

# Characteristics of Soil Aggregates under Mangroves in Comparison with the Aggregates of Upland Forest Soil, Assessed by Density Fractionation

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Abstract: Apart from the well-known ecosystem services of mangroves in the coastal regions, mangroves recently have been recognized as a major repository of carbon. However, little is known about the carbon storage in the mangroves as soil aggregates and, we envisage that studying soil aggregates under mangroves in comparison with upland forest soil aggregates would yield valuable information about the process of carbon storage. Therefore, this study aims to elucidate comparatively, the characteristics of soil aggregates of mangrove and upland forest soil, separated by density fractionation. Samples were collected from a mangrove ecosystem and an upland forest area in the northern region of Okinawa Island. Four density fractions (<1.6, 1.6~2.0, 2.0~2.5 and >2.5 g cm<sup>-3</sup> fractions) were separated by sodium polytungstate with three density levels (1.6, 2.0 and 2.5 g cm<sup>-3</sup>). Carbon and nitrogen content and C/N ratio were highest in the lighter fractions and decreased with increasing density. Clay and silt minerals and sand and silt minerals were included in the low-density fractions (<1.6, 1.6~2.0 g cm<sup>-3</sup>) and high-density fractions (2.0~2.5 and >2.5 g cm<sup>-3</sup>) respectively. In the upland forest soil, highest clay and carbon percentages were observed in the 1.6~2.0 g cm<sup>-3</sup> fraction. However, mangrove soil had the highest carbon content in the 1.6~2.0 g cm<sup>-3</sup> fraction, which, on the contrary, had low clay content. Therefore, it can be suggested that clay minerals are necessary to stock carbon in the upland soil. However, it was not the case for the mangrove soil.

# 1 Introduction

Ecosystem services provided by the mangroves in the coastal areas have long been identified and, sustenance of the biodiversity, protection against soil erosion and conservation of the landscape are to name a few of these services. Recently, as a coastal forest system, the importance of mangroves has become a point of discussion due to its role in barricading the tsunami waves (Dahdouh-Guebas et al. 2005; Kathiresan and Rajendran 2005). Although it is not completely understood, the carbon storage is also being considered as a major ecosystem service of mangrove ecosystems (Donato et al. 2011). Further, these authors recognize mangroves as one of the richest forest systems in terms of carbon in the tropics. They also state that 50% of the total carbon is stored in the soil. Carbon exist in the soil in different forms and carbon bound to soil particles, also known as soil aggregates, is among the major forms. We envisage that a comparison of soil aggregates under mangroves with upland forest soil would give an insight into the process of carbon storage in the mangrove ecosystems.

A soil aggregate is comprised of clay, silt, sand, and organic matter where, the organic matter is comprised of humic substances, plant roots, and fungal hyphae mainly (Denef and Six 2005; Caravaca *et al.* 2001). Generally, soil aggregates are categorized into macroaggregates (>0.25 mm) and micro-aggregates (<0.25 mm) based on the size. Macro-aggregates are composed of micro-aggregates bound to each other by different types of organic matter such as plant roots and fungal

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hyphae. The quality of soil organic matter is an important factor in the formation of soil aggregates. Lu et al (1998) reported that the decomposing organic matter is of great importance for the stability of aggregates in soil. In addition, Al, Fe, and Ca are also necessary to stabilize organic matter in the soil (Barthes et al. 2008; Wada and Higashi 1976; Wada et al. 1987; Egli et al. 2008). The existence of soil aggregates in the environment is important for plant growth (Denef et al. 2007). However, in agriculture, macro-aggregates get disrupted when the soil is cultivated (Bongiovanni and Lobartini 2006). Six et al (2000) reported that there is a relationship between stable micro-aggregates and the carbon stock in the long term. In addition, earthworms are well known to form aggregates and, the microaggregates they form help soil carbon stabilization (Bossuyt et al. 2005).

