

Association of organic matter with iron and aluminum across a range of soils determined via selective dissolution techniques coupled with dissolved nitrogen analysis

Rota Wagai · Lawrence M. Mayer ·
Kanehiro Kitayama · Yasuhito Shirato

Received: 15 February 2011 / Accepted: 5 September 2011
© Springer Science+Business Media B.V. 2011

Abstract Strong correlations of soil total organic carbon (OC) with iron and aluminum phases reported frequently make it important to quantify these organic matter (OM) associations, but selective extractants sometimes contain OC. Soil nitrogen is often predominantly organic and might serve as a proxy for OM. We therefore investigated nitrogen associations with Fe and Al using several selective extractants that use reductive, complexation, and alkaline approaches. Total dissolved nitrogen (TDN) correlated strongly with extracted Fe and Al across seventeen samples, including highly- and weakly-weathered soils, iron-rich ultrabasic soils, podzolic, and volcanic soils. Typically a quarter to a third of total soil nitrogen was dissolved by the various extractions, though higher

fractions (up to 60%) were found in spodic-horizon and volcanic surface-horizon samples. Similar proportions were found for OC, using three OC-free extractants, indicating that TDN provides a useful surrogate for assessing OM partitioning via extractants that contain OC. Use of TDN:metal ratios in extractant solutions allows estimation of extracted OM that could have been sorptively associated with metal oxide/hydroxides and poorly-crystalline aluminosilicates. These ratios were often high in extractions targeted at these adsorbents, and imply that usually most of the extracted TDN consists instead of organo–metal complexes. The dynamics of these complexes may have stronger control on accumulation/remobilization of soil OM than those of metal oxyhydroxides and poorly-crystalline aluminosilicates.

R. Wagai (✉)
National Institute for Agro-Environmental Sciences,
Carbon and Nutrient Cycling Division, 3-1-3, Kannondai,
Tsukuba, Ibaraki 305-8604, Japan
e-mail: rota@affrc.go.jp

L. M. Mayer
School of Marine Sciences, University of Maine,
Walpole, ME 04573, USA

K. Kitayama
Graduate School of Agriculture, Kyoto University,
Kyoto, Japan

Y. Shirato
National Institute for Agro-Environmental Sciences,
Natural Resources Inventory Center, 3-1-3, Kannondai,
Tsukuba, Ibaraki 305-8604, Japan

Keywords Soil organic matter stabilization ·
Organo–mineral interaction · Sorption · Dithionite ·
Oxalate · Pyrophosphate

Introduction

Organic matter (OM) in soil represents the largest carbon pool and a vital nutrient reservoir in terrestrial ecosystems. In most soils other than high-altitude and water-logged areas, major fractions of soil organic C and N are strongly associated with soil minerals (Christensen 1992; Sollins et al. 2009). The C:N ratio of the mineral-associated OM is consistently lower

and less variable than mineral-free OM, which mainly consists of plant detritus (von Lützwow et al. 2007; Wagai et al. 2009), because soil N is retained by microbial mineralization-immobilization processes, in contrast with catabolic loss of C (Paul and Clarks 1996).

In contrast with soil organic C, which is present in many compound classes (Kögel-Knabner 2002), soil organic N exists primarily as amides likely derived from peptidic structures (Knicker et al. 1993; Smernik and Baldock 2005; Mikutta et al. 2010) that are potentially susceptible to enzymatic degradation. Organic N can be stabilized via reactions with humic and phenolic substances (Zang et al. 2000; Nguyen and Harvey 2001), but only limited information is available on its interaction with soil minerals (Schulten and Leinweber 1995; Mikutta et al. 2010). Mineral-bound organic N may have significant influence on the terrestrial biogeochemical cycle. While N commonly limits primary production (e.g., Vitousek and Howarth 1991), terrestrial ecosystems tend to accumulate organic N. For instance, dissolved organic N (DON) formed in or percolated through surface horizons is largely retained in lower soil horizons where Fe and Al oxides accumulate (Qualls and Haines 1992). At the same time, appreciable amounts of N leach out from soil in organic form (e.g., Perakis et al. 2005; Fang et al. 2010; Huygens et al. 2008). Mineral-associated OM may thus be an important N pool, sensitive to N losses (e.g., upon dissolution of sorbents) but also potentially available for soil biota.

Together with aluminosilicate clays, iron (Fe) and aluminum (Al) phases represent key components contributing to soil OM stabilization (Sollins et al. 1996; Baldock and Skjemstad 2000). As cations, Fe and Al can bind with organic ligands (e.g., carboxylic groups) to form organo–metal complexes (Higashi 1983; Boudot et al. 1989). As solid Fe and Al oxides, hydroxides, and oxyhydroxides (collectively termed FeOx and AlOx hereafter), these metals are among the most effective sorbents for dissolved OM (e.g., Kaiser et al. 1997). Bonding between hydroxyls on metal oxide surfaces and OM (especially carboxylic functional groups) can stabilize OM against microbial enzymatic attack (Mikutta et al. 2007; Schneider et al. 2010). Across a wide range of soils, positive correlations of soil Fe and Al phases are found with total organic C (e.g., Tiessen et al. 1984; Skjemstad et al. 1989; Johnson and Todd 1983; Kaiser and

Guggenberger 2000) and ^{14}C -based mean residence time (Torn et al. 1997; Masiello et al. 2004), implying strong contributions of these metal phases (especially less-crystalline ones) to soil OM stability in the field. The importance of organo–metal complexation to OM stability has been suggested in podzolic and volcanic soils (e.g., Wada and Higashi 1976; De Coninck 1980) and, more recently, in a wider range of soils (Percival et al. 2000; Masiello et al. 2004). The amounts of OM associated with various metal phases in field soils are, however, poorly known (Wagai and Mayer 2007).

Extraction techniques are used to dissolve specific phases of Fe and Al in soil (e.g. Parfitt and Childs 1988) because of their importance in soil processes and classification. Most techniques, however, use carbon-based compounds as complexing agents to dissolve Fe and Al, making it difficult to assess release of soil organic C. Many of these extractants are nitrogen-free, nevertheless, allowing us to quantify the N released upon selective dissolution of these metal phases. In this study we used these extractants: (i) to assess the contribution of metal phases on OM stabilization using dissolved N as a proxy for OM, and (ii) to infer the nature of metal–OM associations in a range of field soil samples (Inceptisols, Andisols, Spodosols, Alfisols, Ultisols, Oxisols).

Material and methods

Soil samples

We studied fifteen surface mineral soils and two subsurface spodic horizon samples across a range of soil types (Table 1). These samples included highly-weathered soils rich in well-crystallized FeOx and AlOx, soils developed from Fe-rich ultrabasic rock, and volcanic and podzolic soils that typically contain large amounts of organo–metal complexes and/or poorly-crystalline minerals. Eleven of these samples were previously used to quantify reductively-soluble Fe phases and organic C (Wagai and Mayer 2007). We added five so-called volcanic-ash soils, including allophanic (Tsukuba, Osawa), non-allophanic (Kawatabi), and buried A-horizon (Miyako) Andisols, and one paddy soil on alluvium for this study. The paddy and Tsukuba soils were under cultivation and the rest were under forests.

Table 1 Source, soil order, parent material, sampling depth and chemical characteristics of the soil samples studied

