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Stable Carbon and Nitrogen Isotopes in Woody Plants and Herbs near the Large Copper Smelting Plant

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Abstract—Variations of stable carbon (^{13}C and ^{12}C) and nitrogen (^{15}N and ^{14}N) isotopic composition are analyzed in forest plants subjected to the emissions of large copper smelting plant. The studies were carried out in pine forests at ten test plots near the Karabash copper smelting plant and in the Ilmen State Reserve at South Urals. The ^{13}C/^{12}C and ^{15}N/^{14}N isotopic ratios were analyzed in leaves of plants of different functional groups (with ecto-, ericoid, or arbuscular mycorrhiza; with nitrogen-fixing symbiosis, and non-mycorrhizal). The ^{13}C/^{12}C ratio did not change under technogenic pollution. The low isotopic ^{15}N/^{14}N ratio was established in ectomycorrhizal trees, while the high ratio was found in herbs with arbuscular mycorrhiza, nitrogen-fixing symbiosis, and non-mycorrhizal groups. As compared to nonpolluted habitats, the ^{15}N content in leaves near the copper smelting plant increases by 2.7‰ in the ectomycorrhizal trees and by 3.4‰ in undershrubs with ericoid mycorrhiza, and by 2.2‰ in herbs with arbuscular mycorrhiza. This indicates a significant change in conditions of mineral feeding of plants under heavy metal pollution of natural ecosystems.

Keywords: technogenic pollution, heavy metals, stable carbon isotopes, ^{13}C/^{12}C, ^{15}N/^{14}N, functional groups of plants

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INTRODUCTION

The contents of stable carbon ^{13}C and ^{12}C and nitrogen ^{15}N and ^{14}N isotopes in organisms and other components of ecosystems are successfully used for study of the intensity and tendencies of physiological and ecological processes (Robinson, 2001; Dawson et al., 2002; Tiunov, 2007; Makarov, 2009). The ratio of ^{13}C to ^{12}C (the δ^{13}C value) in plants depends on many factors: types of photosynthesis; biochemical compositions of cells, tissues, and organs; canopy structures of photosynthesizing organs; and external conditions. The ratio of ^{15}N to ^{14}N (the δ^{15}N values) reflects the diversity of nitrogen sources, the presence of symbiotic nitrogen fixation, and other symbioses. The direction and differences in δ^{15}N between diverse species or functional groups of plants could indicate the general level of nitrogen saturation in ecosystem, the degree of nitrogen accessibility, and the degree of competition between species for nitrogen (Martinelli et al., 1999; Robinson, 2001; Makarov, 2009; Menge et al., 2011). Since the nitrogen isotope composition reflects a general change of edaphic conditions in ecosystems (Robinson, 2001), its analysis is successfully applied in studying the ecosystem dynamics (Vitousek et al., 1989; Hobbie et al., 2005; Compton et al., 2007; Menge et al., 2011), including postpyrogenic successions (Hyodo et al., 2013).

Anthropogenic and technogenic pollution of atmosphere causes diverse variations of δ^{13}C values of plants. Tree rings during pollution frequently record a positive shift of δ^{13}C (Niemelä et al., 1997; Savard et al., 2004; a review: Savard, 2010), which is usually explained by photosynthesis at closed stomata (Savard, 2010). However, there are data that the content of ^{13}C may decrease during pollution (Kwak et al., 2009; Cada et al., 2016). Data on dynamics of nitrogen isotope composition under anthropogenic impact are also ambiguous. Urbanization and gaseous pollutants provide both enrichment (Gebauer et al., 1994; Korontzi et al., 2000; Pearson et al., 2000) and depletion (Kwak et al., 2009) of plants in ^{15}N. It is noteworthy that ^{15}N enrichment could be observed in the

