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Variability of the Carbon Isotope Composition of Peat-Forming Plants during the Biochemical Transformation

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Abstract: In this study, we describe the variation in $\delta^{13}C$ value in the litter of two species of peat-forming plants: *Sphagnum fuscum* and *Eriophorum vaginatum,* during 3 years of field decomposition in oligotrophic bog ecosystems drained for the purpose of forest melioration and fire affected and at the stage of post-pyrogenic restoration. Litterbags were periodically retrieved in the autumn and the $\delta^{13}C$ value in the residual litter was related to mass loss, litter chemistry, and hydrothermal conditions. *Sph. fuscum* decomposes much more slowly than *E. vaginatum*. Low rate of transformation for *Sph. fuscum* is observed in drained and post-pyrogenic sites, while for *E. vaginatum* minimal rate of transformation is observed in the native site. During the decomposition of *Sphagnum* residues, ^{13}C enrichment occurs, and during the decomposition of *E. vaginatum,* we observed ^{12}C enrichment. The changes in the isotope composition of carbon for investigation sites are insignificant for *Sphagnum fuscum*, but it was observed for *E. vaginatum*, the largest of ^{13}C depletion is observed in the drained site (−28.3‰) and minimal in the postpyrogenic site (−27.4‰).

Keywords: peat-forming plants; carbon isotope composition; decomposition; oligotrophic bog; drained peatlands; postpyrogenic peatlands

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1. Introduction

Occupying a small area (about 3–5%) of the land surface, wetland ecosystems play a significant role in the biosphere, including the global carbon cycle, being sources and sinks of greenhouse gases [1–3]. On the territory of Western Siberia, the area of bog ecosystems is almost 50%; peat deposits contain about 36% of the total pool of soil carbon in Russia [1,4,5]. The process of carbon accumulation in peat prevails over the process of its emission, due to the slow process of decomposition of plant residues. This causes a constant increase in peat deposits and the development of bog ecosystems. There are relatively few works devoted to the study of the dynamics of decomposition of peat-forming plants in bog ecosystems [6–16]. The rate of decomposition of plant residues depends on the chemical composition of plants and the hydrothermal conditions under which these processes occur [6,17–19]. While humidity and temperature play a leading role in the transformation of plant residues [6,7,17,20], changing weather conditions cause an irregular, intermittent peat-forming process, which is typical for raised bogs that receive their main water supply from precipitation. Climatic changes or anthropogenic impact (drainage) influence the hydrothermal regime of bogs. These variations lead to changes in biogeochemical cycles since warming and a decrease in the level of bog waters stimulate the process of decomposition of organic matter and increase the intensity of CO_2 release into the atmosphere. In addition, it leads to changes in the composition and structure of the vegetation cover [1,21–24].

Melioration, especially for agricultural purposes, leads to the stimulation of microbial decomposition of organic matter and to an increase in the heterotrophic carbon flux into the atmosphere [25,26]. Forest melioration is one of the most sparing options for draining peat soils. Oligotrophic bogs of Western Siberia are in very favorable climatic conditions. Due to the predominance of precipitation over evaporation, there is a rapid recovery of bog ecosystems after forest melioration [27]. Moreover, one of the most significant factors influencing the biogeochemical cycles of bog ecosystems is fires [28]. The restoration of ecosystems and their biogeochemical cycles after fires in natural conditions is of considerable scientific and practical interest. Post-pyrogenic succession is characterized by a gradual change in ecosystems, accompanied by a change in species richness and biomass, and, accordingly, the rate of transformation of organic matter and its chemical composition [29,30].

Many biogeochemical processes are accompanied by a change in the stable carbon isotope ratio, due to which different components of ecosystems and different ecosystems differ in their isotope composition [31]. A change in the stable carbon isotope ratio indicates ongoing biochemical transformations and, therefore, makes it possible to obtain information about the functioning of ecosystems in natural or disturbed conditions, as well as to use it to identify the direction and intensity of processes associated with isotope fractionation [32–34]. A large number of studies are devoted to terrestrial and aquatic ecosystems [35–41]. Changes in $\delta^{13}C$ value during the decomposition of plant residues depend on many factors, including changes in the ratio of chemical components of the plant tissues themselves [42], as a result of which $\delta^{13}C$ value may change non-linearly during transformation. There are many studies devoted to the study of the isotope composition of natural ecosystems. However, data on changes in the isotope composition in bog ecosystems are not so numerous; moreover, these data are often contradictory depending on the study area, types of ecosystems, and plants themselves [35,36,38,41,43–45]. Studies conducted in bog ecosystems are mainly related to the study of the isotope composition of peat organic matter [46–51]. Very rarely have the plants themselves or the early stages of transformation of plant remains been studied [52–57]. On the territory of the southern taiga subzone of Western Siberia, the issue of changes in the isotope composition during the transformation of plant remains has practically not been studied at all [58], especially in bog ecosystems subject to anthropogenic or pyrogenic influence.

