The Journal of Microbiology (2011) Vol. 49, No. 3, pp. 369-375
DOI 10.1007/s12275-011-0421-x
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The Impacts of Excessive Nitrogen Additions on Enzyme Activities and Nutrient Leaching in Two Contrasting Forest Soils

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(Received October 18, 2010 / Accepted February 7, 2011)

Nitrogen (N) deposition has increased dramatically worldwide, which may affect forest soils in various ways. In this study, we conducted a short-term manipulation experiment of N addition on two types of forest soils (urban and rural soils) found in Korea. N addition significantly decreased phenol oxidase activities in urban soil samples; however, it did not affect those in rural soils. Furthermore, N addition did not change β-glucosidase and N-acetylglucosaminidase activities, except for β-glucosidase activities in the O layer of rural soils. Changes in microbial biomass and general activity (dehydrogenase activity) were not induced by N addition, except for dehydrogenase in the A layer of urban soils. Although N addition did not change the extractable soil nutrients, organic matter, and water contents significantly, it enhanced nutrient leaching and resulted in lower pH leachate. These results suggest that excessive N addition to forest soils may induce nutrient leaching in the long-term. Overall results of our study also suggest that N addition may induce retardation of organic matter decomposition in soils; however, such a response may depend on the intensity of previous exposure to N deposition.

***Keywords*:** nitrogen deposition, microbial enzyme activity, soil acidification, temperate forest

Nitrogen (N) deposition from the atmosphere has increased rapidly due to anthropogenic activities such as intensive agriculture and increases in automobile emissions (Holland *et al.*, 1999). East Asian countries including Korea are exposed to high N deposition, comparable to European and North American countries (Galloway, 1998; Bashkin *et al.*, 2002). For example, N deposition in Seoul is reported to be 2.9 to 3.2 g $N \cdot m^{-2} \cdot yr^{-1}$, which is slightly higher or comparable to southern Nevada in USA (2.0 to 3.5 g $N/m^2/yr$) and Wales in the UK (2.0 to 2.5 g $N/m^2/yr$) (Park, 1999). However, there is little information about the impact of N deposition on forest soils in Asian regions.

Since N has been considered a limiting nutrient for terrestrial ecosystems in general, an increase in N deposition was believed to cause positive effects on forest ecosystems through fertilization (Aber *et al.*, 1991). In particular, excessive N deposition can raise the content of foliar N through plant uptake, which may reinforce the activity of ribulose disphosphate carboxylase and hence increase photosynthetic rates and primary production (Schlesinger, 1997).

However, an adverse impact of excessive N addition was noted in many European and North American countries experiencing forest decline in the early 1980s (Van Dijk and Roeloffs, 1998). According to the 'Nitrogen Saturation' hypothesis proposed by Aber *et al.* (1989), added N is absorbed by microbes and plants at an early stage, so that the primary production of microbes and plants tends to increase. However, the long-term addition of excessive N can delay decomposition rates of recalcitrant organic matter due to the inhibition of lignin-degrading enzymes (Carreiro *et al.*, 2000; Micks *et al.*, 2004). As a result, the impeded decomposition rate leads to an insufficient supply of nutrients for microbes and vegetation, accompanied with weakened growth and reduced primary production. The accumulated phenolic material, in turn, could inhibit other enzyme activities related to the degradation of organic matter, which would further limit the nutrient supply for microbes and plants (Wetzel, 1992; Kang and Freeman, 1999).

However, more recent studies conducted in the 2000s have noted that N deposition along with elevated CO_2 have accelerated forest growth, resulting in the retention of 30% of N applied to forests. Further, a similar amount of N is known to be retained in forest soils (Schlesinger, 2009). We hypothesize that such high retention of N in forest soils is related to a decrease in organic matter decomposition and lower enzymatic activity. In addition, the intensity of inhibitory effects of N addition is expected to differ between soils with different historical exposure to N deposition. For example, areas that have already been exposed to high N deposition (e.g., urban soils) may be more negatively affected by N addition than rural soils. To test this hypothesis, we collected soil samples from two forest soils with different N deposition and conducted a short-term N addition experiment.

