TITLE

Nitrate-use traits of understory plants as potential regulators of vegetation distribution on a slope in a Japanese cedar plantation

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ABSTRACT

[Background and Aims]

Plant physiological traits and their relation to soil N availability was investigated as regulators of the distribution of understory shrub species along a slope in a Japanese cedar (*Cryptomeria japonica*) plantation in central Japan.

[Methods]

At the study site, previous studies demonstrated that both net and gross soil nitrification rates are high on the lower slope and there are dramatic declines in different sections of the slope gradient. We examined the distributions of understory plant species and their nitrate (NO_3^--N) use traits, and compared the results with the soil traits.

[Results]

Our results show that boundaries between different dominant understory species correspond to boundaries between different soil types. *Leucosceptrum stellipilum* occurs on soil with high net and gross nitrification rates. *Hydrangea hirta* is dominant on soil with high net and low gross nitrification rates. *Pieris japonica* occurs on soil with very low net and gross nitrification rates. Dominant understory species have species-specific physiological traits in their use of NO_3^--N. *Pieris japonica* lacks the capacity to use NO_3^--N as a N source, but other species do use NO_3^--N. *Lindera triloba*, whose distribution is unrelated to soil NO_3^--N availability, changes the extent to which it uses NO_3^--N in response to soil NO_3^--N availability.

[Conclusions]

Our results indicate that differences in the physiological capabilities and adaptabilities of plant species in using NO_3^--N as a N source regulate their distribution ranges. The identity of the major form of available soil N is therefore an environmental factor that influences plant distributions.

KEYWORDS

Nitrate (NO_3^--N); nitrate reductase activity (NRA); soil NO_3^--N availability; spatial distribution; understory

INTRODUCTION

Topography is among the factors that influence plant distributions on scales of tens to hundreds of meters. Plants often have species-specific distribution patterns along landscape slopes, with some occurring preferentially on upper slopes, others on lower slopes, and yet others spread across the whole gradient. Slope topography does not directly regulate species' distributions; environmental factors that affect plant performance vary across different sections of slope. Soil moisture, nutrient availability (Enoki et al. 1997; Giesler et al. 1998; Small and McCarthy 2005), and ground surface disturbance (Enoki 2003; Sakai and Ohsawa 1993, 1994) are all regulators of plant growth that change with topographic features. Accordingly, there are different types of vegetation on different sections of slope.

Previous analyses of soil N transformations at the site of the present study distinguished spatial patterns of different soil types across a selected gradient (Hirobe et al. 1998, 2001; Tokuchi et al. 1999, 2000). On this gradient, net and gross nitrification rates are high and very low on the lower and upper slope sections, respectively, while net N mineralization rates show no clear gradient along the slope; the soil NO_3^--N pool is larger on the lower slope than on the upper section, while the size of the total inorganic N pool does not change significantly along the gradient; and dramatic changes in NO_3^--N availability occur only in a narrow section of the slope. The changes in soil types across topographic gradients occur elsewhere in connection with the gradient of environmental condition such as soil moisture contents (Giesler et al. 1998; Velthof et al. 2000; Tateno and Takeda 2003). However, the gradient changes in major N forms are especially dramatic at our chosen study site; NO_3^--N is the major inorganic N species on the lower part of the slope, and ammonium (NH_4^+-N) is the major form on the upper slope.

Differences in the major forms of available N in soil likely have a strong impact on plants because there are intrinsic differences between plant metabolic processes that assimilate NO_3^--N and NH_4^+-N. Ammonium is directly assimilated into an organic form, whereas NO_3^--N must first be reduced to NH_4^+-N by enzymes such as nitrate reductase (NR) before incorporation into organic N. NR is a substrate-inducible enzyme, and the capacity to induce NR differs markedly among plant species; some are unable to induce NR, a necessary requirement for the utilization of NO_3^--N as a N source (Al Gharbi and Hipkin 1984; Gebauer and Schulze 1997; Gebauer et al. 1988; Smirnoff et al. 1984).

The change of major form of soil inorganic N with forest succession has been considered as a regulating factor of species establishment in each successional stage through the plant physiological traits about N use (Kronzucker et al. 1997; 2003; Min et al. 1998). Taking into account the spatial heterogeneity of soil NO_3^--N availability and the species-specific mechanisms of NO_3^--N use, it seems very likely that the spatial distribution of different types of soils is a major factor regulating the distribution of plant species across landscapes. However, major N source identity has yet to be

regarded as an important regulating factor in coincidental plant distribution. In this study, we considered soil N transformation as a factor in plant distributions by examining whether plant species had different distribution patterns related to their different responses to the quantity and quality of available soil N. In addition, we examined the possibility that plant species' distributions influence soil N transformation; we postulated that the underlying mechanism lies in differences in litter organic composition among species, which in turn should influence soil N dynamics (Bengtson et al. 2006; Ferrari 1999).

We conducted a vegetation survey of understory species along a slope gradient for comparison with the spatial distribution of soil N transformation mechanisms; we also measured other environmental conditions including light, soil pH, and soil moisture. Dominant understory species were chosen for examination of NO_3^--N use. To do this, we measured N concentration, NO_3^--N concentration, and *in vivo* nitrate reductase activity (NRA) in plant leaves. The presence of NO_3^--N within plant tissue is evidence of NO_3^--N uptake, as plants generally do not have the capacity to synthesize NO_3^--N except that certain legume species is observed generating nitrification (Hipkin et al. 2004). *In vivo* NRA is an important indicator of plant NO_3^--N use, since the reduction of NO_3^--N to nitrite (NO_2^--N) by NR is the first step and the rate-limiting mechanism in the whole sequence of NO_3^--N assimilation processes (Beevers and Hageman 1969; Tischner 2000). Concurrent with plant measurements, we also measured inorganic N (NH_4^+-N and NO_3^--N) pool sizes in the soil samples associated with each sample plant. Measurements were made through the growing period to record seasonal patterns. By comparing spatiotemporal changes in plant leaf traits with soil inorganic N pool sizes, we were able to discern different species' responses and physiological adaptations to NO_3^--N use across the gradient of changing soil inorganic N pool sizes.

MATERIALS AND METHODS

Study site

This study site is located in a plantation of Japanese cedar (*Cryptomeria japonica* D. Don; *ca.* 50 yr old) on Mt. Ryuoh in central Japan (35°1′N, 136°20′E). The mean annual temperature and mean annual precipitation at the site are 10 °C and 2050 mm, respectively (Tokuchi et al. 1993).

The site is on an approximately 135-m-long south-facing slope; elevation ranges from 765 to 851 m. Mean slope inclination is 38.5° (range: 25.3–50.4°) [Fig. 1(a)]. The slope is slightly steeper in the central portion than in other sections. The change in inclination is gradual on the lower slope, but uneven in the upper section. The light conditions for understory species were measured based on the canopy cover ratio determined from hemispherical photographs taken over understory crowns [Fig. 1(b)]. The canopy cover ratio was highest at the bottom of the slope and declined gradually upslope, ranging from 75.21 to 98.36%. There were some canopy gaps that had low canopy cover ratios. The change in canopy cover ratio along the slope was presumably due to changes in the size of canopy

trees (*C. japonica*) [Fig. 1(c); Tokuchi et al. 1999]. The mean diameter at breast height (DBH) of *C. japonica.* at the study site was 190 ± 57 mm, and it was higher on the lower slope than elsewhere. In contrast to aboveground biomass, the fine root biomass of *C. japonica* was lowest on the lower slope [Fig. 1(c); Kasuya and Shimada 1996]. The organic layer was thicker on the upper slope than lower down [Fig. 1(d); Hobara and Tokuchi 1998]. Soil pH was highest on the lower slope, and soil acidity changed gradually [Fig. 1(e); Hirobe et al. 1998; Yoshida unpublished data]. Hirobe et al. (1998) demonstrated a gradual decrease in soil water content with increasing distance from the base of the slope, although previous studies reported highly fluctuating soil water content along the slope [Fig. 1(f); Kasuya and Shimada 1996; Yoshida unpublished data].

In contrast to other environmental factors that change rather gradually along the slope, soil N transformation processes change dramatically in short sections of the gradient (Hirobe et al.1998; Tokuchi et al. 2000). Net soil nitrification rates were found to be much higher on the lower slope than at higher elevations, while net soil N mineralization rates were not clearly related to position on the gradient [Fig. 1(h)]. Net nitrification rates changed dramatically between 60 m and 75 m upward from the base of the slope [see also Fig. 3(d); Hirobe et al. 1998]. Gross nitrification rates were also much higher on the lower slope than at higher elevations [Fig. 1(g); Tokuchi et al. 2000]. However, the section of the slope where the gross nitrification rate changed did not correspond to the zone where the net nitrification rate changed. Hence, the gradient was classifiable into three categories by soil nitrification rates: the lower slope, where both net and gross soil nitrification rates were high; the mid slope, where the net nitrification rate was high and the gross nitrification rate low; and the upper slope, where both net and gross nitrification rates were low.

