



# Carbon and nitrogen pools in aggregate size fractions as affected by sieving method and land use intensification



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## ABSTRACT

Soil organic carbon (C) is a key component regulating grazing land ecosystem production and sustainability. In this study, we investigated the impacts of wet vs. dry physical separation of soil C fractions in representative sandy Coastal Plain Spodosols subjected to different levels of management intensification. X-ray diffraction and solid-state <sup>13</sup>C nuclear magnetic resonance (NMR) spectroscopy were used to characterize the structural composition of the fine (< 53 μm) particle size. Dry sieving resulted in ~10–15% greater total C recovery as compared to wet sieving. Sieving method also affected the proportion of soil C associated with the various size fractions. In general, dry sieving resulted in greater proportion of soil C (48 to 73% of the total C in the 0 to 10 and 10 to 20 cm, respectively) associated with the 150–53 and < 53 μm fractions. Conversely, the proportion of C associated with large aggregates (2000–250 and 250–150 μm fractions) increased 1.5 to 2-fold when wet sieving was performed. X-ray diffraction demonstrated that mineral composition of the fine (< 53 μm) fraction was dominated by quartz and, thus, offers limited protection against mineralization. Results also demonstrated that alkyl C and total O-alkyl C dominated soil C chemical composition of both whole soils and < 53 μm fractions. In whole soils, abundance of alkyl C and total O-alkyl C decreased while methoxyl, carboxylic, and total aromatic-C increased with management intensification (native rangeland < silvopasture < sown pasture). However, in the < 53 μm fraction, total O-alkyl C abundance was greater in native rangeland than that in the silvopasture and sown pasture ecosystems. Both sieving methods were sensitive tools to evaluate the long-term impacts of grazing land intensification on soil C distribution among aggregate size fractions. However, because dry sieving is easier to perform and preserves more C than wet sieving, dry sieving can be a reliable method of soil C separation for determining the impacts of land use management on soil C dynamics in sandy soils.

## 1. Introduction

Globally, grazing lands occupy ~3.6 billion ha and are responsible for offsetting ~20% of the annual C dioxide (CO<sub>2</sub>) emitted as result of land use changes (Follett and Reed, 2010). In the USA, grazing lands play a key role in climate change mitigation as they occupy a large area (258 million ha) (USDA-NRCS, 1997) and have potential to sequester 13 to 70 Mt soil C yr<sup>-1</sup> (Lal et al., 2007). However, grazing land management can affect the equilibrium between C inputs and losses with subsequent effects on soil C sequestration potentials. Soil organic C is a key component for sustaining production as it affects nutrient

supply to plants, soil erosion control, soil physical and chemical properties (e.g., aggregation, cation exchange) and water holding capacity (Li et al., 2007). Therefore, in addition to the positive effect on global C cycle, identifying management strategies that promote soil C accumulation can also enhance grazing land productivity and sustainability.

In humid subtropical ecosystems, soil C decomposition is favored due to the relatively high temperature and precipitation, therefore, enhancing soil C stocks can be a major challenge in this region. In addition, soils in the Coastal Plain region are generally characterized by coarse texture and relatively low soil C content compared with other regions. The combination of warm and moist climate conditions with

Abbreviations: SOC, soil organic carbon

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the lack of clay minerals to protect soil C against microbial mineralization favor fast soil C turnover in these soils.

Physical separation of soil C into particle-size fractions has been extensively used to investigate the changes in soil C dynamics as affected by land use management. Most physical separation procedures are based on the hypothesis that turnover decreases from macro- to micro-aggregates, thereby implying there is a physical protection (Six et al., 1999; Tisdall and Oades, 1982). Although physical methods have routinely been used to fractionate soil C into various size pools with distinct residence times (Six et al., 1999; Solomon et al., 2000; Billings et al., 2006; Dubeux et al., 2006; Qiu et al., 2012; Bliss et al., 2013; Silveira et al., 2014; Trivedi et al., 2015), comparison of different approaches with regards to the most appropriate separation procedure for coarse-textured soils in subtropical regions is still lacking. Since these soils have relatively low clay content ( $< 20 \text{ g kg}^{-1}$ ), we expected poor aggregation and limited protection against soil C degradation. Under these circumstances, although the mineral fraction ( $< 53 \mu\text{m}$ ) represents a relatively small proportion of the soil mass, this fraction may be an important sink for soil C stabilization in Coastal Plain soils (Silveira et al., 2013, 2014).

The success of different separation procedures depends to some extent on the soil characteristics. While wet sieving has been the more frequently used method for physical fractionation of soil C (Cambardella and Elliott, 1993; Six et al., 1998; Marx et al., 2005), preliminary studies in Florida indicated that dry sieving was a more suitable procedure for sandy soils because it minimized loss of water-soluble C pools (Sarkhot et al., 2007a). Additionally, dry sieving has also been suggested as a superior method when examining microbial activity and biologically active pools in aggregates (Sainju, 2006). Therefore, the objectives of this study were to compare the effects of wet vs. dry sieving on soil C distribution into different aggregate size fractions in Coastal Plain Spodosols subjected to different management intensities, and to utilize  $^{13}\text{C}$  NMR spectroscopy techniques to characterize soil C associated with the fine ( $< 53 \mu\text{m}$ ) particle size pool.

