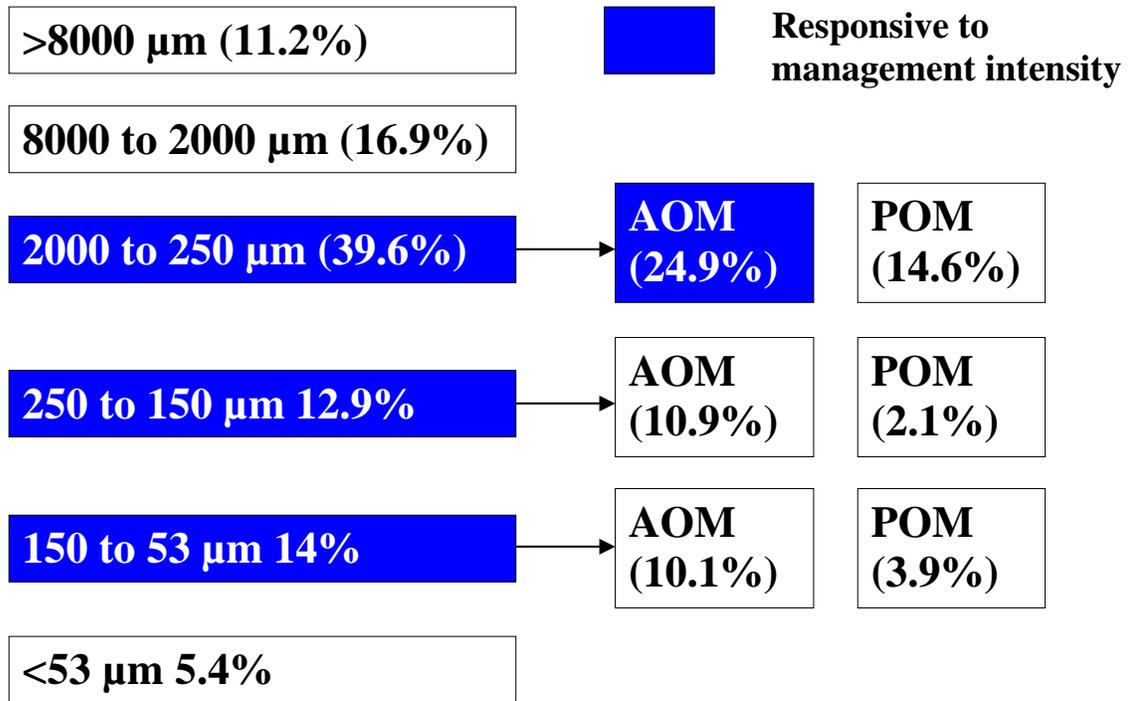


Figure 5.1. Carbon profile at age 4 for a sandy Spodosol in north Florida.

