I. Objectives

- To identify the chemical processes controlling soil organic matter (SOM) formation under continuous rice cropping.

- To compare the elemental composition and chemical structure of SOM fractions extracted from long-term continuously flooded field experiments and fields cropped to upland crops.

- To quantify the effects of such chemical changes on the rate of soil nitrogen (N) mineralization under anaerobic and aerobic conditions.

- To identify any effects of periodic aerobic crop cycles on crop N uptake.

II. Summary of accomplishments

We achieved significant progress on the first, second, and fourth objectives, and we are currently designing studies that address the third objective. Following is a brief summary of the accomplishments:

- We adapted a new SOM extraction and fractionation procedure to fine-textured rice paddy soils. This method had been used in previous studies on soils from cotton-based cropping systems in the San Joaquin Valley, California, to isolate active vs protected (or less active) SOM fractions. The mobile humic acid (MHA) pool extracted in the California work had the chemical characteristics of young, less humified SOM and is hypothesized to be relatively active in nutrient cycling, while the calcium-bound humate (CaHA) pool was more humified and is hypothesized to be less active in nutrient cycling.

- The MHA and CaHA were extracted from several field experiments, including key long-term experiments. One experiment may be the most intensively managed field in the world, with three crops of irrigated rice per year since 1963. Treatments include four inorganic N fertilizer rates which vary from zero to high levels sufficient to achieve maximum potential yield. In another field experiment, N was added to different treatment plots as inorganic N fertilizer or as organic green manures, with
the same total N rate in all cases. Treatments were applied to twenty consecutive crops from 1982 to 1992. A third field had irrigated rice in most wet seasons since 1983 but upland crops or fallow periods in the dry seasons. A fourth field had never been submerged or flood-irrigated during the 34 year history of IRRI, supporting dryland rice nurseries in the dry seasons. These four fields therefore comprise a spectrum of flooding intensity, with the number of irrigated rice crops per year varying from zero to three. Additional fields sampled more recently include long-term fertility trials with different fertilizer (N, P, K) combinations and an experiment that addresses the fourth objective stated above with irrigated rice vs upland maize as dry season treatments, both planted as irrigated rice in the wet season.

- Quantities of carbon (C) and N extracted as MHA and CaHA were more sensitive to the type (organic vs inorganic) and amount of N fertilizer inputs than total soil organic C or N. In the long-term experiment triple-cropped to rice, for example, total soil organic C was 18% greater with high N fertilization than in the control treatment with no N fertilizer, due to greater inputs of crop residue. By contrast, the C extracted as MHA increased by 41%, and C extracted as CaHA by 26%.

- Chemical analyses demonstrated that some chemical properties of the MHA and CaHA change markedly with flooding intensity. Results from nuclear magnetic resonance spectroscopic scans and light absorption intensities at one ultraviolet and two visible light wavelengths support our hypothesis that partially degraded lignin units accumulate in SOM with increased flooding intensity. Similar to the changes in quantities, changes in the chemical nature of the two extracted fractions between field experiments were distinct in the CaHA but even more pronounced in the MHA.

- Several more general and commonly measured properties of extracted SOM changed little with flooding intensity, such as total C and N concentration, hydrolyzable amino acid content (a measure of long-term organic N availability), and the ratio of light absorption at the two visible light wavelengths (considered an index of the chemical structure of SOM). Lack of changes in these general properties, however, emphasize the need for the more detailed analyses that we performed to distinguish important changes in chemical attributes.

- The responsiveness of the MHA and CaHA to flooding intensity and fertilizer input regime suggests that these fractions are useful indicators of the effects of recent crop management practices on SOM cycling and nutrient availability. In addition, these two fractions account for up to 700 kg N ha⁻¹ and 20 to 25% of total soil organic C in the puddled soil layer. We speculate therefore that changes in the sizes of the MHA and CaHA fractions carry significant meaning for the soil N supplying capacity.

- These SOM studies have been integrated with the research programs of associated graduate students at IRRI. One student from Vietnam will characterize the MHA and CaHA extracted from a wide variety of soils. Two students from Germany measured various N and C parameters in the field experiment comparing a rice-rice and a rice-maize system.
Synthesis of our analyses in this field experiment will contribute to a
detailed view of N and C cycling under aerobic vs anaerobic conditions.

- Methods and results of these studies were described in manuscripts that
will be published in 1994 and 1995. The extraction method is described
in detail by Olk et al. (1994a); a preprint copy is enclosed in Appendix 1.
Use of the extracted fractions in understanding nutrient availability in
the California and IRRI projects is outlined by Olk and Cassman (1994);
a preprint copy is enclosed in Appendix 2. Two papers projected to
appear in 1995 will contain most results obtained during this project (Olk
et al., 1995a,b). Abstracts for presentations at two conferences in 1994
are included in Appendix 3 (Olk et al., 1994b,c).

III. Future research directions as follow-up to this project

Although we achieved significant progress in the first, second, and fourth
objectives stated above, we are now only beginning the most important
aspect of this project, understanding how these changes in the chemical
nature of SOM affect the soil N supplying capacity. Some planned activities
in this direction will be funded by permanent "core" funding of IRRI and
special complementary funding by the Swiss Development and Cooperation,
but additional funding will be sought for more specialized studies.

Key experiments will measure rates of N and C mineralization under aerobic
vs anaerobic conditions in soil amended with MHA, CaHA, plant parts, or
model compounds. The rate and nature of SOM formation under the same
treatments will also be studied. Our procedure for measuring N
mineralization will be an improved version of the standard method as
developed by an associated graduate student from Pakistan. Collaborative
work with Dr. William Patrick at Louisiana State University is expected to be
initiated in 1995 to repeat selected treatments with instrumentation
providing highly controlled conditions.

More advanced characterization of the chemical properties of the MHA and
CaHA will continue, both at IRRI and by collaborating specialists overseas.

Rates of turnover of the MHA and CaHA will be studied by gathering
information from different tests. Radiocarbon ages of the MHA and CaHA
in several field experiments will be measured. The rate of incorporation of
maize residues into SOM in the field experiment with irrigated rice/upland
maize treatments can be measured by $^{13}\text{C}$ natural abundance. We will work
with IRRI soil microbiologists to study the role of microorganisms in the
formation and turnover of the MHA and CaHA and other aspects of nutrient
cycling in the irrigated lowland system.

Research made possible by this grant has laid a foundation for understanding
the mechanisms that are major contributors to the yield decline in irrigated
lowland rice, the most productive agroecosystem in Asia (Cassman et al.,
1994). These results and future extensions of the research will lead to
mitigation measures that combine knowledge of basic soil processes with
agronomic practices relevant to Asian rice farming.
References Cited


Characterization of two humic acid fractions from a calcareous vermiculitic soil: implications for the humification process

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Abstract

Increased soil organic matter (SOM) is associated with a reduction in K fixation by a calcareous vermiculitic soil in California. To better understand the mechanism for this effect, humic acids (HA) were extracted from this soil with and without decalcification. The HA extracted without decalcification — the mobile HA (MHA) pool — was dated as modern by 14C-dating, while the radiocarbon age of the HA bound to Ca (CaHA) was estimated at 290 yr. Compared to the MHA, the CaHA had increased aromaticity and C condensation, increased substituent carboxyl groups, and decreased substituent amino acids. Whereas MHA was evenly distributed through a 0–60 cm soil profile, the CaHA was concentrated in the 0–20 cm surface layer. The CaHA pool is probably protected from chemical and biological degradation through development of bonds to exchangeable Ca and possibly structural Fe. The MHA may be bound to the soil through exchangeable monovalent cations, through attachment of peripheral amino acids to exchange sites or fixation sites, or through other SOM molecules. No evidence was found to suggest different source materials for the two HA fractions. Although the MHA and CaHA are chemically distinct, these carbon pools may represent early and late stages of the humification process in aerated soils in temperate climates.

