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ORIGINAL ARTICLE

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Growth, net photosynthesis and leaf nutrient status of *Fagus crenata* seedlings grown in brown forest soil acidified with H_2SO_4 or HNO_3 solution

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Abstract To obtain basic information for evaluating critical loads of acid deposition for protecting Japanese beech forests, growth, net photosynthesis and leaf nutrient status of *Fagus crenata* seedlings grown for two growing seasons in brown forest soil acidified with H_2SO_4 or HNO_3 solution were investigated. The whole-plant dry mass of the seedlings grown in the soil acidified by the addition of H_2SO_4 or HNO_3 solution was significantly less than that of the seedlings grown in the control soil not supplemented with H^+ as H_2SO_4 or HNO_3 solution. However, the degrees of reduction in the whole-plant dry mass and net photosynthetic rate of the seedlings grown in the soil acidified by the addition of H^+ as H_2SO_4 solution at 100 mg l^{-1} on the basis of air-dried soil volume (S-100 treatment) were greater than those of the seedlings grown in the soil acidified by the addition of H^+ as HNO_3 solution at 100 mg l^{-1} (N-100 treatment). The concentrations of Al and Mn in the leaves of the seedlings grown in the S-100 treatment were significantly higher than those in the N-100 treatment. A positive correlation was obtained between the molar ratio of (Ca+Mg+K)/(Al+Mn) in the soil solution and the relative whole-plant dry mass of the seedlings grown in the acidified soils to that of the seedlings grown in the control soil. Based on the results, we concluded that the negative effects of soil acidification due to sulfate deposition are greater than those of soil acidification due to nitrate deposition on growth, net photosynthesis and leaf nutrient status of *F. crenata*, and that the molar ratio of (Ca+Mg+K)/(Al+Mn) in soil solution is a suitable soil parameter for evaluating critical loads of acid deposition in efforts to protect *F. crenata* forests in Japan.

Keywords *Fagus crenata* · Soil acidification · Growth · Net photosynthesis · Leaf nutrient status

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Introduction

Fagus crenata is the most widely distributed broad-leaved deciduous tree species in cool temperate forests in Japan. For example, virgin natural forests of *F. crenata* at the Shirakami Mountains, which are located in northeast Japan, were registered by UNESCO as a World Natural Heritage in December 1993. However, forest decline and dieback of *F. crenata* have recently been observed at several mountainous areas such as the Tanzawa Mountains in central Japan (Totsuka et al. 1997; Maruta et al. 1999). It has been suggested that acid deposition and gaseous air

pollutants are environmental stresses relating to forest decline of *F. crenata* in the Tanzawa Mountains (Totsuka et al. 1997; Yonekura et al. 2001b). *F. crenata* prefers mesic and fertile soil conditions for growth and it associated with ectomycorrhiza. However, edaphic factors affecting its growth have been declining rapidly in recent years. To protect *F. crenata* forests in Japan from various environmental stresses, improved knowledge of the responses of this tree species to various anthropogenic stresses such as acid deposition and gaseous air pollutants such as O_3 and SO_2 is required. Leaf physiological processes such as photosynthesis and nutrient status are considered to be good indicators for the effects of environmental stresses on the vigor and health of *F. crenata* (Izuta et al. 2001; Yonekura et al. 2001a, b).

Acid deposition is a serious environmental problem in East Asian countries such as China, Korea and Japan (Bashkin and Park 1998; Rodhe et al. 2002). Deposition of acids from the atmosphere onto forest floors may gradually increase soil acidity (Driscoll et al. 2001). Soil acidification leads to an increase in the rates of leaching of base cations such as Ca, Mg and K from the rhizosphere soil, which may cause nutrient imbalances in forest tree species (Cronan and Schofield 1990; Driscoll et al. 2001). Furthermore, acid deposition to forest soils with relatively low base saturation increases Al mobilization and shifts chemical speciation of Al from organic to inorganic forms which are toxic to terrestrial biota (Cronan and Schofield 1990). Therefore, trees growing in these conditions may be adversely affected not only by nutrient deficiency, but also by phytotoxic levels of Al (Sverdrup and de Vries 1994; Cronan and Grigal 1995).

In Japan, annual mean concentrations of atmospheric SO_2 dramatically declined from approximately 60 nl l^{-1} (ppb) in the late 1960s to 10 ppb or below in the late 1990s (Ministry of the Environment 2002). However, there is the possibility that long-distance transport of SO_2 from Eurasia will increase the concentration of atmospheric SO_2 and the wet deposition rates of protons (H^+) and sulfate (SO_4^{2-}) in Japan in the near future (Fujita et al. 2001). Therefore, Japanese environmental scientists are greatly concerned about the effects of acidic substances transported from other countries on the atmospheric quality and forest ecosystems in Japan. On the other hand, annual mean concentration of atmospheric NO_2 in Japan has remained stable or has been gradually increasing in recent years (Ministry of the Environment 2002). The wet deposition rates of nitrate (NO_3^-) from the atmosphere have recently been increasing not only in urban and suburban areas, but also in forested areas of Japan (Okita et al. 1993; Iwatsubo et al. 1997; Fujita et al. 2000). As a result, the ratio of nitrate to non-seasalt sulfate (NO_3^-/nss-SO_4^{2-}) in precipitation has recently been increasing in Japan (Takahashi and Fujita 2000).

