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Title:

**Annual growth of invasive *Larix kaempferi* seedlings with reference to microhabitat
and ectomycorrhiza1 colonization on a volcano**

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Abstract

To investigate the seedling growth of a biologically-invasive larch *Larix kaempferi* (Lamb.) Carr. on Mount Koma, Japan, seedlings were excavated from three microhabitats (bareground = BA, *Salix reinii* patch = SP, and *Larix* understory = LU) in three elevational zones. Seedlings showed the highest ectomycorrhizal (ECM) colonization percentage in the most shaded microhabitat, LU. ECM colonization percentages in BA and SP were found to decrease with decreasing elevation. These results inferred that the ECM colonization percentages were related to seedling growth, particularly, on BA and SP. However, the annual seedling growth was not synchronized with increases in either elevational gradients. Although ECM colonization was most evident in LU, the seedling growth was lowest. We concluded that the effects of ECM colonization on seedling growth were reduced mostly by microhabitat characteristics.

Key words: biological invasion; elevational gradient; ECM colonization; Mount Koma; Japanese larch; primary succession.

Introduction

On disturbed and/or stressful habitats, biological invasion often modifies plant community structure and the successional sere (Richardson and Higgins 1998; Titus and Tsuyuzaki 2003). Vegetation on Mount Koma, Hokkaido, northern Japan, was destroyed by the 1929 eruption (Yoshii 1932). Nowadays, an non-native, deciduous larch, *Larix kaempferi* (Lamb.) Carr., is the most common tree on the south-facing slope and increases relative dominance more on higher altitudinal areas, while native pioneer tree species, represented by *Betula ermanii* Cham. and *Alnus maximowiczii* Callier, are less abundant (Kondo and Tsuyuzaki 1999; Nishi and Tsuyuzaki 2004).

Ectomycorrhizal fungi (ECM) often promote the high survival and growth rates of the host tree by improving nutrient and water uptake in the early successional stages (Smith and Read 1997; Nara and Hogetsu 2004). In particular, nitrogen content in leaves increases greatly when ECM colonization is conspicuous (Smith and Read 1997). However, the effects of ECM fungi on the host change from positive to negative along nutrient gradients from low to high (Jones and Smith 2004). To clarify how ECM colonization is related to biological invasion, we measured ECM colonization levels on *L. kaempferi*. Also, we examined how ECM colonization contributes to the seedling growth of *L. kaempferi*. Since seedlings are often more sensitive to stress than adults (Maruta 1983), the seedling stage is highlighted in the present study.

Microhabitats provide diverse abiotic and biotic environmental conditions for plant establishment. Biotic microhabitats, e.g., shrub patches impact on seedling germination and/or seedling growth by altering nutrients, litter accumulation, light quality and quantity, and soil temperature under stressful environments (Schlesinger et al. 1996; Brewer 1998; Uesaka and

Tsuyuzaki 2004). By the facilitative effects of *Salix reinii* shrub patch on Mount Koma, plants beneath shrub patches, including light-demanding species, do not decline their growth rates, although solar radiation decline greatly under the patches (Uesaka and Tsuyuzaki, 2004; Akasaka and Tsuyuzaki, 2005). With increased elevation in mountainous areas, soil temperature decreases and the period for plant growth becomes shorter (Maruta 1983). ECM colonization levels are related to edaphic conditions, such as nitrogen (Aerts and Chapin 2000) and litter accumulation (Schoenberger and Perry 1982; Parke et al. 1983). On Mount Koma, in particular, nitrogen concentration in volcanic deposits declined with increasing elevation (Tsuyuzaki, et al. 2005). Also, nitrogen concentration should be different between microhabitats even in the same elevation.

Based on these considerations, we examined two hypotheses: (1) ECM colonization levels in differ between microhabitats along an elevational gradient. (2) ECM colonization levels influence annual production on shoots and leaves, and nitrogen content in leaves for *L. kaempferi* seedlings.

