Characterization of soil organic carbon pools by acid hydrolysis


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Abstract

Chemically stable fractions of the soil organic carbon (SOC) contribute to the soil C sequestration and may have an important role for the global C budget. Soil organic carbon characterization was studied using acid hydrolysis methods with 1 M and 6 M HCl and hot-water extraction in two coastal plain soils collected from different landscape positions. The residues after the chemical treatments were analyzed by solid state 13C nuclear magnetic resonance (NMR) spectroscopy to investigate the changes in the structural composition. This study demonstrates the effectiveness of acid hydrolysis treatment with HCl for estimating the labile pools of SOC. Between 1 to 4% of total C was extracted by hot water in both soils compared to 18 to 32% of total C hydrolyzed by HCl. Although there were quantitative differences in the amount of C released, both soils showed similar percentages of non-hydrolyzable carbon (18–25% of total C for upland soil, and 24–32% of total C for wetland soil). The NMR spectra of the residues after acid hydrolysis indicated the removal of labile components (i.e. carbohydrates and amino acids), and the ability of 6 M HCl to characterize SOC quality appeared to be superior compared to the 1 M HCl hydrolysis and hot-water extraction. The best two-component model (R2 = 0.994) identified by stepwise regression analysis for hydrolyzable C pool included microbial C biomass and water-extractable C. Our data demonstrate that acid hydrolysis with HCl can provide meaningful estimates of C pools in sandy, low C coastal plain soils.

Keywords: Acid hydrolysis; 13C NMR; C sequestration; Coastal plain soils; Labile C; Soil organic matter

1. Introduction

The increase in greenhouse gas emission and resulting global climate changes have driven the need for a better understanding of C cycling. Soil is a major net C sink and plays an important role in global C cycling. However, simply increasing C in soils is not synonymous with C sequestration. To be sequestered, C should be converted from the active pools to less reactive intermediate or passive pools, and, consequently, sustained in the soil for decades or longer (Wang and Hsieh, 2002). Labile fractions of SOC, such as microbial biomass, exhibit relatively fast turnover rates and, therefore, may not contribute significantly to C sequestration in soils. The more stable (humified and protected) soil organic carbon (SOC) pools are the more appropriate and representative fractions of sequestered C in the soil (Cheng and Kimble, 2001).

Soil organic matter is a complex mixture of organic materials derived from litter and root turnover and microorganisms whose chemical makeup includes carbohydrates, amino acids and proteins, nucleic acids, lipids, lignins and humus; listed in the approximate order of increasing chemical resistance to decomposition (McColl and Gressel, 1995). The components of SOC can be compartmentalized into different fractions or pools. Some C components present in the soil, such as carbohydrates, are considered "labile" because they are easily degradable by microorganisms when they are not protected by physical and chemical processes. More chemically recalcitrant compounds accumulate in the soil as soil organic matter decomposes. The relative amount of recalcitrant and labile materials and the degree to which the organic compounds are protected from decomposition determine soil organic matter degradability (Rovira and Vallejo, 2000).

A variety of methods are used to characterize SOC degradability. Characterization of SOC degradability encompasses chemical, biological and physical techniques. Separation of SOC into pools can be useful in identifying and understanding...
differences in structure, function and bioavailability. This approach allows the identification of labile fractions, which are expected to respond most rapidly to environmental changes (Khanna et al., 2001), and is an indicator of SOC quality. Among the chemical methods used to characterize C pools in soils is acid hydrolysis, which has been employed to distinguish between resistant and active C fractions. The most widely adopted procedure is the refluxing of soil in 6 M hydrochloric acid (Leavitt et al., 1996; Xu et al., 1997; Collins et al., 2000). This technique has been shown to indicate the size of the recalcitrant SOC pool (Rovira and Vallejo 2000). The hydrolyzable fraction is largely comprised of proteins, nucleic acids, and polysaccharides (Schnitzer and Khan, 1972; Schnitzer and Preston, 1983; Rovira and Vallejo, 2002) and some carboxyl C (Preston and Schnitzer, 1984), while non-hydrolyzable residue typically contains mainly lignin and related compounds (Paul et al., 1997), along with fats, waxes, resins and suberins (Rovira and Vallejo, 2002).

According to Preston and Schnitzer (1984) about 90% (wt/wt) of the carbohydrates can be potentially removed after treatment with 6 M HCl, without significant changes in aliphatic, aromatic and remaining carboxyl groups. While more labile components are removed by 6 M HCl, aromaticity of the C increases in the remaining residue (Rovira and Vallejo, 2002). Using radiocarbon dating technique, Leavitt et al. (1996) showed that the fraction resistant to hydrolysis is relatively older than the hydrolyzable fraction.

