

ORIGINAL ARTICLE

Humus composition and the structural characteristics of humic substances in soils under different land uses in Leyte, Philippines

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Abstract

The aim of the present study was to describe the effects of land use on humus composition and the structural properties of humic substances (HS) in degraded soil in Leyte, Philippines. Five adjacent land-use types, primary forest (PF), mahogany plantation (MP), reforestation farming (RF), coffee plantation (CP) and grassland (GR), with comparable geology, parent material, soil type and climate were sampled and examined. The contents of humic acids (HAs), fulvic acids (FAs), polyvinylpyrrolidone (PVP)-adsorbed FAs and PVP-non-adsorbed FAs in the soil, and the elementary composition, infrared (IR) spectra and molecular size distribution of HAs and PVP-adsorbed FAs were compared among the different land uses. Results revealed that conversion of PF into other land uses has led to a decrease in FAs, which were higher under intensive cultivation conditions, such as RF, CP and GR. The amounts of HAs were small and also affected by land use. The amount of PVP-non-adsorbed FAs was more affected by land use than the amount of PVP-adsorbed FAs. Variation in the molecular size of HAs and PVP-adsorbed FAs provided evidence of variations in the structural characteristics of HS as a result of land-use change, although there were no appreciable differences in IR spectra and elementary composition. The relationship between land use and the degree of humification of HAs was poor. The HAs were still in the early stage of humification as indicated by the Type Rp in most soils, the higher contents of elemental H and N, and the high H/C and O/C ratios.

Key words: humification, humus composition, infrared spectra, land use.

INTRODUCTION

Like most tropical South-East Asian countries, land-use change is an important ecological driver in Philippine landscapes and is one of the major causes of the widespread occurrence of degraded land in this humid tropical country. Land use and management are of interest because of their influence on the terrestrial carbon cycle, biodiversity and soil fertility. The latter is a crucial factor in degraded soils, which rely on the cycling of nutrients from soil organic matter (SOM). The National Action Plan (NAP) of the Philippines for 2004–2010 identified soil degradation as a major threat to food security (National Action Plan 2004) and, because of this, control

of soil degradation is one of the major research priorities for the Philippines.

Management of SOM to support sustainable agriculture, particularly in degraded land, has been proposed as a means to control soil degradation in the Philippines. Appropriate management of SOM, however, requires a thorough understanding of the dynamics and changes in SOM composition under different agricultural practices. There have been very few studies examining the effect of land use on humus composition in the Philippines (e.g. Navarrete and Tsutsuki 2008; Ohta 1990). Ohta (1990) reported that conversion of forest into grassland (GR) has resulted in a considerable decrease in soil organic C, humic acids (HAs) and fulvic acids (FAs). Navarrete and Tsutsuki (2008) reported that conversion of secondary forest to other land uses has led to a decrease in soil C and N contents. They reported that land use affected not only the content, but also the composition of neutral sugars. Following a change from primary forest (PF) to other land uses in Indonesia, Watanabe *et al.* (2001) reported that

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the proportion of HAs in the organic matter of the topsoil decreased, whereas that of FAs increased. They reported no clear relationship between land use and the degree of humification of HAs. Despite a clear trend in the influence of land-use change on the content and compositions of humus, additional studies are needed, particularly in degraded soils, to add certainties to the assessment of sustainable land management in the region. In the present study, humus composition and the structural properties of humic substances (HS) in soils under different land uses were evaluated using chemical and spectroscopical techniques.

Humic substances represent a major component of SOM and account for a large pool of recalcitrant C in the terrestrial environment. They are defined by classical extraction and separation procedures based on their solubility in alkali and acidic solutions. Humic substances are grouped into three fractions: HAs, FAs and humin (Swift 1996), and each fraction makes a specific contribution towards soil fertility depending on changes in humus composition and chemical structure (Watanabe *et al.* 2001). Consequently, changes in humus composition and in the structural features of HS induced by land use may serve as a guide to establishing SOM management, which is the core concept of sustainable agriculture, particularly in degraded soil. In the present study, soil samples were collected from four cultivated sites and were compared with an undisturbed site. It was anticipated that changes in humus composition would be more pronounced in sites that had been under intensive cultivation compared with a less-disturbed site.

