^{15}N Natural Abundance of Soil Microbial Biomass in Alpine and Tundra Ecosystems

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Abstract—Isotopic composition of nitrogen in soil microbial biomass ($\delta^{15}\text{N}_{\text{micr}}$) is connected with the transformation of nitrogen compounds and with the balance of carbon and nitrogen availability for microorganisms. We have studied the dependence of $\delta^{15}\text{N}_{\text{micr}}$ on nitrogen isotopic composition in the substrate ($\delta^{15}\text{N}$ of total and extractable nitrogen), as well as the dependence of $\delta^{15}\text{N}_{\text{micr}}$ and ^{15}N -enrichment of microbial biomass ($\Delta^{15}\text{N}_{\text{micr}} = \delta^{15}\text{N}_{\text{micr}} - \delta^{15}\text{N}_{\text{substr}}$) on nitrogen availability parameters (the C/N ratio in soil, the N-mineralization activity, the content of extractable nitrogen, and the nitrogen use efficiency) in soils of four alpine ecosystems in the North Caucasus and four tundra ecosystems in the Khibiny Mountains. It has been shown that $\delta^{15}\text{N}_{\text{micr}}$ varies from -0.2 to $+8.4\text{‰}$ and may be characterized by both ^{15}N -enrichment and depletion (negative $\Delta^{15}\text{N}_{\text{micr}}$ values) relative to the total and extractable soil nitrogen. As a rule, $\Delta^{15}\text{N}_{\text{micr}}$ is $1.5\text{--}3.1\text{‰}$ relative to $^{15}\text{N}_{\text{total}}$ and $0.6\text{--}4.8\text{‰}$ relative to $^{15}\text{N}_{\text{extr}}$. However, under the most N-deficiency conditions in soils of mountain tundra lichen and shrub heaths, N_{micr} does not accumulate an increased amount of ^{15}N . We have not revealed a close correlation of $\delta^{15}\text{N}_{\text{micr}}$ and $\Delta^{15}\text{N}_{\text{micr}}$ with the C/N ratio. The accumulation of ^{15}N in microbial biomass is much stronger related to N-mineralization (positively) and the nitrogen use efficiency (negatively). This testifies to the important role of microbial nitrogen dissimilation in controlling the isotopic composition of soil microbial biomass nitrogen.

Keywords: ^{15}N -enrichment, microbial biomass, nitrogen assimilation-dissimilation, isotope fractionation, N-mineralization, nitrogen use efficiency

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INTRODUCTION

Nitrogen (N) isotopes in the biosphere are fractionated as a result of discrimination of the heavy isotope ^{15}N in most transformation processes of nitrogen-containing compounds. This enables the use of the N isotopic composition of soils and plants to characterize the nitrogen cycle [11, 27] and its particular processes in ecosystems [2, 5, 9, 17]. Soil microorganisms are the key component of ecosystems responsible for the organic matter transformation and the control of the ratio between mineral and organic forms of nitrogen and its availability for plants. However, data on the isotopic composition of nitrogen in soil microorganisms are few, so this parameter is not widely used to characterize the nitrogen cycle and the transformation of nitrogen-containing compounds in ecosystems.

Scientists of the University of Northern Arizona have achieved the greatest success in studying the isotopic composition of soil microbial biomass nitrogen ($\delta^{15}\text{N}_{\text{micr}}$) [10, 13–15]. Prior to these studies, there were data on the enrichment of bacterial cultures with the ^{15}N isotope relatively to amino acid as the only nitrogen source [21], as well as of fruit bodies and

hyphae of ectomycorrhizal and saprotrophic fungi relatively to soil organic matter [18, 19, 29]. The scientists from Arizona have shown for the first time that the total microbial biomass is usually the most enriched with the ^{15}N isotope among nitrogen pools in soil [13, 15]. They also have proposed a model, which explains the variation of the ^{15}N -enrichment of microorganisms, depending on the relative carbon and nitrogen availability. This enabled the characterization of the processes of organic matter transformation in soils on the basis of data on the isotopic composition of carbon and nitrogen in soil microorganisms [10, 14].

The model is based on the concept that heterotrophic organisms, which release nitrogen into the environment during dissimilation, are enriched in the ^{15}N isotope relatively to the food consumed, because its discrimination during dissimilation is higher than during assimilation [12, 23, 25]. Therefore, the ratio between the amounts of the element involved in these metabolic processes determines the ^{15}N -enrichment of an organism. At high carbon and low nitrogen availability (a large C/N ratio in the substrate), microorganisms mainly assimilate nitrogen and are characterized by a relatively low $\delta^{15}\text{N}$ values, which is related to

the nitrogen isotopic composition in its main substrates. When nitrogen availability increases (a decrease in the C/N ratio in the substrate), its excess is mainly removed from the cells as ^{14}N , and $\delta^{15}\text{N}$ of microorganisms increases. This model is confirmed by studying a wide variety of Arizona soils, the soils of elevation and substrate age gradients [10, 13, 14] in particular, and semi-desert soils with different cattle manure input [15]. The model is also confirmed in soils of the substrate age gradient in Hawaii [14].