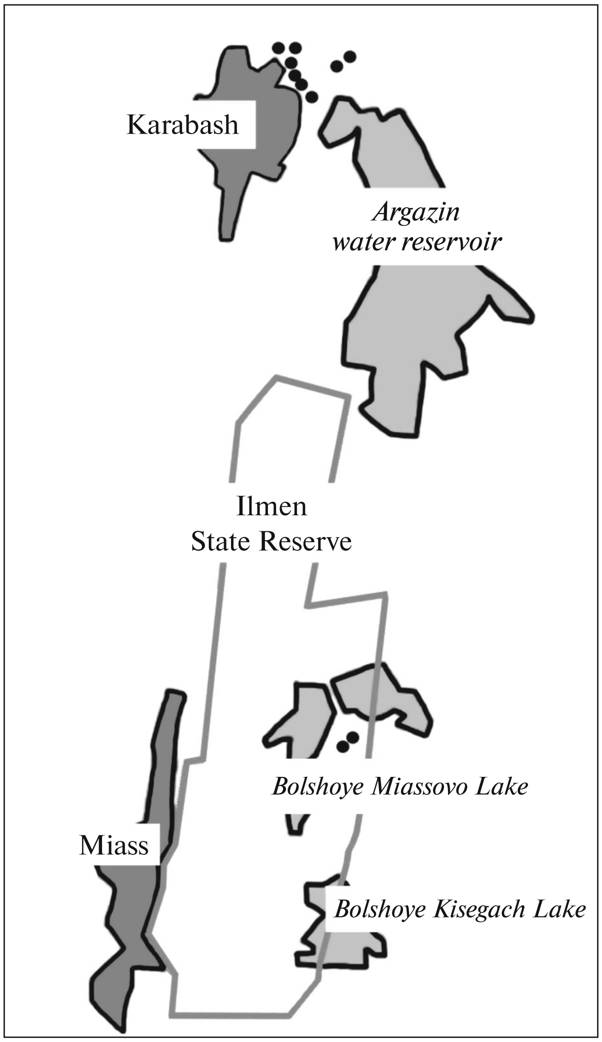

Fig. 1. Area and location of test plots (•).

absence of N-bearing components in emissions (Hofmann et al., 1997). In general, data on peculiar discrimination between stable carbon and nitrogen isotopes under the influence of different types and degrees of pollution are insufficient for unambiguous conclusions. At the same time, the better understanding of mechanisms of $\delta^{13}C$ and $\delta^{15}N$ formation in plants may provide insight into mechanisms of degradation and stability of ecosystems under anthropogenic impacts.

The aim of this work is to analyze the stable carbon and nitrogen isotope variations in forest plants under strong transformations of natural ecosystems by emissions of large copper smelter production in the South Urals. To decrease the uncertainty regarding the influence of industrial pollution on $\delta^{13}C$ and $\delta^{15}N$ in plants based on published data, the following working hypothesis was formulated and testified: heavy metal pollution of natural ecosystems causes an increase of heavy ^{13}C and ^{15}N isotopes in leaves.

MATERIALS AND METHODS

The study area and test plots. The study area is ascribed to the subzone of south taiga pine–birch forests of the eastern macroslope of the South Urals (the vicinities of the city of Karabash and the Ilmen State Reserve of the Ural Branch of the Russian Academy of Sciences, Chelyabinsk oblast). It is characterized by rises usually 250–600 m above sea level. There are brown mountain forest soils, brown forest soils, podzolic gleyey, gray mountain forest soils, mountain forest chernozems, and mountain podzolic thin soils. Climate is continental, moderately cold. The coldest month is January (average monthly temperature of −16...−17°C); the warmest month is July (+18°C); the vegetation period is 160–170 days long; the annual precipitation is about 430 mm per year; the snow cover height up to 40 cm. The predominant types of vegetation are pine–mixed-herb forests and derivative gramineous–mixed herb birch forest.

The ecosystems of the region experienced strong anthropogenic transformations, including industrial pollutions. The Karabash copper smelting plant (KCSP, JSC Karabashmed, Karabash city) is the large source of emissions, the main of which are SO_2 and heavy metal dust. The production was launched in 1910 and emissions reached maximum volumes (up to 140–360 thou. tons per year) in 1970–1980 (Kozlov et al., 2009). During 1990–1998, the copper production was terminated and after repeated opening and renovation the emission volumes decreased to about 10 thou tons (*Complex* ..., 2009). Owing to the accumulated strongest technogenic pollution, zonal ecosystems on the territories closest to the plant were completely destroyed: vegetation and the upper parts of original soils are absent and a vast technogenic wasteland was formed.