Materials and methods

Sampling sites

Mount Koma is an andesite stratovolcano located in southwest Hokkaido Island, Japan (42° 04'N, 140° 42'E, 1131 m in altitude). The 1929 eruption produced 0.53 km³ of ash and pumice, and pumice flows destroyed the former vegetation on the mountain summit (Yoshii 1932). Seeds of the larch, *Larix kaempferi*, which is non-native in Hokkaido, were immigrated from the afforestation of *L. kaempferi* on the foot to the southwestern slope

(Kondo and Tsuyuzaki 1999), and the species has now become dominant and regenerates naturally. Climate of the region is warm-cool temperate. Mean annual temperature is 11.6°C with the maximum of 24.6°C in August and the minimum of -6.9°C in January, and annual precipitation is 973 mm (Mori Climatological Observatory, ca 9 km northwest from Mount Koma, 10 m a.s.l.). The summer climatic conditions were usual in 2003 when the survey was conducted.

Three major microhabitats were recognised: non-native *Larix* understory (hereafter, LU), native *Salix reinii* Franch. et Savat. patch (SP), and bareground (BA) (Akasaka and Tsuyuzaki 2005). The ground surface of LU was shaded greatly by the *Larix* canopy of which diameters were more than 3 m. Photon flux density (PFD) on the ground surface was 1/7 of BA. SP consisted of *Salix reinii* shrub without any other overstory trees and showed 1/4 PFD of BA. Litter in SP consisted mostly of broad-leaved litter, while litter in LU was dominated by *Larix* litter. BA was characterized by un-shaded, dry ground surface with less litter. For the elevation gradient, we classified three zones: high = H (725-735 m a.s.l.), middle = M (625-645 m), and low = L (505-515 m).

Nutrient status of volcanic deposits

Samples of volcanic deposits were collected using a 100 cm³ steel soil tin (5 cm in depth) during August and October, 1998. Ammonium (NH₄⁺), nitrite (NO₃⁻), phosphate (PO₄³⁻) and pH were measured on each sample, and averaged on each microhabitat at each elevational zone. Ten samples were collected from each of three microhabitats at M and H. Twenty samples were collected from L, but one sample from SP was discarded due to collapse. Total nitrogen and available phosphorus measured in 2005 showed similar relationships between

microhabitats along elevational gradient with samples collected in 1998 (Matsuda and Tsuyuzaki, unpublished data). Therefore, nutrient distribution patterns in 1998 could be referred in this study.

ECM sampling and evaluation

To assess ECM colonization intensity on the roots of *Larix*, ten seedlings were excavated haphazardly from each microhabitat at each elevational zone in late August, 2003, when the leaves were fully expanded and the plants retained green leaves. On SP and LU, each seedling was sampled from separated microhabitat. Seedlings sampled from BA were separated more than 5 m in distance. Sampling sites for SP and BA were separated more than 5 m from *Larix* canopies. Seedlings without any stem break, mammal damage and re-sprouts were sampled. The seedling samples were kept in plastic bags with the volcanic deposits, and were transported to the laboratory immediately after collection. The volcanic deposits were rinsed gently with tap water, and then ECM colonization percentage was evaluated by a gridline-intersect method using a 5-mm cross-section sheet (Brundrett et al. 1997). When the roots were overlapped on any lattice points under macro-and/or micro-scopic observations, presence (Pe) or absence (Pn) of ECM was recorded (Ingleby et al. 1990). The total number of points counted (Pt) equals $Pe + Pn$, and total colonization percentage is calculated by $(Pe / Pt) \times 100$.

Seedling growth

Height and the total length of current- (Lc) and previous-year (Lp) shoots on each seedling sampled were measured. The samples were kept at ca 0°C in a refrigerator until measured.

Because internode length on *Larix* seedlings is consistent with the annual growth length of the shoot (Yang et al. 1998), the seedling age could be determined by the number of whorls on the main shoot. The growth patterns of previous-year and current-year shoots were measured based on the whorls. Current-year shoots, leaves and belowground parts were separated from main shoot, and were dried at 70°C in an oven for four to seven days and weighed. Nitrogen content in leaves was measured by an automatic analyzer (Vario EL, Elementar Hanau, Germany). The leaves of three samples in LU at L and one sample in LU at H were under the detection limit for the nitrogen measurement.

