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NUTRIENT LEACHING OF CHINESE FIR (*CUNNINGHAMIA LANCEOLATA*) SEEDLINGS UNDER SIMULATED NITROGEN DEPOSITION

WENFEI LIU[1,2], YANYAN LI[1,2], GUOMIN HUANG[1,2], JIANPING WU[1,2], HONGLANG DUAN[1,2], YINGCHUN LIAO[1,2], RONGZHEN HUANG[1,2], ZHIPENG XU[1,2] AND HOUBAO FAN[1,2*]

[1]*Institute of Ecology & Environmental Science, Nanchang Institute of Technology, Nanchang 330099, China*
[2]*Jiangxi Provincial Key Laboratory for Restoration of Degraded Ecosystems & Watershed Ecohydrology, Nanchang 330099, China*
[*]*Corresponding author's email: hbfan@nit.edu.cn*

Abstract

To investigate the impacts of nitrogen addition on soil nutrient leaching, a one-year greenhouse experiment was conducted on Chinese fir (*Cunninghamia lanceolata*) seedlings with five nitrogen addition treatments: N0 (Control), N1 (60 kg N ha^{-1} yr^{-1}), N2 (120 kg N ha^{-1} yr^{-1}), N3 (240 kg ha^{-1} yr^{-1}) and N4 (480 kg ha^{-1} yr^{-1}). Results showed that pH values of soil leaching solution were significantly decreased by the treatments of N2, N3 and N4, with respective decline of 15.6%, 19.1%, and 25.9%, compared with the N0 treatment. Nitrogen feritilization increased ammoniacal nitrogen and nitrate nitrogen in the soil leaching solution. Nitrogen loss due to soil leaching was also enhanced by nitrogen addition, amounting to 7.37, 20.71, 48.72 and 99.48 mg kg^{-1} for N1, N2, N3, and N4, respectively. Similarly, more K^+, Ca^{2+} and Mg^{2+} were leached into the leaching solution with nitrogen addition. The annual loss of the five nutrient ions into the leaching solution ranked as follows: $Ca^{2+} > NO_3^- > NH_4^+ > K^+ > Mg^{2+}$. The leaching losses of these five nutrient ions increased with across nitrogen gradients. Our study suggests that high-level nitrogen deposition may cause soil nutrient loss through leaching in subtropical forests.

Key words: *Cunninghamia lanceolata*; Greenhouse experiment; Nitrogen deposition; Nitrogen leaching; subtropical China.

Introduction

Human activities, such as burning fossil fuel and applying artificial fertilizer, have led to a three- to five-fold increase in atmospheric nitrogen deposition over the past century (Anon., 2007). For instance, a previous study showed that reactive N produced by human beings increased from 15 Tg in 1860 to 156 Tg in the late 20th century (Galloway *et al.*, 2004). The impacts of nitrogen deposition mainly depended on the deposition rates, time scales and ecosystem types (Höegberg *et al.*, 2006). Low-dose nitrogen deposition often promotes plant growth and stimulates ecosystem carbon storage in temperate forests (Pregitzer *et al.*, 2008; Reay *et al.*, 2008; Phoenix *et al.*, 2012). In contrast, a higher level of nitrogen deposition can negatively affect ecosystem processes and properties in most ecosystem types, resulting in forest ecosystem degradation (Aber *et al.*, 1998; Stevens, 2016), understory diversity losses (Gilliam 2006; Maskell *et al.*, 2010), and soil acidification (Höegberg *et al.*, 2006; Stevens *et al.*, 2009). Therefore, the nitrogen status of ecosystems, such as being saturated with nitrogen, has attracted concern from scientists and the public in recent decades (Anon., 2007).

