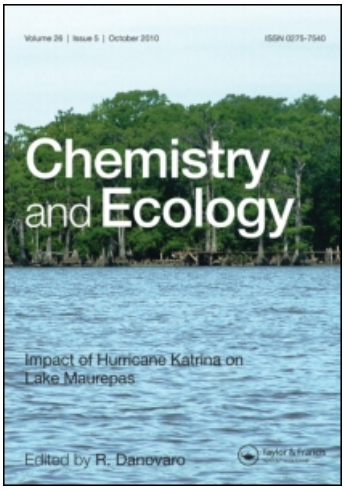

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CHANGES OF SOIL ENZYME ACTIVITIES BY SIMULATED ACID AND NITROGEN DEPOSITION

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Effects of acid and nitrogen depositions on soil microbial activities were studied in a laboratory-based experiment. Five treatments were added to forest soil for five weeks, and soil enzyme activities were determined along with chemical properties. There was little change in pH and nitrogen availability. Dehydrogenase, phosphatase and arylsulphatase activities were decreased by all the acidic treatments compared to the control, while urease activity was increased by the pH 4 treatment. At the same pH treatment, different nitric acid contents induced different urease activities. The results suggest that acid deposition would inhibit microbial activities and that more study is needed to elucidate the impact on nitrogen cycling in forests.

Keywords: Acid deposition; nitrogen deposition; soil enzyme; chemical properties

INTRODUCTION

There has been much concern about effects of acid and nitrogen depositions on terrestrial ecosystems (Johnson and Siccam, 1983; Aber *et al.*, 1989). Many studies have focussed on the relationship between high hydrogen ion concentration in the deposition and the deterioration of vegetation. As nitrogen content (e.g., nitrate) has increased while sulphur oxide compounds have decreased in the deposition (UKRGAR, 1990), so the consequences of acid deposition should be

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assessed from a different perspective. Possible adverse effects of acidity might be modified by the effects of nitrogen addition, which could induce fertilization effect in nitrogen limited forests, or negative effect through nitrogen saturation (Wainwright, 1980). Although soil microorganisms play a key role in nutrient cycles in terrestrial ecosystems, less investigations were given to soil microbial activities than to those in plants regarding their acid deposition effect (Falappi *et al.*, 1994). Microorganisms in soil are believed to be involved in the processes by producing enzymes, which represent a functional process in the soil (Burns, 1982). As such, study of soil enzyme activities impacted by the acid and nitrogen deposition is important in elucidating effects of the deposition on forest ecosystems. In the present study, our objective was to investigate effects of nitrogen and acid additions on soil enzymes and chemical properties in a forest soil.

MATERIALS AND METHOD

Soil cores were taken from the surface (0 – 5 cm), upper A horizon from Mt. Chombong in South Korea. The soil (pH 5.5; total organic carbon 6.6%; CEC 12.3 me/100 g) was sieved (2 < mm) and 400 g of the soil were placed in a polyethylene pot (15 cm diameter and 15 cm height). Treatments were applied as deionised water (c), pH 4 solution with 0.01 M nitric acid (4h), pH 4 with 0.001 M nitric acid (4l), pH 2 with 0.01 M nitric acid (2h), and pH 2 with 0.001 M nitric acid (2l). The treatments were prepared by adjusting pH with hydrochloric acid or sodium hydroxide solutions after adding certain amount of nitric acid. For 5 weeks, 870 ml of the solutions (4.9 cm of rain) were added in twice-weekly spraying. The samples were maintained at 20°Celsius incubator for the duration, after which surface soil (0 – 2 cm) was taken for the assays.

Dehydrogenase activity was determined by adding 2,3,5-triphenyltetrazolium chloride as a terminal proton acceptor, and measuring reduction of the chemical (Casida, 1977). Phosphatase and arylsulphatase activities were assayed by measuring released *p*-nitrophenol over 1 hour incubation from the soils added with *p*-nitrophenyl phosphate and *p*-nitrophenyl sulphate as substrates (Eivazi and

Tabatabai, 1977; Tabatabai and Bremner, 1970). Urease activity was determined by measuring released ammonium from the soil amended with urea, described by Kandeler and Gerber (1988).

