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Nitrogen Nutrition of Plants in an Alpine Lichen Heath under the Conditions of Soil Enrichment with Biogenic Elements

M. I. Makarov[a], *, N. G. Lavrenov[a], V. G. Onipchenko[a], A. V. Tiunov[b], T. I. Malysheva[a], and R. V. Sabirova[a]

[a] *Moscow State University, Moscow, 119991 Russia*
[b] *Severtsov Institute of Ecology and Evolution, Moscow, 119071 Russia*
**e-mail: mmakarov@soil.msu.ru*

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Abstract—The results of a long-term experiment on the enrichment of the soil (Umbric Leptosol) of an alpine lichen heath with biogenic elements have shown that calcium addition and reduction of soil acidity do not change the nitrogen regime of the soil and patterns of nitrogen nutrition of plants. The increase in phosphorus availability contributes to the mobilization of soil organic nitrogen and shifts in the role of mycorrhiza in the nitrogen nutrition of plants, which determines their heavier isotopic nitrogen composition. At the same time, this reduces the intensity of nitrogen fixation by a nitrogen-fixing legume species, *Oxytropis kubanensis*. The increase in the availability of nitrogen leads to its active uptake by all studied plant species except *Oxytropis kubanensis*, as well as to the heavier isotopic nitrogen composition of plants. However, changes in the nitrogen status differ between plant species.

Keywords: alpine ecosystems, nitrogen, phosphorus, $\delta^{15}N$, mycorrhiza

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Nitrogen and phosphorus are among the key biogenic elements that limit the production of many terrestrial ecosystems, including high-mountain ones [1, 2]. The production of temperate-zone mountain phytocenoses is barely limited by phosphorus and potassium, since these elements enter the soil in sufficient amounts during rock weathering. It is more often that deficiency in nitrogen due to its immobilization in soil organic matter is a limiting factor for plant development [3, 4].

High-mountain ecosystems are of interest for researchers as objects that are only slightly affected by human activities and sensitive to climate changes [5]. Rising temperatures may lead to acceleration of soil organic nitrogen mineralization and nitrification, which can significantly change both biogeochemical nitrogen cycles and the nitrogen regime of certain plant species [6, 7]. However, these phenomena have not been sufficiently studied in the high-mountain ecosystems of temperate latitudes, which differ from other terrestrial ecosystems in temperature and nitrogen regimes [5].

The study of the nitrogen regime of plants and nitrogen status of ecosystems often involves experiments based on the addition of inorganic nutrients. A long-term increase in the availability of nutrient elements leads to changes in the biomass and proportion of some species in alpine plant communities [3, 8]. However, it is unknown how the nitrogen regime of individual species and functional plant groups (grasses, sedges, forbs, and legumes) changes in this case. We have assumed that the change in the nitrogen status of plants after the increase in the availability of nutrient elements in soil may result not only from their direct uptake but also from changes in the patterns of the functioning of the microbial community, mycorrhizal symbiosis, and symbiotic nitrogen fixation due to the variable availability of nitrogen and phosphorus. Individual responses of plant species to the increase in the availability of nutrient elements should be particularly clearly expressed in the most limited ecosystems. Therefore, an alpine lichen heath was chosen as a model for testing our hypothesis, since the soil of this community contains the lowest amount of available nitrogen compared to other communities of the alpine zone [9].

OBJECTS AND METHODS

The study was carried out in the alpine zone of the Teberda State Nature Reserve, where a resource manipulation experiment was established in 1998. The experiment includes five variants: (1) control; (2) annual addition of nitrogen (9 g/m^2) in the form of carbamide with $\delta^{15}N = 0.11 \pm 0.09‰$ (variant with N); (3) annual addition of phosphorus (2.5 g/m^2) as double superphosphate (variant with P); (4) annual combined addition of nitrogen and phosphorus at the same doses and in the form of the same compounds (variant with NP); and (5) liming with slaked lime (52 g/m^2) once per 3 years (variant with Ca). All fertilizers are applied to the soil surface in solid form soon after snow cover melting in May. The experimental plots represent a square with a side of 1.5 m. Each variant of the experiment was carried out in four replications. The experiment was described in detail in previous publications [8, 10].