Statistical analysis

Differences in seedling ages were compared among microhabitats and among elevational zones by likelihood rate test after Poisson regression (Fleiss et al. 2003). Absolute and relative differences in ECM colonization on microhabitats, elevational zones and seedling ages were concurrently evaluated by logistic regression. For the evaluation of the relative importance of categorical variables (microhabitats and elevational zones), models that included 25 possible combinations of variables were created by merging variables. The best combination of the selected variables was decided by Akaike's Information Criteria (AIC) in all the cases of model selection (Burnham and Anderson 1998). Differences in nutrient conditions on the volcanic deposits between microhabitats and between elevational zones were tested by post hoc Scheffe's test after ANOVA. Log-transformed values were used for ammonium and nitrite (Zar 1999). Differences in seedling dry mass and leaf nitrogen concentration were compared among microhabitats and among elevational zones by ANOVA. Pearson correlations were determined between total length and mass of current-year shoots, between

belowground mass and total number of counted points (Pt). Two generalized linear models (GLM) were developed with negative binomial errors and a logistic link to evaluate the effect of ECM colonization on the annual growth of seedlings. The masses of current-year shoot, leaf, and current-year shoots and leaves were, respectively, adopted as indices for annual growth. Hereafter, MSL means mass of current-year shoots and leaves. Model I assumes ECM colonization percentage affect annual growth on seedlings, whereas model II does not assume it.

$$\text{Model I: } \mu(Ag_i) = \exp(\beta_0 + \beta_1 Mh_i + \beta_2 El_i + \beta_3 Lp_i + \beta_4 \log(Pn_i) + \beta_5 \log(Re_i))$$

$$\text{Model II: } \mu(Ag_i) = \exp(\beta_0 + \beta_1 Mh_i + \beta_2 El_i + \beta_3 Lp_i + \beta_4 \log(Pt_i))$$

where $\mu(Ag_i)$ = average of annual growth (current year shoot mass, leaf mass, or MSL), Mh_i = microhabitat, El_i = elevational zone, Lp_i = total previous-year shoot length, Rn_i = number of non-ectomycorrhizal root on lattice points counted, Re_i = number of ECM roots on points counted, $Rt_i = Re_i + Rn_i$, and β_0 = constant. β_j is the coefficient on variable j . Total length of previous-year shoots (Lp) was used for the explanatory variable of the previous year shoot growth, because the current year shoot mass for the previous year could not be obtained. Total length of current-year shoots was correlated highly to the mass of current-year shoots ($df = 88$, $r = +0.971$, $p < 0.0001$). Pt on a cross-section sheet was highly correlated to belowground mass ($df = 88$, $r = +0.906$, $p < 0.0001$). Pt was used as an index of belowground mass, because we applied two models to investigate the effects of ECM colonization on annual seedling growth, by using the counted points. Mh_i (microhabitat, BA, SP and LU) and El_i (elevational zone, L, M and H) were categorical variables in the models. The goodness of fit on the two models was compared after the selection of the best variable combination on each model. The statistical analyses were conducted using the statistical program R (R Foundation for

Statistical Computing, Vienna, Austria).

Results

ECM colonization on roots

Seedlings ages ranged from 3-9 years, and there were no significant differences in ages among microhabitats ($df = 2$, $F = 0.14$, $p = 0.866$) and among elevational zones ($df = 2$, $F = 1.29$, $p = 0.274$). Seedling height ranged from 2.9 cm to 19.6 cm (Table 1). In total, ECM colonization percentage on seedlings averaged 67% with a range from 30% to 88%. The averaged colonization percentages ranged from 47% to 71% (Fig. 1) in microhabitats with different elevational zones. The most-plausible dominant ECM was *Boletinus cavipes* (Opat.) Kalchbr., since the fruiting body was the most common on Mount Koma in fall (M. Akasaka personal observation). The best logistic linear model to explain the ECM colonization percentages on seedlings required separate handling of the three microhabitats and three elevational zones (Table 2), i.e., ECM colonization patterns were significantly different between all microhabitats and between all elevational zones. Colonization percentages were lower in SP than in BA, and higher in LU than in BA. Colonization percentages in BA and SP decreased more at lower elevational zones, while the percentages were relatively constant and high in LU at any elevational zones. This indicated that the seedlings in LU succeeded in ECM colonization more than those in SP and BA. Seedling age did not influence ECM colonization percentages (Table 2), and the youngest seedling was three years, indicating that ECM colonization was completed within three years after germination.