Materials and Methods

Site description and soil samplings

The first soil sample was collected at Mt. Nam, Seoul, South Korea, located in the central part of Seoul (39°32' N, 126°08' E). Being located in the center of the city, this area has been exposed to a large amount of N deposition (NO_3^- and NH_4^+, 1008 eq/ha/yr) (Park, 1999).

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Vegetation is dominated by Mongolian oak (*Quercus mongolica*), pines (*Pinus sargentii, P. densiflora*) and five afforestations including *Robinia pseudo-acacia*, *Populus tomentoglandulosa*, *P. koraiensis*, *P. rigida*, and *Metasequoia glyptostroboides* (Lee *et al.*, 1998). The second set of soils was sampled at Mt. Jumbong in Kangwon-Do, South Korea (38°02' N, 128°06' E), which is a well-protected and pristine area with much lower N deposition (456 eq·ha^{-1}·yr^{-1}). The dominant vegetation consists of temperate mixed-hardwood Korean maple (*Acer pseudo-sieboldianum*) and Mongolian oak (*Quercus mongolica*). The texture of both sites is sandy loam and loamy sand. Each soil sample was collected from the O and A layers. In order to exclude vegetation effects, soils were collected at least 2 m away from vegetation and sampled soils were transferred to a laboratory on ice.