Ten sample plots (SPs) were studied. Eight SPs are located at a distance of 6–9 km northeast of the KCSP (impact zone); two SPs are located 48–50 km south of the KCSP (Ilmen State Reserve) in the pine forests with different forest stand ages and last fires of different age (Fig.1; Table 1). The areas were located in the medium landforms at fragmentary mountain and mountain forest brown immature soils. The accumulation levels of heavy metals ejected by KCSP in two-year needles of *Pinus sylvestris* L. are as follows (in μg/g): 8–18 Cu, 70–150 Zn, and 30–105 Pb in the impact zone of KCSP; 2–3 Cu, 40–45 Zn, 1.5–3 Pb in the Ilmen State Reserve (Koroteeva et al., 2015b). An important factor of the state of forest ecosystems of the region are the forest fires (Chibilev et al., 2016). Therefore, SPs were selected to represent a wide time range of last fire. The fire time was determined on the basis of record in the "Accounting Time of Forest Fires" of the Karabash forestry.

Functional groups of plants. The values of $\delta^{13}C$ and $\delta^{15}N$ were determined in leaves of different functional groups, which were distinguished according to the

Table 1. Characteristics of sample plots

Ordinal no.	Coordinates		Time after the last fire, years	Stand composition	Age of the main pine generation, years	Crown cover, %	Cover of herb-subshrub layer, %	Thickness of litter, cm
	N	E						
Ilmen Reserve								
1	55.14124	60.32477	6	10Pinus	190	30–40	50–60	3–5
2	55.13859	60.32898	>60	10Pinus + Betula	170	50–60	70–80	6–8
Impact zone of KCSP								
3	55.51866	60.32880	12	10Pinus + Betula	130	50–60	30–40	8–10
4	55.50965	60.32365	13	7Pinus3Betula	110	50–60	30–40	8–10
5	55.51314	60.27678	7	7Pinus3Betula	105	50–60	30–40	5–7
6	55.51096	60.28785	6	8Pinus2Betula	30	50–60	1–5	3–4
7	55.50222	60.29142	12	10Pinus + Betula	100	30–40	5–10	8–12
8	55.51314	60.27678	7	10Pinus + Betula	72	50–60	5–10	5–9
9	55.49874	60.29126	3	8Pinus2Betula	75	40–50	5–10	3–4
10	55.51096	60.28785	>30	8Pinus2Betula	30	40–50	20–30	5–7

type of symbiosis implemented in the underground sphere. Correspondingly, groups are the groups of plant species: with ectomycorrhiza (ECM); with ericoid mycorrhiza (ER); with arbuscular mycorrhiza (AM); with nitrogen-fixing symbiosis (N_2f); and non-mycorrhizal (noM). These groups can be interpreted as the groups of plants of different life forms: ECM—trees, ER—evergreen undershrub; AM, N_2f, and noM—herbaceous plants. At each SP, we collected samples of plant leaves of each functional group, in some cases, several taxa within group: ECM—*Pinus sylvestris* and *Betula* spp.; ER—*Vaccinium vitis-idaea* L.; AM—*Rubus saxatilis* L.; *Calamagrostis arundinacea* (L.) Roth; one of two Asteraceae species (*Trommsdorffia maculata* (L.) Bernh. or *Saussurea controversa* DC.); N_2f—*Lathyrus vernus* (L.) Bernh.; noM—*Silene nutans* L.

At each SP, 3–5 leaves of one species were collected and united in a mixed sample. Additionally, a mixed sample each of litter (fermentative horizon) and upper layer of mineral part of soil (3–5 cm below the litter) were collected by the envelope method. The samples were dried at first in the shadow up to the air-dried state, and then at 70°C for 48 hours. In total, we analyzed 87 samples: 67 plant samples and 10 samples each from litter and mineral part of the soil.