The purpose of the study was to assess the change in the carbon isotope composition during the transformation of plant residues *Sph. fuscum* and *Eriophorum vaginatum* in oligotrophic bog ecosystems drained for forest melioration and passed through by fire and at the stage of postpyrogenic restoration.

2. Materials and Methods

The measurement site is located in the south of Western Siberia (Russia). The study was carried out on two oligotrophic bogs "Vasuyganskoe" (field station "Vasyuganye" (IMCES SB RAS)) and "Iksinskoe", belonging to the northeastern spurs of the Great Vasyugan bog and located in the Bakcharsky district of the Tomsk region. Since the 1970s, ameliorative canal network has been operating in these bogs, as a result of which cardinal changes in the natural environment occurred in the bogs, in particular, the drainage of significant bog areas, which caused massive forest and peat fires [59].

Two observation sites were chosen for the study at Vasuyganskoe bog—the native pine-shrub-sphagnum phytocenosis 56°52′31.7″ N 82°48′27.3″ E (VASnat) and the pine-shrub-sphagnum phytocenosis located near the drainage canal 56°53′33.3″ N 82°51′08.0″ E (VASdry). The Iksinskoe bog is located between the Iksa and Shegarka rivers (Figure 1). In 1998, a large part of the territory burned out in the drained area of the Iksinskoe bog. The vegetation cover from the near-surface peat layer in the bogs was completely destroyed [59]. The following two sites with different degrees of pyrogenic succession were selected for the study: a pine-shrub-sphagnum phytocenosis with a well-defined undergrowth 56°51′42.1″ N 83°17′53.0″ E (Iksa2) and a pine-birch-cotton grass-sphagnum phytocenosis with a less pronounced degree of pyrogenic succession 56°52′03.4″ N 83°11′52.1″ E (Iksa1).

The data on weather conditions were obtained from the nearest meteorological station, Bakchar [rp5.ru], located 40 km from the study site. An atmospheric soil measuring complex was used to monitor the soil temperature and the level of bog waters [60]. The peat deposit temperature was measured 5; 10; 20 cm deep into the peat. Soil temperature data were obtained for the period from September 2018 to September 2021.

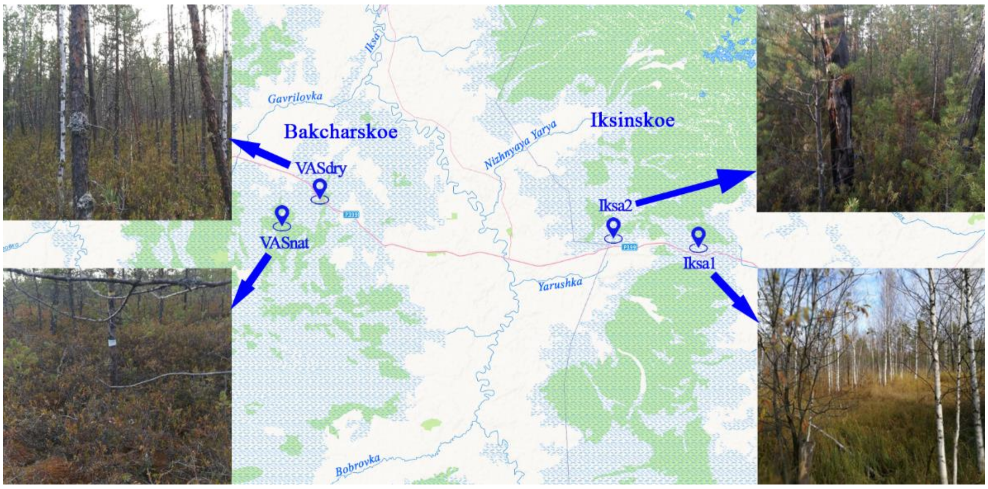

Figure 1. Site locations of studied peatlands, Western Siberia (Tomsk region).