Sample ID	Location	Soil order	Parent material	Horizon: depth (cm)	Total		pH in H ₂ O	Mineral-associated ^a		
					OC (mg g ⁻¹)	N		OC (mg g ⁻¹)	N	
Highly weathered soils										
Ult-4 ^b	Mt. Kinabalu, Borneo	Ultisol	Sedimentary	A: 0–10	27.6	2.2	13	4.4	22.4	1.8
Ox-4 ^b	Mt. Kinabalu, Borneo	Oxisol	Ultrabasic	Ac: 0–10	29.9	2.4	12	4.8	26.3	1.9
Ox-2 ^b	Puerto Rico, USA	Oxisol	Ultrabasic	A: 0–5	64.7	4.5	14	5.6	41.8	2.9
Less weathered soils										
Paddy	Kyoto, Japan	Inceptisol	Alluvium	A: 0–10	17.9	1.5	12	6.1	ND	ND
Inc-6 ^b	Mt. Kinabalu, Borneo	Inceptisol	Sedimentary	A: 0–10	22.4	1.3	17	4.6	20.1	1.1
Alf-4 ^b	Mt. Kinabalu, Borneo	Alfisol	Ultrabasic	A: 0–10	43.1	2.3	19	5.3	39.4	2.1
Inc-5 ^b	Mt. Kinabalu, Borneo	Inceptisol	Ultrabasic	A: 0–10	93.9	3.3	28	4.9	80.5	2.9
Inc-7 ^b	Mt. Kinabalu, Borneo	Inceptisol	Sedimentary	A: 0–10	126	6.6	19	4.5	111	5.3
Podzolic soils										
Spd-2B1 ^b	Bear Brook, Maine	Spodosols	Glacial till	B1: 0–5 ^c	60.9	2.6	23	4.5	48.7	2.3
Spd-3B1 ^b	Bear Brook, Maine	Spodosols	Glacial till	B1: 0–5 ^c	86.8	3.8	23	4.7	69.4	3.2
Volcanic soils										
Ando ^b	Hawaii island, Hawaii	Andisol	Basalt	A: 0–2	151	10.1	15	5.1	ND	ND
Inc-4 ^b	Cascade Head, Oregon	Inceptisol	Mixed volcanic	A: 0–10	105	6.0	17	5.2	73.2	4.2
And-kwt	Kawatabi, Japan	Andisol	Volc.ash (+loess)	A: 0–10	98.5	4.9	20	4.4	95.7	4.6
And-tkb	Tsukuba, Japan	Andisol	Volcanic ash	Ap: 0–20	51.4	4.1	13	6.1	49.3	4.2
And-myk	Miyakonojo, Japan	Andisol	Volcanic ash	2A: 80	130	4.4	29	6.2	ND	ND
And-os1	Osawa, Japan	Andisol	Volcanic ash	A1: 0–2	150	7.5	20	4.9	ND	ND
And-os2	Osawa, Japan	Andisol	Volcanic ash	A2: 2–10	121	6.0	20	5.2	ND	ND

ND not determined

^a High-density fraction (>1.6 g cm⁻³) by shaking without sonication from Wagai and Mayer (2007) and Wagai et al. (2008). Data for Inc-4, And-tkb, And-kwt were newly measured for this study by the same method

^b Archive samples from our previous study. See Table 1 and EA 2 in Wagai and Mayer (2007)

^c Top 0–5 of spodic horizons

Extraction procedures

Overview

Selective extraction techniques show different ability to dissolve various Fe and Al phases (Parfitt and Childs 1988; Reyes and Torrent 1997; summarized in Table 2). The amount of N (and C in case of C-free extractants) co-dissolved by different extractions may therefore reflect the amount of organic N associated with the specific metal phases. For this study we used dithionite-citrate (DC), an inorganic dithionite version (DH) which we previously developed (Wagai and Mayer 2007), ascorbate-citrate (AC), oxalate (OX), sodium pyrophosphate (PP), and carbonate (CO₃) extractions, as described below. We used bulk

samples, which were air-dried or freeze-dried and sieved (2 mm) prior to extraction.

Dithionite-Citrate (DC)

Pedogenic FeOx content is commonly estimated by dithionite-citrate-bicarbonate extraction for 15 min at 80°C (Mehra and Jackson 1960) or by dithionite-citrate for 16 h at room temperature (Holmgren 1967; Loeppert and Inskeep 1996). The latter is operationally simpler yet uses high amounts of sodium citrate which contains small but detectable levels of N. In addition, high dissolved salt contents can reduce analytical combustion efficiency. We thus tested DC extractions varying in concentrations of dithionite and citrate using five samples (Inc-6, Alf-4, Ando, Inc-4,

Spd-3B1). Compared to the standard DC recipe, reduction of dithionite and citrate by one quarter (relative to soil mass) gave slightly higher N recovery (range: 103–126%) without significant changes in Fe (82–100%) and Al (93–112%) recoveries. For this study, we thus used 17.9 mM of sodium dithionite (Sigma-Aldrich, technical grade) and 106 mM sodium citrate (Pure Chemical Industries, Japan, analytical grade). Soil (0.50 g) and sodium dithionite (0.13 g) were weighed into a 60 ml centrifugation tube, 40 ml of 106 mM sodium citrate was added and the tube was capped and sealed with Parafilm. The extraction was shaken (150 rotation min^{-1}) for 16 h at 22–24°C, followed by high-speed centrifugation (29,100g, 40 min, 5°C) without flocculation agents to isolate the dissolved fraction. The same extraction and centrifugation conditions were used for other extractions except for PP and CO_3 (described below).

Dithionite-HCl (DH)

We conducted a fully inorganic dithionite extraction (Wagai and Mayer 2007) for a limited number of samples. Sample was first extracted using 57.4 mM sodium dithionite alone for 16 h and centrifuged. After

sampling an aliquot, the supernatant was discarded. The remaining residue was resuspended in 0.05 M HCl, shaken for 1 h, and centrifuged again. Supernatants from the both extractions were analyzed for C, N, and metals.

Ascorbate-Citrate (AC)

Ascorbate is a weaker reducing agent and thus the AC technique effectively dissolves ferrihydrite with minimal dissolution of more crystalline FeOX (Reyes and Torrent 1997). Compared to the OX extraction, AC also dissolves much less allophane/imogolite (Reyes and Torrent 1997, Table 2). We slightly modified the procedure of Reyes and Torrent (1997). Sodium citrate solution (0.2 M, 45 ml) was added to a 60 ml tube with 0.36 g of L-ascorbic acid (Wako, analytical grade) and 0.3–3.7 g soil sample depending on Fe_{DC} content, sealed with Parafilm, shaken for 16 h and centrifuged (29,100g, 40 min). The solution pH after extraction was 6.6–7.6.

Oxalate (OX)

Acid oxalate extraction, using 0.2 M ammonium oxalate/oxalic acid at pH 3 (Tamm 1922), dissolves

Table 2 Solubility of soil iron and aluminum phases commonly found in soil by commonly-used extraction techniques, based on Parfitt and Childs (1988) together with Ascorbate-citrate extraction (Reyes and Torrent 1997)

Metal phase	Dithionite +HCl rinse (DH) pH 3–7	Dithionite-Citrate (DC) pH 6–8	Ascorbate-Citrate (AC) pH 7–8	Acid Oxalate (OX) pH 3	Pyrophosphate (PP) pH 10
OM-metal complex	$\Delta \sim \bigcirc?$	\odot	\odot	\odot	\odot
Allophane/Imogolite	$\Delta \sim \bigcirc?$	\bigcirc	Δ	\odot	Δ
Ferrihydrite	\odot	\odot	\odot	\odot	#
Goethite	\odot	\odot	\times	\times	#
Hematite	$\bigcirc \sim \odot$	$\bigcirc \sim \odot$	\times	\times	\times
Lepidocrocite	\bigcirc	\bigcirc	$\Delta \sim \bigcirc$	$\Delta \sim \bigcirc$	\times
Magnetite	\bigcirc	\bigcirc	$\Delta?$	$\Delta \sim \bigcirc$	\times
Gibbsite	\times	\times	Δ	Δ	\times
Halloysite	\times	\times	\times	\times	\times
Phyllosilicate clays	\times	\times	\times	\times	\times

Carbon-free dithionite extraction (Wagai and Mayer 2007) was included for comparison despite some uncertainty in solubility of specific phases

\odot 80–100% dissolution of metal phases

\bigcirc 10–80% dissolution

Δ <10% dissolution

\times No dissolution

Possible dispersion of small colloids

? Limited information on its solubility

poorly-crystalline or short-range ordered minerals (Table 2) and is often used to characterize volcanic and podzolic soils (Parfitt and Childs 1988). Dissolution is achieved by the formation of oxalate complexes of Fe and Al (Borggaard 1992). We followed the common procedure (Loeppert and Inskeep 1996) but substituted sodium oxalate (Wako, analytical grade) for ammonium oxalate to allow dissolved N analysis. We compared the two extractions for both volcanic and non-volcanic samples ($n = 10$). Relative to ammonium oxalate, the sodium version dissolved $94 \pm 6\%$ (range: 86–105%) of Fe, $96 \pm 10\%$ (87–117%) of Al, and 112 ± 16 (97–144%) of Si. For this study, we mixed 0.5 g sample with 50 ml of 0.2 M sodium oxalate/oxalic acid solution, shook for 4 h in the dark, and centrifuged (29,100g, 40 min). Refrigerated extracts were prone to precipitate, likely due to high sodium concentration, so we diluted them with Milli-Q water immediately after centrifugation.

