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## Effects of Soil Temperature on the Contents of Nitrogen Compounds in Seedlings of *Larix gmelinii* Regenerated on Permafrost in Central Siberia

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### Abstract

Seasonal changes in nitrogen compounds in vegetative parts of *Larix gmelinii* (Gmelin larch) seedlings regenerated on a permafrost region in Central Siberia were analyzed. Two locations on slopes ("warm" site vs. "cold" site) with populations of larch seedlings of different ages that had regenerated after forest fires were selected as the study sites. The "warm" site included populations of seedlings that were 13, 24, 36, 48, and 60 months old. The seedlings at the "cold" site were all 13 months old. Heights of larch seedlings were ca. 8.1 to 26.3 cm at the "warm" site and 6.3 cm at the "cold" site. However, there was no marked difference between the patterns of allocation of nitrogen compounds in the shoots and roots of seedling in the populations of different ages. During the period of shoot growth, protein nitrogen was present in all parts of the larch seedling but was mostly allocated to the needles, while ammonium nitrogen and nitrogen of free amino acids were accumulated in the roots. At the end of the growing season, before dormancy, there was an accumulation of nitrogen compounds in the roots and stem. The contents of nitrogen compounds in various vegetative parts of larch seedlings at the two sites with different temperature conditions in the rhizosphere were compared. Response of larch seedlings to low temperature in the rhizosphere may be a trigger of change in allocation of nitrogen compounds in the seedlings. This allocation pattern of larch seedlings was exhibited in the accumulation of protein nitrogen and nitrogen of free amino acids, which may serve as an indicator of the plant's capacity to resist low temperature.

**Key words:** Larch (*Larix gmelinii*) seedlings, soil temperature, nitrogen allocation, forest fires, permafrost, Central Siberia

### Introduction

Forests growing in the boreal and subarctic region of continuous permafrost are thought to play an essential role in CO<sub>2</sub> accumulation and its storage function in global ecological cycles (Abaimov *et al.* 1997, 2000, Shvidenko and Nilsson 2000). The existence of Taiga may be regulated by various stress factors, such as water and oxygen deficiency, which are related to the melting and freezing pattern of permafrost, and forest fires (Abaimov *et al.* 2000, Alexeyev *et al.* 2000). In the region of permafrost, in fact, water relations between plants and the rhizosphere, especially at the time of bud sprouting, are thought to be critical factors for the existence of evergreen vs. deciduous leaf habit, e.g., spruce vs. larch (Berg and Chapin 1994). Another factor affecting the growth of woody plants *in situ* is low temperature of the rhizosphere, which is closely connected with the existence of permafrost (Pozdnyakov 1986, Prokushkin *et al.* 2002).

*Larix gmelinii* (Gmelin larch) is most dominant species in Central and Eastern Siberia, and its distribution almost coincides with the zone of permafrost (Dylis 1981, Abaimov and Milutin 1995, Schmidt 1995).

Recently, intensive ecophysiological study on this larch ecosystem in Sakha Republic, Eastern Siberia has revealed that nitrogen might regulate the growth and development of larch ecosystems because of the infertile conditions in the basin of Lena River and its vicinity (Schulze *et al.* 1995). Similar conditions were found in a larch forest in Central Siberia (Matsuura and Abaimov 2000). Plants growing there should have high capacity for utilization of both water and nitrogen under low temperature regimes at rhizosphere. Moreover, there is a great difference between ambient and rhizosphere temperatures.

Several studies have been carried out on larch and pine species in the permafrost region, including studies on the relationship between soil temperature and root growth of two larch species (Prokushkin *et al.* 2002) and on water relations and nitrogen allocation in various organs of larch seedlings (Sudachkova *et al.* 2002). However, there have been few studies on the characteristics of nitrogen utilization of woody species in northern coniferous forests (Gower *et al.* 1995, Koike *et al.* 2001, Pesson and Nasholm 2001). As mentioned above, *Larix* species can grow on permafrost because of their deciduous leaf habit

(Berg and Chapin 1994). Therefore, the turnover rate of nitrogen compounds in a plant body is considered to be one of the most essential factors for adaptation to such a harsh environment (Alekshev 1994, Boothe *et al.* 1995, Pakhomova 1995, Prokushkin *et al.* 1996, Sudachkova *et al.* 1997). Allocation of nitrogen, and utilization of different forms of nitrogen, i.e., nitrate nitrogen ( $\text{NO}_3^-$ -N) and ammonia nitrogen ( $\text{NH}_4^+$ -N), in seedlings of evergreen conifers are affected by soil moisture content (Akama 1991, 1993). The allocation patterns of nitrogen are also strongly affected by leaf habit, i.e., evergreen or deciduous in understorey plants native to northern coniferous forests (Nordin and Nasholm 1997). Under intermediate moisture conditions, evergreen needles of Japanese red pine act as a major storage organ of nitrogen (Akama 1986).

Under infertile conditions with nitrogen limitation, it is thought that the allocation and storage of nitrogen compound are essential metabolism of larch species before winter. However, there have been few studies on the allocation of different forms of nitrogen in larch seedlings regenerated in a permafrost region after a forest fire with a significant change in nutrient balance (Korotkii *et al.* 2000). Regeneration success in larch forests seems to be closely related to the pattern of nitrogen utilization in seedlings and saplings under field conditions. What is the nitrogen allocation in larch seedlings regenerated after a forest fire? What is the most important organ for nitrogen storage in larch seedlings? Does the allocation pattern of nitrogen change according to plant size and growth habitat?