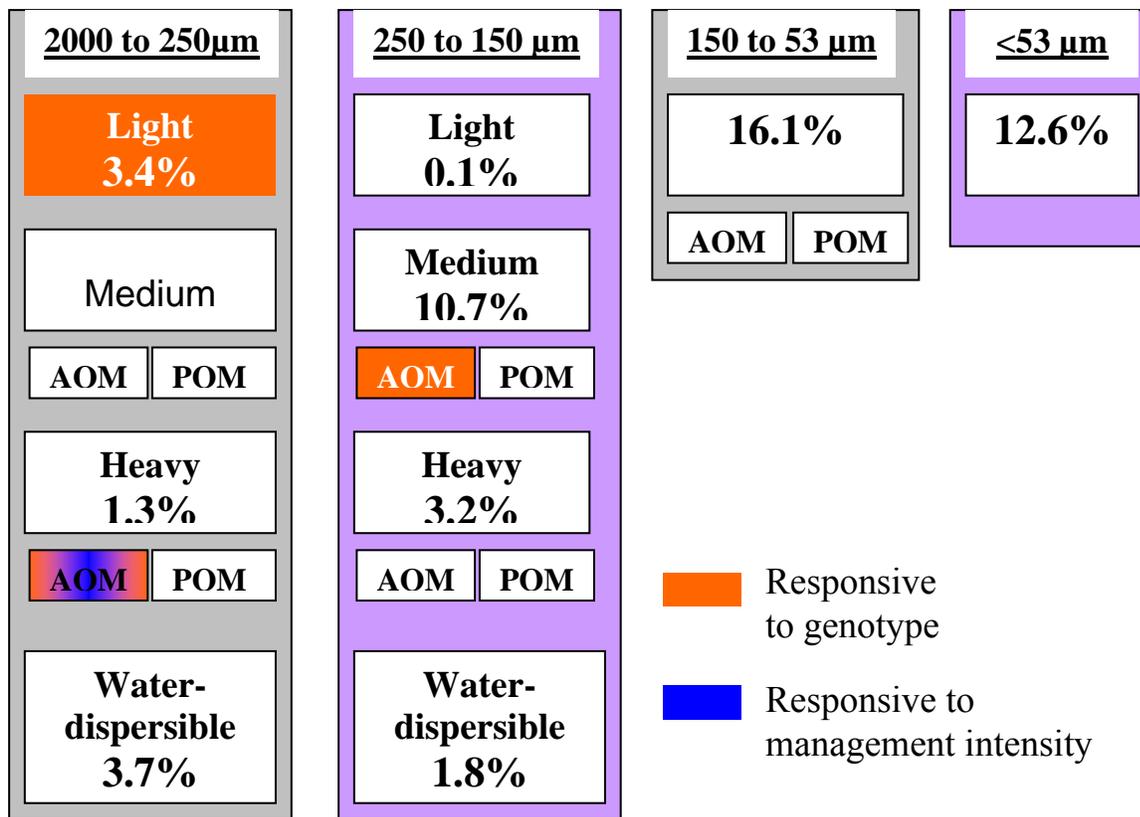


Figure 5.2. Carbon profile at age 6 for a sandy Spodosol in north Florida

APPENDIX A  
EXPERIMENTAL PROTOCOLS

**Wet sieving – Used in Chapter 1**

1. Wet sieving was used for size fractionation in this study. Soil samples obtained from the field were first air-dried and passed through a 2-mm sieve. They were then sieved to separate the aggregates into four size classes, using three 8” diameter brass sieves of sizes 250, 150 and 53  $\mu\text{m}$ ).
2. Place top sieve (250  $\mu\text{m}$ ) in a water tray and pour water into the tray until it stands about 2 cm above the sieve mesh. Any container with sufficient height and diameter/width to fit the sieve can be used as a water tray.
3. Add 100 g of soil to the sieve and swirl the sieve to allow even distribution of the soil. Let it stand for 5 minutes, so that the soil sample is thoroughly soaked.
4. Move the sieve up and down 50 times taking care that every time the water surface is broken. This should take about 2 minutes (Cambardella and Elliot, 1993).
5. Swirl as necessary to avoid collection of soil in the middle of the sieve.
6. Take the sieve out of the water, wash sieve’s side with a spray bottle to ensure that all the finer soil is collected in the tray, then allow the sieve dry for 24 hours.
7. Transfer the soil water mixture in the tray to the next size (150  $\mu\text{m}$ ) sieve and repeat the procedure.

**Dry sieving – Used in Chapters 1, 2, 3**

1. Soil samples obtained from the field were first air-dried and passed through a 2-mm sieve. They were then sieved to separate the aggregates into four size classes, using three 8” diameter brass sieves of sizes 250, 150 and 53  $\mu\text{m}$ ).
2. For this, 100 g of air-dried soil was placed in the large sieve of a stack of sieves with sieve sizes mentioned above. The sieves were placed on a horizontal mechanical shaker for 5 minutes at 75 rpm. This duration was used to achieve size fractionation with minimum disruption of aggregates, and also because, for the soil used in this study, there is very little difference in the size distribution after 5 min and 20 min of shaking.

**Sonication: Used in Chapters 2 and 3**

Sonication was used to breakdown soil aggregates, to measure the strength of aggregates, and to determine the amount of C held inside the aggregates. Three size fractions, 2000 to 250, 250 to 150 and 150 to 53  $\mu\text{m}$ , were used in this study. The same technique can be used for whole soil if desired.

1. Setting the sonicator: Sonic Dismembrator (Fisher Scientific, model 500)
  - a. Adjust the amplitude using the circular knob on left of the control box. The amplitude ranges from 0 to 100%, but for the model mentioned above, a different probe is necessary for amplitude greater than 60%. Choice of amplitude depends on the energy output required. See Table 1 for the values used in this study.

- b. Adjust the ON and OFF timing (59.9 sec maximum) and total duration. Save the settings for future use. In this study, the pulse method (59.9 sec ON and 30 sec OFF) was used to avoid an excessive rise in temperature. However, if temperature is not a concern, using the continuous method will save time.
  - c. Make sure the printer and output settings are enabled. For this, use the arrow keys on the control box to go to the printer/output options and click enter on desired option.
2. Sonication:
- a. Weigh 10 g soil in a 250 mL beaker. Make sure that you use the same shape and size of beaker every time because the height of immersion of probe affects the extent of sonication (North, 1976).
  - b. Add 100 mL distilled water to the beaker slowly to avoid formation of bubbles.
  - c. Put the beaker in the sound box. Immerse the probe in the soil-water suspension taking care that it is in the center and not touching the beaker walls. Use a clamp or another inverted beaker to maintain a constant depth of immersion. Close the sound box securely and push START.
  - d. After the sonicator shows 'experiment completed' message, take out the beaker. Wash the particles on the probe into the beaker using a spray bottle. Dry the probe gently.
3. Post-sonication sieving:
- a. This is done to separate the material released by the aggregates after sonication from the POM and OM still in aggregates (if not all aggregates have been destroyed)
  - b. Pour the contents of the beaker onto the sieve taking care that no particles are sticking to the beaker walls. Use the same size sieve used for sieving before sonication (e.g. 250- $\mu\text{m}$  sieve for the 2000 to 250- $\mu\text{m}$  fraction).
  - c. Spray some water lightly onto the sieve to spread the soil evenly on it and let it dry for 24 hours.
  - d. Measure C remaining after sonication using loss on ignition or any other chosen method of C measurement.