Another technique frequently employed to assess labile pools of SOC is the hot-water extractable C method. Hot water is considered a mild agent that affects the C fraction involved in the short-term binding of aggregates. Studies have shown that the amount of C released after hot-water extraction is strongly related to soil microbial biomass, CO₂ evolution and microaggregation (Haynes and Francis, 1993; Ghani et al., 2003). The fraction of C extractable with hot water has shown to be mainly of microbial origin (Haynes and Francis, 1993). Carbon release by hot-water extraction is, in general, significantly smaller than that measured by acid hydrolysis (Chan and Heenan, 1999; Puget et al., 1999). The latter suggested that polysaccharides are only partly extracted with hot water and that carbohydrate-C recovery was lower compared to diluted and concentrated acid hydrolysis.

Several different procedures have been described in the literature to characterize labile pools of SOC; however there is a clear lack of uniformity in experimental conditions. For instance, Rovira and Vallejo (2000) suggested two methods using either hydrochloric acid (6 M) or sulfuric acid in a two-step procedure (2.5 M and 13 M). They further noted that the first procedure was less affected by the mineral matrix. Schnitzer and Preston (1983) compared hydrolysis of humic and fulvic acids in 6 M hydrochloric acid and 12 M and 0.5 M sulfuric acid and found that both procedures removed amino acids and carbohydrates; however hydrochloric acid was more effective. When using HCl, hydrolysis conditions varied. Acid concentrations ranged from 1 M HCl solution refluxed for 4 h (Xu et al., 1997), 3 M HCl for 18 h (Barriuso et al., 1987), and 6 M HCl from 6 h (Chefetz et al., 2002) to 16 h (Paul et al., 2001) have all been used. The objectives of this study were (i) to assess labile pools of C using different extraction and hydrolysis procedures in upland and wetland soils with contrasting SOC concentrations, and (ii) to characterize changes in the quality of SOC after the treatments using ¹³C NMR analysis, and (iii) to correlate labile and chemically resistant (non-hydrolyzable) C pools with biochemical SOC attributes.

2. Material and methods

2.1. Soil samples

The study site was at Fort Benning military training reservation, in west central Georgia south of the city of Columbus, Georgia and east of Phoenix City, Alabama. The climate is characterized by hot summers and mild winters, with an average annual rainfall of about 1321 mm. The topography of the area is nearly level to gently sloping ridgetops, moderately steep and steep hillsides, and nearly level valleys along stream channels and other tributaries.

The upland soils sampled were Troup loamy sand (loamy, kaolinitic, thermic Gossarenic Kandiudults) (Soil Survey Staff, 1999), covered by longleaf pine sandhill and planted pine communities, while the wetland soils were Bibb sandy loam (coarse-loamy, siliceous, active, acid, thermic Typic Fluvaquents) (Soil Survey Staff, 1999), restricted to bottomlands along streams and creeks, typically covered by hardwood forests (wetlands).

In September 2003, soil samples were collected from eighteen sampling locations across upland and wetland landscape positions. From each sampling location, 3 replicate soil core samples (0–20 cm soil depth) were collected in a 1-m² plot using a one-inch diameter soil probe, combined, and immediately stored on ice. In the laboratory, soil samples were homogenized, passed through a 2-mm sieve, visible roots and plant fragments were removed, and then stored in a refrigerator at 4 °C in airtight, polyethylene containers until analyzed. For total C and N analysis, sub-samples were oven-dried at 60 °C and finely ground to a powder. All data are presented in oven-dry weight basis.

2.2. Acid hydrolysis

Hydrolysis was carried out by reacting 1 g of air-dried soil samples with either 6 M HCl or 1 M HCl at 105 °C. The 6 M HCl was performed at 1:25 and 1:50 soil:solution ratios, while the 1 M HCl was conducted using a 1:50 soil:solution ratio. Reaction time varied from 2 to 24 h. The residue after the hydrolysis was separated from the supernatant by centrifugation (10 min at 6037 g), and washed three times with de-deionized water to remove any chlorine residue. The combined supernatant and washings were diluted to 100 mL with de-ionized water and kept at 4 °C until analyzed for total organic C. The remaining residue was oven-dried at 60 °C. In order to optimize the method efficiency, one representative soil sample from each landscape position was selected and replicated three times. Samples were chosen based on the contrasting organic carbon concentrations and particle size composition (Table 1). Once the method of C hydrolysis was optimized (based on the maximization of the hydrolyzed C fraction), the procedure was run on all the soil samples collected from upland and wetland landscape positions.
Based on the total C concentration in the residues after the treatments, a recalcitrant index (RI) was calculated according to the following equation (Paul et al., 2006):

\[
RI(\%) = \left( \frac{C_{\text{concentration, hydrolyzable residue}} \times \text{Mass}_{\text{chemically resistant residue}}}{C_{\text{concentration, original soil}} \times \text{Mass}_{\text{original soil}}} \right) \times 100
\]

