

# Low-molecular-weight aliphatic carboxylic acids in soils incubated with decomposing forest litter

Masayuki Tani<sup>1</sup>, Masanori Koike<sup>1</sup>, Katsuhisa Kuramochi<sup>1</sup> and Teruo Higashi<sup>2</sup>

(Received: April 17, 2006)

森林リター分解物を添加・培養した土壌における低分子脂肪族カルボン酸

谷 昌幸<sup>1</sup>, 小池正徳<sup>1</sup>, 倉持勝久<sup>1</sup>, 東 照雄<sup>2</sup>

## ABSTRACT

The effects of the addition of decomposing forest litter, collected from Podzolic soil organic layers (Oi, Oe and Oa) and Brown Forest soil Oe layers, on the amounts and composition of low molecular weight aliphatic carboxylic acids (LACAs) in soils were investigated by incubation experiments for 4 weeks. The total amounts of LACAs were greater at the beginning of incubation (384 to 1155  $\mu\text{mol kg}^{-1}$ ), but decreased abruptly after 1 week, becoming relatively stable for a period of 1 to 4 weeks (299 to 377  $\mu\text{mol kg}^{-1}$ ). The average amounts (without 0 weeks) of LACAs, especially acetic acid and citric acid, in soils incubated with forest litter under moderately moist conditions, were smaller than in the blank soil. Larger amounts of LACAs, especially formic acid, succinic acid, and citric acid, accumulated in soils incubated with forest litter under slightly wet conditions than in the blank soil. Changes in soil microflora and their activities induced by the differences in soil moisture conditions and incorporated decomposing forest litter can affect the balance of LACAs between production and decomposition in soils. Two types of decomposing forest litter collected from two Brown Forest soils, which are under almost the same vegetation and climatic conditions, but are from distinctly different parent materials, had nearly similar effects on the dynamics of LACAs. Three types of decomposing forest litter obtained from three layers of the Podzolic soil, which are substantially different in their decomposition degree, induced several changes in the composition of LACAs. The findings in the present study led to the conclusion that the decomposing forest litter derived from the vegetation present, the soil moisture conditions, and accompanying changes in soil microflora greatly influence the amounts and composition of LACAs in forest soils.

Key words: decomposing forest litter, forest soils, incubation experiment, low-molecular-weight aliphatic carboxylic acid (LACA), soil microflora

## INTRODUCTION

It is interesting to evaluate the effects of the dynamics of low-molecular-weight aliphatic carboxylic acids (LACAs) in forest soils, which are essential to soil chemical processes and soil environmental quality (Stevenson, 1967; Fox, 1995). Most LACAs in forest soils probably are produced and brought into the soil of microbial metabolism, leachate from forest litter, and also as root exudates of higher plants (Fox,

1995). In acidic forest soil conditions, LACAs in the soil solution of O horizons, which consist of decomposing forest litter, play an important role in the speciation of aluminum and other metal ions with the ability of forming complexes (Pohlman and McColl, 1988; Tam and McColl, 1991; van Hees *et al.*, 1996). LACAs are also thought to exert an important role on the natural acidity of soils, which is not negligible (Devêvre *et al.*, 1996; Slattery *et al.*, 1998).

Some studies have shown that low-molecular-weight

<sup>1</sup>Department of Agro-Environmental Science, Obihiro University of Agriculture and Veterinary Medicine

<sup>2</sup>Institute of Applied Biochemistry, University of Tsukuba

organic acids were abundant in litter and soil extracts from podzolic soils, and these acids were thought to be concerned with the podzolization process (Fox and Comerford, 1990; Lundström, 1993; van Hees *et al.*, 2000; Bergelin *et al.*, 2000). Fox and Comerford (1990) have reported that oxalate concentrations in soil solutions of Spodosols were greater in the Bh and Bt horizons than in the A horizon. The total amount of LACAs in the soil-water extract of a Typic Podzolic soil in Japan was much larger in the O horizon than in the Bh horizon (Tani *et al.*, 1993). Thus, high concentrations of LACAs in the O horizons enriched by the litter decomposed product might be decomposed and/or immobilized, occurring in the mineral horizons under "dynamic equilibrium" conditions between production and decomposition (Huang and Violante, 1986; Lundström *et al.*, 1995; van Hees *et al.*, 2003a; 2003b).

It has been determined from field experiments that the vegetation and plant materials included in forest litter layers may play an important part in determining the amount and composition of LACAs in the mineral horizons of a Podzolic soil and Brown Forest soils (Tani *et al.*, 1993; Tani and Higashi, 1999). It is essential to prove the effect of decomposing forest litter on the distribution of LACAs in the mineral soil horizons for the further evaluation of the role of LACAs in forest ecosystems. In this study, the amount and composition of LACAs in soils incubated with five different decomposing forest litters under different water regimes were determined. The viable counts of microorganisms were also measured to examine the relationship of LACAs dynamics with soil microflora and their activities.