2.1. Determination of the Decomposition Rate of Peat-Forming Plants

The following two dominant species of the modern plant cover of oligotrophic bogs have been chosen to study the decomposition of peat-forming plants: *Sph. fuscum* and *E. vaginatum*. The decomposition rate of plants was determined by the method of partially isolated samples, which is widely used to study the transformation processes of plant material and peat [Golovatskaya, Nikonova, 2017].

In September 2018, we sampled the moss litter of *Sph. fuscum* (their top 10 cm-long parts) and remains of *E. vaginatum* on each of the studied bogs. The plant samples were air-dried at a laboratory and placed in nylon bags 15 × 15 cm size. Each plant sample weighed 10 g. The prepared bags with plants were placed into peat deposits at a depth of 10 cm from the moss surface (sphagnum fiber) in September 2018. In total, 96 bags were prepared (48 bags for each type of plant residue)

Mass loss (ML, % of the initial mass of plant sample) was calculated by the following equation:

$$\mathrm{ML}(\%) = \frac{\mathrm{M0} - \mathrm{Mt}}{\mathrm{M0}} \times 100 \quad (1)$$

where M0 is dry mass of the initial sample and Mt is dry mass of sample remained in bag over 12-, 24-, and 36-month-long periods after the experiment start.

To assess the change in the content of total C in the initial samples of the studied plants, we used the method of simultaneous determination of the total C content in peat soils elaborated by Anstett's method modified by Ponomareva and Nikolaeva [61,62]. The analyses were performed in three replications.

The carbon isotope composition was determined by isotope ratio mass spectrometry [63] using a DELTA V Advantage isotope mass spectrometer combined with a Flash 2000 elemental analyzer (Thermo Fisher Scientific, Bremen, Germany) equipped with a redox reactor. All samples were milled, after which a sample was placed in tin capsules (tin of a high degree of purity). The optimal weight of a sample for carbon isotope analysis was 450–500 µg. Encapsulated samples were placed in an autosampler of elemental analyzer. The capsule fell into an oxidizing reactor heated to 1020 °C and filled with Cr_2O_3 and Co_3O_4 granules, burned in a carrier gas flow (helium, 250 mL/min) with a simultaneous supply of pure oxygen (180 mL/min). The resulting oxidation products (CO_2) entered the reduction reactor. A magnesium perchlorate trap was used to remove water. Carbon dioxide entered the DELTA V Advantage mass spectrometer via a capillary through a Conflo IV gas distribution system.

The isotope composition was determined by the following formula:

$$\delta^{n}X = \left[\frac{R_{sample} - R_{standard}}{R_{standard}}\right] \times 1000 \quad (2)$$

where ^{n}X are ^{13}C isotopes (‰); R_{sample} is the ratio of the heavy to light isotope in the test sample; $R_{standard}$ is the ratio of heavy to light isotope in the standard.

Laboratory reference gas (CO_2) was calibrated against IAEA Reference Material IAEA-CH-3 Cellulose with a known stable isotope ratio as follows: $\delta^{13}C_{VPDB} = -24.72 \pm 0.04$‰. The absolute measurement error for three repetitions of the analyzed samples is no more than 0.2‰.

2.2. Statistical Analysis

The influence of plant species and site ecosystem type on the rate of decomposition and dynamics of carbon isotope composition was tested using two-way analysis of variance. Student's *t*-test was used to assess significant differences in the chemical composition of the litter within and between plant species. All statistical analyzes were performed using Statistics for Windows v. 6.0.

3. Results

3.1. Weather and Hydrothermal Conditions

During the study period (2018–2021), the average annual temperature was 0.79 ± 1.47 °C, and the average annual cumulative precipitation was 524 ± 92 mm. The average temperature of the growing season from the beginning of May to the end of September was about 13.9 °C, and the amount of precipitation during the growing season (May–September) was 277 mm (Figure 2).