In the 1980s, pioneering work, including the Nitrogen saturation experiments (NITREX) and Experimental Manipulation of Forest Ecosystems in Europe (EXMAN) programmes, as well as nitrogen addition experiments in the Harvard forest, helped the public to understand the concept of nitrogen deposition and improved the investigation on responses of ecosystems to nitrogen deposition (Aber *et al.*, 1998; Tietema *et al.*, 1998). However, most studies focused on temperate forests (Aber *et al.*, 1998; Janssens *et al.*, 2010). Few were conducted in tropical and subtropical regions (Cleveland & Townsend, 2006; Cusack *et al.*, 2011; Fan *et al.*, 2014), where anthropogenic nitrogen deposition has been dramatically increased (Galloway *et al.*, 2004; Mo *et al.*, 2008). For example, the amount of nitrogen deposition has reached 6-9 g N m^2 yr^{-1} in a subtropical region of China due to the rapid expansion of industrial and agricultural activities (Fan *et al.*, 2007b; Wang *et al.*, 2008). Tropical forests usually have high available nitrogen concentrations but low phosphorus availability, whereas temperate and boreal forests are often nitrogen limited under natural conditions (Hall & Matson, 2003). Despite addition rate playing a more important role in ecosystem functions than the cumulative nitrogen input (Höegberg *et al.*, 2006), the differentiated initial nitrogen status among ecosystem types alters the responses of ecosystems to nitrogen deposition (Janssens *et al.*, 2010).

Numerous studies in tropical and subtropical regions showed that nitrogen deposition could reduce soil respiration, increase carbon storage (Pregitzer *et al.*, 2008; Cusack *et al.*, 2011; Wei *et al.*, 2012), stimulate tree growth (Mo *et al.*, 2008; Liao *et al.*, 2010), and change soil microbial community dynamics and litter decomposition (Cusack *et al.*, 2011; Wu *et al.*, 2013). Other studies indicated that experimental nitrogen addition promotes dissolved inorganic and organic nitrogen leaching (Fang *et al.*, 2009). To our knowledge, however, few studies have investigated nutrient leaching under nitrogen deposition in tropical and subtropical regions (Sun *et al.*, 2006).

In this experiment, potted seedlings of *Cunninghamia lanceolata* cultivated in a greenhouse were used to examine nutrient leaching under different nitrogen addition treatments. *C. lanceolata* has been widely planted in China as a reforestation species, and the area of its plantations accounts for 60-80% of the total area of plantations in the southeast of China (Bi *et al.*, 2007). Here, we hypothesized that (1) nitrogen addition would cause soil acidification, and (2) a high level of nitrogen addition would cause soil nutrient loss by leaching.

Materials and Methods

Greenhouse experiment: The experiment was conducted in a greenhouse at the Nanchang Institute of Technology, Jiangxi Province, China. PVC pots (30 L) contained 15 kg air-dried mineral soil collected from a *C. lanceolata* plantation in a suburb of Nanchang City, China. The physical and chemical characteristics of the soil were shown in Fan *et al.*, (2011). A hole was designed at the bottom of each pot and Pledget Degrease pads were placed under the pots to collect the solution that leached from the soil (i.e., the leaching solution). There were five treatments with three replicates for each treatment as follows: N0 (Control), N1 (60 kg N ha^{-1} yr^{-1}), N2 (120 kg N ha^{-1} yr^{-1}), N3 (240 kg N ha^{-1} yr^{-1}), and N4 (480 kg N ha^{-1} yr^{-1}). The five treatments were designed to simulate no nitrogen deposition, natural deposition, medium deposition, and high deposition in greenhouse. Each pot was planted with a 1-year-old *C. lanceolata* seedling with approximate height 40 cm.

Sampling and analyses: The mean monthly precipitation in Nanchang City from 1976 to 2006 was used to calculate the water provided over the experimental period. The mean precipitation from January to December was 56.1, 101.5, 150.9, 224.3, 253.0, 282.0, 122.4, 103.3, 75.2, 57.5, 54.5 and 40.5 mm in Nanchang City. Correspondingly, 3.97, 7.17, 10.67, 15.85, 17.88, 19.93, 8.65, 7.3, 5.32, 4.06, 3.85 and 2.86 L water were applied to each pot from January to December. From April 2007, NH_4NO_3 was dissolved in water and sprinkled on seedlings and soil every three days in summer and autumn, and every ten days in winter and spring. Soil solution was collected from all pots after sprinkling. We combined the solutions from all months and measured nutrient elements (e.g., K^+, Ca^{2+}, Mg^2, N-NO_3^- and N-NH_4^+) in the leaching solution through Dionex ICS-1000 ion chromatography (Dionex Inc, USA).