Nutrient status of volcanic deposits

The low elevational zone (L) showed double the ammonium concentration of the high elevational zone (H) ($df = 2$, $F = 15.83$, $p < 0.001$) (Fig 2). SP showed the highest ammonium concentration of the three microhabitats ($df = 2$, $F = 37.16$, $p < 0.001$). Nitrate concentration was lower in the middle elevational zone (M) than in H ($df = 2$, $F = 4.09$, $p = 0.019$) but was not different from L. While nitrate concentration was not different among microhabitats ($df = 2$, $F = 2.49$, $p = 0.087$). The volcanic deposit was acidic (pH = 4.1-5.5) and showed significant differences among elevational zones ($df = 2$, $F = 23.37$, $p < 0.001$) and among microhabitats ($df = 2$, $F = 14.82$, $p < 0.001$); viz., pH was higher in BA than SP decreasing with increasing elevation. In total, therefore, the ground surface was infertile, in particular on BA, and nitrogenous components (ammonium and nitrate) in the volcanic deposits decreased with increasing elevation. Phosphate concentration did not change along the elevational gradient (Fig. 2), but differed between microhabitats, i.e., the concentration was lowest in M ($df = 2$, $F = 8.17$, $p < 0.001$) and highest in SP ($df = 2$, $F = 16.19$, $p < 0.001$). There were no significant interactions between elevational zones and microhabitats on these four nutrients (ANOVA, $p > 0.17$).

Seedling growth

The maximum seedling dry mass was 38.20 g collected from bareground at low elevational zone. The average masses of collected seedlings were not significantly different among elevational zones ($df = 2$, $F = 0.32$, $p = 0.72$), as well as ages, while these differed among microhabitats ($df = 2$, $F = 14.90$, $p < 0.0001$). The differences in seedling sizes originated mostly from the different growth rates, since the ages were not significantly different between

the habitats. Leaf nitrogen was not different among microhabitats ($df = 2$, $F = 2.57$, $p = 0.08$) and among elevational zones ($df = 2$, $F = 2.41$, $p = 0.10$) (Table 1), while ECM colonization varied greatly between microhabitats. These results indicated that ECM colonization did not explain leaf nitrogen concentration. To evaluate annual growth, we measured total length and mass of annual shoots and total mass of leaves. The maximum mass of annual shoots and leaves (MSL) was less than 105.7 mg in LU, but 2009.2 mg at BA and 1715.8 mg at SP (Table 1). Current-year shoot mass was linearly correlated to leaf mass ($\log_{10}(\text{shoot weight}) = 1.035 \times \log_{10}(\text{leaf weight}) - 1.331$, $df = 88$, $r^2 = 0.896$, $p < 0.0001$), indicating that allocation of photosynthates to current-year shoot and leaf was roughly constant. As a result, the three indexes indicating annual seedling growth, i.e., masses of current-year shoot, leaf, and MSL, selected the same combinations of variables on GLM to explain ECM contribution, and thus only the result for MSL is shown in Table 3. Model II, which did not consider ECM colonization level on roots, was more profitable than model I, indicating that ECM colonization level was not necessary to explain MSL, and belowground mass was more critical. Total previous-year shoot length (Lp) was inserted to the best-fit model, suggesting that the current-year seedling growth was related to production in the previous year.

The best combination of microhabitat variables on model II combined BA and SP with LU as a separate term (Table 3). The coefficient on LU was 0.814 less than that on (BA + SP), indicating that MSL in LU was the lowest of the three microhabitats. For the elevational zones, the best-fit equation was with H and L merged into single variable and M as a single variable. The positive coefficient on M implied that MSL was the highest in M. The relationship between MSL and nitrogen in the volcanic deposits was not detected since the nitrogenous components decreased with increase in elevation. Also, MSL was not related to phosphate

content.