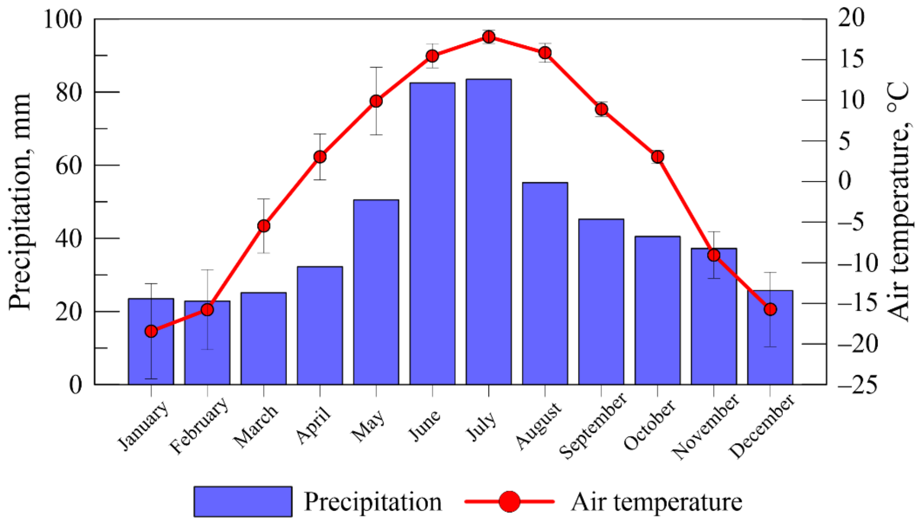

Figure 2. Weather conditions: average monthly air temperature and precipitation, average for 2019–2021.

On average, over the period of the experiment site, VASdry was characterized by the lowest level of bog waters (WTL) −55 cm and the lowest average temperature at a depth of 15 cm during the growing season (+9.5 °C). Iksa1 was the most watered area (WTL = −17 cm) and was characterized by the warmest conditions of the peat deposit (+13.6 °C).

Iksa2 was also quite watered (WTL −27), but the temperature of the peat deposit is much lower (+10.7 °C). VASnat conditions occupied an intermediate position between drained (VASdry) and pyrogenic (Iksa1) peatlands—low WTL (–40 cm), relatively warm conditions of a peat deposit (+12.2 °C).

3.2. Decomposition Rate

After 3 years of decomposition, the average mass loss for sphagnum litter was 25% (from 19.3 to 30.9% depending on the sites), and *E. vaginatum* litter was on average 64% (56.6–67.9%) (Table 1).

Table 1. Percentage of mass loss and chemical composition in initial samples and after 3 years of field decomposition.

Plant Species	Site	Mass Loss, %	C, %	δ^{13}C, ‰	N, %	C/N	Lig	Lig/N
Sph. fuscum	Initial sample		45.1 ± 0.6	−31.2 ± 0.2	0.4 ± 0.0	118 ± 1	12.2	32
	VASnat	30.9 ± 8.9	47.3 ± 0.5	−30.1 ± 0.1	-	-	-	-
	VASdry	27.6 ± 3.7	45.9 ± 1.4	−29.9 ± 0.2	-	-	-	-
	Iksa1	19.3 ± 1.1	44.2 ± 0.8	−30.1 ± 0.2	-	-	-	-
	Iksa2	22.3 ± 5.3	45.5 ± 0.1	−30.0 ± 0.2	-	-	-	-
E. vaginatum	Initial sample		47.0 ± 0.4	−26.1 ± 0.2	1.0 ± 0.0	49 ± 0.1	20.3	22
	VASnat	56.6 ± 5.8	47.4 ± 1.9	−27.9 ± 0.1	-	-	-	-
	VASdry	67.9 ± 1.4	47.8 ± 0.1	−28.3 ± 0.6	-	-	-	-
	Iksa1	67.4 ± 3.4	47.6 ± 0.6	−27.9 ± 0.2	-	-	-	-
	Iksa2	64.9 ± 5.4	47.5 ± 0.6	−27.4 ± 0.2	-	-	-	-

The most intensive decomposition occurred within 1 year of the experiment, with the exception of fuscum in the natural area, in which the maximum losses were obtained within 3 years. On average, the loss of mass of organic matter during 1 year was 56% and 62% of the total loss for the entire period of the experiment (3 years) for *Sph. fuscum* and *E. vaginatum*, respectively (Figure 3). The process of transformation of plant residues of *Sph. fuscum* occurred more intensively in VASnat and VASdry sites, while the decomposition of moss on Iksa1 and Iksa2 postpyrogenic peatlands was slower. On the VASdry and VASnat sites, the mass loss during the 3 years of the experiment was comparable to (VASdry) or even exceeded (VASnat) the weight loss during the 1-year period. We assume that the increase in the rate of decomposition of *Sphagnum fuscum* during the third year of the experiment is associated with the activation of microbiological activity. The decomposition of *E. vaginatum*, on the contrary, proceeded more slowly in natural conditions.