Many experimental studies have been reported on the effects of soil acidification on growth, physiological functions and nutrient status of Japanese forest tree species (Izuta et al. 1990, 1996a, 1997, 2001; Izuta 1998; Miwa et al. 1994, 1998; Lee et al. 1997, 1998; Shan et al. 2000). To evaluate the present and future effects of acid deposition on *F. crenata* forests, it is necessary to clarify the effects of soil acidification due to acid deposition including not only sulfate, but also nitrate on growth, physiological functions such as photosynthesis and nutrient status of this tree species. Furthermore, we must evaluate the critical loads of acid deposition in efforts to protect *F. crenata* forests in Japan.

Are there any differences between the effects of soil acidification due to acid deposition with sulfate and those of soil acidification due to acid deposition with nitrate as the main anion on growth, net photosynthesis and leaf nutrient status of *F. crenata*? What is a suitable soil parameter for evaluating critical loads of acid deposition for protecting *F. crenata* forests in Japan? To obtain answers to the first and second questions, we investigated growth, net photosynthesis and leaf nutrient status of *F. crenata* seedlings grown for two growing seasons in brown forest soil artificially acidified by the addition of H_2SO_4 or HNO_3 solution. Brown forest soil was selected in the present study because this soil is widely distributed in mountainous areas and comprises approximately 40% of the national land area of Japan (The group of Japanese pedologists 1990).

Materials and methods

Soil acidification

In November 1997, brown forest soil originating from granite as the parent rock was collected from the A-horizon below a deciduous broad-leaved tree stand in the University Forest (Kusaki, Gunma Prefecture, Japan). The collected soil was passed through a 5-mm sieve to remove small stones, leaves and roots.

On 1 April 1998, four different amounts of H^+ were gradually added as H_2SO_4 solution to the soil samples at 20, 40, 60 and 100 mg l^{-1} on the basis of air-dried soil volume, these soil treatments being designated as S-20, S-40, S-60 and S-100, respectively. On the same day, four different amounts of H^+ were gradually added as HNO_3 solution to the other soil samples at 20, 40, 60 and 100 mg l^{-1} on the basis of air-dried soil volume, these soil treatments being designated as N-20, N-40, N-60 and N-100, respectively. Control soil and that treated with lime at 1 g l^{-1} on the basis of air-dried soil volume were not supplemented with H^+ as H_2SO_4 or HNO_3 solution. Thus, there were ten soil treatments in the present study. To artificially change the ratio of concentrations of base cations such as Ca, Mg and K to Al concentration in the soil solution, each soil sample was put in a plastic container and soaked for 3 days in deionized water to stimulate leaching of cations from the soil. The soil was air-dried and passed through a 5-mm sieve.

Evaluation of plant growth

On 27 April 1998, 3-year-old seedlings of Japanese beech (*F. crenata* Blume) were obtained from commercial nurseries in Toyama Prefecture, Japan. The seedlings were transplanted to 2-l plastic pots with soil surface areas of 177 cm^2. The amounts of H^+ added to each potted soil in the soil treatment groups S-20 and N-20, S-40 and N-40, S-60 and N-60, and S-100 and N-100 were 40, 80, 120 and 200 mg H^+ pot^{-1}, which corresponds to 2,260, 4,520, 6,780 and 11,300 mg H^+ m^{-2} on the basis of potted soil surface area, respectively. Because the maximum deposition of H^+ in Japan was 0.047–0.113 mol m^{-2} $year^{-1}$ (47–113 mg m^{-2} $year^{-1}$) between 1986 and 1993 (Hara 1997), the amounts of H^+ added to the potted soil in the soil treatment groups S-20 and N-20, S-40 and N-40, S-60 and N-60, and S-100 and N-100 correspond roughly to the cumulative H^+ loads for 20–48, 40–96, 60–144 and 100–240 years, respectively.

Four hundred seedlings were grown in the ten soil treatments (40 seedlings per soil treatment) for 526 days from 27 April 1998 to 10 October 1999. From 27 April to 20 November 1998 and from 11 April to 5 October 1999, the seedlings were grown in a naturally lit phytotron (Koito, Yokohama, Japan) at Tokyo University of Agriculture and Technology (Fuchu, Tokyo, Japan). Air temperature and relative air humidity in the phytotron were maintained at 20.0±1.0/13.0±1.0°C (0600–1800/1800–0600 hours) and 70±5%, respectively. From 21 November 1998 to 10 April 1999, the seedlings were grown under field conditions at an experimental field of Tokyo University of Agriculture and Technology (Fuchu, Tokyo, Japan). To avoid the additional input of ions from wet deposition to the potted soil, the seedlings grown at the experimental field were protected from natural precipitation by a transparent vinyl chloride roof on rainy days. During the growth period of 526 days, all the seedlings were irrigated as necessary with deionized water applied to the potted soil surface without leakage of water from the bottom of the pot.

On 27 April, 8 June and 16 September 1998, and on 7 June and 5 October 1999, the eight seedlings per soil treatment were harvested to determine leaf number, leaf area, stem diameter and dry mass of plant organs. The leaf area was measured with an area meter (AAM-8, Hayashi-denkoh, Japan). The stem diameter was measured with a caliper. The harvested plant organs were dried at 80°C in an oven for 7 days and weighed.

