



Nitrogen acquisition, net production and allometry of *Alnus fruticosa* at a young moraine in Koryto Glacier Valley, Kamchatka, Russian Far East

Koichi Takahashi^{1,2} · Kosuke Homma³ · Jiri Dorezal⁴ · Kotaro Yamagata⁵ · Valentina P. Vetrova⁶ · Toshihiko Hara⁷

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Abstract

Alders (*Alnus* spp.) often dominate at nutrient-poor sites by symbiotic relations with atmospheric nitrogen-fixing bacteria. However, little is known about quantitative relationships between root nodule as a nitrogen acquisition organ and leaf as a carbon acquisition organ. To examine carbon allocation, nitrogen acquisition and net production in nutrient-poor conditions, we examined allocation patterns among organs of shrub *Alnus fruticosa* at a young 80-year-old moraine in Kamchatka. Slopes of double-log allometric equations were significantly smaller than 1.0 for the root mass, leaf mass and root nodule mass against stem mass, and for the root nodule mass against root mass, indicating that smaller individuals invested disproportionately more biomass into resource-acquiring leaf and root tissues than to supportive tissues compared to older individuals. The slope of allometric equation of root depth against stem height was 0.542, indicating that smaller/younger individuals allocate disproportionately more biomass into root length growth than stem height growth. On the contrary, the root nodule mass isometrically scaled to leaf mass. The whole-plant nitrogen content also isometrically scaled to root nodule mass, indicating that a certain ratio of nitrogen acquisition depended on root nodules, irrespective of plant size. Although the net production per plant increased with the increase in stem mass, the slope of the double-log regression was smaller than 1.0. On the contrary, the net production per plant isometrically increased with leaf mass, root nodule mass and leaf nitrogen content per plant. Since the leaf mass isometrically scaled to root nodule mass, growth of each individual occurred at the leaves and root nodules in a coordinated manner. It is suggested that their isometric increase contributes to the increase in net production per plant for *A. fruticosa* in nutrient-poor conditions.

Keywords Allometry · Deglaciation · Growth · Isometry · Primary succession · Root nodule

✉ Koichi Takahashi
koichit@shinshu-u.ac.jp

¹ Department of Biology, Faculty of Science, Shinshu University, Asahi 3-1-1, Matsumoto 390-8621, Japan

² Institute of Mountain Science, Shinshu University, Asahi 3-1-1, Matsumoto 390-8621, Japan

³ Faculty of Agriculture, Field Center for Sustainable Agriculture and Forestry, Niigata University, 94-2 Koba, Sado, Niigata 952-2206, Japan

⁴ Section of Plant Ecology, Institute of Botany, Czech Academy of Sciences, Dukelská 135, 379 82 Třeboň, Czech Republic

⁵ Division of Social Studies, Joetsu University of Education, Joetsu 943-8512, Japan

⁶ Laboratory of Plant Ecology, Kamchatka Branch of Pacific Institute of Geography, Far Eastern Branch of Russian Academy of Sciences, Petropavlovsk-Kamchatsky 683024, Russia

⁷ Institute of Low Temperature Science, Hokkaido University, Sapporo 060-0819, Japan

Introduction

Nutrient-poor soils represent one of the major limitations of primary succession of plants after deglaciation and volcanic eruption (Chapin et al. 1994; Chiba and Hirose 1993; Masuzawa 1985). Of limiting nutrients, nitrogen is the most important element for plant growth because photosynthetic enzymes are nitrogen compounds and therefore photosynthetic capacity positively correlates with nitrogen concentration in both soils and leaves (Chapin 1980; Evans 1989; Hirose and Werger 1987; Reich et al. 1991). To cope with nitrogen deficiency, plants have evolved symbiotic relations with atmospheric nitrogen-fixing bacteria, which effectively increase nitrogen acquisition under nutrient-poor conditions (Huss-Danell 1997; Tjepkema et al. 1986). For instance, alders (*Alnus* spp.) often dominate at early stages of primary succession after deglaciation because of its symbiotic relations with nitrogen-fixing bacteria *Frankia* spp. (Bormann and Sidle 1990; Chapin et al. 1994; Dawson 2008; Jones and del Moral 2005). *Frankia* spp. live in root nodules of actinorhizal plants (Valdés 2008; Wall and Berry 2008; Weber et al. 1987), thereby contributing to nitrogen acquisition, which in turn enables early-successional shrub species such as alder to colonize early stages of primary succession. Although alders are replaced by late-successional species with the progress of succession because alders are shade-intolerant species (Kikuzawa 1983, 1984), carbon and nitrogen accumulations in soil increase with the development of alder forests (Chapin et al. 1994). Therefore, the growth of alders is important for the ecosystem function during primary succession.

Plants provide photosynthetic carbon compounds for symbiotic relationships with nitrogen-fixing bacteria. High carbon allocation to roots and their symbiotic relations increases the acquisition of soil water and nutrients (Markesteijn and Poorter 2009). Although much carbon support for nitrogen-fixing bacteria can be advantageous for nitrogen acquisition, it simultaneously decreases plant growth because plants allocate much photosynthate to root nodules at the expense of growth. For example, Mahon (1977) showed that 17 g of carbohydrate is consumed per gram of nitrogen fixing in *Pisum sativum*. The cost of nitrogen fixation by symbiotic and autotrophic nitrogen fixers is high relative to that of absorbing ammonium or nitrate (Chapin et al. 2011; Minchin and Witty 2005; Schulze et al. 1999). Furthermore, less allocation to leaves would decrease the net production per plant, which in turn decreases the subsequent plant growth (Takahashi et al. 2001). Thus, it is expected that there is an ideal carbon allocation to root nodules and leaves for plants to increase nitrogen acquisition and net production, simultaneously. However, there have been no studies examining

quantitative characteristics of root nodules in relation to nitrogen acquisition and net production.

Allometric relationships between two organs of plants can be used to quantify biomass allocation patterns (Kohyama 1987; Kohyama and Hotta 1990; Takahashi and Obata 2014; Takahashi et al. 2001). The allocation pattern between two organs can be derived from the slope of the double-log linear regression. If a plant organ increases with the same rate of increase of another organ, the slope of the double-log allometric equation between them should be 1.0, i.e., isometric scaling relation. For example, Takahashi and Kohyama (1997) examined the crown development of two small palm species (*Licuala* spp.) in a tropical rain forest. The double-log regression slope of lamina mass against petiole mass was smaller than 1.0, indicating that larger palm individuals invested more in petiole than in leaf lamina to avoid self-shading within a crown. Therefore, the allometric analysis provides useful information for patterns of carbon allocation among plant organs. Tobita et al. (2010) showed that root nodule mass per plant exponentially increased with stem diameter in *Alnus hirsuta* var. *sibirica*. However, there have been no allometric studies that examined quantitative relationships among leaf mass, root nodule mass, whole-plant nitrogen and net production per plant.