Discussion

Since ECM colonization level was highest in LU, the distance from ECM source, i.e., overstory *Larix* trees, must be one of the determinants on ECM colonization level on roots (Nara and Hogetu 2004). Litter accumulation inhibits ECM colonization on roots when allelopathic effects result from the litter (Schoenberger and Perry 1982; Rose et al. 1983). Litter derived from *L. kaempferi* accumulated most on LU, suggesting that ECM colonization was not restricted by the litter accumulation on Mount Koma.

ECM colonization in BA and SP decreased more in the lower elevations where nitrogenous nutrients in the volcanic deposits were higher. With increases in nitrogenous nutrients, ECM colonization often decreases because symbiotic relationships between ECM and seed plants become unnecessary (Aerts and Chapin 2000). The reduction of ECM colonization was not intense even in areas with highest nitrogen concentration on Mount Koma. One possibility is that total nitrogen level is still low even 70 years after the eruption. Phosphate concentration was not related to elevational gradient, probably because of the differences in texture of volcanic deposits and/or macro-scaled topography at M (Tsuyuzaki, personal observation). Anyhow, phosphate concentration influences less to ECM colonization level (Smith and Read 1997). Reduction in light derives decrease in leaf nitrogen concentration on early successional tree (Kaelke et al. 2001), while nitrogen concentration in leaves was not different between microhabitats. This implies that ECM fungi have roles on the nutrient uptake for seed plants, in particular, on nutrient-poor habitats. However, the model not adopted ECM effects on annual

seedling growth indicated that ECM colonization was not the prime factor for determining annual aboveground production for the seedlings.

S. reinii patches, SP, function as seed traps promoting facilitation (Uesaka and Tsuyuzaki 2004). If *S. reinii* traps ECM spores and propagules as well as seeds, ECM colonization percentage in SP would be higher than that in BA. However, ECM colonization percentage in SP was the lowest. *S. reinii* develops high levels of ECM colonization on roots (Nara and Hogetsu 2004; Tsuyuzaki et al. 2005). ECM fungi on the roots of *Betula platyphylla* var. *japonica* compete with endophytic fungi (Hashimoto and Hyakumachi 2001). The ECM on *S. reinii* could exclude the establishment of ECM fungi on *L. kaempferi* seedlings within *S. reinii* patches. However, annual seedling growth in SP, where ECM colonization was the lowest, was not different from that in BA. High nutrients in SP should decrease ECM colonization but promote seedling growth, and thus the patch effects could preclude belowground competition between ECM fungi and/or between *L. kaempferi* and *S. reinii*.

Annual seedling growth of a non-native *L. kaempferi* differed between microhabitats (BA and SP vs LU) and between elevational zones (H and L vs M) on Mount Koma as indicated by model selection. In extremely nutrient-poor conditions, seedling growth patterns differed greatly between microhabitats (Titus and del Moral 1998; Akasaka and Tsuyuzaki 2005). ECM colonization level is not a leading factor to determine the survival and growth of *L. kaempferi* seedlings on Mt. Fuji in central Japan, while the growth of *Salix reinii* seedlings is promoted by ECM colonization (Nara and Hogetsu 2004). Both *L. kaempferi* and *S. reinii* are naturally distributed on Mt. Fuji. Our results indicate that the effects of ECM on *L. kaempferi* seedling growth are weak because the characteristics of microhabitats affected more to seedling growth.

The biological invasion of *L. kaempferi* has doubtlessly changed the distribution patterns of ECM fungi, although the effects of ECM colonization were not reflected in the seedling growth of *L. kaempferi*. Biological invasion changes the nutrient cycles that are related to the distribution of ECM (Ehrenfeld 2003). Also, competition between ECM fungi should be modified by the alteration of distribution patterns of ECM fungi (Read 1991). In conclusion, the colonization of ectomycorrhizal fungi on roots increases with decrease in nitrogenous nutrients. ECM colonization occurs more in the understory of *L. kaempferi* that is the source of ECM fungi. However, the effects of ECM colonization on growth of non-native tree seedling overshadowed mostly by differences in microhabitat conditions such as light in LU. The facultative effects of *S. reinii* patch on seedling growth and the relationships between *L. kaempferi* and *S. reinii* through the colonization of ECM fungi should be clarified.