These outstanding works were followed by a number of new evidences, confirming the ^{15}N -enrichment of soil microbial biomass [20, 22, 26, 30, 32], as well as of some species of microorganisms during their cultivation on different nitrogen-containing media [28, 33]. Single studies confirmed the proposed model by a laboratory incubation experiment with the soil [20] and by the analysis of soils of different land-use systems [32].

However, this model has only been tested on a small number of soils, so it is not completely clear if it is universal for controlling $\delta^{15}\text{N}_{\text{micr}}$ under different soil conditions. In this regard, we tested the hypothesis of assimilation-dissimilation as an important mechanism for control $\delta^{15}\text{N}_{\text{micr}}$ by the example of two gradients of nitrogen availability in the alpine belt of the Caucasus and in the tundra belt of the Khibiny Mountains. According to this hypothesis, we expected that: (1) microbial biomass nitrogen is enriched in the ^{15}N isotope as compared to other soil nitrogen pools and (2) the value of ^{15}N -enrichment depends on the relative carbon and nitrogen availability for microorganisms (the C/N ratio in soil, the N-mineralization activity, and the nitrogen use efficiency).

OBJECTS AND METHODS

We have studied soils along two gradients, which reflect pronounced differences in the transformation of nitrogen compounds and its availability for organisms, depending on the topographic position.

The first object is a well-studied catena in the alpine belt of the North Caucasus on the northeastern slope of Malaya Khatipara Mount in the Teberda State Natural Biosphere Reserve (the Karachay-Cherkess Republic). We have studied soils of four biogeocenoses (alpine lichen heath (ALH), a *Festuca varia* grassland (FVG), a *Geranium-Hedysarum* meadow (GHM), and a snowbed community (SBC)). The biogeocenoses studied are allocated on different landforms: ALH on windward ridge and slope with a small snow accumulation in the winter, FVG on the middle part of slope with a more intensive snow accumulation, GHM on the lower part of slope and in depressions on the slope with a significant snow accumulation, and SBC on high-snow areas at the slope foot.

Mountain-meadow soils (Umbric Leptosols) of these ecosystems have been comprehensively described earlier, including the transformation of nitrogen com-

pounds and N isotopic composition [3–6]. They are characterized by a high content of organic matter and N.

The ammonium form ($\text{N}-\text{NH}_4^+$) predominates among inorganic nitrogen compounds. Smaller content of $\text{N}-\text{NH}_4^+$ and mineralization activities of organic nitrogen are typical for soils of ALH and SBC, which occupy extreme positions in the catena. The C/N ratio varies slightly, being lower in soils of ALH and GHM, where leguminous plants are present in plant communities [3, 6].

The second object is a catena in the tundra belt of Khibiny on the slope of Vud'yavrchorr Mount (Botanical cirque in the Polar-Alpine Botanical Garden-Institute (Murmansk oblast)). Soils of four biogeocenoses (shrub-lichen heath (SLH), shrub heath (SH), grass meadow (GM), and sedge meadow (SM)) have been studied there. The soils are represented by dry-peat humus-illuvial podbur (Folic Leptic Entic Podzols (Siltic)) of SLH, dry-peat raw-humus lithozem (Folic Leptosol) of SH, and dark-humus lithozems (Eutric Leptosols) of GM and SM. Nitrogen transformation processes and N isotopic composition are comprehensively described in our recent studies [1, 2]. These soils differ sharply in the content of extractable inorganic and organic N compounds. In soils of SLH and SH, the extractable N is mainly represented by organic compounds, the concentrations of $\text{N}-\text{NH}_4^+$ are low, and there are trace amounts of nitrates ($\text{N}-\text{NO}_3^-$). At the transition from heath soils to meadow soils, the concentrations of inorganic and organic N compounds increase 36–62 times and 3–8 times, respectively, and N of inorganic compounds begins to predominate, which completely corresponds to tens of times greater N-mineralization and nitrification activities. The C/N ratio in the surface organic horizons is 21–30 and does not pronouncedly depend on the position in the catena. It differs in the upper mineral horizons of soils of heaths (20.8–21.2) and meadows (14.0–15.9) [1].

