## Chapter 18 Effects of Nitrogen Load on Asian Trees

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**Abstract** The effects of increasing nitrogen (N) load on Asian forest trees have been studied mainly in Japan and China during the past decade. In this chapter, we summarize the expected mechanisms of the harmful effects of excessive N load on the eco-physiological function of trees, and introduce experimental studies on the growth responses and foliar nutrient status of young Asian tree seedlings to increasing N loads. By comparing the growth responses to various N loads in 12 tree species in Japan and China, we confirmed that: (1) the threshold of the N load that induced growth reduction was quite different among the species; (2) the threshold value ranged between 50 and 100 kg N ha<sup>-1</sup> year<sup>-1</sup> in relatively sensitive (low-tolerance) species. Furthermore, a significant relationship between foliar nutrient balance and growth indicated that (3) the threshold N/P ratio for growth reduction was slightly higher than that in European tree species, and (4) an Mn/Mg ratio of over 0.8 was observed, together with growth reduction, in the sensitive tree species.

**Keywords** Excessive N load • Growth response • Mn/Mg ratio • N/P ratio • Nutrient imbalance • Species difference • Tree

## 18.1 Nitrogen Saturation in Forest Ecosystems

Generally, nitrogen (N) is a limiting nutrient factor in many temperate forests (Ingestad and Kähr 1985; Crane and Banks 1992). In forest ecosystems where N limits primary production, because most of the N supplied by atmospheric deposition is absorbed and assimilated by microbes and plants, N output as nitrate (NO<sub>3</sub><sup>-</sup>) in stream water is lower than N input by atmospheric deposition (Stoddard 1994).

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However, several researchers have observed relatively high N outputs in stream water, exceeding the N input, and they have suggested that the N status of some forests in Central Europe and North America is changing from 'N-limited' to 'N-saturated' (Skeffington and Wilson 1988; Stoddard 1994; Fenn et al. 1996). In Europe, harmful effects of excessive N load have been expected with increasing N-dominated acid deposition (see Chap. 3). High nitrate deposition is a risk in forest ecosystems, since a high input of ammonium  $(NH_4^+)$  to the soil induces soil acidification and nutrient imbalance, and the risk of excessive ammonium load has also been been predicted in some forested areas (Nihlgård 1985).

## 18.2 Effects of Excessive N Load on Asian Trees

Several researchers have investigated the effects of increasing inputs of nitrate and ammonium on tree health to clarify these phenomena and their mechanisms (e.g. Wilson and Skeffington 1994b; Seith et al. 1996; Izuta and Nakaji 2003; Wang and Liu 2014). Most of these studies dealt with young (few-years-old) tree seedlings, and the N effects on photosynthesis (Nakaji et al. 2002; Azuchi et al. 2004; Guo et al. 2014), growth (Nakaji et al. 2005; Izuta et al. 2005; Mao et al. 2014), mycorrhizal roots, and nutrient status (e.g. Wallenda and Kettle 1998; Seith et al. 1996; George et al. 1999; Sogn and Abrahamsen 1998; Nakaji et al. 2005) were investigated experimentally. Furthermore, based on the results of fertilizer experiments and monitoring studies, mainly in Europe, an empirical critical load of N and a nutrient index were proposed (De Vries et al. 2000a, b; WHO 2000). In European forest ecosystems, the thresholds of the N load for the appearance of N saturation and forest damage were approximately 10 kg N ha<sup>-1</sup> year<sup>-1</sup> and 25 kg N ha<sup>-1</sup> year<sup>-1</sup>, respectively (Wright et al. 1995). Bobbink et al. (2015) have reported that the empirical critical load of N that would not cause nutrient imbalance, reduction in root biomass, or species change showed large variations among ecosystem types, and it ranged from 3 to 20 kg N ha<sup>-1</sup> year<sup>-1</sup>. In Asian countries such as China, Japan, India, and South Korea, high atmospheric N deposition, similar to or higher than that in Central Europe and North America, has been observed (Dentener et al. 2014; Kulshrestha et al. 2014). For example, in Japan, although the mean wet N deposition by precipitation over Japan has been maintained at 7 to 10 kg N ha<sup>-1</sup> year<sup>-1</sup> (Katoh et al. 1990; Hara 1992), the N input by wet deposition in coniferous forests near suburban areas ranged from 10 to 20 kg N ha<sup>-1</sup> year<sup>-1</sup> (Ohrui and Mitchell 1997), and sometimes it reached 40 kg N ha<sup>-1</sup> year<sup>-1</sup> near urban areas (Okita et al. 1993; Baba and Okazaki 1998). In China, wet N deposition has been observed at a range of 9 to 23 kg N ha<sup>-1</sup> year<sup>-1</sup> (Du and Liu 2014), and dry N deposition in North China has been estimated to be about 25 kg N ha<sup>-1</sup> year<sup>-1</sup> (Shen et al. 2014). These reports suggested that the N deposition in these countries would already reach the threshold range of N saturation. In Europe, thresholds of element concentration and element balance in tree leaves were proposed in the 1990s by European Commission -United Nations/Economic Commission for Europe EC-UN/ECE et al. (1997) for evaluating the nutrient status of major tree species, such as spruce, pine, oak, and beech

