Soil Nitrogen Availability as a Controlling Factor of Plant Nitrogen Use and Distribution

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Soil Nitrogen Availability as a Controlling Factor of Plant Nitrogen Use and Distribution

Lina KOYAMA
Graduate School of Natural Science and Technology, Kanazawa University, Kanazawa, Ishikawa 920-1192, JAPAN

Abstract - The relationships among spatial distribution of dominant understory shrubs, physiological characteristics of each species concerning NO_3^--N use, and NO_3^--N use of each species under field condition were investigated in a *Cryptomeria japonica* D. Don plantation. There was considerable difference in the response of plant species to soil NO_3^--N availability. The ability to use NO_3^--N as a N source, the responsiveness to the change of NO_3^--N availability and the dependence on NO_3^--N as a N source of species were closely related the extent of species distribution.

I. Introduction

Nitrogen (N) is one of essential macronutrients for plants, but the availability of N frequently limits plant growth in terrestrial ecosystems [1, 2]. In the study site of this study, it has been shown that soil N availability slightly decreased with the distance from the bottom of a slope, and that the major forms of inorganic N produced in soil were nitrate (NO_3^--N) in the lower part of the slope and ammonium (NH_4^+-N) in the upper part of the slope [3]. As a N source for plants, these two forms of inorganic N differ greatly in the assimilation processes after plant uptake (Fig. 1, [4]); NH_4^+-N can be directly assimilated into an organic form, whereas the assimilation of NO_3^--N requires reduction to NH_4^+-N with enzymes such as nitrate reductase (NR), and the ability to use NO_3^--N as a N source is considerably different among plant species. Since the enzyme NR is substrate inducible, soil NO_3^--N availability is one of the most important regulating factors of plant NO_3^--N use. Accordingly, it is predicted that the change of major form of available N along the slope in the study site strongly influences plant N use, and species distribution as a result. To test this assumption, plant NO_3^--N use was investigated in field condition, and compared with species distribution patterns, and with the response of plant NO_3^--N use to the change of NO_3^--N availability evaluated in cultivation experiments.

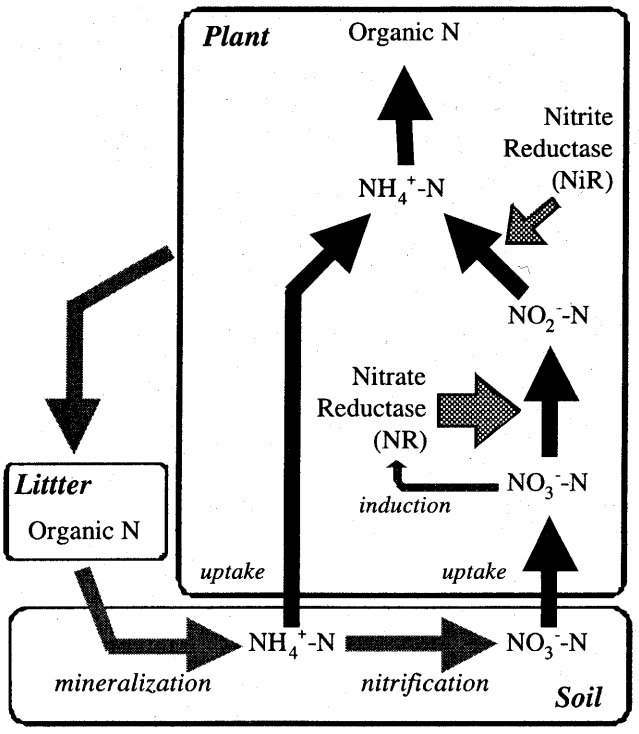

Fig. 1. Processes of plant nitrogen (N) uptake and assimilation..

II. Study Site

A. Environmental Condition

The present study was carried out at Mt. Ryuoh in Shiga prefecture, central Japan (35°10'N, 136°20'E). The elevation of the study area ranges from about 765 m to 845 m, and the mean inclination is about 38.5 degrees [5]. The study area was on a south-facing slope in an about 50-years old *Cryptomeria japonica* D. Don. (Japanese red cedar) plantation. The mean height (± s.d.) and the mean diameter at breast height (± s.d.) of *C. japonica* were 13.7 ± 3.5 m and 20.7 ± 6.1 cm, respectively [5]. The mean annual precipitation and the mean annual temperature in this area from 1987 to 1990 were about 2000 mm and 10 °C, respectively [6].