MATERIALS AND METHODS

Site description

The research sites were located on a 5–15% slope at an altitude of 73–112 m a.s.l. inside the Mt Pangasugan Rain Forest Reserve, 8 km north of Baybay, Leyte, Philippines (10°46'N, 124°50'E). The present climate of Leyte falls within the Köppen's Am type (tropical rainforest climate, monsoon type), with an average annual temperature of 28°C and an average annual rainfall of 3000 mm recorded from 1991 to 2007 (Navarrete *et al.* 2009). The dry and wet seasons occur between March and May and between September and January, respectively. Each month has more than 100 mm rainfall. The soils in the area are classified as Typic Hapludult (Soil Taxonomy) or Haplic Acrisol (WRB System) derived from late Quaternary basaltic volcanic rocks (Asio 1996; Navarrete *et al.* 2009). The soils are generally acidic (pH H₂O: 5.0–5.6) and have a clay loam to heavy clay soil texture. The clay mineralogy is dominated by halloysite and kaolinite with minor amounts of goethite and hematite, and has a gener-

ally high dithionite-extractable Fe content (Asio 1996; Navarrete *et al.* 2009).

Brief land-use history

The original vegetation of the area is derived from dipterocarp rainforest, although the lower part of the rainforest was deforested as early as the 1950s as revealed by aerial photographs, vegetation maps and interviews with local farmers (Navarrete and Tsutsuki 2008). Typical land-use types in the area include; mahogany plantation (MP), reforestation farming (RF), coffee plantation (CP) and grassland (GR) (Navarrete and Tsutsuki 2008; Navarrete *et al.* 2009). Prior to the establishment of MP in the 1980s, the site had been cultivated with root crops since the late 1950s. The RF site was densely planted with various native tree species after a long history of intensive and continuous cultivation. *Imperata cylindrica* grass dominated before reforestation in 1992, which suggests the degraded nature of the area. The CP site was established in the 1990s after many years of rotation farming of annual crops (sweet potato, corn and taro). The GR site had been cultivated for root crops until the 1980s, and then abandoned and left for grassland.

Soil sampling

The sampling methodology used in the present study was described in detail in Navarrete and Tsutsuki (2008). In brief, four adjacent land-use types, MP, RF, CP and GR, were selected in the lower part of the rainforest reserve. A PF that corresponds to secondary forest in Navarrete and Tsutsuki (2008) was also selected as a reference site. The selected land-use types have comparable geology, parent material, soil type and climate (Navarrete and Tsutsuki 2008; Navarrete *et al.* 2009). At each site, 10–15 subsampling areas were randomly selected and soil samples were collected from depths of 0 to 20, 20–40 and 40–60 cm using a soil auger. The subsamples were mixed to make a composite sample, freed of large debris and air-dried. A 0–20 cm depth increment was used to achieve higher resolution in the changes in soil properties among the study sites (Navarrete and Tsutsuki 2008).

Extraction and purification of humic and fulvic acids

The extractions of HAs and FAs were carried out according to the NAGOYA method (Kuwatsuka *et al.* 1992) except for the duration of the period of extraction. In brief, samples were extracted with 0.1 mol L⁻¹ NaOH (300 mL g⁻¹ soil C) under continuous shaking for 24 h at room temperature in a N₂ atmosphere. The extract was recovered by centrifugation (13,000 g for 15 min) after the addition of 30 g L⁻¹ Na₂SO₄ as coagulant, and the supernatant was filtered using filter paper (No. 6;

ADVANTEC, Tokyo, Japan). The residual soil was washed with 50 mL of 0.1 mol NaOH containing $30 \text{ g L}^{-1} \text{ Na}_2\text{SO}_4$, shaken for 2 h and then was centrifuged at $13,000 \text{ g}$ for 15 min. The extract and washing were combined and separated into HAs (precipitate) and FAs (supernatant) by acidifying to pH 1.0 using H_2SO_4 . The precipitated HAs were dissolved in 0.1 mol NaOH, re-precipitated by adding 1.5 mol H_2SO_4 and were centrifuged at $13,000 \text{ g}$ for 15 min. This procedure was repeated until the supernatant became colorless. The supernatant was combined with the FA fraction.

The FA fractions were fractionated by means of a column packed with insoluble cross-linked polyvinylpyrrolidone ([PVP] Polyclar VT; ISP, Wayne, NJ, USA) into PVP-adsorbed and PVP-non-adsorbed fractions (Watanabe and Kuwatsuka 1991, 1992). The PVP-non-adsorbed fractions were discarded after the concentrations of organic C were determined, whereas the PVP-adsorbed fractions were purified by passing through a cation exchange resin (Dowex HCR-W2; Sigma-Aldrich, St Louis, MO, USA) to transform them into the H^+ form; the fractions were then freeze-dried and kept for further analysis.

