Nitrogen use efficiency of *Quercus serrata* seedlings under different soil nitrogen and phosphorus supplies

Benhui ZHU\(^a\), Takeshi IZUTA\(^b\) and Makoto WATANABE\(^b,†\)

\(^a\)Graduate School of Agriculture, Tokyo University of Agriculture and Technology, Fuchu, Tokyo 183–8509, Japan  
\(^b\)Institute of Agriculture, Tokyo University of Agriculture and Technology, Fuchu, Tokyo 183–8509, Japan

Abstract

Nitrogen use efficiency (NUE), defined as the ratio of biomass growth and the nitrogen (N) taken up from a soil, is widely used in the evaluation of plant N economy. We conducted a fertilization experiment with *Quercus serrata* seedlings under different soil N and phosphorus (P) levels to determine the influence of high N deposition levels under different P availabilities upon NUE, plus the NUE components N productivity (NP), and mean residence time of N (MRT). The seedlings were grown for one growing season in the potted soil under three N levels (0, 50, and 100 kg N ha\(^{-1}\)) combined with two P levels (0 and 50 kg P ha\(^{-1}\)). Nitrogen supply to the soil reduced NUE by decreasing NP and MRT, whereas P supply enhanced NUE. Although P supply did not have a significant influence on the reduction of NUE with increasing N levels, it affected the response of the NUE components with increasing N levels. Within leaves, N supply reduced the photosynthetic nitrogen use efficiency (PNUE), P alleviated this reduction. Therefore, P is considered as one of the key factors determining the change of NUE in *Q. serrata* under the influence of high N supply.

**Key words:** Mean residence time, Nitrogen productivity, Nitrogen usage, PNUE

1. Introduction

Increasing nitrogen (N) deposition from the atmosphere has been altering global N availability (Galloway et al., 2013). In recent decades, East Asia has become one of the regions with the highest N deposition levels. In a global analysis of N deposition based on the second phase of Task Force Hemispheric Transport of Air Pollution (HTAP II), Tan et al. (2018) reported that East Asia received the largest N deposition in 2010, with non-coastal deposition levels of 15.1 Tg N year\(^{-1}\), accounting for 13% of the global total N deposition. Xu et al. (2015) quantified data from 43 monitoring sites of the Nationwide Nitrogen Deposition Monitoring Network (NNDMN) established in China from 2010 to 2014 and found the total N deposition ranged from 2.9 to 83.3 kg N ha\(^{-1}\) year\(^{-1}\), with average dry and wet N deposition fluxes of 20.6 ± 11.2 (mean ± standard deviation) and 19.3 ± 9.2 kg N ha\(^{-1}\) year\(^{-1}\) across China, respectively. In Japan, Endo et al. (2011) used data from the Acid Deposition Monitoring Network in East Asia (EANET) to estimate the average of total N deposition from 2003 to 2008 as 3.1 to 18.2 kg N ha\(^{-1}\) year\(^{-1}\). Schwede et al. (2018) calculated the global N deposition in forests and indicated that the largest amount of N deposition was received by Asian forests.

In N-limited forests, N deposition acts as a fertilizer and thus promotes forest productivity at least in the early stages. Many studies have documented stimulated growth of forest tree species with N availability (e.g. Watanabe et al., 2007; Mao et al., 2014). However, continuous high N deposition levels may result in N saturation, which may result in negative effects such as the reduction of plant production, forest decline, and loss of biodiversity (Aber et al., 1989; Vitousek et al., 1997; Matson et al., 2002). Based on the results of the NITREX project (nitrogen saturation experiments), Wright et al. (1995) reported that forest ecosystems in Europe would gradually become N saturated when levels of N input range from 10 to 25 kg N ha\(^{-1}\) year\(^{-1}\). According to this criteria, some forested areas in East Asia may already be suffering from N saturation (Sase et al., 2008; Kimura et al., 2009; Nakaji and Izuta, 2017). Many observational studies and simulated N deposition experiments conducted in East Asia have shown, high N levels having significant effects on soil acidification, plant growth, fluxes of greenhouse gases (e.g. CH\(_4\) and N\(_2\)O), and biodiversity (Liu et al., 2011; Kim et al., 2012; Nakaji and Izuta, 2017).