To accessing these questions, we studied changes in the content of nitrogen compounds in each organ of larch seedlings regenerated on contrasting "warm" and "cold" sites during the growth period, and we examined their roles in the resistance of larch seedlings to low temperature of the rhizosphere.

## Materials and methods

### 1. Study site

The study sites were located 5 km northeast of Tura Town in Evenkia Region, Central Siberia ( $64^{\circ}19'N$ ,  $100^{\circ}13'E$  and ca.170m a.s.l.). Vegetational structures of the study sites are typical of larch forests in the region (Prokushkin *et al.* 1998). The first group of experimental plots seem to be necessary (plots I-V), which included five populations of larch seedlings of different ages, located on a southwest-facing slope. This group of plots was named the "warm" site. The second group of experimental plots (plots I and II) was set on an east-facing slope that was covered mainly with larch seedlings and sparse distributions of grasses, including *Carex* spp. These experimental plots were subjected to litter burning. We called these experimental plots as the "cold" site because of the lower temperature of the rhizosphere compared with that in the "warm" site. A part of the "warm" site had been burned in 1990, and a part of the "cold" sites had burnt by a surface fire of moderate intensity in 1994. The burnt area in the "cold" site was about 120 ha.

### 2. Plant materials

To analyse seasonal changes in nitrogen compounds in each organ (needle, stem and root) of larch (*Larix gmelinii*) seedlings, naturally regenerated seedlings and saplings of 13, 24, 36, 48 and 60 months in age were sampled from the "warm" site populations. For comparisons, 13-month-old larch seedlings were also sampled from the "cold" site.

The following criteria proposed by Elagin (1961) were used to determine the phenological phases of growth and development of larch seedlings and saplings: active development of shoots of seedlings before early July (phase I), termination of needle growth in the middle of July (phase II), formation of winter buds on lateral shoots in early August (phase III), formation of apical buds from middle to late August before needle yellowing (phase IV), and needle shedding after needle yellowing (phase V)

Plant materials for analysis were collected twice, once in phase II and once in phase IV, to determine the seasonal changes in nitrogen compounds in the plant body. During this period, the formation of apical buds of larch seedlings was observed in the latter part of August (Fig. 1).

After careful washing of the seedlings, they were separated into organs (needles, stems and roots) and dried at  $105^{\circ}\text{C}$  for 20 hours. These samples were used for determination of dry mass.

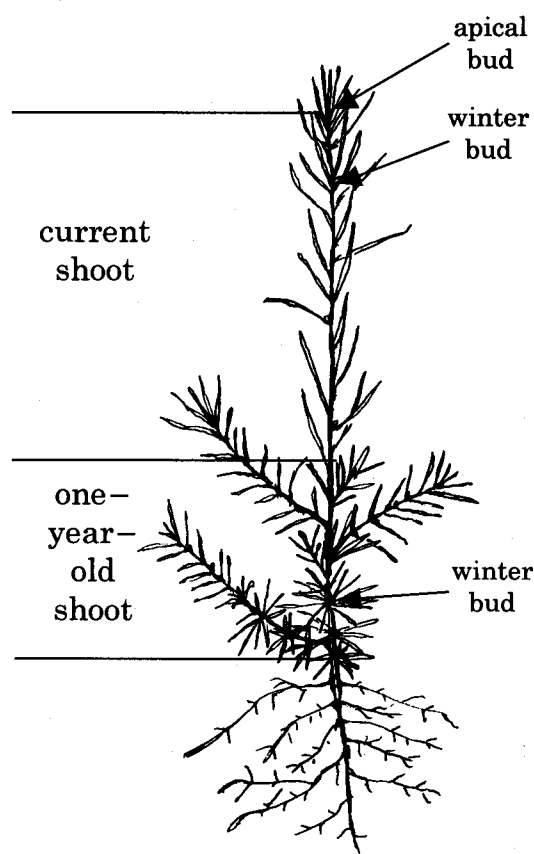


Fig. 1. Schematic representation of two-year-old larch seedlings regenerated after a forest fire in 1994.

### 3. Chemical analysis of nitrogen compounds

The total nitrogen (total-N) content of larch seedlings was determined by the Kjeldahl procedure. Nitrogen contents of plants were expressed here as dry mass bases. For quantitative determination of protein nitrogen (protein-N), precipitation of protein in a solution with 5% trichloro-acetic acid was carried out. The protein-N content was also determined by the Kjeldahl procedure. Non-protein nitrogen (non-protein N) content was estimated by subtracting the value of protein-N from the value of total-N.

