

Spatial patterns of fine root biomass and performances of understory dwarf bamboo and trees along with the gradient of soil N availability in broad-leaved natural forests and larch plantation

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Abstract: The importance of fine roots in forest ecosystem processes is well known. However, the contribution of understory vegetation to underground ecosystem processes is not well understood. We tested the hypothesis that fine-root biomass (FRB) and performance of the overstory and understory independently decrease with increasing soil N availability in cool-temperate deciduous broad-leaved natural forests and larch plantations in Japan. The mean contribution of understory FRB to total FRB (tree + understory) ranged from 4% to 78% (mean 37%). Tree FRB was negatively correlated with understory FRB, and understory FRB was dominant to tree FRB in infertile soil. Understory and total FRB were negatively correlated with soil net N mineralization rate, whereas tree FRB showed a quadratic relationship with soil N mineralization rate with the peak observed at mineralization of 58.4 kg N ha⁻¹ y⁻¹. The low tree FRB at infertile sites may be due to a below-ground competitive effect of understory fine roots on tree FRB. Understory fine-root nitrogen concentration (FRN) and leaf to fine-root (L/FR) ratio were positively correlated with N mineralization rate. However, tree L/FR was not significantly correlated, whereas tree FRN was positively correlated, with soil N mineralization rate, suggesting that the leaf production effi-

ciency of trees might not increase even on infertile soil. We suggest that belowground processes of overstory trees might change depending on understory vegetation, and that understory vegetation might affect the fine roots of overstory trees, which did not increase mass allocation but increased N use efficiency under low FRN.

Keywords: fine root, net nitrogen mineralization rate, *Sasa nipponica*, topography, understory vegetation

Abbreviations: FRB, fine root biomass; FRN, fine root nitrogen concentration; L/FR ratio, ratio of leaf biomass to fine root biomass

Introduction

The biomass allocation to fine roots is important for stand structure, nutrient use efficiency and ecosystem productivity (Tilman 1988, Tateno et al. 2004, Tateno and Takeda 2010). The changes in allocation balance between leaf biomass and fine-root biomass (FRB) in forests are important adaptive responses of plants to different soil conditions (Tilman 1988, Tateno et al. 2004). However, the importance of fine roots for the

understory vegetation has largely been neglected because in terms of biomass the understory is proportionally small relative to that of tree species.

The low aboveground biomass of the understory vegetation relative to that of the dominant tree species in forest ecosystems is because understory plants lack the large trunk of trees. Despite its low biomass, the understory vegetation has important functions in nutrient cycling of forest ecosystems. For example, understory vegetation contributes with a high proportion of net primary production and carbon (C) supply to soil systems (Watanabe et al. 2013), decreases inorganic nitrogen (N) leaching through N uptake (Zak et al. 1990, Fukuzawa et al. 2006), and contributes N to the ecosystem by N fixation in retrogressive successional forest ecosystems under N deficiency (DeLuca et al. 2008). Consequently, the understory is often the driver of ecosystem succession (Nilsson and Wardle 2005). These ecosystem functions of understory vegetation may be attributed to the biomass allocation and performance of fine roots, but few studies have focused on the functions of fine roots in the understory of whole forest ecosystems.

Dwarf bamboo is the dominant understory species in cool-temperate forest in Japan, especially on Hokkaido Island. Dwarf bamboo often contributes up to 30% of the aboveground litter fall (*Sasa senanensis*; Watanabe et al. 2013) and 40% of the aboveground leaf litter material on the forest floor (*Sasa nipponica*; Hasegawa et al. 2013) in deciduous broad-leaved forest. In addition, *S. senanensis* contributes 71% of the total FRB in cool-temperate deciduous forest (Fukuzawa et al. 2007). These contributions suggest that dwarf bamboo might be the main driver of belowground net primary production. In addition, dense populations of dwarf bamboo might be the dominant belowground competitor with overstory tree species. However, differences in belowground processes between overstory species and understory vegetation are poorly understood.

Biomass allocation and performance traits of fine roots are also important for nutrient cycling in forest ecosystems and are influenced by soil resource availability. The FRB is a fundamental index of the cost to plants of soil resource acquisition. The FRB generally increases under low soil water or N availability (Tateno et al. 2004). The fine-root N concentration (FRN) is often used as a performance index of the physiological activity of fine roots. The FRN is associated with root physiological functions and, in general, is positively correlated with root respiration rate, N uptake rate, water uptake rate, and the rates of substrate decomposition in soil systems (Ryan et al. 1996, Eissenstat and Yanai 1997, Chen et al. 2002, Makita et al. 2009). However, roots with

high FRN suffer high C and N costs, although they show high performance (Eissenstat and Yanai 1997). In infertile soils, trees and forest stands with inadequate N acquisition and C productivity cannot meet the high FRN maintenance costs, and thus FRN decreases with decline in soil N availability (Pregitzer et al. 2002, Tateno et al. 2004). The ratio of leaf mass to fine-root biomass (L/FR ratio) is an indicator of the efficiency of soil resource acquisition by fine roots relative to carbohydrate production by leaves. The L/FR ratio is often affected by environmental factors, such as altitude (Körner and Renhardt 1987) or local soil conditions (Tateno et al. 2004, Tateno and Takeda 2010). Nitrogen acquisition by fine roots often limits leaf production and photosynthesis by leaves. On infertile soils, plants need high FRB to acquire N resources, and allocate high amounts of C and N to fine roots, thereby sacrificing C and N allocation to leaves and stems. Therefore, the L/FR ratio is generally low under infertile soil conditions (Tateno et al. 2004).

