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# Effects of land use and long-term organic matter application on low-molecular-weight organic acids in an Andisol

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To clarify the effects of land use and long-term organic matter application on the dynamics of low-molecular-weight organic acids (LOAs), soil samples were collected from an agricultural site under various organic matter managements and an adjacent Oak forest site. The agricultural site was composed of a chemical fertilizer plot, cattle manure applied plots, a crop residue incorporated plot, and both a cattle manure and crop residue incorporated plot. Formic acid was detected in the soils of all plots at the agricultural site; however, the effect of long-term application of organic matter was less clear. Malic acid was also detected in all soils of the arable lands, and the amount was larger in the organic matter-applied plots than in the chemical fertilizer plot. The amount of citric acid in the arable soils was extremely smaller than in the forest soils. No citric acid was detected in the soils without organic matter application. The total amounts of LOAs were considerably smaller in the arable soils than in the adjacent forest soils, except for the plot where both cattle manure and crop residues had been applied. The land use and long-term application of organic matter practically affected the contents of LOAs in an Andisol, and their effects on the dynamics of nutrients and other constituents should be the subject of future research.

**Key words**: Land use, long-term organic matter application, low-molecular-weight organic acids, malic acid, water-extractable organic carbon.

## INTRODUCTION

Soil organic matter is affected by the type of land use which, in turn, determines the organic matter turnover and nutrient cycling. In order to minimize chemical fertilizer input, sustainable agricultural systems often use animal wastes and crop residues as nitrogen (N) and phosphorus (P) sources for plants. Kanchikerimath and Singh (2001) found significant correlations between management practices and soil organic carbon and microbial biomass carbon content in the soil of a 26-year-old fertilization experiment. In addition to its direct contribution to N and P supply, the incorporation of C-rich manure and crop residue to soils has been shown to increase the amount of soluble organic matter in soils (Zsolnay and Gorlitz, 1994) that may affect the bioavailability of soil P.

Low-molecular-weight organic acids (LOAs) are commonly present in soils and are constantly released from root exudates and decayed plant litter, and through microbial organic matter decomposition (Pohlman and McColl, 1988; Fox, 1995; Sandnes et al., 2005; vas Hees et al., 2005). The solubility and acidity of LOAs play important roles in the chemical and biological processes of soils. LOAs function as ligands and through complexation reactions in solution and ligand exchange reactions at the mineral surface they affect metal solubility and P availability (Fox, 1995; Khademi et al., 2010). LOAs also may indirectly affect plant nutrition in acid soils by complexing Al, thus alleviating Al toxicity and improving the growth and physiologic functions of roots (Hue et al.,

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**Abbreviations: LOA,** Low-molecular-weight organic acid; **WEOC,** water-extractable organic carbon.'

1986). A number of different low-molecular-weight aliphatic organic acids have been found in soils: for example, oxalic acid, citric acid, formic acid, acetic acid, malic acid, succinic acid, malonic acid, lactic acid, and fumaric acid (Stevenson, 1967; Fox and Comerford, 1990; Tani et al., 1993). Strobel et al. (1999) reported that the forest soils are generally richer in soil solution LOAs than are arable soils, and the higher LOA content in the forest soils due to the di- and tri-carboxylic acids, while the total concentration of mono-carboxylic acids are comparable. The importance of soil carbon (C), especially the soluble organic carbon fraction, in regulating and influencing ecosystem processes is well recognized. Pohlman and McColl (1988) reported that the proportion of LOAs to dissolved organic carbon (DOC) in soil solutions collected from coniferous forest litter ranged from 0.1 to 4.6%. van Hees et al. (2000) also reported that the proportion of LOAs to the DOC in the soil solutions derived from three podzolic soils ranged from 0.0 to 5.9%, and that the proportion of LOAs was higher in the organic horizons than in the B horizons. Although LOAs were usually minor constituents of DOC, they disproportionately influence soil process because of their small size, relatively high solubility, and ability to form strong complexes with metals. Although many studies focused on the fate of LOAs in the forest ecosystems and soils of Japan, relatively few studies have characterized LOAs in arable soils. In particular, the influence of long-term application of organic matter on the characteristics of LOAs remains unclear. The objectives of the present study were to clarify the effects of land use and the long-term application of organic matter on the composition of water-soluble LOAs.