Pyrophosphate (PP)

Pyrophosphate binds strongly with Fe and Al ions, and 0.1 M of this reagent (16 h, pH 10) has been widely used to estimate the amount of Fe and Al associated with OM in soil (Loveland and Digby 1984). Aluminum dissolved by PP extraction is generally interpreted as deriving from organo–Al complexes (Higashi 1983) with minimal dissolution of allophane/imogolite (Parfitt and Henmi 1982; Farmer et al. 1983). On the other hand, the source of Fe and Al phases (and the OM bound to them) is difficult to specify as pyrophosphate not only dissolves Fe and Al from organic complexes but promotes peptization of FeOx and AlOx colloids (Schuppli et al. 1983; Kaiser and Zech 1996); thus the amount of Fe released depends on solution pH, type of salt (Na vs. K), centrifugation speed, and filtration treatments (Schuppli et al. 1983; Loveland and Digby 1984). Aluminosilicate particles are often effectively removed from the PP extracts, however (Schuppli et al. 1983). High-speed centrifugation (20,000g) without filtration was recommended to improve reproducibility (Loveland and Digby 1984) with a caveat to specify the exact procedure (Loeppert and Inskeep 1996). To reduce colloids, we used a higher speed (40,000g) followed by vacuum filtration with 0.025 μm membrane filter (Millipore, VSWP, 47 mm dia.) following Schuppli et al. (1983). Specifically, 0.2 g of sample was mixed

with 20 ml of 0.1 M sodium pyrophosphate (Wako, analytical grade) solution adjusted to pH 10, shaken for 16 h, and centrifuged (40,000g). A 5 ml aliquot was immediately taken from the supernatant and vacuum-filtered through a 0.025 μm filter that was pre-washed with 0.01 M HCl.

Carbonate (CO₃)

We extracted the samples with 0.1 M sodium carbonate/bicarbonate solution (pH 10) to quantify the amount of OM and metals soluble at this pH in the absence of strong complexing agents for metals. The same extraction and centrifugation procedure as PP was used except that no filtration was done.

Chemical analyses of extracts

Immediately after extraction and centrifugation, supernatants were pipetted into acid-washed tubes for dilution with Milli-Q water. For dissolved organic C and total dissolved N (TDN) analysis, dilution factors of 10 (OX), 15.5 (DC and AC), 18 (CO₃) and 106 (PP) were used. For metal analysis, we used dilution factors of 50 or 100.

To measure DOC and TDN, we used a Shimadzu TOC-V/TN analyzer (Kyoto, Japan). For carbon-free extracts, diluted samples were acidified (pH < 3) and sparged with C-free air. To acidify we added 1 M HCl to 5% (PP) or 1% (DH, CO₃) vol/vol. Solutions were oxidized by catalytic combustion at 720°C, and CO₂ detected by infrared gas analyzer. Combusted N was measured as NO, via reaction with ozone and detection by in-line chemiluminescence. We ran internal standards (hippuric acid N in water) after every 5–10 samples to monitor any sensitivity loss (e.g., by salt accumulation in combustion tube). High concentrations of C-based complexing agents may impede full combustion of the organic N dissolved from soil OM. We confirmed full N recovery using spiked organic N (hippuric acid) in DC and AC solution matrices. The concentrations of Fe, Al, and Si in the diluted extracts were analyzed by inductively coupled plasma-optical emission spectroscopy (Varian Inc., Vista-Pro, USA). Coefficients of variation of TDN, Fe, Al, and Si analyses in the six extractions assessed from replicates of five randomly-chosen samples were <6%.

Other analyses

Subsets of dried, sieved bulk soil samples were used for soil pH in deionized water (soil:water = 10 g:25 ml) after at least 1 h equilibration and for total soil C and N using a CN-900 Analyzer (Sumika Chemical Analysis Service, Tokyo, Japan).

Data analyses

The differences in the concentrations of extractable N, C, and metals among the extractants were tested by paired *t*-test ($\alpha = 0.05$). Correlations between the extractable N vs. metals as well as extractable N vs. C (for C-free extractants) were assessed using Pearson product-moment correlation. For these analyses, we used JMP software (version 8.0.1, SAS Institute, Cary, NC, USA).

Results

Dissolved Fe, Al, and Si

Metal dissolution by the six extractions reflected differences in the solubility of metal phases (Table 2) in these extractants. The strong reductant, dithionite (DH and DC), dissolved more Fe than any other extraction, especially for Oxisols from ultramafic rocks and a Hawaiian volcanic soil ($>100 \text{ mg Fe g}^{-1}$) (Table 3). The eight samples subjected to both DH and DC showed similar dissolution of Fe and Al, as found earlier (Wagai and Mayer 2007). Dissolution of Fe by AC, OX, and PP was significantly lower, averaging 41%, 37%, and 20% of the DC-soluble Fe across all soils. Among extractions not containing dithionite, the only significant difference was higher Fe dissolved by AC than PP ($P < 0.01$). The highly weathered Oxisols showed $\text{Fe}_{\text{AC}}:\text{Fe}_{\text{DC}}$ ratios of 0.01–0.02, indicating dominance of more crystalline FeOx. The other soils had much higher ratios of 0.41–0.85. The CO_3 extraction dissolved little Fe as expected (Table 3). Dissolution of Al and Si also tended to be higher by DC than other extractions. DC dissolved more Al than OX and PP ($P < 0.05$), and more Si than AC, PP, or CO_3 ($P < 0.005$). AC dissolved more Al than OX and PP ($P < 0.02$).

Metal dissolution differed among the soil types. Highly-weathered soils, two less-weathered soils on ultrabasic rock (Alf-4, Inc-5), and the Hawaiian Andisol (Ando) showed the highest release of Fe and, to some extent, Al by the DH and DC extractions only (Table 3), reflecting the abundance of crystalline FeOx and co-precipitated Al in these samples. Spodic-horizon and the remaining volcanic samples showed high dissolution of Fe, Al, Si by all extractions except for CO_3 . Volcanic-ash derived soils (And-tkb, myk, os1, os2) released the highest amounts of Al and Si by the OX extraction (Table 3), with Al:Si mass ratios of 2.2–4.4 that imply allophane/imogolite.

Dissolved nitrogen

In contrast to the large differences in metal dissolution, dissolved N concentrations were rather similar among extractants (Table 3). The average yield of total dissolved N (TDN) across the soils was highest in DC (1.27 mg g^{-1} , normalized to soil weight) and in PP (1.02 mg g^{-1}), followed by AC (0.99 mg g^{-1}) and CO_3 (0.95 mg g^{-1}), and lowest in OX (0.75 mg g^{-1}). These averages represent about a quarter to a third of the total soil N (Fig. 1). Significant differences (by paired *t*-test across all samples) in dissolved N concentrations were found only among the following extraction pairs: DC > AC, DC > CO_3 , DC > OX, PP > OX, and AC > OX ($P < 0.05$). DC extracted $0.3\text{--}4.0 \text{ mg-N g}^{-1}$, accounting for $31 \pm 13\%$ (mean \pm standard deviation, range: 14–59%) of soil total N. The DC extraction released up to 2–3 fold more N than the DH extraction in the spodic and volcanic soils tested (Table 3) but the overall difference between pairs of the eight samples was only marginally significant ($P = 0.07$). AC and OX dissolved $26 \pm 12\%$ (range: 8–44%) and $21 \pm 9\%$ (5–33%) of total N, respectively.