Measurement of net photosynthetic rate

On 15 June 1999, net photosynthetic rate (A) of the seedlings grown in the ten soil treatments was measured using an infrared gas analyzer system (LCA-4, ADC, UK) with a Parkinson leaf cuvette (PLC-4B, ADC, UK). During the measurements, air temperature and relative air humidity in the leaf cuvette were maintained at 20.0 ±0.5°C and 70±5%, respectively. The net photosynthetic rate at 350 μmol mol^{-1} CO_2 (A_{350}) was measured under a photosynthetic photon flux density (PPFD) of 1,600 ±5 μmol m^{-2} s^{-1} at the adaxial leaf surface. The PPFD was supplied by a cold lighting system (PICL-NEX Twin, Nippon P.I., Japan).

The intercellular CO_2-response curve of net photosynthetic rate (A/C_i curve) was generated at a PPFD of 1,600 ±5 μmol m^{-2} s^{-1} by measuring the A at six atmospheric CO_2 concentrations in the leaf cuvette of 20, 100, 200, 350, 700 and 1,200±5 μmol mol^{-1}. The carboxylation efficiency (CE) of photosynthesis was determined as the initial slope of the linear portion of A/C_i curve. The A at 1,200 μmol mol^{-1} CO_2 was regarded as the maximum net photosynthetic rate at saturated CO_2 concentration (A_{max}). The light-response curve of net photosynthetic rate (A/light curve) was generated at 1,200±5 μmol mol^{-1} CO_2 by measuring the A at five PPFDs on the adaxial leaf surface of 0, 100, 170, 400 and 1,600±5 μmol m^{-2} s^{-1}. The quantum yield (QY) of photosynthesis was calculated as the initial slope of the linear portion of A/light curve.

Measurement of total soluble protein and Rubisco concentrations

Immediately after the measurements of net photosynthetic rate on 15 June 1999, the leaves were collected to analyze the concentrations of total soluble protein (TSP) and ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco). The fresh leaves (100 mg) were frozen in liquid nitrogen and then homogenized in 1 ml extraction buffer containing 100 mM HEPES (pH 8.0), 5 mM EDTA, 2% (w/v) polyvinylpolypyrrolidone, 0.7% (w/v) polyethylene glycol 20,000, 1% (v/v) Tween-80 and 24 mM β-mercaptoethanol. These procedures were carried out at 4°C. The homogenate was centrifuged at 9,000g for 30 s, and the supernatant was used in the assays of TSP and Rubisco. The concentration of TSP was measured according to the colorimetric method of Bradford (1976) using the Bio-Rad Protein Assay (Bio-Rad Laboratories, USA). The supernatant was subjected to SDS-polyacrylamide gel electrophoresis (Laemmli 1970) for separation of subunit bands of Rubisco protein. The amount of Rubisco was calculated from the density of subunit bands scanned with a gel image analysis system (Densitograph AE-6920MF, Atto, Japan). A calibration curve of protein content was made with bovine serum albumin.

Chemical analyses of soil solution and leaves

On 27 April 1998, 11 April 1999 and 5 October 1999, soil solutions were taken from the potted soil with a soil moisture sampler (Eijkelkamp, The Netherlands). The pH of the soil solution was measured with a pH meter (M-12, Horiba, Japan). The concentrations of Ca, Mg, K, Mn and Al in the soil solution were determined with an atomic absorption spectrophotometer (AA-670/GV-6, Shimadzu, Japan). On 5 October 1999, the concentrations of C, N and

S of the potted soil were determined with a C/N analyzer (MT-500, Yanagimoto, Japan) and a sulfur analyzer (EMIA-120, Horiba, Japan).

On 5 October 1999, harvested leaves were washed with deionized water and dried at 80°C in an oven for 7 days. The concentrations of C, N and S in the dried leaves were determined with the C/N analyzer and the sulfur analyzer. The dried leaves were ground to fine powders with a vibrating sample mill (TI-100, Heiko, Japan). The powders were digested with HNO_3 and H_2O_2, and then diluted with 100 mM HCl. The concentrations of Ca, Mg, K, Mn and Al in the sample solutions were determined with the atomic absorption spectrophotometer. The concentration of P in the sample solutions was determined by inductively coupled plasma-atomic emission spectrometry (ICPS-5000, Shimadzu, Japan).

Statistical analyses

Analysis of variance (ANOVA) was used to test the effects of soil acidification on *F. crenata* seedlings. Significant differences ($P<0.05$) in the plant growth parameters, photosynthetic parameters and element concentrations of soil solutions or leaves among the ten soil treatments were determined by Duncan's multiple range test. All statistical analyses were performed using SPSS software (Norušis 1993).