The amount of nitrogen-fixing bacteria is predicted to be proportional to the root nodule volume or mass (Mylona et al. 1995; Pawlowski and Bisseling 1996). Tobita et al. (2013) showed that the rate of nitrogen fixation per root nodule mass did not correlate with plant size in *A. hirsuta* var. *sibirica*, indicating that the amount of nitrogen acquisition per plant by nitrogen-fixing bacteria is determined by the amount of nitrogen-fixing bacteria irrespective of plant size. Hooker and Wheeler (1987) also observed a similar pattern in other *Alnus* species. If leaves and nitrogen-fixing bacteria are related as carbon and nitrogen acquisition organs respectively, it is expected that leaf mass, root nodule mass and whole-plant nitrogen will scale isometrically.

Alnus fruticosa Rupr. is a typical early-successional shrub species at young moraines in Kamchatka, Russian Far East (Dolezal et al. 2008). The purpose of this study was to clarify how *A. fruticosa* increases the net production per plant at a young moraine under nutrient-poor conditions. We investigated the pattern of allocation between plant organs including a relationship between whole-plant nitrogen and root nodule mass to reveal whether leaf mass, root nodule mass, whole-plant nitrogen and net production increase isometrically.

Materials and methods

Study site

This study was carried out at the Koryto Glacier Valley (300 m above sea level, 54°45'N, 161°40'E), the Kronotsky

Peninsula, Kamchatka, Russian Far East (Figs. 1, 2). The Kronotsky Peninsula is located on the east coast of Kamchatka as a prominent massif protruding into the Pacific Ocean. The elevation of the Kronotsky Peninsula seldom exceeds 1000 m above sea level, and summit parts alone reach to an elevation as high as 1300 m a.s.l. There are 32 glaciers in this region (Vinogradov 1968), in most of which glacial landforms developed U-shaped valleys, terminal and lateral moraines and draining rivers (Fig. 2). The Koryto Glacier is located in the uppermost part of the Bol'haya Chazhma river, extending from 320 m to 1200 m a.s.l. toward northwest exposure with the total glaciated area of 8.2 km² (Sawaguchi et al. 1999). Mean monthly temperatures of the coldest month (January) and the hottest month (August) at the study site (300 m a.s.l.) were -9.6 and 9.8 °C, respectively, during the 1939–1999 period. Mean annual temperature was -0.3 °C. These data were derived from the nearest weather station Stopozh at the Pacific coast ($54^{\circ}35'01''\text{N}$, $161^{\circ}11'10''\text{E}$) about 50 km from the study site, using the standard lapse rate of -0.6 °C per 100 m of elevation. The study area receives 2.5–3 m winter snowfall and 0.4 m summer rainfall. Snowpack at accumulation zones is 5–10 m deep (Dolezal et al. 2008). The growing season for plants lasted from July to September, because the mean monthly temperatures exceed 5 °C, the effective temperature for plant growth (Kira 1948), during this period.

During the Little Ice Age, the Koryto Glacier extended about 1200 m from the present glacier terminus (Sawaguchi et al. 1999). Several moraines with different ages were formed after its retreat (Fig. 3). This study was conducted in 1999 at a young moraine that was formed ca. 80 years ago

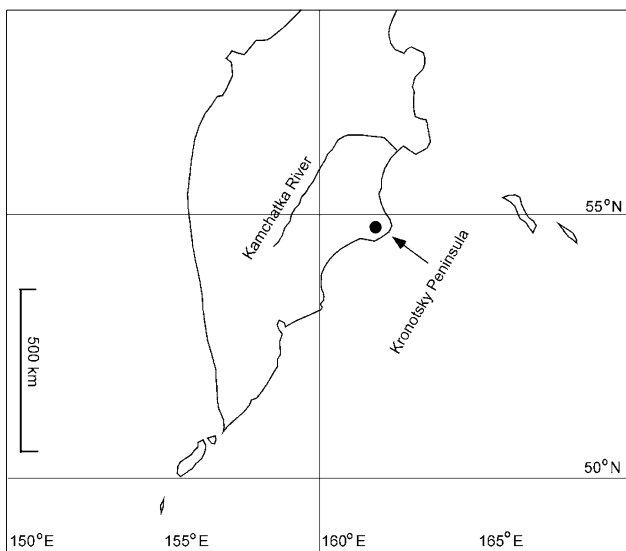


Fig. 1 Location of the Koryto Glacier (filled circle) in the Kronotsky Peninsula, Kamchatka, Russian Far East

(Fig. 3). *Alnus fruticosa* invades new moraines only a few decades after the moraine formation, and remains a dominant species at the oldest moraine (270 years old). *Betula ermanii* is the dominant species on surrounding slopes, but deep snow accumulation prevents the growth of *B. ermanii* at moraines in the valley bottom (Dolezal et al. 2008). The soil thickness was only 4 cm at the moraine examined in this study. Total nitrogen concentrations in soil were 0.014 and 0.072% in the A horizon (0–2 cm depth) and the B horizon (2–4 cm depth), respectively, and total carbon concentrations were 0.098 and 0.72%, respectively. The gravel layer was recognized under the soil layer (Sawaguchi et al. 1999). The details of glacier and vegetation dynamics can be found in several papers (Dolezal et al. 2008, 2013, 2014; Kodama et al. 1996; Muravyev et al. 1999; Sawaguchi et al. 1999; Shiraiwa et al. 1997; Yamagata et al. 1999).