For the three extractants containing no organic carbon, the extracted C percentages were roughly equal to those of N (Fig. 2a–c), implying that N acts as a reasonable surrogate for OM. C:N ratios were thus generally similar between the dissolved fractions and bulk soil (Fig. 3). Compared to bulk soil C:N, however, PP extract of buried A-horizon Andisol (And-myk) and all three extracts of the spodic Spd-3B1 had 8–12 unit higher C:N ratio, whereas the PP and CO_3 extracts of Inc-5 had 15–16 unit lower C:N ratios. Across all samples, the PP extraction accounted

Table 3 The concentration of dissolved N, Fe, Al, and Si by the six types of extractions used in this study

Sample ID	Dissolved total N (mg g^{-1})						Dissolved Fe (mg g^{-1})						Dissolved Al (mg g^{-1})						Dissolved Si (mg g^{-1})											
	DH	DC	AC	OX	PP	CO ₃	DH	DC	AC	OX	PP	CO ₃	DH	DC	AC	OX	PP	CO ₃	DC	AC	OX	PP	CO ₃	DC	AC	OX	PP	CO ₃		
Highly weathered soils																														
Ult-4	0.62	0.76	0.69	0.54	0.69	0.70	25	25	9.9	5.3	7.3	0.28	4.9	4.7	2.4	1.9	1.3	0.16	0.18	0	0.04	0	0	0	0	0	0	0	0	
Ox-4	0.50	0.72	0.43	0.26	0.38	0.43	185	245	2.4	2.4	1.4	0.01	11	5.4	0.23	0.46	0	0	1.2	0.01	0.23	0.16	0	0	0	0	0	0	0	
Ox-2	ND	0.64	0.46	0.23	0.49	0.47	195	132	2.7	9.1	2.1	0.05	14	ND	1.0	2.1	0.58	0.02	ND	0.05	0.96	1.0	0.05	0	0	0	0	0		
Less weathered soils																														
Paddy	ND	0.29	0.31	0.19	0.17	0.21	ND	8.2	2.9	3.6	1.8	0.01	ND	1.1	0.53	1.4	0.33	0.06	0.29	0.01	0.19	0.04	0	0	0	0	0	0	0	
Inc-6	0.23	0.29	0.34	0.25	0.36	0.46	9.7	11.3	3.7	2.9	2.2	0.14	1.3	1.6	0.72	0.54	0.17	0.07	ND	0	0.02	0	0	0	0	0	0	0	0	
Alf-4	0.27	0.51	0.47	0.25	0.31	0.45	32	37	5.4	4.6	4.6	0.06	3.4	4.2	0.87	0.68	0.38	0.04	ND	0	0.08	0.01	0	0	0	0	0	0	0	
Inc-5	ND	1.7	1.4	1.0	1.1	1.5	25	25	12	12	8.3	0.22	2.8	2.4	1.7	1.7	0.93	0.12	0.31	0	0.26	0.04	0	0	0	0	0	0	0	
Inc-7	ND	1.9	0.5	1.0	1.4	2.1	1.2	1.3	1.9	0.7	0.1	0.09	0.51	0.36	1.0	0.48	0.13	0.06	0.35	0.03	0.00	0.28	0	0	0	0	0	0	0	
Podzolic soils																														
Spd-2B1	1.24	1.2	1.1	0.74	1.3	0.8	23	26	14	13	10	0.45	7.9	6.5	12	7.0	3.4	0.52	0.74	0.19	0.69	0.14	0	0	0	0	0	0	0	
Spd-3B1	0.87	2.2	1.7	1.2	1.3	1.0	32	32	18	25	18	0.32	16	16	21	15	10	1.00	2.1	0.26	0.97	0.28	0.01	0	0	0	0	0	0	
Volcanic soils																														
Ando	1.28	4.0	3.1	1.8	1.9	2.0	142	172	72	46	57	0.78	20	29	15	13	12	0.30	1.4	0.25	1.2	0.28	0	0	0	0	0	0	0	
Inc-4	0.68	1.6	1.3	0.8	1.4	1.0	40	47	14	16	12	0.10	16	19	21	15	9.0	0.54	2.1	0.44	0.90	0.52	0	0	0	0	0	0	0	0
And-kwt	ND	1.1	1.5	0.9	2.6	1.9	ND	15	8.8	9.5	8.0	3.7	ND	13	12	17	16	3.65	1.4	0.95	1.3	0.60	3.6	0	0	0	0	0	0	0
And-tkb	ND	0.85	0.57	1.3	0.9	0.4	ND	38	6.7	19	0.8	0.01	ND	14	6.0	44	3.2	0.20	1.9	1.4	20	0.16	0	0	0	0	0	0	0	0
And-myk	ND	ND	ND	1.2	2.2	0.3	ND	ND	ND	18	5.7	0	ND	ND	ND	49	11	0.15	ND	ND	22	0.14	0	0	0	0	0	0	0	0
And-os1	ND	ND	ND	2.4	4.2	1.6	ND	ND	ND	14	7.3	0.05	ND	ND	ND	41	26	0.07	ND	ND	9.2	1.4	0.07	0	0	0	0	0	0	0
And-os2	ND	ND	ND	2.2	2.6	1.0	ND	ND	ND	17	6.5	0.02	ND	ND	ND	46	19	0.11	ND	ND	12	0.41	0.02	0	0	0	0	0	0	0

ND not determined

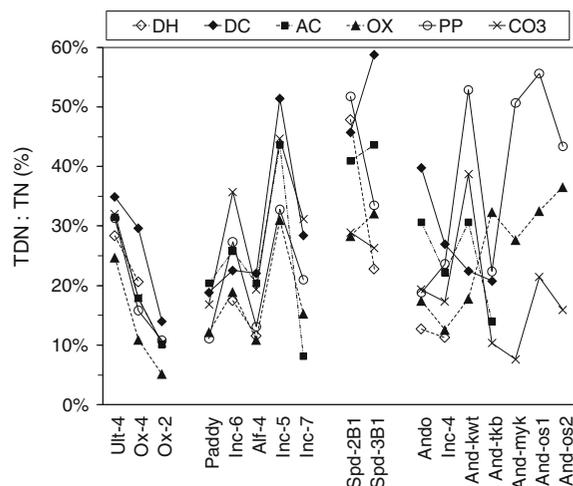


Fig. 1 Fraction of total soil N dissolved (total dissolved N:total N) by the six extraction techniques

for $30 \pm 16\%$ of total N and $33 \pm 19\%$ of total C, and were correlated with each other ($P < 0.0001$) with a mean DOC:TDN ratio of 20.8 ± 7.1 . Similarly, CO_3 dissolved $24 \pm 11\%$ of total N and $25 \pm 11\%$ of total C, respectively, and were correlated with each other ($P < 0.0001$) with a mean DOC:TDN ratio (19.4 ± 6.6) similar to the PP extracts. The OM dissolved by PP and CO_3 was similar for the highly- and less-weathered samples whereas PP tended to dissolve more OM than CO_3 from the spodic and volcanic samples (Table 3).

Co-variation of dissolved metal and nitrogen

Samples yielding more metals tended to release more N in most of the extractions (Fig. 4a–c). Some of the less-weathered samples (Paddy, Inc-6, Inc-7) showed high TDN with low metal dissolution (Table 3) presumably due to the presence of easily-soluble OM, which weakened the overall TDN-metal correlations (Fig. 4). Nevertheless, Fe and/or Al significantly correlated with TDN within each extraction (Table 4). The sum of Fe and Al was a highly significant correlate only in AC and OX extractions, highlighting the important role of poorly crystalline phases of FeOx, AlOx, and/or aluminosilicate. Only Al showed significant correlation with TDN in the DC extracts, suggesting a less important role for dithionite-soluble Fe. Ascorbate-reducible Fe showed much better correlation with TDN in the AC extract,

implying a role for ferrihydrite because (i) AC doesn't dissolve allophane/imogolite (Table 2) and (ii) organo-Fe complexes appeared to be less important than organo-Al complexes (Table 4). Dissolved OC and TDN in the PP extracts correlated well with Al, but not with Fe. Most samples showed minimal Fe and Al dissolution but significant TDN release by the CO_3 extraction (Table 3). Both spodic samples and two of the volcanic samples (Ando, And-kwt), however, released appreciable amounts of metals and N by the CO_3 extraction, which resulted in a moderate TDN-Fe correlation (Table 4). The CO_3 extractant is not, however, designed to dissolve metal oxide phases and likely dissolved a different pool of organic matter.

Discussion

Nitrogen as a proxy for organic matter

Four of the extractions used here (DC, AC, OX, and PP) are well-established techniques to dissolve subsets of Fe and Al phases in soils (Table 2). Using an organic-free version of the dithionite extraction (DH), we previously quantified the organic C co-dissolved upon reductive dissolution of FeOx to estimate the OM sorbed onto these phases (Wagai and Mayer 2007). After accounting for the C dissolved from non-FeOx phases, that study showed that only 2–37% (mean: $12.5 \pm 9.1\%$) of soil total C and mineral-associated C were sorptively stabilized by FeOx, based upon that operational definition. However, the roles of other metal phases considered to stabilize OM, such as poorly-crystalline aluminosilicates, amorphous AlOx, and organo-metal complexes (e.g., Higashi 1983; Kleber et al. 2005), remained less clear.