		Spruce			Pine			Oak			Beech		
$Class^{a}$		1	2	3	1	2	3	1	2	3	1	2	3
Nutrient	z	<12	12-17	>17	<12	12-17	>17	<15	15-25	>25	<15	15-25	>17
concentration	Ρ	<1.0	1.0-2.0	>2.0	<1.0	1.0-2.0	>2.0	<1.0	1.0-1.8	>1.8	<1.0	1.0-1.7	>1.7
$(\text{mg g}^{-1})$	K	3.5	3.5-9.0	>9.0	<3.5	3.5 - 10.0	>10.0	<5.0	5.0 - 10.0	>10.0	<5.0	5.0 - 10.0	>10.0
	Ca	<1.5	1.5-6.0	>6.0	<1.5	1.5-4.0	>4.0	<3.0	3.0-8.0	>8.0	<4.0	4.0-8.0	>8.0
	Mg	<0.6	0.6-1.5	>1.5	<0.6	0.6 - 1.5	>1.5	<1.0	1.0-2.5	>2.5	<1.0	1.0-1.5	>1.5
	S	<1.1	1.1–1.8	>1.8	<1.1	1.1-1.8	>1.8	1	I	1	<1.3	1.3 - 2.0	>2.0
Nutrient ratio	N/P	<6.0	6.0-17.0	>17.0	<6.0	6.0-17.0	>17.0	<8.3	8.3-25.0	>25.0	<10.6	10.6 - 25.0	>17.0
$(g g^{-1})$	N/K	<1.3	1.3-4.9	>4.9	<1.2	1.2-4.9	>4.9	<1.5	1.5 - 5.0	>5.0	<1.8	1.8 - 5.0	>5.0
	N/Ca	<2.0	2.0-11.3	>11.3	<2.0	2.0-11.3	>11.3	<1.9	1.9 - 8.3	>8.3	<2.3	2.3-6.3	>6.3
	N/Mg	<8.0	8.0-28.3	>28.3	<8.0	8.0-28.3	>28.3	<6.0	6.0-25.0	>25.0	<12.0	12.0-25.0	>25.0
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	<b>Table 18.1</b>

Source: European Commission -United Nations/Economic Commission for Europe EC-UN/ECE et al. (1997)  ${}^{a}I = low, 2 = normal or adequate, 3 = optimal to high$ 



Fig. 18.1 Schematic images of the negative effects of increasing N deposition in sensitive forest tree species

(Table 18.1). Because these nutrient indices have not been extensively examined in Asian countries, studies on the critical N load and nutrient balance are needed for evaluating the current status and sufficient management of Asian forests.