Soil pH was measuring using a 5:1 deionised water/soil slurry and a glass pH electrode. Nitrate concentrations and ammonium concentrations in the soils were determined by oxidation method and indophenol method (Emteryd, 1989) followed by extraction with 2 M potassium chloride.

Statistical analysis was carried out by one-way ANOVA followed by Tukey's test to find out differences among treatments.

RESULTS AND DISCUSSION

There are no significant changes in soil pH by the additions (Tab. I). The acid additions in this study were below the buffering capacity of the soil, which is supplied mainly by chemical reactions in the soil (Reuss and Johnson, 1986). Similarly, no significant change in extractable ammonium in the soil was detected (Tab. I). The result supports the idea that acid deposition would not affect the ammonification process significantly, as many different species with a wide range of optimal pH are involved in the process (Olson, 1983). Extractable nitrate concentrations were also not changed significantly between the same nitrate addition treatments, only corresponding to the applied nitrate (Tab. I).

In all the acid additions, dehydrogenase activity decreased compared to the control, but having no difference between pH 4 and pH 2 treatments (Fig. 1-A). Dehydrogenase activity has been used widely as a general index for microbial activity in soil (Trevors *et al.*,

TABLE I Chemical properties of the soils after the treatment. The values are the averages of three replicates with the standard errors in parentheses. Values with different letters significantly different at $p < 0.01$

	Deionised Water	*pH 4* (0.01*M* HNO_3)	*pH 4* (0.001*M* HNO_3)	*pH 2* (0.01*M* HNO_3)	*pH 2* (0.001*M* HNO_3)
pH	5.0 (0.4)	5.3 (0.1)	5.1 (0.2)	4.9 (0.2)	4.7 (0.3)
ammonium ($\mu g\,g^{-1}$)	71 (8)	75 (5)	89 (7)	82 (18)	97 (9)
nitrate ($mg\,g^{-1}$)	153 (20)a	480 (54)b	130 (12)a	493 (35)b	160 (11)a

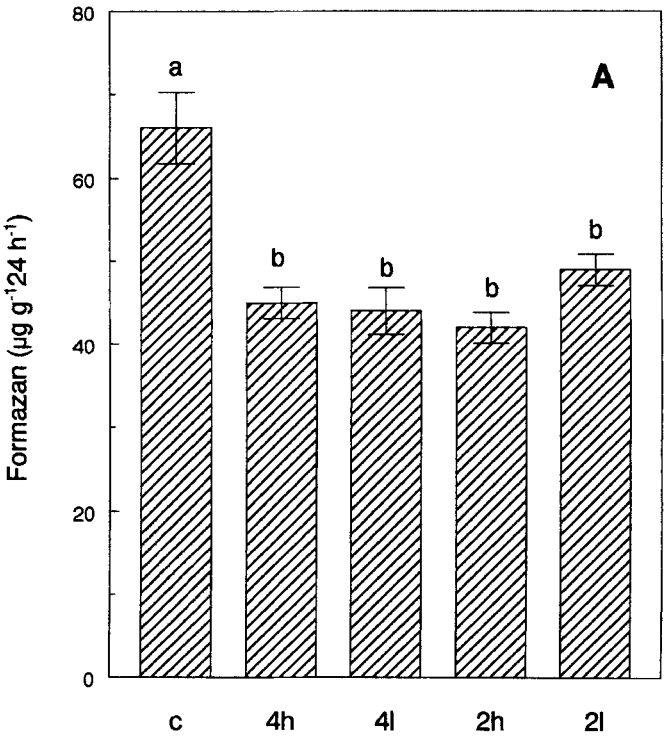

FIGURE 1 Effects of acid and nitrogen additions on dehydrogenase (A), phosphatase (B), arylsulphatase (C) and urease (D) activities (mean ± SE, $n=3$). Bars with different letters are significantly different ($p<0.05$). C = deionised water; 4h = pH 4 solution with 0.01 M HNO_3; 4l = pH 4 with 0.001 M HNO_3; 2h = pH 2 with 0.01 M HNO_3; 2l = pH 2 with 0.001 M HNO_3.