In this study, we used standard extraction techniques that more fully dissolve these other phases, and quantified dissolved N instead of C because most of these extractions use organic C-based complexing agents (i.e. citrate, ascorbate, and oxalate). We anticipated that N would serve as a proxy for OM because organic N accounts for most N in soils, possibly excepting soils rich in 2:1 clays, and is bonded to C in soil OM with narrow range of C:N ratio. Thus TDN likely represents another elemental marker of OM that was bound to metal phases. Inorganic N extracted by 1 M KCl accounted for $<2\%$ of total N across a wide range of soils including

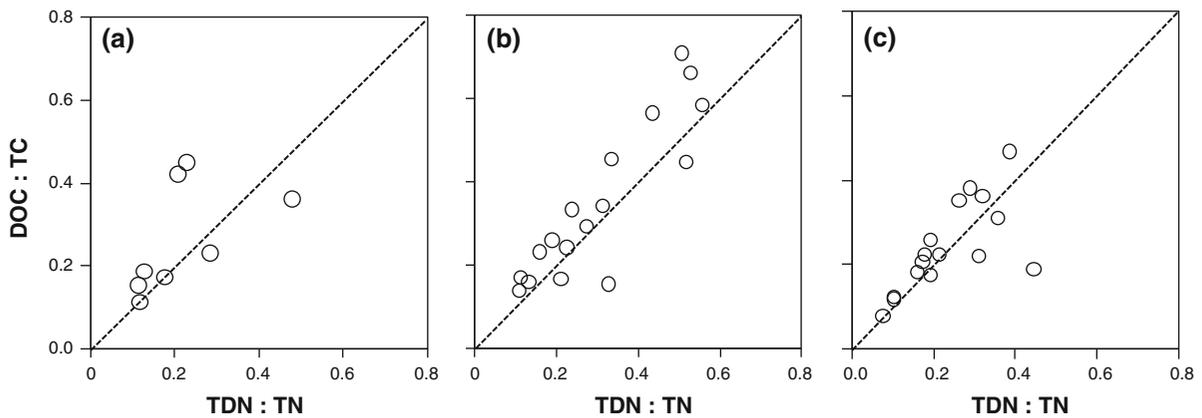


Fig. 2 Fraction of total soil organic C dissolved against the fraction of total soil N dissolved by the three organic carbon free treatments of **a** Dithionite-HCl, **b** Pyrophosphate, and **c** Bicarbonate extractions. *Diagonal lines* represent 1:1 ratio

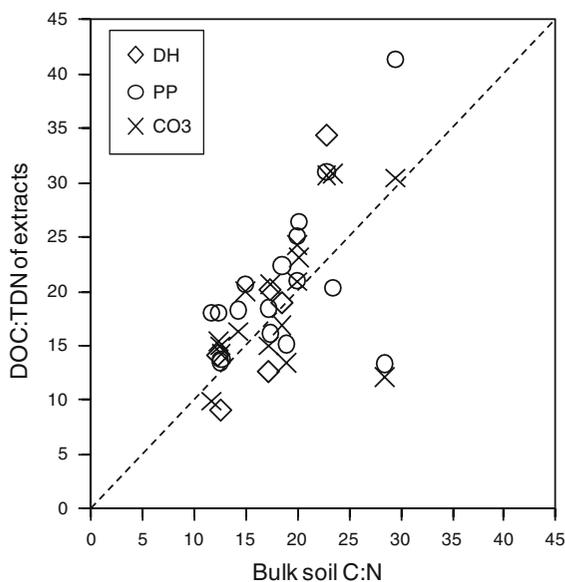


Fig. 3 Scatter plot of C-to-N ratio of bulk phase against dissolved fraction by the three extractants that contained no carbon

volcanic and agricultural soils (e.g., Sano et al. 2006; Mikutta et al. 2010). For the six forest soils used in this study (Ult-4, Ox-4, Inc-5,6,7, Alf-4), the KCl-extractable inorganic N was <1% of total N (Kitayama et al. 1998). The similar C and N extraction yields in the C-free extractions that we employed (Fig. 2a–c) substantiate this expectation. In addition, the proportional extractability of C and N (Fig. 2a–c), and the resulting similar ratios of C:N of bulk vs. dissolved phases (Fig. 3), implies that the dissolved OM

included C-rich compounds derived from decaying litter as well as the low C:N compounds typically found in high-density fractions. In fact, extractable (especially less-crystalline) phases of metals are found in both low- and high-density fractions (Spycher and Young 1979; Sollins et al. 2009; Wagai, unpublished data). Our results also agree with the lack of preferential sorption of DON relative to DOC onto FeOx, AlOx, and field soil samples (Kaiser and Zech 2000; Lilienfein et al. 2004).

Limited extractability of bulk soil OM

Despite the TOC-metal correlations reported from a range of soils (see Introduction), generally minor fractions of total OM (average $26 \pm 13\%$ and range 5–59% of total soil N) dissolved from our samples (Fig. 1). This finding of a significant though minor fraction corroborates our previous work with organic C using the DH extraction only (Wagai and Mayer 2007), with the important exception of some volcanic and spodic samples (discussed below). Similarly limited extractability of total soil C by conventional DCB reagents have been reported based on indirect estimation from the C concentration difference between pre- and post-extraction samples, albeit with the possibility of citrate contamination (Oades et al. 1987; Preston et al. 1994; Schulten and Leinweber 1995). Our direct measurements therefore corroborate the previous reports and extend the conclusion to N for the soils we studied.

The limited extractability of soil N (Fig. 1) and presumably C (Fig. 2) implies two possibilities that

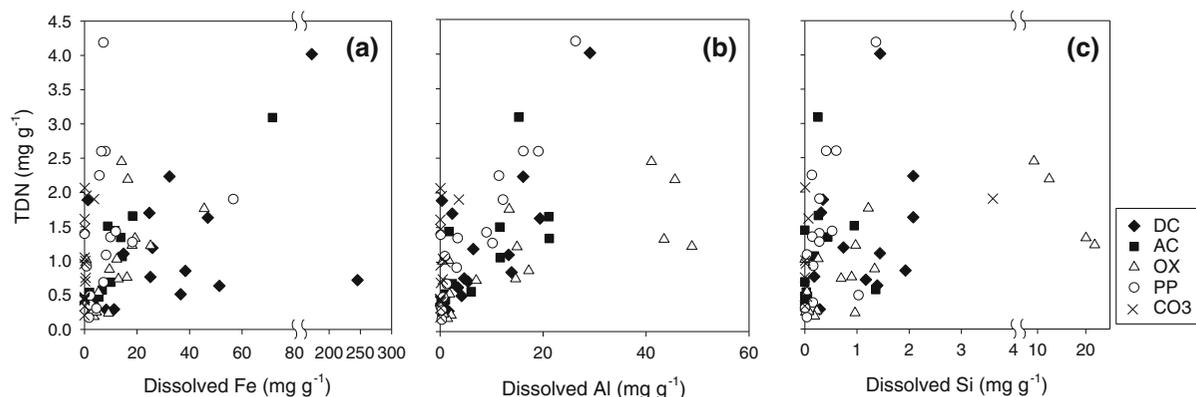


Fig. 4 Scatter plots of total dissolved N against iron (a), aluminum (b), and silica (c) released by dithionite-citrate (DC), ascorbate-citrate (AC), oxalate (OX), pyrophosphate (PP), and bicarbonate (CO_3) extractions across the studied soil samples

Table 4 Correlation coefficients between total dissolved N and Fe, Al, and Fe + Al concentrations (mol g^{-1}) by the five extractions

Extraction type	Fe	Al	Fe + Al
DC	0.32	0.74*	0.39
AC	0.91***	0.71*	0.93***
OX	0.60*	0.76**	0.81***
PP	0.23	0.94***	0.77**
CO_3	0.48*	0.41	0.44

* $P < 0.05$, ** $P < 0.005$, *** $P < 0.0001$

are not mutually exclusive. First, the OM associated with these metal phases might become insoluble against extraction—perhaps via reactions such as post-sorption aging (Kaiser et al. 2007), armoring of organo-metal complexes (Boudot et al. 1989) or microaggregation with fine clay at submicron scales (Tombácz et al. 2004; Chenu and Plante 2006). Second, the correlation of extracted N with these metal phases (Fig. 4, Table 4), suggests that metal abundance limits the pool size of metal-bound OM. Major contributions of the dissolved N to total N in spodic and some of the volcanic samples (Fig. 1), that accumulate Al and Fe ions via weathering and podzolization, highlight this notion. The pool size of older, more stable fractions of bulk C in subsurface horizons of acid soils, isolated via chemical oxidation treatments, was significantly controlled by the abundance or surface reactivity of metal oxides (Kleber et al. 2005; Mikutta et al. 2006). If the metal-bound OM extracted in our study includes very old C pools, then these extractable phases (Fig. 1) might control

the mean age (and apparent residence time) of total soil C as reported previously (Torn et al. 1997; Masiello et al. 2004).