Larger amounts of HAs for further analysis were obtained separately from 100 g soil samples using the procedure described above except for the use of HCl instead of H_2SO_4 during acidification of the extract. The HA precipitates were shaken three times with a 10-fold volume of 0.1 mol HCl : 0.3 mol HF (1:1) for 24 h at room temperature to remove impurities. This procedure was repeated six more times because the ash contents of the HAs were still high. Then the HAs were dialyzed in seamless cellulose tubing (Viskase Companies, Darien, IL, USA) against distilled water until free of chloride in an AgNO_3 test, passed through a column of cation exchange resin (Dowex HCR-W2; Sigma-Aldrich) to transform them into the H^+ form and freeze-dried.

Determination of humic acid and fulvic acid carbon

The concentration of organic C in HAs, FAs (the FA fraction before PVP treatment), PVP-adsorbed FA and PVP-non-adsorbed FA in solutions were determined by a colorimetric method using a potassium dichromate-sulfuric acid solution as the reagent (Tatsukawa 1966).

Expression of the degree of humification

The absorbances at 400 and 600 nm of the HA solutions were measured using a spectrophotometer (UV-2200; Shimadzu, Kyoto, Japan). In the present study, A_{600}/C , the absorbance at 600 nm per mg C mL^{-1} in 0.1 mol NaOH, and $\log(A_{400}/A_{600})$, the ratio of absorbance at 400 nm to that at 600 nm on the logarithmic scale, were used to

express the degree of humification of the HAs (Ikeya and Watanabe 2003). Higher A_{600}/C and/or lower $\log(A_{400}/A_{600})$ values indicate a higher degree of humification (Ikeya and Watanabe 2003). The HAs were grouped into four types: A, B, P and Rp, where Type A HAs are the most humified and Type Rp HAs are the least humified (Kumada 1987). Pg, the green fraction in HAs, has absorption maxima at 615, 570 and 450 nm (Sato 1974). The presence of Pg was judged from the shape of the absorption spectra at these wavelengths and expressed using the symbols +++, ++ and + according to Kumada (1987).

Elementary composition and infrared spectra

After the HAs and PVP-adsorbed FAs were dried under vacuum over P_2O_5 , their C, H, N, O and S contents were determined using a CHNS analyzer (Micro coder JM10; J-Science Laboratory Company, Kyoto, Japan). The ash content was determined by combustion at 950°C and the O content was calculated by subtraction of C, H, N, S and the ash contents from the total weight of the sample.

The IR spectra of HAs and PVP-adsorbed FAs were measured after mixing 1 mg samples with 200 mg KBr and drying sufficiently under vacuum over P_2O_5 at room temperature. The IR spectra were recorded from 400 to $4,000 \text{ cm}^{-1}$ on a spectrometer FT/IR JASCO-420 (JASCO, Tokyo, Japan).

Molecular size distribution

The molecular size distributions of HAs and PVP-adsorbed FAs were determined by size exclusion chromatography using borate buffer (pH 9.2, ionic strength: 0.1) as the eluent (Tsutsuki and Kuwatsuka 1984). Three glass columns (internal diameter 1.5 cm \times length 20 cm; Bio-Rad Laboratories, Hercules, CA, USA) were packed separately with three grades of Toyopearl (Tosoh Corporation, Tokyo, Japan), HW-55F, HW-50F and HW-40F, with pore size diameters of 50, 12.5 and 5 nm, respectively. Approximately 5 mg of HAs or PVP-adsorbed FAs was dissolved in 3 mL of $0.0333 \text{ mol L}^{-1} \text{ Li}_2\text{B}_4\text{O}_7$ buffer and the solutions were passed through a membrane filter (pore size $0.45 \mu\text{m}$; ADVANTEC). Then, a 1 mL portion of the sample solution was eluted at a flow rate of 0.9 mL min^{-1} through the three columns, which were connected in cascade in the following order: HW-55F, HW-50F and HW-40F. No adsorptions of HAs and PVP-adsorbed FAs on the Toyopearl were observed. Elution curves were recorded by monitoring the absorbance at 254 nm. The void volume (V_0 : 39.5 mL) and void volume plus inner volume ($V_0 + V_i$: 163.6 mL) were determined using Blue Dextran 2000 (Pharmacia Fine Chemicals, Uppsala, Sweden) and tryptophan, respectively. The columns were calibrated with proteins of various molecular weights

(Sigma-Aldrich). The weight average molecular weight (M_w) was calculated using the equation: $M_w = \sum(A_i M_i) / \sum A_i$, where A_i and M_i are the absorbance at 254 nm in arbitrary units and the molecular weight estimated from the standard calibration curve, respectively, at elution volume i . The number average molecular weight (M_n) was calculated using the equation: $M_n = \sum A_i / \sum (A_i / M_i)$. Polydispersity (d) was calculated as: $d = M_w / M_n$.