Nitrogen use efficiency (NUE) has been widely used in the evaluation of N economy of plants under various soil nutrient conditions, such as different N supplies or availability gradients, and among different plant species (e.g. Hirose, 1975; Shaver and Melillo, 1984; Birk and Vitousek, 1986; Norby and Iversen, 2006; Friesen and Cattani, 2017). The NUE was originally defined as the ratio of biomass growth to the N taken up from a soil (Hirose, 1971; Vitousek, 1982). Previous studies have revealed that NUE decreased with increasing soil N availability (Vitousek, 1982; Pastor and Bridgham, 1999). The analysis of NUE is considered to be one of the suitable methods to evaluate the forest N status (Tateno et al., 2017). The NUE was later defined as the product of N productivity (NP) and N mean residence time (MRT) by Berendse and Aerts (1987). Generally, plants have a long MRT under infertile conditions, while higher NP is favored under fertile conditions (Berendse, 1985). This approach is applicable for the calculation under steady state conditions where N input is assumed to equal N output such as that found in mature trees and forests. Hirose (2011) further developed the model of NUE, NP and MRT by introducing the
concepts $\bar{N}$ (mean whole-plant N content) and plant nitrogen duration (PND). Using these concepts, NUE of plants under non-steady state conditions such as young trees can be analyzed.

Phosphorus (P) is another key nutrient element for tree growth and forest productivity (Elser et al., 2007; Vitousek et al., 2010). Increasing N to a soil may shift forests from being N-limited to P-limited (Bobbink and Lamers, 2002; Li et al., 2016b). Phosphorus availability is also vital for this shift. Forests with lower P availability may easily shift from N-limited to P-limited than those with higher P availability. At the individual plant level, the combined effects of N and P on growth and photosynthesis have been reported. For example, Mao et al. (2014) reported no significant effects from P supply and the interactions between P and N supplies upon the photosynthesis and growth of hybrid larch $F_t$ seedlings. In a N and P fertilization with two Larix species (Larix olgensis and Larix kaempferi), Li et al. (2016a) reported that N and P had a significant influence on the growth and proposed that P fertilization may alter the N concentration and photosynthetic products. In this context, we consider the response of NUE of trees to N supply may change under different P conditions. However, there are no studies on the effects of N and P supplies to soil on NUE of trees.

In the present study, we had two hypotheses: first that P supply enhances NUE, and second that P supply mitigates reductions of NUE caused by the N supply. To test these hypotheses, we designed a fertilization experiment that used Quercus serrata seedlings grown under different levels of soil N and P supplies. $Q. \text{serrata}$ is a deciduous broad-leaved tree species that is extensively distributed in East Asia (Lincoln, 1986; Nakashizuka and Iida, 1996; Wu and Raven, 1999). In Japan, this tree species grows in urban and sub-urban forested areas where high N deposition has been observed (Kimura et al., 2009).

2. Materials and methods

2.1 Plant materials and nutrient treatments

The experiment was conducted at the Fuchu Campus of Tokyo University of Agriculture and Technology (35°41'N, 139°29'E) with 105 individuals of 2-year-old $Q. \text{serrata}$ seedlings. The seedlings were obtained in February 2018 from a nursery in Shikoku region then they were grown in the field. Bud break was observed in middle April. The stem base diameter (SBD, mm) and height of each seedling were measured in May 2018. Using the product of SBD square and height (D$^2$H, cm$^3$), the seedlings were sorted into seven groups (15 seedlings in each group) with the same mean D$^2$H. Six groups were randomly selected from the seven groups and were used for the fertilization experiment from 12 June to 12 September 2018. The SBD and height of the seedlings at the beginning of the experiment were 4.4 ± 0.6 mm and 41.0 ± 6.8 cm (mean ± standard deviation), respectively. We regularly irrigated the seedlings to keep the soil moist. The SBD and height of the 15 seedlings of the unselected group were measured, followed by the seedlings being harvested on 12 June, the first day of nutrient application. The harvested seedlings were sectioned into roots, stems and leaves, dried in an oven at 80°C for 5 days, and weighed to determine dry mass. The dry mass of roots, stems, leaves and whole plants of the initial samples were 10.1 ± 3.3 g, 6.1 ± 1.6 g, 5.8 ± 1.7 g and 22.0 ± 5.5 g (mean ± standard deviation), respectively. Afterwards, all samples were ground into a powder with a sample mill (Wonder Blender, Osaka Chemical Co., Ltd., Japan). Nitrogen concentrations ($N_{\text{root}}$ %) of the powdered samples were determined with a C/N analyzer (MT700, Yanaco Co. Ltd., Japan).