Contributions of different metal phases to OM stabilization

Comparison among these extractions provides insights into the specific metal phases stabilizing the N. First, the role of well-crystallized FeOx can be inferred by comparing the DC and AC extractions, because dithionite has stronger reducing power than ascorbate (Reyes and Torrent 1997) while both extractions had similar pH (6–8 vs. 7–8, data not shown). Significantly higher Fe and, to a lesser extent, Al dissolution by DC in weathered tropical soils (Table 3), likely represent crystalline FeOx. DC also dissolved slightly more N than AC (but significantly, $P = 0.03$), accounting for $6 \pm 8\%$ of total N, which indicates a discernible, small contribution of well-crystallized FeOx to OM stabilization in these soils. The poorer correlation of TDN with Fe_{DC} than with Fe_{AC} (Fig. 4a, Table 4) further supports the minor importance of crystalline FeOx. Correlation studies in the literature show mixed results on the role of Fe phases—some studies reported higher correlation of soil total C with OX-soluble Fe than dithionite-soluble Fe (Johnson and Todd 1983; Percival et al. 2000) while others showed an opposite pattern (Skjemstad et al. 1989; Masiello et al. 2004). Our approach provides direct evidence that crystalline FeOx is associated with smaller amounts of OM than less-crystalline FeOx and/or organo-metal complexes, confirming experimental

results that OC:Fe ratio of the OM sorbed on crystalline FeOx is lower than that on poorly-crystalline FeOx (Tipping 1981; Kaiser et al. 1997) and organo-Fe complexes (Higashi 1983; Jansen et al. 2005). An important exception is the Kinabalu Oxisol derived from Fe-rich parent material, in which repeated DH extractions released 60–70% of mineral-associated C (Wagai and Mayer 2007). Thus, in situations where well-crystallized FeOx and AlOx dominate, sorption onto these phases can be a major stabilization mechanism (e.g., Shang and Tiessen 1998; Leinweber and Schulten 2000).

Second, the role of less-crystalline FeOx, AlOx, and aluminosilicate phases may be inferred from AC and/or OX extractions in comparison with other extractions. For instance, much higher TDN and Fe releases by AC compared to PP found in the Hawaiian Andisol sample (Ando, Table 3) suggest a contribution of poorly-crystalline FeOx to OM stability. If PP dissolves equal or greater amounts of OM from organo-metal complexes and non-FeOx-phases than AC does (due to higher pH and competitive sorption by the pyrophosphate anion), then the difference in TDN between the two extractions may be attributable to the OM sorbed to ferrihydrite because (i) AC and PP dissolve little allophane/imogolite (Table 1) and (ii) the difference in Al dissolution was small. Similarly, the dissolution patterns of an allophanic Andisol (And-tkb) indicates the importance of poorly-crystalline aluminosilicate phases (allophane/imogolite) that is soluble by OX but not by AC or PP. In this sample, OX released more Al and Si (and, to a less extent, Fe) as well as TDN compared to AC or PP extractions (Table 3). The OX extraction was done at pH 3, so that some of the OM released from these metal phases might have reprecipitated or readsorbed after dissolution. When conducting sequential extractions of OX followed by PP (pH = 10) to fully recover this OM, the OX + PP extraction yielded 43% of total N while single PP and AC released 22% and 14%, respectively, of total N. The difference of roughly 20–30% of total N may represent OM sorptively associated with allophane/imogolite in the And-tkb sample. These comparisons therefore showed detectable but generally small contributions of crystalline and less-crystalline phases of FeOx as well as allophane/imogolite to sorptively stabilize OM.

Third, the role of organo-metal complexes can be inferred from PP extractions. While interpretation of

PP extracts needs caution due to peptization of colloids and alkali-induced OM dissolution from non-metal phases, PP-soluble materials are thought to derive largely from organo-metal complexes in some soils (Higashi 1983; Fujitake et al. 1998). Even if all PP-soluble Fe derived from peptized FeOx which held maximal amounts of OM found in the literature (OC:Fe = 0.18 (wt/wt), Tipping 1981), only $7 \pm 6\%$ of the PP-soluble C in our samples can be accounted for by the peptized FeOx. The very strong correlation of TDN_{PP} with Al_{PP} but not with Fe_{PP} (Fig. 4a–b, Table 4) suggests greater importance of organo-Al complexes. Total soil C also correlated better with Al_{PP} than with Fe_{PP} in our samples ($p = 0.001$ vs. 0.06) and in many other soils (Wada and Higashi 1976; Skjemstad et al. 1989; Percival et al. 2000; Masiello et al. 2004), supporting this interpretation. PP dissolved significantly less metal but similar amounts of N compared to the DC and AC extractions (Table 3), implying that much of the DC- and AC-soluble N may be attributable to organo-metal complexes or to adsorbed OM that is exchangeable by pyrophosphate at pH 10. PP released 11–56% of total soil C and N across all samples; the spodic-horizons and some volcanic samples tended to release higher percentages (Fig. 1). The volcanic-ash samples with highest PP-soluble Al (And-kwt, And-myk, And-os1,2) showed the highest OM dissolution by PP, accounting for 43–56% of total N and 57–71% of total C (Fig. 2b). Similarly, based on solid-phase OM concentrations before and after extraction, Paul et al. (2008) showed that PP-soluble OM accounted for 20–30% of mineral-associated C and N in sedimentary soils and for 40–50% of mineral-associated C and N in volcanic-ash soils. Thus, except for the volcanic-ash samples with large amounts of Al_{PP}, simple complexation between OM and Fe or Al ions likely accounts for less than half of total OM despite significant TOC-Al_{PP} correlations across a range of soils.

Of the two high-pH extractions, PP dissolved much more Fe, Al, and Si than the CO₃ extraction and yet the N dissolution extents were similar (Table 3). We suggest that this result is due to similar alkali-induced OM dissolution but partial reprecipitation of metals at high pH in the CO₃ extraction only. Supporting this hypothesis were positive and significant TDN_{PP} vs. TDN_{CO₃} and DOC_{PP} vs. DOC_{CO₃} correlations and similar DOC:TDN ratio (19 vs. 21) between the two extractions across all samples.

Comparison of the ratio of OM to metal in the extracts to those reported from sorption experiments gives further hints on the nature of metal–OM associations. Due to large difference in atomic weight and density of Fe- and Al-bearing mineral phases, the assessment of OM-to-mineral ratios on a volume basis can be useful (Wagai and Mayer 2007). As maximum reported sorptive capacities, we calculate volumetric OM:metal ratios of 0.63 and 0.23 for allophane and imogolite, respectively (Inoue and Wada 1968), 0.64 for ferrihydrite (Tipping 1981), 0.80 for goethite (Kaiser and Guggenberger 2007), and 0.95 for amorphous gibbsite (Kaiser et al. 1997), based on the assumptions used for Fig. 5. Most of our samples showed volumetric ratios above these maximum sorptive capacities, especially those from the PP and AC extractions (Fig. 5). This approach shows that the

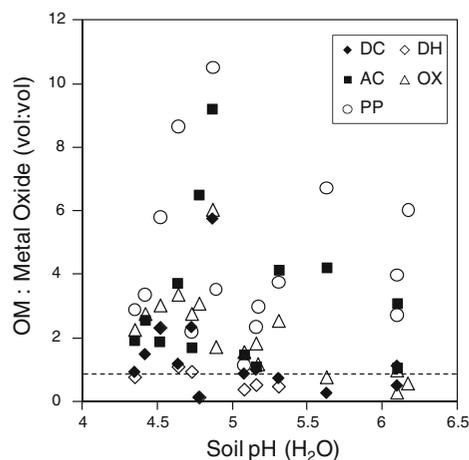


Fig. 5 Ratio of organic matter volume to volume of Fe and Al solid phases against soil pH in water. The dashed horizontal line at 0.95 is the ratio corresponding to the maximum sorptive capacity of the mineral phases considered (see main text). One sample (Inc-7) was omitted as it showed high OM dissolution with very little metal dissolution, giving extremely high OM:metal ratios (60–230). Following assumptions were made to calculate the volumetric ratios. (i) All dissolved N was present as an organic form having the same C:N ratio as mineral-associated OM with a density of 1.4 g cm^{-3} (Mayer et al. 2004). For the five samples (Japanese volcanic-ash soils) where density fraction data were lacking, we used the C:N of bulk soil. (ii) OM consists of 50% C. (iii) Dissolved Fe and Al from DC, AC, and PP originated from goethite and gibbsite with densities of 4.27 and 2.44 g cm^{-3} , respectively. (iv) The mass of allophane/imogolite ($\text{Si}_{\text{OX}} \times 7.1$) and ferrihydrite ($\text{Fe}_{\text{OX}} \times 1.4$) for OX extracts (Shoji et al. 1993) assuming that no other mineral phases dissolved. (v) Densities of allophane/imogolite and ferrihydrite are 1.9 and 3.96 g cm^{-3} , respectively