## 2. Material and methods

### 2.1. Experimental sites and soil sampling

Soil samples (0 to 10 and 10 to 20 cm depths) were collected from existing long-term experimental sites located at the UF/IFAS Range Cattle Research and Education Center in Ona, FL ( $27^{\circ}23'\text{N}$ ,  $81^{\circ}57'\text{W}$ ). Average annual temperature in this area is  $21.5^{\circ}\text{C}$  and mean annual precipitation is 1206 mm. Predominant soils were Ona fine sand (sandy, siliceous, hyperthermic Typic Alaquods) and Smyrna sand (sandy, siliceous, hyperthermic Aeric Alaquod). Treatments consisted of field twice-replicated grazing land biomes that represent a gradient of management intensity ranging from: native rangelands (lowest), pine (*Pinus elliottii* Engelm)–bahiagrass (*Paspalum notatum* Fluegge) silvopasture (intermediate), and sown bahiagrass pastures (highest). Each experimental unit ( $n = 2$ ;  $\sim 6$  ha each) was adjacent to each other but managed independently. All sites exhibited the same topography, soil series, and climate conditions, and have been established and consistently maintained for over 20 years. Detailed information about the management practices have been previously reported in Silveira et al. (2014).

Five equidistant quadrats ( $20 \times 20$  m) were established along a diagonal transect in the middle of each of experimental unit to avoid edge effects. In each quadrat, five soil core samples (3-cm diameter  $\times$  10-cm height) were randomly collected in mid-summer (June and July 2012) from the 0–10 and 10–20-cm depths and combined into a composited sample by depth for and total C and N determinations. For bulk density, one random undisturbed soil core was sampled in each quadrat. Samples were air-dried and sieved through a 2-mm screen. Subsamples were oven-dried ( $65^{\circ}\text{C}$  for 48 h) for bulk density determination.

### 2.2. Soil size fractionation

Air-dried soil samples were fractionated into four particle-size aggregate fractions using either the wet or dry sieving procedure: 2000–250  $\mu\text{m}$ , 250–150  $\mu\text{m}$ , 150–53  $\mu\text{m}$ , and  $< 53 \mu\text{m}$ . Wet sieving followed the method described by Six et al. (1998). Briefly,  $\sim 100$  g of soil was submerged in deionized water ( $\sim 3$  cm above the sieve screen) for 5 min and then the sieve was manually moved up and down 50 times for  $\sim 2$  min. Soil with water that passed through the 250  $\mu\text{m}$  sieve was then transferred to the next sieve size and the same procedure was repeated until the four size fractions were obtained. Soil retained in the various sieve sizes was recovered, oven-dried at  $55^{\circ}\text{C}$ , weighed, and analyzed for total C and N concentrations. Dry sieving was conducted on 100-g subsamples using a series of sieves on a Ro-Tap TM sieve shaker (W.S. Tyler, Mentor, OH) for 5 min, based on the method described by Dubeux et al. (2006). Similar to the wet sieving procedure, soil recovered from each sieve was dried at  $55^{\circ}\text{C}$ , weighed, and analyzed for total C and N concentrations using a Flash EA 1112 Series elemental analyzer (Thermo Fisher Scientific Inc., Waltham, Massachusetts).

### 2.3. X-ray diffraction analysis

Mineral composition of the  $< 53 \mu\text{m}$  fraction derived from wet sieving was investigated by X-ray powder diffraction analysis as performed using a computer-controlled Ultima IV x-ray diffractometer (Rigaku Corporation, Japan) equipped with stepping motor and graphite crystal monochromator. Samples were scanned in cavity mounts from  $2$  to  $60^{\circ}$   $2\theta$  with Cu K $\alpha$  alpha radiation at a rate of  $2^{\circ}$  per minute.

### 2.4. Solid-state $^{13}\text{C}$ NMR spectroscopy

Carbon functional groups associated with the whole soils and the  $< 53 \mu\text{m}$  fraction obtained from wet sieving were characterized using solid-state  $^{13}\text{C}$  NMR spectroscopy. Magic angle spinning (MAS) solid state NMR experiments were carried out on a Bruker 500 MHz Avance III spectrometer equipped with a 3.2 mm E-free H/C/N probe. Soil samples were packed into zirconia rotors with Kel-F drive caps and spun at the magic angle 8 kHz at 233 K. MAS  $^{13}\text{C}$  solid state NMR spectra were collected utilizing a cross polarization with total sideband suppression pulse sequence (COTOSS) sequence (Dixon et al., 1982). A  $2.5 \mu\text{s}$   $^1\text{H}$   $\pi/2$  pulses followed by a 1.5 ms ramped cross polarization pulses at 100 kHz ( $^1\text{H}$ ) and 55 kHz ( $^{13}\text{C}$ ) was used.  $^{13}\text{C}$   $\pi$  pulses were applied at 125  $\mu\text{s}$  intervals for sideband suppression. 100 kHz of  $^1\text{H}$  decoupling was employed during the 40 ms signal acquisition time. The recycle delay time was 3 s. For each sample, approximately 17,500 to 74,500 scans were collected for each sample to obtain sufficient signal/noise ratios. The MAS  $^{13}\text{C}$  solid state NMR spectra were referenced using an external adamantane reference and spectral regions were integrated to determine the percent contribution of each C functional group in the sample based on the assignments from Knicker (2011): 0–45 ppm alkyl C, 45–60 ppm methoxyl C, 60–90 ppm O-alkyl C, 90–110 ppm di-O-alkyl C, 110–140 ppm aromatic C, 140–160 ppm phenolic C and 160–220 ppm carboxylic C.

### 2.5. Statistical analysis

Statistical analysis was performed using SAS PROC MIXED procedure (SAS Institute, 2001). Grazing land type, soil depth, aggregate size fraction, and sieving method were considered fixed effects while replicates were random effect. The PDIF test of the LSMEANS procedure was used to separate means. Main factors and their interactions were considered significant when F-test  $P$  values were  $\leq 0.05$ .

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