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The Rate of Microbial Decomposition of Soil Humic Compounds: the Effect of Acid Rain Induced Al Depletion

Running head: Acid Rain and the Decomposition of Humic Substances

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Abstract

The effect of aluminum complexation on the net decomposition rate of purified humic acid (HA) and fulvic acid (FA), extracted from a podzol B horizon in the Netherlands, were studied at room temperature (20°C). Net decomposition rates were determined from CO_2 evolution from samples as measured by gas-chromatography. Increasing aluminum additions significantly reduced the decomposition rate of HA, of FA ,and of organic matter in podzol B horizons, whereas pH had no notable effect on decompostion rates. Results indicate that at the current high depletion rates of organically-bound soil Al, due to acid rain, the decomposition of soil humic compounds may increase significantly. This may have serious consequences for the ecologically relevant pool of humic compounds in forest soils.

Keywords: aluminum depletion, decomposition of humic substances, soil organic matter, fulvic acid, humic acid.

Introduction

In acidic sandy soils, poor in weatherable base cations acid atmospheric deposition is predominately neutralized in mineral soil by the dissolution of organically-bound aluminum [14, 15]. For example, Mulder *et al.* [15] reported that at the current rate of acidification the organically-bound pool of Al in Dutch forests soils could be depleted significantly within five to ten decades. So far, investigations have focused on Al toxicity, while considerably less attention has been paid to the effect of Al depletion on the stability of organic soil matter. For example, when associated with the depletion of organically bound soil Al, humic substances (HS) may solubilize [13]. Alternatively, a decrease in complexed metal ions (e.g. Al) by HS may increase the rate of microbial decomposition of HS [11].

Humic substances are relatively resistant materials with decomposition rates of 1-2 % during a 28-week period [11]. Earlier, Martin *et al.* [10] showed that the complexes of polysaccharides (as produced by *Azotobacter chrococcum*, *Azotobacter indicus*, *Arthrobacter viscosus*, *Chromobacterium violaceum*, *Bacterium sp.*, and *Karaya* gum with metal cations (Zn, Cu, Fe, Al)) reduced their biodegradation rate in soils. Specific effects of the metals differed for the individual polysaccharides. Carballas *et al.* [5] observed that Al additions decrease mineralization rates of glucose and maize straw, thus giving rise to accumulation of soil organic matter. Similarly, Boudot *et al.* [2] observed a decreased decomposition rate of citric acid and melanin (used as a model for HS) after the addition of Al and Fe. By contrast, the microbial decay of lactic acid was not affected by Al complexation [1].

Increased decomposition rates of HS, as a result of acid rain induced Al leaching, may result in decreasing amounts of soil humus, thus having negative effects on the ecological functioning of the soil. For example, a reduction in soil

HS may result in a decrease of the Cation Exchange Capacity (CEC), thereby limiting the capacity of an ecosystem to store nutrient cations. A loss of organically-based CEC is particularly relevant in sandy soils, which are low in permanent charge. A decrease of soil humus may also result in increased mobilization of soil N [24].

The purpose of this study is to determine

(1) to what extent Al depletion affects the microbial decomposition of soil HS, including HA and FA, and

(2) to evaluate the influence of pH and various Al additions on the decomposition rate of soil organic matter in a podzol B-horizon.

We focus in particular on the decomposition of HA and FA, because these compounds make up 65-75 % of the soil organic matter in podzol B horizons [18]. Both HA and FA are able to form complexes with metal ions (e.g. Al) due to their high content of oxygen-containing groups, mainly COOH, OH and C=O (Table 1) [18, 19, 23]. A podzol B horizon from the Netherlands has been used as a model of acidic, base poor, sandy forest soil with a relatively high content of organically-bound Al [15].

Table 1. Analytical characteristic of model humic (HA) and fulvic (FA) acids [19]

Element	HA (%)	FA (%)
C	56.2	45.7
H	4.7	5.4
N	3.2	2.1
S	0.8	1.9
O	35.5	44.8
Functional groups	cmol kg^{-1}	cmol kg^{-1}
Total acidity	670	1130
COOH	360	820
Phenolic OH	310	310
Alcoholic OH	260	500
Quinonoid C=O + Ketonic C=O	290	270
OCH_3	60	80
E_4/E_6	4.8	9.6

Material and Methods

Soils

For this study we focused on the upper 2 to 3 cm of the podzol B horizon (Bh) at Tongbersven, (near Oisterwyk, the Netherlands). Characteristics of the site have been given elsewhere [6]. Soils are Typic Haplorthods [21] and the vegetation is dominated by *Pinus sylvestris*. Atmospheric strong acid loads at the site are, on average, 4.3 kmol ha^{-1} yr^{-1} and are fully neutralized by Al dissolution in the B horizon.