Vegetation study

To survey understory vegetation, a 1 × 132-m study plot (plot A) was deployed along the length of the slope. The plot was divided into 44 subplots each measuring 1 × 3 m. Species' identities and the numbers of stems were recorded for plants >10 cm in height in each subplot. Nomenclature followed Ohwi and Kitagawa (1983).

Hirobe et al. (1998) concluded that the boundary zone between mid and upper slopes comprises soil patches that are characterized by two completely different net nitrification rates, with no gradational change in soil properties. Accordingly, we set up another study plot (plot B) to overlap the boundary between the mid and upper slopes (45–90 m upward from the base of the slope) to observe relationships between the distributions of soil properties and understory plant species. The plot, which measured 5 × 45 m, was divided into 900 subplots, each 0.5 × 0.5 m, within which we recorded species' identities and the numbers of stems for plants >10 cm in height.

Spatial analyses were performed for selected shrub species based on the results of our vegetation study in plot B. The spatial traits of understory vegetation were described by using spatial

analysis by distance indices (SADIE; Perry, 1995). For this procedure, we used understory shrub stem numbers in each 0.5 × 0.5-m subplot. The degree of non-randomness in spatial distribution was quantified by the index of aggregation (I_a; Perry, 1995). In general, a spatially aggregated data set has $I_a > 1$, a spatially random data set has $I_a = 1$, and a spatially regular data set has $I_a < 1$. Dimensionless indices of clustering (v_i and v_j) were calculated, and the means of v_i and v_j were used to quantify the degree of data clustering into patches (areas with above-average density) and gaps (areas with below-average density). Based on the clustering indices, we calculated the index of local association (X) to explore patterns of spatial association among the selected tree species. The statistical significance of these indices was tested at $\alpha = 0.05$ by comparison with corresponding values obtained from 5967 randomizations. All spatial statistics were performed using SADIE software (http://www.rothamsted.ac.uk/pie/sadie).

Plant and soil sampling

Based on the results of our vegetation study, we chose four dominant understory woody species and examined their N use traits: *Leucosceptrum stellipilum* (Miq.) Kitam. et Murata (Lamiaceae), *Hydrangea hirta* (Thunb.) Siebold (Saxifragaceae), *Pieris japonica* (Thunb.) D. Don (Ericaceae), and *Lindera triloba* (Sieb. et Zucc.) Blume (Lauraceae). Samples were collected five times during the 1998 growing season between late April, when leaves had not fully developed, and early October, immediately before deciduous leaves were shed. On each sampling date, the leaves of 5 plants of *L. stellipilum*, *H. hirta*, and *P. japonica* were collected from across their respective distributional ranges; 10 plants of *L. triloba* were sampled because this species had a wider distribution than the others. To ensure temporal independence in the data, individual plants were never sampled more than once. Soil samples were collected simultaneously with leaves. Triplicate mineral soil samples (0–5 cm layer) were collected with a 100-cc corer (5-cm depth) from areas within a 30-cm radius of the tree trunks. Samples collected within this radius should be considered the rhizosphere soil of the target sample plants because a large proportion of fine roots occurs in the upper 10 cm of soil at the study site (Kasuya and Shimada 1996). Preliminary observations revealed no significant changes in soil inorganic N pool size within a 30-cm radius of individual trees (data not shown).

Plant analysis

In vivo NRA was measured with a modified version of Jaworski's method (Jaworski 1971; Koyama and Tokuchi 2003). Leaf samples were collected between 10:00 and 14:00 on sunny days, and kept at 4°C until analysis. The storage times before laboratory analysis ranged from *ca.* 3 to 4 h, so storage effects were similar across samples; changes in NRA are very slow after the initial decline during the first 30 min (Högberg et al. 1986). Petioles and midribs were removed, and *ca.* 100 mg (fresh weight) of leaf laminae were cut into 2.5-mm-diameter disks or approximately 4-mm^2 segments

and transferred to test tubes. Incubation buffer (5 ml) was added, and the tubes were vacuum infiltrated. The composition of the incubation buffer was 0.1 mol L^{-1} KNO_3, 0.1 mol L^{-1} KH_2PO_4, and 3.0% 1-propanol. pH was adjusted to *ca.* 7.5 using a NaOH solution. The samples were incubated for 1 h at 30°C in the dark. Enzyme activity was stopped by placing sample vials in hot water (80°C). The concentration of NO_2^--N in the incubation buffer was measured colorimetrically by diazotization (Keeney and Nelson 1982). The effect of plant pigments was compensated for by measurement of controls lacking N-naphtylethylene diamine dihydrochloride (Gebauer et al. 1998). A fraction of each leaf sample was oven-dried at 105°C and then weighed to calculate activity per unit dry weight.

The remaining leaves were dried at 40°C and ground in a sample mill (CMT, Ltd., TI-100, Tokyo, Japan). About 100 mg of ground samples 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 using HPLC within about 48 h to avoid the transformation of nitrate in the extract. Nitrate was separated on an anion exchange column (Shim-pack IC-A1 SHIMADZU, Kyoto, Japan) connected to a guard column (IC-GA1 SHIMADZU, Kyoto, Japan); electrical conductivity was measured with a conductivity detector (CDD-6A SHIMADZU, Kyoto, Japan). A potassium hydrogen phthalate solution was used as the mobile phase. The concentrations of N in ground samples were analyzed using an NC analyzer (NC-900; SUMIKA, Osaka, Japan).

Soil analysis

Soil samples were sieved through a 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 concentrations in the soil extracts were determined using the indophenol blue method (Keeney & Nelson, 1982). The NO_3^--N in the extracts was determined by diazotization after reduction to NO_2^--N with zinc powder (Keeney & Nelson, 1982). 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).

Statistical analysis

One-way ANOVA was conducted to detect differences among sampling dates in leaf NRA, NO_3^--N concentration, N concentration, soil inorganic N pool size, and soil NO_3^--N pool size. Pearson's correlation coefficients were calculated between soil N traits and leaf traits. All statistical analyses were conducted in SAS JMP IN (ver. 5.1.1; SAS Institute, Cary, NC, USA).

RESULTS

Vegetation study

In total, we found 1518 tree trunks belonging to 54 species in plot A (Appendix 1). The numbers of both species and trunks increased slightly with increasing distance upward from the base

of the slope; the trend was more marked on the upper slope than in the first 30 m from the base [Fig. 1(i)]. There were four dominant species, which accounted for 67.9% of all trunks, with specific distribution patterns [Fig. 1(j)]. *Leucosceptrum stellipilum* was distributed on the lower slope (<27 m from the base). The mid slope (30–87 m from the base) was dominated by *H. hirta*, which was especially localized in the section 36–66 m from the base. The upper part of the slope (>51 m from the base) was dominated by Ericaceae species, such as *P. japonica*. Another dominant species, *L. triloba*, was distributed across the entire slope.

There were 2382 trunks of 53 tree species in plot B. Three of the dominant species in plot A were also dominant in plot B, i.e., *H. hirta*, *P. japonica*, and *L. triloba* with 434, 602, and 185 stems, respectively. *Hydrangea hirta* was concentrated in a zone <66–69 m from the base of the slope; *P. japonica* was dominant in a zone >63–66 m from the base of the slope [Fig. 2(a),(b)]. *L. triloba* occurred across the entire plot [Fig. 2(c)].

The spatial distributions of *H. hirta* and *P. japonica* were significantly aggregated ($P < 0.05$) with extreme patches and gaps ($P < 0.05$), while that of *L. triloba* was random and without patches or gaps (Table 1). Spatial association pattern analysis demonstrated that the distributions of *H. hirta* and *P. japonica* and of *H. hirta* and *L. triloba* were significantly dissociated ($P < 0.05$); distributions of *P. japonica* and *L. triloba* were independent (Table 2).

Seasonal changes in plant N use traits and soil N pool size

Leaf N concentrations tracked a similar seasonal pattern in all four species [Fig. 3(a), Appendix 2]. Leaf N concentrations were highest at the beginning of the growing season ($p<0.01$), declined markedly during the first month, and remained stable thereafter.

Seasonal changes in leaf NO_3^--N concentrations differed among species [Fig. 3(b), Appendix 2]. There were no significant differences in leaf NO_3^--N concentrations among sampling dates for *L. stellipilum* ($p=0.12$) or *H. hirta* ($p=0.21$). The seasonal patterns of leaf NO_3^--N concentrations in *L. triloba* and *P. japonica* were rather similar to those of leaf N concentrations, which declined during the first month and remained stable thereafter. Leaf NO_3^--N concentrations in *P. japonica* were very low throughout the sampling period.