Belowground competition is important for plant performance as well as aboveground competition (Wilson and Tilman 1993), and often affects total FRB and distribution of FRB of specific species. The FRB in mixed stands containing beech and spruce is higher than that in pure stands of beech and spruce (Schmid 2002). In addition, competitive ability differs among species. For example, FRB of a superior belowground competitor, such as beech, increases and that of an inferior competitor, such as spruce and oak, decreases under co-existence of the two species (Schmid 2002, Leuschner et al. 2001). Though understory species are considered to be regulated by the one-sided competitive effect of shading by overstory trees (Kubota 1977), belowground competition between understory and tree species may often be stronger than aboveground competition (Riegel et al. 1995). With regard to the relationship between strength of competition and soil condition, interspecific belowground competition tends to be experienced in infertile soils relative to fertile soils (Wilson and Tilman 1991, Coomes and Grubb 2000). Therefore, especially under N limitation, belowground competition between overstory and understory vegetation may modify the simple negative relationship between FRB and soil N availability.

In a previous study (Hishi et al. 2014), we investigated changes in N dynamics associated with different aspects and slope positions in Ashoro Research Forest (ARF), Ashoro, Japan, and clarified the factors that control N dynamics. The understory vegetation in ARF consists of dense dwarf bamboo *Sasa nipponica* (Hishi et al. 2014). In the present study, we hypothesized that 1) FRB of both understory and tree species decreases with increasing N availability,

and 2) FRN, as an index of root physiological function, and L/FR ratio, as a measure of the functional efficiency of fine roots, increase with elevated soil N availability in the absence of belowground competition between overstory trees and understory vegetation. Otherwise, 3) belowground competition may reduce the FRB of the inferior competitor under low soil N availability. In addition, 4) competition affects indices of fine-root performance, such as FRN and L/FR. Thus, we investigated the relationship between biomass and performance of fine roots of the understory and tree species along a gradient of soil N availability in broad-leaved natural forests and larch plantations in ARF, which experiences a cool-temperate climate.

Materials and Methods

Study site

The study was located in the ARF, Ashoro, Japan (central Ashoro, eastern Hokkaido Island, 43°15'N, 143°33'E). The ARF has an inland cool-temperate climate with the soil freezing during winter (Hishi et al. 2014). The mean annual precipitation and temperature are 821 mm and 6.24 °C, respectively (2002–2012, Japan Meteorological Agency 2014). Snow cover occurs during late December and early April. The maximum snow accumulation depth during winter is about 0.5 m. The dominant tree species in natural forests in this region are the deciduous broad-leaved species *Quercus crispula*, *Acer pictum* subsp. *mono*, *Tilia japonica* and *Ulmus davidiana* var. *japonica* (Okano 1994). The major plantation tree in this region is *Larix kaempferi*. The soil parent material is volcanic ash, and the soils are classified as Cambisols (IUSS Working Group WRB 2014) with a black surface soil layer.

Ten study sites located in five natural forests and five larch plantations were established in the ARF to include various topographic traits (Table 1). In the natural forest sites, five 400 m² plots were established in the upper and middle parts of a south-facing slope (N-SU and N-SM), in the upper and middle parts of a north-facing slope (N-NU and N-NM), and at the base of the slopes with a gentle gradient (N-L). Similarly, in the plantation sites, five 400 m² square plots were established in the upper and middle parts of a south-facing slope (P-SU and P-SM), in the upper and middle parts of a north-facing slope (P-NU and P-NM), and at the base of the slopes (P-L). The forest type, slope aspect and position, and topographic parameters derived from a digital elevation model are listed in Table 1. These parameters are described in detail by Hishi et al. (2014).