1982). Under the conditions of this study, acid additions inhibited microbial activity due to the dominant toxic effects of the high hydrogen ion concentrations over possible stimulation of micro-organisms by nitrogen additions. Decreased dehydrogenase activity following application of simulated acid rain has been reported in the rhizosphere of loblolly pine (Reddy *et al.*, 1991). However, no change (Wainwright, 1980) or stimulation of the activity (Killham *et al.*, 1983) have also been observed in forest soils, which was attributed to increases in carbon and nitrogen availabilities in the soils. It is likely that the different observations were caused by the amounts and frequency of acid applied, and by the differences in soil characteristics.

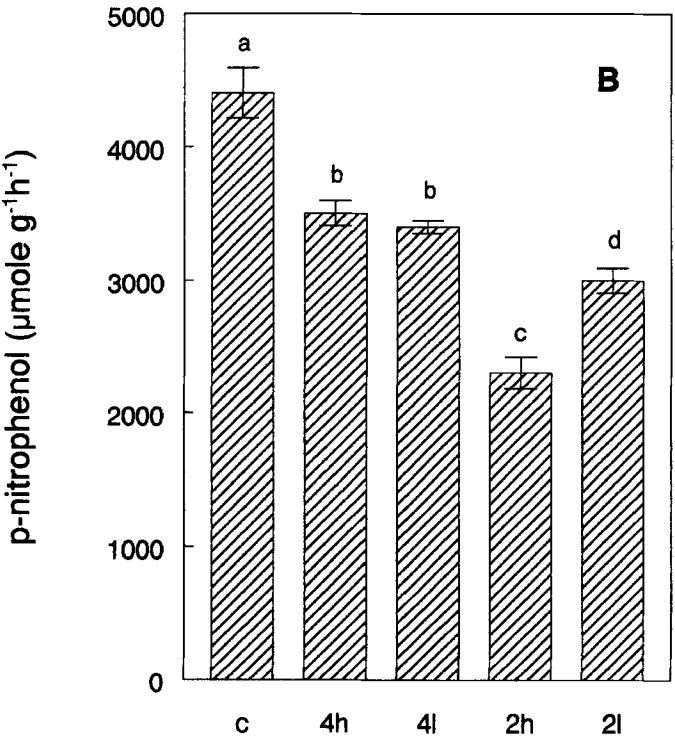

FIGURE 1 (Continued).

Phosphatase and arylsulphatase activities were lowered by pH 4 and pH 2 additions (Figs. 1-B, C). The decrease could be induced by lowered microbial activity (e.g., reduced *de-novo* synthesis of the enzymes). However, different from the response of dehydrogenase, phosphatase and arylsulphatase were inhibited linearly by increased acid additions (i.e., further decrease by pH 2 treatments than pH 4 treatments). The results suggest that the lower activities in pH 2 treatments is more likely caused by destabilisation of immobilised enzymes (e.g., enzymes stabilised on humus and clay), which are believed to persist out of microbial cells (Burns, 1982). Similar decreases of phosphatase (Reddy *et al.*, 1991), arylsulphatase (Press *et al.*, 1985) activities in soils by acid additions have been observed in other studies. The results in the present study implies that acid depositions could impede phosphorus and sulphur cycles through