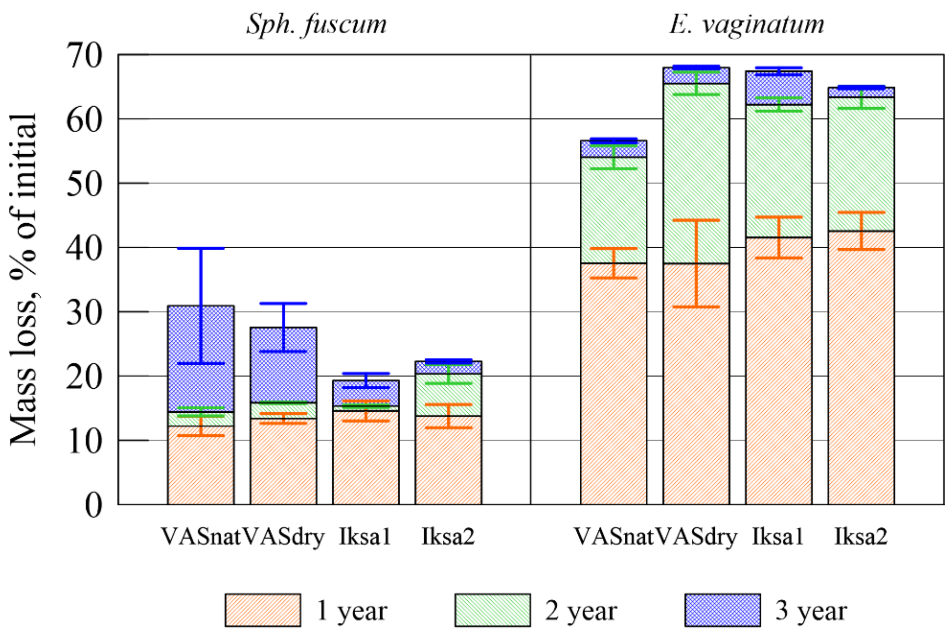

Figure 3. Dynamics of mass loss of *Sph. fuscum* and *E. vaginatum* at different observation points.

3.3. Variability of the Isotope Composition

The carbon content in the litter decreased after one year of the experiment, for *Sph. fuscum* by 3–5% and for *E. vaginatum* by 1.5–4%. By the end of the second year, the content of C had significantly increased in *Sph. fuscum* in the VASnat and Iksa1 (on average, 5%). In *E. vaginatum*, the carbon content also increased, but not so significantly. After 3 years, the carbon content returned to its initial values in all samples, with the exception of *Sph. fuscum* in the VASnat (Figure 4).

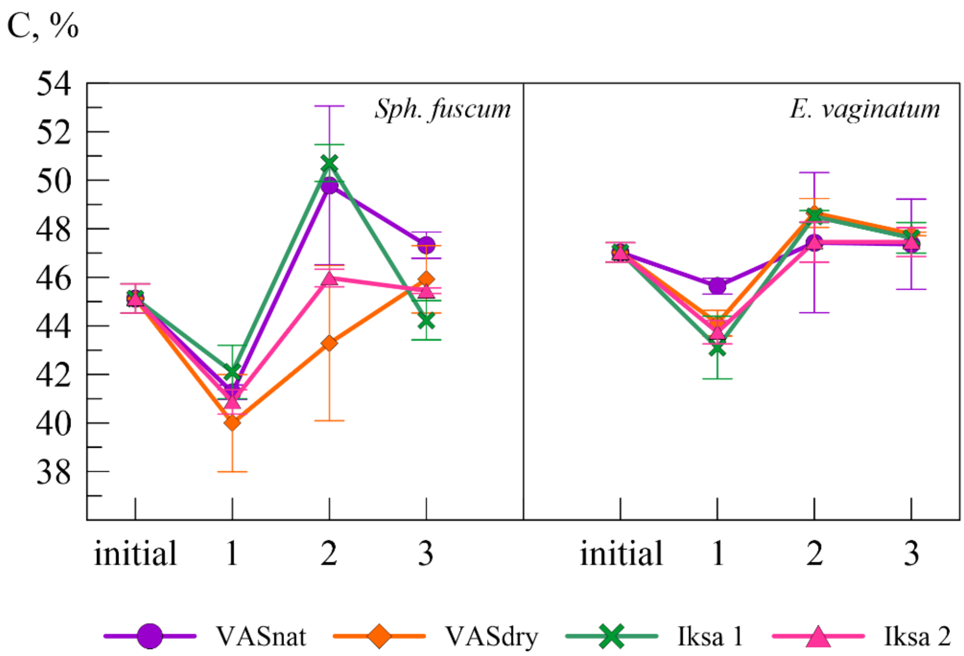

Figure 4. Dynamics of carbon content in the process of transformation of plant residues of *Sph. fuscum* and *E. vaginatum*.