Leaf NRA did not vary significantly across sampling days, except in *L. stellipilum* ($p<0.01$), for which NRA was maximal on the 2nd sampling day [Fig. 3(c), Appendix 2]. Leaf NRA was hardly detected in *P. japonica*.

Inorganic N pool sizes in soils associated with *L. stellipilum* and *H. hirta* tracked similar patterns; they peaked on the 4th sampling day [Fig. 3(d)(e), Appendix 2]. Inorganic N pool size was highest on the 3rd and 4th sampling days in soils associated with *L. triloba*. Seasonal changes in the inorganic N pool size in soils associated with *P. japonica* differed from those in soils associated with other species; the lowest pool size in soils associated with *P. japonica* occurred on the 4th sampling

day.

NO_3^--N pool sizes tracked the temporal pattern seen in soil inorganic N associated with understory plant species other than *P. japonica* [Fig. 3(e), Appendix 2]. The soil NO_3^--N pool size associated with *P. japonica* was an order of magnitude smaller than the pool sizes associated with other species. The pool size associated with *P. japonica* peaked on the last sampling day. Soil NO_3^--N pool sizes associated with the other three species peaked on the 4th sampling day, in late summer. However, in soil associated with *L. triloba*, differences in the pool sizes between the 3rd and 4th sampling days were not significant ($p \geq 0.05$).

Relationships between plant N-use traits and soil N pool sizes

With few exceptions, neither soil NO_3^--N pool size nor soil inorganic N pool size was significantly correlated with leaf N concentration or leaf NO_3^--N concentration in any of the species across the sampling period ($p \geq 0.05$; Table 3). There were significant positive correlations between soil NO_3^--N pool size and leaf NRA for *L. triloba* throughout the sampling period ($p < 0.05$), except on the 1st sampling day ($p = 0.56$). For *L. stellipilum* and *H. hirta*, there were significant positive correlations between soil NO_3^--N pool sizes and leaf NRA only on the 1st sampling day ($p < 0.05$). With one exception, correlation coefficients between soil inorganic N pool sizes and leaf NRA were not significant across the sampling period for any of the species ($p \geq 0.05$). There was a significant correlation for *L. stellipilum* on the 1st sampling day ($p < 0.01$).

DISCUSSION

Correspondence between plant species' distributions and soil N conditions

The dominant understory plants in the study plots had species-specific distribution patterns along the slope [Figs. 1(j), 2(a)–(c), Tables 1, 2]. Spatial analysis by distance indices for the mid slope (plot B) demonstrated that the spatial distributions of dominant species other than *L. triloba* were likely reflections of environmental conditions on the gradient.

As in the classification in the Materials and Methods section, both net and gross soil nitrification rates were high on the lower slope; net nitrification rates were high but gross nitrification rates were low on the mid slope; and both net and gross nitrification rates were low on the upper slope [Figs. 1(g)–(h); Tokuchi et al. 2000, Hirobe et al. 1998]. Interestingly, the boundaries of these three categories corresponded to the points on the slope where the dominant understory species changed [Figs. 1(j), 2(a)–(b), 4]. Furthermore, additional environmental factors, such as microtopography, light conditions, soil pH, and moisture conditions, also changed along the slope, but less dramatically than the soil nitrification rate (Fig. 1). Thus, there was correspondence only between vegetation distribution ranges and soil nitrification rates. In short, soil on the slope was classifiable into three different categories by nitrification rate, and the spatial distribution of these categories corresponded to the

spatial distribution of the dominant understory species (Figs. 1, 2).

Species difference in plant NO_3^--N use

Using NRA analysis, we classified the four study species into three categories based on NO_3^--N use: species that lack the capacity to assimilate NO_3^--N, species able to assimilate NO_3^--N, and species that accumulate NO_3^--N in addition to assimilating NO_3^--N immediately.

Many members of the Ericaceae have extremely low NRA and are presumably unable to use NO_3^--N as a N source (Gebauer et al. 1988; Högbom and Ohlson 1991; Nadelhoffer et al. 1996). In our study, the ericaceous species *Pieris japonica* had very little or no leaf NRA throughout the growing period [Fig. 3(c)]. Furthermore, Koyama and Tokuchi (2003) were unable to detect NRA in either leaves or roots of *P. japonica*, even when considerable concentrations of NO_3^--N were artificially supplied. Thus, *P. japonica* has essentially no capacity to use NO_3^--N as a N source.

The family Lamiaceae includes several nitrophilous species that preferentially use NO_3^--N as a N source (Gebauer et al. 1988). The lamiaceous species at our study site, *L. stellipilum*, ranks among them because of its high NRA [Fig. 3(c)]. Furthermore, leaf NO_3^--N concentrations were much higher in *L. stellipilum* than in the other species [Fig. 3(b)]. At its maximum concentration, NO_3^--N amounted to *ca.* 40% of total leaf N. Rehder (1982) defined nitrate accumulators as species that accumulate NO_3^--N above a fixed value (for example, 0.05% of dry weight or 1% of total N); these amounts of NO_3^--N are detected in leaf laminae, which are believed to have lower NO_3^--N contents than roots, stems, or leaf stalks (cf. Gebauer et al. 1984, Gebauer and Schulze 1997); and such concentrations of NO_3^--N are measured not only at the early stage of development but also at the end of the growing season and with the decay of leaves. *Leucosceptrum stellipilum* fits the definition of a nitrate accumulator, although we have not as yet measured NO_3^--N concentration in decayed leaves. NO_3^--N accumulation is regulated by the balance between NO_3^--N uptake and NO_3^--N reduction (Luo et al. 2006). The very high NO_3^--N concentrations and NO_3^--N reduction rates in *L. stellipilum* leaves indicate that NO_3^--N uptake was also high [Fig. 3(b),(c)]. In earlier N starvation experiments, NO_3^--N was shown to accumulate in plant tissues, and it may be mobilized and utilized (Melzer et al. 1984; Chapin et al. 1990). Even if NO_3^--N stored in the cell vacuole is not utilized, the ion may act as an osmotic solute (Martinoia et al. 1981; Smirnoff and Stewart 1985; Tischner and Kaiser 2007).

Seasonal changes in plant N use

Soil NO_3^--N availability is frequently cited as a factor that regulates temporal changes in plant NRA. For example, temporal NRA changes in the shoots of *Deschampsia flexuosa* (Troelstra et al. 1995) and in *Picea rubens* needles (Tjoelker et al. 1992) correspond to changes in soil NO_3^--N availability. However, we found almost no significant changes in leaf NRA [Fig. 3(c), Appendix 2], while NO_3^--N pool sizes in soil associated with the study species other than *P. japonica* peaked on the

4th sampling day in late summer [Fig. 3(e)]. Thus, temporal changes in the soil NO_3^--N pool size influenced neither NO_3^--N assimilation nor NO_3^--N uptake in any of the dominant understory species.

In a variety of species, leaf NRA is highest in the relatively early stages of leaf growth (Gebauer et al. 1987; Högberg et al. 1986, 1992; Ohlson and Högbom 1993; Pearson and Ji 1994; Stadler and Gebauer 1992; Troelstra et al. 1995). The peak in leaf NRA in selected woody species occurs concurrently with the peak in the leaf-expansion rate at the midpoint of leaf opening (Koyama et al. 2008). We started sampling in late April, when leaves appeared to be not fully developed, and the decline in leaf N concentration in the first month demonstrated that leaves were not fully expanded on the 1st sampling day [Fig. 3(a)]. Therefore, it is very likely that the high N demand during leaf expansion stimulates NRA. Nevertheless, leaf NRA did not change significantly with season except in *L. stellipilum*, and peak NRA in this species did not occur during the leaf expansion period [Fig. 3(c), Appendix 2]. The increase and decrease in NRA during the leaf expansion period occurs rapidly (Koyama et al. 2008), and it is therefore possible that the frequency of our observations was inadequate to resolve the NRA peak during the leaf expansion period.