Experiment design

Soil samples were sieved (2 mm) and placed in PVC cores in the

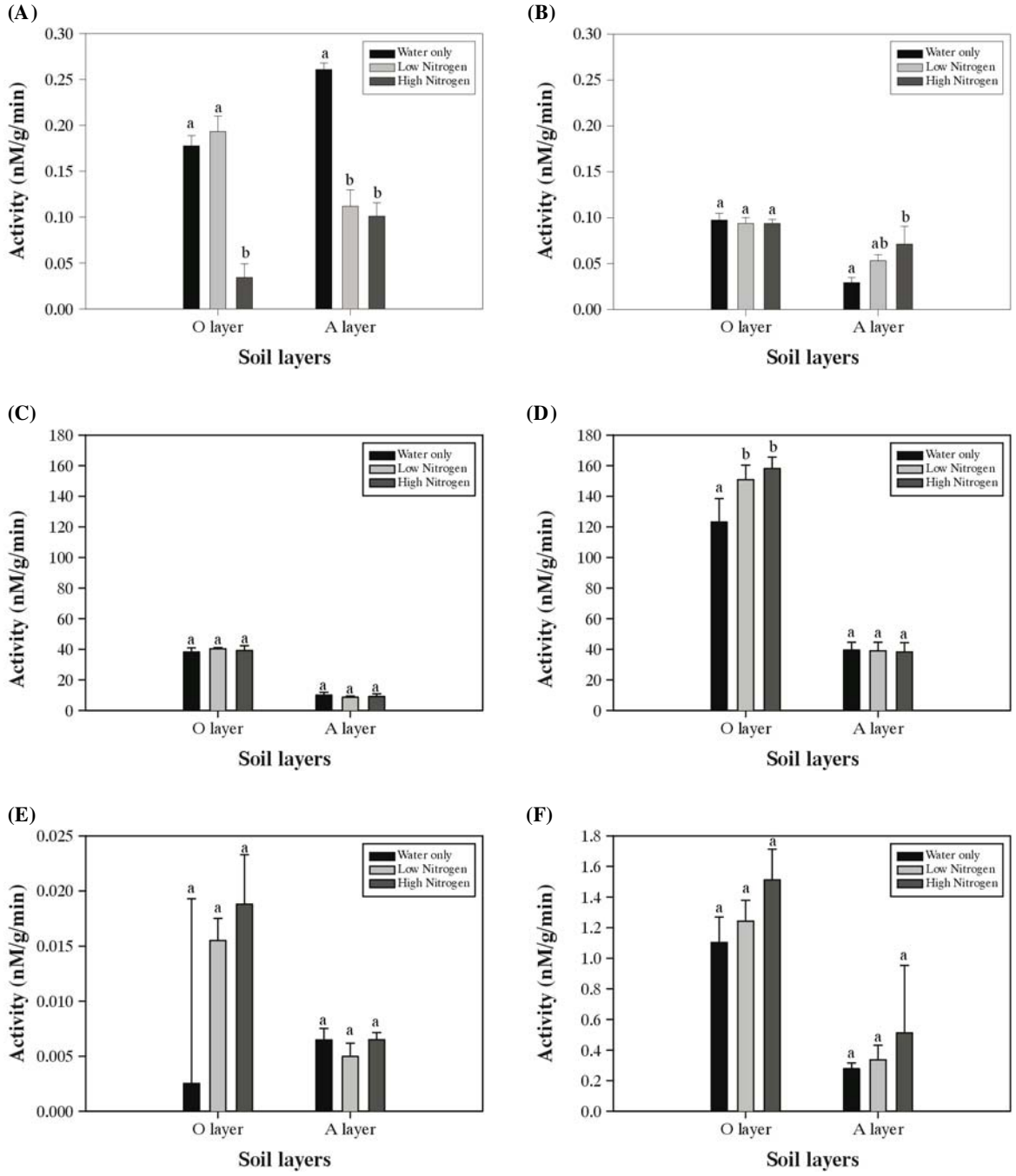

Fig. 1. Influence of N addition on phenol oxidase (A and B), β-glucosidase (C and D), and N-acetylglucosaminidase (E and F) in urban (left panel) and rural (right panel) forest soils in Korea. Bars labeled with different letters are significantly different from each other (one-way ANOVA, $P<0.05$).

laboratory. The area of core surface was 78.5 cm^2, and the depth of each core was 20 cm. Three replicate cores were prepared for each treatment and layer. Three treatments were applied: water only, low N addition (as NH_4NO_3 with a final concentration of 100 kg N/ha/yr/) and high N addition (final concentration of 1000 kg N/ha/yr). In total, 30 ml of N solution were added into cores every three days over a 60 day period, and the water which was leached from soil cores was collected every five days and stored at 0℃ until chemical analysis.

Soil and leachate chemistry

Chemical properties of soils were determined at the beginning and at the end of the experiment. Soil pH was measured using a 10:1 soil slurry with distilled water and a glass pH electrode. Nitrate was extracted from soils using an aqueous method and contents were measured following the modification of the cadmium reduction method using a DR 2000 Auto analyzer (Hach) at 500 nm. Ammonium was extracted from soils using an aqueous method and concentrations were measured using the modification of the Nessler method with a DR 2000 Auto analyzer at 425 nm. Phosphate was measured by the reduction of antimony (III) with ascorbic acid, followed by extraction with Bray P1 solution (Bray and Kurtz, 1945) using a spectrophotometer at 882 nm. Total soil organic matter was estimated by loss-on-ignition (Soil and Plant Analysis Council Inc., 1999).

After each layer sample of soil cores was leached, solution was filtered through #45 Whatman filter paper. Anions (nitrate, phosphate, and sulfate) and cations (ammonium, magnesium, potassium, and calcium) were measured with Dionex DX-120 Ion chromatography (Dionex Corporation, USA), and pH was observed with a glass pH electrode.

Soil microbial activities

Dehydrogenase activity was measured by adding 1 ml of iodonitrozotetrazolium solution (2% v/v) to 1 g of soil, and incubating at 20℃ for 24 h. After incubation, 10 ml of methanol was added to soil samples, mixed for 1 min, and stabilized. After the supernatant was filtered with a glass fiber filter, the red color intensity of the filtered solution was measured at 486 nm with a spectrophotometer (Von Mersi and Schinner, 1991).

Four-methylimbeliferyl (MUF)-β-D-glucoside and 4-MUF-N-acetyl-β-D-glucosaminide were used as model substrates for β-glucosidase and N-acetyl-β-D-glucosaminase, respectively. Five milliliter of substrate (final concentration of 400 μmol) was added to 1 g of soils and incubated for 45 min at 20℃. The reaction was terminated by pipetting 2 ml of solution into a centrifuge tube and centrifuged at 7,200×g for 5 min. Fluorescence in the supernatant was determined by a TD-700 fluorometer (Turner Biosystem, Model TD-700 Laboratory Fluorometer, USA) using 450 nm emission and 330 nm excitation wavelengths with a slit setting of 2.5 (Kang and Freeman, 1999).