## MATERIALS AND METHODS

### Experimental field design and arable soils

Soil samples were collected from the 25-year-old organic matter application field (Typic Hapludands, USDA, 1998) on 26 July 2000, located at the Hokkaido Prefectural Tokachi Agricultural Experiment Station (N 42° 55', E 143° 3'), in Memuro, Hokkaido, Japan. The crop rotation system in the experimental site was potato - sugar beet - soy bean - winter wheat, and soil samples were collected from both winter wheat and sugar beet sites. Management of the experimental field is described in Table 1. The fertilizer and manuring practices were as follows: a chemical fertilizer without organic matter application plot (Plot F), a cattle manure (15 mg ha$^{-1}$ y$^{-1}$) without crop residue incorporation plot (Plot M), a twice the amount of cattle manure (30 mg ha$^{-1}$ y$^{-1}$) without crop residue incorporation plot (Plot 2M), a no cattle manure application and incorporation with crop residue plot (Plot R), and a both cattle manure (15 mg ha$^{-1}$ y$^{-1}$) and incorporation with crop residue plot (Plot RM). In all the plots in which organic matter was applied, the same levels of chemical fertilizers as those in plot F were used.

### Forest soils

Forest soil samples were collected from the wind-break Oak   forest

on 26 July 2000, which was adjacent the 25-years-old organic matter application agricultural field. The vegetation is Oak (*Quercus dentate*) with an understory of bamboo species. Major macro-morphological features of the profile are shown in Table 2. The layers consist of weakly-weathered volcanic ash (Tarumae-a, Tarumae-b, Tarumae-c) in the upper part, well-weathered volcanic ash (Tokachi-c2, Tarumae-d, Eniwa-a) in the middle part, and alluvium in the lowest part. The profile was characterized by a darkish color in the upper 4 horizons and a yellowish or reddish color in the lower 4 horizons. The upper four horizons (A1, A2, 2A/C, and 3AB), which were judged to contain high or medium organic matter based on their field-moist darkish color in the field survey, were designated as upper horizons in the present study. The lower four horizons (3BC, 4BC, 5Bw, and 6C), which were estimated to contain low organic matter, were designated as lower horizons. Field texture was loam in almost all horizons, except for 2A/C. The lower three horizons contained a few rounded and/or sub-rounded alluvial rock fragments.

### Soil water-extracts

Solution samples were extracted with ultra-pure water from field-moist soil samples at the oven-dry soil weight to moisture ratio of 1: 5, respectively (Wolt, 1994), by shaking for 1 h and centrifugation at 10,000 g for 10 min. The extracts were filtered sequentially through a 0.025 μm millipore filter (Millipore typeVS) to remove fine particles and small mineral-humus complexes from extracts in order to avoid interference in the determination of LOAs. Duplicate extractions were applied in the present study.

The amount of water-extractable organic carbon (WEOC) was determined by using an organic carbon analyzer (Simadzu TOC-5000A) and calculated by subtracting the concentration of dissolved inorganic carbon from that of the total dissolved carbon.

### Determination of low-molecular-weight organic acids in water-extracts

LOA concentrations were determined by the method of Tani et al. (2001), in which ten kinds of LOAs, namely oxalic acid, citric acid, malic acid, lactic acid, formic acid, acetic acid, succinic acid, propionic acid, fumaric acid, and butyric acid, were identified. The method includes the following two steps:

1. The soil water-extract was alkalized with diluted NaOH and concentrated by freeze drying. The concentrated solution was passed through a cation-exchange cartridge column, Sep-Pak Plus Accell CM, and a reverse-phase cartridge column, Sep-Pak Plus C$_8$ (Waters Corp., Milford, MA), to remove excess cations and colored hydrophobic organic matter fractions, respectively.
2. LOAs in the above-mentioned solution were identified and quantified using HPLC in the ion-exchange mode and the ion-exclusion mode. Oxalic acid was separated using an ion-exchange column for anions TSKgel IC-ANION-PW$_{XL}$ (TOSOH). The other nine LOAs were separated using an exclusive column apparatus for organic acids, TSKgel OApak-A (TOSOH), in which two columns are connected directly to increase the separation capacity. The LOAs were detected using an electric conductivity detector. The details of the analytical conditions of HPLC were described by Tani et al. (2001).