Isotopic analysis was carried out at the Geonauka Center of Collective Use of the Institute of Geology of the Komi Science Center, Ural Branch of the Russian Academy of Sciences. Carbon and nitrogen isotopic compositions were measured by mass spectrometry in a constant helium flux (CF-IRMS) on an analytical complex including a Flash EA 1112 elemental analyzer connected via a Conflo IV split interface with a Delta V Advantage mass spectrometer (Thermo Fisher Scientific). Nitrogen and carbon isotopic compositions were expressed in per mille deviation from VPDB and AIR (atmospheric air) international standards, δ (‰):

$$\delta X_{\text{sample}} = ((R_{\text{sample}}/R_{\text{standart}}) - 1) \times 100, \qquad (1)$$

where X is the element (nitrogen and carbon), R is the molar ratio of heavy to light isotopes of the corresponding element. The mass spectrometer was calibrated using USGS-40 (L-Glutamic acid) international standard and Acetanilide (C_8H_9NO) laboratory standard. Measurement error is ±0.15‰.

Analysis of data. Statistical analysis was performed using a JMP 10.0.0 package (SAS Institute Inc., USA, 2012). The differences in $\delta^{13}C$ and $\delta^{15}N$ in response to the impact of different factors were estimated using analysis of variance (ANOVA) and general linear models (GLM) with fixed factors. Factors were incorporated in GLM in different combinations: functional group of plants, soil horizon, area (Ilmen State Reserve or impact zone of KCSP) as categorical covariates; the time of last fire as continuous covariate. Units in statistical analysis were values of $\delta^{13}C$ and $\delta^{15}N$ in a mixed soil sample, litter, or leaves of plants of the same species on TP. Their variability was measured in standard error (±*SE*).

RESULTS

Main tendency in the variations of carbon isotopic composition is an increase of ^{13}C content from plant leaves (mean and absolute range: $\delta^{13}C = -29.81 \pm 0.13$; −31.90...−27.08‰) to litters (−27.85 ± 0.29; −29.72...−26.67‰) and humic horizons (−26.28 ± 0.20; −27.08...−25.29‰). These values are highly significant (one-way ANOVA): $F_{(2, 84)} = 62.21$; $P < 0.0001$.

Table 2. $\delta^{13}C$ and $\delta^{15}N$ of litter, soil, and plants of different functional groups in two test plots

Soil horizon, functional group and plant taxon	$\delta^{13}C$, ‰		$\delta^{15}N$, ‰	
	Ilmen State Reserve	Impact zone of KCSP	Ilmen State Reserve	Impact zone of KCSP
Soil and Litter				
Litter	–26.90 ± 0.16	–28.09 ± 0.31	–2.72 ± 0.56	–1.88 ± 0.36
Soil	–26.78 ± 0.22	–26.15 ± 0.22	–1.21*	–0.48 ± 0.23
Plants				
ECM-trees:				
P. sylvestris	–29.77 ± 0.59	–30.56 ± 0.31	–5.03 ± 0.68	–2.07 ± 0.33
Betula spp.	–29.58 ± 0.28	–30.55 ± 0.28	–3.45 ± 1.23	–1.96 ± 0.36
ER-undershrubs (*V. vitis-idaea)*	–29.87 ± 0.26	–30.06 ± 0.31	–4.53 ± 3.36	–1.13 ± 0.69
AM-herbs:				
R. saxatilis	–28.51 ± 0.12	–29.02 ± 0.30	–4.64 ± 1.07	–0.49 ± 0.56
C. arundinacea	–28.38 ± 0.09	–28.48 ± 0.31	–4.25 ± 2.02	–1.36 ± 0.71
T. maculata or *S. controversa*	–31.14 ± 0.44	–30.53 ± 0.30	–1.44 ± 1.58	–0.19 ± 0.91
N_2f-herb (*L. vernus*)	–30.23 ± 0.61	–29.90 ± 0.43	–0.83 ± 0.42	–0.69 ± 0.31
noM-herb (*S. nutans*)	–	–29.40 ± 0.35	–	–0.09 ± 1.33

* Sample from one sample plot only; therefore standard error is not given. Dash means samples were not analyzed.