Soil samples were analyzed in duplicate, after air-drying and sieving (< 2 mm). In brief, soil pH was measured potentiometrically (with a combination electrode) in a 1:5 soil to water suspension. Organic C in the soil samples was determined following the Walkley-Black method, where samples were oxidized with a mixture of potassium dichromate ($K_2Cr_2O_7$) and sulphuric acid (H_2SO_4), without external heating. Excess $K_2Cr_2O_7$ was titrated with ferrous sulphate ($FeSO_4 \cdot 7H_20$). Exchangeable Al was determined colorimetrically in a 1 M KCl extract, using pyrocatechol-violet [9]. Organically-bound Fe and Al were estimated using a 12-hour sodium pyrophosphate ($Na_4P_2O_7 \cdot 10H_2O$) extraction at a high pH (> 9). Subsequently, Fe and Al were determined by atomic absorption spectroscopy [9]. Organic C measured in the pyrophosphate extract was assumed to represent the C fraction involved in binding sesquioxides (Fe and Al). The organic matter content in the pyrophosphate extract was measured by dry combustion at 900°C for 2 hours. The molar Al/C ratio was calculated using $Na_4P_2O_7$ extractable organic C and Al, where organic carbon was assumed to be 50% of the soil organic matter content.

Soil humic substances (HA and FA) were extracted from the podzol B horizon according to an International Humic Substances Society procedure [21]. In brief, HS (HA and FA) were extracted with 0.1 M NaOH under N_2 atmosphere. Subsequently, the extract was acidified with HCl to separate HA (precipitate) and FA (dissolved). Humic acids were purified by means of HF/HCl additions, followed by dialysis and freeze drying. Fulvic acid was purified by adsorption on XAD-8, desorbed by NaOH, and reprotonated by passing it over a H^+- saturated cation-exchanger. Subsequently, FA was also freeze-dried.

Synthesis and Decomposition of Al-HA and Al-FA Complexes

For the decomposition experiments, 40 mg of HA and FA samples were dissolved in 10 ml NaOH (0.1 M) in Erlenmeyer flasks and enriched (except for the control samples) with $AlCl_3$ to obtain molar Al/C ratios of 0.01 and 0.1. Within 2 hours the alkaline extract was brought to pH 5.0 with a few drops of 1.0 M HCl. Samples were dialyzed against distilled water until free of salts (conductivity below 10 nS) and freeze-dried. We did not detect a loss of HA and FA while dializing.

Controls (HA, FA without Al added), Al-HA and Al-FA complexes were inocculated with a few drops of a distilled water extract of the Tongbersven podzol B horizon. These extracts were prepared by shaking a field fresh podzol B sample with distilled water for 5 hours. Subsequently, the extracts were filtered (Schleicher and Schuell filters) and kept at 15°C for 24 hours before use. Incubations were performed in a cool, dark room (8°C) in 130 ml flasks with an airtight rubber septum. After the 5th day the incubations were continued in the dark at room temperature (20°C). Each treatment was carried out in duplicate. The decomposition experiment was conducted for 6 weeks by measuring the concentration of CO_2 by gas-chromatography. Measurements were initially were conducted twice a week. Later, the measuring frequency was reduced to weekly (see results). Air samples were taken from the head space in the bottles with a syringe through the rubber septum. The net decomposition rate was estimated by calculating the fraction of organic C evolved in the sample as CO_2.

Table 2. Characteristics of podzol B horizon of the Tongbersven soil (The Netherlands).

PH (H_2O)	3.7
Organic carbon [% dry soil sample] ($Na_4P_2O_7$)	3.6
Organic Carbon [% dry soil sample] (Walkley Black)	4.4
Exchangeable Al [mmol (+) kg^{-1}]	30
Org. Complexed Al [mmol kg^{-1} soil]	89.8
Org. Complexed Fe [mmol kg^{-1} soil]	51.9
Al/C molar ratio	0.03

Decomposition of Podzol B Samples

Samples of the field fresh podzol B-horizon (5 g each) were enriched with different amounts of $AlCl_3$ to obtain molar Al/C ratios of 0.11 and 0.04. Al enrichment was achieved by adding drops of $AlCl_3$ to the soil suspension. Subsequently, the suspensions, were freeze-dried. The Al/C ratio of the control, i.e. without Al addition, equals 0.03 (see results). To obtain the same pH value (3.7) in all treatments, some drops of NaOH had to be added to the Al -treated samples. To study the effect of pH on the decomposition rate of organic matter, 5 g samples of a field fresh podzol B were treated with HCl or NaOH (0.1 M) to obtain pH values of 2.9, 3.3, 4.1 and 4.4 respectively. The pH of the control sample was 3.7. All decomposition experiments with podzol B samples were conducted in duplicate during a 4 week period.