L-dihydroxy phenylalanine (L-DOPA) was used as a model substrate to measure phenol oxidase activity. The red color of the 2-carboxy-2,

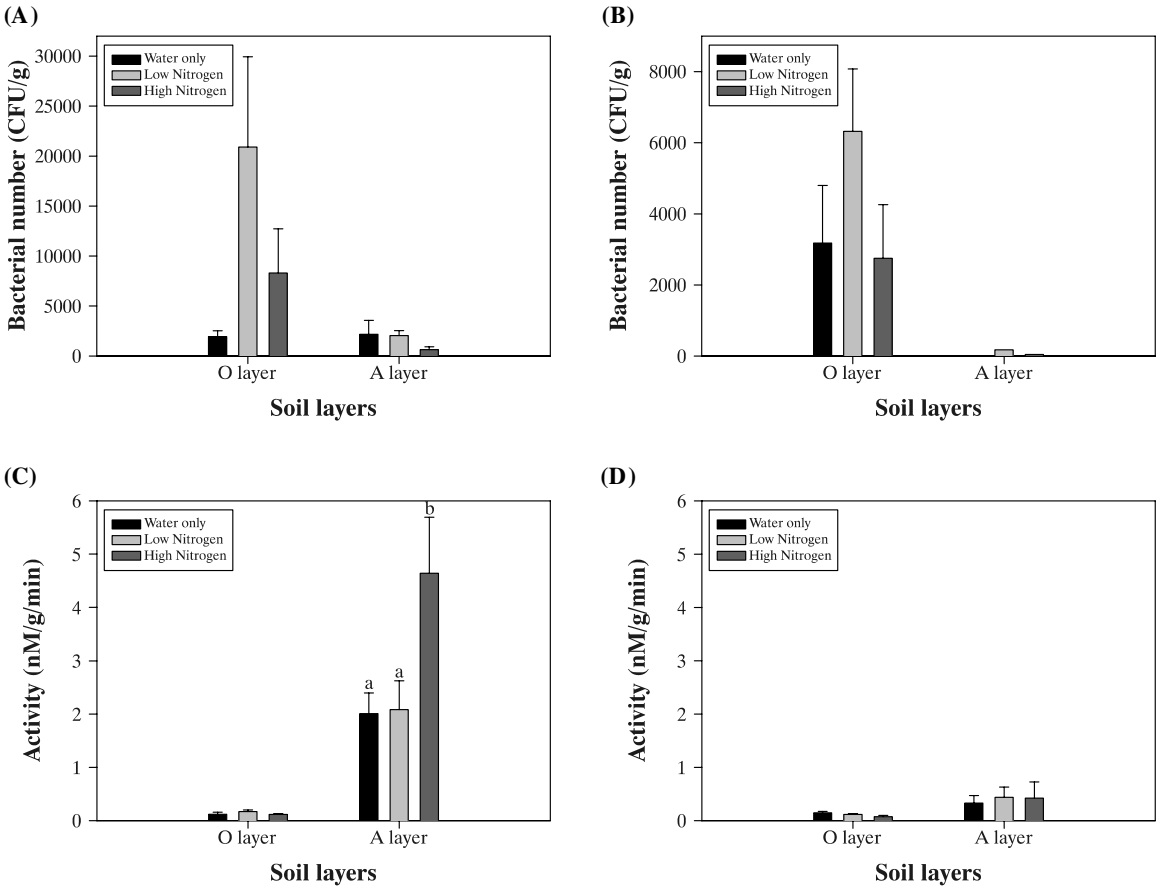

Fig. 2. Influence of N addition on bacterial numbers (A and B) and dehydrogenase (C and D) in urban (left panel) and rural (right panel) forest soils in Korea. Bars labeled with different letters are significantly different from each other (one-way ANOVA, $P<0.05$).

3-dehydroindole-5, 6-quinone compound formed by the enzymatic oxidation of L-DOPA was measured to estimate phenol oxidase activity. Five milliliter of L-DOPA solution (final concentration 10 mmol) was added to 1 g of soil and incubated for 15 min at 20°C. The reaction was terminated by immediate centrifugation at 12,000 rpm for 5 min at 20°C and red absorbance was measured at 460 nm. A control sample of DDI water was also employed (Pind *et al.*, 1994).