1. Introduction

Soil organic matter (SOM) formation and turnover are of fundamental importance to the cycling of N, P, and S in soil because these plant nutrients are structural components of SOM. Although K is not a structural component of SOM and exchange sites in SOM are
not thought to have a high affinity for K\(^+\) (Salmon, 1964), SOM content influences K availability in some soils. For example, in a K-fixing soil under no-tillage cultivation, Evangelou and Blevins (1988) correlated increased available K with greater SOM content despite lower affinity of the soil exchange sites for K\(^+\). In other soils, the effect of increased SOM content with manuring on K\(^+\) selectivity in K–Ca exchange was either positive (Poonia and Niederbudde, 1990) or negative (Goulding and Talibudeen, 1984). Explanations for these various effects emphasize the mode of SOM binding to mineral particles and the low affinity of cation exchange (CE) sites in SOM for K\(^+\).

Cassman et al. (1989) found K fixation to continue during two years of annual cropping with irrigated cotton in a calcareous, highly vermiculitic California soil. Potassium fixation occurred in tandem with an 11% decline in soil organic carbon (SOC). Subsequent addition of manure resulted in increased SOC and reduced long-term fixation, which was reflected by a larger cotton lint yield response to residual K fertilizer (Cassman et al., 1992). No general property of SOM explains this capacity to affect K availability.

The capacity of Ca to bind SOM is of central importance to SOM dynamics in a calcareous soil. Like other polyvalent cations, Ca\(^2+\) stabilizes SOM against microbial degradation (Duchaufour, 1976), probably by occupying functional sites (Theng, 1979) which could lead to intramolecular bridging, folding, and aggregation of these large organic molecules. The principal sites of Ca\(^2+\) adsorption are likely to be carboxyl groups (Baes and Bloom, 1988), which are attached in the SOM to aromatic moieties (Preston and Schnitzer, 1987), or to both aromatic and aliphatic substituents (Wilson et al., 1987). Thus, Ca\(^2+\), as well as Fe and Mn, has been associated with aromatic portions of SOM (Turchenek and Oades, 1979).

Organic matter binds to monovalent cations as well. Vardarachari et al. (1991) demonstrated low levels of humic acid (HA) adsorption to clays saturated with K\(^+\), Na\(^+\), or other monovalent or polyvalent cations. They concluded that exchangeable cations form the predominant bonding link between HA and illite, kaolinite, and montmorillonite. Soil organic matter binds to bivalent cations through water bridging, while SOM probably binds directly to monovalent cations (Sposito, 1984).

Much of the HA in a calcareous soil is bound to Ca\(^2+\), and thus an initial decalcification step is required to obtain maximum yields of extracted HA. This calcium humate pool (CaHA) is thought to be a more inert HA fraction (Campbell et al., 1967; Kononova, 1975). Because HA itself is a relatively recalcitrant pool of SOM, a characterization based on the CaHA pool alone may not properly describe the true chemical activity of SOM. When HA was extracted without initial decalcification, the fraction obtained was called the mobile humic acid pool (MHA) in pioneering work by Tyurin (Kononova, 1966). The MHA had much younger mean residence times than CaHA in two soils as determined by \(^{14}\)C-dating (Campbell et al., 1967).

The purpose of this study was to characterize HA fractions of the calcareous vermiculitic soil in the field studies of Cassman et al. (1989, 1992). Since more than 80% of added fertilizer-K becomes fixed in this soil, SOM must counteract a strong thermodynamic gradient towards K sorption at interlayer sites to influence K availability. Knowledge of SOM properties and SOM formation processes in this soil is essential for explaining the relationship between SOM and K availability. Mechanisms by which extracted HA fractions affect K availability are discussed in a subsequent study (Olk and Cassman, 1994).
2. Materials and methods

Soil used for HA extraction was obtained from a cotton field in the San Joaquin Valley, California. The soil is mapped as a Grangeville sandy loam (coarse-loamy, mixed, thermic Fluvaquentic Haploxeroll). Grangeville soil characteristics at this site were described by Cassman et al. (1989).

2.1. Humic acid extraction

Chemical analyses of SOM properties were performed on HA pools obtained via the standard NaOH extraction method (Schnitzer, 1982) modified to sequentially extract both the MIIA and CaHA. To extract 1 kg soil, 277 ml 0.25M NaOH was added to 111 g soil (1:2.5) in each of nine 500 ml erlenmeyer flasks. The air was replaced by N₂ gas and flasks were stoppered. The suspension was shaken at 180 rpm on a reciprocal shaker for 30 min every 2 h for 24 h and then poured into 250 ml bottles and centrifuged at 16,000g. The supernatant was decanted into 1000 ml graduated cylinders and acidified with HCl to pH 2. The remaining soil in each bottle was washed twice in 200 ml deionized (DI) water, mixing the soil-DI water with a spatula. In each wash cycle, the supernatant was separated through centrifugation and acidified to pH 2 as before. After overnight settling, precipitates from the 0.25M NaOH extract and the two water washes were combined after decanting most of the supernatant, making a slurry of the remainder, and centrifugation to recover the precipitated fraction as the MHA pool.

To reduce inorganic contamination, the MHA was resuspended and stirred in 200 ml of a 0.5% HF and 0.5% HCl solution for 3 days with daily solution replacement. The MHA was then H⁺-saturated by dialyzing for 24 h against 4 liter volumes of 0.01M HCl, 0.001M HCl, and DI water, with replacement of the dialyzing solution every 12 h. Final pH of the DI water dialysis was approximately 5.3. The MHA was then frozen, lyophilized, and stored as a dry powder.

After decantation of solubilized MHA in the final water wash, soil was decalcified with four to five washes in 0.1N HCl until the pH of the wash solution remained below 1.5. The wash pH was then raised above 3.0 by two or three further washes with 200 ml DI water. Soil was then extracted with 0.25N NaOH using the same protocol as for the MHA extraction with two subsequent water washes, treatment in dilute HF/HCl solution, dialysis, and freeze-drying. This HA fraction obtained after decalcification was considered CaHA. The SOM not extracted during either NaOH incubation was considered to be humin (Schnitzer, 1982) and was not examined in our studies.

2.2. Chemical characterization of the humic acids

Elemental composition except for Fe and Al was determined after digestion of 0.1 g lyophilized HA in 5 ml concentrated H₂SO₄ plus 1 g CsCl added to raise the boiling point. Iron and Al were measured in an HClO₄/HNO₃ hydrolysate on an ICP spectrophotometer. Calcium, Mg, K, and Na were determined by atomic absorption spectrophotometry, and N (Dorich and Nelson, 1983) and P (Watanabe and Olsen, 1965) colorimetrically. Total
organic C was measured by a modified Walkley/Black method (Nelson and Sommers, 1982). Inorganic ash content was determined after combustion for 3 h at 700°C.