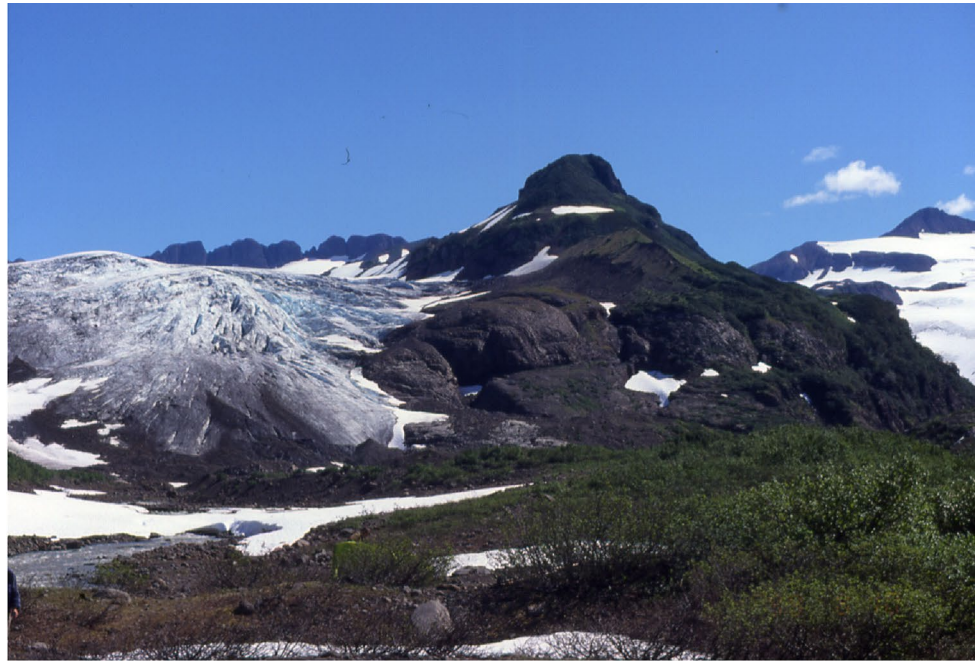
Sampling and measurements

We sampled 27 individuals of shrub *Alnus fruticosa* (shorter than 70 cm in stem height) at the 80-year-old terminal moraine from 15 to 17 August 1999. Although the sampling was done in mid-August, the current-year growth was completed because shoot elongation of *Alnus* spp. ends during the middle of the growing season (Kikuzawa 1983, 1984) and because the tree-ring width of the current-year was greater than that of the previous year in our *Alnus* samples (data not shown).

Vegetation at this moraine was dominated by *Salix arctica* and *A. fruticosa*, and was transition from the initial pioneer stages to *Alnus* dominated sands (Dolezal et al. 2008). Soil-nutrient conditions are more fertile in older moraines, so the maximum plant size of *A. fruticosa* is large in old moraines (Dolezal et al. 2008). *A. fruticosa* grows up to ca. 12 cm in trunk diameter and 2.5 m in trunk height (Dirksen and Dirksen 2007; Dolezal et al. 2008). However, we did not sample large *A. fruticosa* individuals at the other old moraines in this valley. The purpose of this study was to reveal quantitative relationships between leaf mass, net production and plant nitrogen at a nutrient-poor moraine. If we sampled *A. fruticosa* individuals from various moraines with different soil-nutrient conditions, it would cause variations of their quantitative relationships. Therefore, we sampled *A. fruticosa* individuals only from a young moraine. But, our dataset included the full size range of *A. fruticosa* at the moraine, and enabled us to analyze quantitative relationships between leaf mass, net production and plant nitrogen at the nutrient-poor moraine.

As for roots, we carefully dug up individuals with a small shovel. *A. fruticosa* individuals were sparsely distributed at this moraine, and all sampled individuals were open-grown individuals, i.e., not shaded by other individuals. All sampled individuals were measured for stem height, root depth,

Fig. 2 Photos of (top) the Koryto Glacier and (bottom) the U-shaped valley in mid-August, 1999



horizontal crown- and root-projection areas. The latter were estimated as an ellipse from two perpendicular diameters including that of the maximum. Sampled individuals were divided into four organs, i.e., stem, root, leaf and root nodule. *A. fruticosa* forms large multi-lobed root nodule galls, which can be easily detached from roots.

All samples (leaf, root, stem and root nodule) of each individual were brought to the laboratory in Japan, and were weighed after oven-drying at 80 °C for at least 2 days, and

were ground into powder. Their total nitrogen concentrations were determined using a C–N analyzer (Vario EL, Elementar Analysensysteme GmbH, Hanau, Germany). The nitrogen content of each organ of each individual was obtained by the nitrogen concentration multiplied by its mass. Then, the whole-plant nitrogen content was obtained by the sum of nitrogen contents of four organs, i.e., stem, root, leaf and root nodule.

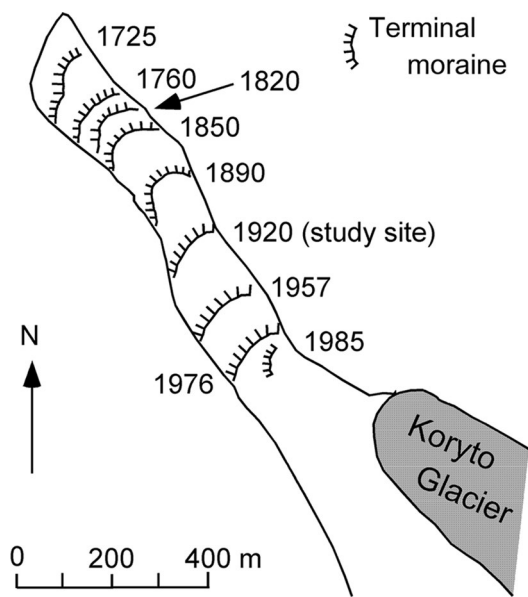


Fig. 3 Distribution of terminal moraines with the formation years at the Koryto Glacier Valley in 2000, modified from Dolezal et al. (2008) and Yamaguchi et al. (2008). This study was conducted at the moraine that was formed in 1920

Estimation of net production

For age dating and radial growth measurement, the cut surface of a main stem at ground level was sanded to count the annual tree-rings and to measure tree-ring width. We measured distances from the pith to the tree ring of the current year (R_{99} , mm) and to the tree-ring of the previous year (R_{98} , mm) using a stereoscopic microscope. The relative growth rate (RGR, year⁻¹) of stem radius was calculated by Eq. (1):

$$RGR = \ln R_{99} - \ln R_{98} \tag{1}$$

where ln is natural logarithm. We assumed that RGR of stem diameter is equal to RGR of stem radius. We estimated the stem diameter of the previous year (D_{98} , mm) by substituting D_{98} and D_{99} (stem diameter of the current year, mm) for R_{98} and R_{99} , respectively, in Eq. (1) as:

$$D_{98} = \exp(\ln D_{99} - RGR) \tag{2}$$

Net production (NP, g year⁻¹) per plant is the current-year increase in dry mass, i.e., total dry mass of leaves (M_L , g) and non-photosynthetic organs (stem, root and root nodule) produced in the current year. Total dry mass of the non-photosynthetic organs produced in the current year was estimated by subtracting the dry mass of the non-photosynthetic organs of the previous year (M_{SRN98} , g) from that of the current year (M_{SRN99} , g). We obtained the following allometric equation of M_{SRN99} against D_{99} :

Table 1 Double-log allometric equations ($\log Y = a \log X + b$, where log is common logarithm) of reduced major axis regression for various dimensions in *Alnus fruticosa* at a young moraine in the Koryto Glacier Valley, Kamchatka, Russia