(1)

2.3. Hot-water extraction

The hot-water extractable C (HWC) was determined on 3 replicates of air-dried samples collected from upland and wetland landscape positions using a modified method of Haynes and Francis (1993). Before extraction, plant materials and highly soluble C compounds were removed by shaking 6 g of air-dried soil sample with 30 mL of distilled water at 20 °C for 30 min, centrifuging for 10 min at 6037 g and filtering. The supernatant was discarded. Subsequently, the remaining residue was extracted with 30 mL of distilled water at 80 °C. Reaction time ranged from 2 to 24 h. Sample were then centrifuged and filtered as described above. The extracts and residues after the treatment were analyzed for total organic carbon.

2.4. Organic C and N analysis

Organic C in hydrolysates was analyzed using a Shimadzu TOC-5050. Total soil organic C of untreated (original) and treated samples was measured by dry combustion using a Carlo Erba NA-1500 CNS analyzer (Haak-Buchler Instruments, Saddlebrook, NJ). Approximately 20 to 40 mg of oven-dried sample was used for total C and N analysis.

Table 1

<table>
<thead>
<tr>
<th>Landscape position</th>
<th>Upland</th>
<th>Wetland</th>
</tr>
</thead>
<tbody>
<tr>
<td>Soil</td>
<td>Ultisol</td>
<td>Inceptsol</td>
</tr>
<tr>
<td>Texture</td>
<td>Sandy</td>
<td>Silt loam</td>
</tr>
<tr>
<td>pH</td>
<td>5.2±0.49</td>
<td>4.9±0.6b</td>
</tr>
<tr>
<td>TN (g kg(^{-1}))</td>
<td>0.57±0.29</td>
<td>2.6±3.0</td>
</tr>
<tr>
<td>TC (g kg(^{-1}))</td>
<td>12.9±6.55</td>
<td>47.3±26.1</td>
</tr>
<tr>
<td>TP (mg kg(^{-1}))</td>
<td>105.6±61.3</td>
<td>231.1±175.5</td>
</tr>
<tr>
<td>NH(_4) (mg kg(^{-1}))</td>
<td>3.7±2.4</td>
<td>12.6±19.2</td>
</tr>
<tr>
<td>WEC (mg kg(^{-1}))</td>
<td>53.6±44.7</td>
<td>135±167.2</td>
</tr>
<tr>
<td>WEP (mg kg(^{-1}))</td>
<td>0.18±0.2</td>
<td>0.24±0.4</td>
</tr>
<tr>
<td>Fe(_{\text{mehlich-1}}) (mg kg(^{-1}))</td>
<td>56.1±103.2</td>
<td>344.2±503.7</td>
</tr>
<tr>
<td>Al(_{\text{mehlich-1}}) (mg kg(^{-1}))</td>
<td>239.7±109.0</td>
<td>718.0±603.2</td>
</tr>
<tr>
<td>P(_{\text{mehlich-1}}) (mg kg(^{-1}))</td>
<td>1.3±0.9</td>
<td>1.2±3.5</td>
</tr>
<tr>
<td>C(_{\text{mehlich-1}}) (mg kg(^{-1}))</td>
<td>426.72±518.6</td>
<td>139.3±152.8</td>
</tr>
<tr>
<td>Mg(_{\text{mehlich-1}}) (mg kg(^{-1}))</td>
<td>131.0±159.3</td>
<td>51.5±62.4</td>
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<tr>
<td>K(_{\text{mehlich-1}}) (mg kg(^{-1}))</td>
<td>51.9±47.3</td>
<td>40.5±29.1</td>
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<tr>
<td>Fe(_{\text{oxalate}}) (mg kg(^{-1}))</td>
<td>2202.1±2347.6</td>
<td>12006.5±12288.8</td>
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<tr>
<td>Al(_{\text{oxalate}}) (mg kg(^{-1}))</td>
<td>1816.4±968.5</td>
<td>2773.3±1986.7</td>
</tr>
<tr>
<td>P(_{\text{oxalate}}) (mg kg(^{-1}))</td>
<td>29.9±28.1</td>
<td>92.5±99.0</td>
</tr>
</tbody>
</table>

WEC = water-extractable C; WEP = water-extractable P; \(p_{\text{mehlich-1}}\) = Mehlich-1 extractable Fe, Al, Ca, Mg, and K; and \(p_{\text{oxalate}}\) = oxalate-extractable Fe, Al and P. Data points represent mean values±standard deviation.