Potentiometric titration of the HA fractions was conducted in a 0.1M KCl solution (Gamble, 1970) made from boiled water. The solution pH was initially raised to 9–10 by KOH addition and then lowered during titration by additions of 0.02M HCl standardized against tris(hydroxymethyl)aminomethane (Bremner and Mulvaney, 1982). A continuous titration curve was generated in which one HA sample was titrated in an open vessel flushed continuously with N₂ gas. A discontinuous titration curve was also generated in which selected H⁺ addition rates were added to samples incubated overnight under N₂ gas. The cation exchange capacity (CEC) was calculated from the discontinuous titration as the amount of H⁺ added between the pH of minimum buffering and the endpoint of 3.20 (Posner, 1966).

Chemical structures of the HA fractions were examined by ¹³C nuclear magnetic resonance (NMR) spectroscopy. Lyophilized HA was dissolved in 0.5M NaOD and flushed with argon to prevent oxidation. All spectra were acquired at 9.4 T (100.61 MHz for ¹³C) and 50°C on a Bruker AM-400 spectrometer using a 10 mm broad-band probe and carbon-specific preamplifier. The acquisition conditions for one-pulse experiments included a 90° excitation pulse (15 μs), 29 kHz spectral width, 0.3 s relaxation delay, 0.2 s acquisition time, 16K complex points, and ¹H composite-pulse decoupling with nuclear Overhauser enhancement (NOE). Spectra were processed with a 30 Hz line-broadening before Fourier transformation and chemical shift was referenced to an internal standard, 2,2'-dimethylsilapentane-5-sulphonate (DSS), at 0 ppm.

To determine the spin–lattice relaxation (T₁) times, spectra were acquired using F1RFT (Gupta et al., 1980) with eight time delays (0.001, 0.025, 0.05, 0.1, 0.2, 0.4, 0.8, and 1.5 s); the remaining acquisition parameters were the same as above. A total of 20,480 scans were acquired for each time delay. The T₁ values were calculated from an exponential curve fitted to the plot of time delay versus ¹³C peak height.

The E₄/E₆ ratio was determined from light absorption at 465 nm and 665 nm by solutions composed of 12 mg lyophilized HA in 0.051 of 0.05N NaHCO₃ (Chen et al., 1977). Hydrolyzable amino acids were determined by the ninhydrin method following 24 h hydrolysis in 6N HCl at 110°C and chromatographic separation. Radiocarbon ages were determined by accelerated mass spectrometry at the Center for Accelerator Mass Spectrometry at the Lawrence Livermore National Laboratory. The nature of inorganic contaminants in the lyophilized HA was studied with an Hitachi scanning electron microscope and an attached Traco Northern energy dispersive spectro-analyzer.

Gapon selectivity coefficients were calculated for the reaction:

\[
(Ca_{0.5})_{x} + K^{+} = K_{x} + 0.5Ca^{2+}
\]  

where "x" represents exchangeable cation concentration in cmol charge g⁻¹ HA and "s" the solution-phase concentration in mM. An amount of HA containing 0.0037 cmol charge (0.0100 g CaHA or 0.0105 g MHA) was placed in dialysis tubing (molecular weight: cutoff 6000–8000) and dialyzed for 24 h against 14 ml 0.36 mM KOH to help solubilize the HA. Solutions of KCl and CaCl₂ were then simultaneously added to create a 20 ml total volume of either 2 mM K with a Ca concentration ranging from 0.15 to 2.0 mM, or 1 mM Ca with a K concentration ranging from 4 to 50 mM. Flasks were covered and swirled at 50 rpm on...
an orbital shaker for 48 h until solution concentrations had equilibrated and aliquots were taken for K and Ca determination. Increases in cmol exchangeable K and Ca were assumed to equal the decrease in cmol solution-phase cations during the 48 h incubation.

3. Results

3.1. Elemental composition and ash content

The final dialysis sequence in the HA extraction allowed recovery of HA that was H⁺-saturated and relatively free of contaminant cations (Table 1). Combined Ca and Mg concentrations represented less than 0.2% of the available CEC sites. The relatively high K contamination may have been released during freezing or lyophilizing from the small amount of K-bearing micas and soil particles detected in the HA with scanning electron microscopy. Soil mineral particles can release K upon freezing or drying (Bertsch and Thomas, 1985). Scanning electron microscopy identified a few K-bearing soil particles in the HA powder, but their numbers amounted to considerably less than 1% of the HA particles counted in numerous scans of several HA samples. The only elements with atomic number greater than 11 that were detected in HA particles were Si, Al, and occasionally Fe, and these were present at low concentrations. Although ash content was lower in the MHA than

<table>
<thead>
<tr>
<th>Humic acid</th>
<th>Ca (mg kg⁻¹)</th>
<th>Mg (mg kg⁻¹)</th>
<th>K (mg kg⁻¹)</th>
<th>Na (mg kg⁻¹)</th>
<th>Ash (g kg⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MHA</td>
<td>100</td>
<td>40</td>
<td>300</td>
<td>0</td>
<td>6</td>
</tr>
<tr>
<td>CaHA</td>
<td>80</td>
<td>20</td>
<td>240</td>
<td>0</td>
<td>35</td>
</tr>
</tbody>
</table>

Table 1
Concentration of contaminant cations and ash in the MHA and CaHA after H⁺-saturation in the extraction process. Fractions were extracted from the 10-30 cm soil layer of a Grangeville sandy loam.

<table>
<thead>
<tr>
<th>Type</th>
<th>Element (in g kg⁻¹)</th>
<th>C/N</th>
<th>Total (in mg kg⁻¹)</th>
<th>CEC (cmol/kg)</th>
<th>Solubility* (H₂O, pH 6)</th>
<th>E₄₆₅/E₆₆₅</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>C</td>
<td>N</td>
<td>P</td>
<td>Al</td>
<td>Fe</td>
<td></td>
</tr>
<tr>
<td>MHA</td>
<td>514</td>
<td>55.0</td>
<td>2</td>
<td>9.3</td>
<td></td>
<td>120</td>
</tr>
<tr>
<td>CaHA</td>
<td>540</td>
<td>40.3</td>
<td>2</td>
<td>13.4</td>
<td></td>
<td>120</td>
</tr>
</tbody>
</table>

* Elemental concentration was based on the extracted HA samples, which included ash content of 6 g kg⁻¹ for the MHA and 35 g kg⁻¹ for the CaHA.

* Moderate solubility was defined as significant but incomplete HA dissolution with an opaque solution. Low solubility was defined as little HA dissolution with a transparent solution.
CaHA (Table 1), the amount of ash in these fractions was relatively low compared with other reports (Stevenson, 1982).

The chemical properties of both HA fractions (Table 2) are comparable to values reported elsewhere (Stevenson, 1982). Total C contents were 500–550 g kg\(^{-1}\) and C:N ratios ranged from 9 in the MHA to 13 in the CaHA. The CEC was 300–400 cmol kg\(^{-1}\) and solubility in water was low for CaHA and moderate for MHA. Both fractions had similar Al content, while Fe content of the CaHA was eight-fold greater than in the MHA.