Statistical analysis

One-way ANOVA was applied to test the impact of nitrogen addition on nutrient leaching of soil via leaching solution, followed by Tukey's HSD tests comparing means among different levels of nitrogen addition for each variable. Relationships between characteristics of leached nutrients and leaching solution pH were analysed. Results were considered significant in all cases if $p<0.05$. All statistical analyses were performed using SPSS 14.0 (SPSS, Inc, Chicago, IL).

Results

Leaching solution pH: The responses of tleaching solution PH to nitrogen deposition varied among different levels of nitrogen addition (Fig. 1A). For the control (N0) and the low addition (N1) treatments, leaching solution pH values were relatively stable during the experimental period. Conversely, for other treatments (i.e., N2, N3, and N4), pH values decreased sharply at the early stage of the experiment, dropping from 7.0 to 4.3. From June 2007 onwards, leaching solution pH values of both N2 and N3 started to fluctuate, exhibiting an increasing trend at the middle stage but decreasing again until the end of the experiment, while the leaching solution pH of N4 remained relatively stable (between 4.0 and 5.0) till the end of the experiment. Consequently, the annual average of leaching solution pH was significantly affected by both medium and high nitrogen additions, with a decrease of 15.6%, 19.1%, and 25.9% in N2, N3, and N4 when compared with the control treatment (Fig. 1B).

NO_3^--N and NH_4^+-N in the leaching solution: Over the study period, NH_4^+-N concentrations of the leaching solution remained stable in the N0 and N1 treatments but fluctuated greatly in other treatments, especially in the N4 treatment (Fig. 2A). Based on the annual averages, elevated nitrogen addition resulted in an increase of NH_4^+-N in the leaching solution (Fig. 2B). The average concentrations of NH_4^+-N increased by 159%, 305%, and 884% for the N2, N3, and N4 treatments when compared with N0, but no significant change was detected for the N1 treatment. NO_3^--N concentration in the leaching solution was stable for the N0 treatment throughout the year but showed an ascending trend over time for the N1, N2, N3 and N4 treatments, especially for the latter two treatments (Fig. 2C). Consequently, the annual average of NH_4^+-N concentrations responded positively to the increasing amount of nitrogen addition, showing an increase of 239%, 522%, 1043%, and 1567% for the N1, N2, N3, and N4 treatments, respectively, compared with the N0 treatment (Fig. 2D).