In this area, a preceding soil investigation showed that net N mineralization rate showed no clear gradient along the slope, while net nitrification rate and percentage of nitrification to N mineralization were high on the lower slope, and very low on the upper slope (Fig. 2-a, [3]). In addition, the spatial pattern of soil N transformation showed that there was no soil of intermediate nitrification rate, but was the transition zone corresponding to the patchiness of two types of soils that had extremely different nitrification rate in the border area of the lower part and the upper part of the slope [3].

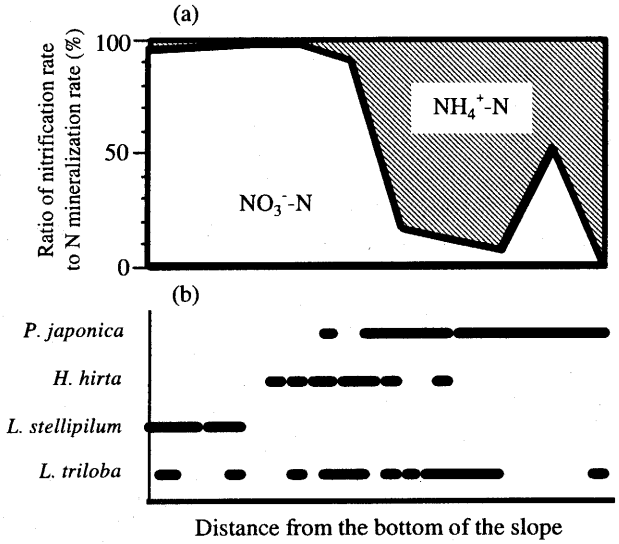

Fig. 2. Changes in (a) major soil inorganic N form [3], and (b) dominant understory species along the slope.

B. Vegetation

There are four dominant understory species in this study site, and the spatial distribution of each species corresponded to a specific type of soil N transformation varying along the slope (Fig. 2-b). *Leucosceptrum stellipilum* (Miq.) Kitam. et Murata (Lamiaceae) and *Hydrangea hirta* (Thunb.) Siebold (Saxifragaceae) were distributed on the lower part and the middle part of the slope, respectively. Net soil nitrification rate was high both in the lower part and in the middle part of the slope. On the other hand, *Pieris japonica* (Thunb.) D. Don (Ericaceae) was dominant on the upper part of the slope where soil nitrification rate was very low. Another dominant species, *Lindera triloba* (Sieb. et Zucc.) Blume (Lauraceae) was found on the entire slope.

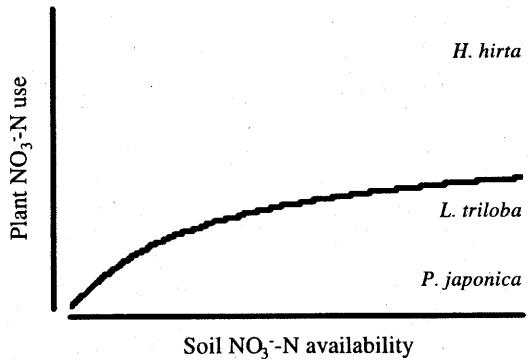

Fig. 3. Schema of responses of plant NO_3^--N use to the change in the soil NO_3^--N availability [7].

The physiological characteristics for three of these dominant understory species (*H. hirta*, *P. japonica* and *L. triloba*) have been described (Fig. 3, [7]). The responses of plant NO_3^--N concentration and nitrate reductase activity (NRA) to the change of NO_3^--N availability were examined in seedlings of these species by using perlite culture method. *Pieris japonica* showed the lack of ability to assimilate NO_3^--N, whereas *H. hirta* and *L. triloba* increased NO_3^--N uptake and assimilation with increase of NO_3^--N supply. Maximally induced NRA were higher in *H. hirta* than in *L. triloba*. Moreover, *H. hirta* responded more sharply to the change of NO_3^--N availability than *L. triloba* did.