The differences in a varying degree are expressed at separate analysis of $\delta^{13}C$ values from two nonpolluted TPs in the reserve or from eight polluted TP near the KCSP (Table 2). Other tendencies in $\delta^{13}C$ variations are not observed. Among plants, the lowered values of $\delta^{13}C$ were found in the species of family Asteraceae (–31.58...–29.30‰), while the elevated values, in *Calamagrostis arundinacea* (–29.70... –27.08‰). The comparison of carbon isotopic composition in the plants of the same taxon on the plots of the Ilmen State Reserve and in the vicinity of KCSP showed that $\delta^{13}C$ value could be shifted in both sides, but does not exceed 1‰.

No common differences in the nitrogen isotopic composition were found between leaves of plants, litters, and humic horizons (ANOVA): $F_{(2, 78)} = 0.77$; $P = 0.4651$. This is likely because we could not determine the $^{15}N/^{14}N$ ratio in some samples from humic horizons probably because of the total low nitrogen content in them. It should be noted that the average $\delta^{15}N$ value increases from leaves of ectomycorrhizal trees forming the leaf fall (–2.46 ± 0.31; –5.71...0.07‰), to soils (litter: –2.05 ± 0.32; –3.39...0.15‰; humic horizon: –0.66 ± 0.24; –1.21...–0.20‰).

The main trend in the variations of nitrogen isotopic composition is an increase of ^{15}N in plants, and to lesser degree, in soil while passing from forests of the Ilmen State Reserve to strongly polluted forests in the impact zone of KCSP. Differences in $\delta^{15}N$ between unpolluted and polluted forests are +0.7...+0.8‰ for litter and soil, and +1.2...+4.2‰ for diverse plant taxa (excluding legumes with nitrogen-fixing symbiosis).

Plants at each TP were sampled systematically from definite taxa. Correspondingly, the results of comparison of $\delta^{13}C$ and $\delta^{15}N$ in the series "plants–soil horizons" performed using ANOVA are not devoid of artifacts. It is more reliable to analyze the reasons of $\delta^{13}C$ and $\delta^{15}N$ variability separately in soil and plants of different functional groups. To take into account several sources of variability, these comparisons were done using GLM (Table 3; a group of non-mycorrhizal plants was excluded for correct assessment of interaction between factors "functional group of plants" and "area").

None of three considered factors (horizon, area, and last fire) affected the ratio of ^{13}C to ^{12}C isotopes in plants. In soils, the values of $\delta^{13}C$ differ only between different horizons: litter and humic ones.

In contrast, significant factor determining the ^{15}N to ^{14}N ratios in soil was not found. The values of $\delta^{15}N$ in plants are defined by a combination of two factors: stronger, by sampling area, and weaker, by functional group. The influence of the first factor is expressed in the total increase of the heavy isotope ^{15}N content near KCSP as compared to the undisturbed forests of the Ilmen Reserve: by 2.7‰ in leaves of ectomycorrhizal trees, by 3.4‰ in cowberry leaves, and by 2.2‰ in herbs with arbuscular mycorrhiza. Differences between plants of different functional groups are expressed in an increase of heavy isotope ^{15}N in the series ECM → ER → AM → N_2f. In other words, the lowest ^{15}N content, on average, is observed in the ectomycorrhizal trees, while the largest contents, in herbs with arbuscular mycorrhiza and with legume–rhizobium symbioses, as well as in non-mycorrhizal herbs (Fig. 2).

Thus, the differentiation of stable isotope proportions in plants in relation with the impact of the Karabash Copper Smelting Plant is statistically significant and well expressed for nitrogen, but is not observed for carbon. The total amplitude in the variations of average isotopic values for the functional group of plants was 1.3 for $\delta^{13}C$ and 4.4‰ for $\delta^{15}N$. This tendency is also expressed for separate consideration of data on unpolluted TPs of the Ilmen State Reserve and polluted TPs near KCSP. In the reserve, the total amplitudes of $\delta^{13}C$ and $\delta^{15}N$ variations were 0.9 and 3.7‰, respectively. In the vicinity of KCSP, the amplitudes of $\delta^{13}C$ and $\delta^{15}N$ values were 1.3 and 1.9‰, respectively.