### **Density separation: Used in Chapter 3**

1. In this study, two size fractions, 2000-250 $\mu\text{m}$  and 250-150 $\mu\text{m}$  were density fractionated, but the same procedure can be used for whole soil.
2. Weigh 10 g of sample in a small (50 mL) beaker.
3. Add approximately 10 mL of water and swirl the beaker to mix it thoroughly.
4. Add approximately an additional 15 mL of water in order to get a clear delineation between heavier mineral soil and the floating soil organic matter.
5. Decant the supernatant into a 600 mL beaker. Repeat the process until no more organic matter can be visually separated. (Twenty-five milliliter water was added to the beaker and the organic matter was separated by swirling and decantation.)
6. The organic matter that cannot be separated from the mineral matter is defined as the heavy fraction (HF).

7. Transfer the contents of the 600mL beaker to the funnel assembly (See the Photograph) and let it settle till the water is clear. It takes 12 to 24 hrs, depending on the fraction size. Finer fractions need more time.
8. After 24 hours, the fraction floating on water is called the light density fraction (LF) and the fraction settled at the bottom of the funnel is called the medium density fraction (MF).
9. Collect the medium-density fraction in a beaker by opening the stopcock and allowing the suspension to pass through.
10. Collect the light fraction in another beaker.
11. The three fractions thus separated can be dried with or without sieving. Sieving is done for measurement of water-dispersible aggregates if desired. Simply pass the fraction through the same size sieve used for sieving. Soil passing through the sieve is called the water-dispersible fraction. Alternatively, the water-dispersible fraction can also be measured by difference (Total soil used for density fraction – [HF + MF + LF])

Table A.1. Amplitude and time combinations and energy outputs used for this study

Amplitude	Time	Energy (J)	Peak Power (W)
20	1	954	16
20	2	1911	16
20	3	2871	16
20	4	3830	16
20	5	4785	16
30	1	1523	26
30	2	3004	26
30	3	4588	26
30	4	5981	26
30	5	7602	26
40	1	2093	36
40	2	4129	36
40	3	6183	36
40	4	8863	36
40	5	10432	36
50	5	13828	48

Note: Energy output can vary depending upon the peak power. The values given here are those observed in this study.

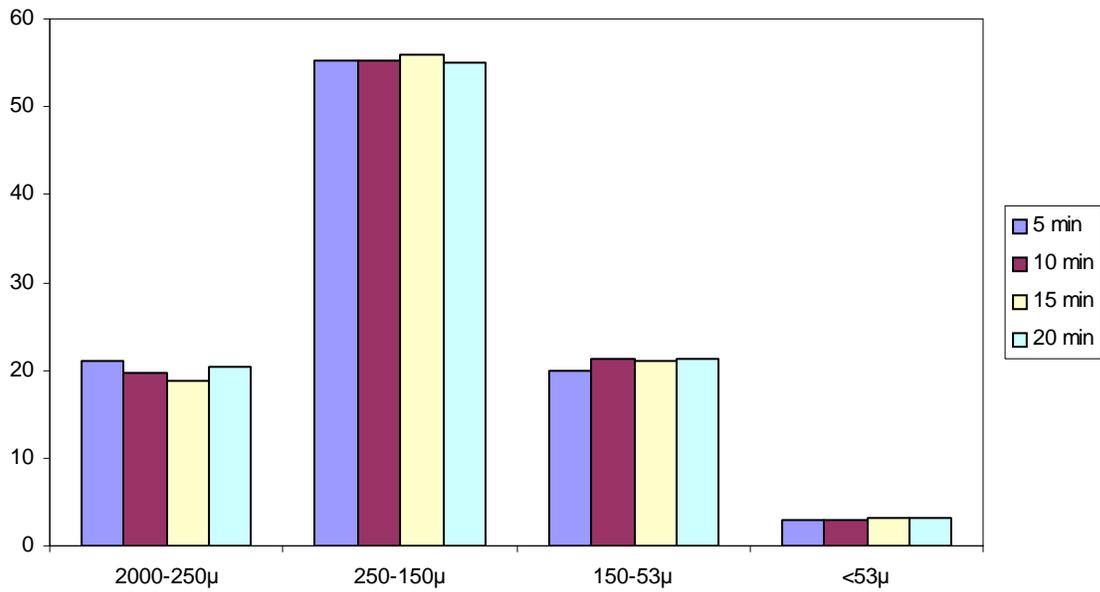


Figure A.1. Effect of sieving time for dry sieving on the weight distribution across size fractions



Figure A.2. Funnel assembly used for density fractionation.