III. Materials and Methods

A. Sample Collection

The NO_3^--N use by four dominant understory species (*L. stellipilum*, *H. hirta*, *P. japonica* and *L. triloba*) were investigated in field condition. Spatial and seasonal changes of leaf N concentration, NO_3^--N concentration and NRA in these species were measured simultaneously with inorganic N pool sizes in soils associated with each individual.

Samples were collected five times during the growing season from late April to early October 1998. At each sampling date, five individuals of *L. stellipilum* and *H. hirta* were chosen on the lower slope, and five individuals of *P. japonica* were chosen on the upper slope because of their narrow distribution on the slope. For *L. triloba, which* occurred along the entire slope, 10 individuals (five on the lower slope and five on the upper slope) were sampled. An individual once chosen was not repeatedly sampled to avoid the effect of sampling.

A sunny day was chosen for sampling to avoid the effect of varying light conditions on leaf NRA [8, 9]. For each individual plant, leaves and soils were sampled simultaneously. Fully developed current leaves were chosen except for the 1st sampling, and collected from 10:00 to 14:00 to avoid the possible effect of diurnal variation in NRA [10]. At the same day of leaf sample collection, triplicate mineral soil samples (0-5 cm layer) were collected with a 100 cc core (5 cm depth) from areas within a 30 cm radius from the tree trunk. It is considered that these samples included the rhizosphere soil of sample individual, since a large part of fine root was found within a depth of 10 cm in the same study site [11]. A preliminary experiment showed there was no significant change in soil inorganic N pool size with distance from the trunk within this area.

B. Plant Analysis

Leaf NRA was measured by a modified version of the in vivo test [7, 12]. Two hundred leaf disks each with a diameter of 2.5 mm were cut out of the leaves from each individual. The leaf disks were incubated with 5 ml of

incubation buffer for 1 h at 30 °C in the dark after vacuum infiltration (6 mm Hg; twice for 30 sec each). The composition of incubation buffer was 0.1 M KNO_3, 0.1 M KH_2PO_4 and 3 % propanol, and pH was adjusted to about 7.5 with NaOH. After the incubation, enzyme activity was stopped by placing sample vials in hot water (80 °C). The leaf disks were subsequently oven-dried at 105 °C, and weighed to calculate the activity per gram dry leaf weight. The concentration of NO_2^--N formed in the incubation buffer was measured colorimetrically by diazotization [13]. The effect of plant pigment on absorbance was compensated for by measurement of complete controls lacking N-naphthylethylene diamine dihydrochloride [14].

The remaining leaves were dried at 40 °C, and a part of the dried leaves was ground with a sample mill. About 100 mg of ground leaves were extracted with 10 ml of deionized water for 1 h at 45 °C. The extract was filtered, and the concentration of NO_3^--N in the extract was analyzed by HPLC within about 48 h to avoid the transformation of nitrate in the extract. Nitrate was separated on the anion exchange column Shim-pack IC-A1 (SHIMADZU, Kyoto, Japan), and the electric conductivity was measured by the conductivity detector CDD-6A (SHIMADZU, Kyoto, Japan).

Thirty disks each with a diameter of 5 mm were cut out from the dried leaves. These leaf disks were weighed, and the concentration of N was analyzed with an NC analyzer NC-900 (SUMIKA, Osaka, Japan). Preliminary experiments revealed no significant differences in the N concentration between the leaf disks and the ground leaves.

C. Soil Analysis

Soil samples were sieved through 2 mm mesh and roots were removed by hand. A 5 g sample was extracted with 50 ml of 2 M KCl and filtered. The NH_4^+-N concentration in the soil extract was determined using the indophenol blue method [13]. The NO_3^--N in the extract was determined by diazotization after reduction to NO_2^--N with zinc powder [13]. Total soil inorganic N (NH_4^+-N + NO_3^--N) and soil NO_3^--N pool size were calculated as N mass per unit area (5 cm depth).

IV. Results

A. Species Difference

Mean leaf N concentrations of *L. stellipilum* were higher than those of other species, while the mean leaf N concentration was lower in *P. japonica* than in the other species throughout the sampling season. The leaf NO_3^--N concentration of *L. stellipilum* was always higher than those of other species by one order of magnitude. Leaf NRA of *L. stellipilum* was also higher than that of other species, while *P. japonica* showed NRA close to zero.