DISCUSSION

Our data show that a change in plant growth conditions near the large copper smelting plant mainly affects the ^{15}N to ^{14}N isotopic ratios in their leaves, and has no affect on ^{13}C and ^{12}C isotopes. The transformation of nitrogen isotopic composition of the south taiga pine forests under industrial impact of emissions of large metallurgical plant is expressed in the increase of $^{15}N/^{14}N$ ratio, i.e., enrichment in ^{15}N. Thus, our working hypothesis was confirmed only for the nitrogen isotopic composition.

Conclusion concerning an increase of $\delta^{15}N$ values in leaves near the metallurgical plants is new, but some other results are well consistent with published data. In particular, the tendency of $\delta^{13}C$ differentiation in the series "plant–litter–soil" is quite expectable. The carbon isotopic composition in the organic matter of soil is directly inherited from plants and the ^{13}C content shows a few per mille increase with increasing soil depth (Morgun et al., 2008; Menyailo et al., 2014), which is observed also in the KCSP region.

Observed features of $\delta^{15}N$ values for plants with different mycorrhiza types also coincide with published estimates. It is known that $\delta^{15}N$ of nitrogen-fixing plants is close to that of atmospheric air (Makarov, 2009). Plants with ericoid mycorrhiza and ectomycorrhiza are usually characterized by low $\delta^{15}N$ (Michelsen et al., 1996; Emmerton et al., 2001; Hobbie et al., 2005), which can be related both to $\delta^{15}N$ levels in substrates used by plants or their symbiotic fungi, and mainly to the well-expressed discrimination of ^{15}N and ^{14}N isotopes during nitrogen transport from mycobionts to phytobionts (Hobbie et al., 2005). Non-mycorrhizal plants are frequently enriched in ^{15}N, i.e., have high $\delta^{15}N$ (Michelsen et al., 1996; Hobbie et al., 2005; Craine et al., 2015) because they adsorb high-^{15}N nitrogen compounds from soil without fungi assistance. Such $\delta^{15}N$ distribution in general was established in the KCSP region and can be interpreted as an indicator of quality of performed study.

Table 3. Significance of influence of factors on $\delta^{13}C$ and $\delta^{15}N$ in soil and leaves of plants (P values, obtained in general linear models)

Source of variability	$\delta^{13}C$	$\delta^{15}N$
Soil and litter		
(1) Soil horizon	**0.0232**	0.1343
(2) Area	0.5665	0.9771
(3) Time after the last fire	0.9815	0.4087
(1) × (2)	0.1654	0.7168
(1) × (3)	0.4788	0.8497
R^{2*}_{adj}	0.54	0.22
Plants		
(1) Functional group of plants	0.1534	**0.0233**
(2) Area	0.6459	**<0.0001**
(3) Time after the last fire	0.9349	0.1271
(1) × (2)	0.9218	**0.0416**
(1) × (3)	0.5104	**0.0232**
R^{2}_{adj}	0.10	0.37

* R^2_{adj} is the determination coefficient corrected for number of parameters; the values of $P < 0.05$ are shown by semi-bold type.

We also suggest that $^{13}C/^{12}C$ ratio does not change under technogenic impact. First, the value of $\delta^{13}C$ is strongly determined by such global factors as the kind of photosynthesis (Smith and Epstein, 1971; Farquhar et al., 1989) or climatic and geographical characteristics (Kovda et al., 2011), which did not change in our study. Second, the different estimates of $\delta^{13}C$ in plants under pollution are weakly consistent with each other. Some data indicate that pollution may cause both positive (Niemelä et al., 1997; Savard et al., 2004; Savard, 2010) and negative shifts (Kwak et al., 2009; Cada et al., 2016) in $\delta^{13}C$. In addition, these assessments were mainly obtained by analyzing $\delta^{13}C$ in wood, not in the pine needles (Niemelä et al., 1997; Savard et al., 2004; Savard, 2010; Cada et al., 2016).