Results

Soil chemical characteristics

The pH of the soil solution decreased with increasing amounts of H^+ added as H_2SO_4 or HNO_3 solution to the soil (Table 1). The addition of lime to the soil significantly increased the pH of soil solution. When the soil was acidified with H_2SO_4 solution, the concentrations of Ca, Mg, Mn and Al in soil solution increased with increasing amounts of H^+ added to the soil. However, the concentration of K in the soil solution was not significantly changed by the addition of H^+ as H_2SO_4 solution to the soil. The concentrations of Ca, Mg and K in the soil solution were significantly greater in the N-100 treatment than the control values. The concentrations of Mn and Al in the soil solution increased with increasing amounts of H^+ added as H_2SO_4 or HNO_3 solution to the soil. The molar ratio of Ca concentration to Al concentration (Ca/Al) and that of total concentration of Ca, Mg and K to Al concentration [(Ca+Mg+K)/Al] in the soil solution significantly decreased with increasing amounts of H^+ added as H_2SO_4 or HNO_3 solution to the soil. The lime treatment induced significant increases in the Ca/Al and (Ca+Mg+K)/Al molar ratios in the soil solution. On the other hand, there were no significant differences in the soil C concentrations among the ten soil treatments. The soil N concentrations tended to increase by the addition of HNO_3 solution to the soil. The soil S concentrations significantly increased with increasing amounts of H^+ added as H_2SO_4 solution to the soil.

Plant growth responses

Immediately after the flush of leaves in the first growing season (8 June 1998), there were no significant differences in the whole-plant dry mass among the ten soil treatments (Fig. 1). On 16 September 1998, however, the whole-plant dry masses of the seedlings grown in the S-40, S-100 and N-100 treatments were significantly lower than the control. Immediately after the flush of leaves in the second growing season (7 June 1999), the whole-plant dry mass of the seedlings grown in the S-20, S-40, S-60, S-100, N-60 and N-100 treatments were significantly less than the control. On 5 October 1999, the whole-plant dry mass of the seedlings grown in the S-60, S-100, N-60 and N-100 treatments were significantly lower than the control.

Table 1 The pH, element concentrations of soil solution and C, N and S concentrations of potted brown forest soil. The values are the means of three chemical analyses of soil solution conducted on 27 April 1998, 11 April 1999 and 5 October 1999 during the growth period of *F. crenata* seedlings for 526 days from 27 April 1998 to October 1999. Each value is the mean of 18 determinations. The values followed by different letters within a column are significantly different according to Duncan's multiple range test ($P<0.05$)

Soil treatment	pH	Ca (mmol l^{-1})	Mg (mmol l^{-1})	K (mmol l^{-1})	Mn (mmol l^{-1})	Al (mmol l^{-1})	Ca/Al (mol mol^{-1})	(Ca+Mg+K)/Al (mol mol^{-1})	C (mg g^{-1})	N (mg g^{-1})	S (mg g^{-1})
Lime	5.60 a	0.870 ef	0.288 b	0.086 b	0.001 d	0.009 c	113.56 a	160.96 a	74.78 a	3.39 ab	0.47 e
Control	5.14 b	0.818 ef	0.257 b	0.130 b	0.006 d	0.028 c	48.61 b	73.77 b	71.34 a	3.46 ab	0.49 e
S-20	4.28 d	1.351 cd	0.274 b	0.089 b	0.150 d	0.332 c	50.10 c	6.34 c	69.87 a	3.51 ab	0.74 d
S-40	4.20 d	1.499 bc	0.337 b	0.117 b	0.533 b	0.700 c	3.66 c	4.98 c	62.27 a	2.55 b	0.89 c
S-60	4.01 de	1.506 bc	0.359 b	0.101 b	0.542 b	1.585 b	1.07 c	1.38 c	75.55 a	5.30 ab	1.16 d
S-100	3.84 ef	1.852 ab	0.508 a	0.143 b	0.718 ab	4.865 a	0.47 c	0.63 c	64.63 a	3.99 ab	1.26 a
N-20	4.79 c	0.465 fg	0.062 c	0.084 b	0.007 d	0.063 c	12.89 c	19.01 c	68.58 a	7.06 ab	0.49 e
N-40	4.73 c	0.304 g	0.061 c	0.122 b	0.009 d	0.091 c	4.91 c	12.45 c	73.85 a	5.80 ab	0.49 e
N-60	4.00 de	1.072 de	0.288 b	0.108 b	0.343 c	0.595 c	1.96 c	2.71 c	62.53 a	4.95 ab	0.48 e
N-100	3.64 f	1.914 a	0.575 a	0.237 a	0.868 a	4.421 a	0.50 c	0.70 c	72.82 a	8.15 a	0.49 e

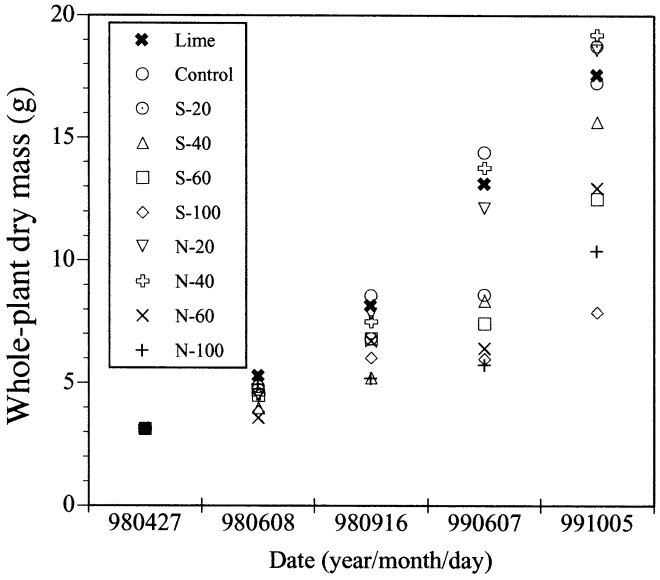

Fig. 1 Trends in the whole-plant dry mass per plant of *F. crenata* seedlings grown in the ten soil treatments during the two growing seasons from April 1998 to October 1999. Each symbol shows the mean of six determinations