The soil properties and O-layer amount were analyzed in our previous study (Table 2, Hishi et al. 2014). Briefly, gravimetric water content, pH (H₂O), total C and total N concentrations, and soil C/N ratio of the surface soil layer (0–5 cm depth) were measured in soil samples collected in 2008. For estimation of the annual net N mineralization rate, the *in situ* buried bag method (Eno 1960) was used at the 10 forest sites from May 2011 to May 2012. The spatial variation in net N mineralization was higher in natural forests than in plantations, whereas the soil C/N ratio varied among plots both in natural and plantation forests. In summary, north-facing and basal slope positions are fertile sites for plants, whereas south-facing and upper-slope positions are infertile sites with respect to soil physical and chemical conditions in ARF. For example, the C/N ratio and net ammonium production of the surface soil layer (0–5 cm depth) on south-facing and upper slopes were higher than those on north-facing and lower slopes both in natural and plantation forests. The soil water content, pH (H₂O),

Table 1. Topographic and forest characteristics at each study site in Ashoro Research Forest (for additional details see Hishi et al. 2014)

Site	Forest type	Position	Aspect	Slope angle (°)	Basal area (m ² ha ⁻¹)	Tree leaf biomass (Mg ha ⁻¹)	Understory leaf biomass (Mg ha ⁻¹)
N-SU	Natural	Upper	SW	20.3	24.6	2.66	1.44±0.07
N-SM		Middle	SW	25.5	44.3	2.06	1.31±0.27
N-NU		Upper	NNE	22.6	38.5	2.49	0.65±0.18
N-NM		Middle	NNE	18.2	25.4	2.51	0.80±0.18
N-L		Lower	NE	11.8	29.4	2.65	0.93±0.13
P-SU	Plantation	Upper	SW	24.4	15.8	3.08	0.75±0.09
P-SM		Middle	SSE	27.5	23.1	3.34	0.51±0.10
P-NU		Upper	NNE	21.7	23.6	4.85	0.35±0.46
P-NM		Middle	NE	22.9	29.4	3.53	0.50±0.23
P-L		Lower	SSW	6.5	27.4	3.40	0.71±0.23

Table 2. Soil characteristics at each study site in Ashoro Research Forest. Different letters within a column indicate a significant difference among sites as indicated by the Tukey–Kramer honestly significant difference test ($p < 0.05$). All data are from Hishi et al. (2014)

Site	WC (%)	O-layer accumulation (Mg ha ⁻¹)	pH (H ₂ O)	Soil TC (%)	Soil TN (%)	Soil C/N	NRminN (kg N ha ⁻¹ y ⁻¹)	NRnit (kg N ha ⁻¹ y ⁻¹)	NRamm (kg N ha ⁻¹ y ⁻¹)
N-SU	25.0±3.6 ^b	30.0±1.7 ^{ab}	4.8±0.4 ^{bc}	12.1±3.3 ^{ab}	0.71±0.08 ^{cde}	17.1±0.6 ^{ab}	21.4±5.4 ^b	2.4±1.0 ^c	19.05±6.16 ^a
N-SM	30.1±6.8 ^{ab}	27.5±8.7 ^{ab}	4.9±0.3 ^{bc}	12.6±0.7 ^{ab}	0.82±0.04 ^{bcde}	15.3±0.7 ^{bc}	22.7±9.7 ^b	13.2±10.3 ^{bc}	9.58±2.88 ^{ab}
N-NU	32.7±5.6 ^{ab}	21.8±2.4 ^b	5.2±0.1 ^b	13.4±1.5 ^{ab}	0.95±0.07 ^{abcd}	14.0±0.7 ^{cd}	55.8±9.1 ^{ab}	55.4±12.2 ^{abc}	0.40±3.67 ^b
N-NM	32.6±2.4 ^{ab}	14.5±0.9 ^b	6.3±0.1 ^a	17.2±5.8 ^a	1.34±0.39 ^a	12.7±0.4 ^{cd}	75.4±7.7 ^{ab}	76.6±7.4 ^{ab}	-1.20±0.33 ^b
N-L	32.0±3.5 ^{ab}	14.0±3.4 ^b	5.3±0.2 ^b	14.6±1.6 ^a	1.10±0.11 ^{abc}	13.2±0.3 ^{cd}	34.8±16.8 ^{ab}	34.1±16.5 ^{abc}	0.72±0.56 ^b
P-SU	25.8±5.0 ^b	33.8±3.5 ^{ab}	4.3±0.1 ^c	12.4±1.1 ^{ab}	0.68±0.06 ^{de}	18.4±0.7 ^a	42.0±7.8 ^{ab}	31.3±11.2 ^{abc}	10.78±5.77 ^{ab}
P-SM	25.1±5.7 ^b	53.4±11.4 ^a	5.0±0.1 ^{bc}	7.5±0.9 ^b	0.51±0.05 ^e	14.7±0.6 ^{bcd}	59.5±6.3 ^{ab}	53.5±7.1 ^{abc}	5.99±2.66 ^{ab}
P-NU	28.4±7.1 ^{ab}	29.3±6.1 ^{ab}	5.1±0.3 ^{bc}	10.7±3.2 ^{ab}	0.76±0.07 ^{cde}	13.9±0.6 ^{cd}	52.9±9.5 ^{ab}	50.8±8.9 ^{abc}	2.16±0.71 ^b
P-NM	34.8±8.8 ^{ab}	28.8±0.9 ^{ab}	4.8±0.2 ^{bc}	12.1±1.1 ^{ab}	0.96±0.05 ^{abcd}	12.6±0.3 ^d	86.6±14.6 ^a	82.8±14.6 ^a	3.81±0.72 ^{ab}
P-L	39.3±11.9 ^a	20.7±2.4 ^b	5.2±0.2 ^b	10.7±3.3 ^b	1.34±0.43 ^{ab}	14.3±0.6 ^{cd}	71.2±17.4 ^{ab}	64.9±14.6 ^{ab}	6.34±3.14 ^{ab}

