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Carbon and nitrogen stable isotope ratios of soils and grasses as indicators of soil characteristics and biological taxa

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ABSTRACT

The use of stable isotope techniques can assist in understanding interactions of plants with various abiotic and biotic processes. In the research, we focused on carbon (C) and nitrogen (N) isotopes because they are the most important resources influencing plant function and the biogeochemical cycles. The $^{13}C/^{12}C$ and $^{15}N/^{14}N$ ratios in plants and in soils and the relationships between these ratios and biological and environmental factors of widely distributed native C_3 plants (couch grass, plantain and yarrow) collected from two sites in St. Petersburg, Russia were studied. The soil characteristics of the sites were rather different. This had a significant effect on the isotope ratios in plants and in soils resulting in a big difference between $^{13}C/^{12}C$ and between $^{15}N/^{14}N$ ratios in the soils, roots and leaves of the plants collected from the two sites. The variability of the C and N isotope ratios was also rather high among different plant species. Two main factors affected this variability: biological (plant species) and ecological (biogeochemical characteristics of soils). The $^{13}C/^{12}C$ and $^{15}N/^{14}N$ ratios of roots and especially leaves were typical for a particular plant species and could differ between different plant species growing simultaneously at the same site. The soil parameters (soil texture, pH, and concentrations of total C and N in the soil) were among main factors influencing the stable isotope ratios in the soil and in different parts of a plant. A strong positive relationship between $^{13}C/^{12}C$ and also between $^{15}N/^{14}N$ ratios in roots and leaves of the plants was observed. On the other hand, the correlation between ratios of $^{13}C/^{12}C$ and $^{15}N/^{14}N$ calculated only for leaves or only for roots was statistically significant and negative.

1. Introduction

Carbon (C) and nitrogen (N) are among major components of all plant tissues. Their metabolism is linked to the plant life function (Zhang et al., 2014). The measurement of naturally occurring stable isotopes is becoming widespread as a useful tool to study plant communities, trophic relationships and environmental factors affecting the plant development. Stable isotopes have been widely used in biogeochemical studies to trace the dominant sources of C and N in the environment (Cloern et al., 2002). Both the elements naturally occur in two stable isotopic forms, light (^{12}C and ^{14}N) and heavy (^{13}C and ^{15}N) (Fry and Sherr, 1989). It was suggested that every biogenic material has its own unique isotopic composition, so-called the “dynamic stable isotope fingerprint” (Wada, 2009). In plant communities, the biologically important stable isotope pairs $^{13}C/^{12}C$ and $^{15}N/^{14}N$ usually vary among plant species. Besides, it is known that C and N isotope ratios of plants may be influenced by environmental factors that can cause certain variations of the isotope ratios, especially in C_3 plants as compared to C_4 plants (Evans et al., 1986; Craine et al., 2015; Wang et al., 2015). The ratios of the heavy and light isotopes of C and N are usually expressed using δ notation in parts per thousand. Relative isotope abundances are denoted as δ-values, which are calculated according to the following equation:

$$\delta^{15}N \text{ or } \delta^{13}C = \left({R_{sample}}/{R_{standard}} - 1 \right) 1000 [‰]$$

where R_{sample} and $R_{standard}$ are the ratios of heavy isotope to light isotope of the sample and the respective standard.

Nitrogen is a key limiting resource in many terrestrial ecosystems. Although N in plant biomass represents a small fraction of the total ecosystem N pool, the isotopic composition of plants can help to trace short-term dynamics of N cycling, as opposed to soil $\delta^{15}N$ which might represent long-term dynamics (Craine et al., 2015). It was reported that the ratio of the $^{15}N/^{14}N$ isotopes changes with trophic level (Hansson et al., 1997). On the other hand, the $^{13}C/^{12}C$ isotope ratio changes very little with trophic position and therefore can reflect sources of primary

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productivity (Roth and Hobson, 2011).

The differences in the stable C isotope ratios of organic and inorganic compounds are useful for studying processes that control C cycling within and between plants and soils. Usually terrestrial plants have $\delta^{13}C$ values lower (more negative, ^{13}C-depleted) than that of atmospheric CO_2 (Tieszen and Boutton, 1989). Besides, within plant species there is a genetic component to the variation in the $\delta^{13}C$ value which may be as great as 3‰ (Tieszen, 1991). Internal partitioning and metabolism of primary assimilation may also produce differences in the $\delta^{13}C$ among plant organs (Yang et al., 2015; Muhammad et al., 2017). An additional point emphasize is that a variety of environmental factors can modify the $\delta^{13}C$ value in plants and in soils.