Table 2 indicates the stem diameter, leaf area per plant, leaf number per plant, dry mass of plant organs, whole-plant dry mass and ratio of shoot dry mass to root dry mass (S/R ratio) of *F. crenata* seedlings after the two growing seasons (5 October 1999). Except for the S/R ratio, all the growth parameters of the seedlings grown in the S-100 treatment were significantly less than the control values. Furthermore, the leaf and root dry mass of the seedlings grown in the S-60 treatment was significantly less than the control values. The leaf, root and whole-plant dry mass of the seedlings grown in the N-60 and N-100 treatments was significantly less than the control values. The stem and branch dry mass of seedlings grown in the N-100 treatment was significantly less than the control values. On the other hand, there were no significant differences in any of the growth parameters between the lime and control treatments.

Photosynthetic responses

The net photosynthetic rate at 350 μmol mol^{-1} CO_2 (A_{350}) of the seedlings grown in the lime treatment was not significantly different from the control value (Table 3). The A_{350} of the seedlings grown in the S-20, S-40, S-60, S-100, N-60 and N-100 treatments was significantly less than the control value. The *QY* and *CE* of the seedlings grown in the S-60 and S-100 treatments were significantly less as compared with the control values (Table 3). In the seedlings grown in the S-60, S-100, N-60 and M-100 treatments, the maximum net photosynthetic rate at saturated CO_2 concentration (A_{max}) was significantly less than the control value (Table 3).

Figure 2 indicates the concentrations of TSP and Rubisco in the leaves of *F. crenata* seedlings on 15 June 1999. No significant differences in the TSP concentrations of the leaves were found among the ten soil treatments. The Rubisco concentrations in the leaves of the seedlings grown in the S-60 and S-100 treatments were significantly less than the control value.

Leaf nutrient status

The addition of H^+ as H_2SO_4 solution to the soil significantly reduced the concentration of Mg and significantly increased the concentrations of Mn, Al and S in the leaves of *F. crenata* seedlings as compared with the control values (Table 4). The addition of H^+ as HNO_3 solution to the soil significantly reduced the concentration of Ca in the N-100 treatment and significantly increased the concentrations of K, Mn and N as compared with the control values. The C/N ratio tended to be reduced by the addition of H^+ as HNO_3 solution to the soil as compared with the control value. No significant differences in the concentrations of P and C were detected among the ten soil treatments.

Table 2 Stem diameter, leaf area per plant, dry mass and ratio of shoot dry mass to root dry mass (S/R) of *F. crenata* seedlings on 5 October 1999. Each value is the mean of six determinations. The values followed by different letters within a column are significantly different according to Duncan's multiple range test ($P<0.05$)

Soil treatment	Stem diameter (cm)	Leaf area (cm^2)	Leaf number	Dry mass (g)				S/R (g g^{-1})
				Leaf	Stem + Branch	Root	Whole-plant	
Lime	0.968 a	463.2 abc	74 ab	2.026 bc	7.354 a	8.465 a	17.545 a	1.16 b
Control	0.892 ab	448.5 abc	77 ab	2.165 ab	7.671 a	7.407 a	17.242 a	1.33 ab
S-20	0.892 ab	454.0 abc	84 ab	2.085 bc	8.219 a	8.422 a	18.726 a	1.28 ab
S-40	0.903 ab	355.2 bcd	72 ab	1.730 bcd	7.255 ab	6.654 ab	15.639 ab	1.44 ab
S-60	0.869 ab	331.6 bcd	57 bc	1.216 de	6.031 ab	5.252 bc	12.499 bc	1.36 ab
S-100	0.661 c	244.4 d	38 c	0.983 e	3.187 c	3.698 c	7.868 d	1.07 b
N-20	0.885 ab	485.0 ab	91 a	2.345 ab	8.325 a	7.900 a	18.570 a	1.28 ab
N-40	0.868 ab	586.8 a	85 ab	2.725 a	8.274 a	8.190 a	19.189 a	1.45 ab
N-60	0.881 b	335.8 bcd	58 bc	1.523 cde	6.328 ab	5.091 bc	12.942 bc	1.56 a
N-100	0.806 b	295.7 cd	58 bc	1.333 de	4.842 bc	4.196 c	10.371 cd	1.59 a

Table 3 Net photosynthetic rates at 350 μmol mol^{-1} CO_2 (A_{350}), quantum yield (QY) of photosynthesis, maximum net photosynthetic rate at saturated CO_2 concentration (A_{max}) and carboxylation efficiency (CE) of photosynthesis in the leaves of *F. crenata* seedlings on 15 June 1999. Each value is the mean of four determinations. The values followed by different letters within a column are significantly different according to Duncan's multiple range test (P<0.05)