WC, Soil water content; NRminN, Net N mineralization rate; NRnit, Net nitrification rate; NRamm, N

earthworm biomass, net nitrification rate and net N mineralization rate on south-facing and upper slopes were lower than those on north-facing and lower slopes both in natural and plantation forests. On south-facing slopes of ARF, compared with the mild soil conditions on north-facing slopes, high frequencies of dry–wet fluctuation during summer and freeze–thaw during winter owing to differences in solar radiation intensity should deplete net N mineralization. These findings are detailed in Hishi et al. (2014).

Biomass sampling

Tree leaf biomass was estimated from the amount of fallen leaves collected in a litter trap of area 0.5 m², because the broad-leaved tree species and larch growing at the study sites were deciduous. A litter trap was placed at the center of each plot. Litterfall was collected monthly or bimonthly between late June and late November in 2008 (except when snow covered the ground). All collected litter was sorted into leaves and other plant organs, and then dried at 75 °C for 72 h. Each fraction was weighed separately for each collection.

Aboveground biomass of the understory vegetation was measured by harvesting all aboveground shoots within an area of 1.0 m² in September, 2012. The leaves and culms of dwarf bamboo (*S. nipponica*) and the other plant species were hand-sorted and weighed after drying at 75 °C for 48 h. Because *S.*

nipponica comprised more than 90% of the understory aboveground biomass in all plots, the leaf biomass of *S. nipponica* was regarded as the total understory leaf biomass.

For fine-root sampling, three organic layers of 100 cm² and three surface soil blocks of 100 cm² and 8-cm deep were sampled at each site in August, 2009. Both the organic layers and soil samples were washed in sequence on 2 mm and 0.5 mm sieves, and fine roots (<2 mm diameter) were separated from the soil. Fine roots were separated into those of dwarf bamboo and those of other woody species on the basis of the following features: the fine roots of *S. nipponica* are yellowish white, the root tip is shorter and narrower, and the branching angle near the root tip is wider than that of woody species. The fine roots of grasses and ferns comprised less than 1% of the total fine roots, therefore understory FRB was regarded as the FRB of *S. nipponica* in this study. The fine-root samples were weighed after drying at 75 °C for 48 h.

For chemical analysis, the dried leaves and fine-root samples were ground and the total C and N concentrations were determined using a CN analyzer (MT-700, Yanaco Co., Kyoto, Japan). The FRN of tree and understory species was determined. Total FRN was calculated as the weighted average of understory and tree FRN based on the understory and tree FRB.

To assess the efficiency of leaf production per unit fine roots, the L/FR ratio was calculated. The tree L/FR ratio was calculated as the tree leaf biomass

divided by the tree FRB. The understory L/FR ratio was calculated as the leaf biomass of *S. nipponica* divided by the FRB of *S. nipponica*. The total L/FR ratio was determined as the total leaf biomass of trees and *S. nipponica* divided by the total FRB.

Statistical analysis

The relationship of root traits, namely FRB, FRN, and L/FR ratio, between trees species and understory vegetation was analyzed using a mixed linear model with the residual maximum likelihood method. Tree FRB and FRN were explained by understory FRB and FRN as a fixed effect, and by forest type and sampling plots as random effects to avoid the differences in effect amplitude of FRB and performance, between larch and broad-leaved trees. For the relationship in L/FR ratio between understory and tree species, forest type was used as a random effect. The relationships between the fine-root traits and soil N availability were examined using a mixed linear model. The net N mineralization rate was a fixed factor, and forest type (natural and plantation forests) was a random factor. To evaluate factors important for FRB, FRN, and L/FR ratio, partial least squares regression analysis was used. The FRB, FRN, and L/FR of tree and understory species in each plot were incorporated into one Y vector, and net N mineralization rate, net nitrification rate, net ammonium production rate, soil C/N ratio, soil pH (H₂O), soil water content, organic layer accumulation, daily temperature range, and frequency of freeze–thaw cycles were set as X vectors. Variable importance projection (VIP) indicated the

relative importance of the X vectors for fine-root traits. These statistical analyses were performed using JMP version 10.0 (SAS Institute, Inc., Cary, NC, USA). To test the quadratic relationships, a mixed linear model using a quadratic equation was applied using the lme4 ver. 1.1-6 package for R (Bates et al. 2014). Quadratic, linear and null mixed models using maximum likelihood were compared using analysis of variance (ANOVA) with R ver. 3.1.0 for Mac OSX (R Development Core Team, 2014).