We have analyzed the surface humus-accumulative horizon in soils of alpine ecosystems and two horizons (the surface organic (T or O) and the upper mineral (BFH or AH)) in soils of tundra ecosystems. The soils were sampled into plastic bags in eight replications in alpine ecosystems and in five replications in tundra ecosystems, were frozen no later than in five hours after the sampling and stored frozen until the analysis.

The nitrogen isotopic composition of soil microbial biomass was characterized by $\delta^{15}\text{N}$ of chloroform-labile nitrogen, which is used to estimate microbial biomass nitrogen by fumigation-extraction method [8]. Although the nitrogen fraction extracted from the chloroform-fumigated soils comprises only about a half of the total nitrogen of microbial biomass, the correspondence of their isotopic composition is proved in publications [13, 14]. Nitrogen was extracted from the initial and chloroform-fumigated

samples, using 0.05 M K₂SO₄ instead of the standard 0.5 M K₂SO₄. It has been shown that this smaller salt concentration does not significantly affect the extractability of chloroform-labile nitrogen, but provides more reproducible results of the isotope analysis due to higher nitrogen concentration in the analyzed sample: salt after evaporation of the K₂SO₄ extract [22].

Nitrogen extracted from non-fumigated (N_{extr}) and fumigated (N_{extr fum}) samples was concentrated by

evaporating 10 mL of the extract in a porcelain cup on the water bath at 60°C. Evaporated salts were homogenized by a metal spatula and ground by a porcelain pestle. We used 30–40 mg of salt for the isotopic analysis of N_{extr}, and 15–20 mg of salt for the analysis of N_{extr fum}. This salt amount contained 20–60 µg of N.

The calculation of δ¹⁵N_{micr} was based on the isotopic mass-balance with the use of data on the concentrations and isotopic composition of N_{extr} and N_{extr fum}:

$$\delta^{15}\text{N}_{\text{micr}} = \frac{\delta^{15}\text{N}_{\text{extr fum}} \times [\text{N}_{\text{extr fum}}] - \delta^{15}\text{N}_{\text{extr}} \times [\text{N}_{\text{extr}}]}{[\text{N}_{\text{micr}}]}$$

Then we calculated the ¹⁵N-enrichment of N_{micr} relative to substrate (N_{total} and N_{extr}):

$$\Delta^{15}\text{N}_{\text{micr-t}} = \delta^{15}\text{N}_{\text{micr}} - \delta^{15}\text{N}_{\text{total}},$$

$$\Delta^{15}\text{N}_{\text{micr-e}} = \delta^{15}\text{N}_{\text{micr}} - \delta^{15}\text{N}_{\text{extr}}.$$

The values of δ¹⁵N_{extr} and δ¹⁵N_{extr fum} were determined in the Center for Research and Analysis of Stable Isotopes at the University of Göttingen on an elemental analyzer with a Delta^{plus} mass spectrometer. The data on the concentrations of different forms of carbon and nitrogen and N-mineralization activity were taken from published studies [1, 3, 4, 6] (Table 1). The nitrogen availability was characterized by C_{total}/N_{total} and C_{extr}/N_{extr} ratios, N-mineralization activity, and concentrations of N_{extr} and C_{extr}. We also calculated the N_{extr} portion of N_{micr} and the C_{extr} portion of C_{micr}, as well as the nitrogen use efficiency (NUE), which reflects the distribution of nitrogen taken up by microorganisms between microbial biomass and dissimilation as inorganic compounds [24]:

$$\text{NUE} = \text{N}_{\text{micr}} / (\text{N}_{\text{micr}} + \text{N-NH}_4^+ + \text{N-NO}_3^-).$$

High NUE indicates that most of nitrogen taken up by microorganisms is fixed in the biomass, and a small inorganic N amount is released into the environment. Low NUE, on the contrary, shows that a significant part of nitrogen is not fixed in the biomass of microorganisms, but is released into the environment.

The nonparametric Kruskal–Wallis test was used to check the significance of the effect of the biogeocenosis factor on the studied parameters. The relationship between δ¹⁵N_{micr}, δ¹⁵N_{total}, and δ¹⁵N_{extr}, as well as between δ¹⁵N_{micr}, Δ¹⁵N_{micr-t}, and Δ¹⁵N_{micr-e}, on the one hand, and the parameters of nitrogen availability (C_{total}/N_{total}, C_{extr}/N_{extr}, the N_{extr} portion of N_{micr}, N-mineralization, and NUE), on the other hand, was assessed by the Spearman correlation coefficient.

RESULTS AND DISCUSSION

In the studied soils, δ¹⁵N_{micr} varies from –0.2 to +8.4‰ (Table 2). In humus horizons of soils in alpine ecosystems, it is 4.5–7.4‰. The values are similar for the upper mineral horizons of tundra soils (4.9–8.4‰), but are significantly lower (from –0.2 to 2.5‰) in organic horizons.