## MATERIALS AND METHODS

### *Soil and decomposing forest litter samples*

The soil sample used for the incubation experiment was collected from the surface 10 cm of a fallow upland soil. The soil was a light-colored Andosol (Umbric-Dystric Andosols in WRB system, 1998), collected at the Agricultural and Forestry Research Center, University of Tsukuba, Ibaraki Prefecture, Japan. The soil sample was air-dried and passed through a 2-mm sieve. The decomposing forest litter samples were collected from the Oi, Oe and Oa layers of a Typic Podzolic soil, which has a thick mor-type humus layer and supports a coniferous forest (Haplic Podzols in WRB system, 1998), and from the Oe layers of two Brown Forest soils, which are found under almost the same vegetation and climatic conditions, but are derived from different parent materials (one from graywacke and another from volcanic ash). They have relatively thin, mull-type humus layers and support a typical deciduous forest (Dystric Cambisols and Fulvic Andosols in WRB system, 1998). A detailed description of these soil profiles and their vegetation were given in previous papers (Tani *et al.*, 1993; Tani and Higashi, 1999). The forest litter samples were ground in a coffee grinder and passed through a 0.5-mm sieve. Their total carbon and nitrogen contents are shown in Table 1. The C/N ratios of the Podzolic soil humus layers were higher in the upper Oi layer than in the lower Oa layer, reflecting the decomposition degrees of forest floor leaves and roots. The C/N ratios of the two Brown Forest soil Oe layers resembled each other, and were lower than the Podzolic soil humus layers.

**Table 1.** Properties of the decomposing forest litter samples.

Soil type	Vegetation	Layer	Depth (cm)	T-C <sup>a</sup> T-N <sup>a</sup>		C/N ratio
				(g kg <sup>-1</sup> )		
Podzolic soil [Haplic Podzols] <sup>b</sup>	Coniferous forest	Oi	+21 - +14	513	14.8	34.6
		Oe	+14 - +4	500	15.6	32.1
		Oa	+4 - 0	461	16.6	27.7
Brown Forest soil 1 [Dystric Cambisols]	Deciduous forest	Oe	+4 - 0	427	17.9	23.9
Brown Forest soil 2 [Humic Cambisols]	Deciduous forest	Oe	+5 - 0	474	20.3	23.4

<sup>a</sup> Total carbon and nitrogen determined by dry combustion method.

<sup>b</sup> WRB system (FAO, 1998)

### *Incubation experiment*

Air-dried soil samples equivalent to 50 g of oven-dried soil were placed in polypropylene vessels (500 ml) and mixed with 2.5 g of each of the forest litter samples. Each vessel was watered with ultra-pure water to maintain the moisture

content of the soil sample at approximately 60 % or 80 % of the maximum water-holding-capacity (WHC). Vessels containing soil samples incorporated with decomposing forest litter were capped with aluminum foil bored to make a small air hole, and were then left in an incubator for 4 weeks

Low-molecular-weight aliphatic carboxylic acids in soils incubated with decomposing forest litter at 28 °C. Every week, ultra-pure water was added to each vessel to maintain the initial water content. The soil samples were served for the analysis of LACAs at 0, 1, 2 and 4 weeks after incubation. Overnight pre-incubation was conducted as for the 0 week's samples. Duplicate vessels on a reduced scale were also prepared to investigate the changes in the viable counts of bacteria and fungi at the same time. Over five kinds of forest litter samples were mixed and incubated at 60 % of the maximum WHC. To investigate the effect of the moisture content on the amounts and composition of LACAs, three kinds of Oe layer samples of each soil type were added at 80 % of the maximum WHC. Both incubation experiments under the different moisture conditions included blank soil samples that were not incorporated with any forest litter samples.

#### ***LACAs analysis***

Phosphate buffer (pH 2) was used to extract the water-soluble LACAs fraction and the weakly adsorbed fraction (Tani *et al.*, 1993). Incubated soil was extracted with ammonium phosphate buffer (pH 2), which was added to each vessel so as to obtain a combined total volume of added extractants and a moisture level reaching 200 ml. The procedures used to obtain the crude extract and concentrate, and to purify the extracted solution, were the same as those used by Tani *et al.* (1993), where ten kinds of LACAs, namely formic acid, acetic acid, propionic acid, butyric acid, lactic acid, oxalic acid, fumaric acid, succinic acid, malic acid, and citric acid were identified and determined by HPLC.

#### ***Viable counts of bacteria and fungi***

The viable counts of microorganisms were determined by the dilution-plate method as an index of microbial activity. Egg-albumin agar medium and rose-bengal agar medium were used for the counts of bacteria and fungi, respectively (Committee of Soil Microorganism Research, 1977).

## **RESULTS**

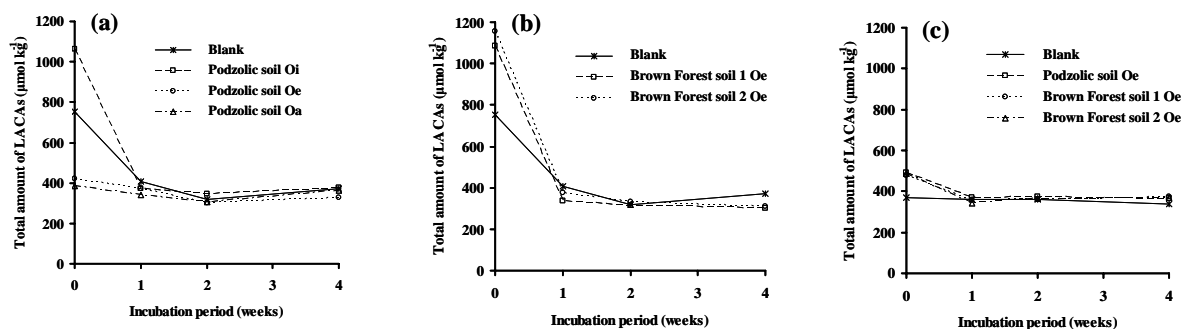
#### ***Amount and composition of LACAs***