Alpine lichen heaths (class *Juncetea trifidi*) are formed on wind-exposed ridges with minimum snow accumulation in winter. They are characterized by a high proportion of fruticose lichens in the plant community. Our study involved seven species of vascular plants: *Anemone speciosa*, *Antennaria dioica*, *Campanula tridentata*, *Carex umbrosa*, *Festuca ovina*, *Oxitropis kubanensis*, and *Trifolium polyphyllum*. The main criteria for selecting these species included their high proportions, uniform occurrence in the experimental plots, and systematic diversity. The species belong to four functional groups: forbs (*Anemone speciosa*, *Antennaria dioica*, and *Campanula tridentata*), grasses (*Festuca ovina*), sedges (*Carex umbrosa*), and legumes (*Oxitropis kubanensis* and *Trifolium polyphyllum*).

Plant leaves were collected in all variants of the experiment in 2016: one sample from each square and one additional sample from the square with the highest occurrence of the species (five samples from each variant, a total of 175 samples). The samples were dried at 45°C for 10 h, ground in a Retsch MM 200 vibratory mill, and analyzed for the total nitrogen and ^{15}N/^{14}N ratio (δ^{15}N) with a Thermo Flash 1112 elemental analyzer and a Thermo Delta V$^{\text{Plus}}$ isotope mass spectrometer at the Severtsov Institute of Ecology and Evolution, Russian Academy of Sciences.

Soil samples were taken simultaneously with leaves from a 0–10 cm layer (two samples per each square, eight samples from each variant, a total of 40 samples) to determine the following parameters: acidity (pH) in the soil-water suspension, total concentration of C and N (C_{total} and N_{total}), concentration of extractable phosphorus (P_{extr}) according to Kirsanov, and concentrations of different forms of C and N, extracted with 0.05 mol K_2SO_4 (organic carbon (C_{org}), total nitrogen (N_{extr}), N-NH_4^+, and N-NO_3^-). The extractable organic nitrogen (N_{org}) was calculated by the difference between N_{extr} and N-NH_4^++N-NO_3^-. The concentration of N-NH_4^+ was determined by the salicylate-nitroprusside method [11], and N-NO_3^- was determined after reducing NO_3^- to NO_2^- on a cadmium column and synthesizing a colored azocompound [12]. Phosphorus was determined by the ammonium molybdate–ascorbic acid method.

The C_{org} and N_{extr} concentrations were measured on a TOC-V_{CPN} analyzer; the inorganic forms of nitrogen and phosphorus, on a GENESYS$^{\text{TM}}$ 10UV spectrophotometer; the total concentrations of C and N, on a Thermo Flash 1112 elemental analyzer at the Severtsov Institute of Ecology and Evolution, Russian Academy of Sciences.

The effect of the factor “variant” on the test parameters was tested for significance using parametric and nonparametric criteria. If the distribution of data did not differ from normal and their variances were equal, an analysis of variance (ANOVA) was used for further research. If the data distribution did not differ from normal but the variances were unequal, ANOVA with Welch’s correction was used. If the distribution differed from normal, the nonparametric Kruskal–Wallis test was used.

RESULTS AND DISCUSSION

Effect of Long-term Application of Elements on Soil Properties

The mountain-meadow soil (Umbric Leptosol) of the alpine lichen heath is acidic (pH 5.06) and rich in organic matter and nitrogen (11% of C_{total} and 0.97% of N_{total}). The C/N ratio is 11.3. Although the concentration of N_{total} is high, nitrogen availability is low. Inorganic N mainly consists of N-NH_4^+ (16.3 mg/kg), while the concentration of N-NO_3^- is only 2.2 mg/kg. The content of extractable N_{org} is also low (9.5 mg/kg). The C/N ratio is lower in the extractable organic matter (9.8) than in the total soil organic matter (11.3). The content of extractable phosphorus is 10.8 mg/kg (Table 1).

The concentration of C_{total} and N_{total} and their ratio did not change in all variants of experiment, which indicates the resistance of organic matter accumulated in the mountain-meadow alpine soil to the long-term fertilizers influence (Table 1). No increase in N_{total} when a large amount of nitrogen was added in the variants with N and NP may be due to the fact that the surface application of urea leads to the volatilization of a significant proportion of ammonia formed during its decomposition [13].