Influence of soil NO_3^--N pool size on plant NO_3^--N use

Since the enzyme NR is substrate inducible (Tischner and Kaiser 2007), soil NO_3^--N availability is considered a factor in regulating plant NRA (Högberg et al. 1986; Koyama and Tokuchi 2003; Melzer et al. 1984). However, we found almost no correspondence between soil NO_3^--N pool sizes and leaf NRA, except in *L. triloba*, which was distributed across the entire slope gradient irrespective of soil nitrification rate [Figs. 1, 2, Table 3]. The relationship between *L. triloba* NRA and soil NO_3^--N availability was different from those of other species. Most of soils associated with *L. stellipilum* and *H. hirta* had relatively large NO_3^--N pool sizes (>30 mg N m^{-2}), and all soils associated with *P. japonica* had NO_3^--N pool sizes <30 mg N m^{-2}. On the other hand, nearly 50% of soils associated with *L. triloba* had NO_3^--N pool sizes <30 mg N m^{-2}, and the other of soils had NO_3^--N pool sizes >30 mg N m^{-2}. Hence, the leaf NRA of species growing where the NO_3^--N availability spectrum was limited (either NO_3^--N rich or NO_3^--N poor) did not respond to changes in NO_3^--N availability. In contrast, the leaf NRA of the species growing across a wide range of NO_3^--N availability responded to changes in NO_3^--N availability. Therefore, species' responsiveness to changes in NO_3^--N availability are a factor that regulates plant distributions.

Another possible mechanism contributing to the lack of a significant correlation between soil NO_3^--N pool size and leaf NRA may be partitioning of NO_3^--N assimilation between leaves and roots. NO_3^--N fertilization increases NRA in the roots of seedlings and cuttings of *L. triloba* and *H. hirta*, but not in the leaves (Koyama and Tokuchi 2003). Thus in our investigation, the soil NO_3^--N pool size may have regulated root NRA but not leaf NRA. However, scaling up from a small experimental configuration, such as that in Koyama and Tokuchi (2003), to our large-scale field study is difficult. In

the fertilization experiment, NRA was very low in *L. triloba* leaves, irrespective of soil NO_3^--N pool size, whereas we detected considerable NRA in the leaves of *L. triloba* under natural conditions. It is possible that such differences are caused by differences in tree age between studies, or in environmental conditions, such as light, moisture, or temperature. Thus, we are presently unable to make unequivocal statements about the potential variability in root NRA when the soil NO_3^--N pool size changes under natural conditions.

Even though soil NO_3^--N availability decreased with distance from the base of the slope and this decrease influenced leaf NO_3^--N assimilation in *L. triloba*, the leaf N content in this species did not vary in concert with changing soil NO_3^--N availability (Table 3). Hence, *L. triloba* may not depend entirely on NO_3^--N, but may also be able to use other species of N, i.e., NH_4^+-N and organic N. Koba et al. (2003) observed that NRA and the natural abundance of ^{15}N ($\delta^{15}N$) in leaves of *L. triloba* were significantly negatively correlated at a NO_3^--N-rich site, but not at a NO_3^--N-poor site. They attributed the negative correlation in this species to a high dependence on NO_3^--N in NO_3^--N-rich soil (cf. Miller and Bowman 2002). The corollary is that *L. triloba* growing in NO_3^--N-poor soils depends on N sources other than NO_3^--N, which is consistent with the results of the present study, suggesting that this species changes major N sources according to the form and amount of available N.

Fujimaki et al. (2001) showed that the arbuscular mycorrhizal colonization of *L. triloba* roots increases with distance from the base of the slope at our study site, but that this is not the case for *L. stellipilum* and *H. hirta*. Both decomposition processes, i.e., the release of mobile N to the rhizosphere, and capture of the less mobile amino acids or NH_4^+-N by plants may be enhanced by arbuscular mycorrhizal colonization (Read and Perez-Moreno 2003). Therefore, it is possible that in our study *L. triloba* depended more on N acquired by mycorrhizae under NO_3^--N-poor conditions than under NO_3^--N-rich conditions.

Furthermore, the thickness of the organic layer increased with distance from the base of the slope [Fig. 1(d); Hobara and Tokuchi 1998], suggesting a slower decomposition rate on the upper slope. This is consistent with the proposition that *L. triloba* under NO_3^--N-poor conditions on the upper slope acquires organic N through mycorrhizal activities that facilitate plant acquisition of organic N. Therefore, mycorrhizal colonization may explain, at least in part, the process by which the leaf N concentrations of *L. triloba* come to be similar across a range of soil NO_3^--N availabilities.

Mycorrhizal colonization very likely contributes to N acquisition in other species. *Pieris japonica* is symbiotic with ericoid mycorrhizae in the study site (Fujimaki et al. 2001). Ericoid mycorrhizal colonization facilitates a direct pathway of organic N to the plant symbiont (Read and Perez-Moreno 2003). The occurrence of *P. japonica* was highest on the upper slope at our study site, where a thick organic layer had accumulated [Figs. 1(d), (j)] and roots likely took up organic N via ericoid mycorrhizae.

Possible counter-effect of plants on soil N transformation

Soil N influences plant N use; conversely, dominant plant species affect N cycling in soil systems. Studies of soils beneath different tree species indicate that plant species might influence soil N dynamics (Bengtson et al. 2006; Chen and Stark 2000; Zeller et al. 2007). Species' capacities for retaining N in ecosystems mirror differences in their preferences for diverse forms of N (Templer and Dawson 2004; Templer et al. 2005). Conversely, the quality of organic matter, i.e., litter quality from different plant species, has a considerable influence on soil N transformation in several ecosystems (Ferrari 1999). Laughlin (2011) demonstrated in a ponderosa pine forest that soil nitrification potential was more strongly linked to the leaf traits of understory species than to functional diversity. Giesler et al. (1998) suggested that the correspondence between vegetation and soil on a scale of tens of meters mirrors feedback effects of plants on soil processes. Correspondence between the distribution of understory species and soil N forms may also reflect feedback effects of plants on the soil at our study site.

Hirobe et al. (1998) concluded that the changes in the net nitrification rate along the slope at our study site is regulated by the amount of readily decomposable organic matter, soil water content, and pH. The tree layer is a monoculture of even-aged Japanese cedar, which likely provides litter that contributes most of the soil organic matter in this study site. The quality and quantity of organic matter supplied by overstory species is unlikely to have changed dramatically along the slope gradient. In contrast, there is considerable variation in the dominant understory species along the gradient, and the distribution of each corresponds to the distribution of different soil types [Figs. 1(g)(h)(j), 2]. Each species likely contributes organic matter in a species-specific manner, which may partly account for differences in soil N transformation, especially nitrification, along the slope. Information on the litter quality of understory species is not available, but the N concentrations of living leaves continued different markedly among the dominant understory species to the late growing season [Fig. 3(a)]. Since leaf C concentration are almost constant, the C/N ratios of living leaves differed among the species (data not shown); consequently, the C/N ratio of leaf litter must vary. Accordingly, it is very likely that the amount of readily decomposable organic matter in soil differs with the dominant plant species growing in the soil. The distinct change in dominant understory species along the slope probably contributes to the formation of spatially differentiated soil types with different N transformation properties. However, the ratio of understory biomass to total biomass is small, and further investigation is required to accurately estimate the contribution of the understory to soil properties and N cycling in this ecosystem.

Relationship between understory plants and soil at the study site

Read and Perez-Moreno (2003) described changes in vegetation and major soil N species across latitudes and altitudes on a global scale; types of mycorrhizae corresponded to changes in

vegetation. Based on the relationships among plants, soil N, and mycorrhizal type, and on the function of mycorrhizal colonization in plant N acquisition, they proposed that mycorrhizal fungi significantly contribute to ecosystem N cycling in ways that are distinctive by latitude and altitude (Read and Perez-Moreno 2003). They further proposed that this perspective is also applicable at more local scales (Read and Perez-Moreno 2003). We believe it probable that similar relationships among vegetation, soil N form, and mycorrhizal type have developed along the slope at our study site.

Based on net nitrification rates, Koba et al. (2003) divided our study area into two sites, the lower and the upper. They demonstrated differences in $\delta^{15}N$ in plants and soil between sites, and attributed these to the relative importance of soil nitrification and NH_4^+-N immobilization (Koba et al. 2003). However, their lower site is divisible into two smaller sections based on dominant understory species and gross soil N transformations. By comparing our work with previous studies at the same study site, we summarize the relationships among position on the slope, soil NO_3^--N availability, dominant understory species identity, and plant species physiological characteristics (Table 4). Based on this comparison among soil N availability, dominant understory species identity, and plant species physiological characteristics , the physiological plasticity of species' response to changes in NO_3^--N availability regulates plant distribution ranges in accordance with soil NO_3^--N availability at the study site.