The seedlings for the fertilization experiment were transplanted to 1/2000 a Wagner pot (0.252 × 0.300 mm) filled with a mixture of Akadama soil and Kanuma soil (volume ratio: 1:1) on 12 June 2018. Six nutrient treatments were used that comprised of three levels of N (0, 50, and 100 kg N ha$^{-1}$, labeled as N0, N50, and N100, respectively) in combination with two levels of P (0 and 50 kg P ha$^{-1}$, labeled as P0 and P50, respectively). For individual seedlings, the nutrient supply rates of N100, N50 and P50 were 40 mg N plant$^{-1}$ week$^{-1}$, 20 mg N plant$^{-1}$ week$^{-1}$, and 20 mg P plant$^{-1}$ week$^{-1}$, respectively. The N0 and P0 respectively meant no N or no P fertilizer was applied. Ammonium nitrate solution was the N source, while the P source for the soil mixture used in the P50 treatment was a solution of 1:1 mixture of potassium dihydrogen phosphate and dipotassium phosphate. Potassium chloride solution was added to the soil mixture used in the P0 treatment to adjust the amount of potassium among the all treatments to a constant level. The nutrient treatments started on the day after transplanting (13 June 2018) and were split into four intervals separated by 3 weeks. Each reagent for each soil treatment was dissolved in 300 ml of tap water before being used to irrigate the soil with the seedlings. The average daily temperature (24 hours) during the experimental period was 26.4°C, and the average PPFD (photosynthetic photon flux density) in the daytime (6:00 to 18:00) was 742 μmol m$^{-2}$ s$^{-1}$.

2.2 Leaf gas exchange measurement

On 27 August 2018, six $Q. \text{serrata}$ seedlings were randomly selected from each treatment (36 seedlings in total) for determining leaf gas exchange rate with an open gas exchange system (LI-6400XT, LI-COR, Lincoln, NE, USA) equipped with 6400-02B LED light source. Measurements were conducted on mature leaves of first and second flush leaves. The photosynthetic photon flux density, CO$_2$ concentration, leaf temperature and vapor pressure deficit at leaf temperature (VPD) during the measurements were maintained at 1500 μmol m$^{-2}$ s$^{-1}$, 400 μmol mol$^{-1}$, 28°C and 1.5 kPa, respectively. We measured the light-saturated net photosynthetic rate ($A_{\text{sat}}$, μmol CO$_2$ m$^{-2}$ s$^{-1}$), stomatal conductance to water vapor ($g_s$, mol H$_2$O m$^{-2}$ s$^{-1}$) and intercellular CO$_2$ concentration ($C_i$, μmol CO$_2$ mol$^{-1}$). After the measurement of leaf gas exchange, four leaf discs (12 mm diameter) were taken using a leaf punch. All leaf discs were dried at 80°C for 5 days and weighed. We measured $N_{\text{mass}}$ of the dry leaf discs with a C/N analyzer (MT700, Yanaco Co. Ltd., Japan) and calculated the area-based N content ($N_{\text{area}}$, mmol N m$^{-2}$). The photosynthetic nitrogen use efficiency (PNUE, μmol CO$_2$ mol$^{-1}$ N s$^{-1}$) was calculated as $A_{\text{sat}}$ divided by $N_{\text{area}}$.

2.3 Growth measurement

The SBD and height of the seedlings were measured monthly, for a total of 4 measurements. After the final measurement of those parameters on 12 September 2018, all seedlings were harvested and divided into root, stem and leaves in each flush.
Dry mass of each plant organ was determined after drying for 5 days at 80°C. Then all samples were ground into powders to determine the $N_{\text{mass}}$ with the C/N analyzer.