Figure 18.1 shows schematic images of the negative effects of excessive N load to the soil on sensitive (i.e., low-tolerant) tree species. Although  $NO_3^-$  and  $NH_4^+$  in soil generally play roles as plant fertilizers, over-nutrition of N by the forms of  $NO_3^-$  and  $NH_4^+$  induces soil acidification by H<sup>+</sup> originating from HNO<sub>3</sub> and the nitrification of  $NH_4^+$  (Nilsson 1986). Soil acidification causes leaching of base cations such as Ca and Mg from soil to the watershed and enhances the solubility of Mn and Al (Van Breemen et al. 1982; Ulrich and Sumner 1991). This change can induce a lack of mineral nutrients and cause the excessive accumulation of Mn and toxic Al ions in plants. High concentrations of soil  $NO_3^-$  and acidity affect the species component of mycorrhiza and reduce the mycorrhizal infection rate in fine roots (i.e., mycorrhizal roots), as well as reducing mycorrhizal lifespan (Majdi and Nylund 1996; Wallenda and Kottke 1998; Wöllecke et al. 1999). Mycorrhizal roots play important roles in the uptake of water and nutrients such as Mg and phosphorus (P) (Marschner and Dell 1994). Because high concentrations of  $NO_3^-$  and  $NH_4^+$  can cause chemical antagonism with  $Mg^{2+}$  and  $PO_3^-$  at root uptake sites (Boxman and Roelofs 1988; Wilson and Skeffington 1994a), these biotic and chemical changes in the soil environment could cause deteriorated nutrient status in trees via the excessive accumulation of Mn and/or deficiencies in Mg and P. This nutrient imbalance may also increase sensitivity to other environmental stressors such as drought and frost (Nihlgård 1985; Cowling et al. 1988; Schulze 1989; Izuta and Nakaji 2003). In sensitive tree species such as *Pinus densiflora* (Japanese red pine), excessive N-induced low foliar concentrations of Mg and P reduced photosynthetic activity, due to the reduction of foliar chlorophyll and ribulose-1,5-bisphosphate (RuBP) carboxylase/oxygenase (Rubisco), despite a high foliar N concentration (Nakaji et al. 2001, 2002). Furthermore, a high foliar Mn concentration compared with Mg (i.e., a high Mn/Mg ratio) tends to inhibit the activation of Rubisco (Nakaji et al. 2005). Consequently, in sensitive tree species and acid soil, excessive N will reduce tree growth via an imbalance of nutrients.

The nutrient imbalances and growth reduction of below-ground organs have been reported in many experimental studies of European tree species. For example, Seith et al. (1996) investigated growth and foliar nutrients in Picea abies (Norway spruce) seedlings grown in two N treatments (150 mg and 300 mg N kg<sup>-1</sup> soil), and they reported N-induced reductions in needle concentrations of N, Ca, Mg, and Mn and increases in those of P and K. They also reported that experimental N addition to the soil induced reduction of fine root growth in *Picea abies* seedlings (Seith et al. 1996; George et al. 1999). Sogn and Abrahamsen (1998) reported that a 5-year experiment with varied N supply at 30 kg and 90 kg N ha<sup>-1</sup> year<sup>-1</sup> reduced needle concentrations of K, Mg, and P in Pinus sylvestris (Scotch pine) seedlings without growth reduction. Figure 18.2 shows comparisons of growth response to increasing N load in 12 tree species in Japan and China (Nakaji et al. 2005; Izuta et al. 2005; Guo et al. 2010, 2014; Liu et al. 2011; Mao et al. 2014; Wang and Liu 2014). These studies conducted manipulation experiments during one to three growing seasons, using natural forest soil and 1- to 2-year-old seedlings. In this comparison, the relative growth of whole-plant dry mass was calculated in each experiment. The results show interesting trends in regard to threshold and species differences in tolerance to excessive N load. None of the 12 tree species showed growth reduction by N addition below 50 kg N ha<sup>-1</sup> year<sup>-1</sup>, but the threshold for growth reduction was quite different among the tree species. For example, the whole-plant dry mass of three species, Fagus crenata (Japanese beech), Castanopsis sieboldii (Sudajii, a Japanese evergreen oak), and Pinus densiflora (Japanese red pine) tended to be reduced at values between 50 and 100 kg N ha<sup>-1</sup> year<sup>-1</sup> (Fig. 18.2). These species can be classified as N-sensitive (low-tolerant) species. On the other hand, Cryptomeria japonica (Japanese cedar), Picea asperata (dragon spruce), and Quercus acuta (Japanese red oak) can be classified as N-tolerant tree species, because they showed enhanced growth rates even with very high N loads, over 200 kg N ha<sup>-1</sup> year<sup>-1</sup> (Fig. 18.2). Although the threshold for two tree species was not known, the four remaining species, Acer truncatum (purple brow maple), Pinus tablaeformis (Chinese pine), Lithocarpus edulis (Matebashii, a Japanese evergreen oak species), and Quercus glauca (ring-cup oak) seemed to be intermediate in response, with a threshold of