An understanding of the reasons of the variations is often difficult because this is rather complex process. Various factors affecting fractionation of N and C can make it difficult to precisely quantify their contributions (McClelland et al., 1997). Comparison of the $\delta^{15}N$ and $\delta^{13}C$ signatures, however, can produce valuable information on the source contributions. The differences in the $\delta^{15}N$ and $\delta^{13}C$ values between places of sampling, in addition to absolute $\delta^{15}N$ and $\delta^{13}C$ values in soil and in different plant parts, can be used to assess the N and C contributions from different sources.

The ratios of stable isotopes can change in the course of transfer of the isotopes from soil to a plant. This may be due to differential fractionation during assimilation and metabolic processes. Metabolic fractionation may also result in variation in the isotope ratios of different plant parts. Although in plants these fractionations are often quite small, they are very important and should be taken into account for proper data interpretation (Dawson et al., 2002). Besides, it is necessary to remember that these processes may occur differently in different plant species. The plant and soil $\delta^{13}C$ and $\delta^{15}N$ values may be used as indicators that can help to predict the biogeochemical interactions between soil and plants and to appreciate the biochemical processes that take place in different botanical taxa.

The main aim of the research was to study environmentally induced and also phylogenetic factors affecting the C and N isotopic composition of plants and soil collected from the rhizosphere of the plants. The objectives of the experiment were the following: (1) to examine the ability of three widely distributed in the natural environment plant species (couch grass, plantain and yarrow) to differently fractionate C and N isotopes; (2) to assess the differences in the $\delta^{13}C$ and $\delta^{15}N$ values of the soil where the plants grow resulting from differences in the soil characteristics; (3) to study the effects of the soils on the $\delta^{13}C$ and $\delta^{15}N$ values in the plants, (4) to estimate the contribution of different soil parameters on the stable isotope ratios in different parts of the plants.

2. Materials and methods

2.1. Sampling

Three native plant species: couch grass *(Elytrigia repens* L. Nevski), plantain *(Plantago major* L.*)*, and yarrow (*Achillea millefolium* L.) were collected in October 2017 simultaneously from two sites in St. Petersburg, Russia. Several small (1 m^2) plots were selected at each site. The two sites differed in the soil characteristics (Table 1). The date of sampling was chosen because development of plants is usually completed by middle of autumn. Therefore, we could exclude, at least partially, the variations in the physiological state of the plants that may occur at the initial stages of the plant growth. Several plants of each species were collected to provide the reproducibility of the analyses. Soil samples were taken from surface of the plant roots. Just in this zone we can expect the influence of root exudates on the soil. Plants were washed carefully immediately after sampling to remove dust and soil particles from surface of leaves and especially roots. Then the plant and soil samples were air-dried up to constant weight.

2.2. Analysis

The plant samples were ground. The soil samples were sieved through a 2 mm mesh to remove non-soil materials including plant fragments and then ground to fine powder. The pH values (1:2.5 H_2O) of soil samples were determined and total amounts of C, N, and H in the soils were measured with an elemental analyzer CHN628 (LECO). The samples were weighed into tin capsules (weight of a sample was ~1 mg) and then analyzed in duplicate on an elemental analyzer EA3024 (EuroVector) coupled to a continuous flow isotope ratio mass spectrometer Nu-horizon (Nu Instruments). An internal standard sample (glutamic acid) was used to check reproducibility of the stable isotope ratio determination. The standard deviations were 0.07‰ (n = 9) for $\delta^{13}C$ and 0.23‰ (n = 8) for $\delta^{15}N$.

2.3. Data analysis

For multivariate statistical analysis Statistica for Windows 6.0 Software packages were used. The calculation of mean concentrations of C, N, and H in soils, mean values of the $\delta^{13}C$ and $\delta^{15}N$ in plant and soil samples and analysis of variances to estimate differences between groups of the samples were carried out. Additionally, correlation analysis was applied to the experimental data sets to assess the contribution of specific factors that may have an effect on the variations of the $\delta^{13}C$ and $\delta^{15}N$ values in different plant parts and in soil. This can help to better appreciate the mechanisms affecting the C and N isotope ratios in each plant species and in different types of soil.