Soil treatment	A_{350} (μmol m^{-2} s^{-1})	QY (10^{-2} mol mol^{-1})	A_{max} (μmol m^{-2} s^{-1})	CE (10^{-2} mol m^{-2} s^{-1})
Lime	5.47 a	3.10 a	8.94 ab	3.33 a
Control	5.39 a	3.06 a	7.74 ab	3.45 a
S-20	2.80 bc	2.58 abc	7.22 b	2.51 ab
S-40	3.10 bc	2.43 abc	6.82 b	2.40 ab
S-60	1.55 bc	1.84 c	4.80 c	0.77 c
S-100	2.08 c	2.14 bc	5.21 c	1.50 c
N-20	4.55 ab	2.79 a	7.47 b	3.46 a
N-40	5.41 a	2.55 a	10.65 a	4.02 a
N-60	2.47 c	1.72 ab	5.27 c	2.49 ab
N-100	3.07 bc	1.99 ab	5.17 c	2.41 ab

Discussion

Effects of soil acidification on *F. crenata* seedlings

In the present study, the whole-plant dry mass of *F. crenata* seedlings grown in the S-60, S-100, N-60 and N-100 treatments was significantly less than the control value after the two growing seasons (Fig. 1). However, the degree of reduction in the whole-plant dry mass when compared to the control was greater in the S-100 treatment than in the N-100 treatment. Although no significant differences in the pH and concentrations of Al and Mn in the soil solutions were found between the S-100 and N-100 treatments (Table 1), the concentration of Al in the leaves of the seedlings was significantly higher in the S-100 treatment than in the N-100 treatment (Table 4). The degree of reduction in the A_{350} when compared to the control was greater in the S-100 treatment than in the N-100 treatment (Table 3). This tendency was also observed in other months of the second growing season, especially from spring to summer (data not shown). These results suggest that the greater degree of reduction in the whole-plant dry mass of the seedlings grown in the S-100 treatment as compared with the N-100 treatment can be explained by the greater degree of Al-induced reduction in the net photosynthetic rate.

In the present study, net photosynthetic rate was significantly reduced in the seedlings grown in the S-20, S-40, S-60, S-100, N-60 and N-100 treatments as compared with the control value (Table 3). In the seedlings grown in the S-100 treatment, the QY, A_{max} and CE were significantly less than the control values (Table 3). In the seedlings grown in the N-100 treatment, the A_{max} in June and CE in September were significantly less as compared with the control values (Table 3). The QY reflects the activity of PSII in the chloroplasts and depends on the quantity of chlorophyll and/or light-harvesting chlorophyll-binding protein in PSII (Gabrielsen 1948; Larcher 2003). The A_{max} corresponds to the regeneration rate of RuBP in the Calvin cycle and depends on the concentration of recyclable Pi (von Caemmerer and Farquhar 1981; Sharkey 1985). The CE reflects the activity of CO_2 fixation in the Calvin cycle and mainly depends on the activity and/or quantity of Rubisco (von Caemmerer and Farquhar 1981). Therefore, the reduction in net photosynthetic rate of the seedlings in the S-100 treatment is considered to be mainly due to the reductions in the quantity of chlorophyll and/or light-harvesting chlorophyll-binding protein in PSII, the regeneration rate of RuBP in the Calvin cycle and the quantity and/or activity of Rubisco. In the N-100 treatment, the reduction in net photosynthetic rate is considered to be mainly caused by the reductions in the regeneration rate of RuBP in the Calvin cycle and the quantity and/or activity of Rubisco. Although the concentrations of TSP in the leaves of the seedlings were not significantly different among the ten soil treatments, the concentrations of Rubisco were significantly reduced in the seedlings grown in the S-60

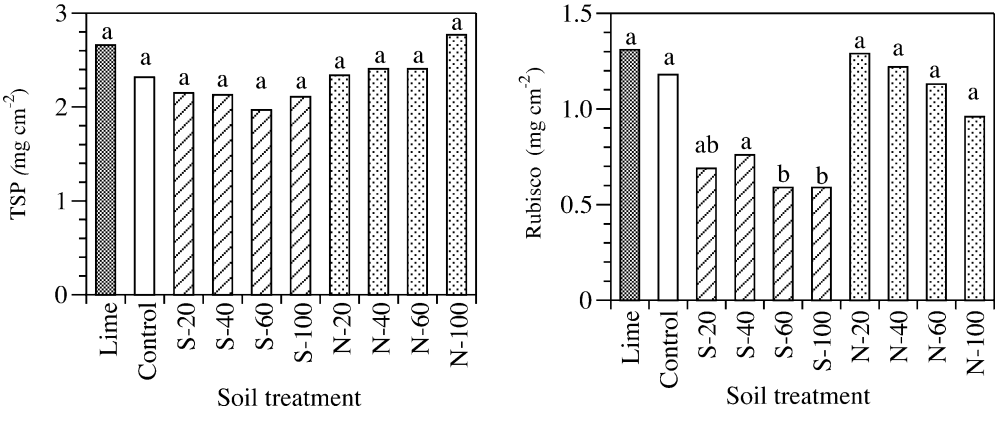

Fig. 2 The concentrations of total soluble protein (TSP) and Rubisco in the leaves of *F. crenata* seedlings on 15 June 1999. Each bar shows the mean of six determinations. Different letters above a bar indicate significant differences among the ten soil treatments (Duncan's multiple range test; P<0.05)