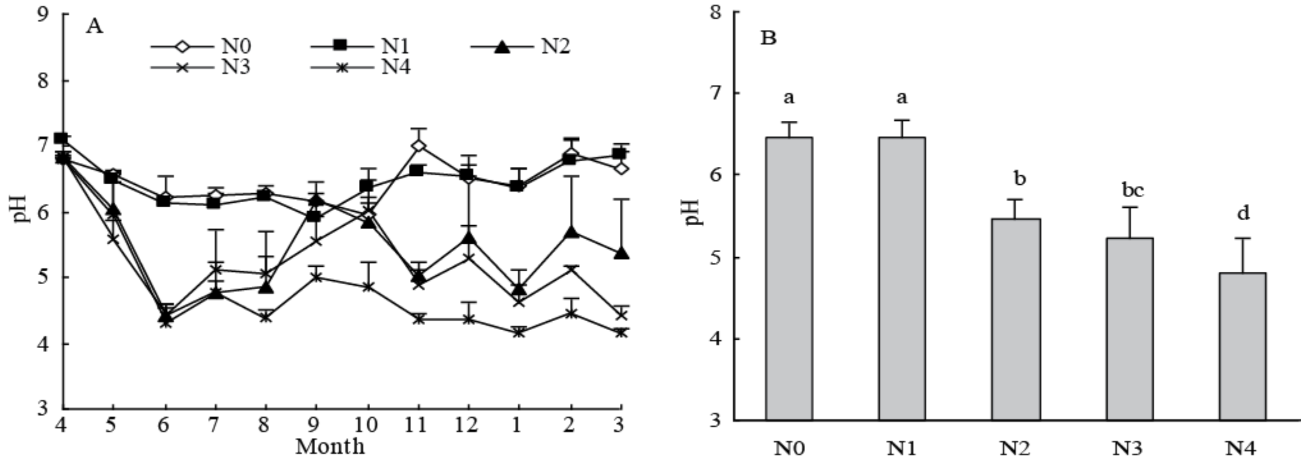

Fig. 1. Monthly dynamics (A) and annual averages (B) of soil leaching solution pH under different levels of nitrogen addition. N0, N1, N2, N3 and N4 stand for different nitrogen addition treatments. Values represent means ± 1 SE (n = 3). Different lowercase letters above the bars depict significant differences among nitrogen deposition treatments ($p<0.05$) determined by Tukey's HSD tests.