3. Results and discussion

3.1. Soil parameters that can affect the C and N isotope ratios

The concentrations of total C, N, and H were always lower in the soil taken from roots of the plants collected from site 1 than those in the soil taken from roots of the plants collected from site 2 (Table 1). The differences were statistically significant ($P < 0.05$). The C/N ratio of the soil collected from site 1 was less than that of the soil collected from site 2. The soil at site 1 had a blocky structure, while the soil at site 2 was a well-structured with plenty of pore space to allow water and air movement and healthy root development.

The pH values of the soil taken from surface of roots of plantain and especially couch grass collected from site 1 were higher as compared to the pH of the rhizosphere soil of the same plant species collected from site 2 (Table 1). The least pH values were observed for the rhizosphere soil of yarrow. Besides, there was no difference between the pH values of the rhizosphere soil of the yarrow collected from both sites. Thus, plants were capable of influencing the soil pH, and these effects were species-dependant.

3.2. Variability of the plant and soil isotopic composition

Our results demonstrated a large variability of the isotope ratios of soils and plants (Table 2). Analysis of variance showed a statistically significant ($P < 0.05$) difference between mean $\delta^{13}C$ and $\delta^{15}N$ values of different plant species, confirming that the isotopic composition of plants can reflect differences in the biogeochemical pathways through which C and N are assimilated into biomass.

3.2.1. The $\delta^{15}N$ values

A significant difference was found between the $\delta^{15}N$ values of soils, roots and leaves of the plants collected from a particular site (Table 2). In plants, the largest $\delta^{15}N$ values were measured in leaves of the plants collected from site 2, the smallest $\delta^{15}N$ values were measured in roots of the plants collected from site 1. The least values of the $\delta^{15}N$ were typical for the rhizosphere soil.

It is known that soil $\delta^{15}N$ has wide temporal and spatial variations

Table 1
Main characteristics of the soils taken from surface of roots of the plants collected from site 1 and site 2. The C/N ratio is a mean value of the ratio of C to N calculated using concentrations of C and N in all soil samples collected from a particular site.

	Site 1			Site 2		
Texture	clay loam soil with blocky structure			sandy loam well-structured soil		
Plant species	Couch grass	Plantain	Yarrow	Couch grass	Plantain	Yarrow
pH	7.31 ± 0.02	7.09 ± 0.04	6.91 ± 0.02	6.95 ± 0.02	6.91 ± 0.04	6.88 ± 0.07
H, %	5.70 ± 0.24	4.94 ± 0.28	5.25 ± 0.25	6.63 ± 0.34	6.96 ± 0.28	6.44 ± 0.24
C, %	2.37 ± 0.07	2.06 ± 0.05	2.24 ± 0.05	3.97 ± 0.05	4.33 ± 0.10	3.92 ± 0.04
N, %	1.73 ± 0.06	1.34 ± 0.06	1.58 ± 0.07	2.30 ± 0.10	2.31 ± 0.11	2.03 ± 0.12
C/N	1.4	1.5	1.4	1.7	1.9	1.9

Table 2
The $\delta^{15}N$ and $\delta^{13}C$ values of roots, leaves and the rhizosphere soil of the plants collected from two sites.

	$\delta^{15}N$		$\delta^{13}C$	
	Site 1	Site 2	Site 1	Site 2
Rhizosphere soil				
Couch grass	−10.3 ± 0.6	−7.1 ± 2.0	−27.3 ± 0.3*	−26.0 ± 0.2
Yarrow	−14.4 ± 1.4	−15.6 ± 0.6	−27.0 ± 1.4	−27.1 ± 0.1c
Plantain	−14.2 ± 1.5	−6.9 ± 3.3	−27.8 ± 0.8	−26.8 ± 0.1
Roots				
Couch grass	−3.9 ± 0.1*	−0.17 ± 0.21	−30.4 ± 0.1*ab	−34.9 ± 0.2b
Yarrow	−2.8 ± 1.4	−0.17 ± 0.54	−29.9 ± 0.1*c	−33.9 ± 0.3c
Plantain	−3.8 ± 1.6	−0.33 ± 0.57	−31.5 ± 0.2*	−31.5 ± 0.2
Leaves				
Couch grass	−0.23 ± 0.23*ab	6.4 ± 0.2ab	−32.1 ± 0.2*	−35.6 ± 0.1ab
Yarrow	1.1 ± 0.1*c	3.5 ± 0.1c	−31.1 ± 0.5*	−34.9 ± 0.1c
Plantain	−0.89 ± 0.03*	1.2 ± 0.2	−31.9 ± 0.1*	−33.4 ± 0.1