Table 4 The element concentrations in the leaves of *F. crenata* seedlings at the final harvest on 5 October 1999. Each value is the mean of four determinations. The values followed by different letters within a column are significantly different according to Duncan's multiple range test ($P<0.05$)

Soil treatment	Ca (mg g^{-1})	Mg (mg g^{-1})	K (mg g^{-1})	P (mg g^{-1})	Mn (mg g^{-1})	Al (mg g^{-1})	S (mg g^{-1})	C (mg g^{-1})	N (mg g^{-1})	C/N
Lime	10.10 abc	2.35 a	1.51 b	0.43 ab	0.37 f	0.08 c	2.00 d	0.52 a	16.33 ab	0.04 a
Control	10.64 ab	2.20 ab	1.47 b	0.52 ab	1.27 ef	0.12 bc	2.52 bc	0.52 a	14.95 b	0.04 a
S-20	11.29 a	1.92 abcd	1.74 ab	0.67 ab	6.74 bc	0.15 bc	2.45 c	0.48 a	17.81 ab	0.03 ab
S-40	10.85 ab	2.02 abcd	1.49 b	0.54 ab	8.57 a	0.23 bc	2.85 b	0.49 a	20.22 ab	0.03 ab
S-60	9.60 abc	1.64 cd	1.54 b	0.52 b	7.10 bc	0.28 b	3.33 a	0.50 a	17.61 ab	0.03 ab
S-100	8.49 bc	1.62 d	1.80 ab	0.68 ab	5.00 b	0.64 a	3.46 a	0.52 a	19.37 ab	0.03 ab
N-20	8.77 abc	2.05 abcd	1.35 b	0.60 ab	2.57 e	0.21 bc	2.31 cd	0.52 a	22.46 ab	0.02 ab
N-40	8.89 abc	1.82 bcd	1.38 b	0.64 ab	1.94 e	0.23 bc	2.40 cd	0.52 a	23.84 ab	0.02 ab
N-60	9.77 abc	1.82 bcd	1.87 ab	0.65 ab	8.00 ab	0.24 bc	2.29 cd	0.50 a	24.39 a	0.02 b
N-100	7.54 c	2.11 abc	2.29 a	0.73 a	5.78 cd	0.32 b	2.26 cd	0.49 a	23.17 a	0.03 ab

and S-100 treatments (Fig. 2). These results suggest that soil acidification by the addition of H_2SO_4 solution inhibited the biosynthesis of Rubisco in the leaves of *F. crenata* seedlings. On the other hand, because the addition of HNO_3 solution to the soil increased the concentration of N in the leaves as compared with the control value (Table 4), the degree of reduction in the concentration of Rubisco may be relatively low in the seedlings grown in the soil acidified by the addition of HNO_3 solution (Fig. 2). In general, the concentration of Rubisco increases with increasing leaf N content (Evans 1989; Makino 1997; Nakano et al. 1997), and the photosynthetic capacity rises with increasing leaf N content in linear proportion until limitation by other factors evokes saturation (Larcher 2003). In the present study, a positive correlation was obtained between the relative value of Rubisco concentration in the soil acidification treatments to that in the control treatment and the concentration of N in the leaves of *F. crenata* seedlings (data not shown). Therefore, there is the possibility that nitrate in the soil acted as N fertilizer in *F. crenata* seedlings grown in the soil acidified by the addition of HNO_3 solution.

Soil acidification by the addition of H_2SO_4 solution and excess Al and/or Mn in the nutrient solution induce a disturbance of nutrient status in several Japanese forest tree species such as *Cryptomeria japonica* (Izuta et al. 1996b), *Pinus densiflora* (Lee et al. 1997) and *F. crenata* (Izuta et al. 2001). In the present study, the addition of H^+ as H_2SO_4 or HNO_3 solution to the soil significantly reduced the concentration of Mg or Ca in the leaves of *F. crenata* seedlings as compared with the control values (Table 4). Therefore, nutrient deficiency in the leaves may be one of the limiting factors relating to the reduction in the net photosynthetic rate of the seedlings grown in the acidified soils (Izuta et al. 2001).

Based on the results obtained in the present study, we conclude that the negative effects of soil acidification on growth, net photosynthesis and nutrient status of *F. crenata* due to acid deposition with sulfate are greater than those of soil acidification due to acid deposition with nitrate. Therefore, when we evaluate the effects of soil acidification due to acid deposition on *F. crenata* forests in Japan, we should take the main anion in acid deposition into consideration.

Soil parameters for evaluation of critical load of acid deposition

In general, important factors relating to the reduction in dry matter production of woody plants grown in acidic soil are considered to be soil acidity itself and the concentration of Al in the soil solution. As shown in Fig. 3, the relative whole-plant dry mass per plant of *F. crenata* seedlings grown in the acidified soils to that of the seedlings grown in the control soil (Relative DM) declined when the pH of the soil solution was less than approximately 4.2. However, when the pH of the soil solution was below 4.0, there was a variation in the relative DM of the seedlings grown in the acidified soils. Furthermore, although negative correlations were obtained between the relative DM of the seedlings and the concentration of Al or Mn in the soil solution, there were great variations in the relative DM of the seedlings when the concentrations of Al and Mn in the soil solution were less than 0.7 mmol l^{-1} and above 0.5 mmol l^{-1}, respectively (data not shown). These results indicate that the growth response of the seedlings to soil acidification cannot be completely explained by low soil solution pH or the concentration of Al or Mn in the soil solution.