3.2. Structural components

The \(^{13}\)C NMR scan patterns of the MHA and CaHA in the spectral region of 20 to 80 ppm suggested slightly greater contents of aliphatic compounds in the MHA (Fig. 1). Two notable differences were a distinct peak at about 58 ppm in the MHA that was absent in the CaHA and a larger peak at 40 ppm for the MHA than for the CaHA. These peaks have been attributed to C in amino acids (Piccolo et al., 1990). Neither fraction contained significant phenolic structures, as the signal intensity in the 140 to 160 ppm region was weak (Baldock et al., 1991; Kögel-Knabner et al., 1991).

Differences between the NMR scans were also apparent in the region of 100 to 190 ppm, where the CaHA possessed relatively more aromatic-C and perhaps olefinic-C (110–140 ppm), and carbonyl- and carboxyl-C (170–190 ppm) than the MHA. While it is difficult to quantify the contribution from each peak, a large difference in peak size and definition in the aromatic/olefinic-C region (110–130 ppm) is clear in the scans of the two HA fractions. The location of the central aromatic peak at about 130 ppm indicates that a large proportion of the aromatic rings is not substituted by oxygen or nitrogen; significant N and O substitution would result in a chemical shift at about 150 ppm (Hatcher et al., 1980).

Although these two spectra were acquired under partially saturated conditions, a direct comparison of their relative peak intensity could still be made because the \(^{13}\)C \(T_1\) times of the MHA and CaHA were similar (Table 3). The \(T_1\) times were slightly longer, however, for the MHA than for the CaHA. One possible explanation is that the various structural...
Table 3

\(^{13}\text{C} \) Spin-lattice relaxation time \((T_1)\) of the MHA and CaHA. Fractions were extracted from the 10-30 cm soil layer of a Grangeville sandy loam.

<table>
<thead>
<tr>
<th>Chemical shift (ppm)</th>
<th>MHA ((s))</th>
<th>CaHA ((s))</th>
</tr>
</thead>
<tbody>
<tr>
<td>25</td>
<td>0.76 (0.17)*</td>
<td>0.45 (0.09)</td>
</tr>
<tr>
<td>27</td>
<td>0.48 (0.06)</td>
<td>0.29 (0.08)</td>
</tr>
<tr>
<td>33</td>
<td>0.43 (0.08)</td>
<td>0.38 (0.05)</td>
</tr>
<tr>
<td>43</td>
<td>0.37 (0.18)</td>
<td>0.21 (0.07)</td>
</tr>
<tr>
<td>64</td>
<td>0.21 (0.09)</td>
<td>0.14 (0.08)</td>
</tr>
<tr>
<td>122</td>
<td>0.54 (0.19)</td>
<td>—</td>
</tr>
<tr>
<td>132</td>
<td>0.39 (0.10)</td>
<td>0.29 (0.11)</td>
</tr>
<tr>
<td>180</td>
<td>0.91 (0.34)</td>
<td>0.76 (0.31)</td>
</tr>
</tbody>
</table>

* Numbers in parentheses are the fractional errors.

Groups in the MHA experienced a slightly higher degree of freedom than those in the CaHA. Such flexibility could be attributed to a smaller molecular weight or a less rigid structure. Alternatively, the higher Fe content of the CaHA (Table 2) could account for its shorter \(T_1\) values (Breitmaier and Voelter, 1987).

Greater aromaticity and condensation of the CaHA was also suggested by the \(E_s/E_0\) ratio (Table 2). This value decreases with increasing molecular weight, aromaticity, and condensation, and is thought to represent an inverse index of humification (Stevenson, 1982). The ratio for the CaHA was below 5.0, which is the typical upper limit for HA (Stevenson, 1982). The value for the MHA was 6.0 which is at the lower limit of the normal range of 6.0-8.5 for fulvic acid (FA). The high N concentration of the MHA (Table 2) clearly distinguishes it from the FA pool, however, which typically has an N content less than 3% (Stevenson, 1982).

The two HA fractions differed in other respects. The radiocarbon age of the CaHA was measured at 290 ± 60 yr, while the MHA was modern, which indicates that much of its C was incorporated into the MHA after 1950 (Stout et al., 1981). The CaHA was composed of small, relatively dense particles and had a dark, almost black color. Dark color of HA has been interpreted as abundant conjugated double bonds and high aromaticity (Kumada, 1965). In contrast, the MHA was reddish-brown, with a loose fluffy consistency.

The CaHA had 54% less hydrolyzable amino acids than the MHA (Table 4). This difference, about 14 g N kg\(^{-1}\) HA, accounted for nearly all of the difference in total N concentration of the two fractions. Concentrations of individual amino acids were all lower in the CaHA. Amino acids that are enriched in microbial cell walls such as aspartic acid, glycine, glutamic acid, alanine, and lysine (Stevenson, 1982) represented a larger proportion of hydrolyzable amino acids in the CaHA than in the MHA, while the proportions of all other amino acids were lower in the CaHA.

3.3. CEC and cation adsorption selectivity

While the continuous titration curves had comparable shapes (Fig. 2), the MHA had slightly more buffering than the CaHA in the pH range of 9 to 9.75 (Table 5), the pH range
Table 4
Hydrolyzable amino acids in the MHA and CaHA. Fractions were extracted from the 10-30 cm soil layer of a Grangeville sandy loam.

<table>
<thead>
<tr>
<th>Amino acid</th>
<th>Amount (nmol mg⁻¹)</th>
<th>Percent of total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>MHA</td>
<td>CaHA</td>
</tr>
<tr>
<td>Aspartic acid + asparagine*</td>
<td>237 215</td>
<td>15.8 14.3</td>
</tr>
<tr>
<td>Glycine*</td>
<td>161 79</td>
<td>10.7 11.5</td>
</tr>
<tr>
<td>Glutamic acid + glutamine*</td>
<td>156 75</td>
<td>10.4 10.8</td>
</tr>
<tr>
<td>Alanine*</td>
<td>56 29</td>
<td>3.8 4.3</td>
</tr>
<tr>
<td>Lysine*</td>
<td>120 51</td>
<td>8.0 7.4</td>
</tr>
<tr>
<td>Valine</td>
<td>110 46</td>
<td>7.4 6.6</td>
</tr>
<tr>
<td>Leucine</td>
<td>99 40</td>
<td>6.6 5.8</td>
</tr>
<tr>
<td>Threonine</td>
<td>93 39</td>
<td>6.2 5.7</td>
</tr>
<tr>
<td>Proline</td>
<td>81 36</td>
<td>5.4 5.3</td>
</tr>
<tr>
<td>Serine</td>
<td>70 29</td>
<td>4.6 4.2</td>
</tr>
<tr>
<td>Isoleucine</td>
<td>59 21</td>
<td>3.9 3.0</td>
</tr>
<tr>
<td>Phenylalanine</td>
<td>49 22</td>
<td>3.3 3.3</td>
</tr>
<tr>
<td>Arginine</td>
<td>33 9</td>
<td>2.2 1.3</td>
</tr>
<tr>
<td>Tyrosine</td>
<td>22 8</td>
<td>1.5 1.1</td>
</tr>
<tr>
<td>Total</td>
<td>1498 689</td>
<td></td>
</tr>
</tbody>
</table>

* Indicates amino acids that are enriched in microbial cell walls (Stevenson, 1982).

in which amine groups would be active (Posner, 1964). The CaHA had more buffering than the MHA in the acidic pH range where carboxyl groups deprotonate. Similar trends were obtained from the discontinuous curves (data not shown).