For cropping arable soils, oxalic acid could not be separated with an ion-exchange column for anions, because excess sulfate ions, which might be derived from ammonium sulfate fertilizer, impeded the separation of the oxalate peak. Therefore, the present paper could not address oxalic acid in arable soils. Although there is plenty

**Table 1.** Description of experimental design for fertilization and organic materials application in the agricultural site.

| Plot name | Applied and/or incorporated materials |
|---|---|
| F | Chemical fertilizers |
| M | Chemical fertilizers, cattle manure (15 mg ha$^{-1}$ y$^{-1}$) |
| 2M | Chemical fertilizers, cattle manure (30 mg ha$^{-1}$ y$^{-1}$) |
| R | Chemical fertilizers, crop residue |
| RM | Chemical fertilizers, cattle manure (15 mg ha$^{-1}$ y$^{-1}$), crop residue |

**Table 2.** Major macro-morphological features of the soil profile in the forest site.

| Horizon | Depth (cm) | Moist soil color [a] | Field texture [b] | Rock fragment [c] | Structure [d] | Hardness [e] |
|---|---|---|---|---|---|---|
| A1 | 0 – 5 | 10YR 3/1 | L | NO | MO ME GR | VL |
| A2 | 5 -11 | 10YR 2/1 | L | NO | MO VF GR | VL |
| 2A/C | 9 - 11 | 10YR 2/3 | SiL | NO | WE ME GR | L |
| 3AB | 11 - 16 | 10YR 3/3 | L | NO | WE FI SB | L |
| 3BC | 16 - 23 | 7.5YR 4/4 | L | NO | WE VF SB | VL |
| 4BC | 23 - 32 | 7.5YR 4/5 | L | FG RO FE | WE FI SB | VL |
| 5Bw | 32 - 43 | 7.5YR 4/5 | L | FG RO FE | WE ME SB | L |
| 6C | 43 - 52 | 10YR 4/6 | L | MG SR FE | WE FI SB | L |

[a] Based on Munsell soil color chart. [b] L=loam; SiL=silt loam. [c] NO=none; FG=fine gravel; MG=medium gravel; RO=rounded; SR=sub-rounded; FE=few. [d] MO=moderate; WE=weak; VF=very fine; FI=fine; ME=medium; GR=granular; SB=sub-angular blocky. [e] VL=very loose; L=loose.

of scope for improvement in the determination methods for LOAs in soil-water extracts, we confirmed that the present results were still deserving of special attention.

## RESULTS AND DISCUSSION

### Some chemical properties of soil sample

Some chemical properties of the soils collected from the forest profile and experimental fields are listed in Table 3. In the forest site, the total carbon content was highest in the surface A1 horizon, and decreased with increasing soil depth. The vertical distribution pattern of total nitrogen content was similar to that of total carbon content. Soil pH ranged from 5.2 to 5.8, and substantially increased from the upper horizons to the lower horizons. In the agricultural site, soil pH ranged from 5.5 to 6.1 and from 5.7 to 6.1 in the wheat field and sugar beet field plots, respectively. Soil pH is higher in the organic matter application plots than in plot F in each field. The total carbon and nitrogen contents were higher in the organic matter application plots, especially in the plots where cattle manure had been applied.