The species difference in total soil inorganic N pool sizes under the four species was smaller than that of soil NO_3^--N pool sizes. However, the total soil inorganic N pool size under *P. japonica* was generally smaller than under the other three species. Among the four species, the soil NO_3^--N pool sizes under *L. stellipilum* and *H. hirta* were larger than those of other species almost throughout the sampling season. Soil NO_3^--N pool sizes under *P. japonica* were very small compared with those under the other three species by one order of magnitude; soil NO_3^--N pool size under *L. triloba* was intermediate throughout the sampling season.

B. Seasonal Change

Leaf N concentrations declined markedly during the first one month, and remained almost unchanged thereafter. Seasonal patterns of leaf NO_3^--N concentration and leaf NRA were different among species. The seasonal pattern of the leaf NO_3^--N concentration was similar to that of the leaf N concentration in *L. triloba* and *P. japonica*, although the leaf NO_3^--N concentration in *P. japonica* was very low throughout the sampling period. With regard to the leaf NRA, *H. hirta* and *L. stellipilum* showed maximum NRA at the 1st and 2nd sampling date, respectively; while *L. triloba* showed maximum NRA at the 4th sampling date (Fig. 4).

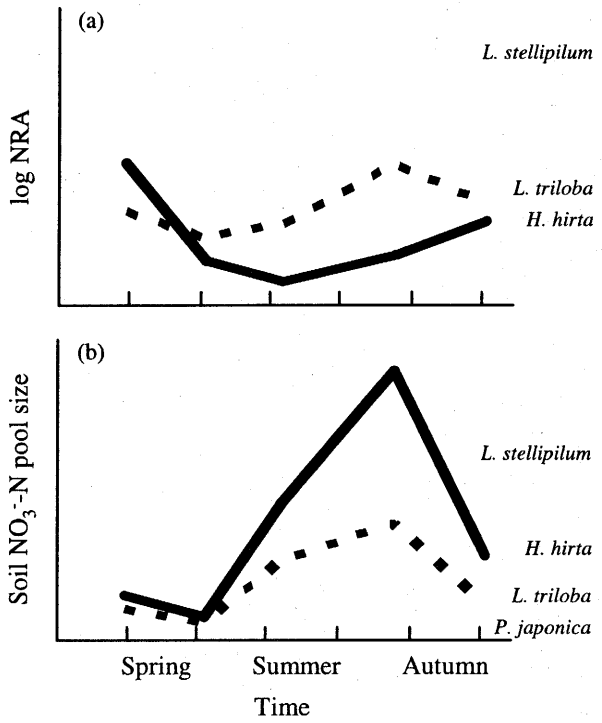

Fig. 4. Schematic diagrams of seasonal changes of (a) plant NRA and (b) soil NO_3^--N availability.

Inorganic N pool sizes in soils associated with *L. stellipilum* and *H. hirta* showed a similar seasonal pattern. Soil NO_3^--N pool sizes of *L. stellipilum* and *H. hirta* peaked at late summer (Fig. 4), and peaks of total soil inorganic N pool sizes of these species were also found at the same sampling date. Total soil inorganic N and soil NO_3^--N pool sizes in soils associated with *L. triloba* showed a pattern similar to those associated with *L. stellipilum* and *H. hirta*, but the peaks were not as clear. Nitrate was a minor form in inorganic N in soils associated with *P. japonica*. The pool sizes of total soil inorganic N and soil NO_3^--N under *P. japonica* were at the maxima at the 3rd and 5th sampling date, respectively, although the fluctuation of soil inorganic N pool sizes was small compared to that in other species.

C. Relationships between plant and soil

There was few significant correlation between leaf N concentrations and soil conditions in all four species. In addition, even in case the correlations were significant, they were not consistently positive or negative through the growing season. No consistent correlation of leaf NO_3^--N concentration and any soil condition was detected throughout the growing season in all species. Leaf NRA in *L. triloba* showed significant correlations with soil NO_3^--N pool size from the 2nd to the 5th sampling dates, while leaf NRA in the other three species rarely showed significant correlations with any soil condition.