According to the analysis of the isotope composition of plant residuals (Figure 5), during the transformation of *Sph. fuscum* at all sites, the carbon isotope composition became heavier by an average of 1‰. For *Sph. fuscum* enrichment in the heavy carbon isotope was observed throughout the period at all sites, except for the VASdry one, where during the 1st year of the experiment a low $\delta^{13}C$ value was observed, followed by an increase. For *E. vaginatum*, the reverse process was observed as follows: the carbon isotope composition changed by an average of −1.8‰. A significant change in the isotope composition of *E. vaginatum* occurred during 1 year of the experiment, when the average $\delta^{13}C$ value was −28‰. Low $\delta^{13}C$ values occurred in the *E. vaginatum* at all sites during the first and second years of incubation. During the third year, there is a slight enrichment by ^{13}C; however, the $\delta^{13}C$ value remains significantly lower than in the initial samples. An exception is the *E. vaginatum* placed at the dried site, where, as a result of the leaching regime, continues despite rather high mass losses.

The results of the correlation analysis showed a close relationship between weight loss and changes in the isotope's composition (Figure 6). For *Sph. fuscum,* the relationship was positive (correlation coefficient 0.81), and for *E. vaginatum,* it was negative (−0.79).

According to a two-way analysis of variance, the δ13C value of the residual plants significantly varied depending on the plant species and not depending on the location of the samples (Table 2).

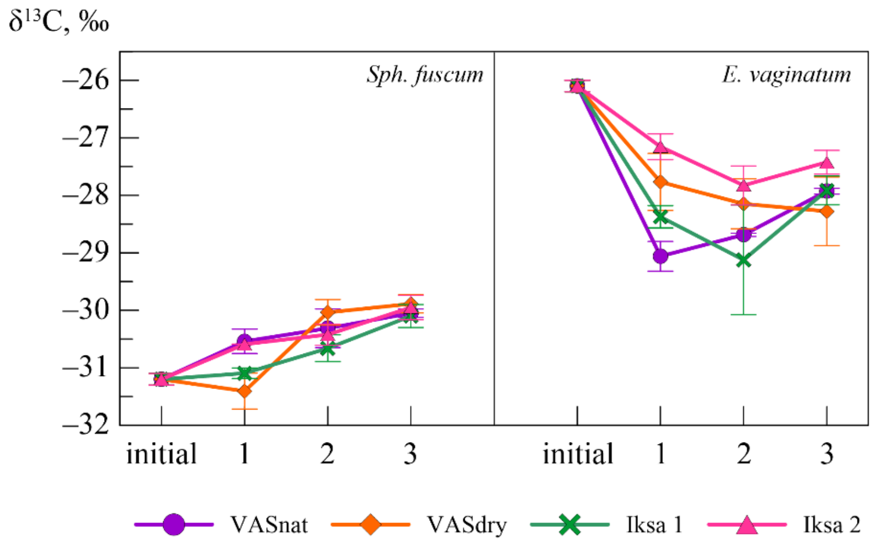

Figure 5. Variability of the carbon isotope composition of peat-forming plants during the biochemical transformation.