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Table 1. Stem height, mass of current-year shoots and leaves (MSL), nitrogen concentration in leaves on *Larix kaempferi* seedlings on Mount Koma.

Values are mean $\pm$ SD of ten seedlings in each microhabitats at each elevation, with range shown in parentheses.

Elevational zones	Bareground (BA)			<i>Salix reinii</i> patch (SP)			<i>Larix kaempferi</i> understory (LU)		
	Height	MSL	Nitrogen	Height	MSL	Nitrogen	Height	MSL	Nitrogen
	(cm)	(mg)	($\mu\text{g}/\text{mg}$)	(cm)	(mg)	($\mu\text{g}/\text{mg}$)	(cm)	(mg)	($\mu\text{g}/\text{mg}$)
High	8.4 ± 3.4	570.8 ± 543.4	12.3 ± 1.7	9.7 ± 4.3	312.4 ± 507.2	11.0 ± 2.7	5.8 ± 2.5	25.5 ± 31.7	9.2 ± 1.8
	(3.2-13.1)	(21.9-1599.6)	(9.2-14.2)	(3.6-18.2)	(14.7-1715.8)	(6.0-14.8)	(3.3-11.9)	(3.7-105.7)	(6.6-12.0)
Middle	9.4 ± 2.3	854.1 ± 595.9	10.8 ± 3.1	12.3 ± 2.2	384.5 ± 282.0	12.5 ± 1.3	7.0 ± 1.7	28.7 ± 19.8	10.5 ± 2.7
	(6.2-12.5)	(54.4-1771.0)	(5.5-15.7)	(7.6-15.0)	(113.7-929.1)	(10.1-14.9)	(5.1-10.7)	(6.6-66.0)	(6.8-14.1)
Low	9.2 ± 3.4	661.5 ± 791.8	9.3 ± 2.8	12.4 ± 4.3	296.6 ± 324.1	10.6 ± 0.6	7.6 ± 3.2	32.0 ± 29.5	10.0 ± 2.3
	(4.5-14.6)	(23.4-2009.2)	(5.3-13.1)	(7.4-19.6)	(13.4-847.5)	(9.7-11.7)	(2.9-13.3)	(1.8-104.3)	(7.2-13.7)

Table 2. Coefficients and standard errors of the best fit logistic linear model applied to ECM colonization levels for *Larix kaempferi* on Mount Koma ($n = 90$). SE = standard error. See also Fig. 1 for the average ECM colonization percentages in each microhabitat at each elevational zone.

Variable		Coefficient	SE
Microhabitat†	Bareground (BA)	(0)	-
	<i>Salix reinii</i> patch (SP)	-0.398*	0.025
	<i>Larix kaempferi</i> understory (LU)	0.317*	0.039
Elevational zone†	High elevational zone (H)	(0)	-
	Middle elevational zone (M)	-0.453*	0.030
	Low elevational zone (L)	-0.691*	0.028
Seedling age		-0.011 ^{NS}	0.009
Intercept		0.996*	0.068
AIC		1174.1	

†: Coefficients on microhabitats and elevational zones are calculated with respect to bareground and to high elevational zone, respectively. * = Significantly different at $p < 0.01$, NS; not significant.

Table 3. Coefficients of the best fit negative binomial generalized linear models applied to mass of current-year shoots and leaves for *Larix kaempferi* seedlings on Mount Koma. BA = bareground, SP = *Salix reinii* patch, LU = *Larix kaempferi* understory. H = high elevational zone, M = middle, L = low.