growth reduction at over 100 kg N ha<sup>-1</sup> year<sup>-1</sup>. The responsible mechanism has not yet been clarified; however, this result suggests that we must pay attention to species differences in N sensitivity when estimating the empirical critical load of N in Asian forests for maintaining tree growth and biodiversity.

The growth reduction in sensitive tree species caused by excessive N load has been explained mainly by the depression of photosynthesis due to nutrient imbalances of N/P and Mn/Mg appearing with reduced mycorrhizal infection (Nakaji et al. 2001, 2002; Izuta and Nakaji 2003; Izuta et al. 2005). Figure 18.3 shows the relationship between foliar nutrient status and growth response to an increasing N load in eight Japanese tree species (Izuta et al. 2005; Nakaji et al. 2005; Izuta et al. 2002, unpublished data). When the data were pooled for all tree species, significant correlations were observed in the relationships of dry-mass responses and P (r=0.41, P=0.010), the N/P ratio (r=-0.34, P=0.035), Mn (r=-0.53, P<0.001), and Mn/Mg (r=-0.53, P<0.001). There was no significant relationship in regard to other foliar elements such as K and Ca. This suggests that P and Mn are important nutrient factors related to the N sensitivity of Japanese tree



**Fig. 18.3** The relationship between foliar nutrient status and growth response to increasing N load in eight Japanese tree species. The nutrient concentration and nutrient balance were investigated in current-year leaves (**a**) N; (**b**) P; (**c**) N/P; (**d**) Mn; (**e**) Mg; (**f**) Mn/Mg. Correlation coefficients for all the plant species and significance levels of the relationship are shown in each panel (\* P < 0.05, \*\*\* P < 0.001). *Vertical dashed lines* indicate threshold values showing optimal-to-high nutrient status for N or low nutrient status for P and Mg in European pine (P), oak (O), and beech (B) species (see Table 18.1) (EC-UN/ECE et al. 1997). Symbols: + *L. gmelinii* × *L. kaempferi* (Mao et al. 2014), • *F. crenata* (Izuta et al. unpublished data), **C**. *sieboldii*,  $\Box$  *L. edulis*, • *Q. glauca*,  $\diamond$  *Q. acuta* (Izuta et al. 2005),  $\triangle$  *C. japonica*, **A** *P. densiflora* (Nakaji et al. 2005)

seedlings. When we compared the criteria for nutrient balance for European tree species and these data, we found that foliar N levels in the Japanese tree species were lower than the European criteria for high nutrient balance (pine = 17 mg N g<sup>-1</sup>, oak and beech = 25 mg N g<sup>-1</sup>), and the foliar P concentration was lower than the criteria for P deficit in European tree species (1 mg g<sup>-1</sup>) (Fig. 18.3a, b). Lower P in Japanese tree species could be related to the fact that the origin of most soil in Japan is volcanic ash. As for the nutrient balances, 10% growth reduction in *P. densiflora* (pine), *F. crenata* (beech), and *C. sieboldii* (evergreen oak) was observed with higher N/P ratios of about 20, 35, and 55, respectively. Mn/Mg ratios over 0.8, 1.0, and 1.3 were observed with growth reduction in *P. densiflora*, *F. crenata*, and *C. sieboldii* (Fig. 18.3). Since the Mn/Mg ratio is related to the activation of Rubisco (Nakaji et al. 2001; Manter et al. 2005), this imbalance is also important. As the species are different in Europe and Japan, European data would not be appropriate, and these comparison results indicate that an original N threshold value is needed for Japanese tree species.

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