2.4 Calculation of nitrogen use efficiency

$NUE$ (g g\(^{-1}\) N), $NP$ (g g\(^{-1}\) N day\(^{-1}\)), and $MRT$ (day) were determined following Hirose (2011). The $NUE$ of all seedlings during an experiment was calculated as follows:

$$NUE = \frac{\Delta W}{\Delta N}$$

(1)

where $\Delta W$ (g) and $\Delta N$ (g) are the dry mass growth and the amount of $N$ uptake between initial and final samples, respectively. The $NUE$ comprises $NP$ and $MRT$ as:

$$NUE = NP \cdot MRT$$

(2)

and $NP$ and $MRT$ were calculated from the following equations:

$$NP = \frac{\Delta W}{\bar{N}} \cdot \Delta T$$

(3)

$$MRT = \frac{\bar{N}}{\Delta N} \cdot \Delta T$$

(4)

where $\Delta T$ is the experimental duration (90 days in this study) and $\bar{N}$ (g) is the mean plant nitrogen, which is determined by:

$$\bar{N} = \frac{1}{\Delta T} \int_{0}^{T} [N_{0} + f(t) - g(t)] \, dt$$

(5)

where $N_{0}$ is the initial N content, and the functions $f(t)$ and $g(t)$ represent N uptake and loss, respectively. The conceptual model of this calculation is shown in Fig. 1. Because no litterfall was found in the present experiment, we assumed $g(t) = 0$.

The objectives of the calculations were to determine the whole-plant dry mass and N content in the initial and final samples, and to estimate the whole-plant N content during the entire experimental period. To estimate the initial state dry biomass, we established an allometric equation between the log-transformed dry mass and D\(^{15}\)H of the 15 seedlings harvested during the initial sampling. The dry mass of each plant organ from the seedlings used in the fertilization experiment was calculated. The difference between the whole-plant dry mass of the seedlings between initial and final samples was considered as $\Delta W$.

Using the average N concentrations in roots, stems, and leaves of the 15 seedlings at the initial sampling, we calculated the N content of each plant organ and for the whole plant at the initiation of nutrient treatment (i.e. $N_{0}$). We also determined the amount of N in roots, stems, and leaves of the seedlings harvested at the final sampling. The difference between whole-plant N content between the initial and final sampling was considered as $\Delta N$.

In the determination of $\bar{N}$, we applied following logistic model (Fig. 1):

$$N_{i} = N_{0} + f(t) - g(t) = \frac{K}{1 + e^{-r(T - t)}}$$

(6)

where $N_{i}$ is whole-plant N content at a given time point, $K$, $a$, and $r$ are parameters (Hunt, 1982; Paine et al., 2012). We estimated the dry mass and N content from each plant organ of the seedlings on 12 July and 12 August 2018 with an allometric equation that used SBD and height, which were obtained from initial and final sampling. Consequently, whole-plant N content of each seedling was derived from four different time points. The three parameters ($K$, $a$, and $r$) of equation (6) were determined by fitting the model to the data on whole-plant N content at the four time points. In the fitting process, we used the solver function add-in of Microsoft Excel to arrive at a minimum value for the sum of square error. After the three parameters in equation (6) were obtained, the specific function of N amount over time was obtained. We integrated N amount with respect to time, and then divided it by the total experimental time $\Delta T$; then $\bar{N}$ was determined with equation (5). The $NP$ and $MRT$ were calculated from $\Delta W$, $\Delta T$, $\Delta N$, and $\bar{N}$ with equations (3) and (4), and $NUE$ was calculated from $NP$ and $MRT$ using equation (2).

Using a similar method for determining $\bar{N}$, we calculated mean leaf N ($N_{L}$, g) during the experiment. NP was factored into the product of leaf N productivity (LNP, g g\(^{-1}\) N day\(^{-1}\)) and leaf N ratio (LNF, %) according to following equation,

$$NP = \frac{\Delta W}{\bar{N}_{L}} \cdot \Delta T$$

(8)

where LNP and LNF were determined with following equations,

$$LNP = \frac{\Delta W}{\bar{N}_{L}} \cdot \Delta T$$

(9)

2.5 Statistical analysis

Statistical analyses were performed with the R software, version 3.51 (R Development Core Team, 2018). The effects of N and P supply on each parameter were tested by an analysis of deviance with a generalized linear model. In the model,
Table 1. Dry mass of plant organs, ratio of root to shoot dry mass (R/S ratio), and mass-based nitrogen concentration (N\(_{\text{max}}\)) of Quercus serrata seedlings at the end of the experiment.