There is a good agreement of δ¹⁵N_{micr} in humus horizons with previously published results. There are evidence of the values in the range 6.3–7.2‰ for mountain-meadow alpine soils [22]; and within 4.7–5.9‰ for soils of coniferous and mixed forests in Austria [26], coniferous plantations in Central China [32], and grass ecosystems in Kansas prairies [31]. Higher values (7–11‰) were obtained for plowed Luvisol in France [20] and for soils of the elevation gradient in northern Arizona [14].

However, even higher values of δ¹⁵N_{micr} were also published: 13–18‰ for semidesert soils of Arizona with different input of cattle manure [13, 15]; 12.4–15.9‰ for plowed soils of central China [32]; and 10.4–16.5‰ for Luvisols, Chernozems, and Kastanozems of the Russian Plain [22].

Low δ¹⁵N_{micr} values (1.8–2.3‰) similar to those obtained for organic horizons are also recorded, for example, in soils under grass communities in Kansas [30].

Soil microbial biomass nitrogen in alpine and tundra ecosystems is mainly characterized by ¹⁵N-enrichment relative to N_{total} and N_{extr} (Δ¹⁵N_{micr-t} is 1.5–3.1‰, and Δ¹⁵N_{micr-e} is 0.6–4.8‰). Humus horizons in soils of SLH and SH and the organic horizon in soil of SLH in the Khibiny mountain tundra are characterized by absence of ¹⁵N-enrichment of microbial biomass relative to N_{total} (Δ¹⁵N_{micr-t} is from –0.2 to +0.3‰) and even by ¹⁵N-depletion of microbial biomass relative to N_{extr} (Δ¹⁵N_{micr-e} is from –0.6 to –1.6‰).

In published studies, N_{micr} is also usually characterized by ¹⁵N-enrichment. For example, in soils of Arizona formed under different bioclimatic condi-

Table 1. The content of C and N fractions and N-mineralization in soils of mountain-tundra and alpine ecosystems, mean $\pm$ standard deviation (according to [1, 3, 4, 6])

Biogeo- cenosis	C _{total} , %	C _{extr}	C _{micr}	N _{total} , %	N _{extr}	N _{micr}	N–NH ₄ ⁺	N–NO ₃ [–]	N-mine- ralization, mg N/kg per day
		mg/kg			mg/kg				
Alpine ecosystems, the A horizon									
ALH	11.0 ± 1.3	91 ± 20	1074 ± 185	0.97 ± 0.12	28.0 ± 7.3	120 ± 25	16.3 ± 4.8	2.2 ± 0.5	0.13 ± 0.02
FVG	10.3 ± 0.9	227 ± 43	1110 ± 126	0.79 ± 0.06	53.2 ± 10.3	94 ± 11	28.3 ± 9.5	1.0 ± 0.3	0.63 ± 0.16
GHM	10.6 ± 2.1	189 ± 39	1016 ± 269	0.89 ± 0.17	77.4 ± 16.5	105 ± 38	49.7 ± 9.9	0.9 ± 0.3	1.07 ± 0.04
SBC	11.8 ± 1.5	248 ± 59	1011 ± 377	0.88 ± 0.08	49.2 ± 17.3	86 ± 30	23.2 ± 10.1	1.0 ± 0.4	0.33 ± 0.05
Mountain-tundra ecosystems, organic horizon (T or O)									
SLH	23.6 ± 5.2	166 ± 41	1015 ± 202	1.12 ± 0.20	11.7 ± 1.3	78 ± 25	1.8 ± 1.0	0.12 ± 0.00	0.35 ± 0.04
SH	32.2 ± 2.2	466 ± 106	3205 ± 316	1.23 ± 0.04	28.1 ± 8.6	285 ± 50	2.4 ± 3.3	0.22 ± 0.00	0.79 ± 0.12
GM	40.7 ± 2.6	1402 ± 333	3991 ± 869	1.38 ± 0.19	160 ± 26	357 ± 58	86 ± 16	0.96 ± 0.77	15.9 ± 1.1
SM	36.0 ± 1.5	1290 ± 303	1765 ± 284	1.72 ± 0.21	197 ± 57	195 ± 51	113 ± 40	4.64 ± 2.94	14.9 ± 3.1
Mountain-tundra ecosystems, upper mineral (organic–mineral) horizon (BFH or AH)									
SLH	10.8 ± 4.3	101 ± 21	247 ± 113	0.50 ± 0.16	9.7 ± 0.9	23 ± 7	1.6 ± 1.0	0.11 ± 0.00	0.31 ± 0.03
SH	9.3 ± 2.2	124 ± 19	119 ± 20	0.43 ± 0.08	10.4 ± 2.7	14 ± 4	1.9 ± 0.7	0.11 ± 0.00	0.50 ± 0.05
GM	14.6 ± 4.6	248 ± 40	410 ± 86	0.91 ± 0.26	32.9 ± 7.1	35 ± 6	13.7 ± 3.8	2.72 ± 1.01	4.7 ± 0.7
SM	11.0 ± 0.7	461 ± 41	131 ± 18	0.79 ± 0.04	39.7 ± 3.5	15 ± 3	7.7 ± 1.0	1.93 ± 0.43	3.8 ± 0.5