Results

Tree leaf biomass ranged from 2.06 to 4.85 Mg ha⁻¹ (mean ± S.D. 3.06 ± 0.79 Mg ha⁻¹) (Table 1). Understory leaf biomass ranged from 0.35 to 1.44 Mg ha⁻¹ (mean ± S.D. 0.79 ± 0.35 Mg ha⁻¹). Tree leaf biomass was not linearly correlated with understory leaf biomass (data not shown, $n = 10$, mixed linear model, $p = 0.0973$). Tree leaf biomass was not significantly correlated with soil net N mineralization rate ($p = 0.5856$). The understory leaf biomass was negatively correlated with soil N mineralization rate (mixed linear model, $n = 10$, understory leaf biomass = $-0.0097 \times N$ mineralization + 1.32, $p = 0.0386$). Total leaf biomass (tree leaf biomass + understory leaf biomass) was negatively but not significantly correlated with soil N mineralization rate (mixed linear model, $n = 10$, total leaf mass = $-0.020 \times N$ mineralization + 5.88, $p = 0.0849$).

Total FRB of each plot ranged from 1.34 to 4.93 Mg ha⁻¹ (mean ± S.D. 3.07 ± 0.43 Mg ha⁻¹) (Table 3). Tree FRB for each stand ranged from 0.64 to 4.16 Mg

Table 3. Fine-root biomass (FRB, $n = 3$), fine-root nitrogen concentration (FRN, $n = 3$), and leaf biomass to fine-root biomass ratio (L/FR ratio, $n = 1$) of trees, understory, and total vegetation in each stand (mean ± S.D.). Different letters within a column indicate a significant difference among sites as indicated by the Tukey–Kramer honestly significant difference test ($p < 0.05$).

Site	FRB (t ha ⁻¹ , $n = 3$)			FRN (% , $n = 3$)			L/FR ratio ($n = 1$)		
	Tree	Understory	Total	Tree	Understory	Total	Tree	Understory	Total
N-SU	1.05±0.65 ^c	3.88±0.78 ^a	4.93±1.37 ^a	1.06±0.13 ^{cd}	1.24±0.21 ^{cd}	1.20±0.17 ^{cde}	2.54	0.37	0.83
N-SM	1.03±0.39 ^c	3.62±1.48 ^a	4.65±1.21 ^{ab}	0.92±0.18 ^d	0.92±0.06 ^d	0.93±0.09 ^e	1.99	0.36	0.72
N-NU	4.16±1.32 ^a	0.17±0.07 ^c	4.34±1.25 ^{abc}	1.17±0.13 ^{bcd}	1.18±0.08 ^{cd}	1.17±0.13 ^{cde}	0.60	3.76	0.72
N-NM	1.81±0.23 ^{bc}	0.26±0.11 ^c	2.07±0.17 ^{bcd}	1.52±0.08 ^{abc}	1.39±0.08 ^{abc}	1.50±0.08 ^{abcd}	1.39	3.10	1.60
N-B	3.18±0.63 ^{ab}	0.79±0.42 ^{bc}	3.98±0.61 ^{abcd}	0.97±0.13 ^d	1.03±0.10 ^{cd}	0.98±0.11 ^e	0.83	1.17	0.90
P-SU	1.30±0.60 ^{bc}	2.36±0.96 ^{ab}	3.67±0.42 ^{abcde}	1.19±0.09 ^{bcd}	1.16±0.22 ^{cd}	1.15±0.16 ^{de}	2.36	0.32	1.04
P-SM	1.68±0.91 ^{bc}	0.58±0.22 ^c	2.27±0.87 ^{bcd}	1.24±0.10 ^{bcd}	1.29±0.04 ^{bcd}	1.27±0.06 ^{bcd}	1.98	0.87	1.70
P-NU	1.71±0.80 ^{bc}	0.25±0.10 ^c	1.96±0.81 ^{cde}	1.65±0.34 ^{ab}	1.03±0.10 ^{cd}	1.58±0.32 ^{abc}	2.83	1.38	2.65
P-NM	1.21±0.43 ^{bc}	0.30±0.23 ^c	1.51±0.52 ^{de}	1.66±0.11 ^{ab}	1.65±0.13 ^{ab}	1.65±0.79 ^{ab}	2.91	1.68	2.67
P-B	0.64±0.46 ^c	0.71±0.47 ^{bc}	1.34±0.42 ^e	2.03±0.32 ^a	1.70±0.13 ^a	1.85±0.06 ^a	5.34	1.00	3.05

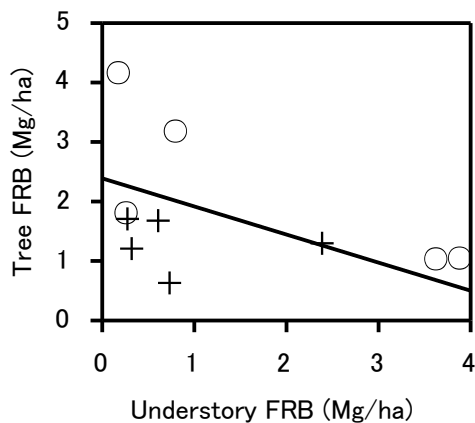


Fig. 1. Relationship between tree and understory fine-root biomass (FRB). The significant regression line is derived from a mixed linear model regression analysis. Circles indicate natural forest, crosses indicate larch plantation.

ha^{-1} (mean \pm S.D. $1.77 \pm 0.34 \text{ Mg ha}^{-1}$). Understory FRB ranged from 0.17 to 3.88 Mg ha^{-1} (mean \pm S.D. $1.29 \pm 0.46 \text{ Mg ha}^{-1}$). Understory FRB was negatively correlated with tree FRB (Fig. 1, $n = 10$, tree FRB = $2.388 - 0.472 \times$ understory FRB, $p = 0.0468$).