The labile indicators of soil state (availability of mineral forms of nutrients, acidity, and extractable organic matter) changed. These changes are mainly expectable and result from the direct effect of the added elements on their concentrations in the soil. Thus, the addition of nitrogen to the soil led to an increase in the concentration of N_{extr} from 28.0 to 51.1 mg/kg in the variant with N and 43.3 mg/kg in the variant with NP. The main component responsible for this increase was N-NH_4^+, with its concentration increasing approximately twofold (up to 34.5 mg/kg in the variant with N and 28.5 mg/kg in the variant with NP). The same trend was observed for the concentration of N-NO_3^-; i.e., its concentration significantly increased in the variant with N (up to 5.2 mg/kg),

Table 1. Soil properties of the alpine lichen heath in different variants of experiment, mean ± standard deviation ($n = 8$)

Variant	C_{total}	N_{total}	C_{total}/N_{total}	P_{extr}, mg/kg	pH	$N\text{-}NH_4^+$	$N\text{-}NO_3^-$	C_{org}	N_{org}	C_{org}/N_{org}
	%					mg/kg				
Control	11.0 ± 1.3^a	0.97 ± 0.12^a	11.3 ± 0.8^a	10.8 ± 6.0^a	5.06 ± 0.15^a	16.3 ± 4.8^a	2.2 ± 0.5^a	91.4 ± 19.8^{ab}	9.5 ± 2.5^a	9.8 ± 1.3^a
Ca	11.1 ± 1.8^a	0.97 ± 0.15^a	11.4 ± 0.7^a	9.5 ± 2.7^a	5.44 ± 0.13^b	23.5 ± 9.3^{ab}	2.9 ± 1.1^a	124.4 ± 42.1^a	12.0 ± 4.2^a	10.5 ± 2.2^{ab}
P	10.8 ± 1.7^a	0.94 ± 0.12^a	11.4 ± 0.6^a	133.6 ± 99.7^b	4.98 ± 0.14^{ac}	18.2 ± 3.1^a	2.1 ± 1.1^a	85.7 ± 5.8^b	7.3 ± 0.9^b	11.8 ± 1.0^b
N	11.1 ± 2.0^a	0.99 ± 0.21^a	11.3 ± 1.1^a	6.9 ± 4.7^a	4.88 ± 0.12^c	34.5 ± 12.9^b	5.2 ± 3.8^b	107.3 ± 29.1^{ab}	11.4 ± 3.9^a	9.7 ± 1.7^{ab}
NP	10.9 ± 1.5^a	0.95 ± 0.15^a	11.5 ± 0.4^a	73.8 ± 37.3^b	4.91 ± 0.28^{ac}	28.5 ± 11.8^b	3.1 ± 1.2^{ab}	105.0 ± 22.6^a	11.8 ± 3.5^a	9.2 ± 1.2^a

The same alphabetic notations within one column show the absence of significant differences; $P < 0.05$.

while this increase was less in the variant with NP (3.1 mg/kg) (Table 1).

The concentration of P_{extr} increased by a factor of 12 (133 mg/kg) in the variant with P and by a factor of almost 7 in the variant with NP (74 mg/kg) (see Table 1). The lower concentration of P_{extr} (as well as inorganic nitrogen compounds) in the second case may be due by greater involvement of this element in the growth of plant biomass upon simultaneous N and P addition [3] and its subsequent fixation in the soil organic matter.

The soil acidity significantly decreased after liming: the pH increased to 5.44. On the contrary, the soil was acidified in the variant with nitrogen (the pH significantly decreased to 4.9), which was due to the potential physiological acidity of NH_4^+ formed from carbamide and subsequent nitrification.