### Water-extractable organic matter in forest soils

The amounts of WEOC and LOAs in the horizon-wise

soils of the forest site are shown in Table 4. The amount of WEOC was largest in the surface A1 horizon, and generally larger in the upper horizons than in the lower horizons. The amounts of WEOC decreased with the increase in soil depth. Dissolved organic carbon (DOC) in soil solution is controlled and replenished by C inputs from plant litter, root exudation, and the decomposition of organic matter in the soil (Qualls and Haines, 1991). The constant supplies of organic matter from the surface litter layer probably explain the vigorous production of soluble organic compounds. However, a decomposition study of the forest floor suggested that dissolved organic matter (DOM) consists of easily degradable organic compounds, and that the turnover of DOM can be rapid (Yano et al. 2000), while in mineral soil, the mobility of DOM is controlled by sorption on mineral surfaces (Qualls and Haines, 1992; Kaiser et al., 1997; Nambu and Yonebayashi, 2000). Thus, the quantified WEOC in the lower horizons is a net result of processes that can cause WEOC to decrease, such as decomposition by microbes in the upper horizons or adsorption by mineral surface.

The amounts of LOAs in the soil water-extracts are shown in Table 4. Formic acid was detected in all horizons throughout the profile. The amount of formic acid was relatively larger in the upper horizons than in the lower horizons. Malic acid and lactic acid were also detected in most of the horizons. Citric acid was detected only in the upper horizons. Although acetic acid was not detected in the upper horizons, trace amounts were observed   in the

**Table 3.** Some chemical properties of the soil samples.

| Horizon or plot | pH(H$_2$O) | Total-C (g kg$^{-1}$) | Total-N (g kg$^{-1}$) | C/N Ratio |
|---|---|---|---|---|
| **Forest site** | | | | |
| A1 | 5.45 | 116 | 8.43 | 13.8 |
| A2 | 5.21 | 60.9 | 4.27 | 14.3 |
| 2A/C | 5.59 | 38.7 | 2.42 | 16.0 |
| 3AB | 5.65 | 37.3 | 2.29 | 16.3 |
| 3BC | 5.61 | 29.8 | 2.10 | 14.2 |
| 4BC | 5.78 | 11.0 | 0.94 | 11.7 |
| 5Bw | 5.80 | 9.34 | 0.96 | 9.77 |
| 6C | 5.71 | 7.35 | 0.96 | 7.68 |
| **Agricultural site: Winter wheat** | | | | |
| F | 5.54 | 31.5 | 2.56 | 12.4 |
| M | 5.68 | 36.5 | 3.19 | 12.0 |
| 2M | 5.80 | 39.7 | 3.66 | 11.9 |
| R | 5.88 | 35.1 | 2.98 | 12.4 |
| RM | 6.12 | 37.8 | 2.96 | 12.8 |
| **Agricultural site: Sugar beet** | | | | |
| F | 5.70 | 28.1 | 2.84 | 9.89 |
| M | 5.91 | 30.8 | 3.16 | 9.75 |
| 2M | 6.14 | 41.3 | 3.66 | 11.3 |
| R | 5.78 | 33.4 | 2.82 | 11.8 |
| RM | 6.10 | 35.6 | 3.12 | 11.4 |

**Table 4.** The amounts of water-extractable organic carbon (WEOC) and low-molecular-weight organic acids (LOAs) in soils collected from the forest site.

| Horizon | WEOC (mmol C kg$^{-1}$) | LOAs (µmol kg$^{-1}$) | | | | |
|---|---|---|---|---|---|---|
| | | Formic acid | Acetic acid | Lactic acid | Malic acid | Citric acid |
| A1 | 9.51 | 9.65 | N.D. | Tr. | 1.35 | 1.40 |
| A2 | 3.36 | 4.80 | N.D. | 13.1 | 2.45 | 0.50 |
| 2A/C | 2.61 | 6.75 | N.D. | 1.35 | 1.05 | 0.45 |
| 3AB | 1.76 | 5.10 | N.D. | 1.23 | 1.10 | 0.45 |
| 3BC | 0.46 | 3.33 | Tr. | 1.20 | 1.15 | N.D. |
| 4BC | 0.35 | 2.62 | Tr. | 3.10 | 0.40 | N.D. |
| 5Bw | 0.33 | 1.56 | Tr. | 2.75 | Tr. | N.D. |