N-mineralization was determined by incubation in a laboratory during 20 days at natural soil moisture and temperature of 22°C for soils of Khibiny and in field conditions during 46 days in July–August for soils of the Caucasus.

tions, ^{15}N -enrichment averages 3.2‰ relative to N_{total} and 3.7‰ relative to N_{extr} [13]. Mountain-meadow soils of the Teberda Reserve were also previously characterized by $\Delta^{15}\text{N}_{\text{micr}}$ close to our present data (2.3–2.4‰ relative to N_{total} and 1.2–3.7‰ relative to N_{extr}) [22].

Slightly higher ^{15}N -enrichment of microbial biomass relative to N_{total} is recorded in soils of coniferous and mixed forests in Austria (4–5‰) [26] and in soils of Central China (1.8–6.8‰) [32]. Much higher ^{15}N -enrichment was obtained for very contrasting soils. For example, very high enrichment (17‰) relative to N_{total} is recorded in tundra soils in northern Norway [7], but in Luvisols, Chernozems, and Kastanozems of the Russian Plain, this parameter does not exceed 10‰. Relative to N_{extr} , ^{15}N -enrichment of microbial biomass in these soils reaches 11.9–14.5‰ [22].

However, soil microbial biomass is not always characterized by enrichment with the ^{15}N isotope in comparison with other nitrogen pools. For example, N_{micr} in plowed Luvisol in France was not ^{15}N -enriched relative to both N_{total} and N_{extr} . This parameter reached 2 and 3.5‰ relative to N_{total} and N_{extr} , respectively, only as a result of the laboratory incubation of soil [20].

In soils under grass communities in Kansas, different researchers revealed either a very small ^{15}N -enrichment of microbial biomass relative to N_{total} (0.1–0.6‰) [31]

or its depletion (from –0.8 to –1.4‰) as well as enrichment (1.3–1.8‰) [30]. The enrichment relative to N_{extr} was more pronounced in the both cases: 2.2–3.0‰ [31] and 0–3.5‰ [30].

The ^{15}N -enrichment of microorganisms relative to substrate was also shown during the cultivation of fungus (*Saccharomyces cerevisiae*), bacterium (*Escherichia coli*), and archaea (*Sulfolobus tokodaii* and *Halo-bacterium salinarum*) on media with casamino acids as a nitrogen source. It comprised 3.6 ± 0.2 , 0.6 ± 0.2 , and 3.5 ± 0.7 ‰ for fungus, bacterium, and archaea, respectively. Individual amino acids of microorganisms were characterized by a wide range of ^{15}N -enrichment (from –3.0 to 9.0‰) [33].

The high $\delta^{15}\text{N}_{\text{micr}}$ and ^{15}N -enrichment of soil microbial biomass seems to be related to the greater discrimination of the ^{15}N isotope during nitrogen dissimilation as compared to its assimilation, and, therefore, these parameters should correlate with nitrogen involvement into metabolic processes of microorganisms [10, 13–15]. At the same time, $\delta^{15}\text{N}_{\text{micr}}$ is affected by the isotopic composition of nitrogen substrates used by microorganisms, which is integrally characterized by $\delta^{15}\text{N}_{\text{total}}$ and $\delta^{15}\text{N}_{\text{extr}}$ and is more precisely described by $\delta^{15}\text{N}$ of individual compounds, which are mainly absorbed by microorganisms.

A positive correlation between $\delta^{15}\text{N}_{\text{micr}}$ and $\delta^{15}\text{N}_{\text{total}}$ enabled to conclude that there is a direct effect of the

Table 2. Parameters of relative C and N availability for microorganisms, $\delta^{15}\text{N}$ of nitrogen fractions, and ^{15}N -enrichment of microbial biomass (‰) in soils of mountain-tundra and alpine ecosystems, mean $\pm$ standard deviation