| Treatment     | Whole plant (g) | Roots (g) | Stems (g) | Leaves (g) | R/S ratio | N\(_{\text{max}}\) (%) |
|---------------|-----------------|-----------|-----------|------------|-----------|------------------------|
|               | Dry mass        |           |           |            |           |                        |
|               | Total           | 1st flush | 2nd flush | 3rd flush  | 4th flush | Roots | Stems | Leaves |
| N0P0          | 58.4 (8.6)      | -         | -         | -          | -         | 0      | 1.71 | 1.3 | 0.71 |
| N50P0         | 66.2 (12.3)     | -         | -         | -          | -         | 0.2    | 1.21 | 0.9 | 0.27 |
| N100P0        | 73.2 (11.4)     | -         | -         | -          | -         | 0.2    | 1.17 | 0.9 | 0.27 |
| N50P50        | 52.2 (15.8)     | -         | -         | -          | -         | 0.2    | 1.43 | 1.0 | 0.37 |
| N50P0         | 63.8 (12.0)     | -         | -         | -          | -         | 0.1    | 1.41 | 1.2 | 0.44 |
| N100P50       | 64.6 (10.6)     | -         | -         | -          | -         | 0.4    | 1.18 | 0.9 | 0.73 |

Each value is the mean of 15 samples. Standard deviation is shown in parentheses.
Significance: ***, P < 0.001; **, P < 0.01; *, P < 0.05; ns, not significant. The actual P value is shown when 0.05 < P < 0.10.

Table 2. Components of nitrogen use efficiency (NUE): whole-plant biomass growth (ΔW), nitrogen uptake (ΔN), mean whole-plant nitrogen content (\(\bar{N}\)), mean leaf nitrogen content (\(\bar{N}_L\)), leaf nitrogen productivity (LNP), leaf nitrogen fraction (LNF, ratio of \(\bar{N}_L\) to \(\bar{N}\)), and initial plant nitrogen (N\(_{\text{ini}}\)) of Quercus serrata seedlings during the experimental period.

| Treatment     | ΔW (g) | ΔN (mg) | \(\bar{N}\) (mg) | \(\bar{N}_L\) (mg) | LNP (g g\(^{-1}\) Nday\(^{-1}\)) | LNF (%) | N\(_{\text{ini}}\) (mg) |
|---------------|--------|---------|-------------------|-------------------|-----------------------------|---------|------------------|
| N0P0          | 36.9 (5.5) | 187 (28) | 281 (37) | 115 (9) | 3.57 (0.45) | 41.1 (2.8) | 188 (30) |
| N50P0         | 43.4 (8.2) | 387 (65) | 413 (94) | 180 (35) | 2.73 (0.47) | 44.0 (2.3) | 211 (54) |
| N100P0        | 51.0 (10.3) | 525 (106) | 491 (88) | 212 (34) | 2.71 (0.53) | 43.3 (3.0) | 213 (26) |
| N50P50        | 30.8 (11.1) | 148 (59) | 260 (58) | 115 (24) | 2.94 (0.64) | 44.6 (4.0) | 190 (41) |
| N50P0         | 42.7 (10.2) | 330 (79) | 355 (64) | 152 (27) | 3.16 (0.78) | 42.8 (1.5) | 194 (45) |
| N100P50       | 42.7 (8.8) | 419 (67) | 406 (88) | 168 (30) | 2.88 (0.68) | 42.0 (3.4) | 198 (50) |

Each value is the mean of 15 samples. Standard deviation is shown in parentheses.
Significance: ***, P < 0.001; **, P < 0.01; *, P < 0.05; ns, not significant. The actual P value is shown when 0.05 < P < 0.10.
3.3 Leaf gas exchange traits

Nitrogen supply significantly increased $N_{area}$ of the first and second flush leaves, whereas significant decreases in $g_s$, $C_i$, and PNUE were observed (Table 3). Phosphorus supply did not exhibit significant effects on any leaf traits determined in this experiment. Nitrogen levels decreased $A_{sat}$ for the first and second flush leaves in treatments without a P supply, while $A_{sat}$ increased with P supply under high N levels; however, neither the main effect of N nor P was significant and the significance of the interactions between N and P supplies were marginal ($P=0.087$ and 0.089 in first and second flush leaves, respectively). We observed significant and marginally significant interactions of N and P supplies in PNUE in the first and second flush leaves, respectively. Phosphorus supply alleviated the N supply-induced decrease of PNUE.