Y	X	a	b	R ²	n
D_R	H_S	0.542 (0.413–0.713)	0.680	0.571	26
A_R	A_C	1.160 (1.000–1.350)	-0.480	0.868	26
M_R	M_S	0.869 (0.834–0.905)	-0.035	0.991	26
M_L	M_S	0.809 (0.752–0.870)	-0.034	0.968	27
M_N	M_S	0.792 (0.716–0.875)	-0.543	0.946	25
M_N	M_R	0.901 (0.813–0.997)	-0.491	0.947	24
M_N	M_L	0.987 (0.874–1.114)	-0.509	0.920	25
N_P	M_N	0.989 (0.855–1.144)	-0.644	0.924	18
N_L	M_N	1.030 (0.920–1.150)	-0.928	0.932	25
NP	M_S	0.850 (0.820–0.882)	0.470	0.993	24
NP	M_L	1.045 (0.980–1.114)	0.513	0.979	24
NP	M_N	1.114 (1.006–1.234)	1.045	0.952	22
NP	N_L	1.031 (0.987–1.076)	2.015	0.991	24

Values in parentheses indicate 95% confidence intervals of slope *a*. All regression equations were significant at $P < 0.001$

H_S stem height (cm), D_R root depth (cm), A_C crown-projection area (cm²), A_R root-projection area (cm²), M_L leaf mass (g), M_S stem mass (g), M_R root mass (g), M_N root nodule mass (g), N_P whole-plant nitrogen (g), N_L leaf nitrogen per plant (g), NP net production per plant (g year⁻¹)

$$\log M_{SRN99} = 2.602 \log D_{99} - 1.593 \tag{3}$$

$(R^2 = 0.981, P < 0.001, n = 26)$

where log is common logarithm (base 10). Then, we calculated M_{SRN98} by substituting D_{98} for D_{99} . NP per plant in the current year (1999) was calculated by Eq. (4):

$$NP = M_L + M_{SRN99} - M_{SRN98} \tag{4}$$

Allometric analysis

Warton et al. (2006) recommended to use the least square (LS) regression and the reduced major axis (RMA) regression to predict a *Y* value at a given *X* value and to evaluate a slope of a bivariate relationship between *X* and *Y*, respectively. Therefore, this study used the LS and RMA regressions for Eq. (3) and the other allometric relationships listed in Table 1, respectively. Whether two variables show isometric scaling or not was determined by the 95% confidence interval (CI) of the slope of the RMA regression equation on the logarithmic scale. If the 95% CI of the slope includes 1.0, the bivariate relation is isometrically scaled. The present study analyzed allometry using the program (S)MATR, version 2 (Warton et al. 2006).

Results

Stem height and diameter of *Alnus fruticosa* increased with tree age (Fig. 4a, b). Stem height attained about 40 cm by 10 years after germination. The slope of the double-log allometric equation of root depth against stem height was 0.542 (95% CI 0.413–0.713) (Fig. 5a; Table 1), indicating that smaller/younger plants allocate disproportionately more biomass into root length growth than stem height growth. The regression slope for the root-projection area against crown-projection area included 1.0 (95% CI 1.00–1.35) (Fig. 5b; Table 1), indicating that the root-projection area isometrically increased with the crown-projection area.

Slopes of double-log allometric equations were significantly smaller than 1.0 for the allometry of root mass, leaf mass and root nodule mass against stem mass and for the allometry of root nodule mass against root mass, i.e., 95% CI of these slopes did not include 1.0 (Fig. 6a–d; Table 1), indicating that smaller plants invested disproportionately more biomass into resource-acquiring leaf and root tissues than to supportive tissues. On the contrary, root nodule

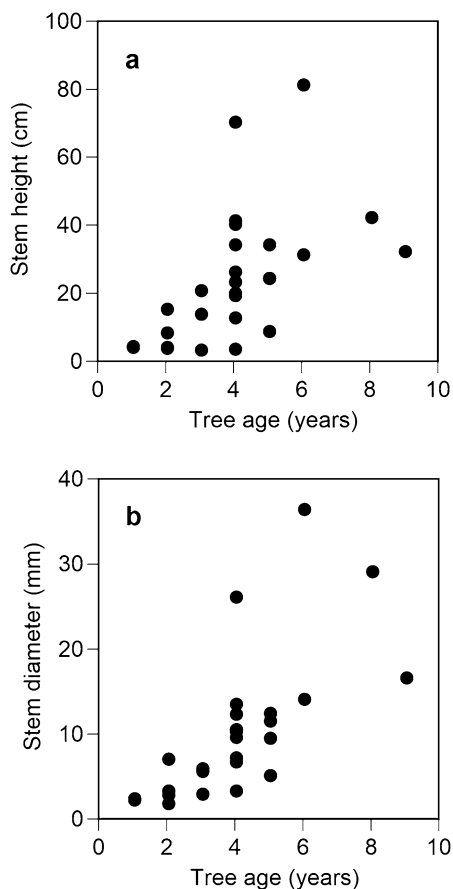


Fig. 4 Relationships of **a** stem height and **b** stem diameter to tree age of *Alnus fruticosa*