Total FRN of each plot ranged from 0.93% to 1.85% (mean \pm S.D. $1.27 \pm 0.28\%$) (Table 3). Tree FRN for each stand ranged from 0.92% to 2.03% (mean \pm S.D. $1.30 \pm 0.29\%$). Understory FRN ranged from 0.92% to 1.70% (mean \pm S.D. $1.28 \pm 0.28\%$). Understory FRN was positively correlated with tree FRN (data not shown, $n = 10$, tree FRN = $0.203 + 0.861 \times$ understory FRN, $p = 0.0009$).

Total L/FR ratio of each plot ranged from 0.72 to 3.05 (mean \pm S.D. 1.59 ± 0.90) (Table 3). Tree L/FR ratio for each stand ranged from 0.60 to 5.34 (mean \pm S.D. 2.23 ± 1.33). Understory L/FR ratio ranged from 0.32 to 3.76 (mean \pm S.D. 1.40 ± 1.17). Understory L/FR ratio was not significantly correlated with tree L/FR ratio ($n = 10$, $p = 0.3461$).

Partial least squares regression analysis showed that indices associated with N fertility, such as net N mineralization and net nitrification rates, were the most important variables for FRB, FRN, and L/FR ratio of understory and tree species (Table 4). Surface soil pH (H_2O), water content, accumulation of the organic layer, and daily temperature range were not selected as important factors.

In relation to N availability, total FRB was negatively correlated with net N mineralization rate (Fig. 2a, mixed linear model, $n = 10$, total FRB = $-0.0452 \times$ N mineralization + 5.502, $p = 0.0029$). Understory FRB was negatively correlated with net N mineralization rate (Fig. 2b, mixed linear model, $n = 10$,

Table 4. Variable importance projection (VIP) values from partial least squares regression analysis for the Y vector incorporating fine-root biomass, fine-root nitrogen concentration, and leaf biomass to fine-root biomass ratio. Important variables with VIP > 1 are highlighted in bold.

X vectors	VIP
net N mineralization rate	1.328
net nitrification rate	1.358
net ammonium production rate	1.040
soil C/N ratio	1.085
soil pH (H_2O)	0.601
water content	0.958
forest floor accumulation	0.346
daily temperature range	0.820
frequency of freeze-thaw cycles	1.032

understory FRB = $-0.0586 \times$ N mineralization + 4.441, $p = 0.0014$), whereas tree FRB was not significantly correlated with net N mineralization rate (Fig. 2c, mixed linear model, $n = 10$, $p = 0.5724$). The relationship between tree FRB and N mineralization significantly fitted a quadratic relationship (tree FRB = $-0.0015 \times$ N mineralization² + $0.1751 \times$ N mineralization - 2.5980, $p < 0.05$) rather than a linear or constant null model (ANOVA model comparison, quadratic regression vs linear regression vs null model, $p = 0.01295$).

Both tree and understory FRN were positively correlated with net N mineralization rate (Table 5, trees, $n = 10$, FRN = $0.793 + 0.0095 \times$ N mineralization, $p = 0.0220$; understory, $n = 10$, FRN = $0.784 + 0.0092 \times$ N mineralization, $p = 0.0253$), whereas total FRN was not significantly correlated with soil N mineralization (Table 5, $n = 10$, $p = 0.0644$).

Total L/FR ratio was not significantly correlated with net N mineralization rate (Table 5, mixed linear model, $n = 10$, L/FR = $0.021 \times$ N mineralization + 0.448, $p = 0.0660$). Understory L/FR was positively correlated with net N mineralization rate (Table 5, mixed linear model, $n = 10$, L/FR = $0.043 \times$ N mineralization - 0.937, $p = 0.0172$), whereas tree L/FR was not significantly correlated with net N mineralization rate (Table 5, mixed linear model, $n = 10$, $p = 0.9763$).