Kronzucker et al. (1997) suggested that a species with reduced capacity to use NO_3^--N presented a critical impediment to seedling establishment on disturbed site where NO_3^--N availability was high. Similarly, a species that certainly lacks the capacity to use NO_3^--N in the study site, *P. japonica*, is under disadvantage on NO_3^--N rich soil. Therefore, it is highly possible that *P. japonica* has tolerance to the toxicity of NH_4^+-N and preference to NH_4^+-N as frequently found in ericaceous species (e.g. Nordin et al. 2001); consequently, it become dominant on NH_4^+-N dominated soil as suggested with late-successional species that has high tolerance to NH_4^+-N toxicity and high efficiency of NH_4^+-N transport (Kronzucker et al. 2003).

Combining the results of our field investigation and an earlier fertilization experiment suggesting that *H. hirta* is more responsive to changes in NO_3^--N availability and more dependent on NO_3^--N than *L. triloba* (Koyama and Tokuchi 2003), we can summarize the relationship between a species' physiological traits and its distribution as follows. A species highly dependent on NO_3^--N as a N source and sensitive to NO_3^--N deficiency, namely *H. hirta*, has a narrow distribution range. A species less dependent on NO_3^--N as a N source and insensitive to changes in NO_3^--N availability, i.e., *L. triloba*, has a larger distribution range under natural conditions. Therefore, we conclude that physiological traits and the plasticity of plant species in their use of NO_3^--N are among the factors that regulate species' distribution ranges; thus, soil NO_3^--N availability is an environmental factor that influences plant distribution.

However, the spatial differentiation of *L. stellipilum* and *H. hirta* cannot be explained by

differences in their NO_3^--N use. In terms of net nitrification rates, both the lower slope dominated by *L. stellipilum* and the mid slope dominated by *H. hirta* have high NO_3^--N availability [Figs. 1(h), 4]. However, gross nitrification rates are markedly different between the two slope sections [Figs. 1(g), 4], suggesting a difference in soil microbial activity between the two portions of the slope. A possible explanation for the spatial differentiation of *L. stellipilum* and *H. hirta* is that there may be differences in their ability to compete with soil microbes for NO_3^--N in the soils of the two slope sections.

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FIGURE CAPTIONS

Figure 1 Changing environmental conditions for understory plant species and vegetation along a slope gradient (plot A). (a) Microtopography depicted by slope inclination in each 1 × 3-m subplot; (b) canopy cover ratio as an index of light conditions for the understory layer in each subplot; (c) diameter at breast height (DBH) of overstory *Cryptomeria japonica* [●; modified from Tokuchi et al. (1999)] and fine root biomass [hatched bar; modified from Kasuya and Shimada (1996)]; (d) thickness of the organic layer. Mean thickness at the eastern and western ends of the plot is depicted for the L (litter; closed area) and F+H (humus) layers (hatched area). Figure modified from Hobara and Tokuchi (1998); (e) soil pH (H_2O) measured in August 1995 [△; Hirobe et al. (1998)], and July 1992 (■; Yoshida unpublished data); (f) soil water content measured in August 1995 [△; Hirobe et al. (1998)], May 1993 [▲; Kasuya and Shimada (1996)], and July 1992 (■; Yoshida unpublished data); (g) gross soil N mineralization (hatched bar) and nitrification rates (closed bar); (h) net soil N mineralization (hatched bar) and nitrification rates (closed bar). (g) and (h) are modified from Tokuchi et al. (2000). (i) Numbers of trunks (●) and species (○) in 1 × 3-m subplots; and (j) distributions of four dominant understory species, *viz., L. triloba*, *P. japonica*, *H. hirta*, and *L. stellipilum*. The sizes of circles reflect numbers of stems, as indicated in the figure key.

Figure 2. Spatial distribution of dominant understory species and soil trait regarding N transformation on the mid slope (plot B). The number of stems is represented by the sizes of circles for (a) *H. hirta*, (b) *P. japonica*, and (c) *L. triloba*. Circle size represents (d) % soil nitrification. Figure (d) is modified from Hirobe et al. (1998).

Figure 3. Seasonal changes in (a) leaf N concentrations, (b) leaf NO_3^--N concentration, (c) leaf NRA in four dominant understory species along the study slope, and (d) inorganic N pool sizes and (e) NO_3^--N pool sizes in soil samples associated with the four species. Leaves of the four dominant species and associated soils were investigated simultaneously for *L. stellipilum* (○), *H. hirta* [(●), *P. japonica* (△), and *L. triloba* (×). Bars represent SDs. See Appendix 2 for the results of statistical analysis.

Figure 4. Soil nitrification along different sections of the slope gradient [data from Tokuchi et al. (2000)]. Relationships between gross and net nitrification rates are depicted for soil from the lower slope where *L. stellipilum* was dominant (○), soil from the middle slope where *H. hirta* was dominant (●), and soil from the upper slope where *P. japonica* was dominant (△). Bars show SEs for net nitrification rate.

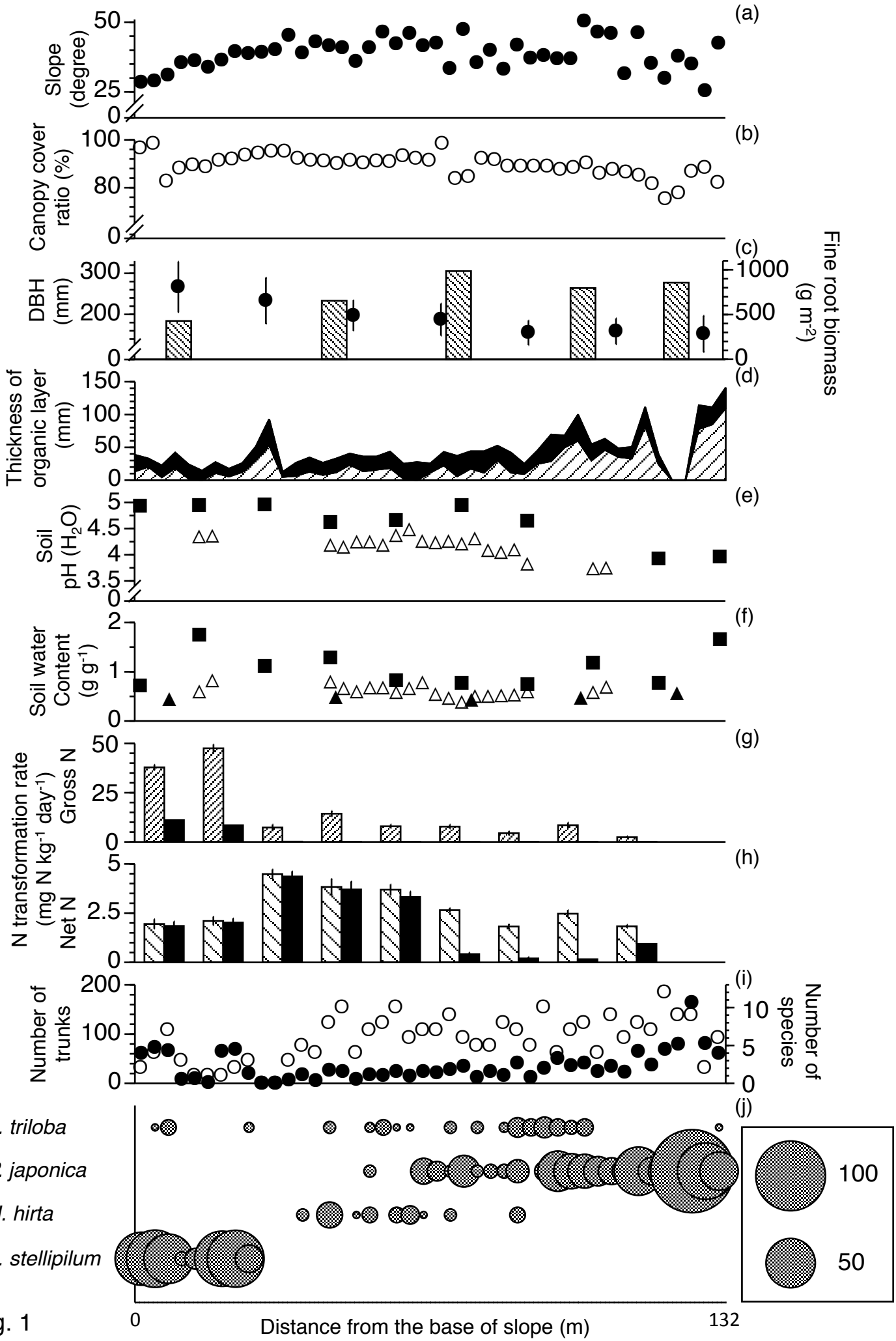
(a)
Slope (degree)
50
25
0
(b)
Canopy cover ratio (%)
100
80
0
(c)
DBH (mm)
300
200
0
1000
500
0
Fine root biomass (g m-2)
(d)
Thickness of organic layer (mm)
150
100
50
0
(e)
Soil pH (H2O)
5
4.5
4
3.5
0
(f)
Soil water Content (g g-1)
2
1
0
(g)
N transformation rate (mg N kg-1 day-1)
Gross N
50
25
0
(h)
Net N
5
2.5
0
(i)
Number of trunks
200
100
0
10
5
0
Number of species
(j)
L. triloba
P. japonica
H. hirta
L. stellipilum
100
50
0
132
Distance from the base of slope (m)