APPENDIX B  
AGGREGATES OBSERVED IN THE SANDY SPODOSOLS

This appendix shows the morphology and chemical nature of the aggregates in a sandy Spodosol of north Florida under forest management. Aggregates were observed using a Scanning electron microscope (SEM) (JEOL JSM 6400) equipped with an energy-dispersive x-ray fluorescence elemental microanalysis (EDX) system. The elemental dot maps were used to determine the internal structure, that is, the spatial distribution of the different elements in the aggregates. The edx graphs were used to determine the relative concentration of the different elements in the aggregates.

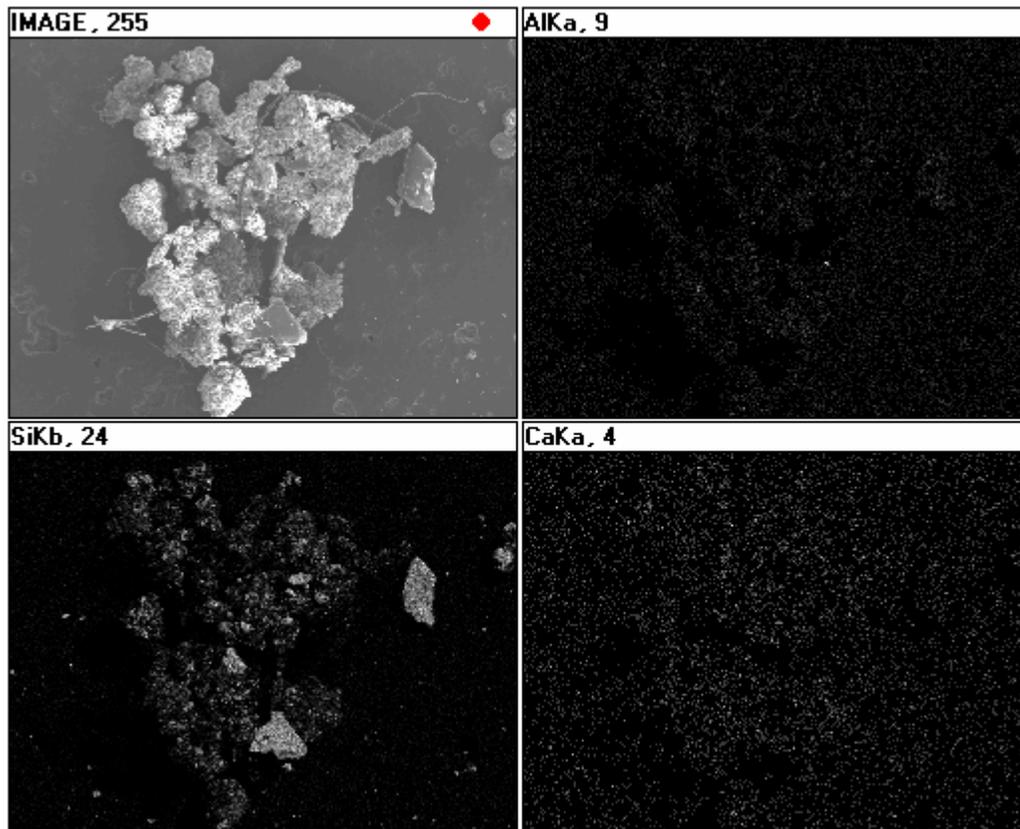


Figure B.1. Image and elemental dot map of aggregate (2000 to 250  $\mu\text{m}$ ). The upper left panel is the SEM photo, the upper right panel is the dot map for aluminum, the lower right panel is the dot map for silica and the lower right panel is the dot map for calcium.

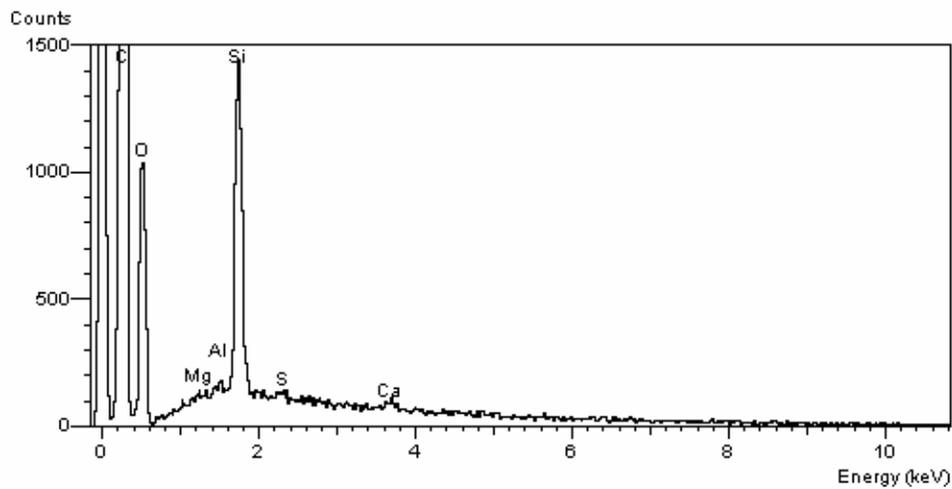


Figure B.2. EDX spectra for the aggregate shown in B.1.