The amounts of LACAs in the incubated soil samples, where the soil moisture content was maintained at 60 % of the maximum water-holding-capacity (WHC), as obtained by the extraction with a phosphate buffer solution are given in Table 2. Formic acid, acetic acid, and lactic acid were

dominant among the volatile monocarboxylic acids in almost all the soil samples. The amounts of formic acid ranged from 79.6 to 122.8  $\mu\text{mol kg}^{-1}$ , being not so changeable throughout the incubation period as those of the acetic acid. The amounts of acetic acid were quite variable, ranging from 33.3 to 712.7  $\mu\text{mol kg}^{-1}$ . These amounts were generally larger at the beginning of incubation, and were drastically reduced after 1 week in the soils incubated with forest litter from the Podzolic soil Oi and the Brown Forest soil Oe layers. Propionic acid and butyric acid were less than the other monocarboxylic acids or not detected. The nonvolatile dicarboxylic and tricarboxylic acids consisted mainly of oxalic acid, succinic acid, and citric acid. Sharp fluctuations were not observed for the amounts of oxalic acid and succinic acid during the whole period of incubation, and ranged from 39.2 to 69.6 and from 31.2 to 101.8  $\mu\text{mol kg}^{-1}$ , respectively. The pattern of changes in the amounts of citric acid was relatively similar to that of the acetic acid. They were the largest in the soil pre-incubated soils at 0 week, decreasing abruptly after 1 week, and gradually after 2 or 4 weeks.

The total amounts of LACAs in the blank soils and the incubated soils with forest litter from the Podzolic soil Oi and the Brown Forest soil Oe layers were considerably larger at the beginning of incubation at 0 week, since they contained large amounts of acetic acid and citric acid (Fig. 1a and 1b; Table 2). Almost all soil samples incubated at 60 % of the maximum WHC revealed a similar range of the total amounts of LACAs at 1 week. At the end of 4 weeks of incubation, the total LACAs in the incubated soils with forest litter from the Podzolic soil organic layers slowly increased like in the blank soil, while those from the Brown Forest soil Oe layers continued to gradually decrease.

The amounts of LACAs in the incubated soils, where the soil moisture contents were maintained at 80 % of the maximum WHC, are given in Table 3. Like in Table 2, formic acid, acetic acid, and lactic acid predominated among the volatile monocarboxylic acids in almost all of the soils. The amounts of both formic acid and acetic acid were not as variable throughout the whole incubation period, compared to those in Table 2, and ranged from 85.8 to 124.8 and from 45.0 to 68.6  $\mu\text{mol kg}^{-1}$ , respectively. The amount of acetic acid was not always larger at the beginning of the incubation at 80 % of the maximum WHC. The amount of oxalic acid, which was one of the major nonvolatile acids, ranged from



**Fig. 1.** Changes in the total amounts of LACAs in soils incubated with or without decomposing forest litter of (a) Podzolic soil (Oi, Oe and Oa layers), and (b) Brown Forest soils (Oe layers) at 60 % of maximum water-holding-capacity, and (c) Podzolic soil and Brown Forest soils (Oe layers) at 80 % of maximum water-holding-capacity.

**Table 2.** LACAs in the incubated soil samples where moisture content was maintained at 60% of maximum water-holding capacity.