N.D., not detected. Tr., trace amount.

lower horizons. lower horizons. Propionic acid, butyric acid, fumaric acid, and succinic acid were undetected in virtually all the horizons. The total amount of LOAs was larger in the upper horizons (7.9 to 20.9 µmol kg$^{-1}$) than in the lower horizons (4.31 to 7.4 µmol kg$^{-1}$). Major sources of LOAs in soil environments are metabolites from the decomposition of soil organic matter in the upper horizons (Stevenson, 1967), root exudation (Smith, 1976), and leaching from decomposing litter in the forest floor (Fox and Comerford, 1990). As LOAs originate from several biotic sources, it was reasonable to find that the amount of LOAs differed greatly between the upper part and the lower part of the profile studied.

The composition of LOAs in the upper horizons differed entirely from that in the lower horizons. In the upper horizons, di-carboxylic acid and tri-carboxylic acid, such as malic acid and citric acid, were major constituents of the identified LOAs. In the lower horizons, mono-carboxylic acids, such as formic acid and lactic acid, were dominant. Di-carboxylic acids and tri-carboxylic acids have more affinity to metal oxide surfaces than do mono-carboxylic acids (Fox, 1995), thus di-carboxylic

**Table 5.** The amounts of water-extractable organic carbon (WEOC) and low-molecular-weight organic acids (LOAs) in soils collected from the agricultural site.

| Plot | WEOC (mmol C kg$^{-1}$) | LOAs (μmol kg$^{-1}$) | | | | |
|---|---|---|---|---|---|---|
| | | Formic acid | Acetic acid | Lactic acid | Malic acid | Citric acid |
| Winter wheat | | | | | | |
| F | 0.45 | 2.78 | Tr. | 0.95 | 0.34 | N.D. |
| M | 0.85 | 2.83 | Tr. | 2.36 | 0.60 | Tr. |
| 2M | 1.48 | 4.44 | Tr. | 1.60 | 0.55 | 0.22 |
| R | 0.86 | 4.71 | Tr. | 0.79 | 0.69 | Tr. |
| RM | 0.96 | 2.90 | N.D. | N.D. | 13.5 | N.D. |
| | | | | | | |
| Sugar beet | | | | | | |
| F | 0.80 | 3.14 | N.D. | 1.78 | Tr. | N.D. |
| M | 0.90 | 3.44 | N.D. | 1.69 | 0.53 | Tr. |
| 2M | 2.25 | 1.92 | Tr. | Tr. | 3.83 | Tr. |
| R | 0.65 | 4.05 | Tr. | N.D. | 1.05 | N.D. |
| RM | 1.41 | 2.26 | Tr. | Tr. | 12.3 | 0.30 |

N.D., not detected. Tr., trace amount.

acids and tri-carboxylic acids are more influenced of sorption phenomena on the metal oxide surfaces than are mono-carboxylic acids. Moreover, the moisture status of the lower horizons could be attributed to the production of volatile organic acids, such as formic acids and lactic acid, by bacteria under relatively aerobic conditions (Stevenson, 1967). Therefore, the different compositions of LOAs between in the upper horizons and in the lower horizons could be the net result of processes such as adsorption by mineral surfaces or production by microbes.

The proportion of total LOAs to WEOC ranged from 0.25 to 8.85%, and it was higher in the upper horizons than in the lower horizons. This result was practically similar to those reported for Inceptisols, Alfisols, and Spodosols (Pohlman and McColl, 1988; van Hees et al., 2000). The proportion of total LOAs to WEOC substantially increased with increasing soil depth, reaching its highest value in the bottom-most 6C horizon. The contribution of LOAs to the total WEOC in the water extract of a Light-colored Andosol under an Oak forest was significant, especially in the lower horizons.