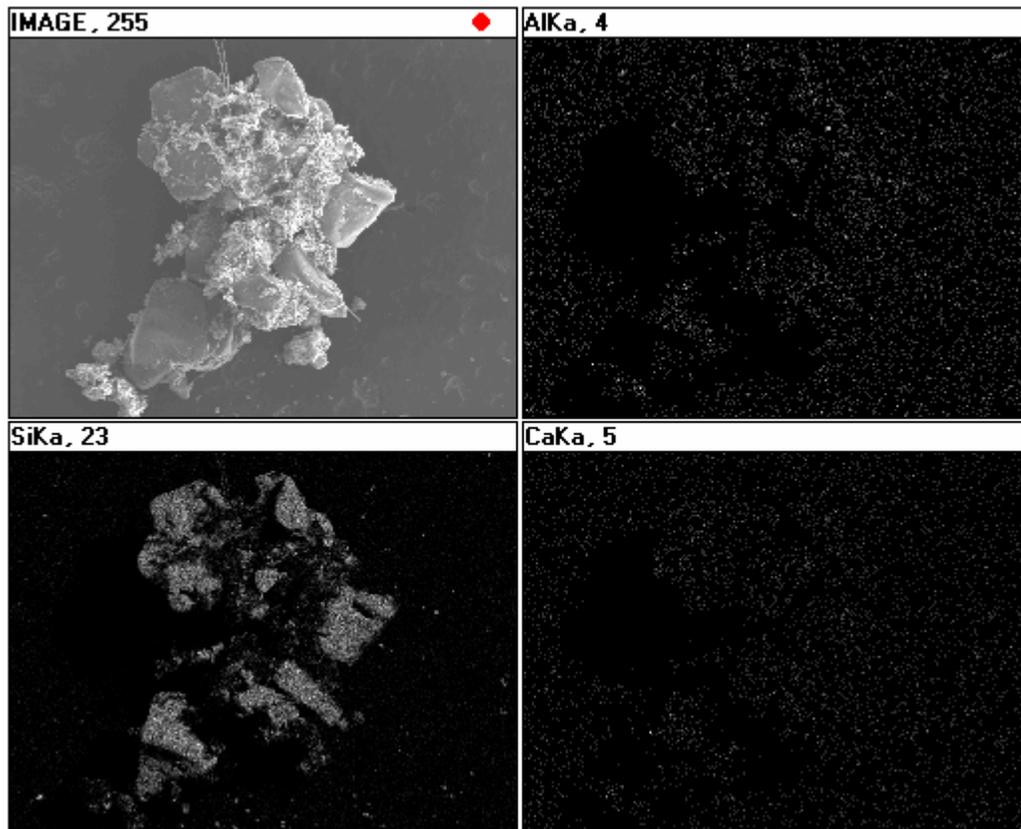


Figure B.3. Image and elemental dot map of aggregate (2000 to 250  $\mu\text{m}$ ). The upper left panel is the SEM photo, the upper right panel is the dot map for aluminum, the lower right panel is the dot map for silica and the lower right panel is the dot map for calcium.

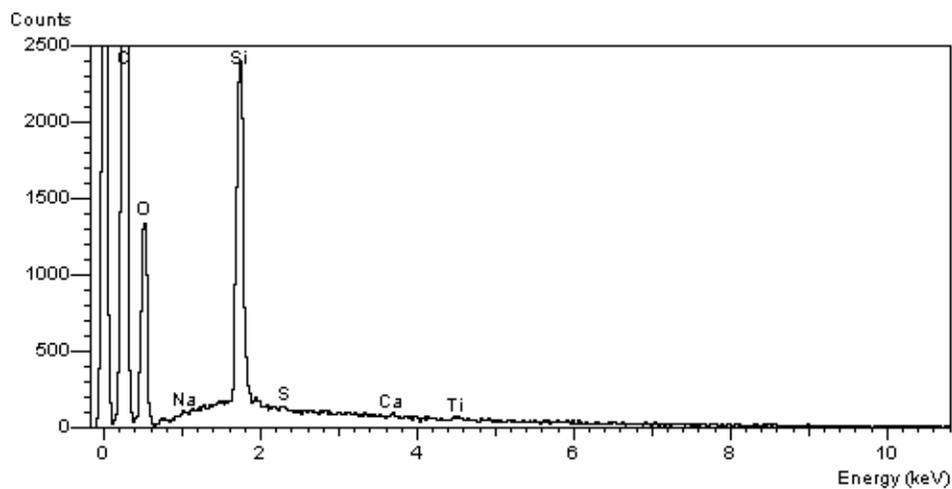


Figure B.4. EDX spectra for the aggregate shown in B.3.