The proportions of ^{15}N and ^{14}N frequently depend on the local factors. An established increase of $^{15}N/^{14}N$ ratio in plants in the vicinity of copper smelting plant or positive shift of $\delta^{15}N$ in leaves, first, has an analogue, i.e., was observed during other anthropogenic impacts (Gebauer et al., 1994; Hofmann et al., 1997; Korontzi et al., 2000; Pearson et al., 2000), and, second, can be relatively logically explained. The explanations can be based on the concepts of a change of physiological mechanisms of nitrogen metabolism

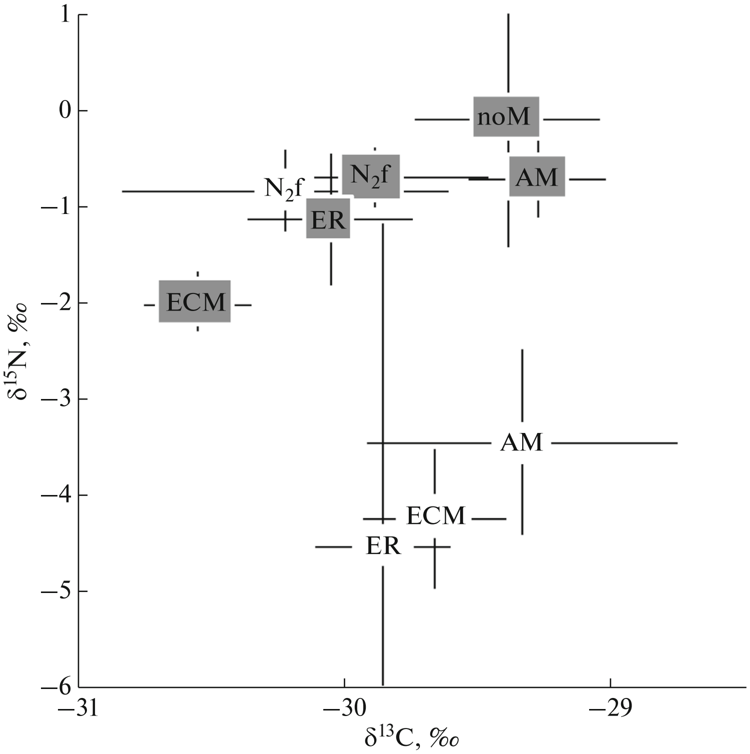

Fig. 2. $\delta^{13}C$ and $\delta^{15}N$ in leaves of plants of different functional groups in the Ilmen State Reserve (empty symbols) and in the impact zone of the Karabash copper smelting plant (gray symbols) ($m \pm$ SE).

under direct pollutant affect (Hofmann et al., 1997), and on the ecological mechanisms. With allowance for an increase of $\delta^{15}N$ in plants of different groups near KCSP by the similar manner, it is more reasonable to find common ecological explanations.

The known phenomenon, which can be related to the increase of $\delta^{15}N$ in plants in response to heavy metal pollutions, is an increase of root depths as compared to undisturbed forests. This phenomenon was strictly described in the vicinity of other metallurgical production of the Urals, the Sredneural'skii copper smelting plant (Veselkin, 2002). But unusually deep position of roots *Pinus sylvestris* was observed also near KCSP: in 2009, the occurrence of thin roots of pine in forests 15–30 km from KCSP was about 50–80%, and reached 0–25% at a distance less than 10 km from KCSP (Veselkin, 2013); moreover, in some open test pit in the impact zone of KCSP, the pine roots were absent even in humic horizons, lying at depth more than 12–15 cm, which is not typical of this species.