Microbial counting was measured using the *PROFILE^{TM}-1 Biluminometer* (New Horizon Corporation, Model 3550i, USA), which quantifies the amount of ATP. The amount of bacterial ATP present in soil samples was converted to bacterial biomass by employing the conversion factor reported by Lee and Deininger (2001). Eukaryotic ATP was eliminated by filtering soil extract and applying agents to remove extracellular ATP according to the manufacturer's manual.

Statistical analysis

A one-way ANOVA with a *post hoc* test was used to find any differences among the treatments. Significant differences were reported at the $P<0.05$ level.

Results and Discussion

Enzyme activities and microbial numbers

In urban forest (Mt. Nam) soils, N addition negatively affected phenol oxidase activities in the O and A layer soil cores (Fig. 1A), while bacteria numbers and dehydrogenase activities were not influenced by N addition (Figs. 2A and C). However, the inhibitory effects of N addition on phenol oxidase were absent in the O layer soils of the rural forest (Mt. Jumbong) (Fig. 1B), and even positive effects were observed in the A layer soils (Fig. 1B). In addition, no changes in bacterial numbers and dehydrogenase activities were found in soils for the same site, except for dehydrogenase of the A layer from urban forest soils (Figs. 2B and C).

Repression of phenol oxidase activity by N addition has been observed in various ecosystems, including forest soil (McAndrew and Malhi, 1992; Smolander *et al.*, 1994), forest litter (Carreiro *et al.*, 2000; Saiya-Cork *et al.*, 2002), and grassland (Matocha *et al.*, 2004; Waldrop and Zak, 2006). According to Fog (1988) and Berg *et al.* (1997), excessive N can inhibit the growth of fungal community that produces enzymes for the degradation of lignin. It was also reported that excessive N addition could shift a soil microbial community from fungi-dominated systems to bacteria-dominated systems (Holland and Coleman, 1987). N inhibition of ligninolytic enzyme activities occurred in litter with high lignin contents and abundant white rot fungi (Dilly and Munch, 1996; Carreiro *et al.*, 2000). More recent studies revealed that bacterial dominated soils also have a negative response of phenol oxidase from N addition (Saiya-Cork *et al.*, 2002; De Forest *et al.*, 2004b; Waldrop and Zak, 2006). However, other studies have reported that N addition did not lead to changes in soil microbial community and number of bacterial populations (De Forest *et al.*, 2004a, 2004b; Waldrop and Zak, 2006; Lucas *et al.*, 2007). The results of our study suggest that N addition can inhibit phenol oxidase; however, such inhibition only appears in sites with previous exposure to high N deposition. Differences in inorganic N content at two sites are presented in Table 1, where both NO_3^- and NH_4^+ were substantially higher in urban soils than rural soils, except for NO_3^- in the A layer (Table 1). As such, previous exposure to N deposition may determine whether a site will be strongly affected by excessive N addition or not. Furthermore, such changes are not accompanied by changes in microbial numbers and general activity (e.g., dehydrogenase), suggesting that added N could inhibit phenol oxidase directly.

Contrary to the response of phenol oxidase to N addition, other enzyme activities such as β-glucosidase and N-acetylglucosaminidase were not negatively affected in our study. On the other hand, β-glucosidase increased with N addition in the O layer of rural forest soils (Fig. 1D). It is likely that N addition appeared to alleviate N limitation in rural soils, resulting in an increase in β-glucosidase activity in the O layer soil of rural forest. This is in accordance with a previous report where only cellulose-degrading enzyme activities increased with N addition, while oxidative enzyme activities tended to decline with the same treatments (Carreiro *et al.*, 2000; Saiya-Cork *et al.*, 2002; Michel and Matzner, 2003; Gallo *et al.*, 2004; De Forest *et al.*, 2004a).

Leachate chemistry

N addition induced leaching of cations (NH_4^+, Mg^{2+}, and Ca^{2+}) and an anion (NO_3^-) in both forest soils and both layers (Table 2). Cation leaching was more prominent in rural soils than urban soils, except for Ca^{2+}. With each site, high N addition induced a stronger response of nutrient leaching than low

Table 1. The changes in chemical properties of O and A layer soil cores from Mt. Nam and Mt. Jumbong, caused by N addition.