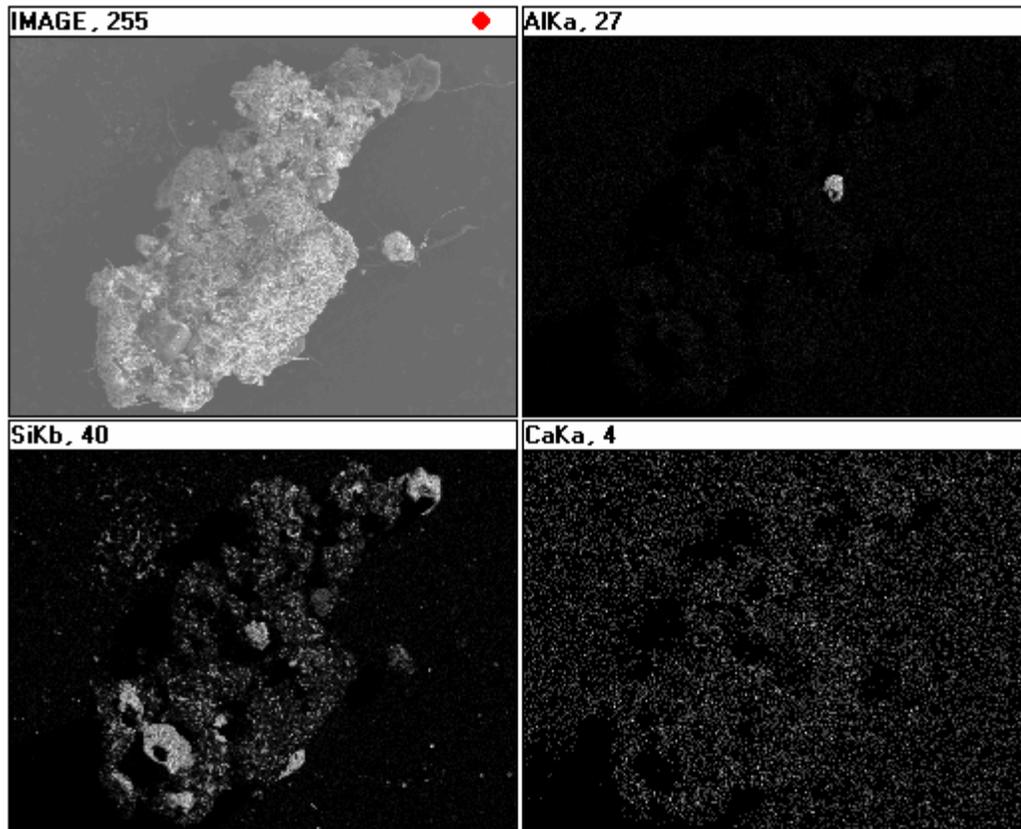


Figure B.5. Image and elemental dot map of aggregate (2000 to 250  $\mu\text{m}$ ). The upper left panel is the SEM photo, the upper right panel is the dot map for aluminum, the lower right panel is the dot map for silica and the lower right panel is the dot map for calcium.

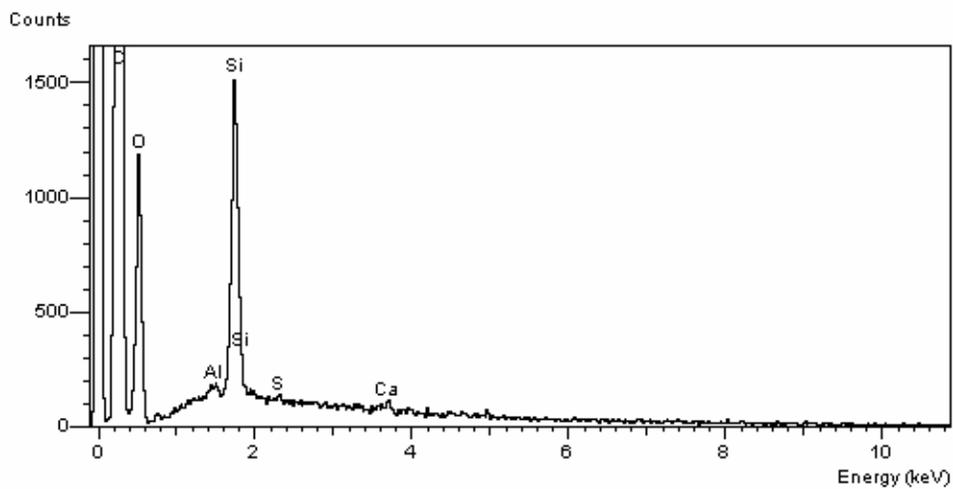


Figure B.6. EDX spectra for the aggregate shown in B.5.