The content of free-amino-acid nitrogen (FAA-N) was determined by a colorimetric procedure using sodium diethyl-di-thiocarbamate. One ml of the plant material for analysis was added to 2.5 ml of  $\text{Na}_2\text{HPO}_4$  and 2.5ml of a complex solution ( $\text{Na}_3\text{PO}_4:\text{CuCl}_2:\text{Na}_2\text{HPO}_4=1:1:4$ ). After 30 min at  $24^\circ\text{C}$ , the mixture was centrifuged (10 000rpm for 10min) to separate Cu, and 3ml of the supernatant was incubated at  $24^\circ\text{C}$  with 0.1ml 2% solution of sodium diethyl-di-thiocarbamate ( $\text{C}_3\text{H}_{10}\text{NS}_2\text{Na}_3\text{H}_2\text{O}$ ) (appear specific yellow color). After 10min, 10ml  $\text{C}_4\text{H}_9\text{OH}$  was added to the experimental solution, and the reaction was stopped immediately by centrifugation (5.000 rpm for 10min). The content of FAA-N in the supernatant was determined by a calorimetric procedure (Absorptance=440 nm;  $\check{S}=10.05$ ).

Ammonium nitrogen ( $\text{NH}_4\text{-N}$ ) content was measured by the Konvey micro-diffusive method (Ermakov 1972, Pleshkov 1976). Three ml of 1.5%  $\text{H}_3\text{BO}_3$  and 2 drops of 0.1% of a special indicator (methyl red ( $(\text{CH}_3)_2\text{NC}_6\text{H}_4\text{N}=\text{NC}_6\text{H}_4\text{CO}_2\text{H}$ ) and methylene blue ( $\text{C}_{16}\text{H}_{18}\text{ClN}_3\text{S}\cdot 3\text{H}_2\text{O}$ ) = 4:1) were carried in the inside compartment of the Konvey cap. Color of the solution becomes red. Then 10 ml of plant material for analysis and 2 drops of a special indicator were carried in the outside compartment of the Konvey cap. After the

addition of 5ml-saturated MgO to the outside compartment of the Konvey cap (Color of the solution becomes green.), the mixture was incubated at  $38^\circ\text{C}$  for 24hrs. After the cap was closed. The remaining  $\text{H}_3\text{BO}_3$  in the inside compartment of the Konvey cap (green color) was titrated with 0.01n  $\text{H}_2\text{SO}_4$  until the color of solution had changed from green to red (where 1ml 0.01n  $\text{H}_2\text{SO}_4 = 0.14\text{mg NH}_4\text{-N}$ ).

Nitrate nitrogen ( $\text{NO}_3\text{-N}$ ) in an aqueous extract of powdered plant material was measured calorimetrically (Pleshkov 1976).

### Results

The average depth of melting of permafrost in July was 0.7 to 0.8 m. Soil temperature at the "warm" site was  $8^\circ\text{C}$  higher on average than that at the "cold" site throughout the period of growth from early July to late August. In early August, the soil temperatures at a depth of 5 cm were  $19^\circ\text{C}$  at the "warm" site and  $11.5^\circ\text{C}$  at the "cold" site (Fig. 2). In comparison to the "warm" site, edaphic conditions of the "cold" site were characterised by lower temperatures during the entire growing season ( $7 - 9^\circ\text{C}$ ).

There was phenological phase shift between the two sites. Phenological events in seedlings, e.g., formation of winter buds, were delayed at the "warm" site. These phenomena were taken into account for responses of larch seedlings to changing temperatures at rhizosphere. Lengths of shoots and roots of larch seedlings in populations of different ages that regenerated at the "cold" and "warm" sites are shown in Figure 3. The seedling size increased with increase in age, and root length increased with increase in shoot length. However, there was no difference between the root lengths of seedlings at the two sites.

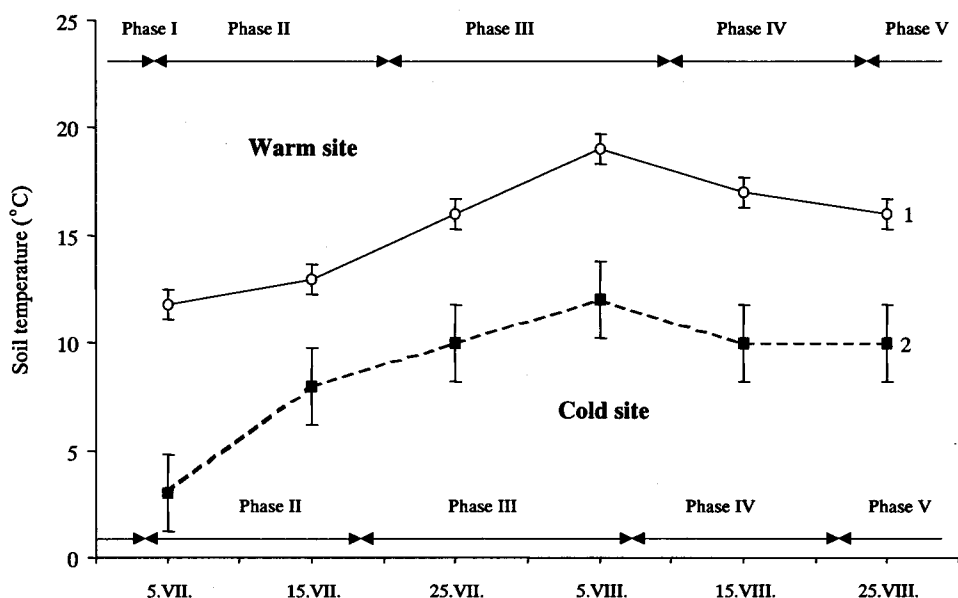


Fig. 2. Temperature of the rhizosphere at a depth of 5cm during the growing season. Phase II ~ V represent the development stage of seedlings.