Calcium was preferentially adsorbed by both fractions over K⁺, but the preference was stronger in the CaHA (Table 6). The number of CE sites exchanged by the two cations was

![Fig. 2. Continuous titration curves for the calcium humate (Ca-HA) and mobile humic acid (MHA) fractions. Points are the means of two replicates. Fractions were extracted from the 10-30 cm soil layer of a Grangeville sandy loam.](image)
Table 5
Buffering capacity for the MHA and CaHA, as the cmol H⁺ kg⁻¹ required to lower the pH by 0.5 units. Fractions were extracted from the 10–30 cm soil layer of a Grangeville sandy loam.

<table>
<thead>
<tr>
<th>Change in pH</th>
<th>Buffering capacity (cmol H⁺ kg⁻¹)</th>
<th>MHA</th>
<th>CaHA</th>
</tr>
</thead>
<tbody>
<tr>
<td>9.75–9.50</td>
<td>10.7 (0.1)</td>
<td>10.4 (0.4)</td>
<td></td>
</tr>
<tr>
<td>9.50–9.00</td>
<td>16.0 (0.0)</td>
<td>15.8 (0.3)</td>
<td></td>
</tr>
<tr>
<td>9.00–8.50</td>
<td>11.8 (0.2)</td>
<td>12.7 (0.1)</td>
<td></td>
</tr>
<tr>
<td>8.50–8.00</td>
<td>10.6 (0.2)</td>
<td>11.3 (0.1)</td>
<td></td>
</tr>
<tr>
<td>8.00–7.50</td>
<td>10.8 (0.5)</td>
<td>12.0 (0.1)</td>
<td></td>
</tr>
<tr>
<td>7.50–7.00</td>
<td>12.3 (0.2)</td>
<td>15.1 (0.0)</td>
<td></td>
</tr>
<tr>
<td>7.00–6.50</td>
<td>16.3 (2.4)</td>
<td>21.3 (0.6)</td>
<td></td>
</tr>
<tr>
<td>6.50–6.00</td>
<td>21.8 (0.6)</td>
<td>28.3 (0.8)</td>
<td></td>
</tr>
<tr>
<td>6.00–5.50</td>
<td>26.7 (2.6)</td>
<td>33.6 (1.2)</td>
<td></td>
</tr>
<tr>
<td>5.50–5.00</td>
<td>34.5 (3.2)</td>
<td>40.5 (2.7)</td>
<td></td>
</tr>
<tr>
<td>5.00–4.50</td>
<td>42.9 (0.8)</td>
<td>47.0 (1.5)</td>
<td></td>
</tr>
<tr>
<td>4.50–4.00</td>
<td>51.0 (0.2)</td>
<td>57.4 (1.7)</td>
<td></td>
</tr>
<tr>
<td>4.00–3.50</td>
<td>73.0 (0.2)</td>
<td>79.0 (2.2)</td>
<td></td>
</tr>
<tr>
<td>3.50–3.20</td>
<td>72.8 (1.0)</td>
<td>79.4 (1.8)</td>
<td></td>
</tr>
</tbody>
</table>

* Numbers in parentheses represent the standard error.

Greater for the CaHA and increased with solution-phase cation concentration. The maximum proportion of CE sites exchanged by Ca and K was 66% for the CaHA at 1 mM Ca and 50 mM K. Cation exchange onto the H⁺-saturated HA caused a decline in the dialyzing solution pH to below 4, which inhibited further deprotonation.

Table 6
Gapon selectivity coefficients* for the MHA and CaHA and the percent cation exchange sites exchanged by Ca and K. Fractions were extracted from the 10–30 cm soil layer of a Grangeville sandy loam.

<table>
<thead>
<tr>
<th>Initial concentration (mM)</th>
<th>K₀</th>
<th>Percent CE sites exchanged</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>MHA</td>
<td>CaHA</td>
</tr>
<tr>
<td>K</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ca</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2.0</td>
<td>0.15</td>
<td>7.5</td>
</tr>
<tr>
<td>2.0</td>
<td>0.30</td>
<td>7.1</td>
</tr>
<tr>
<td>2.0</td>
<td>0.50</td>
<td>8.8</td>
</tr>
<tr>
<td>2.0</td>
<td>1.00</td>
<td>6.7</td>
</tr>
<tr>
<td>2.0</td>
<td>2.00</td>
<td>4.6</td>
</tr>
<tr>
<td>4.0</td>
<td>1.00</td>
<td>9.6</td>
</tr>
<tr>
<td>8.0</td>
<td>1.00</td>
<td>7.2</td>
</tr>
<tr>
<td>16.0</td>
<td>1.00</td>
<td>5.3</td>
</tr>
<tr>
<td>50.0</td>
<td>1.00</td>
<td>5.7</td>
</tr>
</tbody>
</table>

* Selectivity coefficients are for the reaction $K_{Ca} + 0.5Ca^{2+} = (Ca_{0.5})_{x} + K^{+}$.

* Ratio is MHA coefficient/CaHA coefficient.
Table 7
Recovery of the MHA and CaHA and characteristics of soil layers in a Grangeville soil profile

<table>
<thead>
<tr>
<th>Layer (cm)</th>
<th>MHA Recovery (g kg⁻¹)</th>
<th>Total organic C (g kg⁻¹)</th>
<th>Total C recovery (%)</th>
<th>Soil clay content (%)</th>
<th>IM NH₄⁺ extractable Ca (cmol kg⁻¹)</th>
<th>pHb</th>
</tr>
</thead>
<tbody>
<tr>
<td>0-20</td>
<td>0.6a</td>
<td>4.0a</td>
<td>22</td>
<td>21</td>
<td>9.3</td>
<td>8.0</td>
</tr>
<tr>
<td>20-40</td>
<td>0.6a</td>
<td>3.9b</td>
<td>23</td>
<td>22</td>
<td>9.4</td>
<td>8.1</td>
</tr>
<tr>
<td>40-60</td>
<td>0.7b</td>
<td>3.1c</td>
<td>19</td>
<td>36</td>
<td>11.5</td>
<td>8.3</td>
</tr>
</tbody>
</table>

* MHA contains 514 g C kg⁻¹; CaHA contains 540 g C kg⁻¹.

** pH was measured in 0.01 M CaCl₂.

* Means are of two extraction replications for one bulk sample. Within a column, means followed by the same letter are not statistically different at p = 0.05 by Fisher’s LSD.

3.4. Humic acid fractions in a soil profile

In the sampled profile of a Grangeville soil, clay content was 72% greater in the 0.40 to 0.60 m depth than in the surface 0 to 0.20 m layer (Table 7). Total organic C was relatively constant at all depths, while MHA content increased slightly at the lowest depth interval sampled. In contrast, CaHA content decreased markedly with depth, in opposition to soil exchangeable Ca.