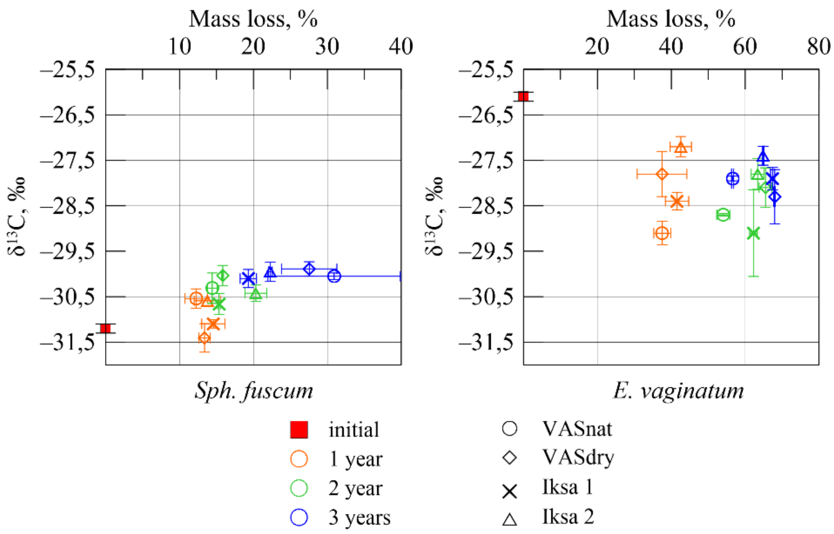

Figure 6. Dependence of the carbon isotope composition on mass loss.

Table 2. Summary of the ANOVA for the effect of plant species and location of the samples on value δ13C in plant residuals.

Factor	Df	F Value	*p*
Plant species	1	168.238	<0.05
Sites	3	1.3	<0.05
Plant species × Sites	3	0.892	<0.05

The influence of the location on the $\delta^{13}C$ value most clearly appears during the 1st year of the experiment for both *Sph. fuscum* and *E. vaginatum*. At the same time, samples of *Sph. fuscum* incubated in the VASnat and in the Iksa2 area have similar $\delta^{13}C$ values throughout the period of investigation, significantly differing from the VASdry and Iksa1. For *E. vaginatum*, significant differences in the $\delta^{13}C$ value were found in the location for all sites, and, unlike sphagnum, the maximum difference was observed between samples on VASnat and Iksa2 during the first year. By the end of the third year, the differences in the location of the samples are not significant.

4. Discussion

The obtained differences in the rate of mass loss of *Sph. fuscum* and *E. vaginatum* are significant and are consistent with previously obtained data [12,19]. Sphagnum mosses contain the least amount of bitumen and many easily hydrolyzable and water-soluble compounds of the carbohydrate complex; therefore, sphagnum mosses should be the least resistant to decomposition. However, they also contain a specific phenolic compound, sphagnol, which prevents rapid bacterial decomposition [64]. The low content of lignin can also contribute to slow decomposition. Nutrient-enriched plant residues with a high nitrogen content, a minimal C/N ratio, and a low lignin content usually quickly decompose. For plant residues with a low nitrogen content, a wide C/N ratio, and a high lignin content, the decomposition rate is much lower. In this case, not only the lignin content but also the lignin/nitrogen ratio is an important factor, since it has been shown that the higher the lignin/nitrogen ratio, the slower the decomposition occurs [65,66]. We assume that due to the low nitrogen content (0.4%) with a rather high lignin content (12%), a high lignin/nitrogen ratio (32) was obtained, which apparently contributes to a lower decomposition rate of *Sphagnum fuscum*. Sphagnum mosses and in some species of herbaceous plants (Carex, Eriophorum, Scheuchzeria) hemicellulose predominates among carbohydrates, which is also more resistant to decomposition [64]. Thus, sphagnum mosses have chemical and mechanochemical immunity, which allows them to remain in deposits. The rapid decomposition of plant residues at the initial stages is associated with the processes of leaching of easily soluble substances from fresh plant litter, as well as the intensive activity of microorganisms [67–72]. Microorganisms destroy the easiest available components of the carbohydrate and polypeptide complexes, the content of which decreases with time, and, accordingly, the loss of organic matter decreases [8,73]. Sphagnum organic matter can contain up to 20% labile water-soluble components such as carbohydrates [74]. The loss of these substances explains the large mass loss in the earliest stages of peat formation [75].

The influence of the location on the change of $\delta^{13}C$ value can be assessed through such parameters as peat temperature and the water table level. The results of the correlation analysis showed a weak relationship between the temperature of the peat deposit and the $\delta^{13}C$ value ($r = -0.29$, $p < 0.05$ and -0.41 $p < 0.05$ for *Sph. fuscum* and *E. vaginatum*, respectively). The dependence dynamics of the $\delta^{13}C$ value on temperature were noted in the article by Bragazza and Iacumin [15]. They studied seasonal dynamics and observed ^{13}C enrichment during the summer period and ^{13}C depletion in the winter. In addition, studies of litter decomposition under laboratory conditions show changes in the $\delta^{13}C$ value depending on temperature [76].