**Water-extractable organic matter in arable soils**

The amounts of WEOC ranged from 0.45 to 1.48 mmolC kg$^{-1}$ and from 0.65 to 2.25 mmolC kg$^{-1}$ in soils from the wheat field and the sugar beet field, respectively (Table 5). These amounts were comparatively smaller to a degree than those of the forest surface and upper soils. In the wheat field, the amounts of WEOC were larger in the organic matter application plots, M, 2M, R, and RM, than in plot F. On the other hand, the amount of WEOC in soils from the sugar beet field was smallest in plot R, followed by plot F. In either case, the amount of WEOC was largest

in plot 2M of each field, followed by plot RM, indicating that the amount of WEOC in the cropping arable soils was affected mostly by the amount of applied cattle manure rather than the incorporation of crop residues.

The amounts of LOAs in the soil water-extracts are shown in Table 5. Formic acid was detected in the soils of all plots, ranging from 2.78 to 4.71 μmol kg$^{-1}$ and from 1.92 to 4.05 μmol kg$^{-1}$ in the wheat field and the sugar beet field, respectively. The amount of formic acid was largest in the plot R of both fields. The amount of formic acid was also larger in the soils of plot 2M in the wheat field; however, in the sugar beet field, the amounts of formic acid were smaller in plots 2M and RM than in plot F, and the effect of organic matter application was less clear. In any case, the amounts of formic acid in the arable soils were rather smaller than those found in the upper soils of the forest site.

Malic acid was also detected and quantified in most soils except for plot F in the sugar beet field. The amounts of malic acid were larger in the organic matter application plots than in plot F of each field. Especially, the amounts of malic acid in the soils of the RM plots in both fields were unexpectedly and enormously large, as compared with other plots in the agricultural site and even with the surface soils of the forest site. This result was interesting and significant; however, it was difficult to determine why the amounts of malic acid were extremely large only in the RM plots at the present time. Although it remains no more than speculation, the combination of the application of cattle manure and the incorporation of crop residues probably induced the specific and considerable change of microorganism-flora, resulting in an excessive release from microbes into soils. This constitutes a subject for future research.

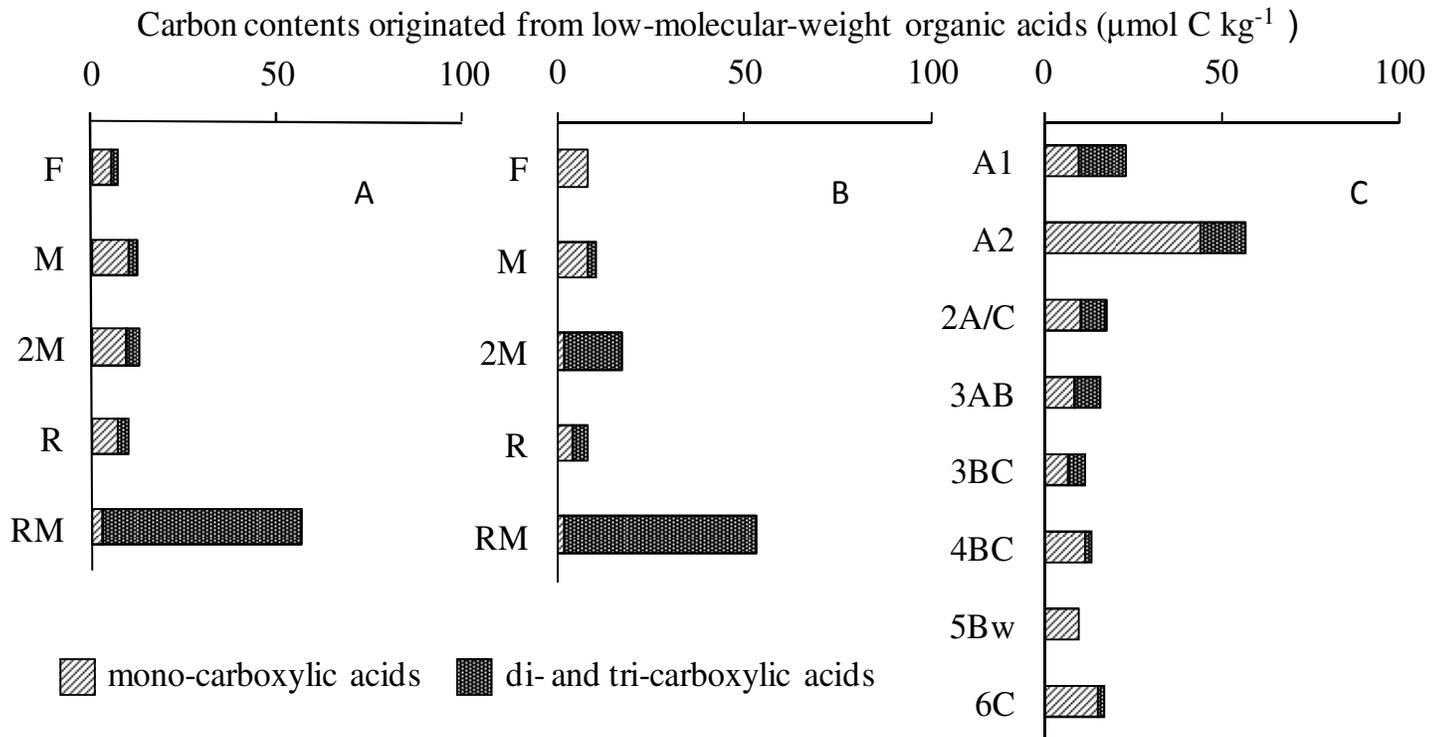The amount of citric acid in the arable soils was