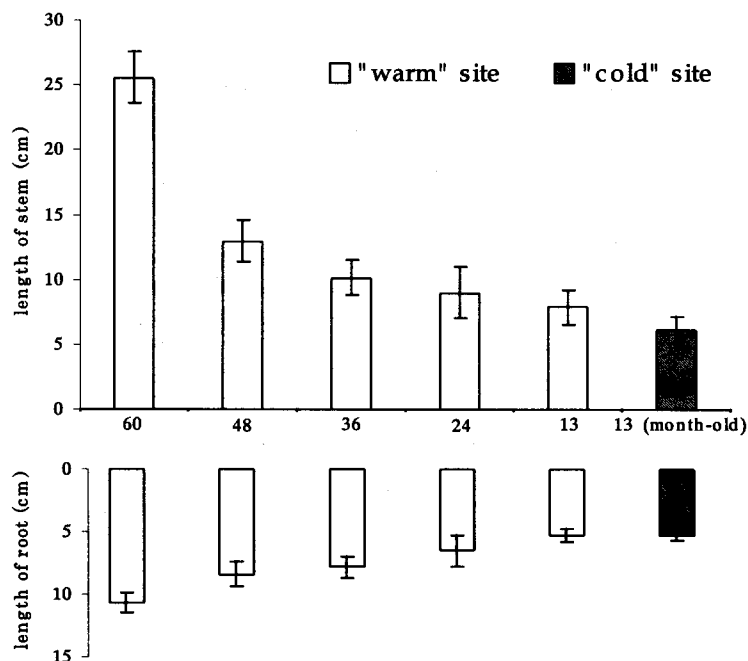


Fig. 3. Lengths of shoots and roots of larch seedlings in populations of different ages at the "warm" and "cold" sites.

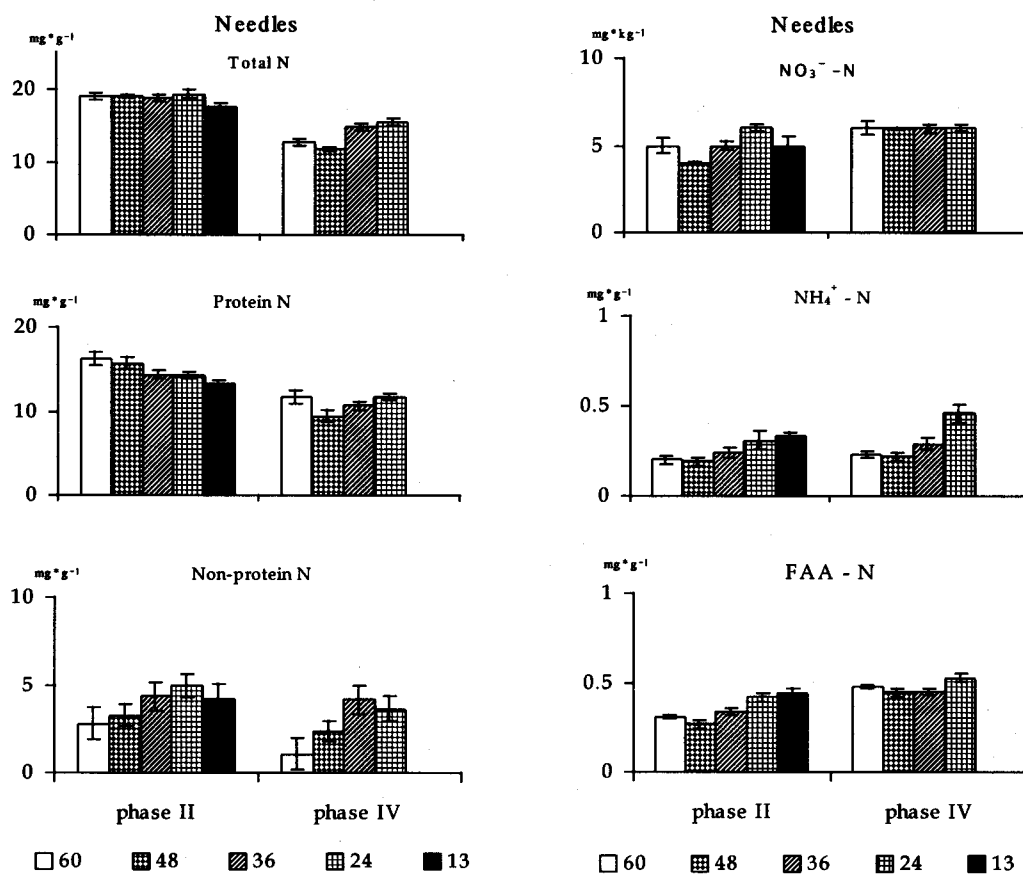


Fig. 4. Contents of total-N, protein-N and non-protein-N in needles of larch seedlings in populations of different ages in phases II and IV ("warm" site). (Ages of seedlings are shown in bars with months)

Fig. 5. Contents of NO<sub>3</sub><sup>-</sup>-N, NH<sub>4</sub><sup>+</sup>-N and FAA-N in needles of larch seedlings in phases II and IV ("warm" site). (Ages of seedlings are shown in bars with months)

In seedlings of 13 months of age, shoot length at the "warm" site was slightly larger than root length at the "cold" site. At 48 months of age, increasing rate of shoot and root was estimated to be ca. 2.0-2.5cm per year. However, at 60 months of age, the growth rate of shoot lengths of seedlings at the "warm" site was two-times greater than that of 48 month of age.