minor dissolution of total N by AC (Fig. 1), despite its strong correlation with Fe (Table 4), is mostly due to non-sorptive causes. Even for the OX extraction conducted at pH 3 (i.e. alkali-induced OM dissolution is unlikely), all samples except for one Oxisol (Ox-2) and two volcanic samples (And-tkb, -myk) showed volumetric ratios >1 . These high ratios suggest that the OM derived not only from sorbed phases but also from organo–metal complexes or even non-metal-associated OM, which can have much higher OM:metal ratios than adsorptive associations. Similarity in TDN pool sizes among the extractions (Fig. 1) further suggests that the extracted TDN consists largely of organo–metal complexes for most samples. The higher OM:metal ratios found for acidic samples (soil pH < 5) in all extractions (Fig. 5) agrees with the pattern previously found (Wagai and Mayer 2007) and is consistent with the common pedogenic process (i.e. podzolization) where lower pH promotes production of organic acids and free metals, leading to organo–metal complexation.

Conclusions

The literature pointing out the importance of Fe and Al phases for the stabilization of OM in field soils is based largely on correlations between the amounts of OM and metals released by selective extractions. Our results show that N provides a reasonable surrogate with which to explore OM–metal associations using carbon-containing selective extractants. Only rarely did these extractants dissolve more than half of the total soil OM, indicating other causes for the majority of OM stabilization in these soils. These results therefore extend our previous conclusion that was based on an inorganic dithionite extraction (Wagai and Mayer 2007). Comparison among the extractions agrees with other studies finding that poorly-crystalline Fe phases contribute more to soil OM stability than crystalline FeOx. Nevertheless, OM:metal volumetric ratios indicate that OM adsorbed on ferrihydrite accounts for fairly minor fractions for N in reductive extractions despite excellent OM:Fe correlation. Organo–metal complexes appear to be more important components in these extraction solutions, and accumulation/remobilization of soil OC and N may be more dependent on their dynamics than those of FeOx, AlOx, and poorly-crystalline aluminosilicates.

Acknowledgments We thank S. Hiradate for sharing some of the volcanic samples, R. Daimun, W. Umetsu, and M. Kajiura for lab assistance, and two anonymous reviewers for helpful comments. We also thank N. Yamaguchi and S. Hiradate for valuable discussions. Initial part of the project was supported by postdoctoral fellowship from Center for Ecological Research at Kyoto University, and conducted by the use of laboratory facilities provided by Dr. N. Majalap. This work was supported by Grant-in-Aid for Young Scientists (B21310016) of Japan Society for the Promotion of Science.

References

- Baldock JA, Skjemstad JO (2000) Role of the soil matrix and minerals in protecting natural organic materials against biological attack. *Org Geochem* 31:697–710
- Borggaard OK (1992) Dissolution of poorly crystalline iron oxides in soils by EDTA and oxalate. *Zeitschrift für Pflanzenernährung und Bodenkunde* 155:431–436
- Boudot JP, Bel Hadj Brahim A, Steiman R, Seigle-Murandi F (1989) Biodegradation of synthetic organo-metallic complexes of iron and aluminium with selected metal to carbon ratios. *Soil Bio. Biochem.* 21:961–966
- Chenu C, Plante AF (2006) Clay-sized organo-mineral complexes in a cultivation chronosequence: revisiting the concept of the ‘primary organo-mineral complex’. *Eur J Soil Sci* 57:596–607
- Christensen BT (1992) Physical fractionation of soil and organic matter in primary particle size and density separates. In: *Advances in soil science*, vol 20, Springer, New York, pp 1–89
- De Coninck F (1980) Major mechanisms in formation of spodic horizons. *Geoderma* 24:101–128
- Fang Y, Yoh M, Koba K, Zhu W, Takebayashi YU, Xiao Y, Lei C, Mo J, Zhang WEI, Lu X (2010) Nitrogen deposition and forest nitrogen cycling along an urban–rural transect in southern China. *Glob Change Biol* 17:872–885
- Farmer VC, Russell JD, Smith BFL (1983) Extraction of inorganic forms of translocated Al, Fe and Si from a podzol Bs horizon. *J Soil Sci* 34:571–576
- Fujitake N, Kusumoto A, Tsukamoto M, Kawahigashi M, Suzuki T, Otsuka H (1998) Properties of soil humic substances in fractions obtained by sequential extraction with pyrophosphate solutions at different pHs: I. yield and particle size distribution. *Soil Sci Plant Nutr* 44:253–260
- Higashi T (1983) Characterization of Al/Fe-humus complexes in dystrandepts through comparison with synthetic forms. *Geoderma* 31:277–288
- Holmgren GG (1967) A rapid citrate-dithionite extractable iron procedure. *Soil Sci Soc Am J* 47:210–211
- Huygens D, Boeckx P, Templer P, Paulino L, Van Cleemput O, Oyarzun C, Muller C, Godoy R (2008) Mechanisms for retention of bioavailable nitrogen in volcanic rainforest soils. *Nature Geo* 1:543–548
- Inoue T, Wada K (1968) Adsorption of humified clover extracts by various clays. *Trans 9th Int Congr Soil Sci* 3:289–298
- Jansen B, Nierop KGJ, Verstraten JM (2005) Mechanisms controlling the mobility of dissolved organic matter, aluminium and iron in podzol B horizons. *Eur J Soil Sci* 56:537–550
- Johnson DW, Todd DE (1983) Relationship among iron, aluminium, carbon and sulphate in a variety of forest soils. *Soil Sci Soc Am J* 47:792–800
- Kaiser K, Guggenberger G (2000) The role of DOM sorption to mineral surfaces in the preservation of organic matter in soils. *Org Geochem* 31:711–725
- Kaiser K, Guggenberger G (2007) Sorptive stabilization of organic matter by microporous goethite: sorption into small pores vs. surface complexation. *Eur J Soil Sci* 58:45–59
- Kaiser K, Zech W (1996) Defects in estimation of aluminum in humus complexes of podzolic soils by pyrophosphate extraction. *Soil Sci* 161:452–458
- Kaiser K, Zech W (2000) Sorption of dissolved organic nitrogen by acid subsoil horizons and individual mineral phases. *Eur J Soil Sci* 51:403–411
- Kaiser K, Guggenberger G, Haumaier L, Zech W (1997) Dissolved organic matter sorption on subsoil and minerals studied by ¹³C-NMR and DRIFT spectroscopy. *Eur J Soil Sci* 48:301–310
- Kaiser K, Mikutta R, Guggenberger G (2007) Increased stability of organic matter sorbed to ferrihydrite and goethite on aging. *Soil Sci Soc Am J* 71:711–719
- Kitayama K, Aiba SI, Majalap-Lee N, Ohsawa M (1998) Soil nitrogen mineralization rates of rainforests in a matrix of elevations and geological substrates on Mount Kinabalu, Borneo. *Ecol Res* 13:301–312
- Kleber M, Mikutta R, Torn MS, Jahn R (2005) Poorly crystalline mineral phases protect organic matter in acid subsoil horizons. *Eur J Soil Sci* 56:717–725
- Knicker H, Fründ R, Lüdemann HD (1993) The chemical nature of nitrogen in native soil organic matter. *Naturwissenschaften* 80:219–221
- Kögel-Knabner I (2002) The macromolecular organic composition of plant and microbial residues as inputs to soil organic matter. *Soil Biol Biochem* 34:139–162
- Leinweber P, Schulten H-R (2000) Nonhydrolyzable forms of soil organic nitrogen: extractability and composition. *J Plant Nutr Soil Sci* 163:433–439
- Lilienfein J, Qualls RG, Uselman SM, Bridgham SD (2004) Adsorption of dissolved organic carbon and nitrogen in soils of a weathering chronosequence. *Soil Sci Soc Am J* 68:292–305
- Loeppert RH, Inskeep WP (1996) Iron. In: Sparks DL (ed) *Methods of soil analysis. Part 3, chemical methods*. Soil Sci. Soc. Am. Book Series 5, Madison, WI
- Loveland PJ, Digby P (1984) The extraction of Fe and Al by 0.1 M pyrophosphate solutions: a comparison of some techniques. *J Soil Sci* 35:243–250
- Masiello CA, Chadwick OA, Southon J, Torn MS, Harden JW (2004) Weathering controls on mechanisms of carbon storage in grassland soils. *Global Biogeochem Cycles* 18:GB4023
- Mayer LM, Schick LL, Hardy KR, Wagai R, McCarthy J (2004) Organic matter in small mesopores in sediments and soils. *Geochim Cosmochim Acta* 68:3863–3872
- Mehra OP, Jackson ML (1960) Iron oxide removal from soils and clays by a dithionite-citrate system buffered with sodium bicarbonate. *Proceedings 7th Nat Conf Clays*, vol 5, pp 317–327