Recently, there is a trend of using sequential density fractionation in soil aggregate research. The term "fractionation" here, is referred to the separation of aggregates into low and high-density fractions by using sodium polytungstate (Swanston et al. 2005; Sollins et al. 2006). The low density fraction usually has less stable organic and soil particles with high carbon content where high density fraction is comprised of stable organo-mineral complexes more comparatively lower carbon content (Gregorich et al. 1996: Golchin et al. 1995a and 1995b). Properties of soil minerals and humic substances of density fractions can be assessed by sequential density fractionation and subsequent X-ray diffraction analysis and NMR analysis (Golchin et al. 1995a and 1995b, Sollins et al. 2006, Helfrich et al. 2006, Sollins et al. 2009, Wagai et al. 2008).

A previous study reported that some metal elements have a connection to the carbon stabilization under different types of soils. In a mangrove soil the clay-humus complexes were formed by a combination of clay, humic substances and some metals (Al and Fe) (Kinjo *et al.* 2006). Furthermore, Al, Fe and Ca affected the stabilization of two types of humic substances in

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upland forest soil (Teruya and Kinjo 2014). These findings suggest that the process of carbon storage in the soils as clay-humus complexes may vary from soil to soil. Soil aggregates are formed a result of clay humus complexes. Therefore, the properties of soil aggregates could also vary from soil to soil. Some studies have compared the properties of soil aggregates under different soil conditions (Elliott, 1986). However, the studies on the soil aggregation under mangrove systems are scarce. Soil aggregation under the reduced conditions of the mangroves could be different from that of the oxidized upland soils.

Therefore, the objective of this study is to elucidate the properties of soil aggregates of mangrove and upland forest soil, separated by density fractionation, in view of understanding the relationships between mineralogy and carbon storage in the mangrove soil.

# 2 Materials and methods

# 2.1 Sampling site

Sampling sites were selected in a mangrove ecosystem and a mountainous area in the northern part of Okinawa Island (Fig.1). Mangrove site is frequently flooded with seawater as the area was low-lying and close to the seashore. The distance to the mangrove vegetation from open sea was approximately 200 m. Data related to stand characteristics of the mangroves in this area are scarce. Nakasuga and Kobashigawa (1976) reported that the original stands located in sampling site were comprised of Kandelia candel and Bruguiera conjugata (gymnorhyza). Currently the dominant plant species is Bruguiera gymnorhiza while Kandelia spp was found scattered in some parts of the mangrove forest. There was no noticeable litter accumulation in the sampling sites, mainly due to the frequent flushing of falling litter with seawater. A dense root layer was observed in the sampling sites. Therefore, the main source of the organic matter is expected to be the decaying roots. In addition, deposition of river water sediments and relocated organic material could also contribute to the organic matter input.

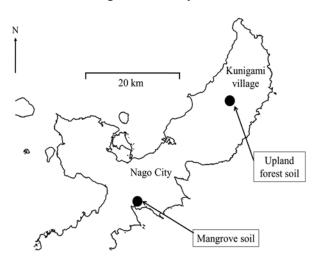


Figure 1: Sampling sites

The upland site is approximately 150 m above the sea level with Castanopsis sieboldii as the dominant tree species. Although detailed historical information about the forest development in the sampling site is not available, the sampling site possesses characteristics of the typical forests in the northern mountainous areas of Okinawa. The forest stand is a broad-leaved evergreen secondary climax forests left intact for more than 40-50 years (Hirata 1994; Ito 1997). Ito (1997) also shows that the tree species diversity of these forests is substantially high and, is comparable with the tropical forests. Other than the outstanding C. seiboldii, Distylium racemosum and Schefflera octophylla are also found as secondary dominant trees. Although undergrowth is relatively dense, the shrub layer in the sampling site is almost absent. Dense mat of fine roots can be seen just beneath the soil surface with substantial contribution from the main tree species. A litter layer of about 2-3 cm thick in average can be found covering the forest floor. The thickness of the A horizon ranges from 2.5 to 4 cm. The sampling site had a gentle slope facing North West.

Soils from both sites were collected from 5 points inside a 2 m  $\times$  2 m square at 1 cm depth. Five samples were pooled to prepare a composite soil sample. During the sampling point selection, places with large roots were avoided. Collected soils were ground and sieved through 2 mm mesh after removing the roots and other debris.