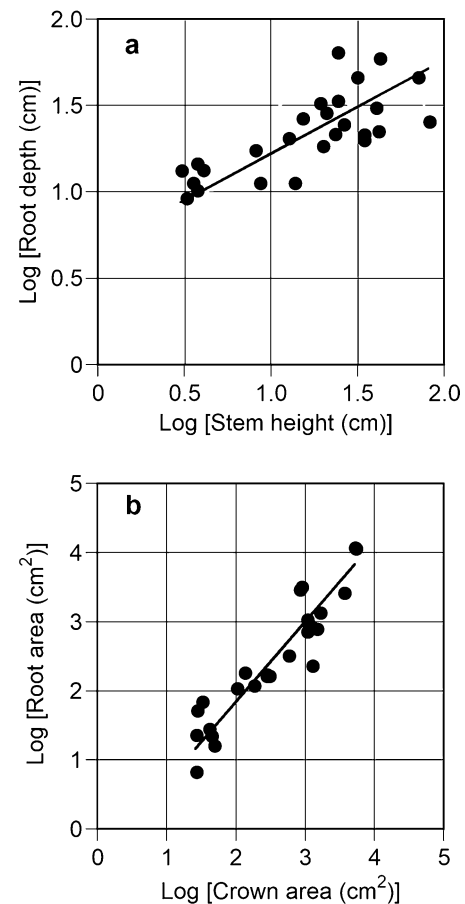
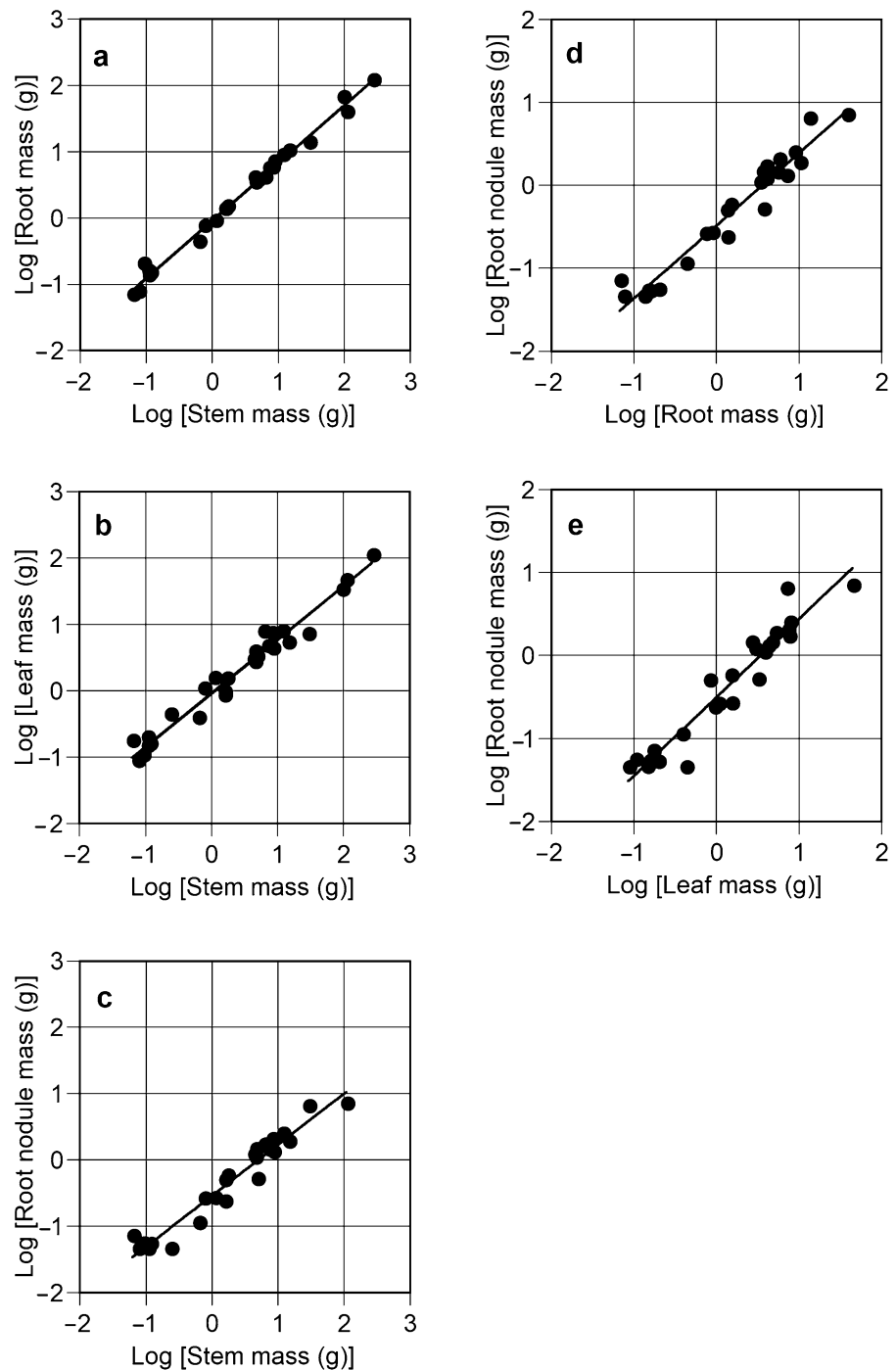


Fig. 5 Allometric relationships **a** between root depth and stem height and **b** between root-projection area and crown-projection area of *Alnus fruticosa*. Allometric equations are listed in Table 1

mass increased proportionally with leaf mass on the isometric scaling, i.e., 95% CI (0.874–1.114) of the slope of the allometric equation included 1.0 (Fig. 6e; Table 1).

Whole-plant nitrogen and leaf nitrogen per plant increased isometrically with root nodule mass (Fig. 7a, b; Table 1), i.e., 95% CI of these slopes of allometric equations included 1.0 (0.855–1.144 and 0.92–1.15, respectively). Net production per plant increased with stem mass, and the slope of the double-log allometric equation was significantly smaller than 1.0 (95% CI 0.820–0.882, Fig. 8a; Table 1), indicating that net production per plant was smaller relative to the stem mass in larger *A. fruticosa*. On the contrary, net production per plant isometrically increased with leaf mass and leaf nitrogen per plant, i.e., 95% CI of slopes of double-log allometric equations included 1.0 (Fig. 8b; Table 1). Although 95% CI of the slope of the allometry between the net production per plant and root nodule mass did not include 1.0, the lower limit (1.006) of the 95% CI was almost equal to 1.0, indicating isometric scaling.

Fig. 6 Allometric relationships of **a** root mass, **b** leaf mass and **c** root nodule mass to stem mass, **d** root nodule mass to root mass and **e** root nodule mass to leaf mass of *Alnus fruticosa*. Allometric equations are listed in Table 1



Discussion

It has been often observed that soil nutrients are scarce at young moraines and increase with vegetation development (Bormann and Sidle 1990; Uri et al. 2014). This is also true in the Koryto Glacier Valley. Nitrogen and carbon concentrations in the soil were considerably low at the young moraine examined in this study (about 80 years after the formation), and these concentrations were greater at older

moraines (Dolezal et al. 2008). The nitrogen concentration at the moraine examined in this study was almost equal to that at the alpine zone with scarce vegetation in Japan (Masuzawa 1985; Singh et al. 2012). *Alnus* spp. dominate at young moraines with nutrient-poor soil conditions in other sites (Chapin et al. 1994; Jones and del Moral 2005). Therefore, there is no doubt that *A. fruticosa* gained dominance at the studied moraine by acquiring much of the nitrogen necessary for growth from the atmosphere rather than from the soil.