2.5. Solid-state \(^{13}\text{C}-\text{CPMAS-NMR spectroscopy}

The chemically resistant (insoluble and/or non-hydrolyzable) residues were characterized by solid-state \(^{13}\text{C}-\text{CPMAS-NMR spectroscopy employing cross polarization (CP), magic angle spinning (MAS) and high power proton decoupling. Samples were recorded using a Varian Unity-Inova 500 MHz spectrometer operating at 125 MHz for carbon. Because of the relatively low carbon contents of these soils, samples were treated with HF/BF\(_3\) to reduce the effects of paramagnetic sites and increase carbon content through dissolution of the mineral matrix. These paramagnetic sites occur mainly as inorganic cations or organic free radicals, and their impact on the spectra of whole soils or soil components generated utilizing the cross polarization magic angle spinning technique (CP-MAS) can be manifested by broadened resonances and signal loss (Preston and Schnitzer, 1984; Oades et al., 1987). The HF/BF\(_3\) procedure and its effects on NMR spectral quality have been described previously (Schilling and Cooper, 2004). Briefly, ~15 g of original and treated soil was mixed with 30 mL of 2% HF/BF\(_3\) reagent, and the slurry then stirred for 1 h. The mixture was then vacuum filtered through 0.45 μm filters and washed several times with distilled, deionized water, and then the entire treatment repeated four more times. After the final treatment, residue was oven-dried overnight at 60 °C and then stored in glass vials at 4 °C until NMR analysis.

Approximately 250 mg of treated and dried residue were packed into a 7.5 mm solid state rotor (Doty Scientific, Columbia, SC) and spun at the magic angle at 4 KHz. Spinning sidebands were eliminated using the total suppression of sidebands (TOSS) sequence (Dixon, 1982). A series of variable contact time experiments were carried out to determine the optimum cross polarization time, and these experiments indicated that a 750 μs CP contact time yielded the most representative spectra. A 6.5 ms 90° pulse width and a recycle delay time of 2 s were used throughout. Each sample required between 30,000 and 40,000 scans before sufficient signal/noise ratios could be obtained, resulting in spectral acquisition times of 15–20 h. Chemical shifts were externally referenced to the 31 ppm methyl resonance of \(p\)-di-\(t\)-butylbenzene. Intensities over defined chemical shift windows were integrated to quantify selected soil carbon structures; these spectral windows and the structures they represent were (Kögel-Knabner 2002); 0–30 ppm (CH\(_3\) alkyl), 30–50 ppm (CH\(_2\) alkyl), 50–60 ppm (N-alkyl and methoxy), 60–70 ppm (alcohol and ether C), 75–105 ppm (O-alkyl and acetal), 110–150 ppm (unsubstituted and alkyl-substituted aromatic), 150–160 ppm (O-substituted aromatic or phenolic), 160–220 ppm (carbonyl C found in carboxylic acids, esters, amides, ketones and aldehydes).

2.6. Soil biochemical C parameters

Soil total C and N concentrations were determined in samples from eighteen sampling locations across upland and wetland landscape positions. Soil potential respiration (CO\(_2\) efflux) was performed using approximately 5 g of field-moist soil placed in a 150 ml glass serum bottle and sealed with butyl
rubber septa. Three replicates of control blanks (no soil) and samples were incubated in the dark at 25 °C. Carbon dioxide measurements were taken every 12 h for a period of seven days. A Shimadzu gas chromatograph (GC) equipped with a Poropak N (Supelco, Bellefonte, PA) column and thermal-conductivity detector was used for all CO₂ measurements. Oven, injector, and detector temperatures were set to 60, 140, and 200 °C, respectively. Before analysis, headspace pressure was determined by a digital pressure meter for calculation of CO₂ partial pressure in the serum bottles and 200 μl of headspace gas were analyzed.