Discussion

In this study, understory FRB largely contributed of the total FRB (Table 3), accounting $37 \pm 28\%$ (mean \pm S.D.) of the total FRB. This suggests that, in terms of

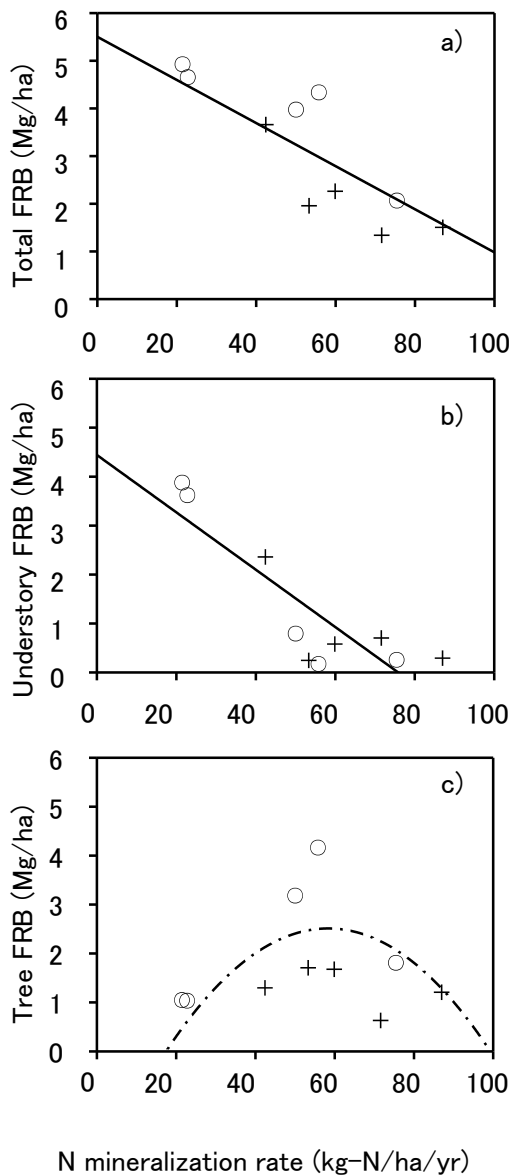


Fig. 2. Relationship between net nitrogen mineralization rate and fine-root biomass of (a) total, (b) understory and (c) tree vegetation. Circles indicate natural forest, crosses indicate larch plantation. Solid lines represent significant linear regression lines, the dashed line represents a significant quadratic regression line.

biomass, the contribution of understory vegetation to ecosystem belowground processes is appreciable. This result is comparable with the substantial contribution (50%) of understory *S. senanensis* to total FRB of forest in northern Japan (Fukuzawa et al. 2006). Soil N availability negatively affects FRB in forest ecosystems (Tateno et al. 2004). In the present study, however, the negative relationship between FRB and soil N availability was only observed for understory

species and not for overstory tree species (Fig. 2). The FRB, FRN, and L/FR ratio of the understory changed with soil N availability, consistent with our expectations, whereas for trees only FRN altered with soil N availability (Table 5). The relationships between soil N availability and total FRB, FRN, and L/FR ratio were similar to those for the understory vegetation, indicating that patterns of the understory fine roots contributed to the patterns of whole-ecosystem belowground processes in ARF.

Tree FRB was not linearly correlated with soil N availability (Fig. 2c). Tree FRB and N mineralization showed a quadratic rather than linear relationship, indicating that tree FRB was highest in the mid-range of N availability. We suggest that this result may be due to a positive effect of understory FRB on tree FRB in infertile sites, and a negative effect on tree FRB in fertile sites, resulting in high FRB in the mid-range of N availability. In general, upper-slope dry sites or high soil N availability decreases FRB in forest ecosystems, whereas lower-slope humid sites or low N availability increases FRB (Enoki et al. 1996, Tateno et al. 2004, Noguchi et al. 2007). In the current study, in fertile sites (N mineralization rate $> 50 \text{ kg N ha}^{-1} \text{ yr}^{-1}$), tree FRB was low (Fig. 2a), which was consistent with previous studies. On the other hand, under low soil N availability with high density of understory roots, tree fine roots may show inadequate growth and functioning. In general, under inadequate soil nutrient availability, belowground competition among plant species is enhanced (Wilson and Tilman 1991, 1993), and total FRB and the FRB of superior competitors are often higher than that of inferior competitors (Leuschner et al. 2001, Schmid 2002). These responses might result in high tree FRB in the mid-range of soil N availability.

In ARF, *S. nipponica* might be a superior belowground competitor to tree species, especially in N-poor soils, such as at a south-facing, upper-slope site, although previous reports indicate that the forest understory vegetation is often primarily regulated by the one-sided effect of competition with overstory trees for light resources (Kubota 1977). However, Riegel et al. (1995) showed that overstory trees (*Pinus ponderosa*) negatively affect biomass of understory vegetation not via aboveground competition for light, but via belowground competition for nutrients, which suggests that belowground competition between understory and tree species often may be stronger than aboveground competition. Our present results showed that understory FRB increased and tree FRB decreased on infertile sites, which indicated that the understory might affect trees through belowground competition in infertile soils. In addition, a negative relationship between understory and tree FRB was observed (Fig. 1), whereas the leaf area index (LAI)

Table 5. Mixed linear model regression analysis of relationships between the fixed effect (soil nitrogen mineralization rate) and fine-root nitrogen concentration (FRN) and leaf biomass to fine-root biomass ratio (L/FR ratio) of the total vegetation, trees, and understory. The functions are described only when the model is significant.