Soil samples	Incubation periods (weeks)	LACAs ( $\mu\text{mol kg}^{-1}$ )									
		Formic acid	Acetic acid	Propionic acid	Butyric acid	Lactic acid	Oxalic acid	Fumaric acid	Succinic acid	Malic acid	Citric acid
Blank	0	116.0	350.3	9.3	Tr. <sup>b</sup>	35.2	50.9	0.7	48.8	3.6	137.1
	1	114.2	105.3	N.D.	Tr.	17.3	46.5	0.4	37.6	3.9	81.6
	2	84.2	50.4	N.D. <sup>a</sup>	Tr.	14.2	54.8	0.2	34.2	2.0	77.1
	4	105.9	69.7	N.D.	N.D.	15.6	69.6	0.3	31.2	2.7	78.9
Podzolic soil Oi	0	122.8	556.6	26.1	Tr.	47.7	51.5	1.2	82.1	2.8	170.1
	1	104.7	70.9	14.4	Tr.	14.6	41.7	0.5	37.4	3.8	82.2
	2	85.5	71.8	14.6	Tr.	15.7	44.2	0.3	37.5	1.9	73.7
	4	112.4	72.8	10.8	Tr.	15.5	60.6	0.3	35.5	2.1	67.3
Podzolic soil Oe	0	91.5	49.8	8.0	Tr.	22.1	50.0	0.4	62.1	1.2	134.5
	1	108.5	64.8	10.7	Tr.	13.9	49.9	0.4	43.0	4.1	77.0
	2	83.4	44.5	N.D.	Tr.	13.6	47.4	0.2	38.8	1.9	71.9
	4	94.7	54.6	9.1	N.D.	13.3	58.7	0.3	41.7	2.3	54.9
Podzolic soil Oa	0	88.0	44.9	N.D.	Tr.	17.7	45.9	0.3	51.6	1.5	134.1
	1	102.0	74.1	N.D.	Tr.	15.2	39.2	0.4	35.4	3.8	72.6
	2	85.1	39.6	N.D.	Tr.	13.8	49.5	0.3	41.2	1.9	73.5
	4	93.7	47.8	N.D.	N.D.	12.2	66.6	0.3	87.1	2.0	51.9
Brown Forest soil 1 Oe	0	119.0	662.0	Tr.	Tr.	26.9	40.5	1.0	92.7	2.0	139.6
	1	100.1	53.2	N.D.	Tr.	12.7	45.4	0.4	63.8	3.1	59.0
	2	97.4	41.0	N.D.	Tr.	9.7	46.4	0.3	59.5	3.3	57.7
	4	82.2	39.7	N.D.	N.D.	10.5	56.9	0.2	53.8	2.3	53.8
Brown Forest soil 2 Oe	0	118.2	712.7	Tr.	Tr.	32.0	44.2	1.1	101.8	3.0	141.7
	1	109.2	57.8	N.D.	N.D.	13.1	45.0	0.5	82.5	3.6	64.4
	2	98.7	43.4	N.D.	N.D.	11.2	49.7	0.3	62.3	2.6	62.3
	4	79.6	33.3	N.D.	N.D.	7.3	59.7	0.2	79.9	1.7	46.3

<sup>a</sup>N.D., no peak detected; <sup>b</sup>Tr., trace amount

**Table 3.** LACAs in the incubated soil samples where moisture content was maintained at 80% of maximum water-holding capacity.

Soil samples	Incubation periods (weeks)	LACAs ( $\mu\text{mol kg}^{-1}$ )									
		Formic acid	Acetic acid	Propionic acid	Butyric acid	Lactic acid	Oxalic acid	Fumaric acid	Succinic acid	Malic acid	Citric acid
Blank	0	100.1	46.9	N.D. <sup>a</sup>	Tr. <sup>b</sup>	11.6	54.9	0.3	60.8	2.6	90.9
	1	106.9	56.6	N.D.	Tr.	11.7	49.4	0.4	69.4	3.2	63.0
	2	102.9	62.7	N.D.	N.D.	5.5	51.2	0.4	67.8	3.4	66.2
	4	85.8	45.8	N.D.	N.D.	10.1	70.6	0.2	68.5	2.6	54.5
Podzolic soil Oe	0	111.4	68.6	N.D.	Tr.	22.6	54.1	0.4	107.4	2.6	123.0
	1	107.6	65.3	N.D.	N.D.	14.8	49.1	0.4	68.6	2.6	63.0
	2	107.1	50.9	N.D.	N.D.	6.2	54.6	0.4	82.5	3.8	67.7
	4	108.0	57.1	N.D.	N.D.	7.9	62.0	0.3	68.1	3.8	60.0
Brown Forest soil 1 Oe	0	118.9	45.0	N.D.	Tr.	16.4	57.0	0.4	119.0	3.0	117.3
	1	107.3	45.4	N.D.	Tr.	12.6	46.7	0.4	74.7	3.5	64.4
	2	99.7	45.2	N.D.	Tr.	11.0	57.7	0.4	80.7	3.1	61.3
	4	105.0	53.7	N.D.	N.D.	6.6	75.5	0.4	63.1	3.3	67.6
Brown Forest soil 2 Oe	0	124.8	46.4	N.D.	Tr.	14.0	62.7	0.5	122.0	2.5	113.0
	1	103.9	45.8	N.D.	N.D.	11.5	45.6	0.4	75.9	3.0	58.3
	2	104.5	59.0	N.D.	N.D.	6.9	50.7	0.4	77.1	3.4	63.0
	4	103.6	48.9	N.D.	N.D.	6.5	71.8	0.4	68.8	3.4	65.4

<sup>a</sup>N.D., no peak detected; <sup>b</sup>Tr., trace amount

Low-molecular-weight aliphatic carboxylic acids in soils incubated with decomposing forest litter

45.6 to 75.5  $\mu\text{mol kg}^{-1}$ , and was always larger in the soils incubated for 4 weeks than in those at the beginning of incubation, 0 week. In contrast, the amounts of abundant succinic acid and citric acid were larger in the 0 week incubated soils, and decreased at 1 week. In most of the incubated soils, the amounts of succinic acid were relatively larger than those of citric acid, which differed from the results in Table 2. Thus, different soil moisture regimes can alter the balance between the production and decomposition of LACAs, resulting in different compositions of LACAs.

The total amounts of LACAs in the blank soil incubated at 80 % of the maximum WHC barely changed during the period of incubation (Fig. 1c). The pattern of changes in the total amounts of LACAs in the incubated soils with decomposing forest litter closely resembled each other. They were slightly larger at the beginning of incubation 0 week. They decreased after 1 week, and remained stable until 4 weeks. The difference between the incubated soils in the amended forest litter was slight.