Fig. 1

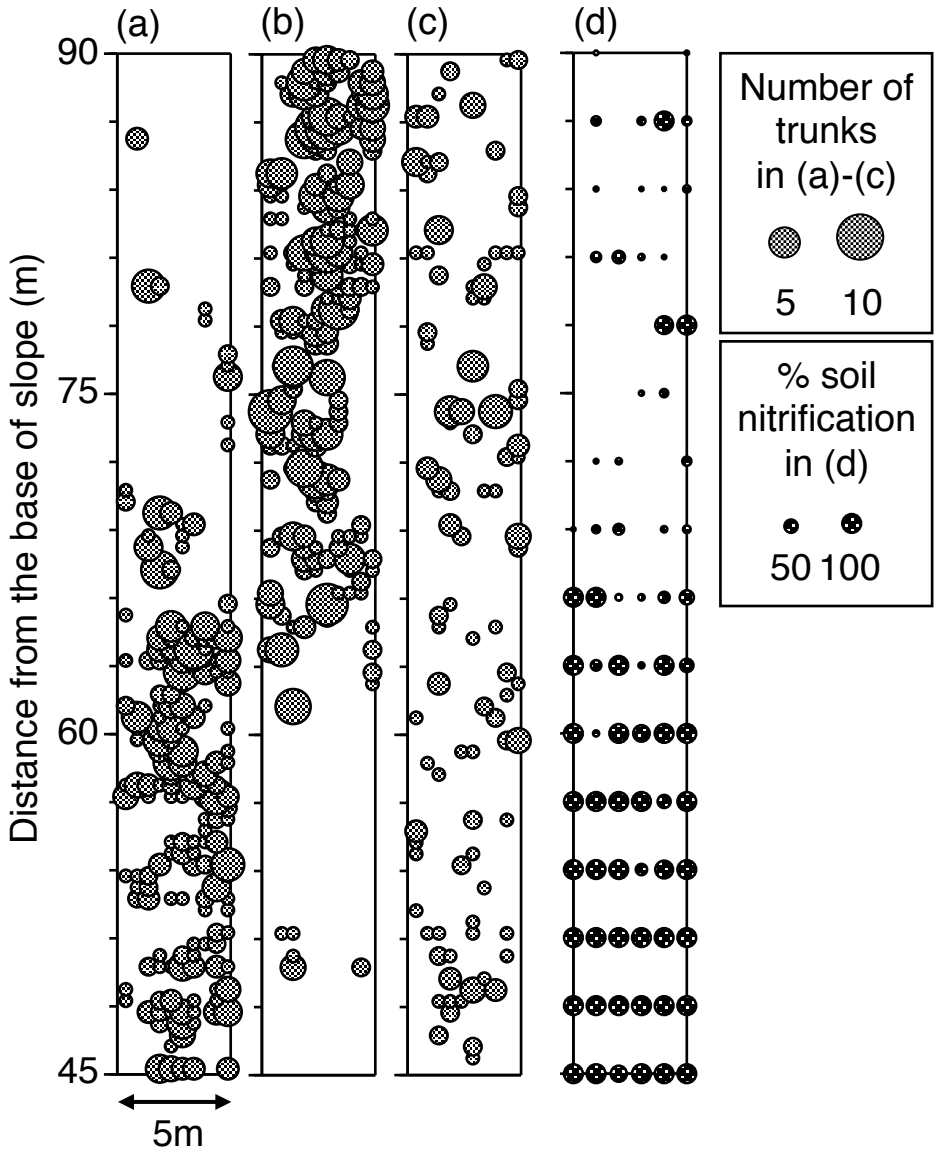
(a)
(b)
(c)
(d)
90
75
60
45
Distance from the base of slope (m)
5m
Number of trunks in (a)-(c)
5
10
% soil nitrification in (d)
50
100

Fig. 2

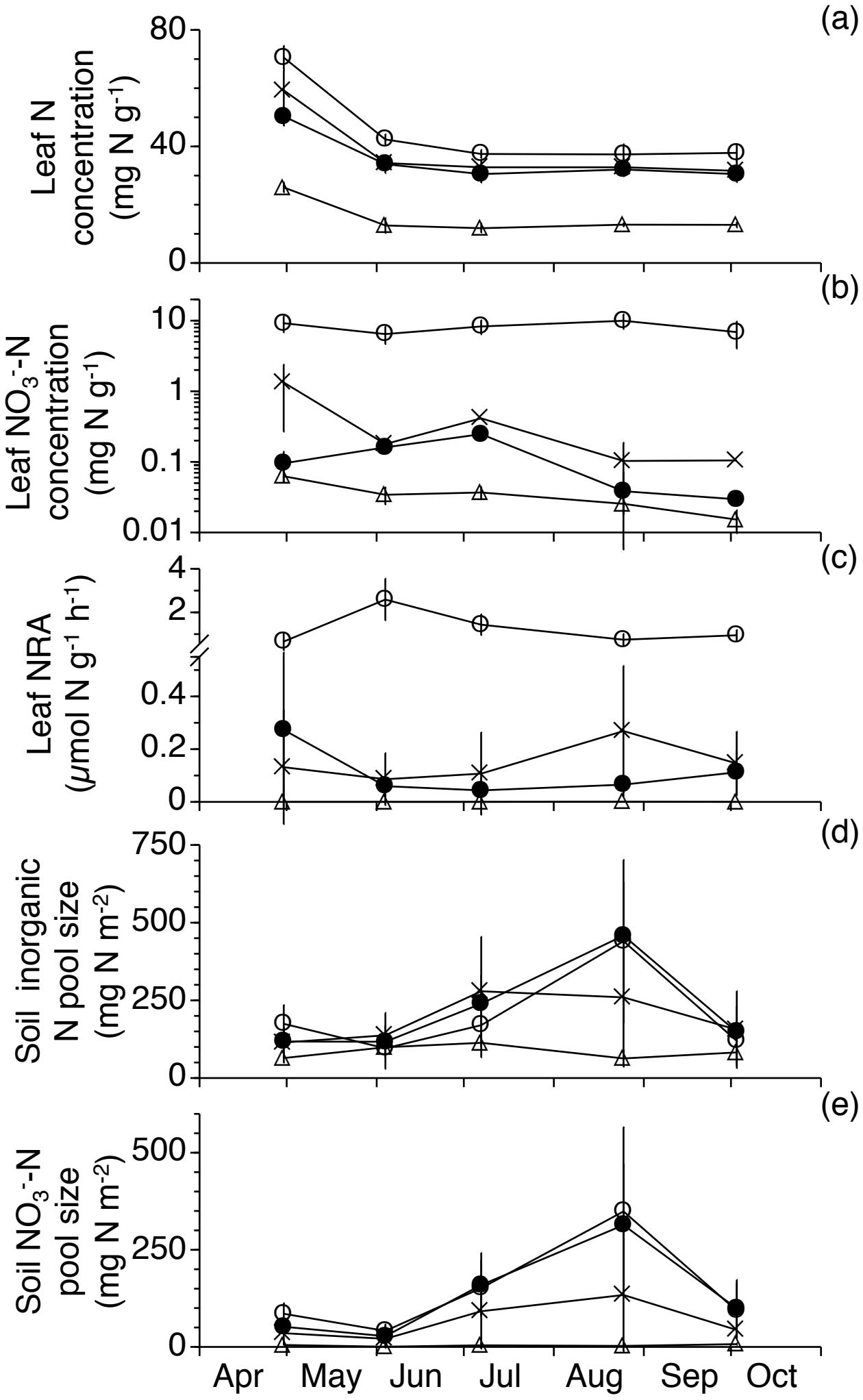
(a)
Leaf N concentration (mg N g-1)
80
40
0
(b)
Leaf NO3--N concentration (mg N g-1)
10
1
0.1
0.01
(c)
Leaf NRA (μmol N g-1 h-1)
4
2
0.4
0.2
0
(d)
Soil inorganic N pool size (mg N m-2)
750
500
250
0
(e)
Soil NO3--N pool size (mg N m-2)
500
250
0
Apr
May
Jun
Jul
Aug
Sep
Oct