# 2.2 Soil chemical and mineralogical properties

Soil pH (soil: water = 1:2.5) and EC (soil: water = 1:5) were measured by respective electrodes (HORIBA). Carbon (C) and nitrogen (N) contents were analyzed by NC analyzer (SUMIGRAPH NC-220F). Exchangeable cations (Ex-Ca, Ex-Mg, Ex-K and Ex-Na) were extracted with ammonium chloride solution (Sarks 1996) and analyzed by ICP-AES (Shimadzu ICPE-9000).

Clay, silt and sand fractions of both soils were separated by sedimentation and decantation method and expressed on weight basis. Silt and sand fractions were directly analyzed by X-ray diffraction (Rigaku, Ultima+,  $2\sim60^{\circ}$  (20), 30kV, 15mA, XCuK $\alpha$ ) using random direction method. Clay fraction was treated with potassium acetate and magnesium acetate. Treated clay fractions were air-dried, heated at  $105^{\circ}$ C,  $300^{\circ}$ C, and  $550^{\circ}$ C for one hour, allowed to cool down and saturated with glycerol. Each treated clay fraction was analyzed by X-ray diffraction (Rigaku, Ultima+,  $2\sim30^{\circ}$  (20), 30kV, 15mA,  $XCuK\alpha$ ).

# 2.3 Density fractionation

The physical separation of the soil organic matter as well as mineral particles by density fractionation technique has been used in understanding organic matter—mineral interactions such as bondages between organic matter and different soil minerals and the degree of protection within aggregates. Further, density fractionation also aid in separating different pools of organic matter based on their state of decomposition.

Principally, the lighter fractions contain plant like substances and clay minerals. The denser fractions contain sand, silt and organic matter in the advanced stages of decomposition bound to soil minerals. Among the various solutions used in the density fractionation, sodium polytungstate is a less hazardous solution with densities reaching as high as 2.9 g cm<sup>-3</sup> (Crow *et al.* 2007). Therefore, the density fractionation technique was identified as the best-suited method for the present study and, sequential density fractionation was carried out by slightly modifying the methods described by Sollins *et al.* (2006) and Sollins *et al.* (2009).

Four density fractions were separated by sodium polytungstate with three density levels. During the separation, air-dried soil (<2 mm) was placed in a centrifuge tube and sodium polytungstate (d=1.6 g cm<sup>-3</sup>) was added. The tube was shaken for 24 hours and allowed to stand for 30 min before centrifuging at 2000 rpm for 30 min. The supernatant was extracted as <1.6 g cm<sup>-3</sup> fraction. Particles of the supernatant were extracted by a glass fiber filter (Membrane filter, pore size=0.45 μm) under a vacuum. Sodium polytungstate was washed off with distilled water and filter was dried at 30°C for 24 hours. The dried fraction on filter was separated by a brush and weighed. After extracting the <1.6 Mg m<sup>-3</sup> fraction, next sodium polytungstate solution with d=2.0 Mg m<sup>-3</sup>was added to the residue left from the previous step. The supernatant was comprised of 1.6~2.0 g m<sup>-3</sup> fraction and it was extracted by the same method explained above in extracting <1.6 g m<sup>-3</sup> fraction. Finally, sodium polytungstate solution with 2.5 g m<sup>-3</sup> density was used to separate 2.0~2.5 g m<sup>-3</sup> fraction and the remainder was >2.5 g cm<sup>-3</sup> fraction. Each fraction obtained by density fractionation was sieved with 0.25 mm mesh. Carbon and nitrogen of each sieved fraction were measured by NC analyzer (SUMIGRAPH NC-220F). To assess the mineralogical properties, first, the organic matter of each fraction was removed by oxidizing with 6% peroxide. After the oxidation, suspensions of each fraction were prepared by adding a little water. Suspensions were applied on glass slides and air-dried. Mineralogical properties of each fraction were analyzed by X-ray diffraction (Rigaku, Ultima+, 2~30° (2θ), 30kV, 15mA, XCuKα). Sand, silt and clay fraction of each density fraction in both types of soils were separated by sedimentation and decantation and expressed on weight basis.