Fine-root performance	Function (y: performance, x: fixed effect)	F-value
Tree FRN	$y=0.793+0.0095x$	8.13 *
Understory FRN	$y=0.784+0.0092x$	7.53 *
Total FRN		4.66 ns
Tree L/FR		0.00 ns
Understory L/FR	$y=-0.937+0.043x$	9.39 *
Total L/FR		4.64 ns

*: significant at $p < 0.05$; ns: nonsignificant at $p > 0.05$

and leaf biomass did not significantly affect understory leaf biomass and understory aboveground biomass (LAI vs understory aboveground biomass, $P = 0.1399$; LAI vs understory leaf biomass, $P = 0.2153$, tree leaf biomass vs understory aboveground biomass, $P = 0.9280$; tree leaf biomass vs understory leaf biomass, $P = 0.3789$). These results suggest that belowground competition between overstory trees and understory species was stronger than the shading effect of overstory trees on understory vegetation in the present study.

Vertical niche differentiation in fine roots among species is often reported (Parrish and Bazzaz 1976). In the present study, we investigated fine roots only in the shallow (0–5 cm depth) surface soil layer and did not measure the contribution of FRB in the upper 5 cm soil layer to whole fine-root systems in the study plots. In ARF, we measured tree and understory FRB in 30 cm depth at different stands in secondary forests of *Q. crispula* and in plantations of *L. kaempferi* from those of current study. In broad-leaved natural forests, the mean FRB of the surface soil layer (5 cm depth) in the current study accounted for 92% of tree FRB and 87% of understory FRB within the upper 30 cm depth in five secondary stands of *Q. crispula* in ARF. In five *L. kaempferi* plantations in ARF, the mean FRB of the surface soil layer in the current study accounted for 97% of tree FRB and 63% of understory FRB within the upper 30cm depth in plantations (Hishi T, unpublished). Therefore, we consider that FRB in the present study adequately reflected the whole-site FRB.

The FRN is often associated with physiological functions, such as nutrient uptake ability, respiration rate, or root turnover (Eissenstat and Yanai 1997, Hishi 2007), and increases with soil N availability (Pregitzer et al. 2002, Tateno and Takeda 2010). As in previous studies, both tree and understory FRN increased with elevated soil N mineralization rate

(Table 5), suggesting that fine-root physiological function per unit biomass increases with increasing soil N availability. Leaf N concentration at an infertile site is lower than that at a fertile site (Tateno and Takeda 2010). Indeed, leaf N concentration of natural and plantation forests was 0.91% and 0.88% at infertile south-facing upper-slope sites, and 1.44% and 0.93% at fertile north-facing mid-slope sites, respectively. Leaf N uptake of natural forests and plantations at south-facing upper-slope sites was 66% and 73%, respectively, of that at north-facing mid-slope sites (Hishi T, unpublished data). This pattern was similar to that shown by FRN in the present study. These results suggest that trees, which did not produce adequate FRB because dwarf bamboo is a superior competitor, show increased nitrogen use efficiency with decreasing N concentration both in leaves and fine roots under infertile soil conditions.

In forest ecosystems, the proportion of belowground biomass allocation to leaf production or whole-ecosystem net primary productivity generally increases under nutrient-poor conditions (Enoki et al. 1996, Tateno et al. 2004). We predicted that the L/FR ratio would decrease with increasing soil N availability. In the present study, only the understory L/FR ratio decreased with increasing soil N availability (Table 5, $p < 0.05$), whereas the total and tree L/FR ratios did not show such a relationship. This difference in fine-root performance between tree and understory species might not be due to the difference in their leaf mass, because both tree and understory leaf biomass were not positively correlated with soil N availability. The leaf production per unit roots of understory species decreased with soil N availability, because high allocation to roots, which increases acquisition of soil resources, may be needed to produce leaf biomass under infertile soil conditions. On the other hand, tree L/FR did not change in response to soil N availability. Belowground competition often decreases fine-root

allocation of inferior competitors (Leuschner et al. 2001, Schmid 2002), suggesting that belowground competition with understory vegetation inhibited the growth of fine roots even under infertile conditions in the present study.

Conclusion

The biomass and performance of understory fine roots showed a linear relationship with soil N availability, whereas tree fine roots did not show such a relationship except for FRN. We suggest that the decline in tree FRB in infertile soil might be due to belowground competition with understory fine roots. In addition, total fine-root biomass and performance were similar to those of the understory, but not to those of trees. We suggest that belowground processes of the understory vegetation are not negligible and often play a key role in whole-ecosystem processes. To understand whether belowground competition between overstory trees and understory species affects stand productivity and N cycling at the ecosystem level, the study of ecosystem C and N cycles in experiments that involve manipulation of understory and overstory species is needed.

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