*- differences between sites were statistically significant ($P < 0.05$).
[a] – differences between couch grass and yarrow collected from the same site were statistically significant ($P < 0.05$).
[b] - differences between couch grass and plantain collected from the same site were statistically significant ($P < 0.05$).
[c] - differences between yarrow and plantain collected from the same site were statistically significant ($P < 0.05$).

due to heterogeneous distribution of soil N, as well as due to nitrification, denitrification and mineralization occurring in the soil (Khadka and Tatsumi, 2006). There were no statistically significant differences between the $\delta^{15}N$ values of the soils collected from the two sites, although the $\delta^{15}N$ values on the average were lower at site 1 as compared to site 2. This correlated with concentration of N in the soils; it was also lower in the soil collected from site 1 than in the soil collected from site 2. The $\delta^{15}N$ values of roots of the plants collected from site 1 were always lower than the $\delta^{15}N$ values of roots of the plants collected from site 2. However, a significant ($P < 0.01$) difference in the $\delta^{15}N$ values was found only between roots of couch grass collected from different sites. In leaves, a statistically significant difference was observed between the $\delta^{15}N$ values of all the plant species that were collected from the two sites. Besides, we found a significant ($P < 0.05$) difference in the $\delta^{15}N$ values of leaves of the three plant species collected from a particular site (Table 2). Jang et al. (2015) suggested that possible reason for the large variation in $\delta^{15}N$ values among plants at different sites could be different N-availability and uptake of different N forms (i.e., NO_3^- and NH_4^+).

There was a sufficiently large difference between the $\delta^{15}N$ values of soil and roots (usually 6–11‰) as well as between the $\delta^{15}N$ values of roots and leaves (~4‰). It may be assumed that the differences in the $\delta^{15}N$ values of roots and leaves could be explained by different sources of N for these plant parts. It is known that the ^{15}N abundance of plant-available soil N may be significantly different from that of air N_2 (Wanek and Arndt, 2002). Therefore, it may be suggested that in roots major part of N is accumulated from soil, while in leaves N is assimilated mainly from air. It was reported that this variation in the $\delta^{15}N$ signatures between parts of a single plant may be due to ammonium or nitrate acquisition, preferential nitrate reduction in roots or shoots, and N_2 fixation (Kahmen et al., 2008; Marschner, 2012; Ariz et al., 2015). An intra-plant variation in the $\delta^{15}N$ may also be caused by the organ-specific loss of N, different patterns of N assimilation, and reallocation of N. (Evans, 2001; Dawson et al., 2002). For example, it was shown (Evans et al., 1996; Yoneyama et al., 2001) that if NO_3 is the only source of N and it is partially assimilated in the roots, there could be an isotopic difference between the roots and leaves.

3.2.2. The $\delta^{13}C$ values

Compared to $\delta^{15}N$, the largest $\delta^{13}C$ values were observed in soil (Table 2). Similar findings were reported by other researchers; it was found that the $\delta^{13}C$ values of soil were higher compared to the $\delta^{13}C$ of the plant leaves (Ma et al., 2009; Gerschlauer et al., 2019). The $\delta^{13}C$ values of the soil taken from roots of the plants collected from site 1 were usually lower as compared to those of the soil taken from roots of the plants collected from site 2, although statistically significant ($P < 0.05$) difference was found only between the soils taken from roots of couch grass. This also correlated with concentration of C in the soils; it was lower in the soils taken from site 1. There were no statistically significant differences between the $\delta^{13}C$ values of the rhizosphere soil of different plant species growing at the same place, except the values of the $\delta^{13}C$ of the soils taken from roots of yarrow and plantain collected from site 2. More differences were observed between the $\delta^{13}C$ values of roots and leaves of the plants collected from sites 1 and 2. The plants collected from site 1 had the $\delta^{13}C$ values statistically significantly ($P < 0.05$) higher than the plants collected from site 2. Besides, there were certain differences between the $\delta^{13}C$ values of roots and between the $\delta^{13}C$ values of leaves of different plant species collected from the same site (Table 2).