V. Discussion

The four species can be divided into three categories according to their NO_3^--N use in field condition: (1) species that cannot utilize soil NO_3^--N as N source, *P. japonica*, (2) species that can utilize soil NO_3^--N as N source, *H. hirta* and *L. triloba*, and (3) species that accumulate NO_3^--N besides assimilation of soil NO_3^--N, *L. stellipilum*.

Comparison among three species that can use NO_3^--N as a N source (*L. stellipilum*, *H. hirta* and *L. triloba*) showed that they differentiated peak season of NO_3^--N use. Seasonal changes of NO_3^--N pool size soils associated with these three species showed similar pattern (Fig. 4-b). However, seasonal correspondence between soil NO_3^--N availability and plant NO_3^--N use was found only in *L. triloba*, but not in two other species. These two species (*L. stellipilum* and *H. hirta*) showed the peak NRA in the earlier season than *L. triloba* did. It has been suggested that the seasonal changes of NO_3^--N use was not regulated only by the seasonal changes of soil NO_3^--N availability, but also by other factors such as the seasonal changes in plant demand for N. It is plausible that *L. stellipilum* and *H. hirta* increased their demand for N during the period of leaf expansion, and that caused rises in NO_3^--N uptake and assimilation in early growing season. These results suggested that the spatially coexisting species absorbed and assimilated NO_3^--N at the different stages in the growing period; moreover, *H. hirta* effectively responded to NO_3^--N availability only at the peak period. Thus, the results of this study were compatible with the temporal differentiation of NO_3^--N use by understory herbaceous species in nutrient rich swamp forest [15].

The comparison between plant distribution and the physiological characteristics derived from cultivation experiments [7] showed the close relationships between the extent of species distribution and the physiological characteristics of each species: the ability to use NO_3^--N as a N source, the responsiveness to the change of NO_3^--N availability and the dependence on NO_3^--N as a N source of species. The lack of the ability to use NO_3^--N in a species should apparently put the species at a disadvantage in NO_3^--N rich condition; and corresponding to that, the distribution of a species which cannot use NO_3^--N in this study site, *P. japonica*, was limited in the area where soil nitrification rate was very low. On the other hand, a species highly responsive to the change of NO_3^--N availability or a species greatly dependent on NO_3^--N is likely to be at a disadvantage in NO_3^--N poor condition; *H. hirta* showed high responsiveness to the change of NO_3^--N availability and high dependence on NO_3^--N in cultivation experiment, and the distribution of this species was limited in the area where soil nitrification rate was high. *Lindera triloba* showed moderate responsiveness to the change of NO_3^--N availability and moderate dependence on NO_3^--N, and this species occurred both in NO_3^--N rich and NO_3^--N poor condition.

However, the results showed that the response of plant NO_3^--N use to soil NO_3^--N availability in the field condition differed from the plant NO_3^--N use in cultivation experiment that mirrored the physiological characteristics of NO_3^--N use by each species. Only *L. triloba* showed a significant correlation between leaf NRA and soil NO_3^--N pool size through the growing season among the three species that can utilize soil NO_3^--N. This could be ascribed that only small number of *L. stellipilum* and *H. hirta* was associated with soils in which NO_3^--N pool size were very low, though the range of NO_3^--N pool sizes in soils associated with *L. stellipilum*, *H. hirta* and *L. triloba* did not markedly differ among species. Consequently, most of *L. stellipilum* and *H. hirta* might be associated with soils that held sufficient amount of available NO_3^--N, and with such degree of NO_3^--N availability, clear relationship of plant NRA to the NO_3^--N availability might be reduced under the influence of the variable environmental condition. Interestingly, these two species showed significant correlations between leaf NRA and soil NO_3^--N pool size in the 1st sampling date. Moreover, mean leaf NRA of *H. hirta* was highest at the same time. It seems possible that these species respond to the heterogeneity of NO_3^--N availability only in early growing season; consequently, plants can maximally acquire N resource to develop current leaves and shoots.

Thus, the results suggested that species distribution were closely related to species specific N use patterns. Spatially, temporally or qualitatively differentiated plant N use pattern is probably an important factor that enables the dominant

species to coexist under natural condition in the study site. In addition, it is also presumed that spatial differentiation of species that have distinct N use pattern facilitates or at least maintains local N cycling in the habitat of each species.

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