Microbial biomass C (MBC) was determined using a modification of the procedure of DeBusk and Reddy (1998). Ethanol-free chloroform was added directly to approximately 5 g of field-moist soil to enhance distribution of chloroform within the sample (Ocio and Brookes 1990), and samples placed in a vacuum desiccator with a beaker containing 20 ml of chloroform and a few boiling chips. The desiccator was evacuated five times to 600 mm Hg, the desiccator valve was closed under vacuum, and left in darkness. After 24 h of exposure to chloroform, the beaker was removed from the desiccator and residual chloroform was removed from the soils by evacuating the desiccator 10 times to 600 mm Hg. Twenty-five milliliters of 0.5 M potassium sulfate was then added to each sample, which was shaken horizontally for 1 h and centrifuged for 10 min at 6037 g at 21 °C. Supernatant was filtered through Whatman # 41 filter paper and preserved with one drop of concentrated sulfuric acid. A second set of samples was extracted in the same manner without prior fumigation, and MBC was calculated as the difference between the fumigated (F) and non-fumigated (NF) samples. Carbon extracted by 0.5 M potassium sulfate (NF samples) was also used as an indicator of labile C.

Statistical analysis was performed using SAS statistical package (SAS Institute Inc., 1990). Treatments were considered different when \( P < 0.05 \). Stepwise multivariate regression (Proc Reg) analysis was used to investigate the relationship between hydrolyzable C pools and soil biochemical parameters.

3. Results and discussion

3.1. Efficiency of different procedures to extract organic C

For all treatments, extracted soluble C increased rapidly during the initial 2 h of hydrolysis (Fig. 1). Increasing the extraction time (>2 h) did not significantly increase C release, except for the HWC treatment. Xu et al. (1997) also observed larger soluble SOC pulses in the initial 2 h of hydrolysis in 6 M HCl. Increasing the soil:solution ratio from 1:25 to 1:50 for the 6 M HCl treatment did not significantly increase the efficiency of the hydrolysis, and similar amounts of C were released at both soil:solution ratios. Hydrolysis and hot-water extraction resulted in the recovery of varying amounts of C. The overall efficiency of 1 M HCl in hydrolyzing C was statistically lower \( (P \leq 0.05) \) to that of 6 M HCl.

![Fig. 1. Soluble organic C released by different treatments as a function of time. HWC = hot-water C extraction.](image)
HCl for both soils. These results again support those obtained by Xu et al. (1997), who found that 6 M HCl could release more organic C than 1 M HCl, especially in the initial 2 h of hydrolysis. The greater proportion of C released in 6 M hydrolysates suggests that the more chemically resistant compounds were hydrolyzed by this strong reagent than that in 1 M HCl hydrolysates. The ratio of soluble C to N was greater in 6 M HCl hydrolysate (C:N ratio = 31 for both soils) compared to the 1 M HCl (C:N ratio = 20 and 28 for wetland and upland soil, respectively). Conversely, the hot-water extraction was less effective in removing soluble C than the acid hydrolysis. Approximately 1 to 4% of total C was extracted by hot water in both soils compared to 18 to 32% of total C hydrolyzed by hydrochloric acid.

For the upland soil, 18 to 25% of the total C was hydrolyzed in 6 M and 1 M HCl, while for wetland soil, this amount varied from 24 to 32% of the total soil C. These amounts agree with Xu et al. (1997), who found 29 to 34% of soil organic carbon hydrolyzed in 6 M HCl. Paul et al. (2001) reported that the residue of acid hydrolysis contained between 23 and 70% of total C, and the great variability could be due the distinctive organic matter quality and land use among the studied soils. Despite the higher total C concentration exhibited by the wetland soil (Table 1), there was no prevailing difference in the percentages of soil C retained in the non-hydrolyzable fractions.

Soils showed a significant effect on the amount of C hydrolyzed. Wetland soils released much more soluble organic C than upland soils (Fig. 1), which was consistent with the total C concentrations of the soils.

3.2. Solid-state $^{13}$C-CPMAS-NMR

$^{13}$C NMR spectra of the untreated soils are presented in Figs. 2a and 3a. It should be noted that 30,000–40,000 discreet scans and 15–20 h of spectral acquisition time were necessary to acquire these and the following spectra, even after pretreatment to remove paramagnetics and concentrate the organic carbon present. This illustrates the difficulty in characterizing the carbon pool in low-carbon soils such as those studied here. However, the HF/BF$_3$ pretreatment used here, which is absolutely essential in order to acquire the NMR spectra, has been shown to remove <15% of the original organic carbon present. Furthermore, the effects are predictable and will impact each sample in an equivalent and reproducible manner (Schilling and Cooper, 2004). $^{13}$C NMR spectroscopy after chemical pretreatment should thus be considered at least a semi-quantitative technique for comparing soil carbon residues after acid and hot-water extractions.