Among indirect effects, changes in the labile organic matter are of particular interest. Thus, the concentrations of extractable C_{org} and N_{org} tended to increase in the variants with liming, nitrogen, and combined nitrogen and phosphorus (Table 1). This could probably result from the mobilization of organic matter due to the intensification of microbial activity in soils after neutralizing acidity (Ca) and increasing the availability of nutrients (N and NP). However, the most significant change in C_{org} and N_{org} occurred after phosphorus addition. In this case, the concentration of N_{org} significantly decreased (from 9.5 to 7.3 mg/kg) and the C_{org}/N_{org} ratio increased from 9.8 to 11.8. Although the concentration of C_{org} did not significantly differ from the control, it was significantly lower than in the variants with Ca and NP. Probably, nitrogen deficiency for soil microorganisms increases under high phosphorus availability, and they more actively utilize labile organic compounds (in particular, nitrogen-containing ones) under these conditions.

Concentration and Isotopic Composition of Nitrogen in Alpine Plants

The plant species studied in the alpine lichen heath differ markedly in the content and isotopic composition of nitrogen in the leaves (Table 2). Lower nitrogen concentrations (1.14–1.29%) are characteristic of grasses and sedges (*Festuca ovina* and *Carex umbrosa*); its higher concentration (1.42–2.31%) is contained in the leaves of forbs (*Anemone speciosa*, *Antennaria dioica*, and *Campanula tridentata*) and its concentrations reach a maximum (2.29–3.25%) in legume leaves. At the same time, the nitrogen concentration of 2.29% recorded in the leaves of *Trifolium polyphyllum* (which does not have symbiotic nitrogen fixation [14, 15]) is close to that in forbs species (*Anemone speciosa* and *Antennaria dioica*). In turn, the nitrogen concentration of 3.25% in the leaves of *Oxitropis kubanensis* (which actively fixes atmospheric nitrogen [14]) is significantly higher than in all other species.

The isotopic composition of nitrogen ($\delta^{15}N$) also significantly varies in the leaves of different plant species, including forbs and legumes. The heaviest nitrogen (the least negative (–0.59‰) $\delta^{15}N$ value) among all studied plant species is characteristic of *Carex umbrosa*, while it is much lighter in the leaves of *Festuca ovina* ($\delta^{15}N = -3.57$‰). A significant variation in the $\delta^{15}N$ value is characteristic of forbs, including species with a light isotopic composition of nitrogen (*Antennaria dioica* and *Campanula tridentata*) and a heavier composition (*Anemone speciosa*).

This diversity can be explained by the dependence of nitrogen isotopic composition in plants on a number of factors: the use of different sources of nitrogen (NH_4^+, NO_3^-, or N_{org}) or one source from different soil horizons by different plants; different discriminations of the heavy isotope during its uptake and assimilation by different plant species; the effect of mycorrhiza [16–19].

The isotopic composition of nitrogen also markedly differs in legumes. The $\delta^{15}N$ value for *Trifolium polyphyllum*, which does not have symbiotic nitrogen fixation, is close to that for most of the other studied species with arbuscular mycorrhiza (–3.08‰). In contrast, the $\delta^{15}N$ value for *Oxitropis kubanensis* with an active nitrogen-fixing symbiosis (–0.78‰) is much closer to the atmospheric nitrogen value, which allowed us to calculate the contribution of symbiotic nitrogen fixation to the nitrogen nutrition of this legume species (it was over 70%) [14].

Effect of Long-term Application of Elements on N and $\delta^{15}N$ in Plants

Changes in mineral nutrition conditions influenced the concentration and isotopic composition of nitrogen in the plant leaves (see Table 2). Different experimental variants had different effects on the studied parameters. For instance, liming did not change the concentrations and isotopic composition of nitrogen in any of the studied plant species. This is consistent with the fact that the decrease in soil acidity (by about 0.4 pH units) did not significantly influence any indicators of its nitrogen state (see Table 1).