# 2.4 Degree of humification of humic acids

The method of humic acid extraction and subsequent estimation of the degree of humification was

adopted from Kumada (1977). Density fractions were treated with a mixture of 0.1 M NaOH and 0.1 M Na<sub>4</sub>P<sub>2</sub>O<sub>7</sub> solutions for 30 minutes in a hot water bath (100°C). Soluble and insoluble fractions were separated by centrifugation. The soluble fraction was mixed with concentrated sulfuric acid and resulting supernatant and the precipitate were separated. The precipitate was dissolved in the mixture of 0.1 M NaOH and 0.1 M Na<sub>4</sub>P<sub>2</sub>O<sub>7</sub> solutions again and the resulting soluble fraction was extracted as humic acids. The degree of humification of the humic acids was estimated by the parameters ( $\Delta$  log K and RF value) derived from optical density (K) of the solution.

$$\Delta \log K = \log K_{400nm} - \log K_{600nm}$$

RF value =  $K_{600nm} \times 1000$  / [Amount of 0.1N KMnO<sub>4</sub> (ml) consumed by 30 ml of humic acid solution]

#### 3 Results and discussion

# 3.1 Soil chemical and mineralogical properties

Table 1 shows the chemical and physical properties of both upland forest and mangrove soils. The pH of upland forest and mangrove soils were 4.3 and 7.8 respectively. Mangrove soil was rich in exchangeable cations (Ex-Ca, Ex-Mg, Ex-K and Ex-Na) and alkaline due to the salts from the seawater. Upland forest soil had high clay content while mangrove had high sand content. Sand from seaside may have washed into the mangrove area. Carbon contents of upland forest soil and mangrove soils were 33.9 and 19.3 g kg<sup>-1</sup>, respectively. High carbon accumulation in the upland forest soil compared to mangrove soil, could be attributed to the comparatively high clay content in the upland soil.

Figure 2 and 3 shows the X-ray diffraction patterns of sand, silt and clay fractions of upland forest and mangrove soil. Quartz (0.336, 0.24, 0.22, 0.19 and 0.18 nm), monohydrate calcite (0.43 nm) and K-feldspar (0.330 nm) were identified in the sand fractions of both upland forest and mangrove soils. Quartz (0.33, 0.24, 0.22, 0.19, 0.18 and 0.16 nm), illite (1.04, 0.55 and 0.26 nm) and kaolin minerals (0.70 and 0.35 nm) were identified in the silt fractions of both soils. In the clay fraction of upland and mangrove soil, illite and kaolin minerals were identified by using the variation and shift of main peaks of each mineral (Fig.3). Vermiculite-chlorite intergrade was also identified in the clay fraction of mangrove soil and upland forest soil by the 1.17 nm and 1.47 nm peaks. Therefore, upland and

Table 1: Soil chemical and physical properties

|                    |                          |   |   |     | Exchar      | ngeable |      |      |                               |      |
|--------------------|--------------------------|---|---|-----|-------------|---------|------|------|-------------------------------|------|
|                    | рН<br>(H <sub>2</sub> O) | Organic-C<br>(x 10 <sup>-1</sup> g kg <sup>-1</sup> ) | Total-N<br>(x 10 <sup>-1</sup> g kg <sup>-1</sup> ) | Ca  | Mg<br>(cmol |         | Na   | Clay | Silt<br>(g kg <sup>-1</sup> ) | Sand |
| Mangrove soil      | 7.8                      | 19.3  | 1.6   | 3.2 | 1.5         | 1.1     | 20.1 | 269  | 162                           | 569  |
| Upland forest soil | 4.3                      | 33.9  | 1.3   | 1.2 | 0.2         | 0.3     | 0.1  | 437  | 271                           | 292  |