It is clear from Figs. 2a and 3a that the original organic matter in these two soil classes (up- and wetland) is quite different. The upland SOC exhibits a broad alkyl peak in the 30–50 ppm range, indicative of polymethylene carbon in aliphatic structures. Conversely, much of the alkyl carbon in the wetland SOC appears to be terminal methyl carbon (0–30 ppm), possibly due to input from algae. The wetland SOC also contains a very prominent methoxyl and/or N-alkyl fraction (50–60 ppm) not seen in the upland SOC. Both soils show large relative abundances of carbohydrate (75–105 ppm) and aromatic (110–160 ppm) carbon. O-substituted aromatic carbon

![Fig. 2. $^{13}$C NMR spectra of upland soil sample residues. (a) untreated (control), (b) hot-water extraction, (c) 1 M HCl, (d) 6 M HCl 1:50 solution ratio.](image-url)
representative of lignin input is also observable in the wetland soil (150–160 ppm). Neither soil appears to contain much carbonyl carbon (160–220 ppm). These differences in carbon functional types are quantitatively summarized in Fig. 4.

$^{13}$C NMR spectra of the original (untreated) soils and residues after hot-water and acid extractions are presented in Figs. 2 and 3. These spectra support the previous conclusions regarding effectiveness of the various extraction procedures. Hot-water extraction seems to have little effect, as the spectra of these residues (Figs. 2(b) and 3(b)) are quite similar to the spectra of the original soils (Figs. 2(a) and 3(a)). This trend is completely consistent with the very small removal of C from the soils by hot-water treatment (1–4%). As a result, no significant difference in chemical composition would be expected. It should be noted that the similarity in spectra of soils before and after hot-water extractions, when considered along with the data summarized in Fig. 1, is a good indication of the reliability and precision of the NMR technique for characterizing carbon in low-carbon soils when it is combined with HF/BF$_3$ pretreatment.

Acid extraction, however, has a pronounced effect, producing residues with significantly different distributions of carbon functional groups than observed in the original soils and suggesting the removal of labile components (i.e. carbohydrates and amino acids) (peaks at 50–110 ppm) without major changes in the chemically resistant C fractions. For the upland soil, one of the most dramatic effects is the appearance of a strong terminal methyl peak at $\sim$15 ppm in the residue that was refluxed with 1 M HCl (Fig. 2(c)). This change is likely due to extraction of labile carbohydrate and aromatic material, resulting in selective enrichment of lipid-like alkyl C. Additionally, conversion of some O-type carbon to terminal methyl carbon upon hydrolysis of the bonds that link the labile material to the unextractable residue may also be occurring. There is also a discernable methoxy/N-alkyl peak at 55 ppm (Preston and Schnitzer, 1984) in the residue refluxed with 1 M HCl not observed in the original soil. Extraction with 6 M HCl produces similar if somewhat more clear effects (Fig. 2(d)), although the methoxy/N-alkyl peak is greatly reduced compared to the 1 M HCl non-hydrolyzable residue.

Even though the qualitative character of the wetland SOC is quite different than the upland soil, acid hydrolysis appears to have similar effects, with residues that are altered from the original SOC in the same way. Comparison of the spectra in Fig. 3(a) and (c) demonstrates loss of carbohydrate (75–105 ppm) and aromatic (110–160 ppm) carbon and concentration of aliphatic carbon, particularly in the terminal methyl form (0–30 ppm). The same intense methoxy/N-alkyl peak seen in the spectrum of the 1 M HCl non-hydrolyzable upland soil residue (50–60 ppm) is also present in the wetland spectrum. For both soils, this peak is diminished after 6 M HCl extraction (Figs. 2(d) and 3(d)).
These results are consistent with other observations reported in the literature (Chefetz et al., 2002). Acid hydrolysis also removed the peaks at 85 ppm in both soils, which are primarily associated with carbohydrates (Schnitzer and Preston, 1983), and the total amount of polysaccharides (60–110 ppm region of the spectra) was considerably reduced.

Comprehensive changes in the composition of recalcitrant soil carbon can be more easily identified by integrating over spectral windows representative of the main carbon functional groups present and summarizing these areas in a histogram (Fig. 5). Several previous studies have identified alkyl, O-alkyl and aromatic C contents as key indicators of soil organic matter decomposition (Coûteaux et al., 1998; Huang et al., 1998; Webster et al., 2000; Kogel-Knabner, 2002), and we will use the same parameters here; integration of peak areas between 0–50, 50–105, and 110–160 ppm generated relative abundances of alkyl, O-alkyl and aromatic carbon, respectively.