Effect of Phosphorus

The addition of phosphorus to the soil led to more pronounced changes in the nitrogen status of plants. They could be clearly seen in the isotopic composition of nitrogen, while its concentration changed to a much lesser extent. The only species in which the increase in phosphorus availability led to changes in nitrogen concentration (it significantly decreased from 3.25 to 2.67%) was *Oxitropis kubanensis*. Its $\delta^{15}N$ value also significantly decreased (from –0.78 to –2.32‰), thereby becoming closer to the values that are characteristic of forbs species. The trend of change in both

Table 2. Concentration and isotopic composition of nitrogen ($\delta^{15}N$) in the leaves of plants of the alpine lichen heath in different variants of experiment, mean ± standard deviation ($n = 5$)

Species	Variant									
	Control		Ca		P		N		NP	
	N, %	$\delta^{15}N$, ‰	N, %	$\delta^{15}N$, ‰	N, %	$\delta^{15}N$, ‰	N, %	$\delta^{15}N$, ‰	N, %	$\delta^{15}N$, ‰
Anemone speciosa	1.96 ± 0.12^{ac}	-1.59 ± 0.43^{A}	1.89 ± 0.07^{a}	-1.40 ± 0.36^{AB}	1.85 ± 0.16^{a}	-0.81 ± 0.26^{B}	2.47 ± 0.05^{b}	0.14 ± 1.22^{BC}	2.28 ± 0.25^{bc}	1.31 ± 1.71^{C}
Antennaria dioica	1.42 ± 0.05^{a}	-4.63 ± 0.48^{A}	1.41 ± 0.05^{a}	-4.37 ± 0.52^{AB}	1.43 ± 0.08^{ab}	-3.73 ± 0.50^{AB}	1.86 ± 0.40^{b}	-2.45 ± 1.56^{B}	1.95 ± 0.47^{b}	-3.99 ± 0.27^{AB}
Campanula tridentata	2.31 ± 0.21^{a}	-4.34 ± 0.82^{A}	2.34 ± 0.11^{a}	-4.55 ± 0.55^{A}	2.35 ± 0.30^{a}	-3.45 ± 1.15^{AC}	3.55 ± 0.85^{b}	1.47 ± 2.53^{B}	2.35 ± 0.55^{a}	-1.17 ± 2.42^{BC}
Carex umbrosa	1.29 ± 0.03^{a}	-0.59 ± 0.51^{A}	1.31 ± 0.07^{a}	0.32 ± 0.33^{B}	1.31 ± 0.06^{a}	0.74 ± 0.61^{B}	1.91 ± 0.16^{b}	0.14 ± 1.34^{AB}	1.68 ± 0.23^{b}	1.57 ± 2.04^{AB}
Festuca ovina	1.14 ± 0.10^{a}	-3.57 ± 0.41^{A}	1.16 ± 0.05^{a}	-3.59 ± 0.41^{A}	1.07 ± 0.11^{a}	-1.77 ± 0.70^{B}	1.72 ± 0.38^{b}	-1.33 ± 2.36^{ABC}	2.40 ± 0.30^{c}	2.42 ± 1.68^{C}
Oxitropis kubanensis	3.25 ± 0.41^{a}	-0.78 ± 0.17^{A}	3.17 ± 0.31^{a}	-0.90 ± 0.24^{A}	2.67 ± 0.17^{b}	-2.32 ± 0.80^{B}	2.86 ± 0.25^{ab}	-0.87 ± 0.55^{A}	–	–
Trifolium polyphyllum	2.29 ± 0.34^{a}	-3.08 ± 0.76^{A}	2.15 ± 0.11^{a}	-2.93 ± 0.77^{A}	2.28 ± 0.28^{a}	-2.28 ± 1.29^{A}	3.45 ± 0.25^{b}	-1.28 ± 1.64^{A}	2.78 ± 0.17^{c}	-1.45 ± 1.2^{A} 9

The same alphabetic notations within one line show the absence of significant differences in the concentration of N (lowercase) or $\delta^{15}N$ (uppercase); $P < 0.05$.

indicators shows that the increase in phosphorus availability might lead to a decrease in the activity of symbiotic nitrogen fixation. Since it is believed that the low availability of phosphorus, on the contrary, can limit the activity of nitrogen fixation [20], additional research is required to explain the result. As concerns another legume species (*Trifolium polyphyllum*), we assumed that the low availability of phosphorus in the soil might cause the failure of symbiosis with nodule bacteria. However, *T. polyphyllum* does not begin to show signs of nitrogen fixation (an increase in the nitrogen concentration and $\delta^{15}N$ value) upon a significant increase in soil phosphorus availability, which indicates that the absence of nitrogen fixation in this legume species is explained differently.