Biogeocenosis	$C_{\text{total}}/N_{\text{total}}$	$C_{\text{extr}}/N_{\text{extr}}$	$C_{\text{micr}}/N_{\text{micr}}$	$C_{\text{extr}}/\%$ of C_{micr}	$N_{\text{extr}}/\%$ of N_{micr}	NUE	$\delta^{15}\text{N}_{\text{total}}$	$\delta^{15}\text{N}_{\text{extr}}$	$\delta^{15}\text{N}_{\text{micr}}$	$\Delta^{15}\text{N}_{\text{micr-t}}$	$\Delta^{15}\text{N}_{\text{micr-e}}$
Alpine ecosystems, the A horizon											
ALH	11.3 ± 0.8	3.3 ± 0.6	9.0 ± 1.0	9 ± 2	23 ± 4	0.87 ± 0.02	3.0 ± 0.4	3.9 ± 1.0	4.5 ± 1.2	1.5 ± 0.7	0.6 ± 1.0
FVG	13.0 ± 0.4	4.3 ± 0.7	11.8 ± 0.5	21 ± 3	59 ± 8	0.76 ± 0.04	4.4 ± 0.6	3.1 ± 0.9	6.8 ± 0.9	2.4 ± 0.9	3.7 ± 0.5
GHM	12.0 ± 0.5	2.4 ± 0.3	9.7 ± 1.6	19 ± 7	74 ± 12	0.67 ± 0.06	4.6 ± 0.8	2.6 ± 0.4	7.4 ± 1.3	2.8 ± 1.1	4.8 ± 1.1
SBC	13.4 ± 0.5	5.0 ± 1.2	11.8 ± 0.8	24 ± 8	57 ± 11	0.78 ± 0.09	4.2 ± 0.7	3.0 ± 2.2	6.6 ± 1.3	2.4 ± 1.0	4.3 ± 1.1
Mountain-tundra ecosystems, organic horizon (T or O)											
SLH	21.0 ± 1.1	14.2 ± 2.8	12.8 ± 1.2	16 ± 2	15 ± 2	0.97 ± 0.04	2.4 ± 1.4	2.8 ± 0.8	2.2 ± 1.3	-0.2 ± 0.7	-0.6 ± 0.6
SH	26.3 ± 2.0	16.6 ± 3.2	11.4 ± 1.5	15 ± 1	10 ± 2	0.99 ± 0.02	-0.3 ± 0.8	1.3 ± 0.4	2.5 ± 1.1	2.8 ± 0.4	1.2 ± 0.9
GM	29.8 ± 3.8	8.8 ± 1.6	11.1 ± 1.4	35 ± 6	45 ± 8	0.80 ± 0.05	-2.1 ± 0.9	-1.5 ± 0.9	-0.2 ± 0.8	1.9 ± 0.7	1.3 ± 1.0
SM	21.1 ± 1.9	6.5 ± 1.3	9.3 ± 1.2	73 ± 5	101 ± 16	0.62 ± 0.08	-1.7 ± 0.7	-1.1 ± 0.7	1.0 ± 1.2	2.7 ± 0.9	2.1 ± 1.2
Mountain-tundra ecosystems, upper mineral (organic-mineral) horizon (BH or AH)											
SLH	20.8 ± 3.4	10.4 ± 1.8	10.8 ± 0.9	41 ± 8	42 ± 8	0.93 ± 0.03	4.6 ± 0.8	6.5 ± 0.4	4.9 ± 2.0	0.3 ± 1.3	-1.6 ± 1.4
SH	21.2 ± 1.2	11.9 ± 1.9	9.1 ± 1.0	104 ± 25	74 ± 17	0.87 ± 0.08	5.4 ± 0.3	5.9 ± 0.7	5.2 ± 1.3	-0.2 ± 1.1	-0.7 ± 0.6
GM	15.9 ± 0.7	7.5 ± 0.4	11.7 ± 1.4	60 ± 8	94 ± 22	0.68 ± 0.05	5.3 ± 0.5	5.1 ± 1.1	8.4 ± 1.7	3.1 ± 1.0	3.3 ± 0.5
SM	14.0 ± 0.2	11.6 ± 0.6	8.6 ± 1.1	352 ± 58	264 ± 45	0.61 ± 0.03	5.2 ± 0.1	6.2 ± 0.2	8.0 ± 1.1	2.8 ± 1.1	1.8 ± 1.1

Fig. 1. The relationships between isotopic compositions of soil microbial biomass nitrogen and other soil nitrogen pools and nitrogen availability. Here and in Figs. 2 and 3: the solid trend line is for all the samples analyzed; gray squares and dashed trend line are for the A horizon of alpine soils; white circle and dashed trend line are for organic horizons of mountain-tundra soils; and black circle and dashed trend line are for the upper mineral horizons of mountain-tundra soils. Statistically significant approximation coefficients are only given ($p < 0.05$).

source of nitrogen nutrition of microorganisms on $\delta^{15}\text{N}_{\text{micr}}$ in soils of Central China [32]. However, there is an opinion that the fractionation of isotopes during nitrogen uptake and metabolism in microorganisms may significantly affect the $\delta^{15}\text{N}_{\text{micr}}$, and therefore this parameter can hardly reliably identify nitrogen substrate used by microorganisms under natural conditions [16].