RESULTS

Amounts of humic and fulvic acids and the degree of humification

The amounts of HAs (on a C basis) were small (<1 g C kg⁻¹), regardless of the land-use type, whereas the amounts of FAs (FA fraction before PVP treatment) were 4–21-fold larger than those of HAs (Table 1). The amounts of FAs increased in the following order: PF > CP > MP = RF > GR ($P < 0.05$). The amounts of HAs and FAs were slightly larger in the surface soil (0–20 cm) compared with the subsoil. The HA/FA ratio had a narrow range, although it tended to be larger in the surface soil than in the subsoil under all land-use types.

The $\log(A_{400}/A_{600})$ values of the HAs ranged widely from 0.54 to 1.00 and were slightly higher at the MP and RF sites than at the PF, GR and CP sites (Table 1). A slight variation in $\log(A_{400}/A_{600})$ values was detected with depth, although it appears that $\log(A_{400}/A_{600})$ values in the subsoil HAs were slightly higher compared with the surface soil HAs at the MP and RF sites ($P < 0.05$). The A_{600}/C values of HAs were slightly lower ($P < 0.05$) at

the MP and RF sites than at the PF, GR and CP sites. However, the A_{600}/C value at the CP site increased with depth owing to the relatively higher content of Pg. The HAs with higher $\log(A_{400}/A_{600})$ values showed lower A_{600}/C values, suggesting lower degrees of humification (Ikeya and Watanabe 2003; Kumada 1987). According to the Kumada classification, most HAs belonged to Type Rp, and the HAs in the lower layers in the CP site were Type P₊₊₊. The presence of Pg in the HAs in the soils under all land-use types was revealed by their ultraviolet-visible spectra (data not shown), and the intensity of Pg was more appreciable in the lower layer soils at the CP site than at the other land-use sites.

Recovery of carbon in PVP-adsorbed and PVP non-adsorbed fulvic acids

As FAs were the dominant humus fraction in the soils studied, they were fractionated into PVP-adsorbed and PVP-non-adsorbed fractions. Regardless of the land use, the amount of C recovered in the PVP-non-adsorbed FAs was larger (0.55–3.79 g C kg⁻¹) than that recovered in the PVP-adsorbed FAs (0.39–1.24 g C kg⁻¹) (Fig. 1). The amount of C recovered in the PVP-adsorbed FAs was slightly larger ($P < 0.01$) at the PF and CP sites than at the RF, GR and MP sites. The amount of C recovered in the PVP-non-adsorbed FAs was relatively greater at the PF site (2.01–3.79 g C kg⁻¹) compared with the MP (1.49–2.29 g C kg⁻¹), CP (0.68–1.26 g C kg⁻¹), RF (0.55–1.08 g C kg⁻¹) and GR (0.72–0.81 g C kg⁻¹) sites.

Elemental composition

In general, there were slight differences in the elementary compositions of HAs and PVP-adsorbed FAs among the

Table 1 Amounts of humic and fulvic acids and the spectrophotometric properties of humic acids under different land uses

Site	Depth (cm)	C _{org}			HA/FA	Degree of humification		Type of HA
		HA	FA			$\log(A_{400}/A_{600})$	A_{600}/C	
Primary forest (PF)	0–20	23.5	0.97	6.24	0.16	0.79	1.70	Rp ₊
	20–40	13.5	0.20	3.33	0.06	0.82	1.03	Rp ₊
	40–60	12.4	0.10	1.78	0.05	0.81	0.29	Rp ₊
Mahogany plantation (MP)	0–20	22.1	0.98	4.82	0.20	0.94	1.00	Rp
	20–40	11.6	0.62	2.63	0.24	1.00	0.95	Rp
	40–60	9.0	0.26	2.07	0.12	0.97	0.87	Rp
Rainforestation farming (RF)	0–20	16.3	0.90	4.66	0.19	0.80	1.88	Rp ₊₊
	20–40	10.9	0.38	3.10	0.12	0.77	1.88	Rp ₊₊
	40–60	8.9	0.21	2.40	0.09	0.87	1.13	Rp ₊₊
Coffee plantation (CP)	0–20	21.5	0.92	5.58	0.16	0.77	2.26	Rp ₊
	20–40	16.8	0.21	4.60	0.05	0.58	2.17	P ₊₊₊
	40–60	15.0	0.14	2.73	0.05	0.54	2.20	P ₊₊₊
Grassland (GR)	0–20	13.5	0.55	3.41	0.16	0.87	1.23	Rp ₊
	20–40	11.8	0.26	3.28	0.08	0.79	1.08	Rp ₊₊
	40–60	11.9	0.14	3.00	0.05	0.80	0.84	Rp ₊₊