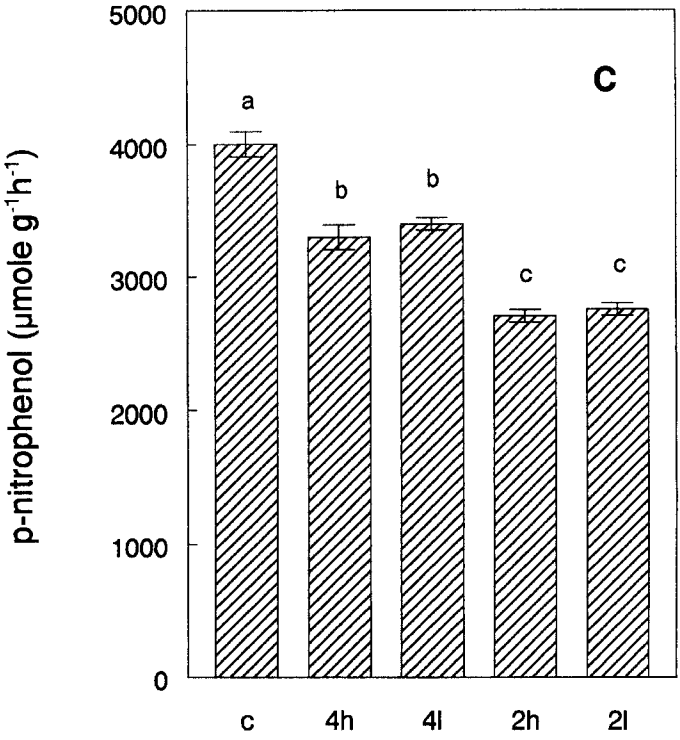

FIGURE 1 (Continued).

lowering the enzyme activities, as these enzymes are believed to play pivotal roles in mineralisation of organic phosphorus and sulphur in soil (Speir and Ross, 1978).

Interestingly, urease activity was increased by the pH 4–0.01 M addition (4h) compared to the control (Fig. 1-D). Different from other enzymes, micro-organisms were activated in terms of urease production by the nitrate additions in the pH 4 treatment. Killham *et al.* (1983) have reported increased urease activity in a soil applied with pH 4 simulated rain. In an other study, however, repression of urease production was reported by nitrate addition in soils amended with organic carbon (McCarty *et al.*, 1992). Kang and Freeman (1997) have also observed that ammonium addition decreased urease activity, while no change was induced by nitrate addition in a Sitka spruce forest floor. It seems that the contradictory responses of urease are due

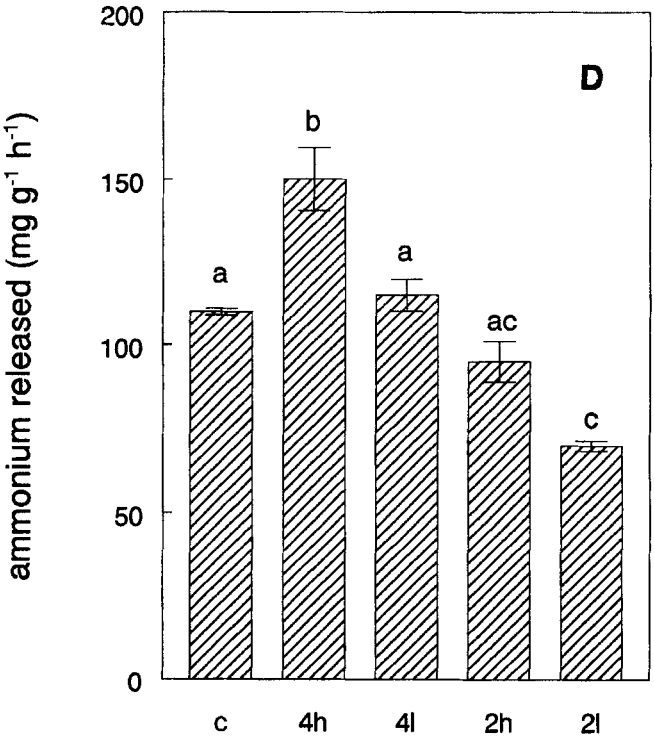

FIGURE 1 (Continued).

to differences in nitrogen status and carbon availability in the soils. For example, nitrogen addition could be a signal for the induction of urease in a nitrogen limited soil (*in sensu* Burns, 1982), while the addition might inhibit the production of the enzyme when nitrogen is not limited in the system. Although there was no change in nitrogen availability of the soil in the short-term (Tab. I), the different response of urease to the acid deposition suggests that acid deposition with high contents of nitrogen might affect nitrogen cycles in forest ecosystems, if we consider the long-term effect of nitrogen deposition, predicted to increase further (Galloway *et al.*, 1994).

In this short-term experiment, soil pH and other chemical properties (extractable ammonium and nitrate) showed little responses to the acid additions, while general microbial activities decreased even by the

intermediate acid additions. The sensitivity of the soil enzymes to acid deposition suggest that dehydrogenase, phosphatase, and arylsulphatase could be used as monitors of acidic deposition in forest soils. The different response of urease implies that further investigation should focus on nitrogen-related processes in forest, if we are to elucidate the long-term effects of acid and nitrogen deposition on forest ecosystems.

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