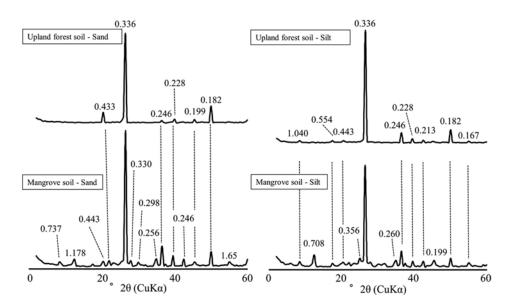


Figure 2: X-ray diffraction patterns of sand and silt fractions of upland forest and mangrove soils

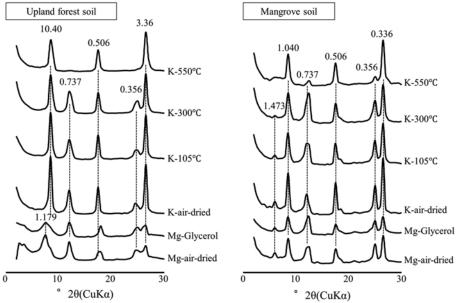


Figure 3: X-ray diffraction patterns of clay fractions of upland forest and mangrove soils

mangrove soil were similar in terms of soil mineralogical properties.

# 3.2 Properties of density fractions

Figure 4 shows the distribution of clay, silt and sand across the density fractions of each soil. In both soils, sand content in 2.0~2.5 and >2.5 g cm<sup>-3</sup> fractions was higher than the other fractions. Clay content did not differ across four density fractions of the mangrove soil. However, clay and silt contents in 1.6~2.0 g cm<sup>-3</sup> fraction were higher than that of the other density fractions in upland forest soil. As the conditions of mangrove and upland forest soil are different, particle size distribution in each fraction also may vary.

Figure 5 shows the X-ray diffraction pattern of each density fraction of upland and mangrove soils. In the mangrove soil, the dominant mineral quartz (0.336,

0.246, 0.225, 0.199 and 0.185 nm) was found in  $1.6\sim2.0$ ,  $2.0\sim2.5$  and >2.5 g cm<sup>-3</sup> fractions. As the sand and silt contents of the mangrove soil was high (Fig. 4), these fractions may have contributed more to the overall quartz content. The dominant minerals of <1.6 g cm<sup>-3</sup> fraction were kaolin minerals (0.737, 0.363, 0.267 nm), illite (0.104, 0.506, 0.356 and 0.256 nm), and vermiculite-chlorite intergrade (0.804 nm). From the current data, it can be said that the light-density fraction of mangrove soil is mainly comprised of clay minerals. Quartz (0.336 and 0.228, 0.182 and 0.169) was the dominant mineral in the >2.5 g cm<sup>-3</sup> and  $2.0\sim2.5$ fractions of the upland forest soil. Kaolin minerals (0.737, 0.363 and 0.267) and quartz (0.336 and 0.246 0.228, 0.182 and 0.169) dominated the  $1.6\sim2.0~{\rm g~cm^{-3}}$ fraction. Kaolin minerals (0.737 and 0.363 nm), vermiculite-chlorite intergrade (1.179 and 0.804 nm) and quartz (0.336 and 0.169 nm) were identified in <1.6 g cm<sup>-3</sup> fraction. The light-density fraction of the upland forest soil had high clay mineral content compared to mangrove soil. In the X-ray diffraction pattern of the upland soil, a peak was observed at 0.535 nm in <1.6 g cm<sup>-3</sup> fraction. However, the mineral corresponding to this peak could not be identified. Even though illite was identified in the clay fraction (Fig.2) of the upland soil, it was not identified in any of the four density fractions. As the main peak corresponding to illite was not observed (Fig.5), the mineral contributed to the unknown peak could have been illite. In both soils, clay minerals tended to appear with decreasing density.