The concentration of nitrogen in all other studied plant species remained unchanged after adding phosphorus to the soil; however, the $\delta^{15}N$ value increased in this case (especially in four species). The direct correlation between the phosphorus concentration and $\delta^{15}N$ value recorded in the leaves of some marsh plants was previously interpreted as a result of the influence of phosphorus availability on the fractionation of nitrogen isotopes by the plants [21]. However, our data indicate that the change in the $\delta^{15}N$ value may be due to changes in the nitrogen nutrition of plants. This is confirmed by the transformation of the nitrogen state of the soil after adding phosphorus: the reduction of N_{org} concentration and depletion of extractable organic matter by nitrogen (the increase in the C_{org}/N_{org} ratio) adequately explain the change in the isotopic composition of nitrogen in the plants. It is known that labile organic nitrogen and its important source, microbial biomass nitrogen, are the most isotopically heavy nitrogen pools in soil [19, 22, 23]. Presumably, the nitrogen deficiency for plants increases when phosphorus is highly available, and they begin to utilize organic fractions in this case. However, it is hardly probable that plants utilize products of mineralization of organic fractions, since microorganisms also suffer from nitrogen deficiency and generally immobilize it when phosphorus is highly available. It is more likely that the $\delta^{15}N$ value increased due to the shift in the role of mycorrhiza in the nitrogen nutrition of plants. There can be two opposite explanations here.

On the one hand, mycorrhiza can take up heavy organic nitrogen when its deficiency is high. Although arbuscular mycorrhiza (ARM), which is characteristic of most plants in the alpine lichen heath [24], is traditionally considered in the context of its involvement in the phosphorus nutrition of plants, there is growing evidence that it can be also involved in nitrogen nutrition. In particular, ARM can take up amino acids and amino sugars, especially when the availability of inorganic nitrogen is low [25, 26].

On the other hand, the participation of ARM in the nitrogen nutrition of plants may decrease when phosphorus is highly available. The significant reduction of colonization of ARM roots after 6-year addition of phosphorous fertilizers is shown by the example of steppe plant communities of Inner Mongolia [27]. As in plants with ericoid mycorrhiza and ectomycorrhiza [28, 29], the prevailing transport of ^{14}N from ARM to host plants and the formation of the light isotopic composition of nitrogen in leaves are confirmed by the decrease of the $\delta^{15}N$ in the leaves of plants with ARM and its increase in their roots [30, 31]. In turn, if the participation of ARM decreases in the plants nitrogen nutrition, the fractionation of isotopes and formation of the light isotopic composition of nitrogen in the leaves also decreases.

This uncertainty indicates the necessity of additional studies for determining the cause of formation of the heavier isotopic composition of nitrogen in plants of the alpine lichen heath upon increase in the availability of phosphorus.

Effect of Nitrogen

As could be expected, the nitrogen status of plants changed most significantly after adding nitrogen alone or combined with phosphorus. Both the concentration of nitrogen and its isotopic composition changed in the plant leaves.

The nitrogen concentration increased in all studied species except *Oxitropis kubanensis,* and its increase was much higher in the variant with N than in the variant with NP. This indicates active consumption of nitrogen by plants, while its lower concentrations in the variant with NP can be explained by its dilution due to the greater biomass growth [3]. At the same time, the response to the soil enrichment with nitrogen differs in different species. Thus, the increase in the nitrogen concentration is lower and its isotopic composition changes less significantly in *Antennaria dioica* than in other plant species. This is probably due to the lower competitiveness of this forb species, whose biomass remained unchanged after increase in nitrogen availability [8]. In contrast, *Carex umbrosa* actively accumulates additional nitrogen, which is well correlated with the maximum growth in the biomass of this species [8].

Trifolium polyphyllum, which does not have symbiotic nitrogen fixation, responds to the nitrogen enrichment of soil in a similar way as forbs: it effectively intakes additional nitrogen, the concentration of which increases by almost 1.5 times in the variants with N and NP. In turn, the concentration of nitrogen in *Oxitropis kubanensis* did not change. This shows that the increase in nitrogen availability in soil probably leads to a decrease of nitrogen fixation contribution to the nitrogen balance of *O. kubanensis*.