There was a sufficiently large difference between the $\delta^{13}C$ values of

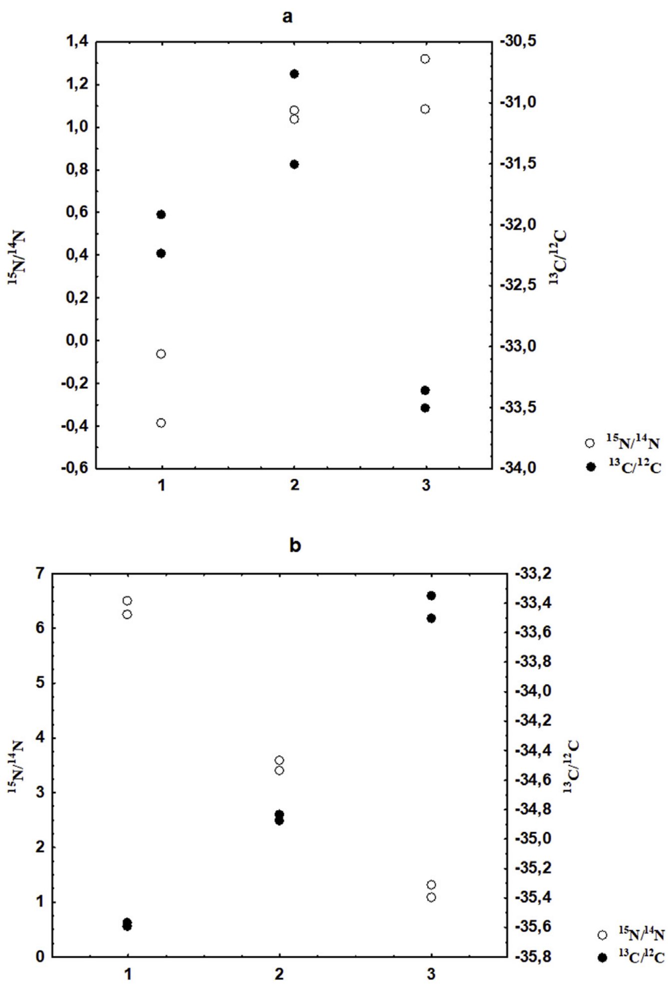

Fig. 1. The $^{15}N/^{14}N$ and $^{13}C/^{12}C$ ratios of leaves of different plant species collected from site 1 (a) and site 2 (b). 1 – couch grass, 2 –yarrow, 3 – plantain.