Table 2 shows the percentage of each density fraction in each soil along with the percentages of carbon and nitrogen and C/N ratio in each density fraction. In the mangrove soil, the proportion of the material included in the density fractions increased with increasing density and the percentage of carbon and nitrogen in each density fraction decreased as the density increases. Therefore, compared to the other fractions, <1.6 g cm<sup>-3</sup> fraction had the highest carbon and nitrogen content and C/N ratio in the mangrove forest. Wagai et al (2008) reported that the low-density fraction, which includes plant detritus, was easily separable from soil minerals. Therefore, <1.6 g cm<sup>-1</sup> fraction of the soil seems to have plant detritus and separable soil particles. The upland forest soil also follows a similar pattern. The result obtained for the upland soil of this study are comparable with the data reported in other similar studies conducted under upland forests occupied by Tsuga heterophylla, Thuja plicata, Taxus brevifolia, Acer circinatum, Cornus nuttallii, Vaccinium spp. and Polystichum munitum (Sollins et al 2009; Grunewald et al 2006; Sollins et al 2006).

Figure 6 shows the distribution of total carbon

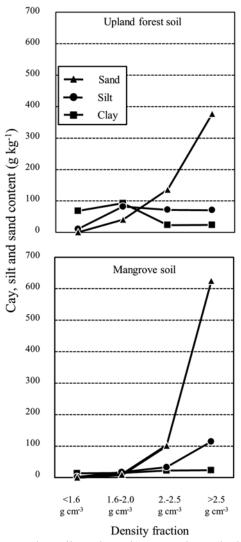


Figure 4: Clay, silt and sand content in each density fraction

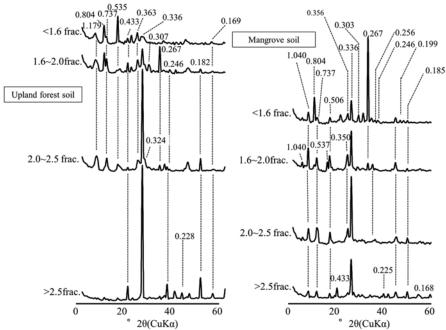


Figure 5: X-ray diffraction patterns of each density fraction of upland forest and mangrove soils

| Table 2: | Percentage of each | density fraction | in each soil | type and carbon | , nitrogen and C/I | N ratio of each density |
|----------|--------------------|------------------|--------------|-----------------|--------------------|-------------------------|
|          | fraction           |                  |              |                 |                    |                         |

| naction            |                      |          |      |      |      |  |  |  |
|--------------------|----------------------|----------|------|------|------|--|--|--|
|                    |                      | Fraction | С    | N    | C/N  |  |  |  |
|                    |                      |          |      |      |      |  |  |  |
| Mangrove soil      | <1.6 frac.           | 1.5      | 31.7 | 1.1  | 29.6 |  |  |  |
|                    | $1.6 \sim 2.0$ frac. | 4.3      | 26.4 | 1.2  | 21.8 |  |  |  |
|                    | $2.0 \sim 2.5$ frac. | 16.0     | 5.9  | 0.5  | 12.3 |  |  |  |
|                    | > 2.5 frac.          | 78.2     | 0.7  | 0.05 | 7.3  |  |  |  |
|                    | <1.6 frac.           | 8.0      | 32.0 | 1.2  | 26.7 |  |  |  |
| Upland forest soil | $1.6 \sim 2.0$ frac. | 21.6     | 18.5 | 0.9  | 19.8 |  |  |  |
|                    | $2.0 \sim 2.5$ frac. | 23.2     | 9.3  | 0.6  | 14.8 |  |  |  |
|                    | > 2.5 frac.          | 47.2     | 2.2  | 0.2  | 14.6 |  |  |  |
|                    |                      |          |      |      |      |  |  |  |