The possible explanation of deep position of roots in forest ecosystems highly polluted by heavy metal is related to the toxicity of upper soil layers accumulated through the accumulation of atmospheric fallouts (Koroteeva et al., 2015a, 2015b). This gives grounds to suggest that the deep position of roots near KCSP is typical not only of pine, but also likely of other plants. In turn, since $\delta^{15}N$ of soils increases with increasing depth, the deeper position of roots could lead to the increase of heavy ^{15}N isotope in nitrogen sources used by plants. Such mechanism can cause a shift of $\delta^{15}N$ in plants by approximately 1–1.5‰ toward the higher values, because a regression coefficient for the correlation between $\delta^{15}N$ in soil and in plants almost equal +1 (Craine et al., 2015). One more explanation of $\delta^{15}N$ increase in plants near KCSP is inferred but statistically unproved increase of $\delta^{15}N$ in litter and humic horizons near KCSP. The thorough substantiation of this effect will provide the explanation for the positive shift of $\delta^{15}N$ in plants near KCSP by approximately 0.8–1‰.

The assurance that the observed change of nitrogen isotopic composition in plants near KCSP was caused by a change of soil conditions is also supported by one more fact: dynamics of $\delta^{15}N$ values depending on the level of technogenic pollution in peavine *Lathyrus vernus* – representative of the legume family with rhyzobial nitrogen-fixing symbiosis. The nitrogen isotopic composition of this species was the same in the polluted and background territories. Legumes likely used as a nitrogen source in atmosphere are relatively independent of soil nitrogen sources. Therefore, the absence of $\delta^{15}N$ change in peavine leaves under the heavy metal pollution is well understood.

In addition to the deep position of roots, one more hypothetical explanation can be proposed for the increase of heavy ^{15}N in plants under heavy metal pollution. Since the highest ^{15}N content is observed in non-mycorrhizal plants as compared to plants with different mycorrhiza (Michelsen et al., 1996; Hobbie et al., 2005), an increase of δ^{15}N in the vicinity of KCSP could be caused by weakened development of mycorrhiza (ecto and arbuscular). Such an assumption has certain substantiation. It is known that the arbuscular mycorrhiza in herbaceous plants in technogenic habitats could be less developed than in the absence of technogenic impact (Betekhtina and Veselkin, 2013). However, ectomycorrhiza of pine trees in the vicinity of metallurgical plants are very stable (Veselkin, 2005, 2006, 2013). Thus, an assumption of a change in the mycorrhizal state cannot serve as the only explanation of a δ^{15}N shift in plants near KCSP, but likely could be responsible for some of established differences.

The degree of isotopic fractionation, especially nitrogen isotopes, can potentially change during post-fire recovery of ecosystems (Hyodo et al., 2013). Therefore, we analyzed the influence of factor of "fire time" as additional to factors "area" and "functional group of plants". It was found however that the values of δ^{13}C and δ^{15}N in soil and plants are not correlated with time passed after last fire. This is likely caused by the short period of post-fire recovery, maximum up to 60 years. In the study area located closely to the zonal ecotone "south taiga–forest-steppe", fires in pine forests are very frequent and forests that have not burn for more than 60 years almost never occur. At the same time, most of described cases in literature, when stable isotopic composition is correlated to the duration of succession, the studied successions varied from hundreds (Compton et al., 2007; Hyodo et al., 2013) to thousands (Vitousek et al., 1989; Menge et al., 2011) years.

CONCLUSIONS

The main result of our study is revealing an increase of heavy isotope ^{15}N in plants of several functional groups growing in the vicinity of the copper smelting plant. We suggest that such effect in response of heavy metal pollution of natural ecosystems has not been described yet. The established difference in δ^{15}N values between unpolluted and polluted forests in leaves of the same functional groups (ectomycorrhizal, with ericoid and arbuscular mycorrhiza) is about 2–3.5‰. These differences are comparable with difference in δ^{15}N between different soil horizons (Martinelli et al., 1999; Menyailo and Hungate, 2006), and are slightly lower than typical difference in δ^{15}N between ectomycorrhizal and non-mycorrhizal plants, but are comparable with typical difference in δ^{15}N between arbuscular mycorrhizal and non-mycorrhizal plants (Craine et al., 2015). Thus, a 2–3.5‰ shift in δ^{15}N is significant and indicates notable changes in mechanisms and sources of nitrogen feeding of plants under the impact of industrial emissions of metallurgical plant.

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