- Mikutta R, Kleber M, Torn MS, Jahn R (2006) Stabilization of soil organic matter: association with minerals or chemical recalcitrance? *Biogeochemistry* 77:25–56
- Mikutta R, Mikutta C, Kalbitz K, Scheel T, Kaiser K, Jahn R (2007) Biodegradation of forest floor organic matter bound to minerals via different binding mechanisms. *Geochim Cosmochim Acta* 71:2569–2590
- Mikutta R, Kaiser K, Dörr N, Vollmer A, Chadwick OA, Chorover J, Kramer MG, Guggenberger G (2010) Mineralogical impact on organic nitrogen across a long-term soil chronosequence (0.3–4100 kyr). *Geochim Cosmochim Acta* 74:2142–2164
- Nguyen RT, Harvey HR (2001) Preservation of protein in marine systems: hydrophobic and other noncovalent associations as major stabilizing forces. *Geochim Cosmochim Acta* 65:1467–1480
- Oades J, Vassallo A, Waters A, Wilson M (1987) Characterization of organic matter in particle size and density fractions from a red-brown earth by solid state ^{13}C NMR. *Aust J Soil Res* 25:71–82
- Parfitt RL, Childs CW (1988) Estimation of forms of Fe and Al: a review, and analysis of contrasting soils by dissolution and Moessbauer methods. *Aust J Soil Res* 26:121–144
- Parfitt RL, Henmi T (1982) Comparison of an oxalate-extraction method and an infrared spectroscopic method for determining allophane in soil clays. *Soil Sci Plant Nutr* 28:183–190
- Paul EA, Clarks FE (1996) *Soil microbiology and biochemistry*. Academic Press, San Diego
- Paul S, Veldkamp E, Flessa H (2008) Differential response of mineral-associated organic matter in tropical soils formed in volcanic ashes and marine Tertiary sediment to treatment with HCl, NaOCl, and $\text{Na}_4\text{P}_2\text{O}_7$. *Soil Biol Biochem* 40:1846–1855
- Perakis SS, Compton JE, Hedin LO (2005) Nitrogen retention across a gradient of ^{15}N additions to an unpolluted temperate forest soil in Chile. *Ecology* 86:96–105
- Percival HJ, Parfitt RL, Scott NA (2000) Factors controlling soil carbon levels in New Zealand grasslands is clay content important? *Soil Sci Soc Am J* 64:1623–1630
- Preston CM, Newman RH, Rother P (1994) Using ^{13}C CPMAS NMR to assess effects of cultivation on the organic matter of particle size fractions in a grassland soil. *Soil Sci* 157:26–35
- Qualls RG, Haines BL (1992) Biodegradability of dissolved organic matter in forest throughfall, soil solution, and stream water. *Soil Sci Soc Am J* 56:578–586
- Reyes I, Torrent J (1997) Citrate-ascorbate as a highly selective extractant for poorly crystalline iron oxides. *Soil Sci Soc Am J* 61:1647–1654
- Sano S, Yanai J, Kosaki T (2006) Relationships between labile organic matter and nitrogen mineralization in Japanese agricultural soils with reference to land use and soil type. *Soil Sci Plant Nutr* 52:49–60
- Schneider MPW, Scheel T, Mikutta R, van Hees P, Kaiser K, Kalbitz K (2010) Sorptive stabilization of organic matter by amorphous Al hydroxide. *Geochim Cosmochim Acta* 74:1606–1619
- Schulten H-R, Leinweber P (1995) Dithionite-citrate-bicarbonate-extractable organic matter in particle-size fractions of a Haplaquoll. *Soil Sci Soc Am J* 59:1019–1027
- Schuppli PA, Ross GJ, McKeague JA (1983) The effective removal of suspended materials from pyrophosphate extracts of soils from tropical and temperate regions. *Soil Sci Soc Am J* 47:1026–1032
- Shang C, Tiessen H (1998) Organic matter stabilization in two semiarid tropical soils: size, density, and magnetic separations. *Soil Sci Soc Am J* 62:1247–1257
- Shoji S, Nanzyo M, Dahlgren RA (1993) Volcanic ash soils—Genesis, properties and utilization. *Developments in Soil Science* 21, Elsevier, Amsterdam
- Skjemstad JO, Bushby HVA, Hansen RW (1989) Extractable Fe in the surface horizons of a range of soils from Queensland. *Aust J Soil Res* 28:259–266
- Smernik RJ, Baldock JA (2005) Does solid-state ^{15}N NMR spectroscopy detect all soil organic nitrogen? *Biogeochemistry* 75:507–528
- Sollins P, Homann P, Caldwell BA (1996) Stabilization and destabilization of soil organic matter: mechanisms and controls. *Geoderma* 74:65–105
- Sollins P, Kramer M, Swanston C, Lajtha K, Filley T, Aufdenkampe A, Wagai R, Bowden R (2009) Sequential density fractionation across soils of contrasting mineralogy: evidence for both microbial- and mineral-controlled soil organic matter stabilization. *Biogeochemistry* 96:209–231
- Spycher G, Young JL (1979) Water-dispersible soil organic-mineral particles: II. Inorganic amorphous and crystalline phases in density fractions of clay-size particles. *Soil Sci Soc Am J* 43:328–332
- Tamm O (1922) Eine Methode Zur Bestimmung der anorganischen Komponente des Gelkomplexes im Boden. *Meddelanden fran Statens skogsforsoksanstalt Stockholm* 19:387–404
- Tiessen H, Stewart JWB, Cole CV (1984) Pathways of phosphorous transformations in soils of differing pedogenesis. *Soil Sci Soc Am J* 48:853–858
- Tippling E (1981) The adsorption of aquatic humic substances by iron oxides. *Geochim Cosmochim Acta* 45:191–199
- Tombácz E, Libor Z, Illés E, Majzik A, Klumpp E (2004) The role of reactive surface sites and complexation by humic acids in the interaction of clay mineral and iron oxide particles. *Org Geochem* 35:257–267
- Torn MS, Trumbore SE, Chadwick OA, Vitousek PM, Hendricks DM (1997) Mineral control of soil organic carbon storage and turnover. *Nature* 389:170–173
- Vitousek PM, Howarth RW (1991) Nitrogen limitation on land and in the sea: how can it occur? *Biogeochemistry* 13:87–115
- von Lütow M, Kögel-Knabner I, Ekschmitt K, Flessa H, Guggenberger G, Matzner E, Marschner B (2007) SOM fractionation methods: relevance to functional pools and to stabilization mechanisms. *Soil Biol Biochem* 39:2183–2207
- Wada K, Higashi T (1976) The categories of aluminium- and iron-humus complexes in Ando soils determined by selective dissolution. *J Soil Sci* 27:357–368
- Wagai R, Mayer LM (2007) Sorptive stabilization of organic matter in soils by hydrous iron oxides. *Geochim Cosmochim Acta* 71:25–35
- Wagai R, Mayer LM, Kitayama K, Knicker H (2008) Climate and parent material controls on organic matter storage in

- surface soils: A three-pool, density-separation approach. *Geoderma* 147:23–33
- Wagai R, Mayer LM, Kitayama K (2009) Nature of the “occluded” low-density fraction in soil organic matter studies: a critical review. *Soil Sci Plant Nutr* 55:13–25
- Zang X, van Heemst JDH, Dria KJ, Hatcher PG (2000) Encapsulation of protein in humic acid from a histosol as an explanation for the occurrence of organic nitrogen in soil and sediment. *Org Geochem* 31:679–695