We have found a direct correlation between $\delta^{15}\text{N}_{\text{micr}}$ and $\delta^{15}\text{N}_{\text{total}}$ for all the studied samples of alpine and tundra soils and for particular groups of soil horizons (Fig. 1). Low $\delta^{15}\text{N}_{\text{micr}}$ in organic horizons may be related to a lighter nitrogen isotopic composition of substrate used by microorganisms. However, $\delta^{15}\text{N}_{\text{micr}}$ in the organic horizons of tundra soils of SLH and SH

characterized by low nitrogen availability is greater than in the corresponding horizons of soils of GM and SM, which are characterized by much higher N-mineralization activity. This unexpected result may be presumably explained by a large portion of mycelium of ericoid mycorrhizal fungi in the microbial biomass of organic horizons in SLH and SH soils. It is known that under conditions of low nitrogen availability, mycorrhizal fungi intensively fractionate nitrogen isotopes and accumulate ^{15}N [16].

This untypical regularity of $\delta^{15}\text{N}_{\text{micr}}$ formation in organic horizons of tundra soils results in its slighter correlation with such parameters of nitrogen availability as the N_{extr} portion of N_{micr} and NUE for all the studied soil samples. In addition, the relationship with

N-mineralization is not direct, but inverse. These correlations are much closer in mineral horizons of soils of alpine and tundra ecosystems (Fig. 1). This corresponds to smaller $\delta^{15}\text{N}_{\text{micr}}$ in soils of ALH, SLH, and SH characterized by low nitrogen availability. There is a negative correlation between $\delta^{15}\text{N}_{\text{micr}}$ and the $C_{\text{total}}/N_{\text{total}}$ ratio, which corresponds to a decrease in the ^{15}N accumulation in microorganisms on nitrogen-poor substrates.

The positive correlation between $\delta^{15}\text{N}_{\text{micr}}$ and $\delta^{15}\text{N}_{\text{extr}}$ is considerably weaker and even negative within the groups of the upper mineral horizons. This confirms the opinion that the integral characteristic of the nitrogen cycle is usually well reflected in isotopic data by $\delta^{15}\text{N}_{\text{total}}$, while the characterization of particular processes by isotopic parameters of more labile pools of soil nitrogen under natural conditions is rather difficult [11, 27]. For example, the difference in $\delta^{15}\text{N}$ between various fractions of extractable nitrogen in the studied soils may exceed 10–20‰ [2, 5], and it is not known, which of them are mainly absorbed by microorganisms under different soil conditions.

The correlation between $\delta^{15}\text{N}_{\text{micr}}$, the nitrogen isotopic composition of substrate, and the nitrogen dissimilation activity is not always obvious, also because the effect of physiological fractionation of isotopes and of the isotopic composition of substrate can be both unidirectional and partially compensating. When nitrogen availability is low, the fractionation of isotopes during assimilation-dissimilation and during the absorption of a limited resource is small. As a result, $\delta^{15}\text{N}_{\text{micr}}$ becomes close to $\delta^{15}\text{N}$ of the main nitrogen sources. In case of high nitrogen availability, strong fractionation of isotopes during the assimilation-dissimilation causes an increase in $\delta^{15}\text{N}_{\text{micr}}$, on the one hand. On the other hand, the fractionation during nitrogen absorption also increases, and the predominating ^{14}N adsorption results in the compensation of the increase in $\delta^{15}\text{N}_{\text{micr}}$. This may be seen in organic horizons of soils of mountain-tundra meadows (GM and SM), which are characterized by high concentration of inorganic N and N-mineralization activity, but lower $\delta^{15}\text{N}_{\text{micr}}$ as compared to organic horizons in of SLH and SH soils (Table 2).

In soils of alpine and tundra ecosystems, ^{15}N -enrichment of microbial biomass relative to N_{total} and N_{extr} may well or slightly correspond to the known regularities, which include a negative correlation with the $C_{\text{total}}/N_{\text{total}}$ and $C_{\text{extr}}/N_{\text{extr}}$ ratios [10, 14, 30, 32] and a positive correlation with the N-mineralization activity [14]. The former correlation in the studied soils is mainly insignificant: there is only a weak tendency for all the studied samples (the correlation is only statistically significant for $\Delta^{15}\text{N}_{\text{micr-e}}$ and $C_{\text{extr}}/N_{\text{extr}}$) (Figs. 2, 3). It is the most pronounced for the mineral horizons of mountain-tundra soils. Thus, contrary to a close neg-

ative correlation of the $C_{\text{total}}/N_{\text{total}}$ ratio with $\delta^{15}\text{N}_{\text{micr}}$, such a relationship with $\Delta^{15}\text{N}_{\text{micr-o}}$ and $\Delta^{15}\text{N}_{\text{micr-e}}$ is not revealed. This is explained by the relative nitrogen enrichment of organic matter in soil of ALH, which is characterized by low nitrogen availability, and by the absence of such a relationship in organic horizons of tundra soils.