Larch seedlings at the "warm" site allocated total-N to needles during the early stage of the growing season (Fig. 4). Thus, total-N content was ca.  $19 \text{ mg} \cdot \text{g}^{-1}$  regardless of plant age. At the "warm" site, total-N in needles, especially in needles of the 60-month-old population, decreased to  $15 \text{ mg} \cdot \text{g}^{-1}$  ( $P < 0.05$ ) at the beginning of needle yellowing. Similar changes during the growing season were observed for protein-N, but the amount of protein-N decreased with decrease in seedling age (Fig. 4). Protein-N in needles of populations of all ages decreased significantly at phase IV. The content of non-protein-N in needles increased with decrease in age of the seedlings at phenological phases II and IV.

The content of  $\text{NO}_3\text{-N}$  in needles was nearly 100-times less than that of  $\text{NH}_4\text{-N}$  and FAA-N in needles (Fig. 5). The content of  $\text{NO}_3\text{-N}$  in needles showed an almost constant value of  $5 \text{ mg} \cdot \text{kg}^{-1}$  from phase II to phase IV. In contrast, even though the amount of  $\text{NO}_3\text{-N}$  was smaller than that of  $\text{NH}_4\text{-N}$ , needle  $\text{NH}_4\text{-N}$  content

decreased with increase in age of the seedlings in both phases II and IV. In short, needles of young larch seedlings had a higher content of  $\text{NH}_4\text{-H}$ . In phase II, the content of FAA-N in the needles increased with decrease in age of the seedlings. However, there was no age-related difference in the contents of FAA-N during phase IV, when needles started to turn yellow.

In contrast, regardless of seedling age, the contents of total-N, protein-N, and non-protein-N of the stems of larch seedlings increased throughout the growing period (Figs. 6, 7). With increase in age of the larch seedlings, the contents of total-N, protein-N and  $\text{NH}_4\text{-N}$  in the stems decreased. The concentrations of total-N, protein-N and  $\text{NH}_4\text{-N}$  in the stems of seedlings in the 13-month-old population were  $11.8 \text{ mg} \cdot \text{g}^{-1}$ ,  $9.0 \text{ mg} \cdot \text{g}^{-1}$  and  $0.4 \text{ mg} \cdot \text{g}^{-1}$ , respectively. The contents of total-N, protein-N and  $\text{NH}_4\text{-N}$  in the stems of 60-month-old seedlings, on the other hand, were lower:  $9.0 \text{ mg} \cdot \text{g}^{-1}$ ,  $6.0 \text{ mg} \cdot \text{g}^{-1}$  and  $0.2 \text{ mg} \cdot \text{g}^{-1}$ , respectively. These tendencies were found for all phases of the growing season. The contents of non-protein-N in the stems were about  $3.2 \text{ mg} \cdot \text{g}^{-1}$  in populations of all ages. The contents of  $\text{NO}_3\text{-N}$  in the stems were higher in 36-month-old and 48-month-old populations in both phases II and IV (Fig. 7).

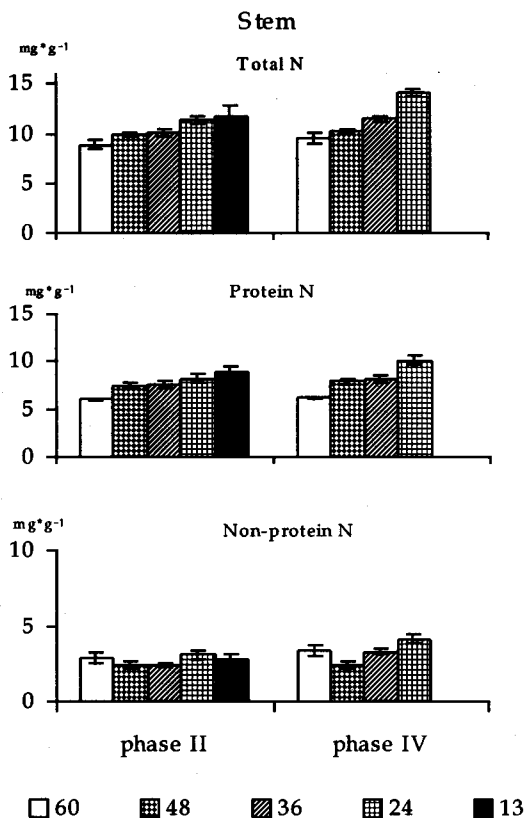


Fig. 6. Contents of total-N, protein-N and non-protein-N in stems of larch seedlings in populations of different ages in phases II and IV ("warm" site). (Ages of seedlings are shown in bars with months.)