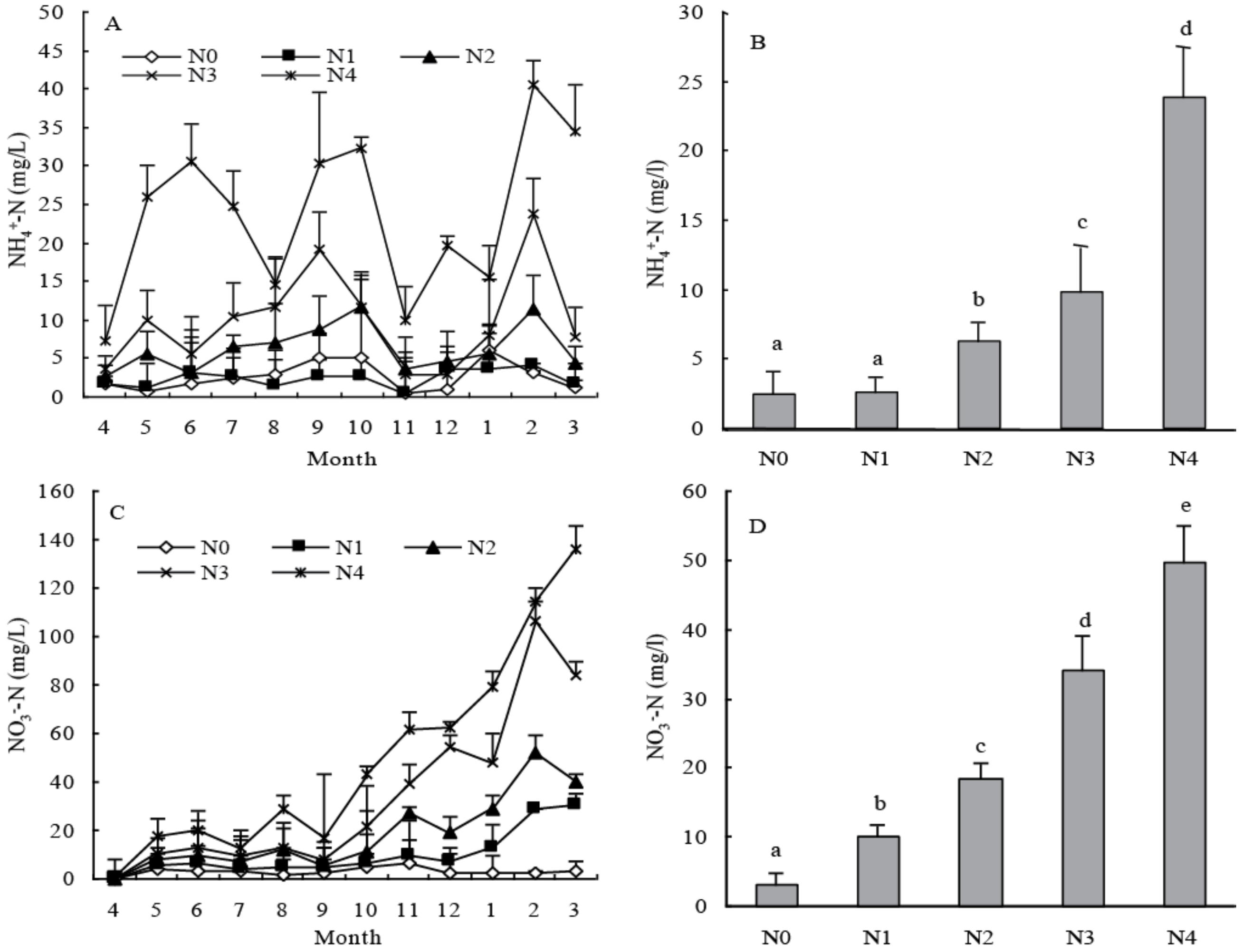

Fig. 2. Monthly dynamics and annual averages of NH_4^+-N (A and B) and NO_3^--N (C and D) concentrations in soil leaching solutions under different levels of nitrogen addition. Values represent means ± 1 SE (n = 3).

Table 1. The annual losses of nutrient ions under simulated nitrogen addition.

Treatments	The amount of leached ion (mg/ barrel)				
	K^+	Ca^{2+}	Mg^{2+}	NO_3^-	NH_4^+
N0	16.35 ± 1.54 c	133.09 ± 8.30 c	9.58 ± 1.32 c	47.09 ± 11.80 d	10.79 ± 3.94 d
N1	20.80 ± 1.71 c	147.52 ± 12.97 c	15.39 ± 2.12 b	68.84 ± 12.88 d	16.78 ± 6.30 d
N2	32.04 ± 1.27 b	235.56 ± 6.34 b	24.01 ± 1.35 a	102.57 ± 18.48 c	45.16 ± 10.35 c
N3	37.98 ± 5.37 b	241.65 ± 15.82 ab	26.97 ± 8.52 a	177.06 ± 23.58 b	82.21 ± 11.54 b
N4	39.60 ± 3.61 a	296.80 ± 17.82 a	28.61 ± 2.84 a	265.33 ± 40.02 a	244.27 ± 15.41 a

K^+, $Mg^{2+}$and Ca^{2+} in the leaching solution: Concentrations of K^+, Mg^{2+} and Ca^{2+} in the leaching solution fluctuated over the study period, but showed no clear consistent pattern with time (Fig. 3A, 3C, and 3E). However, based on the annual averages, concentrations of these three elements all showed an uptrend with increasing nitrogen addition (Fig. 3B, 3D, and 3F), increasing by 51.40%, 48.76%, 90.14% and 92.92% for K^+, 45.77%, 51.26%, 105.25% and 121.26% for Ca^{2+}, and 53.21%, 59.03%, 104.57% and 158.12% for Mg^{2+} for the N1, N2, N3 and N4 treatments, respectively when compared with the N0 treatment. These results indicated that higher nitrogen addition would lead to more K^+, Ca^{2+} and Mg^{2+} leached in the solution, with a significant difference between the N0 and other treatments ($p<0.05$).

Impact of nitrogen deposition on nutrient leaching: The annual nutrient loss of soil through leaching was calculated by the nutrient concentration in the leaching solution and the leachate volume over the study period. As shown in Table 1, the amount of annual loss of the five nutrient ions in the leaching solution occurred in the following order: $Ca^{2+} > NO_3^- > NH_4^+ > K^+ > Mg^{2+}$. In addition, their annual leaching loss increased with increasing nitrogen addition (Table 1).