4. Discussion

4.1 Effects of nitrogen supply

Nitrogen supply decreased the NUE of *Q. serrata* seedlings (Fig. 2). This result is consistent with previous reports (Vitousek, 1982; Reich and Schoettle, 1988; Pastor and Bridgham, 1999). Reductions of NP and MRT that were caused by N supply contributed to the reduction of NUE. The difference in increments of $\Delta N$, $\bar{N}$, and $\Delta W$ caused by N supply resulted in changes in NP and MRT. The reduction of MRT under higher N availability was due to smaller increases of $\bar{N}$ as compared to the increases of $\Delta N$ (Equation 4 and Table 2). According to the N accumulation model shown in Fig. 1, this may be related to the N uptake rate. Lower nutrient availability resulted in an increase of the R/S ratio (Table 1). Increase of the R/S ratio under lower nutrient or N availability has been found in other studies (Chapin III, 1980; Ågren and Franklin, 2003). In addition to increasing the R/S ratio, plants with limited N generally show compensatory enhancements of the absorbing potentials for nitrate and ammonium (Lambers et al., 2008). Equation (4) suggests that MRT is proportional to $\bar{N}$ and inversely proportional to $\Delta N$. And equation (5) suggests that $\bar{N}$ is proportional to integral of N amount, since the experimental time and initial N amount of each group were the same, MRT actually reflects the N uptake rate (litterfall is ignored in this study). If total N absorption amount $\Delta N$ equals, the faster the plants absorb N, the larger the $\bar{N}$, and correspondingly the larger the MRT (see Fig. 1). Thus, *Q. serrata* seedlings at lower N levels possibly depleted available N in the soil sooner and/or earlier than at higher N levels. Therefore, the ratio of $\bar{N}$ to $\Delta N$ at lower N availability is relatively higher than that at higher N availability, where MRT becomes bigger. However, this inference about the relationship between the N availability and N uptake pattern needs to be further studied.

The decrease of LNP was more important for explaining the response of NP in *Q. serrata* seedlings in present study (Table 2), since no main effects of N and P on LNF were observed. Although an increase of N availability induced an increase in the whole-leaf N content (Table 1), the extent of biomass increase was less than that in whole-leaf N content (equation 8). Garnier et al. (1995) demonstrated clear relationships between PNUE and LNP, and between PNUE and NP. They concluded PNUE had a strong impact on LNP, and thus on NP. In our study, PNUE did decrease with increasing N availabilities (Table 3), with a trend that was similar with LNP (Table 2) indicating this change of leaf level photosynthetic N use is one of the determinant factors for LNP and thus NP in the response of *Q. serrata* seedlings to increasing N availability. Generally, high N concentrations in

![Figure 2](image-url)
leaves are accompanied with high $A_{sat}$ values (Sage and Pearcy, 1987; Poorter and Evans, 1998; Wright et al., 2004). In our experiment, however, the leaves with higher N concentrations did not exhibit higher $A_{sat}$ values, especially for treatments without P supply. This may be due to a lower $C_i$, which was caused by a smaller $g_s$. (Table 3, Farquhar and Sharkey, 1982; Evans et al., 1986; Lambers et al., 2008). In addition, there is a possibility that re-translation of nutrients from older leaves to younger leaves occurred. When we carried out the leaf gas exchange experiment at the end of August, the number of seedlings with third or fourth flush leaves under higher nitrogen treatments was larger than that under lower nitrogen treatments. Some nutrients in the first and second flush leaves might have been translocated to third and/or fourth flush leaves to support greater growth. Therefore, $A_{sat}$ may become lower under higher N treatment, especially N100P0. We consider the response of $g_s$ to N supply similarly explained as $A_{sat}$.