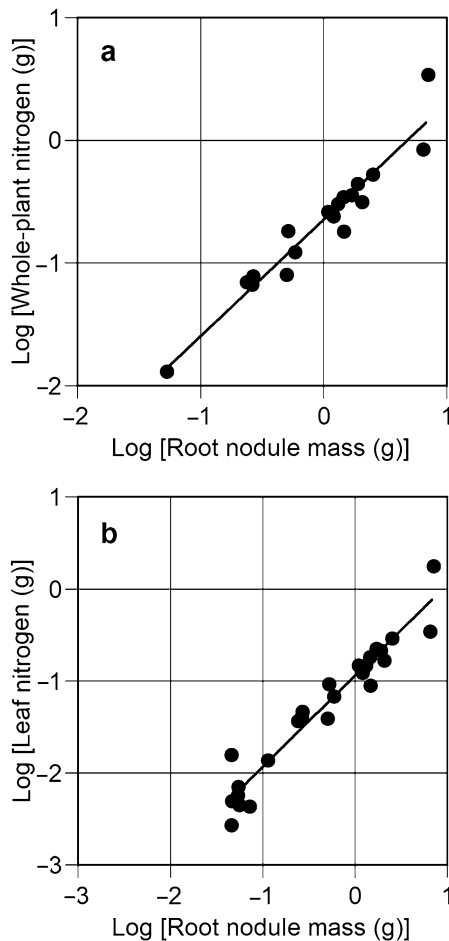


Fig. 7 Allometric relationships of **a** whole-plant nitrogen and **b** leaf nitrogen per plant to root nodule mass of *Alnus fruticosa*. Allometric equations are listed in Table 1

This study showed smaller plants invest disproportionately more biomass into resource-acquiring leaf and root tissues than to supportive stem tissue. Also, smaller/younger *A. fruticosa* individuals have disproportionately more root nodules per root biomass than larger individuals. These allocation patterns of *A. fruticosa* contribute to anchoring small individuals and to getting enough carbon and soil nutrients in unstable nutrient-poor soil conditions.

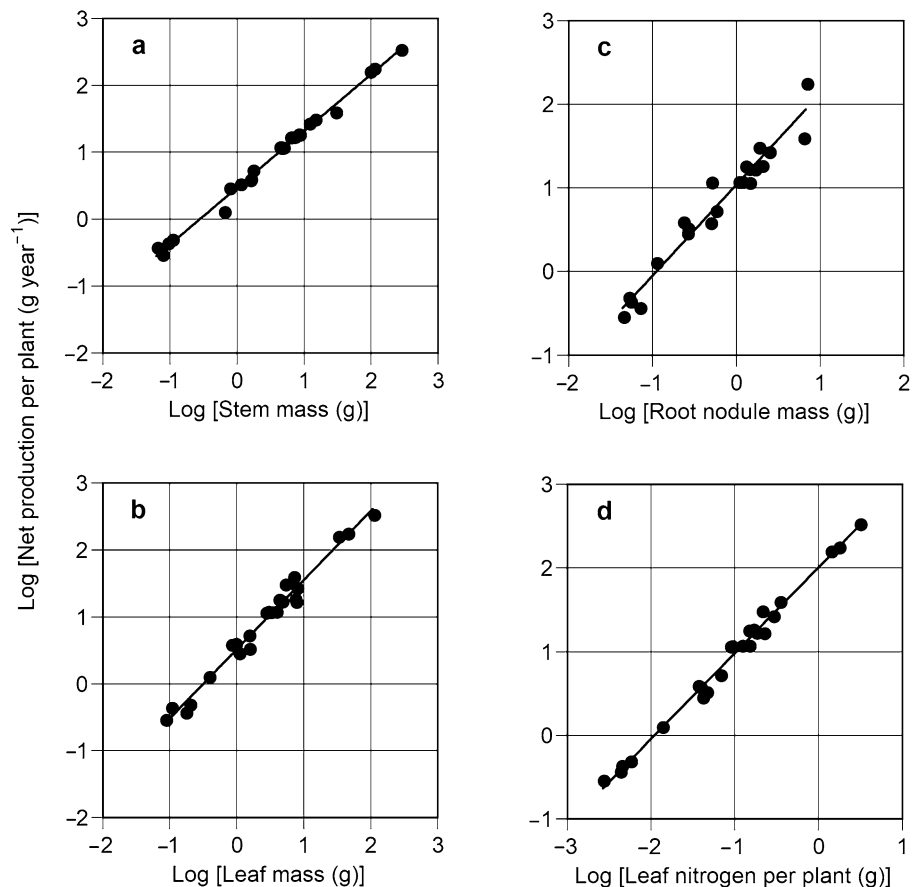
Tobita et al. (2010) showed that the root nodule mass (M_N , g) was proportional to the square of trunk diameter (DBH, cm) for *Alnus hirsuta* var. *sibirica* ($M_N = 0.442 \text{ DBH}^{2.01}$). In the present study, the logarithmic allometric equation of root nodule mass against stem diameter (D , cm) was expressed as $M_N = 1.004 D^{2.16}$ ($R^2 = 0.919$). The slope (2.16) of the allometric equation of the present study was almost equal to that (2.01) of Tobita et al. (2010) on the logarithmic graph, although the intercept (1.004) of the present study was greater than that (0.442) of Tobita et al. (2010). The rate of atmospheric nitrogen fixation per

root nodule mass did not correlate with plant size (Tobita et al. 2013), indicating that the rate of atmospheric nitrogen fixation per plant was determined mainly by the root nodule mass per plant. Johnsen and Bongarten (1991) showed that the nitrogen fertilization treatment decreased root nodule mass and rates of atmospheric nitrogen fixation. Berninger et al. (2000) also showed, using a model, that the proportion of atmospheric nitrogen acquisition by nitrogen-fixing bacteria to the total nitrogen acquisition per plant decreased when the nitrogen absorption from the soil was large. The alder stand of Tobita et al. (2010) was established after artificial disturbances by road construction in the subalpine zone (1100 m a.s.l.) in Japan. It seems that the study site of Tobita et al. (2010) was not extremely nutrient-poor conditions because vegetation originally developed there before artificial disturbances. Therefore, it is thought that *A. fruticosa* is more dependent on nitrogen-fixing bacteria for nitrogen acquisition in the present study because of considerable nutrient-poor conditions in the soil, compared with the study site of Tobita et al. (2013).