C_{org}, organic C; FAs, fulvic acids; HAs, humic acids; $\log(A_{400}/A_{600})$, the ratio of absorbance at 400 nm to that at 600 nm on the logarithmic scale; A_{600}/C , the absorbance at 600 nm per mg C mL⁻¹ in 0.1 mol NaOH.

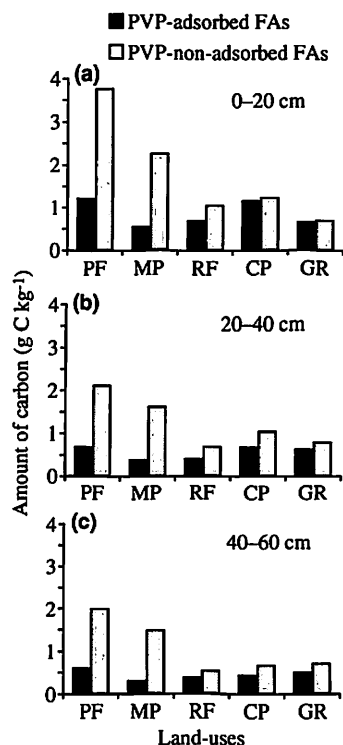


Figure 1 Amounts of C recovered in the polyvinylpyrrolidone (PVP)-adsorbed and PVP-non-adsorbed fulvic acid (FA) fractions in soils under different land uses. (a) 0–20 cm soil, (b) 20–40 cm soil and (c) 40–60 cm soil. PF, primary forest; MP, mahogany plantation; RF, rainforestation farming; CP, coffee plantation; GR, grassland.

different land-use types (Table 2). The H, N and S contents were higher in HAs than in PVP-adsorbed FAs ($P < 0.05$), whereas the O content was higher in PVP-adsorbed FAs than in the HAs ($P < 0.05$). These results are in agreement with previously reported data (e.g.

Watanabe and Kuwatsuka 1992). The C and H contents in the HAs and PVP-adsorbed FAs were slightly higher at the MP site compared with the other land uses, whereas the N content in the HAs was slightly higher at the PF and RF sites (6.3%) compared with the MP and CP (5.7%) and GR sites (5.1%); Kuwatsuka *et al.* (1978) reported that N is enriched into the HA molecules at the early stage of humification, 80–90% of which is peptide/amide N according to Abe *et al.* (2007). Abe and Watanabe (2004) suggested that the amount of peptide/amide N decreases, whereas the amount of aromatic N is maintained with decreasing N content in HAs during the progression of humification. The O contents in the HAs were slightly higher at the RF and GR sites under intensive cultivation compared with the other land uses. The ash contents of the HAs were high, despite six cycles of HF : HCl treatments.

The H/C ratio (Table 2) was greater in the HAs than in the PVP-adsorbed FAs ($P < 0.05$), whereas the O/C, O/H and C/N ratios were greater in the PVP-adsorbed FAs than in the HAs ($P < 0.05$). The H/C (1.45–1.54) and O/C (0.61–0.72) ratios observed for the HAs in the present study were greater than the reported values for Type Rp HAs (H/C, 1.07–1.37; O/C, 0.47–0.49) by Kuwatsuka *et al.* (1978).

Infrared spectra

The IR spectra of the HAs (Fig. 2a) and PVP-adsorbed FAs (Fig. 2b) showed similar patterns, with a broad peak centered at 3300–3400 cm^{-1} , which was assigned to the O–H stretching of various hydroxylated functional groups. A peak at 2964 cm^{-1} was associated with the asymmetric and symmetric stretching of aliphatic C–H (CH_2 and CH_3) bands. The main difference in the IR spectra between the HAs and PVP-adsorbed FAs was a peak

Table 2 Elemental composition of the humic acids and polyvinylpyrrolidone-adsorbed fulvic acids in the topsoil (0–20 cm) under different land uses