### 4.2 Effects of phosphorus supply on the response of NUE to nitrogen supply

Our results confirmed the first hypothesis that P supply enhances NUE of *Quercus serrata* seedlings (Fig. 2). However, the second hypothesis that P supply mitigates the N supply-induced reduction of NUE was rejected, although the decrease of MRT that was induced by N supply was alleviated by P supply (Fig. 2). Phosphorus supply alone reduced the N uptake, a component of MRT as shown in equation (4). However, it promoted the N supply-induced increase of N uptake, although this interaction was marginal. Several reports suggest that higher P availability within a certain extent promotes N uptake (e.g. Ali et al., 2002; Xu et al., 2002). The amount of P supply in the present study was relatively higher as a balance to N supply (Güsewell, 2004; Knecht and Göransson, 2004; Garrish et al., 2010). For example, Knecht and Göransson (2004) summarized the suggested optimum nutrients ratios from several laboratory experiments and proposed that the optimum N:P supply mass ratios for some terrestrial plants are between 7 : 1 to 13 : 1. In a N and P fertilizer experiment on *Ficus insipida* (a tropical pioneer tree) seedlings, Garrish et al. (2010) reported the maximum dry mass growth corresponded to a soil solution N:P mass ratio of 4.1 : 1. In our experiment, the treatments which were both supplied with N and P were N50P50 and N100P50, and their N:P supply mass ratios were 1 : 1 and 2 : 1, respectively. In a N and P fertilization experiment on *Eucalyptus grandis*, Graciano et al. (2006) reported that the effect of P supply on N uptake of *E. grandis* was greater than the effect of N supply. In our experiment, however, the reason for the effect of P supply on the response of N uptake to N availability is not clear. Further research is needed to clarify these mechanisms.

Although P supply did not change the response of NP to the N supply of *Quercus serrata*, the components of NP (i.e. LNF and LNP) showed significant interactions with N and P supplies (Table 2). The LNF increased with increasing N availability in the absence of P supply, while it decreased at higher N availability under a P supply condition. Meanwhile, P alleviated the N supply-induced decrease of LNP as we mentioned above. This phenomenon
was also found in PNUE, one of the most important factors for explaining LNP (Garnier et al., 1995). Phosphorus supply did not modify the increase of $N_{\text{area}}$ with N supply. However, N-induced changes in $A_{\text{sat}}$ were opposite between two P treatments (i.e. P0 and P50, Table 3). Generally, $A_{\text{sat}}$ is highly correlated with $N_{\text{area}}$ (Wright et al., 2004; Watanabe et al., 2018) because large amount of N in leaves is used for photosynthesis (Evans and Clarke, 2018). Actually, $A_{\text{sat}}$ increased with increasing $N_{\text{area}}$ under high P availability in the present study. However, the response of $A_{\text{sat}}$ to $N_{\text{area}}$ under low P availability was unclear. As mentioned above, P is considered an important nutrient along with N for tree growth (Bobbink and Lamers, 2002). If N availability increases to too much, P may limit photosynthetic activity. Therefore, we consider the unclear response of $A_{\text{sat}}$ to $N_{\text{area}}$ in the absence of a P supply was due to the limited availability of P.

The results of present study indicate the effects of N deposition on N use traits of forest trees may be different under different soil P availabilities. It should be noted, however, whether these results in present study using potted seedlings and nutrient poor soils can be extended to larger scales (e.g. actual forests) remains uncertain. In the future, more studies combined with field surveys and/or experiments are needed.

5. Conclusions

This study with *Q. serrata* seedlings is an initial trial on the effect of P supply on the response of NUE of plants to N supply. Our two hypotheses were that P supply enhances NUE, and that P mitigates the reduction of NUE due to a higher N supply. According to the results obtained from this study, the first hypothesis was supported, while the second hypothesis was rejected. Nitrogen supply-induced reduction of NUE was not modified by P supply. However, P supply mitigated reductions in some components of NUE of *Q. serrata* seedlings by increasing N availability. Therefore, we consider P plays an important role in determining the change of NUE under high N supply. Further studies are needed to understand the details of N use of tree species under increasing N deposition with different soil P availability.

Acknowledgments

The authors are greatly indebted to Mr. Masanari Norisada and Ms. Xiaomei Gao (Tokyo University of Agriculture and Technology) for the technical support. We thank the anonymous reviewers for their valuable comments and suggestions. This study was supported by JSPS KAKENHI, Type B (17H01868) and Type C (18K11664) programs.

References

Aber JD, Nadelhoffer KJ, Steudler P, Melillo JM, 1989: Nitrogen saturation in northern forest ecosystems. *BioScience* 39(6), 378–386.