Several researchers showed positive correlations between photosynthetic rates and rates of atmospheric nitrogen fixation by nitrogen-fixing bacteria (e.g., Lawn and Brun 1974). For example, it has been reported that the amount of nitrogen fixation by nitrogen-fixing bacteria decreased as photosynthetic rate decreased due to shading and defoliation treatments because plants could not supply carbohydrates necessary for nitrogen-fixing bacteria (Denison et al. 1992; Grove and Malajczuk 1987; Ruess et al. 2006; Vance et al. 1979). Williams et al. (1982) also showed that high photosynthetic rate per plant increased the rate of atmospheric nitrogen fixation per plant by increasing root nodule mass. These previous studies showed that the photosynthetic rate per plant, root nodule mass and nitrogen-fixing rate are closely related (Bormann and Gordon 1984; Gordon and Wheeler 1978). Therefore, it is necessary for plants to pay maintenance costs in order to maintain many nitrogen-fixing bacteria in root nodules (Chapin et al. 2011; Minchin and Witty 2005; Schulze et al. 1999), but keeping root nodules above the necessary level prevents the growth of plants. In the present study, the net production of *A. fruticosa* isometrically increased with leaf mass, root nodule mass and leaf nitrogen content per plant. Nitrogen is necessary to increase leaves, and root nodules are necessary to obtain nitrogen. Since the scaling between leaf mass and root nodule mass was isometric, it is suggested that they are mutually dependent and that the increase in both is important for increasing the net production of *A. fruticosa*.

This study showed that leaf mass, root nodule mass and total nitrogen content per plant were isometrically scaled with each other. This study is the first one to demonstrate the quantitative relationships of whole-plant nitrogen, root

Fig. 8 Allometric relationships of net production per plant to **a** stem mass, **b** leaf mass, **c** root nodule mass and **d** leaf nitrogen per plant of *Alnus fruticosa*. Allometric equations are listed in Table 1



nodule mass, net production per plant from an allometric point of view. Shade-intolerant alders dominate at early stages of primary succession, but are replaced by shade-tolerant late-successional species with the progress of succession, accompanied with the increase in soil nutrients and the decrease in light intensity on the forest floor (Bormann and Sidle 1990; Chapin et al. 1994; Jones and del Moral 2005). The symbiotic relationship with nitrogen-fixing bacteria is difficult for plants in dark conditions because photosynthetic production is not enough to support the nitrogen-fixing bacteria (Chapin et al. 2011). The change of the limiting resource from nitrogen to light causes the successional change of dominant plant species. Therefore, the examination of the carbon allocation, net production and nitrogen acquisition of alders along successional gradients would provide useful information to understand the role of alder invasion at early-successional stages in the ecosystem function from the viewpoint of carbon and nitrogen economy.

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References

- Berninger F, Nikinmaa E, Sievanen R, Nygren P (2000) Modelling of reserve carbohydrate dynamics, regrowth and nodulation in a N_2 -fixing tree managed by periodic prunings. *Plant Cell Environ* 23:1025–1040
- Bormann BT, Gordon JC (1984) Stand density effects in young red alder plantations: Productivity, photosynthate partitioning, and nitrogen fixation. *Ecology* 65:394–402
- Bormann BT, Sidle RC (1990) Changes in productivity and distribution of nutrients in chronosequence at Glacier Bay National Park, Alaska. *J Ecol* 78:561–578
- Chapin FS III (1980) The mineral nutrition of wild plants. *Ann Rev Ecol Syst* 11:233–260
- Chapin FS III, Walker LR, Fastie CL, Sharman LC (1994) Mechanisms of primary succession following deglaciation at Glacier Bay, Alaska. *Ecol Monogr* 64:149–175
- Chapin FS III, Matson PA, Vitousek PM (2011) Principles of terrestrial ecosystem ecology, 2nd edn. Springer, New York
- Chiba N, Hirose T (1993) Nitrogen acquisition and use in three perennials in the early stage of primary succession. *Funct Ecol* 7:287–292
- Dawson JO (2008) Ecology of actinorhizal plants. In: Pawlowski K, Newman WE (eds) Nitrogen-fixing actinorhizal symbioses. Springer, Dordrecht, pp 199–234
- Denison RF, Hunt S, Layzell DB (1992) Nitrogenase activity, nodule respiration, and O_2 permeability following detopping of alfalfa and birdsfoot trefoil. *Plant Physiol* 98:894–900