Site	C	H	N	S	O	Ash (%)	H/C	O/C	O/H	C/N
	(weight %) [†]						atomic ratios			
Humic acids										
Primary forest (PF)	45.6	5.9	6.3	2.2	40.0	6.62	1.54	0.66	0.43	8.46
Mahogany plantation (MP)	48.2	6.1	5.7	1.0	39.0	3.59	1.50	0.61	0.41	9.87
Rainforestation farming (RF)	44.1	5.7	6.3	1.6	42.2	5.30	1.54	0.72	0.46	8.18
Coffee plantation (CP)	46.5	5.7	5.7	1.3	40.9	4.10	1.45	0.66	0.46	9.59
Grassland (GR)	46.1	5.7	5.1	1.3	41.7	3.18	1.47	0.68	0.46	10.47
PVP-adsorbed fulvic acids										
Primary forest (PF)	45.8	4.3	2.4	1.1	46.4	2.50	1.13	0.76	0.67	22.44
Mahogany plantation (MP)	49.9	4.9	3.5	0.7	40.9	2.35	1.19	0.61	0.52	16.22
Rainforestation farming (RF)	44.7	4.3	2.3	0.4	48.4	1.89	1.14	0.81	0.71	23.03
Coffee plantation (CP)	44.5	4.3	2.0	1.1	48.1	2.08	1.16	0.81	0.70	26.08
Grassland (GR)	46.0	3.9	1.3	0.3	48.5	1.45	1.02	0.79	0.77	40.04

[†]Moisture and ash-free basis. PVP, polyvinylpyrrolidone.

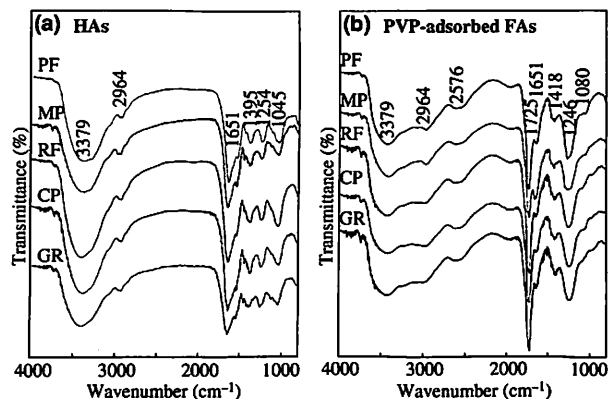


Figure 2 Infrared spectra of (a) humic acids (HAs) and (b) polyvinylpyrrolidone (PVP)-adsorbed fulvic acids (FAs) in soils under different land uses. PF, primary forest; MP, mahogany plantation; RF, rainforestation farming; CP, coffee plantation; GR, grassland.

at 1720 cm^{-1} that resulted from C=O stretching of COOH, which was stronger in the PVP-adsorbed FAs than in the HAs. Corresponding to this difference, a peak at 2576 cm^{-1} resulting from O–H stretching from strongly H-bonded COOH (Piccolo *et al.* 1992) was observed only in the spectra of the PVP-adsorbed FAs. A peak centered at 1651 cm^{-1} resulting from the C=O stretching of amide groups (amide I band), and C=O and/or C=O of H-bonded conjugated ketones was very strong and sharp in the IR spectra of the HAs, but weak in spectra of PVP-adsorbed FAs. A small peak resulting from O–H deformation and C–O stretching of phenol at 1395 cm^{-1} was also clearer in the IR spectra of the HAs. A peak at 1254 or 1246 cm^{-1} was assigned to C–O stretching and O–H bending of COOH groups, whereas a peak at 1045 cm^{-1} or a shoulder at 1080 cm^{-1} was assigned to the C–O stretching of polysaccharide (Stevenson 1994). The IR spectra of the HAs and PVP-adsorbed FAs were similar regardless of land use, and this result is in agreement with previous reports (González Pérez *et al.* 2004; Olk *et al.* 1999).

Molecular size distribution

The molecular size distributions of HAs and PVP-adsorbed FAs extracted from the surface soil (0–20 cm) are shown in Fig. 3a,b, respectively. The elution curves of the HAs showed two peaks at 39.5 mL, corresponding to V_0 , and around 52 mL, whereas the curves of the PVP-adsorbed FAs showed a broad peak between 50 and 60 mL. The molecular size distributions of HAs and PVP-adsorbed FAs varied only slightly with land use.