The combined mass of the MHA and CaHA represented only 10-20% of the total SOC in this soil. Typically, about 80% of SOM in a mollic epipedian cannot be extracted by any chemical method (McKeague et al., 1986). Chemical properties of the unextracted humin are not described here, but this more recalcitrant fraction may be less important to nutrient cycling.

4. Discussion

Although effects of SOM content on K availability have been demonstrated in previous studies, a mechanism to account for such effects has not been established, and the SOM fractions responsible for this phenomenon have not been identified. The extraction method described here distinguishes HA fractions by their binding to the dominant stabilizing soil cations. In comparison to analysis of the aggregate SOM pool, characterization of the MHA distinguishes a younger SOM fraction that is likely to be more active in nutrient cycling.

Chemical properties of the CaHA and MHA suggest that the MHA represents an early stage and the CaHA a later stage in SOM evolution. Humification in temperate climates is thought to involve increasing aromaticity accompanied by increased condensation and cross-linking of aromatic rings, increasing molecular weight, development or concentration of carboxyl groups, and cleavage of amino acids (Schnitzer and Desjardins, 1966; Tsutsuki and Kuwatsuka, 1978a, Tsutsuki and Kuwatsuka, 1978b; Anderson, 1979; Stevenson, 1982; Kumada, 1987; Tate, 1987; Wilson, 1987). The younger MHA characterized in our studies was less aromatic and had fewer carboxyl groups than the CaHA. The MHA also had longer $T_1$ times and a higher $E_2/E_0$ ratio which suggest lower molecular weight and less condensation. The MHA had greater total N and hydrolyzable amino acid content than the CaHA,
especially of those amino acids from nonmicrobial origin. Hydrolyzable amino acids are thought to be attached as peripheral groups to aromatic cores of HA (Hayes, 1991).

Campbell et al. (1967) also found that MHA was much younger and had higher $E_4/E_6$ ratios, which were interpreted as less aromaticity and condensation. In their studies on two Canadian soils, however, the mean residence times of the MHA were 785 and 85 years in Chernozemic and Podzolic soils, respectively. Both values are greater than the radiocarbon age of MHA in the Grangeville soil. Age differences may reflect the greater intensity of SOM degradation in the warmer climate of California.

Differences in the chemical structure of MHA and CaHA affect their behavior in soil. The increase in MHA content in the fine-textured subsoil at lower depth may reflect the greater solubility of the MHA and deposition of solubilized MHA carried downward by irrigation water. Because CaHA content and exchangeable Ca had different distribution patterns in the soil profile, precursors of the CaHA may be more limiting to development of this fraction at depth than is Ca. The stabilized CaHA in the surface would be less likely than the MHA to solubilize significantly and eluviate.

It is not clear whether the MHA and CaHA evolve from different organic molecule precursors or whether the MHA itself is a precursor of CaHA. More carboxyl-laden SOM precursors may be attracted to calcareous mineral surfaces (Tsutsuki and Kuwatsuka, 1979). Calcium binding can, however, retard microbial degradation of many organic molecules. Calcium addition reduced gaseous evolution of $^{14}$C added to soil as glucose under laboratory and field conditions (Mencer and Oades, 1989a, Mencer and Oades, 1989b). Decalcification of organo-Ca mineral complexes led to dissociation of the complexes and biodegradation of the SOM (Gaiffe et al., 1984). The most important condition for development of SOM precursors into CaHA may therefore simply be binding to Ca$^{2+}$. In the Grangeville soil, Ca represented about 75% of extractable cations. Calcium was also present as precipitated CaCO$_3$ and in the soil solution. Thus SOM precursors would likely encounter Ca$^{2+}$ and be subject to stabilization.

The relatively high Fe content of the CaHA (Table 2) agrees with the results of Posner (1966) and suggests that Fe may also contribute to the stabilization of the CaHA fraction. McKeague et al. (1986) suggested a possible role by Fe oxides in cation bridging to SOM in mollic epipedons.

If the MHA and CaHA did develop from similar precursors, then the much younger age of the MHA may reflect greater biodegradability due to weaker binding to mineral surfaces by associations with cations other than Ca, or via mechanisms other than cation linkages. The MHA precursors may bind to mineral surfaces through monovalent cations held at exchange sites. Evidence exists for monovalent-selective sites in SOM (Zadmard, 1939) and for organic ionophores that have three-dimensional configurations which allow highly specific binding of K$^+$ (Bruening et al., 1990). The best known example, valinomycin, has structural similarities to the MHA, both with amino acid moieties and potentially greater structural flexibility (Dobler, 1981). Gamble (1973) interpreted titration curves of FA to postulate that similar structural configurations of FA caused binding of Na and K.

The MHA could also be bound via its peripheral amino acid substituents to exchange sites or to the fixation sites found on the edges of expanding 2:1 layers in vermiculitic soils. Large proportions of biotic K can be exchanged within hours by dodecylammonium chloride, an aminated 12-carbon aliphatic chain (MacKintosh and Lewis, 1968). Extensive
interlayer expansion can also occur in vermiculite by adsorption of amino acids (Theng, 1974). Adsorption by peripheral amino acid groups of the MHA onto fixation sites would most likely protect them from degradation, thus accounting for the unusually high N content of the MHA.

Alternatively, MHA molecules could simply be the most recent additions to the periphery of the older, more recalcitrant SOM constituents. In this scenario, the bonds that link MHA to older SOM would be broken by the NaOH extraction before soil decalcification. Shinkarev et al. (1987) studied the kinetics of HA dissolution and concluded that the MHA molecules were attached to the CaHA in the A horizon of a Chernozem, while the MHA molecules were held directly to the mineral surface in a Sod-Podzolic soil.

In summary, it is not possible to specify how the MHA and CaHA evolve, and particularly whether the MHA is a precursor pool of the CaHA, but it is clear that the MHA can be extracted without decalcification, is a relatively young HA fraction, has a lower Gapon selectivity coefficient for Ca–K exchange, and does not appear to form stable complexes with Ca and Fe. These traits contrast sharply with CaHA which is much older and apparently protected by Ca and possibly Fe binding. The MHA would therefore appear to have potentially greater activity in nutrient cycling and nutrient availability than CaHA. In a related study, for example, addition of MHA reduced K fixation in vermiculitic soils, whereas addition of CaHA had no effect (Olk and Cassman, 1994). Behavior of extracted MHA is likely to represent the effect of younger SOM fractions in whole soils. The differences in chemical natures of the MHA and the CaHA and their effects on nutrient availability indicate the need to study separate HA pools rather than aggregate HA obtained by the standard HA extraction procedure.