Relationships between leached nutrients and leaching solution pH: For each of the five nutrient ions investigated in this study, the amount of its loss through leaching increased with decreasing leaching solution pH that resulted from increased nitrogen addition ($p<0.05$ in all cases; Fig. 4). These trends confirmed that nutrient loss from soil was negatively correlated with the level of nitrogen deposition.

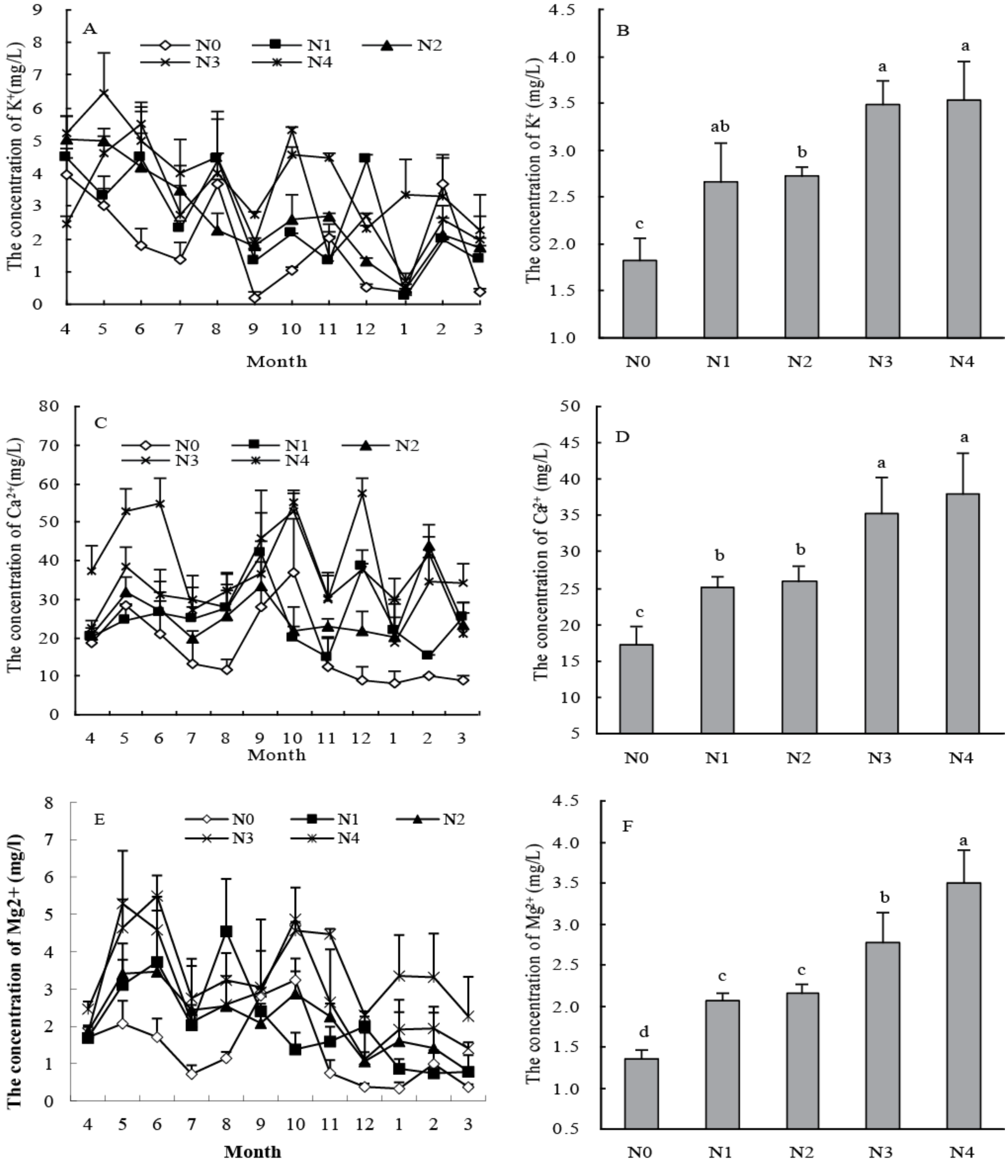

Fig. 3. Monthly dynamics and annual averages of K^+ (A and B), Ca^{2+} (C and D), and Mg^{2+} (E and F) concentrations in soil leaching solutions under different levels of nitrogen addition. Values represent means ± 1 SE (n = 3).