Variables		Model I		Model II	
		Coefficients	SE	Coefficients	SE
Microhabitat (<i>Mh</i>)†	BA+SP	(0)	-	(0)	-
	LU	-0.757***	0.137	-0.814***	0.124
Elevational zone (<i>El</i>)†	H+L	(0)	-	(0)	-
	M	0.274**	0.102	0.291***	0.101
Total previous-year shoot length (<i>Lp</i>)		0.611***	0.076	0.604***	0.076
Total number of lattice points counted with ECM (<i>Pe</i>)		0.227*	0.103	-	-
Total number of lattice points counted without ECM (<i>Pn</i>)		0.271*	0.115	-	-
Total number of lattice points counted (<i>Pt</i>)		-	-	0.498**	0.076
Intercept (β_0)		6.549***	0.397	6.181***	0.458
AIC		1805.2		1801.8	

†: Coefficients on microhabitat LU and elevational zone M were calculated to BA+SP and H+L, respectively. Codes of significance: ***; $p < 0.001$, **; $p < 0.01$, *; $p < 0.05$, NS; not significant.

Figure captions

Fig. 1. Difference in ECM colonization percentages ($= Pe / Pt \times 100$) for *Larix kaempferi* between microhabitats and between elevational zones on Mount Koma. Means are shown with standard deviations. BA = bareground, SP = *Salix reinii* patch, LU = *Larix kaempferi* understory.

Fig. 2. Differences in edaphic factors between microhabitats and between elevational zones on Mount Koma. Different capital and small letters indicate significant differences between elevational zones ($p < 0.05$) and between microhabitats, respectively. Interactions between microhabitats and elevational zones are not significant in all the edaphic factors. Upper margin of each box represents 75th percentile; lower margin represents 25th percentile. Line in the middle of box represents the median. Circles indicate outliers. Abbreviations of microhabitats, see Fig. 1.

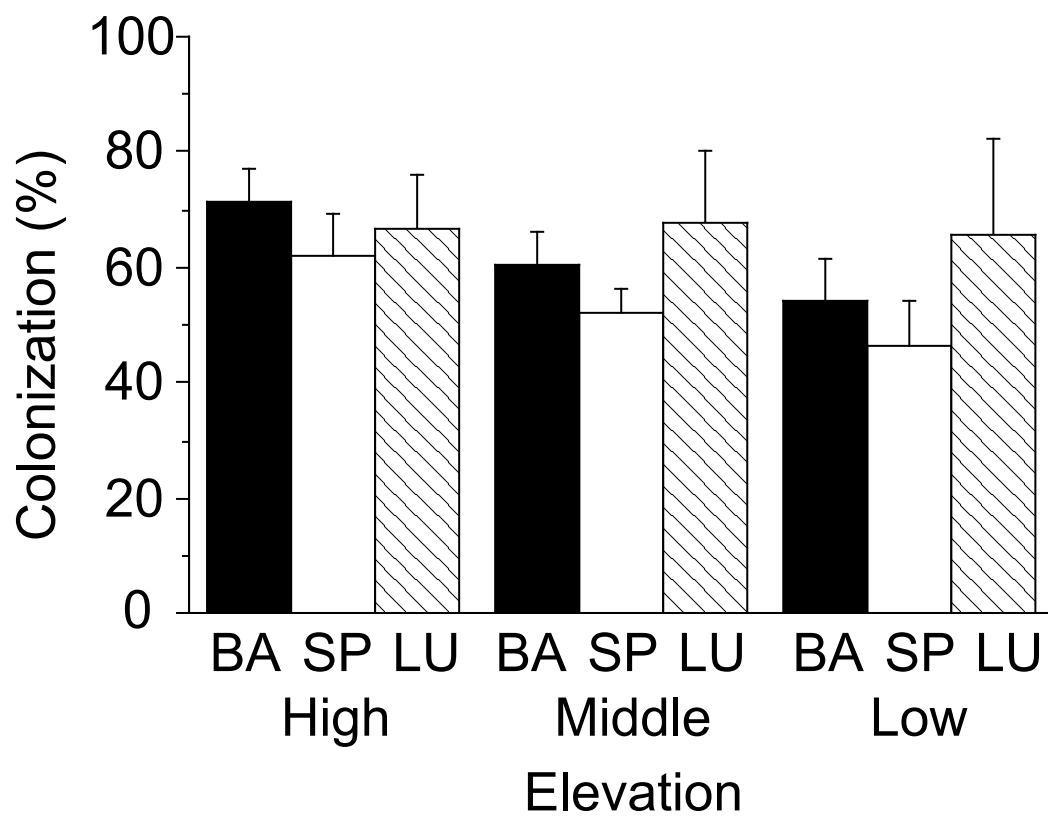


Fig. 1 (Akasaka et al.)