Results

The Tongbersven Bh horizon was acidic, having a pH (H_2O) of 3.7 (Table 2). The content of organic C (Walkley-Black method) was 4.4%, while the $Na_4P_2O_7$ extractable organic carbon was 3.6%. This suggest that the majority of the organic matter in the podzol B horizon (more than 80%) is bound with metal. The molar Al/C ratio of 0.03 for the Bh was well within the range (0.1-0.03) commonly found for this type of Bh horizon [13]. Organically complexed Fe amounted to 51.9 mmol kg^{-1}, while complexed Al was, 89.8 mmol kg^{-1}. Exchangeable Al was, with 30 mmol kg^{-1}, only a small fraction of the pyrophosphate extractable Al.

The decomposition rate of HA and FA was low during the first eight days of the experiment, when the temperature was kept at 8°C (Figs. 1, 2). In this phase of the experiment no significant difference between control and Al-treated FA (Al/C = 0.01) ($Z = 1.00$, $p < 0.05$) and (Al/C = 0.1) ($Z = 0.371$, $p < 0.05$) nor between control and Al treated HA (Al/C = 0.01) ($Z = 0.371$, $p < 0.05$) and (Al/C = 0.1) ($Z = 0.371$, $p < 0.05$) could be detected. Three days after changing the temperature to 20°C the decomposition of HA and FA increased significantly.

The decomposition rate of the FA control was statistically significant ($Z = 0.022$, $p < 0.05$) and higher than that of the FA-Al complexes. In the FA control 5.1% of the

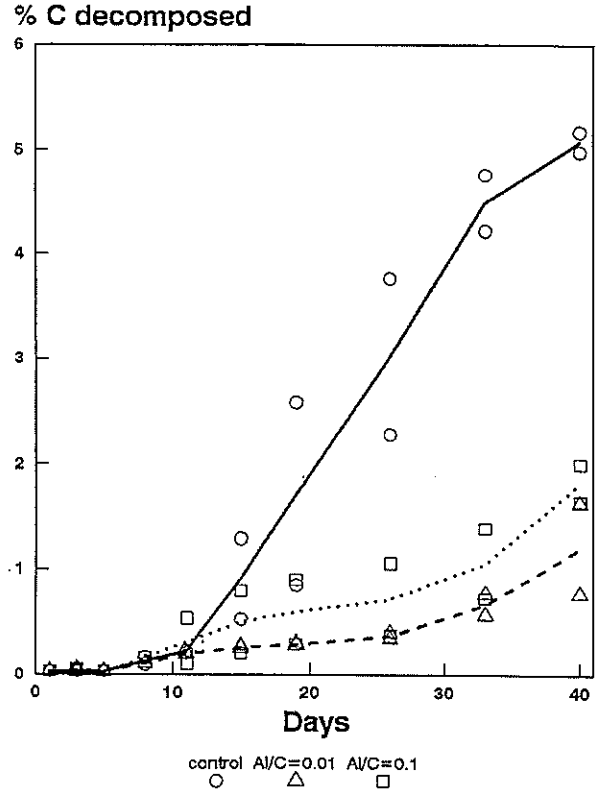


Fig. 1. The decompositon of control FA and Al - FA complexes during 40 days at 20°C

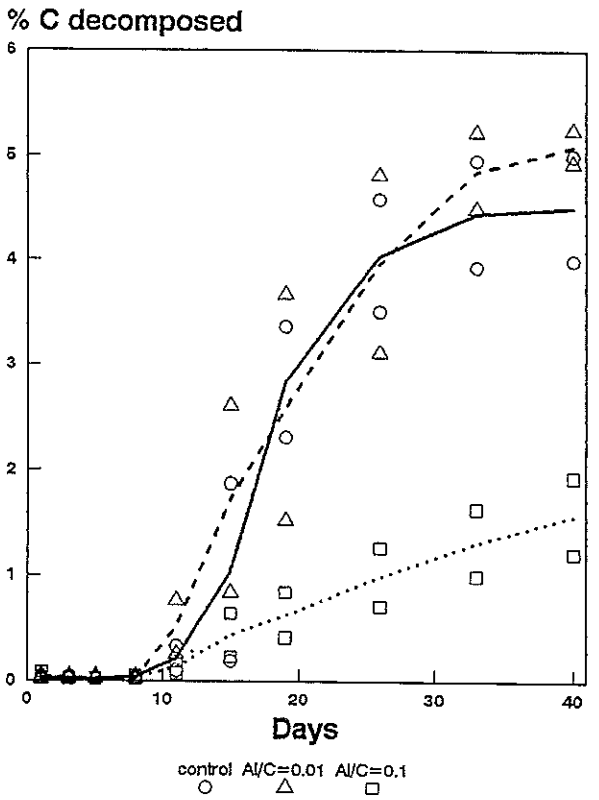


Fig. 2. The decompositon of control HA and Al - HA complexes during 40 days at 20°C