Discussion

Our study demonstrated that nutrient losses (K^+, Ca^{2+}, Mg^{2+}, NO_3^-, and NH_4^+) increased with declines in PH. In addition, significant linear relationships were found between nutrient ion contents and leaching solution pH. The similar results were reported in southeast China, where a soil column experiment was conducted to investigate the effects of nitrogen deposition on soil nutrient leaching. The treatments were designated as N0 (0 mg $N \cdot column^{-1} \cdot month^{-1}$), N1 (7.8 mg $N \cdot column^{-1} \cdot month^{-1}$), N2 (26 mg $N \cdot column^{-1} \cdot month^{-1}$) and N3 (52 mg $N \cdot column^{-1} \cdot month^{-1}$) (Sun *et al.*, 2006). The results from this study indicated that nitrogen addition can lead to soil nutrient losses and increase soil acidity. Nakaji *et al.*, (2001, 2002) found that elevated nitrogen deposition can cause nitrogen leaching from the soil in the form of NO_3^-, while K^+,

Mg^{2+} and Ca^{2+} may be leached from the soil due to the charge balance of NO_3^-. Other simulated nitrogen deposition experiments showed that the leaching of NO_3^- from soil increased with nitrogen deposition and that soil acidification was mainly caused by this process (Nihlgård, 1985; Bergkvist & Folkeson, 1992).

Nitrogen leaching is the major form of nitrogen loss in the circumstances of saturated nitrogen in soils and plants. The ratios of leached nitrogen to total input nitrogen were 26%-44% in this experiment, showing a descending trend with increasing nitrogen addition. The large amount of losses indicated that the ecosystem does not always respond positively to nitrogen input (Höegberg *et al.,* 2006; Aber *et al.,* 1998; Cleveland & Townsend, 2006). The similar results were also reported in a forest ecosystem investigated in southern China, which showed that the output of nitrogen from the ecosystem accounted for 25-66% of the artifical nitrogen addition (Fang *et al.,* 2009).

The increased nitrogen leaching would cause the loss of base cations and consequently leading to soil acidification (Höegberg *et al.,* 2006). Emmett *et al.,* (1998) suggested that the decrease in soil pH was related to the increased nitrogen leaching from the system. Our results supported this finding because soil pH was negatively correlated with NO_3-N and NH_4-N leaching. In addition, the pattern of nitrogen leaching is also regulated by forest types. For example, tropical and subtropical forest soils possess lower capacities for nitrogen retention than those in temperate zones (Fang *et al.,* 2009). This is mainly in that the precipitation in the rainy season impairs the contact opportunities of added nitrogen with the soil. In the experimental region, the precipitation in the rainy season (March to September) accounted for 74% of the total annual precipitation. Furthermore, the soil organic carbon content of the experimental soil was 30 g kg^{-1}. It is commonly believed that the small organic carbon pool limits nitrogen retention in the soil because soil organic matter is a very critical pool of fertilizer nitrogen.