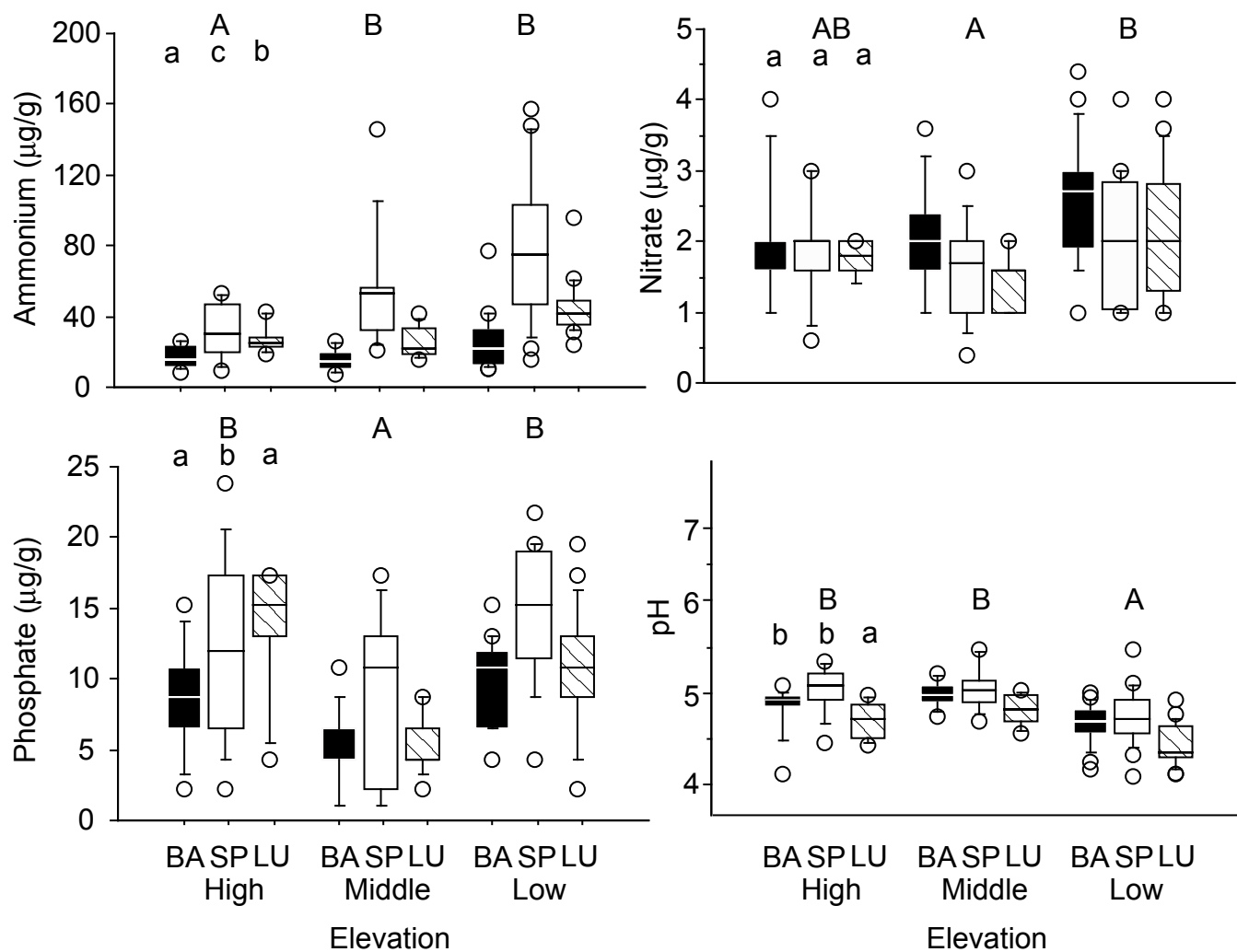


Fig. 2 (Akasaka et al.)