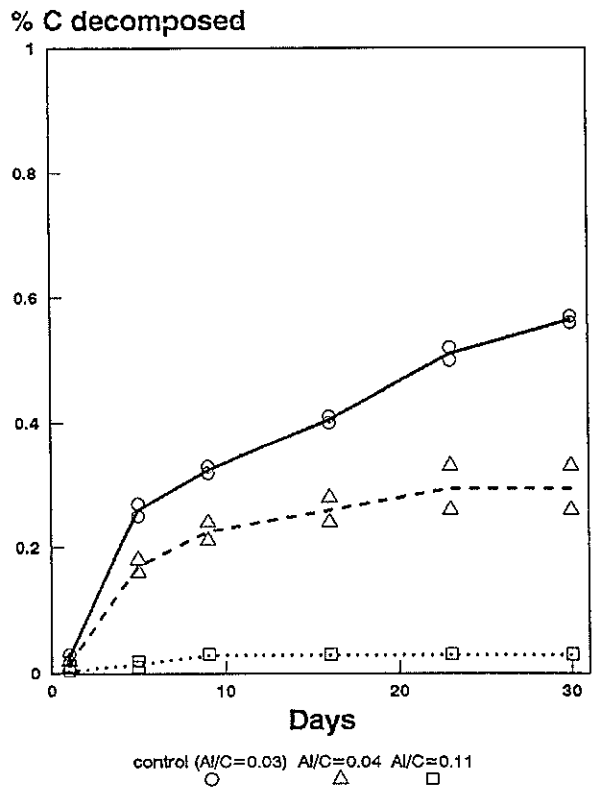


Fig. 3. The decompositon of organic matter in the podzol B horizon (Tongbersven) at different Al concentration during 30 days at 20°C

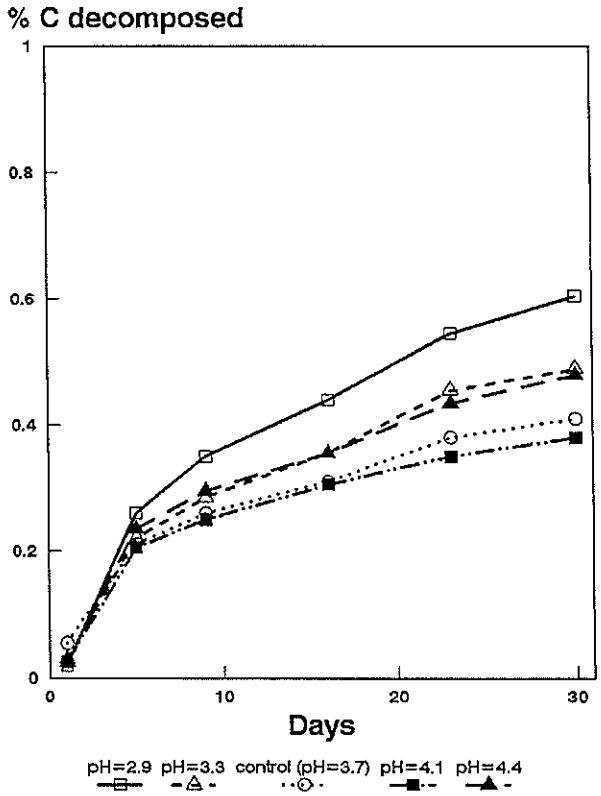


Fig. 4. Decompositon rate of organic matter in the podzol B horizon at various pH level during 30 days at 20°C

organic C was converted to CO_2 during the 40 days of the experiment, whereas for Al complexed FA these numbers were 1.8% (Al/C = 0.1) and 1.2% (Al/C = 0.01) (Fig. 1). Note, however, that CO_2 evolution in the Al/C = 0.01 and Al/C = 0.1 were not significantly different ($Z = 0.173$, $p < 0.05$).