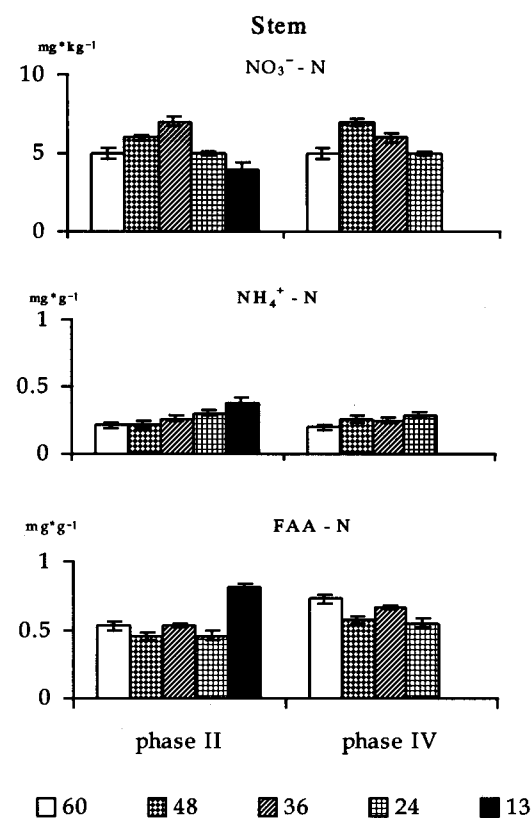


Fig. 7. Contents of  $\text{NO}_3\text{-N}$ ,  $\text{NH}_4\text{-N}$  and FAA-N in stems of larch seedlings in phases II and IV ("warm" site). (Ages of seedlings are shown in bars with months)

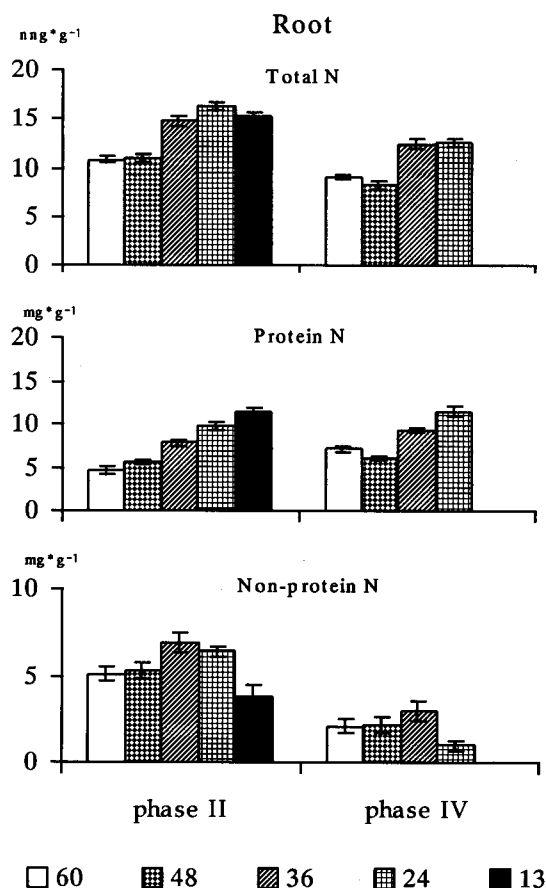


Fig. 8. Contents of total-N, protein-N and non-protein-N in roots of larch seedlings in populations of different ages in phase II and IV ("warm" site). (Ages of seedlings are shown in bars with months)

A comparison of seasonal changes and age-related changes in total-N and protein-N contents in the roots of larch seedlings demonstrated that there was a positive correlation between these changes during the growing season (Fig. 8). The contents of total-N and protein-N increased with decrease in age of the seedlings, as was found for protein-N in the roots. The maximum content of non-protein-N in the roots was found in phase II of the growing season. At this time, the soil nutrient-absorption activity of the larch seedlings was maximum.

At the end of the growing season, reductions in the contents of non-protein-N compounds were observed (Fig. 9).  $\text{NO}_3\text{-N}$  in the roots decreased with increase in  $\text{NH}_4\text{-N}$  in the roots in phase II, but these changes were not clearly observed in phase IV. Among the non-protein-N compounds in the roots, the amount of FAA-N reached ca. 12% of total-N (Figs. 8, 9). The content of FAA-N in the roots peaked in the 24-month-old and 36-month-old populations in phase II.

Accumulation and allocation of nitrogen compounds in various parts of larch seedlings were found to depend on soil temperature. The maximum contents of total-N in the needles and roots of 13-month-old larch seedlings at the "cold" site were  $20.3\text{mg}\cdot\text{g}^{-1}$  and  $18.3\text{mg}\cdot\text{g}^{-1}$ ,

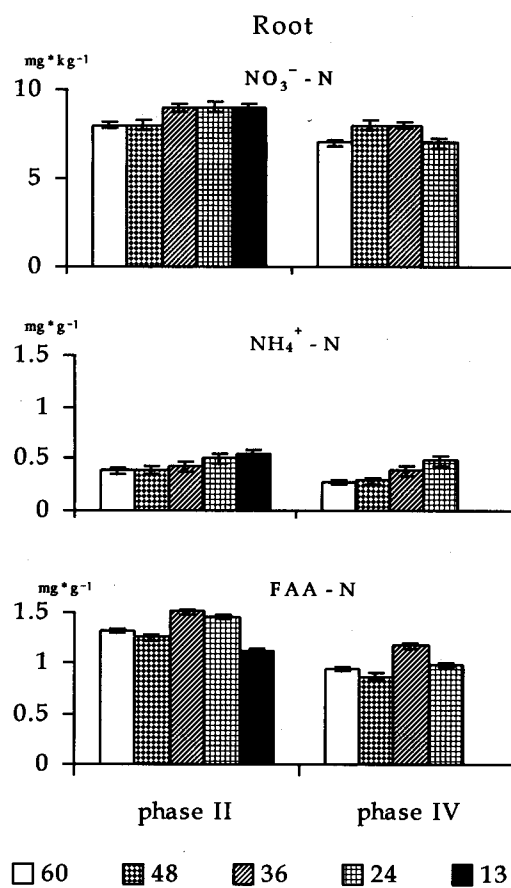


Fig. 9. Contents of  $\text{NO}_3\text{-N}$ ,  $\text{NH}_4\text{-N}$  and FAA-N in roots of larch seedlings in phases II and IV ("warm" site). (Ages of seedlings are shown in bars with months)