**Effect of decomposing forest litter and soil moisture regime**

It was suggested that the "dynamic equilibrium" conditions under the production and decomposition of LACAs had become stable in an earlier stage of incubation. As a wide variation in the total amounts of LACAs and each individual acid was observed in the pre-incubated soils at 0 week (Fig. 1), the average amounts of the predominant five LACAs, which were formic acid, acetic acid, oxalic acid, succinic acid and citric acid from 1 week to 4 weeks were compared to assess the effect of decomposing forest litters and soil moisture regimes on the amounts and composition of LACAs.

The amounts of formic acid ranged from 93.2 to 107.6  $\mu\text{mol kg}^{-1}$ , and were similar to each other (Fig. 2a). There was a slightly larger amount of formic acid in the blank soil than in the soils incubated with decomposing forest litters at 60 % of the maximum WHC, while the amended soils contained larger amounts of formic acid to some extent at 80 % of the maximum WHC. The average amounts of acetic acid were relatively lower than those of formic acid, ranging from 44.6 to 75.1  $\mu\text{mol kg}^{-1}$  (Fig. 2b). At 60 % of the maximum WHC, the largest amount was observed in the blank soil. The soils incubated with forest litter from the Podzolic soil contained larger amounts of acetic acid than those from the two Brown Forest soils. Among the forest

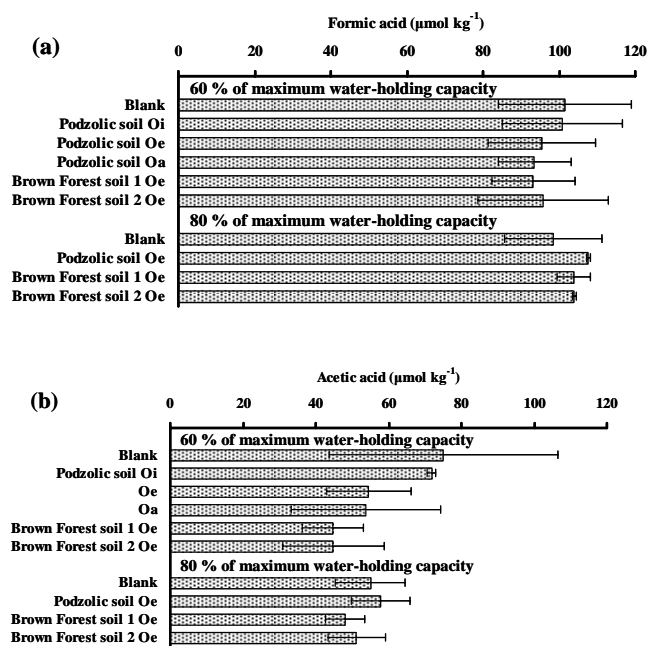


Fig. 2. Average amounts of (a) formic acid and (b) acetic acid in soils incubated for the period of 1 to 4 weeks.

litter collected from three organic layers of the Podzolic soil, the amount of acetic acid decreased from the Oi to Oa layer, being especially larger in the soil amended with the Oi layer litter. The soils incubated with intermediately decomposing forest litter obtained from three Oe layers contained a relatively larger amount of acetic acid at 80 % of the maximum WHC than at 60 %.

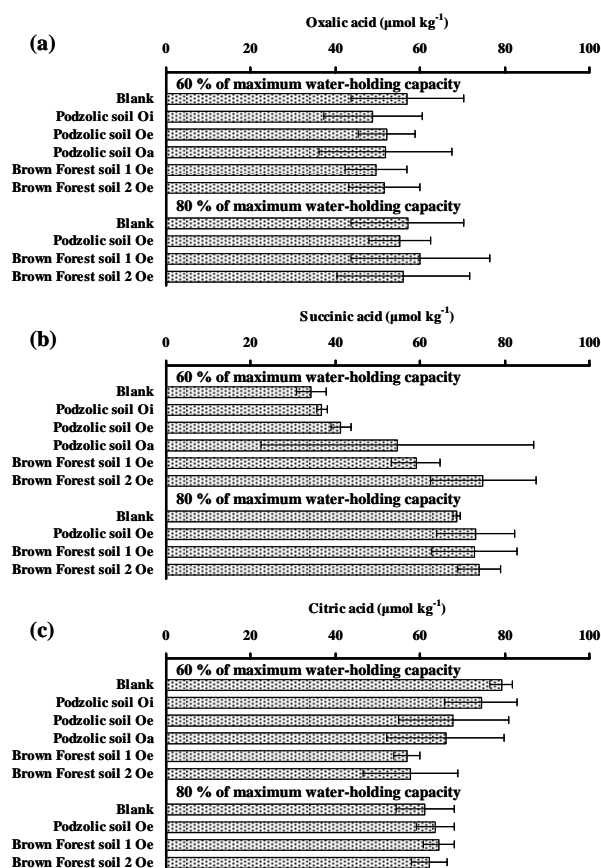


Fig. 3. Average amounts of (a) oxalic acid, (b) succinic acid and (c) citric acid in soils incubated for the period of 1 to 4 weeks.