In the HA control, 4.5% of the organic C was converted to CO_2 after 40 days, while for the Al-HA complexes this was 1.6% (Al/C = 0.1) and 5.1% (Al/C = 0.01) (Fig. 2). Note that in the case of HA there was no significant difference in decomposition between the control and the Al/C = 0.01 treatment ($Z = 0.142$, $p. < 0.05$). It should be stressed that on the 20th day of the experiment fungi started to grow in duplicates of the control HA and the Al-HA (Al/C = 0.01), as well as in the control FA (i.e. those substrates where decomposition rates were higher).

For the experiment with the podzol B (Al/C = 0.03), which was conducted at 20°C for the complete 30-day period, CO_2 production for the control amounted to 0.6% of the organic C. In the case of the Al addition only 0.3% (Al/C = 0.04) and 0.03 % (Al/C = 0.11) of the organic C was converted to CO_2 (Fig. 3). Only a higher amount of Al addition significantly decreased decomposition rate ($Z = 0.04$, $p. < 0.05$), while the case of Al/C = 0.04 did not differ significantly from the control ($Z = 0.06$, $p. < 0.05$). The decomposition rate of organic matter in podzol B samples was not affected by pH (Fig. 4) and ranged from 0.35% to 0.6% ($Z = 0.142$, $p. < 0.05$).

Discussion

Our study illustrates the recalcitrant nature of soil HS (FA and HA) with decomposition rates of ca 5% after 40 days. The lower decomposition rates of ^{14}C labelled of 2% within 28 weeks as found by Martin and Haider [11] could be due to the different type of soil (sandy loam topsoil) used for incubation, resulting in different microorganisms. Also, the decomposition rate of organic matter in the Tongbersven podzol B horizon decreased significantly from 0.33% to 0.03% in 30 days upon further addition of Al.

By contrast, the higher mineralization rates reported by Boudot et al. [2] (over 25% purified FA and of 20% purified HA after 44 days) could be due to the different nature of their "model" humic material which was derived from fungal melanins. Melanins are largely phenolic polymers synthesized by many soil organisms. Although it has been suggested that some fungal melanins have a structure similar to that of HA [7], their decomposition rate cannot be transfered directly to that of soil HA and FA.

The relative resistance of HS to microbial decomposition as compared to other organic compounds is probably due to their extremely complex structure. Consequently, a variety of enzymatic activities are needed to depolymerize or catabolize humic substances. Probably phenol oxidases, etherases, peroxidases and also enzymatic activities responsible for depolymerization of humic proteins and polysaccharides are involved in the mineralization process

[18]. However, the exact mechanisms of decomposition of HS are still unknown. Also, the synthesis of a diversity of enzymes seems to be a barrier to rapid decomposition because of very high energetic requirements for such processes [18, 23]. Consequently, many microorganisms have a limited capability to decompose aromatic components of HS for energy and carbon [12, 20, 22].

In general complexes of Al with HS in our experiments decreased their decomposition rate. Decomposition of FA was already significantly reduced at the lowest Al addition. By contrast, only the highest Al addition had a significant negative effect on the decomposition rate of HA.

In natural conditions the decomposition rate of HS is lower than indicated by our incubation experiments. For example, Campbell *et al.* [4] extracted humic materials from forest soil in Saskatchewan and measured a weighted mean ^{14}C age of 250-940 years (a decomposition rate of ca. 0.3-0.07% per year). According to Schlesinger [16], the turnover time of organic carbon in the soil may be in the order of thousands of years for humus.

Concerning the effect of metal complexation, similar results were reported by Boudot *et al.* [2] for fungal melanins, where the decomposition rate decreased with an increasing metal to carbon ratio. Klemmedson and Blaser [8] suggested that the relative resistance of Al-organic matter complexes to the microbial breakdown is caused by the protection of reactive groups by complexed Al.

Klemmedson and Blaser [8] found no influence of Al content of Al in the soil matrix on the decomposition rate of chestnut leaves (litter bag study). However, in most recent studies concerning decomposition of more simple organic substances (polysaccharides, glucose, citric acid, melanin), the addition of metals (Zn, Cu, Fe, Al) markedly reduced decomposition rates [2, 5, 10].

Conclusions

Our results indicate that the current depletion of organically-bound Al in Dutch forest soils will decrease the decomposition rate of soil HS. Consequently, acid rain could result in a significant reduction of the pool of soil HS, thereby affecting the soil's chemical (CEC, N binding) and hydrological (water retention) characteristics. Decomposition rates appeared particularly sensitive to temperature. At 8°C rates were extremely low, whereas a significant increase in rates occured at 20°C. Therefore, decomposition of soil HS is probably limited to the summer months, when temperatures in the mineral soil horizons range between 8 and 14°C [3].

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