Fig. 3

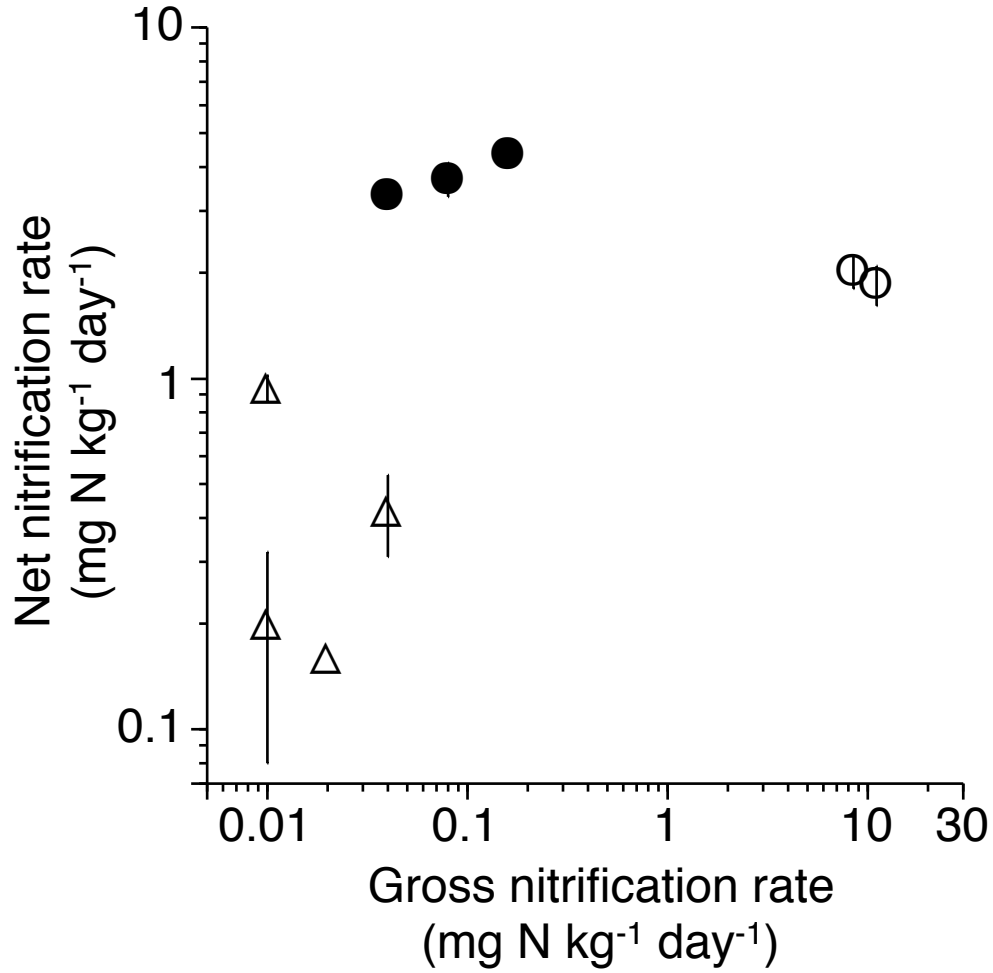
10
1
0.1
Net nitrification rate
(mg N kg-1 day-1)
0.01
0.1
1
10
30
Gross nitrification rate
(mg N kg-1 day-1)

Fig. 4

Table 1. Spatial distribution parameters for *H. hirta*, *P. japonica*, and *L. triloba* in plot B

Species	I_a	Mean v_i	Mean v_j
H. hirta	5.91†	6.01*	-6.38*
P. japonica	7.44†	7.44*	-7.90*
L. triloba	1.08$^{\#}$	0.97^{ns}	-1.03^{ns}

†, significantly aggregated distribution at $P < 0.05$; #, not significantly different from random distribution.
*, significant patch or gap, $P < 0.05$; ns, no significant patch or gap.

Table 2 Spatial associations among *H. hirta*, *P. japonica*, and *L. triloba* in plot B.

Species	X
H. hirta vs. *P. japonica*	-0.48††
H. hirta vs. *L. triloba*	-0.20††
P. japonica vs. *L. triloba*	0.04$^{\#\#}$

††, significantly dissociated from one another, $P < 0.05$; ##, not significantly different from independent relationship.

Table 4 Relationship between the distribution of soil and plant species along the slope in the study site.

Position on the slope (Distance from the base of the slope)		Position on the slope in the study site: Lower slope 0-27 m	Mid slope 27-66 m	Upper slope 66-132 m	Whole slope	Comments	Data source
Soil	Net nitrification rates	High	High	Low			
	(mgN kg soil^{-1} day^{-1})	0.21-2.14	0.11-1.15	0.00-0.63		Min-Max; Mineral soil 0-5 cm; Aug. 1995	Hirobe et al. 1998
		1.85-2.02	3.32-4.36	0.16-0.94		Min-Max; Mineral soil 0-5 cm; Nov. 1995; Fig. 1 (d)	Tokuchi et al. 2000
	Gross nitrification rates	High	Low	Low			
	(mgN kg soil^{-1} day^{-1})	8.48-11.07	0.04-0.16	0.01-0.04		Min-Max; Mineral soil 0-5 cm; Nov. 1995; Fig. 1 (c)	Tokuchi et al. 2000
	NO_3^--N pool sizes	Large	Large	Small			
	(mgN m^{-2})	10.58-781.82	0.00-699.78	0.00-27.94		Min-Max; Mineral soil 0-5 cm; Figs. 3 (m)-(p), and 6	the current study
	Total C/N ratio	Low	Medium (varied)	High			
		11.3-16.0	14.0-18.3	14.5-21.5		Min-Max; Mineral soil 0-5 cm; Aug. 1995	Hirobe et al. 1998
		18.2-19.5	17.3-21.2	20.4-21.5		Min-Max; Mineral soil 0-5 cm; Nov. 1995	Tokuchi et al. 2000
	Extractable organic C/N ratio	Low	Medium (varied)	High			
		data not available	4.3-8.4	4.0-6.3		Min-Max; Mineral soil 0-5 cm; Aug. 1995	Hirobe et al. 1998
		6.7-7.8	7.8-9.3	9.0-14.2		Min-Max; Mineral soil 0-5 cm; Nov. 1995	Tokuchi et al. 2000
	soil pH	High	Medium	Low			
		3.7-4.7	4.0-4.7	3.5-4.5		pH (H_2O); Min-Max; Mineral soil 0-5 cm; Aug. 1995	Hirobe et al. 1998
		4.92-4.93	4.61-4.95	4.94-3.35		pH (H_2O); Min-Max; Mineral soil 0-5 cm; Jul. 1992	Yoshida unpublished data
Plant	Dominant understory species	*L. stellipilum*	*H. hirta*	*P. japonica*	*L. triloba*	Fig2. 1 (b), 2 (a)-(c)	the current study
	Physiological characteristics of dominant understory species about NO_3^--N use	- NO_3^--N preference - NO_3^--N storage	-NO_3^--N preference	-Lack of capacity to use NO_3^--N	-Change with soil NO_3^--N availability	Fig. 3 (e)-(l); Table 1	the current study; Koyama and Tokuchi 2003
	Mycorrhizal colonization	-AM colonization	-AM colonization	-EM colonization	-AM colonization -Change with the position on the slope -Low AM colonization on the lower slope -High AM colonization on the upper slope		Fujimaki et al. 2001

*AM colonization: Arbuscular Mycorrhizal colonization, **EM colonization: Ericoid Mycorrhizal colonization

Table 3 Relationships between soil N pool size and leaf traits examined by Pearson's correlation coefficients.