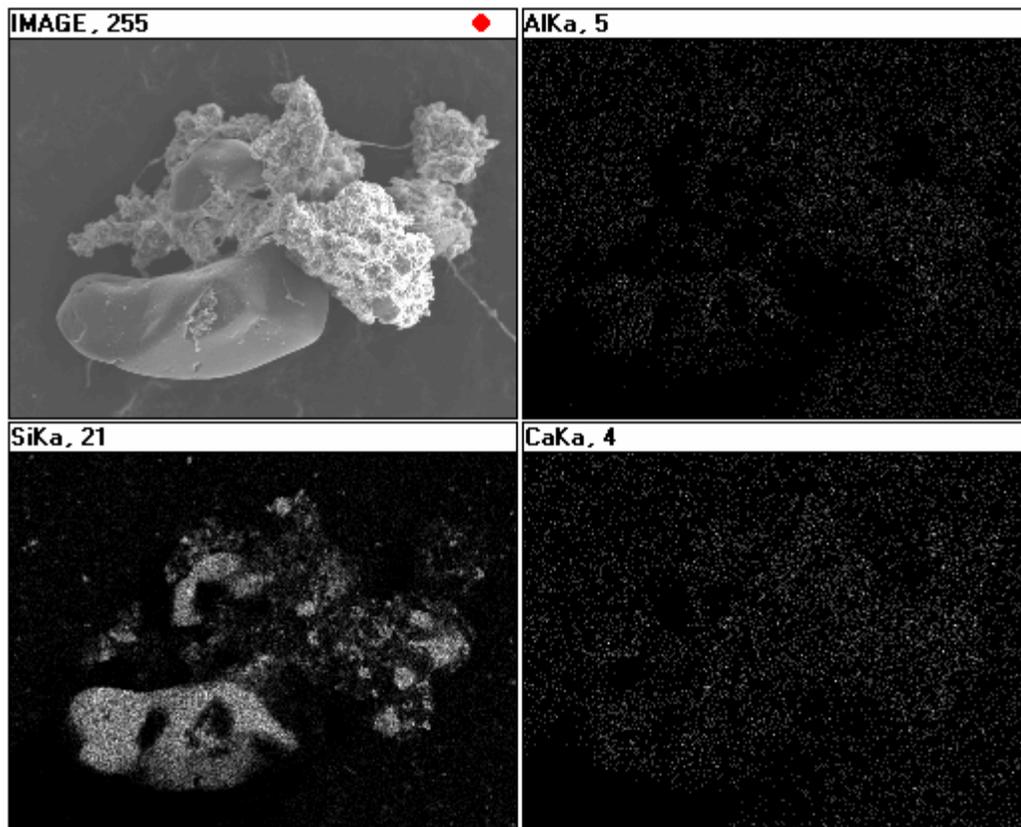


Figure B.7. Image and elemental dot map of aggregate (250 to 150  $\mu\text{m}$ ). The upper left panel is the SEM photo, the upper right panel is the dot map for aluminum, the lower right panel is the dot map for silica and the lower right panel is the dot map for calcium.

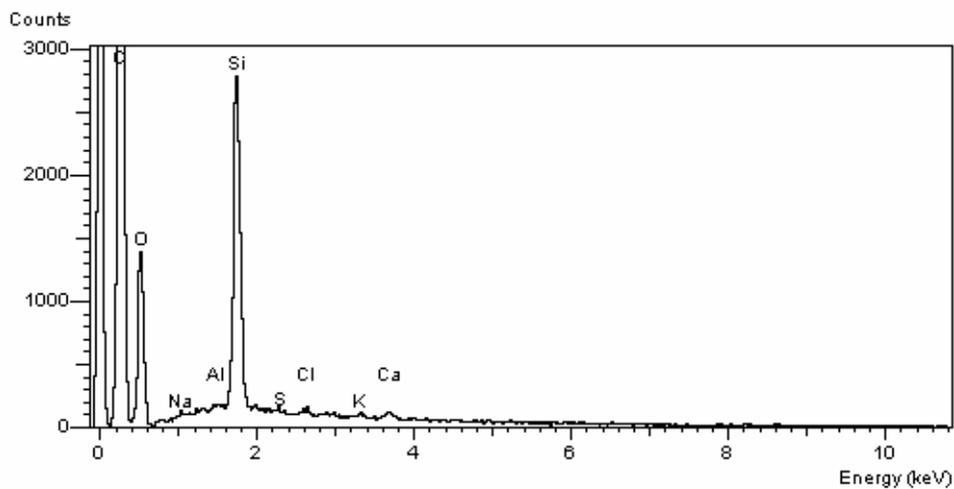


Figure B.8. EDX spectra for the aggregate shown in B.7.