Acknowledgements

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Characteristics of Two Chemically Extracted Humic Acid Fractions in Relation to Nutrient Availability*

D.C. Olk and K.G. Cassman

*Citation:

Abstract

Differentiation of less chemically protected soil organic matter (SOM) from more protected SOM may allow better characterization of the SOM molecules active in nutrient cycling. Using a sandy loam from California, USA, a Philippine irrigated lowland clay, and a Philippine clay planted to upland crops, NaOH-extractable SOM was separated into the calcium (Ca)-bound humates (CaHA) and the non Ca-bound mobile humic acid (MHA) fraction. In all soils, the MHA was less humified than the CaHA in terms of total nitrogen (N) and hydrolyzable amino acid content, $E_4/E_6$ ratio, and degree of aromaticity. In previous research, MHA addition to the vermiculitic California soil reproduced the positive effect of manure addition on potassium availability. Current research at IRRI is studying the effects of recent crop intensification in the irrigated lowland rice system on properties of the MHA and CaHA and resulting changes in N cycling.
Despite decades of research, chemically extracted humic acid (HA) fractions of SOM have not been clearly related to SOM dynamics or other soil processes in cultivated systems (Cambardella and Elliott, 1992; Feller, 1993). To isolate HA fractions meaningful to nutrient cycling, SOM could be extracted based on the degree of chemical protection: more protected SOM should be less active in nutrient cycling. The longevity of chemically protected SOM is well known (Jenkinson and Rayner, 1977) and has been attributed to binding with exchangeable or structural cations (Martin and Haider, 1986). Binding to calcium (Ca) could be one definition of chemical protection because Ca is generally the dominant exchangeable cation and the stabilizing effect of Ca on SOM is well known (Kononova, 1975; Muneer and Oades, 1989).

Humic acids extracted by NaOH were separated into Ca-bound and non Ca-bound fractions in earlier characterizations of SOM properties (Posner, 1966; Campbell et al., 1967; Mather and Paul, 1967; Shinkarev et al., 1987). The non Ca-bound fraction was named the mobile humic acids (MHA) in pioneering work by Tyurin (Kononova, 1966). To our knowledge, however, no published studies have related the properties of the MHA to nutrient availability.

The objective of this paper is to compare the properties of the MHA and the Ca-bound humates (CaHA) in three cropping systems: a temperate climate, cotton-based
rotation; tropical irrigated rice; and tropical dryland rice. Some implications for nutrient availability will also be discussed.

Materials and Methods

The MHA and CaHA were extracted from a sandy loam of the San Joaquin Valley, California, USA, mapped as a Haploxeroll, and from two IRRI clay soils, one mapped as a Tropudalf and the other mapped as a Tropaquept (Table 1). The California soil was formed in granitic alluvium and was highly calcareous. The IRRI soils developed in recent volcanic tuff and their most common exchangeable cation was also Ca, but precipitated Ca was not present. The California soil has been in a cotton-based rotation since 1945 (Cassman et al., 1989). The Tropaquept at IRRI has been triple-cropped to irrigated lowland rice since 1968 and the Tropudalf has been planted to dryland rice for a similar period of time.

The California soil was extracted following the procedure described by Olk et al. (1995). Briefly, air-dried soil was incubated under N₂ for 20 h in 0.25 M NaOH at a solution:soil ratio of 2.5:1. The solutions were centrifuged, the solubilized MHA decanted and acidified to pH 2. The soil was washed twice more in water to maximize HA recovery; these precipitates were combined with the
precipitate of the NaOH wash and considered MHA. The soil was decalcified by 0.1 M HCl washes until the pH of the supernatant remained below 1.5, then incubated under N₂ for 20 h in 0.25 M NaOH. As with the MHA extraction, the solutions were centrifuged, the solubilized CaHA decanted and acidified to pH 2. Both HA fractions were then stirred in 200 ml of a 0.5% HF and 0.5% HCl solution for 3 d with daily solution replacement. The HA were H⁺-saturated by dialyzing for 24 h against 0.01 M HCl, 0.001 M HCl, and water. The HA were frozen and lyophilized. Both HA fractions typically had ash contents of 1-2% or less.

The extraction procedure was modified for the IRRI soils in consideration of their high clay content. The initial solution:soil ratio was 10:1. After decantation of the first NaOH wash, both soils were washed twice with 0.0025 M CaCl₂ and the lowland rice soil four additional times with water to continue MHA solubilization while inducing flocculation and settling of fine clays. The precipitates from the CaCl₂ and water washes had high ash contents and were not combined with that of the NaOH wash. The soils were then decalcified and extracted for the CaHA as above. The IRRI soils were stored at 4 °C from sampling until extraction, and their moisture contents were maintained at field levels during storage.

The ratio of light absorbances at 465/665 nm (E₄/E₆) was determined on solutions of 12 mg HA solubilized in 50 ml 0.05 M NaHCO₃ (Chen et al., 1977). Total carbon (C) and
nitrogen (N) concentrations of the HA extracted from the California soil were measured by a modified Walkley-Black method (Nelson and Sommers, 1982) and colorimetric analysis (Dorich and Nelson, 1983) of an acid digest, respectively. Total C and N of the HA extracted from the IRRI soils were determined on a CHN automated elemental analyzer. Hydrolyzable amino acids were determined by the ninhydrin method following 24 h hydrolysis in 6 M HCl at 110\(^{\circ}\) C. Radiocarbon ages were measured at the Center for Accelerator Mass Spectrometry at Lawrence Livermore National Laboratory, CA, USA.

Results

In each of the three soils, the C concentration was similar for both HA fractions, while the MHA was enriched in N and had lower C:N ratios than the CaHA (Table 2). The MHA had a higher hydrolyzable amino acid content than the CaHA in all soils, but this difference was smaller in the IRRI soils because of lower amino acid concentration in the MHA. The amino acids in all HA fractions were dominated by glutamine + glutamic acid, asparagine + aspartic acid, glycine, alanine, and lysine (data not shown), which are often the most common amino acids in microbial cell walls (Stevenson, 1982). The relative proportions of individual amino acids were similar for MHA extracted from the
California and IRRI irrigated lowland soils (Fig. 1) and the IRRI dryland rice soil (data not shown).

The lower E$_4$/E$_6$ ratios of the CaHA suggest that this fraction has a greater molecular weight and may be more condensed and aromatic than the MHA (Stevenson, 1982). $^{13}$C nuclear magnetic resonance scans confirmed the greater aromaticity of the CaHA in all soils as reported elsewhere (Olk et al., 1994; Olk et al., 1995).

Together the MHA and CaHA accounted for about 20% of the total soil organic C in all soils despite different soil organic C contents (Table 1). However, proportions of total soil organic C extracted as MHA and CaHA varied by soil. The amount of C extracted as MHA from the IRRI soils was 4 to 12 times greater than from the California soil. The amount of C extracted as CaHA was lowest in the IRRI dryland soil. Total N extracted as MHA and CaHA followed similar patterns.

The MHA was dated as modern for all soils (data not shown). The radiocarbon age of the CaHA was 290 yr in the California soil and modern in both IRRI soils.

Discussion

In each of these three soils and cropping systems, the MHA represent an early stage and the CaHA a more advanced stage of humification, based on the higher total N
and hydrolyzable amino acid content, the higher \( \frac{E_4}{E_6} \) ratio, and lower aromaticity of the MHA. This interpretation is consistent with chemical changes in SOM due to humification as described by Tsutsuki and Kuwatsuka (1978), Anderson (1979), and Stevenson (1982). Differences in the degree of humification of MHA vs CaHA are attenuated, however, in the IRRI soils. The modern \(^{14}C\) dates of the CaHA in the IRRI soils suggest that the CaHA turns over more quickly in these soils than in the California soil. Differences in climate, soil mineralogy, clay content, and amount of precipitated Ca in the California soil may influence the rate of turnover of the CaHA. These differences may also influence the proportions of MHA and CaHA.