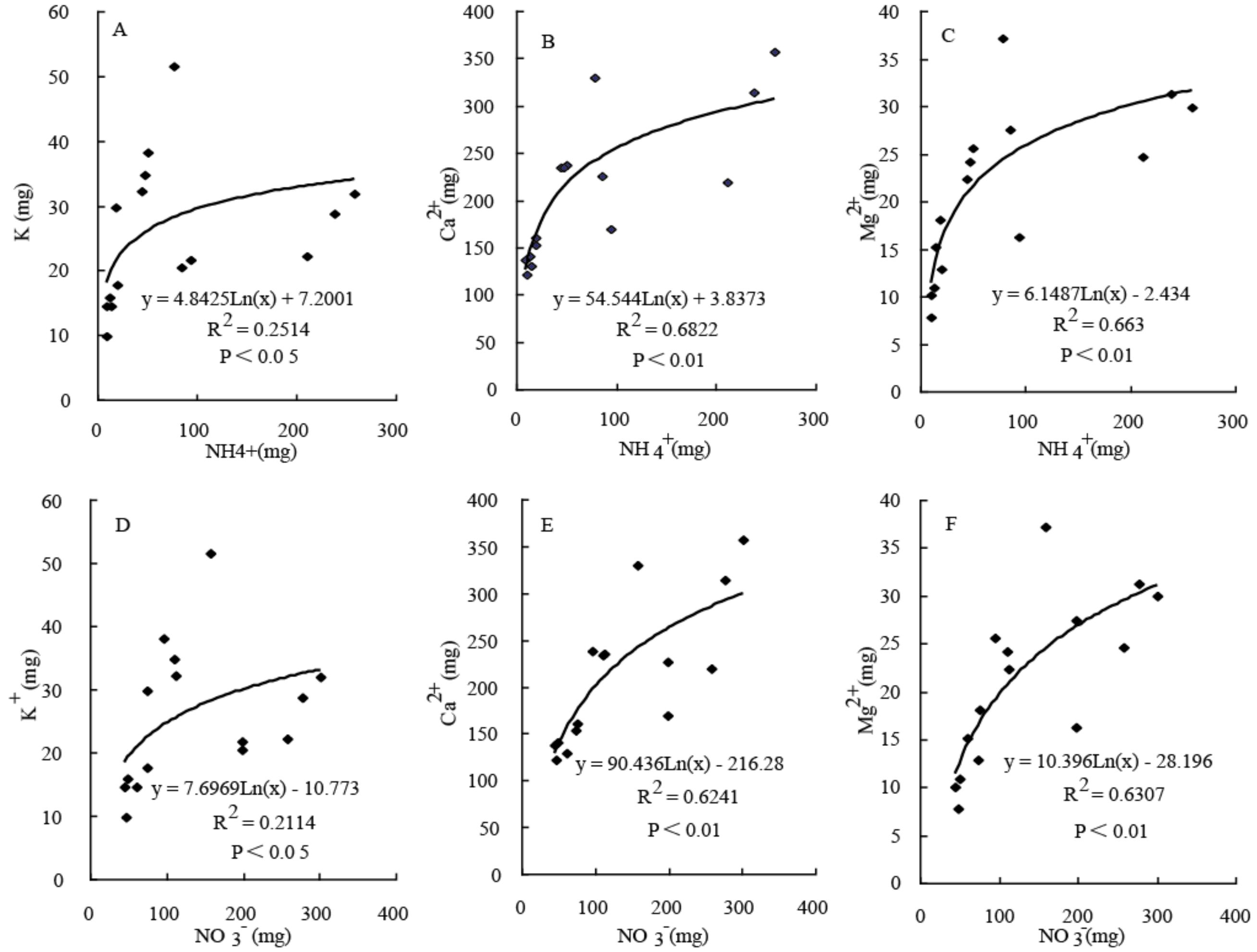

Fig. 4. The relationships between leaching solution pH and the annual losses of K^+ - NH_4^+-N (A), Ca^{2+}- NH_4^+-N (B), Mg^{2+} - NH_4^+-N (C), K^+ -NO_3^--N (D) , Ca^{2+}-NO_3^--N (E) and Mg^{2+}- NO_3^--N (F) under different levels of nitrogen addition.

Conclusion

Results of this study showed that soil leaching solution pH decreased with the increasing level of nitrogen deposition, on the contrary, nutrient losses from soil tended to increase, suggesting that high-level of nitrogen addition would lead to soil acidification and nutrient loss. Therefore, it is expected that high-level nitrogen deposition would have negative effects on soils of subtropical forests where nitrogen being relatively saturated, and leading to soil nutrient loss through leaching.

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