Fig. 5(a) indicates that the upland soil is largely unaffected by the hot-water extraction. However, the 1 M HCl hydrolysis removes some aromatic material, while hydrolysis with 6 M HCl at a 1:50 soil to solution ratio is required to significantly reduce the carbohydrate content of the upland soil. After acid hydrolysis with the 6 M HCl at a 1:50 soil to solution ratio, the soil carbon is predominately alkyl, with a significant amount of aromatic C and much less carbohydrate C than the original sample. A similar pattern was also observed by Rovira and Vallejo (2002), who suggested that aliphatic carbons are associated with the 0–45 ppm region of the spectra. These alkyl carbons are strongly resistant to degradation (Preston, 1996) and hardly solubilized by acid hydrolysis.

The trends in carbon functional types in the wetland soil after various treatments are somewhat different. Although O-alkyl carbon is significantly reduced by the acid extractions, it appears that the 1 M HCl hydrolysis is about as efficient as the stronger 6 M treatments, unlike what was observed with the upland soil. In addition, acid extractions appear to increase aromaticity (calculated by using the integrated areas associated with aromatic C (110–160 ppm) as percentage of the total areas (0–220 ppm) in the wetland soil with little change in alkyl carbon content, again in contrast to the upland soil. The aromaticities of the upland residues were less affected by acid hydrolysis and remained similar to that of the control soil. Possibly the aromatic units of the upland soil were more resistant to acid hydrolysis than those in the wetland soil. Differences in total C concentrations, degree of C protection by soil minerals, soil quality inputs and landscape position may also affect the composition of the SOC in the upland soil compared to the wetland soil. It appears that for the upland soil the acid hydrolysis with HCl concentrated principally the aliphatic C fraction (peaks at 13–14 ppm), whereas the same extraction concentrated principally the aromatic C fraction in the wetland soil.

The ability of 6 M HCl to characterize SOC quality was superior to the other procedures. Moreover, because acid hydrolysis protocols could be greatly improved by reducing the hydrolysis time, the 1:50 soil:solution ratio, which was not affected by
the length of time, was chosen to assess SOC degradability in a large set of samples \((n=18)\) collected from different landscape positions.

### 3.3. Relationship between hydrolyzable organic C and soil C parameters

Spatial variability associated with total C, microbial biomass C, CO\(_2\) evolution and water and potassium sulfate-extractable C was high across the sites (Table 2). The highest values for most of the attributes were found in wetlands, due to their hydrological regime, which results in accumulation of organic matter (Vepraskas and Faulkner, 2001). In general, sites that exhibited higher total C also showed higher microbial biomass C and CO\(_2\) production, as well as water and potassium sulfate-extractable C.

Recalcitrant C index (RI\(_C\)) ranged from 20 to 70\% (Fig. 6). Despite the greater total C found in wetland sites (Table 2), uplands exhibited similar RI\(_C\), suggesting that the non-hydrolyzable C pool did not depend upon the total C content, but possibly the quality and the chemical characteristics of the organic materials. On average, RI\(_C\) in the upland sites was 36\% compared to 39\% in the wetlands. Additionally, it can be inferred based on the relative abundance of the unhydrolyzable pool that RI\(_C\) did not correlate with the landscape position. Possibly because sites were under similar environmental conditions, such as temperature and precipitation, this could be affecting and leading to similar proportion of recalcitrant and labile pools of SOC.

Stepwise multivariate regression analysis revealed that MBC and WEC contributed significantly \((P<0.0001)\) for the variance in the hydrolyzed C pool. Microbial biomass C and WEC explained 99.4\% of the variability (Eq. 2), while total C, potassium sulfate-extractable C and CO\(_2\) evolution did not significantly improve the prediction of hydrolyzable C pool.

\[
\text{Hydrolizable C pool} = 0.023 \text{ MBC} + 0.095 \text{ WEC} \quad (2)
\]

Our results indicated that hydrolyzable C pool was not significantly dependent on the initial soil organic matter concentration.

![Fig. 5. Relative distributions of major carbon functional groups in soils before and after hot-water and acid extractions.](image-url)
This finding further confirmed that acid hydrolysis can be useful for estimating the more bioreactive pool of soil C, and the results are comparable to those estimated by biochemical methods. Rovira and Vallejo (2000, 2002) found significant correlation between the percentage of C unhydrolyzable and mineralization rates. These authors also suggested that recalcitrant index was more adequate than classic indices (C:N and lignin:N ratios) for predicting organic matter decomposition. Collins et al. (2000) found a significant relationship between CO$_2$ evolution and mean residence time of the non-hydrolyzable residue. Based on our results, acid hydrolysis with 6 M HCl appeared to reflect the bioavailable pool of SOC estimated by biochemical procedures, with the advantages of being less time-consuming and easier to perform.