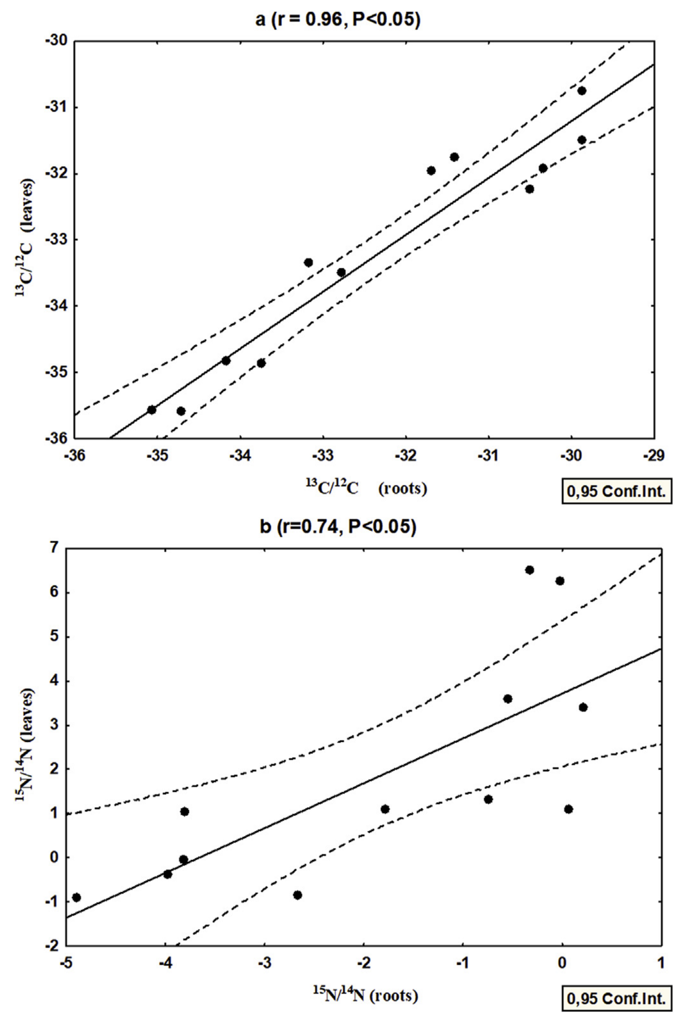

Fig. 2. Relationship between $^{13}C/^{12}C$ (a) and $^{15}N/^{14}N$ (b) ratios in roots and leaves.

soil and roots (~3.7‰ in site 1 and ~7.4‰ in site 2). But compared to $\delta^{15}N$, differences between the $\delta^{13}C$ values of roots and leaves were not so significant (~1.1‰–1.2‰). The $\delta^{13}C$ values in roots were higher than in leaves. Such an enrichment of $\delta^{13}C$ in roots relative to leaves may suggest an isotopic fractionation between leaves and roots (Yang et al., 2015). Besides, a loss of CO_2 can occur during post-photosynthetic metabolism (Yoneyama, 2017).

3.3. Differences between isotope ratios of soils and plants collected from two sites

Data presented in Table 2 indicate that there was a certain difference between the isotope ratios of soils and plants collected from the two sites. The mean $\delta^{15}N$ values of soil, roots and leaves collected from site 1 were ~3.1‰–3.7‰ lower than corresponding mean $\delta^{15}N$ values of the soil and plant samples collected from site 2. The mean $\delta^{13}C$ values of the soil samples collected from site 1 were also lower (~0.8‰) than the mean $\delta^{13}C$ values of the soils collected from site 2. However, the $\delta^{13}C$ values of the plants collected from site 1 were higher (~2.9‰) as compared to the $\delta^{13}C$ values of the plants collected from site 2. As one can see, these differences were due to different types of soils at the two sites and probably, also fractionation during transport of C and N isotopes from the different soils to plants. The differences between these two sites were marked not only for $\delta^{15}N$ and $\delta^{13}C$ values but also for main soil characteristics. This confirms that soil plays a leading part in providing plants with required C and N isotopes and the differences in $\delta^{13}C$ and $\delta^{15}N$ values between the sites appear to be largely due to soil textural differences.

3.4. Differences between isotope ratios of soils and roots and leaves of the plants collected from the same site

Plants, both roots and especially leaves, were usually enriched with $\delta^{15}N$ as compared to soil. On the other hand, the plant $\delta^{13}C$ values were depleted in comparison to the $\delta^{13}C$ values of the rhizosphere soil. Thus, there were different trends in the ratios of stable N and C isotopes in the soil – plant system.

It was reported that leaves were on the average 0.96‰–1.91‰ more depleted in the $\delta^{13}C$ values as compared to roots (Badeck et al., 2005). In some cases roots may be enriched in the $\delta^{13}C$ relative to leaves even by 2.3‰ (Bowling et al., 2008). The authors suggested that the inter-organ differences can be caused by fractionation associated with transport of metabolites across organ boundaries. In our case, the differences between mean $\delta^{13}C$ values of roots and leaves were 1.1‰–1.2‰. More significant differences were observed between $\delta^{15}N$ values of roots and leaves: ~3.5‰ (site 1) and ~3.9‰ (site 2). As was shown (Evans, 2001), variations of the $\delta^{15}N$ values in field studies were ecosystem specific. The differences in the $\delta^{15}N$ values between leaves and roots were often less than 3‰ in deciduous forest and tallgrass prairie ecosystems, but could be as great as 7‰ in warm and cold desert ecosystems.