- Dirksen VG, Dirksen OV (2007) Vegetation dynamics and ecological consequences of the 1996 eruption in Karymsky volcanic center, Kamchatka. *J Volcanol Seismol* 1:164–174
- Dolezal J, Homma K, Takahashi K, Vyatkina MP, Yakubov V, Vetrova VP, Hara T (2008) Primary succession following deglaciation at Koryto Glacier valley, Kamchatka. *Arc Antarct Alp Res* 40:309–322
- Dolezal J, Yakubov V, Hara T (2013) Plant diversity changes and succession along resource availability and disturbance gradients in Kamchatka. *Plant Ecol* 214:477–488
- Dolezal J, Altman J, Vetrova VP, Hara T (2014) Linking two centuries of tree growth and glacier dynamics with climate changes in Kamchatka. *Clim Change* 124:207–220
- Evans JR (1989) Photosynthesis and nitrogen relationships in leaves of C_3 plants. *Oecologia* 78:9–19
- Gordon JC, Wheeler CT (1978) Whole plant studies on photosynthesis and acetylene reduction in *Alnus glutinosa*. *New Phytol* 80:179–186
- Grove TS, Malajczuk N (1987) Nitrogen fixation (acetylene reduction) by forest legumes: sensitivity to pre-harvest and assay conditions. *New Phytol* 106:115–127
- Hirose T, Werger MJA (1987) Nitrogen use efficiency in instantaneous and daily photosynthesis of leaves in the canopy of a *Solidago altissima* stand. *Physiol Plant* 70:215–222
- Hooker JE, Wheeler CT (1987) The effectivity of *Frankia* for nodulation and nitrogen fixation in *Alnus rubra* and *A. glutinosa*. *Physiol Plant* 70:333–341
- Huss-Danell K (1997) Tansley review No. 93 Actinorhizal symbioses and their N_2 fixation. *New Phytol* 136:375–405
- Johnsen KH, Bongarten BC (1991) Allometry of acetylene reduction and nodule growth of *Robinia pseudoacacia* families subjected to varied root zone nitrate concentrations. *Tree Physiol* 9:507–522
- Jones CC, del Moral R (2005) Patterns of primary succession on the foreland of Coleman Glacier, Washington. *Plant Ecol* 180:105–116
- Kikuzawa K (1983) Leaf survival of woody plants in deciduous broad-leaved forests. 1. Tall trees. *Can J Bot* 61:2133–2139
- Kikuzawa K (1984) Leaf survival of woody plants in deciduous broad-leaved forests. 2. Small trees and shrubs. *Can J Bot* 62:2551–2556
- Kira T (1948) On the altitudinal arrangement of climate zones in Japan. *Kanti Nogaku* 2:143–173 (in Japanese)
- Kodama Y, Shiraiwa T, Kobayashi D, Matsumoto T, Yamaguchi S, Muravyev YD, Glazirin GE (1996) Hydrometeorological and glaciological observations in the Koryto and Ushkovsky Glaciers, Kamchatka, 1996. *Low Temperature Sci. Hokkaido Univ Ser A* 55:107–136
- Kohyama T (1987) Significance of architecture and allometry in saplings. *Funct Ecol* 1:399–404
- Kohyama T, Hotta M (1990) Significance of allometry in tropical saplings. *Funct Ecol* 4:515–521
- Lawn RJ, Brun WA (1974) Symbiotic nitrogen fixation in soybeans. I. Effect of photosynthetic source-sink manipulations. *Crop Sci* 14:11–16
- Mahon JD (1977) Respiration and the energy requirement for nitrogen fixation in nodulated pea roots. *Plant Physiol* 60:817–821
- Marksteijn L, Poorter L (2009) Seedling root morphology and biomass allocation of 62 tropical tree species in relation to drought- and shade-tolerance. *J Ecol* 97:311–325
- Masuzawa T (1985) Ecological studies on the timberline of Mt. Fuji. I. Structure of plant community and soil development on the timberline. *Bot Mag Tokyo* 98:15–28
- Minchin FR, Witty JF (2005) Respiratory/carbon costs of symbiotic nitrogen fixation in legumes. In: Lambers H, Ribas-Carbo M (eds) *Plant respiration. Advances in photosynthesis and respiration*, vol 18. Springer, Dordrecht
- Muravyev YD, Shiraiwa T, Yamaguchi S, Matsumoto T, Nishimura K, Kohshima S, Ovsyannikov AA (1999) Mass balance of glacier in condition of maritime climate-Koryto Glacier in Kamchatka, Russia. *Cryospheric Stud Kamchatka II*:51–61
- Mylona P, Pawlowski K, Bisseling T (1995) Symbiotic nitrogen fixation. *Plant Cell* 7:869–885
- Pawlowski K, Bisseling T (1996) Rhizobial and actinorhizal symbioses: what are the shared features? *Plant Cell* 8:1899–1913
- Reich PB, Uhl C, Walters MB, Ellsworth DS (1991) Leaf lifespan as a determinant of leaf structure and function among 23 amazonian tree species. *Oecologia* 86:16–24
- Ruess RW, Anderson MD, Mitchell JS, McFarland JW (2006) Effects of defoliation on growth and N fixation in *Alnus tenuifolia*: Consequences for changing disturbance regimes at high latitudes. *Ecoscience* 13:404–412
- Sawaguchi S, Yamagata K, Muravyev YD, Solomina ON (1999) Holocene glacier advances in Koryto Glacier, Kamchatka, Russia. *Cryospheric Stud Kamchatka II*:79–84
- Schulze J, Adgo E, Merbach W (1999) Carbon costs associated with N_2 fixation in *Vicia faba* L. and *Pisum sativum* L. over a 14-day period. *Plant Biol* 1:625–631
- Shiraiwa T, Muravyev YD, Yamaguchi S, Glazirin GE, Kodama Y, Matsumoto T (1997) Glaciological features of Koryto Glacier in the Kronotsky Peninsula, Kamchatka, Russia. *Bull Glacier Res* 15:27–36
- Singh D, Takahashi K, Kim M, Chun J, Adams JM (2012) A hump-backed trend in bacterial diversity with elevation on Mount Fuji, Japan. *Microb Ecol* 63:429–437
- Takahashi K, Kohyama T (1997) Crown architecture of two understory palm species of the genus *Licuala* in a tropical rain forest. *Plant Species Biol* 12:35–41
- Takahashi K, Obata Y (2014) Growth, allometry and shade tolerance of understory saplings of four subalpine conifers in central Japan. *J Plant Res* 127:329–338
- Takahashi K, Seino T, Kohyama T (2001) Responses to canopy openings in architectural development of saplings in eight deciduous broad-leaved tree species. *Can J For Res* 31:1336–1347
- Tjepkema JD, Schwintzer CR, Benson DR (1986) Physiology of actinorhizal nodules. *Ann Rev Plant Physiol Plant Mol Biol* 37:209–232
- Tobita H, Hasegawa SF, Tian X, Nanami S, Takeda H (2010) Spatial distribution and biomass of root nodules in a naturally regenerated stand of *Alnus hirsuta* (Turcz.) var. *sibirica*. *Symbiosis* 50:77–86
- Tobita H, Hasegawa SF, Yazaki K, Komatsu M, Kitao M (2013) Growth and N_2 fixation in an *Alnus hirsuta* (Turcz.) var. *sibirica* stand in Japan. *J Bioscience* 38:761–776
- Uri V, Aosaar J, Varik M, Becker H, Ligi K, Padari A, Kanal A, Lõhmus K (2014) The dynamics of biomass production, carbon and nitrogen accumulation in grey alder (*Alnus incana* (L.) Moench) chronosequence stands in Estonia. *For Ecol Manage* 327:106–117
- Valdés M (2008) *Frankia* ecology. In: Pawlowski K, Newman WE (eds) *Nitrogen-fixing actinorhizal symbioses*. Springer, Dordrecht, pp 49–71
- Vance CP, Heichel GH, Barnes DK, Bryan JW, Johnson LE (1979) Nitrogen fixation, nodule development, and vegetative regrowth of alfalfa (*Medicago sativa* L.) following harvest. *Plant Physiol* 64:1–8
- Vinogradov VN (1968) *Katalog Lednikov SSSR, t.20. Kamchatka, ch.2–4* (Catalog of the glaciers of the USSR, vol 20, Kamchatka, Pt. 2–4), Gidrometeoizdat, Leningrad (in Russian)
- Wall LG, Berry AM (2008) Early interactions, infection and nodulation in actinorhizal symbiosis. In: Pawlowski K, Newman WE (eds) *Nitrogen-fixing actinorhizal symbioses*. Springer, Dordrecht, pp 147–166
- Warton DI, Wright IJ, Falster DS, Westoby M (2006) Bivariate line fitting methods for allometry. *Biol Rev* 33:1–33

- Weber A, Nurmiäho-Lassila EL, Sundman V (1987) Features of the intrageneric *Alnus-Frankia* specificity. *Physiol Plant* 70:289–296
- Williams LE, DeJong TM, Phillips DA (1982) Effect of changes in shoot carbon-exchange rate on soybean root nodule activity. *Plant Physiol* 69:432–436
- Yamagata K, Sawaguchi S, Muravyev YD, Solomina ON (1999) Soil development in relation to vegetation and topography at the Koryto Glacier basin, Kamchatka. *Cryospheric Stud Kamchatka* II:85–92
- Yamaguchi S, Naruse R, Shiraiwa T (2008) Climate reconstruction since the Little Ice Age by modelling Koryto glacier, Kamchatka Peninsula, Russia *J Glaciol* 54:125–130