			NH_4^+ (μg/g)	NO_3^- (μg/g)	PO_4^{3-} (μg/g)	OM (%)	WC (%)
Mt. Nam	O layer	Control	68 (16)	14 (5)	67 (8)	25 (6)	53 (4)
		Low N	57 (15)	10 (4)	69 (8)	25 (3)	53 (4)
		High N	70 (8)	19 (8)	64 (4)	30 (4)	53 (4)
	A layer	Control	21 (10)	1 (1)	83 (10)	6 (1)	28 (2)
		Low N	16 (12)	5 (3)	90 (16)	5 (1)	29 (1)
		High N	19 (12)	2 (1)	114 (7)	7 (1)	28 (2)
Mt. Jumbong	O layer	Control	6 (4)	7 (5)	96 (5)	12 (4)	43 (2)
		Low N	6 (4)	12 (4)	100 (6)	12 (0)	43 (1)
		High N	7 (3)	16 (4)	96 (5)	13 (1)	44 (2)
	A layer	Control	7 (3)	6 (3)	110 (6)	7 (0)	32 (1)
		Low N	8 (4)	5 (1)	115 (10)	6 (0)	31 (1)
		High N	11 (2)	11 (2)	129 (4)	6 (0)	31 (1)

Table 2. Relative amount of ions leached from soils with low and high N supplementation over the experimental period. Numbers are relative to the control, set as 1. Numbers labeled with asterisks are significantly different from control values.

			NH_4^+	K^+	Mg^{2+}	Ca^{2+}	NO_3^-	SO_4^{2-}
Mt. Nam	O layer	1.0	1.0	1.0	1.0	1.0	1.0	1.0
		Low N	1.5*	0.4	0.6	0.7	0.7	0.7
		High N	7.8*	1.0	1.2*	1.4*	1.3*	0.6
	A layer	Control	1.0	1.0	1.0	1.0	1.0	1.0
		Low N	1.4*	0.7	1.1*	1.0	1.1*	1.0
		High N	12.6*	0.8	2.7*	2.5*	2.6*	0.7*
Mt. Jumbong	O layer	Control	1.0	1.0	1.0	1.0	1.0	1.0
		Low N	0.9	1.4*	0.8	0.7	0.7	1.1*
		High N	2.2*	2.9*	1.3*	1.3*	2.3*	0.9
	A layer	Control	1.0	1.0	1.0	1.0	1.0	1.0
		Low N	2.5*	0.4	0.9	0.8	1.4*	1.4*
		High N	35.3*	0.9	2.1*	1.6*	4.7*	0.4

N addition (Table 2). This appears to be related to the decrease in leachate pH from soils that were exposed to N addition (Fig. 3). N addition did not significantly decrease soil pH in either soil type. Soil pH ranged from 3.8 to 4.5 for the Mt. Nam soils and from 4.3 to 5.8 for the Mt. Jumbong soils. However, N addition decreased leachate pH from soils, which accompanied nutrient leaching (Fig. 3). Nitrate is known to easily drain with base cations because nitrate attracts positively charged ions such as hydrogen, calcium, magnesium, and potassium, and carries them into a nearby water system (William *et al.*, 1999). This phenomenon can bring about the deficiency of fundamental nutrients for forest growth and the rising soil