The amounts of oxalic acid ranged from 48.8 to 60.0  $\mu\text{mol kg}^{-1}$ , and not much difference was observed between each soil sample (Fig. 3a). The amount of oxalic acid in the soils incubated with forest litter at 80 % of the maximum WHC was slightly larger than at 60 %. The effect of the decomposing forest litter addition on the amounts of succinic acid at 60 % of the maximum WHC was obviously observed, being smallest in the blank soil (34.3  $\mu\text{mol kg}^{-1}$ ), and larger in the soils incorporated with the forest litter from the Podzolic soil Oa layer and the Brown Forest soil Oe layers (54.6 - 74.9  $\mu\text{mol kg}^{-1}$ ; Fig. 3b). Among the forest litter layers, the amounts of succinic acid were larger in the soils incubated with the Brown Forest soil Oe layers than the Podzolic soil organic layers, and they increased from the Oi to Oa layer of the Podzolic soil. While at 80 % of the maximum WHC the amounts of succinic acid were generally larger than at 60 %, there was little difference between the blank soil and the incubated soils with forest litter (Fig. 3b). The average amounts of citric acid were comparatively similar to those of succinic acid, ranging from 57.7 to 79.2

$\mu\text{mol kg}^{-1}$  (Fig. 3c), although the effect of the addition of decomposing forest litter at 60 % of the maximum WHC was in sharp contrast to that of succinic acid. Namely, the blank soil was the largest in the whole soil samples; the soils added with forest litter from the Podzolic soil contained larger citric acid than from the Brown Forest soils, and the amounts of citric acid decreased from the Oi to Oa layer of the Podzolic soil. At 80 % of the maximum WHC, a great difference in the amounts of citric acid was not found.

The average amounts of total LACAs in the soils incubated with the intermediately decomposing forest litter of Oe layers from the Podzolic soil, and the Brown Forest soil 1 and 2, at 60 % of the maximum WHC, for a period from 1 to 4 weeks (i.e. without 0 week), were 335, 318 and 338  $\mu\text{mol kg}^{-1}$ , respectively. Those incubated at 80 % of the maximum WHC were larger, being 371, 363 and 359  $\mu\text{mol kg}^{-1}$ , respectively, so that 6.3 to 14.4 percent increases were observed when the soils were incubated with forest litter under slightly wet conditions.

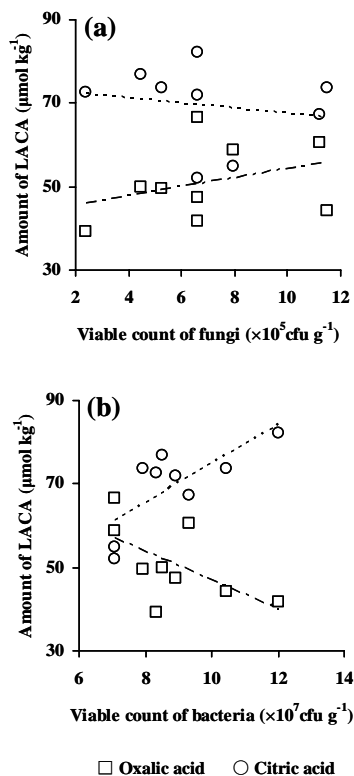
## DISCUSSION

The total amounts of LACAs in the whole soil samples were larger at the beginning of incubation (0 week), abruptly decreasing after only 1 week and remaining relatively unchanged in the latter half of incubation (2 and 4 weeks). These patterns were consistent with those of dissolved organic carbon biodegradation in the forest floor leachates obtained in a beech - fir mixed forest (Boissier and Fontvielle, 1993). The LACAs are assimilated by various heterotrophic microorganisms, and so may occur in soils under dynamic equilibrium conditions between their production and decomposition (Huang and Violante, 1986; Lundström *et al.*, 1995). Almost all soil samples revealed a similar range of the total LACAs after 1 week, suggesting that the production and decomposition of LACAs in the incubated soils had already equilibrated in a short period.

The addition of decomposing forest litter caused a negative effect on the amounts of acetic acid and citric acid under the moderately moist conditions. Their reductions were more remarkable in the soils in which the forest litter from the Brown Forest soils was incorporated. Among the Podzolic soil organic layers, the soil added with the Oi layer contained slightly larger amounts of acetic acid and citric acid than the Oe and Oa layers. It is inferred from these results that the C/N ratio of the decomposing forest litter will

Low-molecular-weight aliphatic carboxylic acids in soils incubated with decomposing forest litter

be relevant to the balance between the production and decomposition of acetic acid and citric acid. In contrast, the addition of decomposing forest litter, especially that obtained from the Podzolic soil Oa layer and the Brown Forest soil Oe layers, leads to an increase in the amount of succinic acid.



**Fig. 4.** Correlation of viable counts of (a) fungi and (b) bacteria with amounts of oxalic acid and citric acid in soils incubated with decomposing forest litter obtained from Podzolic soil organic layers at 60 % of maximum water-holding-capacity for the period of 1 to 4 weeks.