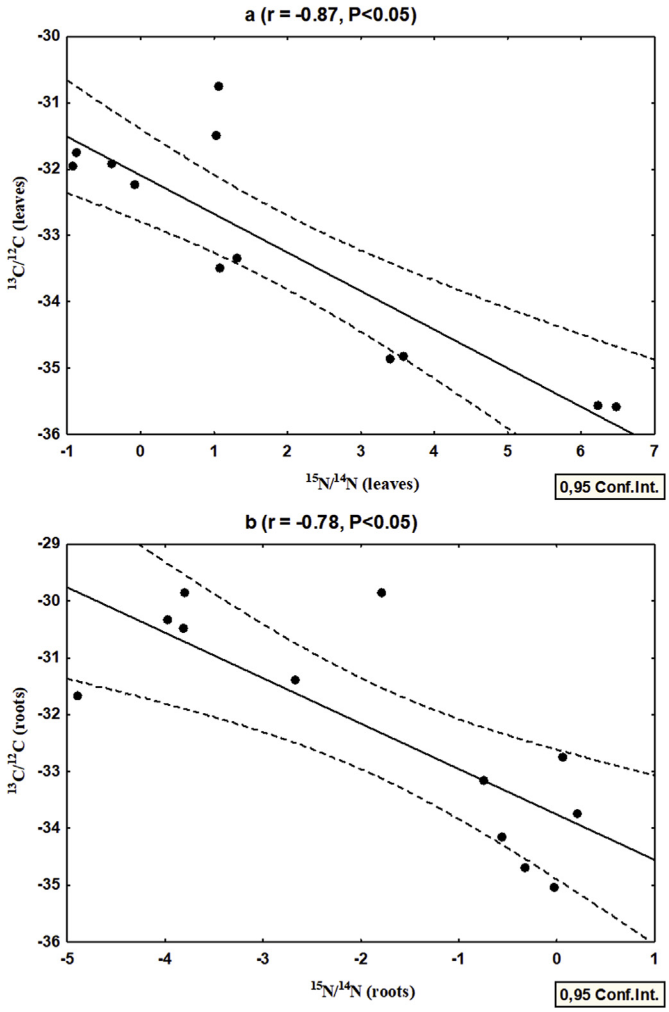

Fig. 3. Relationship between $^{13}C/^{12}C$ and $^{15}N/^{14}N$ ratios in leaves (a) and roots (b).

3.5. Effects of biological taxa on distribution of stable isotopes in plants (taxonomic patterns)

One of the selected plants, couch grass, belongs to Monocotyledons, while others are Dicotyledons. We may expect certain differences in the biochemical processes occurring in the different plant species, and the reason of the differences might be that they fall into different botanical classes. However, as is seen from Table 2, basically certain differences between couch grass (monocot) and yarrow and plantain (dicots) were observed in leaves. In roots, the differences were not so clearly seen and were found only between the $\delta^{13}C$ values of couch grass and plantain and yarrow. For example, the $\delta^{15}N$ values of leaves of couch grass were statistically significantly higher and the $\delta^{13}C$ values were lower ($P < 0.05$) than the values of leaves of yarrow and plantain (when the plants were collected from site 2).

Similar phenomenon (differences between the $\delta^{15}N$ values of leaves of different plant species are much higher than differences between the $\delta^{13}C$ of leaves of the plants) was also observed by other researchers. It was shown that the variation in the $\delta^{15}N$ may be almost twice as large as that in the $\delta^{13}C$ (Yang at el., 2015). On the other hand, Goldman (2010) reported about statistically significant differences in the $\delta^{13}C$ values of *P. Acaulis*, *P. Sericea*, and *F. Lenensis*, while there were no significant differences in the $\delta^{15}N$ values among the species.

It was previously reported (Smith and Epstein, 1971) that on the average the $\delta^{13}C$ values of dicots were slightly more negative than those of monocots. As we can see, the differences can actually be observed. However, in our experiment the $\delta^{13}C$ values of roots and leaves of plantain and yarrow (dicots) were usually higher than those of couch grass (monocot).

As it is seen, external factors are able to provide a certain influence on the process of the isotope distributions among different plant species. For example, in leaves of the plants collected from site 1 the highest $\delta^{15}N$ was found in yarrow, while in leaves of the plants collected from site 2 the highest $\delta^{15}N$ value was observed in couch grass (Table 2).