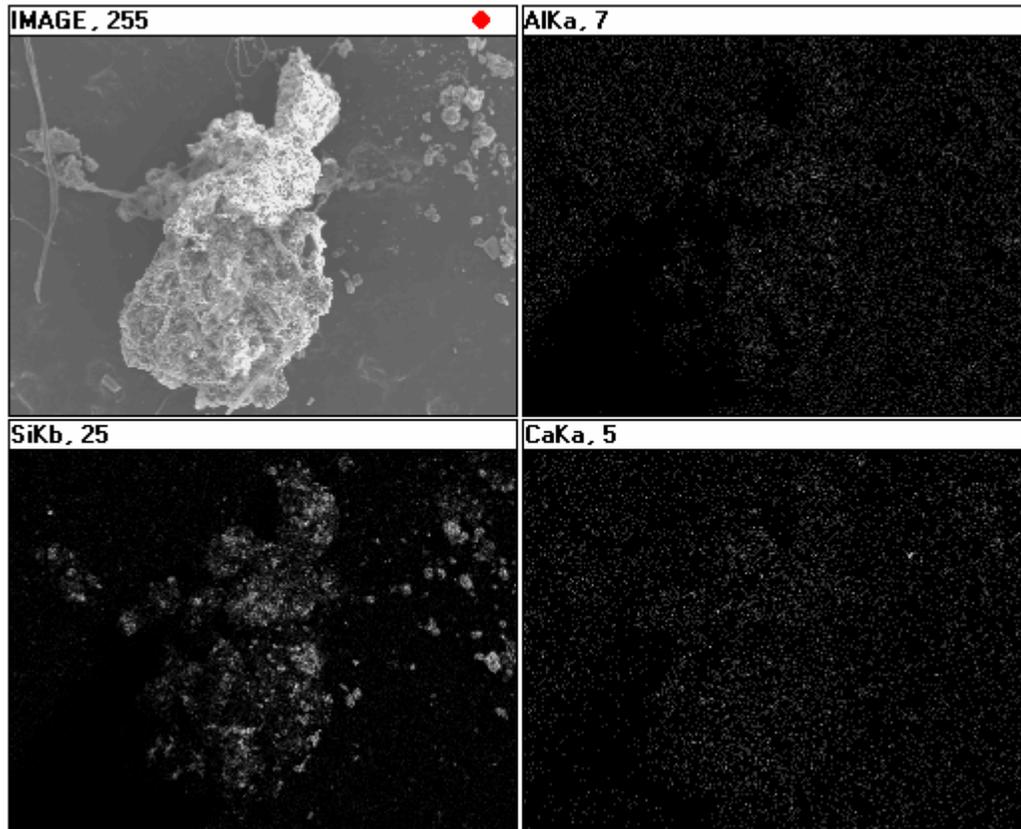


Figure B.9. Image and elemental dot map of aggregate (250 to 150  $\mu\text{m}$ ). The upper left panel is the SEM photo, the upper right panel is the dot map for aluminum, the lower right panel is the dot map for silica and the lower right panel is the dot map for calcium.

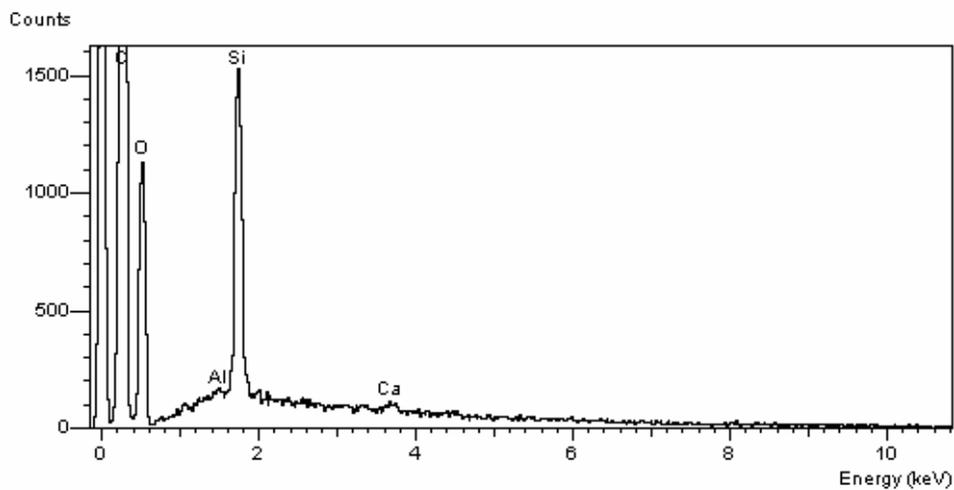


Figure B.10. EDX spectra for the aggregate shown in B.9.

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Deoyani Vinayak Sarkhot has an undergraduate degree in agriculture and a master's degree in soil science, both earned in India. A love for nature and for the plants led her to agriculture, and her interest in soil and water conservation, which deepened with every passing year, naturally led to soil science. She has worked as a teacher, teaching basic soil science in a farmers' school, as an extension worker in a saline land reclamation project and as a journalist in an agri-business publication. Her main ambition is doing some work that will make a difference for the farmers and for her beloved nature. She is immensely grateful for all the opportunities she has had so far and hopes that this good fortune will continue to be with her as she begins the new phase of her life.