	Sampling date				
	1st	2nd	3rd	4th	5th
L. stellipilum (n=5)					
soil NO_3^--N × leaf N	-0.681	0.148	0.854	-0.861	0.474
soil inorg N × leaf N	-0.621	-0.437	0.813	-0.908 *	0.512
soil NO_3^--N × leaf NO_3^--N	0.641	0.628	0.287	0.333	-0.156
soil inorg N × leaf NO_3^--N	0.509	-0.122	0.368	0.374	-0.062
soil NO_3^--N × leaf NRA	0.962 **	0.087	0.570	0.283	0.039
soil inorg N × leaf NRA	0.906 *	0.601	0.568	0.264	0.124
H. hirta (n=5)					
soil NO_3^--N × leaf N	-0.350	0.348	0.580	0.694	-0.641
soil inorg N × leaf N	-0.597	0.156	0.604	0.755	-0.642
soil NO_3^--N × leaf NO_3^--N	-0.047	0.215	0.736	-0.185	-0.503
soil inorg N × leaf NO_3^--N	0.228	0.555	0.725	-0.287	-0.600
soil NO_3^--N × leaf NRA	0.945 *	0.627	0.611	-0.345	0.440
soil inorg N × leaf NRA	0.828	0.642	0.648	-0.341	0.598
P. japonica (n=5)					
soil NO_3^--N × leaf N	0.298	0.265	-0.125	0.269	-0.440
soil inorg N × leaf N	-0.142	0.543	0.480	0.388	0.358
soil NO_3^--N × leaf NO_3^--N	0.388	0.131	-0.608	-0.786	0.335
soil inorg N × leaf NO_3^--N	0.522	0.384	-0.022	-0.926 *	0.475
soil NO_3^--N × leaf NRA	-	-	-	-0.829	-
soil inorg N × leaf NRA	-	-	-	-0.427	-
L. triloba (n=10)					
soil NO_3^--N × leaf N	0.131	0.227	0.580	0.592	0.694 *
soil inorg N × leaf N	0.020	0.102	0.479	0.381	0.129
soil NO_3^--N × leaf NO_3^--N	0.365	0.771 **	-0.065	0.410	0.280
soil inorg N × leaf NO_3^--N	0.030	0.536	0.809 **	0.263	-0.121
soil NO_3^--N × leaf NRA	-0.209	0.836 **	0.674 *	0.670 *	0.816 **
soil inorg N × leaf NRA	0.379	-0.044	-0.068	0.575	0.133

*: $p<0.05$, **: $p<0.01$

Appendix 1 Plant species distribution in plot-A. The number of trunks was recorded for the plants taller than 10 cm in each subplot.

Subplot number	3	6	9	12	15	18	21	24	27	30	33	36	39	42	45	48	51	54	57	60	63	66	69	72	75	78	81	84	87	90	93	96	99	102	105	108	111	114	117	120	123	126	129	132	Total
Pieris japonica (Thunb.) D. Don																		3				14	8	3	21	3	4	4	11		8	34	26	24	18	15	14	49	16	24	36	145	67	31	578
Leucosceptrum stellipilum (Miq.) Kitam. et Murata	59	69	51	4	9		64	68	16																																			340
Lindera triloba (Sieb. et Zucc.) Blume		1	5						2						3			2	5	1	1			3		3		2	8	6	9	6	4	6										1	68
Tripetaleia paniculata Sieb. et Zucc.																							1				4							2	1	3						4	13	21	49
Hydrangea hirta (Thunb.) Siebold													3		14		1	5		5	7	1		3					5																44
Smilax china Linn.		2	3									1	1	2		1		2	1	3		1	3		1			1		1	1		1				2	2	4		7				40
Clethra barbinervis Sieb. et Zucc.																				2		1				1	7	1		1		1		2		2	1	5	2	1	10				37
Illicium religiosum Sieb. et Zucc.													4		3				4				5		6		6							3											31
Rhododendron kaempferi Planch.																																		1		6			12				4	23
Akebia trifoliata (Thunb.) Koidz.	1											4	8	1	1	1				1	1			2							2														22
Callicarpa japonica Thunb.		1															2	3		3	3	5											1												18
Eurya japonica Thunb.																								3	2										4		4	2	2	1					18
Osmanthus heterophyllus (G. Don) P. S. Green																																							18					18
Rubus crataegifolius Bunge																																							3	15				18
Hamamelis japonica Sieb. et Zucc. var. *obtusata* Matsum.																									3		3				1		1		1		1	1	2	4					17
Ilex pedunculosa Miq.																													1			9		1							1	1		1	15
Pertya scandens (Thunb.) Sch. Bip.																										2			10	2															14
Rubus palmatus Thunb.																				1														1						2	9				13
Abelia serrata Sieb. et Zucc.								1					1		2	3			1			1							3																12
Callicarpa mollis Sieb. et Zucc.																1	4					1						4		1	1														12
Lyonia ovalifolia (Wall.) Drude var. *elliptica* (Sieb. et Zucc.) Hand.-Mazz.																																				1			7				2	10
Schizophragma hydrangeoides Sieb. et Zucc.			1												1	7				1																								10
Cryptomeria japonica (Linn. fil.) D. Don						1								1	1	2	1	1											2																9
Lindera umbellata Thunb.															1	2			1	5																									9
Vaccinium japonicum Miq.																																						4		1	1	3			9
Viburnum phlebotrichum Sieb. et Zucc.														1									1			2					1				3									8
Symplocos coreana (H.Lév.) Ohwi																					1			2							3		1												7
Magnolia salicifolia (Sieb. et Zucc.) Maxim.																						2									3					1									6
Pourthiaea vilosa (Thunb.) Decne. var. *laevis* (Thunb.) Stapf.																																				2		1		3					6
Rhododendron reticulatum D. Don																								5																		1			6
Fraxinus lanuginosa Koidz.																								5																					5
Parabenzoin praecox (Sieb. et Zucc.) Nakai			3															1	1																										5
Viburnum dilatatum Thunb.																									1																4				5
Castanea crenata Sieb. et Zucc.																																	1							2	1			4
Robinia pseudoacacia L.			2	2																																								4
Rhus trichocarpa Miq.																																					1		1		1				3
Viburnum erosum Thunb.																3																													3
Wisteria brachybotrys Sieb. et Zucc.																2			1																										3
Ilex crenata Thunb.			1																	1																									2
Ilex macropoda Miq.																							1	1																					2
Styrax japonica Sieb. et Zucc.																												1														1			2
Acanthopanax sciadophylloides Franch. et Savat.																																					1								1
Acer palmatum Thunb. var. *matsumurae* (Koidz.) Makino																												1																	1
Chamaecyparis obtusa (Sieb. et Zucc.) Sieb. et Zucc., apud Endl.									1																																				1
Clematis japonica Thunb.				1																																										1
Clerodendrum trichotomum Thunb.												1																																		1
Corylus sieboldiana Blume.																															1														1
Elaeagnus pungens Thunb.																			1																											1
Euonymus alatus (Thunb.) Sieb. f. *striatus* (Thunb.) Makino																					1																									1
Quercus mongolica Fischer var. *grosseserrata* (Blume) Rehd. et Wils.																																										1			1
Quercus serrata Thunb.																																								1					1
Stachyurus praecox Sieb. et Zucc.																												1																	1
Symplocos chinensis Druce var. *leucocarpa* (Nakai) Ohwi f. *pilosa* (Nakai) Ohwi																1																													1
Symplocos prunifolia Sieb. et Zucc.																																				1									1
Number of trunks	60	73	66	7	9	1	64	69	19	0	0	6	17	5	26	23	8	17	15	23	14	24	21	27	34	11	24	15	40	11	30	50	35	40	24	34	22	65	37	69	79	164	80	60	1518
Number of species	2	4	7	3	1	1	1	2	3	0	0	3	5	4	8	10	4	7	8	10	6	7	7	9	6	5	5	8	7	5	10	4	7	8	4	9	6	8	7	12	9	9	2	6	54

Subplot number is represented by the distance from the bottom of the slope to the top of each subplot measuring 1m x 1m.

Appendix 2 Results of one-way ANOVA among sampling dates to show seasonal changes of leaf traits and soil N pool sizes.

				Sampling date				
Species	df	F	p	1st	2nd	3rd	4th	5th
Leaf N concentration								
L. stellipilum	4	120.62	< 0.01	a	b	b	b	b
H. hirta	4	55.11	< 0.01	a	b	b	b	b
P. japonica	4	63.93	< 0.01	a	b	b	b	b
L. triloba	4	81.77	< 0.01	a	b	b	b	b
Leaf NO_3^--N concentration								
L. stellipilum	4	2.06	0.12			-		
H. hirta	4	1.61	0.21			-		
P. japonica	4	21.43	< 0.01	a	b	b	bc	c
L. triloba	4	8.55	< 0.01	a	b	b	b	b
Leaf NRA								
L. stellipilum	4	10.71	< 0.01	b	a	b	b	b
H. hirta	4	2.28	0.10			-		
P. japonica	4	1.00	0.43			-		
L. triloba	4	1.62	0.19			-		
Soil inorganic N pool size								
L. stellipilum	4	17.25	< 0.01	b	b	b	a	b
H. hirta	4	33.38	< 0.01	c	c	b	a	bc
P. japonica	4	3.62	< 0.01	b	ab	a	b	ab
L. triloba	4	9.68	< 0.01	b	b	a	a	b
soil NO_3^--N pool size								
L. stellipilum	4	18.83	< 0.01	b	b	b	a	b
H. hirta	4	25.98	< 0.01	c	c	b	a	bc
P. japonica	4	5.74	< 0.01	ab	c	abc	bc	a
L. triloba	4	10.05	< 0.01	bc	c	ab	a	bc

Different letters indicate significant difference within a single species after Tukey-Kramer test at the $p<0.05$ level.