respectively. The total-N content in the stems of seedlings at the "cold" site increased significantly (Table 1). The concentration of FAA-N in comparison with "warm" site, increased to 65.6 % (Table 2). The accumulation of FAA-N in the roots at the "cold" site was notably large. Accumulation of FAA-N in the needles and stems was accompanied by a considerable decrease in the protein-N content. On the other hand, there was no difference between root and needle contents of  $\text{NO}_3\text{-N}$  at the "warm" site and "cold" site.

## Discussion

Nitrogen is one of the most important elements for regulation of forest dynamics in Eastern Siberian (Schulze et al. 1995). This major element in soil is distributed usually with a large degree of heterogeneity in both time and topography (Matsuura and Abaimov 2000, Hirobe et al. 2001). After a forest fire, the balance of elements in the soil is greatly changed. A large amount of  $\text{NO}_3\text{-N}$  may be washed away by rain if the development of vegetation cover is poor. However, the regeneration of larch (*Larix gmelinii*) after a forest fire is relatively good (e.g., Abaimov et al. 2000), and the regenerated larch may act as a trap for inorganic

Table 1. Contents of nitrogen compounds in 13-month-old larch (*Larix gmelinii*) seedlings grown at the "cold" site. The unit is mg·g<sup>-1</sup>, dry mass base.

Forms of nitrogen	Needle	Stem	Root	Whole plant
Total	20.3±0.4	11.9±0.4	18.3±0.6	17.1±0.8
Protein	14.9±0.3	8.6±0.2	13.7±0.2	12.7±0.4
Non-protein	5.4±0.7	3.2±0.7	4.6±0.8	4.4±0.9
Free amino acids	0.6±0.2*10 <sup>-1</sup>	0.9±0.2*10 <sup>-1</sup>	3.5±0.4*10 <sup>-1</sup>	1.6±0.4*10 <sup>-1</sup>
Ammonia	0.5±0.1*10 <sup>-1</sup>	0.6±0.4*10 <sup>-1</sup>	0.6±0.3*10 <sup>-1</sup>	0.5±0.4*10 <sup>-1</sup>
Nitrate	0.1*10 <sup>-2</sup> ±0.1*10 <sup>-3</sup>	0.7*10 <sup>-2</sup> ±0.5*10 <sup>-3</sup>	0.1*10 <sup>-2</sup> ±0.4*10 <sup>-3</sup>	0.2*10 <sup>-2</sup> ±0.5*10 <sup>-3</sup>

Table 2. Contents of nitrogen compounds in 13-month-old larch (*Larix gmelinii*) seedlings grown at the "cold" site compared with these in seedlings of the same age grown at the "warm" site.

Unit is percentage (%). Figures in the table are percentages of values at the "cold" site to those at the "warm" site.

Forms of nitrogen	Needle	Stem	Root	Whole plant
Total	116.00±7.20*	100.80±5.50	120.40±8.30	117.70±11.60
Protein	98.00±9.60	95.60±6.40	120.20±9.90	111.10±10.20
Non-protein	234.80±13.40	114.30±9.50	121.10±8.70	142.70±13.80
Free amino acids	145.50±12.60	114.80±10.30	165.60±8.50	130.30±10.40
Ammonia	118.60±6.70	125.00±8.90	87.70±8.60	96.40±10.10
Nitrate	100.00±9.50	175.00±10.30	100.00±13.10	90.50±13.30

\* - Number - % "warm" site

nitrogen.

The utilization and storage capacity of nitrogen in evergreen and deciduous plants are greatly different (e.g. Gower *et al.* 1995). Nitrogen contents in foliage organs are large because more than 70% of leaf nitrogen is allocated to photosynthetic organs (Kozłowski and Pallardy 1997). The pattern of allocation of nitrogen in the plant body is critical for survival of the plant under harsh environmental conditions (Akama 1986, Korotkii *et al.* 2000). Therefore, re-mobilization of foliage nitrogen to the stem and root is essential for plants with a deciduous leaf habit.

The observed increase in needle nitrogen content in phase II may reflect intensive formation of chloroplasts (Fig. 4). Soluble proteins are thought to localize in the chloroplast. At the end of growing season, i.e., phases IV-V, the processes of destruction and re-mobilization of nitrogen compounds from needles may cause an increase in nitrogen in the stem. This process seems to be closely

correlated with the pattern of degradation of photosynthetic organs. In fact, it has been shown that non-protein-N and FAA-N in leaves of deciduous trees decrease from leaf unfolding to mid August and then increase before autumn coloration and again decrease sharply as the growing period approaches the leaf shedding stage (Kozłowski and Pallardy 1997). This decrease may be due to the degradation of chloroplasts because protein N is mainly localized in the chloroplast (e.g., Körner 1999). Nitrogen contents of roots in understorey evergreen shrubs in a north European forest increased at the end of growing season, indicating that roots may also act as a storage organ of nitrogen in the form of FAA-N (Nordin and Nasholm 1997). However, the seasonal changes in the content of foliage nitrogen have only been reported for deciduous trees.