In the present study, M_w values should be regarded as an index for comparing the average size of HAs and PVP-adsorbed FAs measured under similar conditions because the presence of elution at V_0 made precise determination

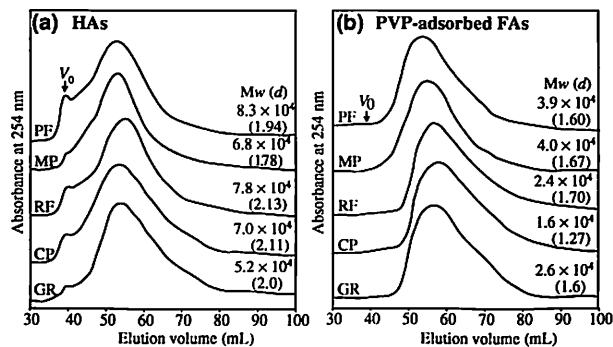


Figure 3 Elution curves of humic acids (HAs) and (b) polyvinylpyrrolidone (PVP)-adsorbed fulvic acids (FAs) in soils under different land uses. The void volume (V_0) and void volume + inner volume ($V_0 + V_i$) were 39.5 and 163.6 mL, respectively. M_w and d refer to the weight average molecular weight and polydispersity, respectively. PF, primary forest; MP, mahogany plantation; RF, rainforestation farming; CP, coffee plantation; GR, grassland.

of M_w difficult. There were marked differences in the M_w values of HAs between the PF site and the MP, CP, RF and GR sites (Fig. 3a).

DISCUSSION

Conversion of PF to other land uses has led to a decrease in FAs (Table 1), which was more appreciable at the RF and GR sites under intensive cultivation (Navarrete and Tsutsuki 2008). In contrast, the HA contents in the soils were small even in the PF. Ohta (1990) observed higher contents of HAs and FAs ($0.86\text{--}18.7\text{ g C kg}^{-1}$ and $6.40\text{--}18.7\text{ g C kg}^{-1}$, respectively) in soils under natural forest and GR in central Luzon, Philippines. The organic C content in their soils was larger than that in the Leyte soils used in the present study, although the soil types were the same. A small soil C content may be related to the small proportion of HA C in the total soil C in our soil. Despite the small content of HAs, the results of the present study revealed that land use had influenced the humus content of the soils.

In the present study, the dominant HA type was Type Rp (Table 1). The relative content of Pg in the HAs was larger under intensive cultivation, and the subsoil HAs at the CP site were Type P₊₊₊. The relative content of Pg was greater in the HAs under intensive cultivation. Watanabe *et al.* (2001) reported that the relative strength of Pg absorption was greater in agricultural field soil HAs than in PF soil HAs in Indonesia. Their results and our results suggest that Pg is more resistant to the effect of land use than other HA components in the soil. The results of the present study revealed no clear relationship between land use and degree of humification (A_{600}/C and $\log(A_{400}/A_{600})$) of HAs (Table 1). Watanabe *et al.* (2001) also found a similar trend for Indonesian soils.

The contents of the PVP-non-adsorbed FAs in the Leyte soils were greater than those of the PVP-adsorbed FAs on a C basis (Fig. 1). The contents of the PVP-non-adsorbed FAs in the RF, CP and GR soils were smaller than those in the PF and MP soils. As the major components of the PVP-non-adsorbed FAs are polysaccharides and proteinaceous materials (Watanabe and Kuwatsuka 1992), they are expected to be the most decomposable fraction among the humus fractions. Our results were in contrast to the results reported by Watanabe *et al.* (2001) that showed faster loss of HS rather than the non-HS in the FA fraction in Indonesian soils.

The M_w of the HAs and PVP-adsorbed FAs may have decreased after the conversion of PF to MP, RF, CP or GR. The difference in the molecular size of HAs among land-use types provided evidence of variations in the structural characteristics of HS resulting from land use. However, the IR spectra of the HAs and PVP-adsorbed FAs (Fig. 3a,b) did not show structural changes in the functional groups with land use. Olk *et al.* (1999) also found that land use cannot be distinguished by the IR spectra of HAs. The IR spectra also failed to detect any influence of different tillage on structural changes in HAs in Brazilian Oxisols (González Pérez *et al.* 2004). Therefore, structural changes in HAs and PVP-adsorbed FAs as affected by land use appear to be too small to be detected using IR spectroscopy.

The high turn-over rate of organic matter under warm, but humid conditions in Leyte, where high temperatures and favorable moisture conditions prevail, may not allow for the occurrence of HAs with a high degree of humification. This inference is supported by the results of the present study, that is, the dominance of Type Rp, the higher contents of H and N as well as the high ratios of H/C and O/C, all indicating that the HAs are still under early stages of humification, resulting in small differences in SOM quality.