Thus, the addition of decomposing forest litter could induce changes in the amount and composition of LACAs in the soils, as Alin *et al.* (1997) suggested when they pointed out that the amendment of plant residues also resulted in similar changes. It is expected that microbial activities will relate to these changes. In Fig. 4 you can see a diagram which shows the relationship between viable counts of microorganisms and the amounts of oxalic acid and citric acid in the soils incubated with the forest litter of the Podzolic soil at 60 % of the maximum WHC from 1 to 4 weeks. As for oxalic acid, the positive correlation with viable counts of fungi and the negative correlation with those of bacteria were found, despite the fact that they were not always statistically significant. However, just the opposite relationship was observed in citric acid. When these parameters were tested with multiple regression analysis, a statistically significant correlation was observed as below:

$$A_{\text{oxa}} = 1.86 \times 10^{-5} C_f - 4.51 \times 10^{-7} C_b + 77.9 \quad (r^2 = 0.665, p = 0.038)$$

$$A_{\text{cit}} = -1.60 \times 10^{-5} C_f + 5.62 \times 10^{-7} C_b + 30.8 \quad (r^2 = 0.761, p = 0.014)$$

$A_{\text{oxa}}, A_{\text{cit}}$  : Amounts of oxalic acid and citric acid ( $\mu\text{mol kg}^{-1}$ ), respectively.

$C_f, C_b$  : Viable counts of fungi and bacteria ( $\text{cfu g}^{-1}$ ), respectively.

For example, the positive regression coefficient between the amounts of oxalic acid and viable counts of fungi ( $1.86 \times 10^{-5}$ ) suggests that fungi contribute to the production of oxalic acid in the soil environment, as pointed out by many researchers (Graustein *et al.*, 1977; Cromack *et al.*, 1979; Malajczuk and Cromack, 1982; van Hees *et al.*, 2003a; 2003b).

Larger amounts of LACAs, especially formic acid, succinic acid, and citric acid, accumulated in the soils incubated with decomposing forest litter under the slightly wet moisture condition (at 80 % of the maximum WHC). Since viable counts of fungi were generally lower at 80 % of the maximum WHC than at 60 %, it was supposed that the decomposition of LACAs by fungi could be suppressed, and would result in the change of balance between the production and decomposition of LACAs.

Two types of decomposing forest litter collected from two subtypes of the Brown Forest soils, which are about 200 m apart and under the almost same vegetation and climatic condition, however from distinctly different parent materials, had nearly similar effects on the amount and composition of LACAs. Three types of decomposing forest litter obtained from the Oi, Oe and Oa layers of the Podzolic soil, which are substantially different in their decomposition degree and C/N ratio, induced several changes in the composition of LACAs under experimental conditions.

As Fox (1995) suggested, factors affecting the types and amounts of low molecular weight organic acids present in the soil include the vegetation present, soil type, depth in the profile, proximity to roots, and soil aeration. We conclude that the decomposing forest litter derived from the vegetation present, accompanying soil microflora and their activity, and the soil moisture conditions, which control the balance between production and decomposition of LACAs in soils, exert a great influence on the amounts and composition of LACAs in forest soils.