Although the content of non-protein-N in the stem decreased from phase II to phase IV, the contents of protein-N, NO<sub>3</sub>-N and NH<sub>4</sub>-N remained relatively



constant during these phases. However, FAA-N in the stem also decreased from phase II to phase IV (Figs. 6, 7). The decrease in the content of FAA-N in the stem may be due to the processes of cell lignifications and suberizations because lignin originates from amino acids (Kozłowski and Pallardy 1997). The total protein-N content in the whole plant did not depend on soil temperature. However, when soil temperature was low, protein-N accumulated mainly in the roots, and decreases in the contents of protein-N in needles and stems were also observed ( $P < 0.05$ ). Roots may act as a storage organ when soil temperature is low.

In phase II, the low soil temperature caused an increase in the  $\text{NH}_4\text{-N}$  content in the stem to 75% (Table 2). The  $\text{NH}_4\text{-N}$  content of the whole plant and particularly that of the roots decreased when soil temperature was low. At the same time, the concentrations of  $\text{NH}_4\text{-N}$  in needles and stems increased. Thus, all the examined forms of nitrogen compounds in the whole larch seedling increased when soil temperature was low. Edaphic conditions may also influence the allocation forms of nitrogen in the plant body. In fact, it has been shown that the concentration of  $\text{NO}_3\text{-N}$  in the roots of seedlings of Sugi-cedar (*Cryptomeria japonica*), an evergreen conifer, but not that in the roots of seedlings of Japanese red pine (*Pinus densiflora*), increased when there was large concentration of  $\text{NO}_3\text{-N}$  in the soil (Akama 1991, 1993). A similar phenomenon under the condition of low soil temperature was found for Scotch pine (*Pinus sylvestris*) seedlings (Prokushkin 1982). Therefore, it seems that the capacity of reducing  $\text{NO}_3\text{-N}$  to  $\text{NH}_4\text{-N}$  may be a specific characteristic of each tree species.

Before winter, the contents of protein-N in both the stems and roots increased in phase IV, a finding that may reflect the pattern of allocation of different forms of nitrogen compounds to prepare for the cold winter season. In this study, seedlings until 48 months of age showed similar trends in the nitrogen allocation pattern. However, the stems of 60-month-old seedlings may play an important role in nitrogen storage. In fact, the shoot size of 60-month-old seedlings was two times larger than those of seedlings in younger populations. Thus, larch species may change the site of nitrogen storage from the roots to the stem in relation to the size of the seedling.

Nitrogen absorption capacity of larch plants is generally strongly related to the activity of symbiotic micro-organisms (Smith and Read 1997). Therefore, further studies are needed to determine the role of ectomycorrhiza in the rhizosphere of larch plants in a permafrost region.

### Conclusions

Seasonal changes and age-related changes in the composition of nitrogen compounds in each part of larch seedlings were determined.  $\text{NH}_4\text{-N}$  and FAA-N accumulated mainly in the roots regardless of seedling size. At the end of the growth period, when larch is preparing for winter, there was an accumulation of nitrogen compounds in the roots and stem. Low soil

temperature caused an increase in total-N concentrations in the needles and roots. Accumulation of FAA-N and  $\text{NH}_4\text{-N}$  was found in the needles of larch seedlings at the "cold" site. The contents of  $\text{NO}_3\text{-N}$  in the stems, particularly in the stems of 60-month-old larch seedlings, were remarkably high. The response of larch to low soil temperature may be a trigger of change in the turnover of nitrogen compounds in larch seedlings.

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## Appendix



Photo. 1.  
A view of damaged stands after forest fires. At this side, forest fire occurred in 1994 (photo was taken in 1998). Spruce and larch trees survived along the riverside while the dead larches remained. Fires could not spread beyond the stream at opposite hills.



Photo. 2.  
Photo shows the dead larch trees fell down with shallow root system (the depth was ca. 35cm). Dominant herbaceous plants after fires is *Epilobium angustifolium* L.



Photo. 3.  
Two-year-old seedlings of larch after forest fires. Density of larch seedlings was approximately 2 millions per hecter. Height of seedlings was ca. 13.5cm.



Photo. 4.  
A view of "warm" site located at the middle part of hills after forest fire. Density of 4-year-old larch seedlings was ca. 20 thousand per hecter. Two persons in the photo squatted themselves for surveying seedling size. Height of seedlings ranged between 45 and 68cm.



Photo. 5.  
A view of regenerated larch seedlings with mother trees in the "warm" site after forest fire in 1990. Age of seedlings was approximately 6-year-old. Height of seedlings ranged between 50 and 70cm.



Photo. 6.  
Larch seedlings were regenerated at the bottom part of earth hammock where water condition was well. However, the temperature at soil surface was low (ca. 2-4°C) because the distance of front part of permafrost was within 10 cm.