In Europe and USA, molar ratios of cations such as Ca, Mg and/or K to Al in soil or nutrient solutions have been regarded as a more important limiting factor for growth and nutrient status of tree species (Sverdrup and de Vries 1994; Cronan and Grigal 1995). In Europe, therefore, the critical load of acid deposition for protecting forest ecosystems has already been evaluated by several models based on a (Ca+Mg+K)/Al molar ratio in nutrient or soil solutions of 1.0 (Sverdrup and de Vries 1994). In the present study, relatively clear positive correlations were obtained between the Ca/Al molar ratio or the (Ca+Mg +K)/Al molar ratio in the soil solution and the relative DM of *F. crenata* seedlings grown in the acidified soils (data not shown). The addition of H^+ as H_2SO_4 or HNO_3

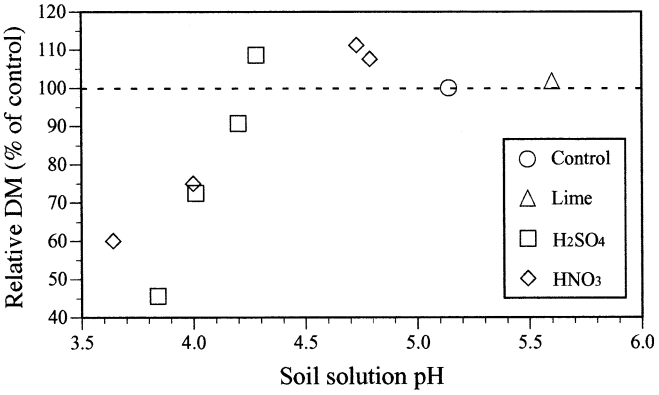

Fig. 3 The relationship between the pH of soil solution and relative whole-plant dry mass per plant of *F. crenata* seedlings grown in acidified soil to that of the seedlings grown in the control soil (Relative DM). The seedlings were grown in brown forest soil acidified by adding H^+ as H_2SO_4 (*square*) or HNO_3 solution (*diamond*). Control soil (*circle*) and that treated with lime (*triangle*) were not supplemented with H^+ as H_2SO_4 or HNO_3 solution

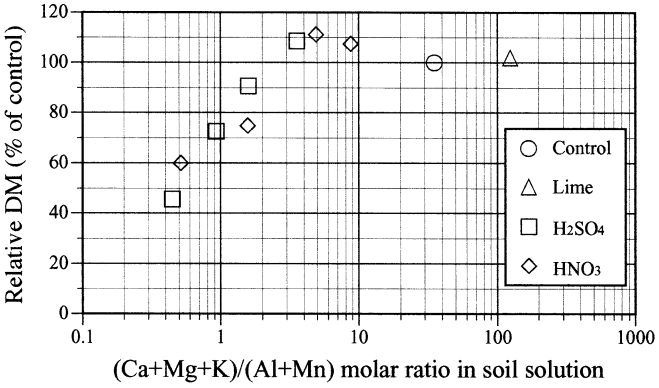

Fig. 4 The relationship between the molar ratio of total concentration of Ca, Mg and K to Al concentration in soil solution [(Ca+Mg+K)/(Al+Mn)] and relative whole-plant dry mass per plant of *F. crenata* seedlings grown in acidified soil to that of the seedlings grown in the control soil (Relative DM). The seedlings were grown in brown forest soil acidified by adding H^+ as H_2SO_4 (*square*) or HNO_3 solution (*diamond*). Control soil (*circle*) and that treated with lime (*triangle*) were not supplemented with H^+ as H_2SO_4 or HNO_3 solution

solution to the soil significantly increased the concentration of Mn in the soil solutions and leaves of the seedlings (Tables 1, 3). Excessive Mn induces several detrimental effects on growth, physiological functions such as photosynthesis and nutrient status of Japanese deciduous broad-leaved tree species (Kitao et al. 1997a, b). Therefore, we investigated the relationship between the molar ratio of (Ca+Mg+K)/(Al+Mn) in the soil solution and the relative DM of *F. crenata* seedlings. As shown in Fig. 4, a very clear positive correlation was obtained between the molar ratio of (Ca+Mg+K)/(Al+Mn) in the soil solution and the relative DM of the seedlings. Based on the results obtained in the present study, we conclude that the molar ratio of (Ca+Mg+K)/(Al+Mn) in the soil solution is a suitable soil parameter to evaluate the critical loads of acid deposition for protecting *F. crenata* forests in Japan. The evaluation of long-term buffering capacity of Japanese forest soils is an important problem to be solved for evaluating critical loads of acid deposition for protecting *F. crenata* forests in Japan. Furthermore, the effects of acute deposition of acidity are likely to be different from those of chronic deposition of acidity on the buffering capacity of soils and tree growth. Therefore, further studies are needed on the long-term effects of acid deposition on chemical characteristics of Japanese forest soils and growth of *F. crenata*.

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