## REFERENCES

- Alin, S., Xueyuan, L., Kanamori, T., Ono, S., and Arai, T. 1997: Low-molecular-weight aliphatic acids in soils incubated with plant residues under different moisture conditions. *Pedosphere*, **7**, 79-86
- Bergelin, A., van Hees, P. A. W., Wahlberg, O., and Lundström, U. S. 2000: The acid-base properties of high and low molecular weight organic acids in soil solution of podzolic soils. *Geoderma*, **94**, 223-235
- Boissier, J. M. and Fontvieille, D. 1993: Biodegradable dissolved organic carbon in seepage waters from two forest soils. *Soil Biol. Biochem.*, **25**, 1257-1261
- Committee of Soil Microorganism Research 1977: Methods for counting microorganisms in soils. In *Experimental Methods for Soil Microorganisms*, Eds. T. Suzuki *et al.*, p. 21-42, Yokendo LTD., Tokyo (in Japanese)
- Cromack, Jr. K., Sollins, P., Graustein, W. C., Speidel, K., Tood, A. W., Spycher, G., Li, C. Y., and Tood, R. L. 1979: Calcium oxalate accumulation and soil weathering in mats of the hypogeous fungus *Hysterangium Grassum*. *Soil Biol. Biochem.*, **11**, 463-468
- Devêvre, O., Garbaye, J., and Botton, B. 1996: Release of complexing organic acids by rhizosphere fungi as a factor in Norway spruce yellowing in acidic soils. *Mycol. Res.*, **100**, 1367-1374
- FAO 1998: World Reference Base for Soil Resources, FAO, ISRIC and ISSS, Rome
- Fox, T. R. 1995: The influence of low-molecular-weight organic acids on properties and processes in forest soils. In *Carbon Forms and Functions in Forest Soils*, Eds. W. W. McFee and J. M. Kelly, p. 43-62, SSSA, Madison, WI
- Fox, T. R. and Comerford, N. B. 1990: Low-molecular-weight organic acids in selected forest soils of the southeastern USA. *Soil Sci. Soc. Am. J.*, **54**, 1139-1144
- Graustein, W. C., Cromack, Jr. K., and Sollins, P. 1977: Calcium oxalate: Occurrence in soils and effect on nutrient and geochemical cycles. *Science*, **198**, 1252-1254
- van Hees, P. A. W., Anderson, A-M. T., and Lundström, U. S. 1996: Separation of organic low molecular weight aluminium complexes in soil solution by liquid chromatography. *Chemosphere*, **33**, 1951-1966
- van Hees, P. A. W., Lundström, U. S., and Giesler, R. 2000: Low molecular weight organic acids and their Al-complexes in soil solution – composition, distribution and seasonal variation in three podzolized soils. *Geoderma*, **94**, 173-200
- van Hees, P. A. W., Godbold, D. L., Jentschke, G., and Jones, D. L. 2003a: Impact of ectomycorrhizas on the concentration and biodegradation of simple organic acids in a forest soil. *Eur. J. Soil Sci.*, **54**, 697-706
- van Hees, P. A. W., Jones, D. L., and Godbold, D. L. 2003b: Biodegradation of low molecular weight organic acids in a limed forest soil. *Water, Air, Soil Pollut. Focus*, **3**, 121-144
- Huang, P. M. and Violante, A. 1986: Influence of organic acids on crystallization and surface properties of precipitation products of aluminum. In *Interaction of Soil Minerals with Natural Organics and Microbes*, SSSA Spec. Publ. 17, Eds. P. M. Huang and M. Schnitze, p. 159-221, SSSA, Madison, WI
- Lundström, U. S. 1993: The role of organic acids in the soil solution chemistry of a podzolized soil. *J. Soil Sci.*, **44**, 121-133
- Lundström, U. S., van Breemen, N., and Jongmans, A. G. 1995: Evidence for microbial decomposition of organic acids during podzolization. *Eur. J. Soil Sci.*, **46**, 489-496
- Malajczuk, N. and Cromack, Jr. K. 1982: Accumulation of calcium oxalate in the mantle of ectomycorrhizal roots of *Pinus radiata* and *Eucalyotus marginata*. *New Phytol.*, **92**, 527-531
- Pohlman, A. A. and McColl, J. G. 1988: Soluble organics from forest litter and their role in metal dissolution. *Soil Sci. Soc. Am. J.*, **52**, 265-271
- Slattery, W. J., Edwards, D. G., Bell, L. C., Coventry, D. R., and Helyar, K. R. 1998: Soil acidification and the carbon cycle in a cropping soil of north-eastern Victoria. *Aust. J. Soil Res.*, **36**, 273-290
- Stevenson, F. J. 1967: Organic acids in soil. In *Soil Biochemistry*, Vol. 6, Eds. A. D. McLaren and G. H. Peterson, p. 119-146, Marcel Dekker, NY
- Tam, S. C. and McColl, J. G. 1991: Aluminum-binding ability of soluble organics in Douglas fir litter and soil. *Soil Sci. Soc. Am. J.*, **55**, 1421-1427
- Tani, M., Higashi, T., and Nagatsuka, S. 1993: Dynamics of low-molecular-weight aliphatic carboxylic acids (LACAs) in forest soils: I. Amount and composition of



LACAs in different types of forest soils in Japan. *Soil Sci. Plant Nutr.*, **39**, 485-495

Tani, M. and Higashi, T. 1999: Vertical distribution of low-molecular-weight aliphatic carboxylic acid (LACAs) in some forest soils of Japan. *Eur. J. Soil Sci.*, **50**, 217-226

## 摘 要

ポドゾル性土の0i層, 0e層および0a層, 母材が異なる2種類の褐色森林土の0e層から採取した森林リター分解物を土壤に添加し, 水分含量を最大容水量の60%(適潤条件)および80%(加湿条件)に調整して4週間の培養を行った。森林リター分解物の種類や水分条件などが低分子脂肪酸カルボン酸(LACA)の量と組成に及ぼす影響を調べた。LACA総量は培養開始時に極めて高く(384~1155  $\mu\text{mol kg}^{-1}$ ), 1週間後には急激に減少した。培養1~4週間の間は, LACA総量の変動が比較的小さかった(299~377  $\mu\text{mol kg}^{-1}$ )。培養開始時を除く期間中におけるLACA量の平均値は, とくに酢酸とクエン酸について, 森林リター分解物を添加し適潤条件で培養した土壤において, リター無添加土壤よりも低かった。一方, 加湿条件で培養した場合には, リターを添加した土壤でギ酸, コハク酸およびクエン酸が増加した。土壤水分条件と添加した森林リター分解物の違いは土壤微生物相とその活性に変化を引き起こし, 土壤中におけるLACAの生産と分解の平衡状態に影響を及ぼした。母材が異なる2種類の褐色森林土は, 植生および気候条件がほぼ同一であり, これらの0e層から採取したリター分解物が培養土壤中のLACAの量および組成に及ぼす影響に違いは認められなかった。一方, ポドゾル性土0層から採取した分解程度が異なる3種類のリター分解物は, 培養土壤中のLACA組成に影響を及ぼした。本研究の結果より, 森林リター分解物の分解程度, 土壤水分条件, それらの違いに起因する土壤微生物のフローラと活性は, 森林土壤における低分子脂肪酸カルボン酸の量と組成に重要な影響を及ぼしていることが明らかとなった。