Ågren GI, Franklin O, 2003: Root: shoot ratios, optimization and nitrogen productivity. *Annals of Botany* 92(6), 795–800.

Ali J, Bakht J, Shafi M, Khan S, Shah WA, 2002: Uptake of nitrogen as affected by various combinations of nitrogen and phosphorus. *Asian Journal of Plant Sciences* 1(4), 367–369.

Berendse F, 1985: The effects of grazing on the outcome of competition between plant species with different nutrient requirements. *Oikos* 44, 35–39.

Berendse F, Aerts R, 1987: Nitrogen-use-efficiency: a biologically meaningful definition? *Functional Ecology* 1(3), 293–296.

Birk EM, Vitousek PM, 1986: Nitrogen availability and nitrogen use efficiency in loblolly pine stands. *Ecology* 67(1), 69–79.

Bobbink R, Lamers LPM, 2002: Effects of increased nitrogen deposition. In *Air Pollution and Plant Life* (ed. by Bell JNB, Treshow M). John Wiley and Sons, Chichester, UK, pp. 201–235.

Chapin III FS, 1980: The mineral nutrition of wild plants. *Annual Review of Ecology and Systematics* 11(1), 233–260.

Elsea JJ, Bracken MES, Cleland EE, Gruner DS, Harpole WS, Hillebrand H, Ngai JT, Seabloom EW, Shurin JB, Smith JE, 2007: Global analysis of nitrogen and phosphorus limitation of primary producers in freshwater, marine and terrestrial ecosystems. *Ecology Letters* 10(12), 1135–1142.

Endo T, Yagoh H, Sato K, Matsuda K, Hayashi K, Noguchi I, Sawada K, 2011: Regional characteristics of dry deposition of sulfur and nitrogen compounds at EANET sites in Japan from 2003 to 2008. *Atmospheric Environment* 45(6), 1259–1267.

Evans J, Sharkey T, Berry J, Farquhar G, 1986: Carbon isotope discrimination measured concurrently with gas exchange to investigate CO$_2$ diffusion in leaves of higher plants. *Functional Plant Biology* 13(2), 281–292.

Evans JR, Clarke VC, 2018: The nitrogen cost of photosynthesis. *Journal of Experimental Botany* 70(1), 7–15.

Farquhar GD, Sharkey TD, 1982: Stomatal conductance and photosynthesis. *Annual Review of Plant Physiology* 33(1), 317–345.

Friesen PC, Cattani DJ, 2017: Nitrogen use efficiency and productivity of first year switchgrass and big bluestem from low to high soil nitrogen. *Biomass and Bioenergy* 107, 317–325.

Galloway JN, Leach AM, Bleeker A, Erisman JW, 2013: A chronology of human understanding of the nitrogen cycle. *Philosophical Transactions of the Royal Society B: Biological Sciences* 368(1621).

Garnier E, Gobin O, Poorter H, 1995: Nitrogen productivity depends on photosynthetic nitrogen use efficiency and on nitrogen allocation within the plant. *Annals of Botany* 76(6), 667–672.

Garrish V, Cernusak LA, Turner BL, Winter K, 2010: Nitrogen to phosphorus ratio of plant biomass versus soil solution in a tropical pioneer tree, *Ficus insipida*. *Journal of Experimental Botany* 61(13), 3735–3748.

Graciano C, Goya JF, Frangi JL, Guiamet JJ, 2006: Fertilization with phosphorus increases soil nitrogen absorption in young plants of *Eucalyptus grandis*. *Forest Ecology and Management* 236(2), 202–210.

Gäsewold S, 2004: N: P ratios in terrestrial plants: variation and functional significance. *New Phytologist* 164(2), 243–266.

Hirose T, 1971: Nitrogen turnover and dry-matter production of a *Solidago altissima* population. *Japanese Journal of Ecology* 21(1–2), 18–32.

Hirose T, 1975: Relations between turnover rate, resource utility and structure of some plant populations: A study of the matter budgets. *Journal of the Faculty of Science, the University of Tokyo* 11, 355–407.

Hirose T, 2011: Nitrogen use efficiency revisited. *Oecologia* 166(4), 863–867.

Hunt R, 1982: *Plant Growth Curves. The Functional Approach to Plant Growth Analysis*. Cambridge University Press, UK.