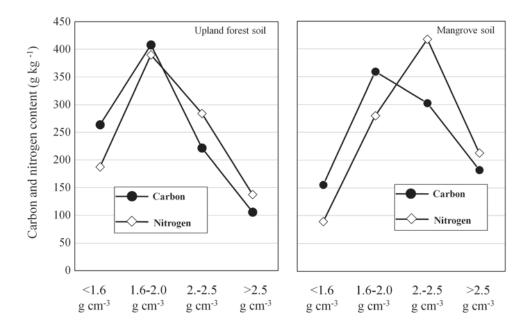


Figure 6: Distribution of the total soil carbon and nitrogen across the density fractions

across the density fractions. Compared to the other density fractions, 1.6-2.0 g cm<sup>-3</sup> fraction of both mangrove and upland soil had the highest carbon content. As 1.6~2.0 g cm<sup>-3</sup> fraction was comprised of silt and clay mostly (Fig.4), it is possible that silt and clay contribute more to the formation of clay-humus complexes. Therefore, the reason for the low carbon contents in the <1.6 g cm<sup>-3</sup> and >2.5 g cm<sup>-3</sup> fractions could be the low clay content. Although the variation of the carbon was similar, the variation of nitrogen was dissimilar in the two soils. In the mangrove soil, nitrogen content in 2.0~2.5 g cm<sup>-3</sup> fraction was higher than in other fractions, whereas 1.6~2.0 g cm<sup>-3</sup> fraction had the highest nitrogen content in upland soil.

The degree of humification of humic acids in each density fraction of mangrove and upland forest soils is shown in figure 7. The categorization was based on the criteria stated by Kumada (1977). Generally, the degree of humification increases when the RF value is increasing and the  $\Delta$  log K value is decreasing. Therefore, Rp region indicated in the figure has the less-humified material. Four density fractions of the

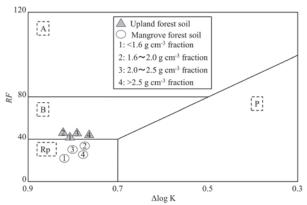


Figure 7: The degree of humification of humic acids in each density fraction of mangrove and upland soil

mangrove soil represented type Rp. On the other hand, all the fractions of the upland forest soil were categorized into type B and therefore, the degree of humification was higher than the mangrove soil.

Although the chemical and physical properties of the organic matter, biota and the organo-mineral interactions could be decisive factors in controlling the degree of humification, the difference in the redox status of the two soil types could be a dominant factor in the overall context.

# 4 Conclusions

The organic matter dynamics of the two systems vary in several aspects. As the litter of the mangrove ecosystem is frequently flushed, the accumulation of litter layer is very much limited. Relatively high litter accumulation was observed in the upland soil. However, due to the presence of favorable conditions for rapid decomposition, large litter buildup cannot be observed. Furthermore, the redox status of the two sites could lead to the differences in overall biochemically-driven processes such as litter decomposition, integration of litter to the mineral fraction and organic matter storage. The assessment of soil aggregates under the mangrove system in comparison with upland forest soil, by density fractionation yielded important information about the interactions between organic matter and minerals under and mangrove ecosystem hence, about characteristics of the carbon storage. Four fractions (<1.6, 1.6~2.0, 2.0~2.5 and >2.5 g cm $^{-3}$  fractions) were successfully separated by sodium polytungstate solutions with three different densities. Lighter fractions had the highest carbon and nitrogen content and C/N ratio and, all of them decreased with increasing density. Clay and silt minerals and sand and silt minerals were included in low-density fractions (<1.6 and 1.6~2.0 g cm<sup>-3</sup>) and high-density fractions  $(2.0\sim2.5 \text{ and } > 2.5 \text{ g cm}^{-1})$ <sup>3</sup>) respectively. In upland forest soil, highest clay and carbon percentages were observed in the 1.6~2.0 g cm<sup>-3</sup> fraction. Mangrove soil also had the highest carbon content in the 1.6~2.0 g cm<sup>-3</sup> fraction but had low clay content. The result of the upland soil of this study agrees with the results reported in similar studies conducted under other upland forests (Sollins et al 2009; Grunewald et al 2006; Sollins et al 2006). However, similar studies on mangrove soil were not found.

Therefore, the data from this study suggest that the clay minerals are necessary to stock carbon in the upland soil. However, such relationship is not visible in the mangrove soil, signifying the important differences in the organo-mineral interactions of the mangrove soil compared to the upland soils. We suggest that these differences can be further clarified by assessing the other influential parameters such as litter quality, biotic and abiotic factors affecting the litter decomposition in the mangrove ecosystem.

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