The analysis of the influence of the water table level in the studied areas did not reveal a relationship between the $\delta^{13}C$ value and the water table level. Obviously, this is because even at sufficiently low water levels, the moisture content of the upper horizons of the peat deposit remains quite high (80–85%). Therefore, the decrease in the water table level is less critical for the dynamics of the $\delta^{13}C$ value in comparison with the change in temperature. At the same time, a decrease in the water table level leads to an increase in the rate of transformation of plant residues under drier conditions, decomposition proceeds more intensively, which is consistent with other studies [10,18,19,53]

The change in $\delta^{13}C$ value was different for the two species, the $\delta^{13}C$ value of *Sph. fuscum* litter was enriched, while the $\delta^{13}C$ value of *E. vaginatum* litter decreased. We assume that this also depends on the chemical composition of the plants. Eriophorum has a higher content of cellulose, lignin and lignin-like substances, alcohol-soluble compounds (including aromatic and aliphatic carbohydrates, terpenes, carbolic acids, resins, and fatty acids, essential oils, fats, and phytosterols) [58]. Since soluble elements are more enriched in the heavy carbon isotope than lipids and lignins, the removal of the soluble fraction as a result of leaching will lead to low $\delta^{13}C$ values [36,77,78]. Our data are consistent with previous studies in which low $\delta^{13}C$ values were noted at the early stages of the decomposition of plant residues [38,41]. Over time, the rate of transformation significantly decreases, probably due to the transformation process proceeds largely due to the activity of microorganisms. Probably, as a result of the activation of microorganisms, the accumulation of a heavy isotope can occur due to the contribution of microbial organic compounds [15]. Earlier studies also noted that enrichment by the heavy isotope can be caused by the inclusion of carbon from soil organic matter by decomposers Wedin et al., [36], in addition, it was found that significant changes occur in microfungal communities [79].

5. Conclusions

Quantitative estimates of the rate of decomposition of two species of peat-forming plants (*Sph. fuscum* and *E. vaginatum*) were obtained during 36 months of a field experiment. The highest resistance to decomposition, whose mass loss over the three years of the experiment was 19–31%, characterized the samples of *Sph. fuscum*. *E. vaginatum* showed the least resistance to decomposition with the maximum mass loss—56–68% after three years. The most intensive decomposition of plant residuals was observed during the first year of the experiment; further, the rate of decomposition of organic matter decreased.

The rate of decomposition is determined by environmental conditions, but primarily the rate depends on the chemical composition of plants. The next most important factor is the temperature, the higher the temperature. The influence of the water level on the transformation process is unreliable. Our study revealed significant differences in the dynamics of the $\delta^{13}C$ value during the decomposition of plant residues. After 3 years, *Sph. fuscum* showed a high $\delta^{13}C$ value, while *E. vaginatum* showed a low $\delta^{13}C$ value relative $\delta^{13}C$ values of the initial samples. Due to the peculiarities of the morphological and chemical structure of *Sphagnum fuscum*, the $\delta^{13}C$ value in plant residues is associated with microbiological activity, and the enrichment by the ^{13}C isotope most likely occurs due to microbial biomass. *E. vaginatum* was characterized by rapid mass loss. This leads to a sharp decrease in the $\delta^{13}C$ value and subsequent slight enrichment in the ^{13}C isotope with a decrease in the transformation rate due to a decline in the role of leaching and an increase in the role of microbial decomposition, which leads to an increase in the content of ^{13}C isotope due to microbial biomass. The changes in the isotope composition of carbon at the investigation sites are insignificant for *Sphagnum fuscum*, but they were observed for *E. vaginatum*. The largest decrease in the $\delta^{13}C$ value is observed in the drained site VASdry (the $\delta^{13}C$ value was −28.3‰ for the third year of the experiment relative −26.1‰ for the initial sample) and minimal in the post-pyrogenic site Iksa2 (the difference between the $\delta^{13}C$ value in the third year of the experiment and the $\delta^{13}C$ value of the initial sample was −1.3‰).

Author Contributions: E.G., L.N.—organized field work and collected plant samples; L.N.—mass loss analysis of plant residues, G.S., D.K.—determination of carbon content and isotopic composition of samples; E.G.—wrote a draft version of the manuscript, which was commented on by all co-authors. All authors have read and agreed to the published version of the manuscript.

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Conflicts of Interest: The authors declare no conflict of interest.

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