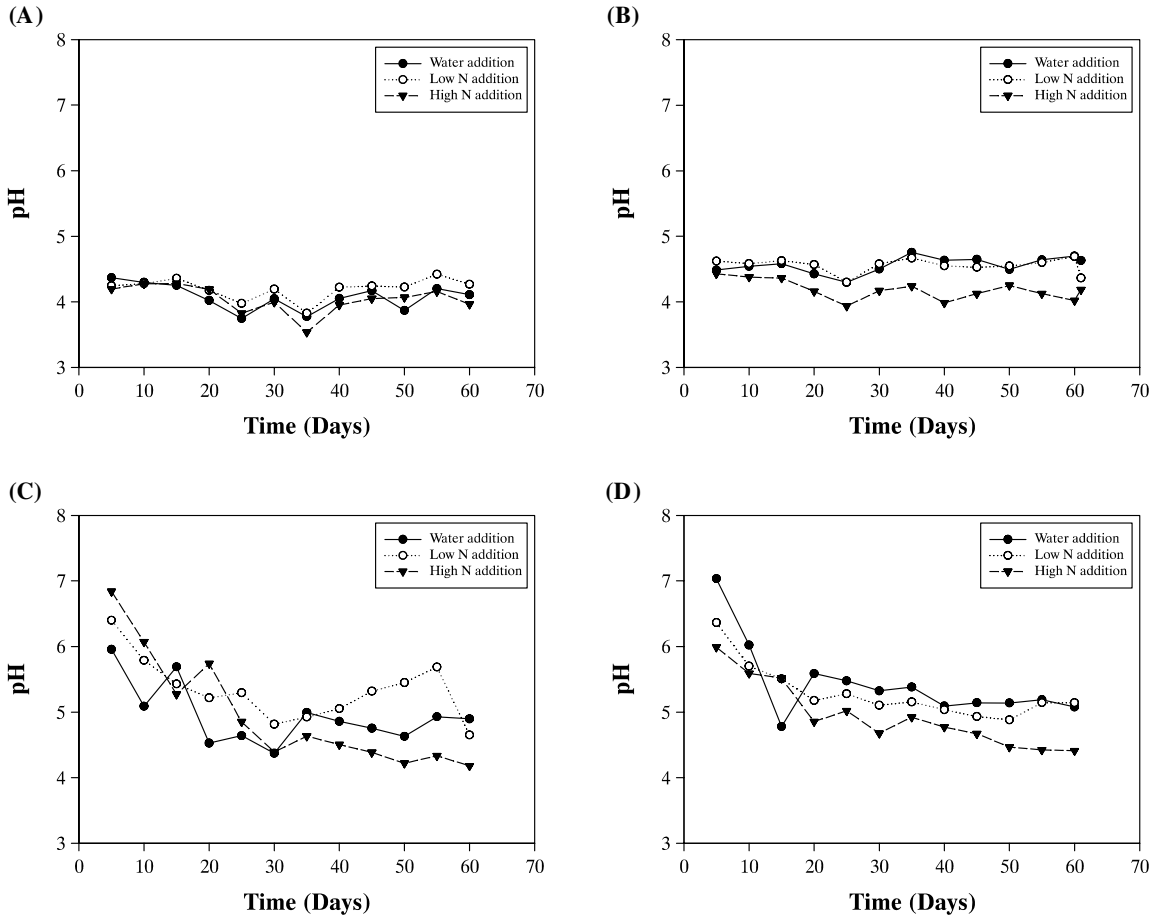

Fig. 3. pH of leachate from soil cores of the O layer of Mt. Nam (A), A layer of Mt. Nam (B), O layer of Mt. Jumbong (C), and A layer of Mt. Jumbong (D).

acidity can cause a loss in biodiversity in forest ecosystems (Aber *et al.*, 1989). In addition, low pH in leached water may give rise to accelerated acidification of water systems, which would adversely affect aquatic ecosystems (Grennfelt and Hultberg, 1986). Recent studies indicate that nitrate concentrations have increased in many lakes and rivers in northern Europe and North America. At sites showing this phenomenon, increases in base cations such as Al^{3+}, Ca^{2+}, Mg^{2+}, and K^{+} was observed in water systems, which contributed to the acidification of lakes (Henrikse and Brakke, 1988). At Lochnagar in northeast Scotland, one of the UK's watershed monitoring network sites, sulfate concentrations in the lake have decreased for the past ten years, while nitrate concentrations have increased significantly, resulting in the lowering of pH and the acidification of the lake (Jenkins, 1999).

Our results suggest that excessive N addition to forest soil induces lower activity of phenol oxidase without changes in microbial biomass and general activity in urban forest soils. This could result in the accumulation of organic matter in forest soil in the future because inhibition of phenol oxidase is reported to play a key role in consequent inhibitions of various hydrolases (Freeman *et al.*, 2001). Elevated CO_2, along with an increase in N deposition, may result in the accumulation of organic carbon, both as tree biomass and soil organic matter from fertilization effects (Schlesinger, 2009). Our results suggest that inhibition of phenol oxidase by N deposition may be an additional mechanism for such accumulation.

Acknowledgements

This project was supported by NRF (2010-0028708), Eco-STAR and EcoRiver 21.

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