Carbon contents originated from low-molecular-weight organic acids ($\mu$mol C kg$^{-1}$ )



**Figure 1.** Carbon contents originated from low-molecular-weight organic acids in the soil water-extracts from (a) winter wheat field, (b) sugar beet field and (c) Oak forest.

extremely smaller than in the forest surface soils. No citric acid was detected in the soils of plots F to which no organic matter had been applied. The total amounts of organic carbon originated from LOAs ranged from 7.0 to 56.8 $\mu$molC kg$^{-1}$ and from 8.5 to 53.4 $\mu$molC kg$^{-1}$ in the soils of the wheat field and the sugar beet field, respectively (Figure. 1). The proportion of total carbon contents of LOAs to WEOC was less than 1.5 %, except for plots RM, and comparable to that of the forest upper horizons. The total amount of carbons in soil LOAs were larger in the organic matter application plots, M, 2M, R, and RM than in plot F of each filed. This might be due to the relatively high contribution of di- and tri-carboxylic acids to the water-extractable total carbon, while the total amounts of mono-carboxylic acids were comparable. Malic acid shared dominancy with di- and tri-carboxylic acids. The larger amounts of malic acid under organic matter application could be attributed to enrichment with metabolic products.

Even by omitting the results of the RM plots, the upper horizons of forest soil were richer in LOAs than were the cropping arable soils (Figure 1), as also found by Hue et al. (1986) and Strobel et al. (1999). In addition to LOAs, the amounts of WEOC were significantly larger in the forest surface A1 horizon than in the all plots of crop fields. In general, forest topsoils show larger WEOM amounts than do arable soils (Ellert and Gregorich, 1995; Delprat et al., 1997; Campbell et al., 1999; Strobel et al., 1999).

The constant supply of organic matter from the organic litter layer in tandem with high microbiological activities probably explain the vigorous production of soluble organic compounds and the large WEOM amounts in the uncultivated forest topsoils. When a forest is cleared and tilled to produce annual crops, the proportion of actively cycling carbon declines (Ellert and Gregorich, 1995), and more aero-biodegradation conditions could be stimulated. Thus, cropland soils contain less WEOM, in spite of the long-term application of organic amendments, than does forest topsoil.

The amounts of WEOC and water-extractable LOAs in the cropping arable soils were larger in the organic matter application plots, M, 2M, R, and RM than in plot F without any organic matter application. These results indicated that the land use and long-term application of organic amendments practically affected WEOC and water-extractable LOAs contents in this experimental field, and that their effects on the dynamics of nutrients and other soil constituents should receive more attention.

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