### 4. Conclusions

This study demonstrates that acid hydrolysis with HCl can provide meaningful estimates of labile C pools size in soils. This technique is easy to perform and gives repeatable and unambiguous results. Solid state $^{13}$C NMR spectra indicate that the acid hydrolysis removes primarily the more labile components of the SOC and the residue left after the chemical treatment was composed of more chemically resistant compounds. There was a strong correlation between biochemical parameters and the labile C pool estimated by acid hydrolysis, suggesting that this procedure is a promising approach to characterize SOC bioavailability.

### Table 2

<table>
<thead>
<tr>
<th>Site</th>
<th>Total C G kg$^{-1}$</th>
<th>MBC$^a$ mg kg$^{-1}$</th>
<th>CO$_2$ efflux mg CO$_2$ C kg$^{-1}$ h$^{-1}$</th>
<th>WEC$^b$ mg kg$^{-1}$</th>
<th>K$_2$SO$_4$–C g kg$^{-1}$</th>
<th>C hydrolyzed $\frac{g}{kg}$</th>
</tr>
</thead>
</table>
| Upland
| 1    | 4.3±0.14            | 18.2±3.6             | 0.1±0.03                                 | 1.3±0.1              | 142.8±33.0              | 1.5±0.1                |
| 2    | 3.4±0.04            | 13.1±3.5             | 1.0±0.36                                 | 2.0±0.05             | 116.3±14.1              | 0.9±0.9                |
| 3    | 5.6±0.04            | 33.0±6.3             | 0.05±0.00                                | 3.0±0.2              | 93.2±9.8               | 1.1±0.2                |
| 4    | 1.8±0.02            | 8.1±4.5              | 0.8±0.45                                 | 1.1±0.1              | 112.2±3.6              | 0.7±0.04               |
| 5    | 2.7±0.01            | 20.7±3.0             | 0.4±0.02                                 | 3.6±0.3              | 138.7±14.7             | 1.1±0.02               |
| 6    | 4.5±0.02            | 51.0±14.9            | 2.8±0.08                                 | 4.1±0.3              | 158.5±12.3             | 1.5±0.1                |
| 7    | 5.7±0.18            | 36.7±23.2            | 7.8±0.73                                 | 10.6±1.0             | 202.8±11.6             | 2.1±0.1                |
| 8    | 5.0±0.07            | 33.5±11.0            | 3.8±0.49                                 | 4.9±0.4              | 189.8±9.5              | 1.8±0.2                |
| 9    | 4.2±0.11            | 16.8±4.3             | 8.6±1.45                                 | 13.3±1.0             | 203.5±8.9              | 2.3±0.3                |
| Wetland
| 1    | 4.5±0.04            | 13.6±6.5             | 11.8±0.79                                 | 18.4±4.1             | 83.1±4.2               | 1.9±0.1                |
| 2    | 3.5±0.07            | 26.9±1.0             | 8.0±1.55                                 | 2.6±0.2              | 47.3±4.1               | 2.4±0.05               |
| 3    | 4.2±0.13            | 25.6±6.4             | 10.9±1.22                                 | 5.5±0.5              | 79.9±7.8               | 1.4±0.1                |
| 4    | 13.4±0.05           | 29.1±4.5             | 14.2±1.34                                 | 9.8±1.1              | 124.5±16.2             | 3.6±0.2                |
| 5    | 10.1±0.02           | 67.2±17.0            | 26.5±2.27                                 | 9.2±0.9              | 155.1±15.4             | 4.6±0.3                |
| 6    | 16.2±0.07           | 88.2±16.0            | 21.8±1.32                                 | 7.3±0.6              | 104.8±5.0              | 3.6±0.1                |
| 7    | 64.0±2.41           | 512.3±62.5           | 81.3±6.34                                 | 69.6±5.6             | 558.4±54.3             | 25.3±1.1               |
| 8    | 55.2±1.95           | 364.8±34.5           | 81.9±7.29                                 | 38.6±3.9             | 465.8±42.5             | 16.7±0.8               |
| 9    | 61.5±1.79           | 614.4±54.0           | 85.5±1.43                                 | 58.0±4.9             | 475.8±43.9             | 26.6±1.8               |

$^a$ MBC = Microbial biomass C.  
$^b$ WEC = water-extractable C.

Data points represent mean values±standard deviation.

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**Fig. 6.** Recalcitrant C index estimated for eighteen sites.
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