Nevertheless, each extracted fraction was relatively similar for all three soils in such chemical characteristics as C:N ratio, \( \frac{E_4}{E_6} \) ratio, and molar fractions of individual amino acids. Such similarities across this spectrum of soil environments support the usefulness of this extraction method for studying SOM properties in diverse cropping systems and soils. The reproducible chemical properties also underline the apparent ubiquity of basic soil processes controlling SOM formation in different soil environments.

Characterization of the MHA and CaHA may help elucidate the role of SOM in nutrient cycling. Addition of MHA to the highly vermiculitic California soil reduced K
fixation, increased 1 M NH$_4$Cl-extractable K by 30% in K adsorption isotherm incubations, and increased plant K uptake by 42% in a pot experiment (Olk and Cassman, 1993; Olk and Cassman, submitted). These laboratory results reproduced the positive effect of steer manure addition on the availability of previously applied K fertilizer in a field experiment (Cassman et al., 1992). Addition of CaHA had no significant effect on K availability.

We are currently studying the effects of intensified irrigated rice cultivation on MHA and CaHA properties in tropical lowland soils. In preliminary results the intensity of irrigated rice cropping in submerged soil markedly affected certain HA properties while having little effect on others. Changes in HA properties with flooding intensity were greater for the MHA than for the CaHA.

Subsequent research will address the effects of these altered SOM properties on N mineralization and availability in the irrigated lowland system.

This experimental approach can be useful for other systems. Characterization of young, labile SOM may become more necessary for understanding nutrient availability in Asian agriculture given the recent trend toward crop intensification: the SOM fractions most active in nutrient cycling in these new systems may also be the same fractions most affected by intensification. Moreover soil extraction for MHA and CaHA may be appropriate even when the soil exchangeable cations are not dominated by Ca, as the CaHA
may also contain SOM bound by Ca and Fe (Posner, 1966; Olk et al., 1995).

Acknowledgements

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Figure 1. Molar fractions of hydrolyzable amino acids in the mobile humic acids (MHA) extracted from the IRRI irrigated lowland soil vs molar fractions of the MHA extracted from the California cotton soil. Molar fractions are expressed as percent of total hydrolyzable amino acids. Amino acids are represented by the following abbreviations: A (alanine); B (asparagine + aspartic acid); F (phenylalanine); G (glycine); H (histidine); I (isoleucine); K (lysine); L (leucine); P (proline); R (arginine); S (serine); T (threonine); V (valine); Y (tyrosine); and Z (glutamine + glutamic acid). Amino acids were hydrolyzed by 6 M HCl at 110° C and measured by the ninhydrin method.
Table 1. Selected physical and chemical characteristics of soil extracts for humic acid fractions.+

<table>
<thead>
<tr>
<th>Site</th>
<th>Cropping system</th>
<th>Great group</th>
<th>Percent clay</th>
<th>Soil organic C++</th>
<th>pH++</th>
<th>Exchangeable cations</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Ca       Mg       K</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>g kg(^{-1}) soil</td>
</tr>
<tr>
<td>California cotton</td>
<td>Haploxeroll</td>
<td>21</td>
<td>11.3</td>
<td>8.0</td>
<td>20</td>
<td>2.8      0.27</td>
</tr>
<tr>
<td>IRRI dryland rice</td>
<td>Tropudalf</td>
<td>40</td>
<td>13.0</td>
<td>5.8</td>
<td>15</td>
<td>9.0      1.34</td>
</tr>
<tr>
<td>IRRI irrigated rice</td>
<td>Tropaquept</td>
<td>66</td>
<td>28.8</td>
<td>6.2</td>
<td>22</td>
<td>16.3     0.75</td>
</tr>
</tbody>
</table>

+ Soil was from the 0-10 cm layer of the California soil, 0-14 cm layer in the IRRI dryland rice soil and 0-15 cm layer in the IRRI irrigated rice soil.

++ Total soil organic C was measured by a modified Walkley-Black method (Nelson and Sommers, 1982), and pH in a 1:2 suspension of soil:0.01 M CaCl\(_2\).
Table 2. Elemental composition and some chemical properties of the mobil... humic acid (MHA) and calcium humate (CaHA) fractions.

<table>
<thead>
<tr>
<th>Fraction</th>
<th>Site</th>
<th>C concentration</th>
<th>N concentration</th>
<th>C:N</th>
<th>Hydrolyzable amino acids</th>
<th>E4/E6</th>
<th>Total C weight</th>
<th>Total N weight</th>
</tr>
</thead>
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<tr>
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<td>14.5</td>
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<td>4.3</td>
<td>2.82</td>
<td>0.19</td>
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Hydrolyzable Amino Acids in MHA
(Molar percent)

IRRI irrigated lowland rice soil

California cotton soil

\[ Y = 1.12 + 0.79X \]
\[ r^2 = 0.93^{**} \]

In long-term irrigated lowland rice experiments under intensified cropping, the soil N supplying capacity has declined despite constant fertilizer-N inputs. Total soil organic C (SOC) and N levels have been maintained, suggesting instead a change in the chemical nature of SOM. We characterized two humic acid fractions extracted from aerated, double-cropped irrigated, and triple-cropped irrigated lowland soils. Together the mobile humic acids (MHA) and the Ca-bound calcium humates (CaHA) represent 20-27% of SOC and 17-24% of total N. The CaHA were more humified than the MHA but both fractions were dated as modern by 14C dating. The C and N concentrations and the ratios of light absorption at 465/665 nm of the MHA and CaHA changed little with increased cropping intensity. 

13C nuclear magnetic resonance spectroscopy demonstrated relatively more prominent phenolic/N-substituted aromatic peaks in both fractions with increased irrigated cropping intensity. The known capacity of phenolic compounds to abiotically complex nitrogenous compounds suggests a possible mechanism for decreased soil N supplying capacity under intensified irrigated rice cropping.

D.C. Olk, (63-2) 818-1926
CHEMICAL PROPERTIES OF TWO HUMIC ACID FRACTIONS IN TROPICAL LOWLAND RICE SOILS

D.C. Olk, K.G. Cassman*, E.W. Randall, and P. Kinchesh
International Rice Research Institute and University of London

Soil organic matter was extracted from soil with different histories of intensive rice cropping under submerged soil conditions at the IRRI Research Farm in the Philippines. The extraction procedure isolated two humic acid fractions: mobile humic acid (MHA) which is relatively soluble, N rich, and younger than the calcium humates (CaHA) which are older, low in solubility, and low in N. Crop management history in the past 10-25 years had small effects on the C, N, hydrolyzable amino acid content, and $E_4/E_6$ ratio of each HA fraction. In contrast, N rates of 0 or 360 kg/ha-yr in a triple-crop system since 1968 had a large effect on the proportion of MHA versus CaHA. Spectra from $^{13}$C NMR indicated that unsubstituted aromatic C dominated the CaHA which suggests advanced stages of humification. Methoxyl- and phenolic-C were enriched in the MHA which indicates the incorporation of partially degraded lignin subunits into this fraction. Although HA is generally considered relatively old and recalcitrant, these results indicate changes in chemical composition, quantities, and proportions of HA fractions due to management within a 10-25 yr period, which suggests that these humic pools are more active in submerged, reduced soils than in aerated soil.

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