The $\delta^{15}N$ value increased in all plants except *O. kubanensis*; i.e., the content of the heavy nitrogen isotope increased. However, statistically significant differences from the control were not necessarily observed for all species due to fairly high variation in this indicator. This trend of change in the isotopic composition of nitrogen is understandable. Thus, the $\delta^{15}N$ of ammonium nitrogen, which prevails among the inorganic forms of the element in the soil of the alpine lichen heath, is –4 to –7‰ [18, 32]. Being the main source of nitrogen for plants, it possibly controls the isotopic composition of the element for many species. The $\delta^{15}N$ value is about 0‰ for carbamide, which is used in the experiment as an additional source of nitrogen. When carbamide is decomposed on the soil surface and isotopically light nitrogen is volatilized with ammonia, ammonium nitrogen entering the soil should be even heavier. The contribution of this heavy nitrogen to plant nutrition determines the increase of $\delta^{15}N$ in the plants. We recorded no significant differences of the $\delta^{15}N$ in the leaves of *Trifolium polyphyllum*; however, the trend towards increase in the variants with N and NP is clearly observed, which also indicates the intensive consumption of additional nitrogen by this legume plant.

Theoretically, the heavier isotopic composition of nitrogen in plant leaves with increase of nitrogen availability may also be due to changes in the functioning of mycorrhiza (a decrease of participation in plant nutrition), similarly to the $\delta^{15}N$ increase after increasing phosphorus availability. Since the involvement of mycorrhiza in transporting nitrogen to host plants can decrease when the element is highly available [25, 26, 31], its role in the formation of the light isotopic composition in leaves also decreases. For instance, the decrease of ^{15}N concentration in the leaves of some herbaceous plant species with ARM was observed when the soil was less rich, while there was no difference in the isotopic composition between the roots and leaves when the nitrogen availability increased [33]. In the experiment on the colonization of *Alnus incana* roots by ARM fungus *Rhizophagus irregularis*, the decrease of the ^{15}N isotope concentration in leaves and its increase in roots were also characteristic only of the variant with nitrogen deficiency [31]. Although the experiment using fertilizers in Inner Mongolia showed no significant effect of nitrogen addition on the colonization of ARM roots, it revealed strong effect on the composition of the mycorrhiza community [27], which can also be reflected in the involvement of mycorrhiza in the nitrogen nutrition of plants. However, at the expected significant $\delta^{15}N$ increase of the carbamide, mycorrhiza still appears to play a secondary role in changing the $\delta^{15}N$ value in plant leaves in variants with N and NP.

The $\delta^{15}N$ value did not change for *Oxitropis kubanensis* after adding nitrogen. As in other species, presumably, the isotopic composition of nitrogen in this species should become heavier, since the contribution of the additional heavy nitrogen isotope to the nutrition should increase. However, the stable concentration of nitrogen in the leaves of *Oxitropis kubanensis* owing to the increased availability of the element in the soil may indicate a change in the balance of soil and atmospheric nutrition, which requires further research.

CONCLUSIONS

The increase in nitrogen and phosphorus availability in the mountain-meadow soil of the alpine lichen heath leads to changes in the nitrogen nutrition of plants not only due to the direct uptake of inorganic compounds but also presumably due to changes in the patterns of microbial community activity, mycorrhizal symbiosis, and symbiotic nitrogen fixation. This indirect effect on the nitrogen nutrition of plants is most clearly observed when the availability of phosphorus is increased, since this contributes to the mobilization of nitrogen from soil organic compounds by microorganisms and, possibly, to changes in the role of mycorrhiza in the nitrogen nutrition of plants. The effect of the increased availability of phosphorus on symbiotic nitrogen fixation proved to be unexpected: the intensity of nitrogen fixation decreased in the nitrogen-fixing legume species (*Oxytropis kubanensis*), while no signs of its appearance were detected for *Trifolium polyphyllum*, which does not fix nitrogen. The response to the nitrogen enrichment of the soil differs between plant species and may be due to their different competitive abilities for the resource and, possibly, to the shift in the role of mycorrhiza in the nitrogen nutrition of plants.

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