According to available literature, the most important factors affecting the stable isotope values are soil texture (Bird et al., 2003), soil moisture (Goldman, 2010), and microbial activity in the rhizosphere soil (Wang et al., 2015). The differences in soil parameters between sites 1 and 2 might result in differences in water-holding capacity, nutrient status, and root penetration resistance. Consequently, various physical and chemical processes in the soil might be affected and this could produce a certain change in the distribution of stable isotopes in a particular plant species.

3.6. Main reasons influencing variability of the $\delta^{13}C$ and $\delta^{15}N$

The variability of the $^{13}C/^{12}C$ and $^{15}N/^{14}N$ ratios was rather high among different plant species (Fig. 1). The main factors affecting this variability were (1) biological (plant species) and (2) ecological (biogeochemical characteristics of soils). For example, the higher were concentrations of N in the rhizosphere soil, the higher were $\delta^{15}N$ values in plants. Such a positive linear relationship between soil $\delta^{15}N$ and leaf $\delta^{15}N$ was also reported by other researchers (Mgelwa et al., 2019). This may be due to an increasing contribution of soil N to the plants associated with increasing soil N availability.

On the other hand, the higher were total amount of C in the soil taken from the plant roots, the lower were the $\delta^{13}C$ values of roots and leaves of the plants that grow in the soil. This probably was due to discrimination of C isotope during process of its transfer from soil to a plant. Until now it is widely believed that organic C in plant tissues originates from atmospheric CO_2. This plant organic C is supplied by the vegetation to the soil as litter and residues (Yoneyama, 2017). The ^{13}C-enrichment of soil organic matter relative to original plants may occur through isotopic fractionation during organic matter decomposition (Wang et al., 2008).

The correlation between the $^{13}C/^{12}C$ as well as between $^{15}N/^{14}N$ ratios in roots and leaves was statistically significant ($P < 0.05$) and positive (Fig. 2). Similar effect (a strong positive relationship between isotope ratios of different plant parts) was also observed by other researchers (West et al., 2009; Kaler et al., 2018). It was suggested that this phenomenon might be associated with photosynthetic differences between different plant organs (Kaler et al., 2018). This may also indicate that the changes in the ratios of C and N isotopes in roots and in leaves occur similarly. On the other hand, the correlation between $^{13}C/^{12}C$ and $^{15}N/^{14}N$ ratios calculated only for leaves (Fig. 3a) and only for roots (Fig. 3b) was statistically significant and negative. Negative relationships between $\delta^{13}C$ and $\delta^{15}N$ in leaves of 38 plant species of 8 families were also reported by Vitória et al. (2018). Zhao et al. (2010) found that plant $\delta^{15}N$ and $\delta^{13}C$ values were regulated by some concurrent factors. A significant ($P < 0.001$) negative correlation was observed between the two isotopic signatures in non-leguminous plants, while a significant ($P < 0.003$) positive correlation was found in leguminous plants. These results indicate that the C and N discrimination are dependent at certain extent and besides, may partially be controlled by the common environmental factors.

4. Conclusions

The primary objective of this study was to estimate the contribution of environmentally induced and phylogenetic factors influencing the C and N isotopic composition of plant species and rhizosphere soil. The main conclusions of the research are the following:

- Each plant species can respond to variations in the environmental conditions in accordance with its sensitivity to the current environmental situation. The $\delta^{13}C$ and $\delta^{15}N$ values of the plants examined in the experiment were species-specific and varied strongly with sampling location.
- The ratios of stable N and C isotopes of roots and especially leaves were typical for a particular plant species and vary among different plant species growing simultaneously at the same place. This effect was more pronounced when we compare Dicotyledonous and Monocotyledonous plants.
- The soil parameters (soil texture, pH values, and chemical composition of the soil) were among main factors affecting C and N isotope ratios of the soils and different plant parts.
- The differences in the plant $\delta^{15}N$ most likely reflect access to different soil N sources. The variations in $\delta^{13}C$ might result from different discrimination regimes (both physical and biochemical) during photosynthesis and in the course of transfer of C isotope between plant and soil.
- The analysis of stable C and N isotope ratios may be used to study how environmental (first of all characteristics of soil) and biological (different taxa) factors can determine the differences in C and N dynamics in the soil – plant system.

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