The direct correlation of $\Delta^{15}\text{N}_{\text{micr-t}}$ and $\Delta^{15}\text{N}_{\text{micr-e}}$ with N-mineralization is closer, in mineral horizons in particular. There is also a direct relationship between the ^{15}N -enrichment of microbial biomass and the absolute and relative N_{extr} concentrations, as well as a negative correlation between the ^{15}N -enrichment and NUE. Thus, all the parameters of the nitrogen dissimilation activity of microorganisms are related to the ^{15}N -enrichment of microbial biomass. Our result confirms the efficiency of the earlier proposed approach to the control of the isotopic composition of microbial biomass in soil based on the variation in the relative carbon and nitrogen availability for microbial nutrition, which determines the level of nitrogen dissimilation by microorganisms [10, 14]. This hypothesis was also confirmed by the results of the field experiment with the fertilization of grass ecosystems in Kansas: the ^{15}N -enrichment of soil microbial biomass was significantly higher in soils with the application of nitrogen fertilizers as compared to soils without them. This parameter was also in positive correlation with the amount of inorganic nitrogen and in negative correlation with the C/N ratio [30].

The dissimilation hypothesis of the ^{15}N -enrichment of microbial biomass was also recently confirmed by the cultivation of *Aspergillus oryzae* on five media with different C/N ratios (from 5 to 100), using glycine as the only nitrogen source. Intensive release of NH_4^+ , depleted of the ^{15}N isotope during the growing of *A. oryzae* on media with the C/N ratio <30 was accompanied by an increase in $\delta^{15}\text{N}_{\text{micr}}$. On media with C/N > 30, nitrogen was fixed in the biomass of *A. oryzae*, and $\delta^{15}\text{N}_{\text{micr}}$ was not changed by more than 1‰. There was a negative correlation between the ^{15}N -enrichment of N_{micr} relative to glycine and NUE and the C/N ratio in the substrate [28].

Thus, nitrogen availability parameters are good predictors of $\delta^{15}\text{N}_{\text{micr}}$ and ^{15}N -enrichment of microbial biomass, while the C/N ratios in soil and in extractable components indirectly characterize the relative carbon and nitrogen availability and to a smaller extent determine the ^{15}N -enrichment. Contrary to them, the expected relationship of carbon availability parameters (C_{extr} and the C_{extr} portion of C_{micr}) with the parameters of the isotopic composition of microbial biomass is absent (Figs. 2, 3). The high availability of carbon should favor greater assimilation and lower dissimilation of nitrogen and result in low $\delta^{15}\text{N}_{\text{micr}}$ and ^{15}N -enrichment. However, in the studied

Fig. 2. The relationships between ^{15}N -enrichment of microbial biomass relative to total soil nitrogen and parameters of nitrogen and carbon availability.

soils, the concentrations of extractable carbon and nitrogen are in direct correlation ($R^2 = 0.84$, $p < 0.001$). As a result, the parameters of carbon availability are positively related with the parameters of the isotopic composition of microbial biomass nitrogen. A positive correlation between the ^{15}N -enrichment of microbial

biomass and the concentration of C_{extr} is also typical for soils of grass ecosystems in Kansas [30]. We have found only one study [15], where a negative correlation between the ^{15}N -enrichment of microbial biomass relative to extractable nitrogen and the concentration of extractable carbon is shown.

Fig. 3. The relationships between ^{15}N -enrichment of microbial biomass relative to extractable soil nitrogen and parameters of nitrogen and carbon availability.

CONCLUSIONS

The soil microbial biomass in alpine and mountain-tundra ecosystems is usually enriched in the ^{15}N isotope relative to the total and extractable nitrogen. The enrichment is related to the rate of nitrogen dissimila-

tion by microorganisms, depending on the nitrogen availability. The parameters of N availability include the absolute and relative concentrations of extractable nitrogen, the N-mineralization activity, and the nitrogen use efficiency by microorganisms. The C/N ratio

in soil and extractable compounds is not always a reliable indicator of the ^{15}N -enrichment of soil microbial biomass, and the absolute and relative concentration of extractable carbon is not related to high carbon and low nitrogen availability in terms of the isotopic composition of microbial biomass nitrogen.

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CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest.

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