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REFERENCES

Abe T, Katayama A, Watanabe A 2007: Temporal changes in distribution and composition of N from labeled fertilizer in

- soil organic matter fractions. *Biol. Fertil. Soils*, 43, 427–435.
- Abe T, Watanabe A 2004: X-ray photoelectron spectroscopy of nitrogen functional groups in soil humic acids. *Soil Sci.*, 169, 35–43.
- Asio VB 1996: Characteristics, Weathering, Formation, and Degradation of Soils from Volcanic Rocks in Leyte, Philippines, vol. 33. Hohenheimer Bodenkundliche Hefte, Stuttgart, Germany.
- González Pérez M, Martin-Neto L, Saab SC *et al.* 2004: Characterization of humic acids from a Brazilian Oxisol under different tillage systems by EPR, ^{13}C NMR, FTIR and fluorescence spectroscopy. *Geoderma*, 118, 181–190.
- Ikeya K, Watanabe A 2003: Direct expression of an index for the degree of humification of humic acids using organic carbon concentration. *Soil Sci. Plant Nutr.*, 49, 47–53.
- Kumada K 1987: Chemistry of Soil Organic Matter, 241 pp, Elsevier, Tokyo, Amsterdam.
- Kuwatsuka S, Tsutsuki K, Kumada K 1978: Chemical studies on soil humic acids. I. Elementary composition of humic acids. *Soil Sci. Plant Nutr.*, 24, 337–347.
- Kuwatsuka S, Watanabe A, Itoh K, Arai S 1992: Comparison of two methods of preparation of humic and fulvic acids, IHSS method and NAGOYA method. *Soil Sci. Plant Nutr.*, 38, 23–30.
- National Action Plan 2004: The Philippine National Action Plan to combat desertification, land degradation, drought, and poverty for 2004–2010, Department of Agriculture, Department of Environment and Natural Resources, Department of Science and Technology, and Department of Agrarian Reform, Manila, Philippines.
- Navarrete IA, Tsutsuki K 2008: Land-use impact on soil carbon, nitrogen, neutral sugar composition and related chemical properties in a degraded Ultisol derived from volcanic materials in Leyte, Philippines. *Soil Sci. Plant Nutr.*, 54, 321–331.
- Navarrete IA, Tsutsuki K, Asio VB, Renzo K 2009: Characteristics and formation of rain forest soils derived from late Quaternary basaltic rocks in Leyte, Philippines. *Environ. Geol.*, 58, 1257–1268.
- Ohta S 1990: Influence of deforestation on the soils of Pantabangan Area, Central Luzon, the Philippines. *Soil Sci. Plant Nutr.*, 36, 561–573.
- Olk DC, Brunetti G, Senesi N 1999: Organic matter in double cropped lowland rice soils: chemical and spectroscopical properties. *Soil Sci.*, 164, 649–663.
- Piccolo A, Zaccheo P, Genevini PG 1992: Chemical characterization of humic substances extracted from organic waste-amended soils. *Bioresour. Technol.*, 40, 275–282.
- Sato O 1974: Methods for estimating Pg content in P type humic acid and for calculating $\Delta\log K$ of its Pb fraction. *Soil Sci. Plant Nutr.*, 20, 343–351.
- Stevenson FJ 1994: Humus Chemistry: Genesis, Compositions, Reactions, 2nd edn. John Wiley & Sons, New York.
- Swift RS 1996: Organic matter characterization. In Methods of Soil Analysis. Part 3. Chemical Methods, Ed. DL

- Sparks *et al.*, pp. 1011–1069, SSSA. Book Ser. 5. SSSA, Madison, WI.
- Tatsukawa R 1966: Rapid determination of soil organic matter with special reference to sugars, uronic acids, and amino sugars. *J. Sci. Soil Manure, Jpn.*, 37, 28–33 (in Japanese).
- Tsutsuki K, Kuwatsuka S 1984: Molecular size distribution of humic acids as affected by the ionic strength and the degree of humification. *Soil Sci. Plant Nutr.*, 30, 151–162.
- Watanabe A, Kuwatsuka S 1991: Fractionation of soil fulvic acids using polyvinylpyrrolidone and their ionization difference spectra. *Soil Sci. Plant Nutr.*, 37, 611–617.
- Watanabe A, Kuwatsuka S 1992: Chemical characteristics of soil fulvic acid fractionated using polyvinylpyrrolidone (PVP). *Soil Sci. Plant Nutr.*, 38, 31–41.
- Watanabe A, Sarno, Rumbanraja J, Tsutsuki K, Kimura M 2001: Humus composition of soils under forest, coffee, and arable cultivation in hilly areas of south Sumatra, Indonesia. *Eur. J. Soil Sci.*, 52, 599–606.