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SOIL CHEMISTRY

Changes in Labile and Microbial Pools of Carbon and Nitrogen in Forest Litter Samples under Different Methods of Storage

M. N. Maslov[a], *, O. A. Maslova[a], and O. A. Tokareva[a]

[a] *Lomonosov Moscow State University, Leninskie gory 1, Moscow, 119991 Russia*

**e-mail: maslov.m.n@yandex.ru*

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Abstract—The influence of conservation procedures (drying, freezing, and storage at low positive temperatures) and preliminary aerobic incubation on the result of determination of labile and microbial pools of carbon and nitrogen has been analyzed using forest litter samples from the northern, middle, and southern taiga spruce forests. It was established that drying of litter samples and their subsequent moistening before analyzing them resulted in a decrease of microbial carbon and nitrogen concentrations against the background of increasing concentrations of elements of the labile pool. Freezing of the samples had similar effect on the labile pool, but it did not lead to a statistically significant change in concentrations of C and N in microbial biomass. Storage at low positive temperatures resulted in decrease of microbial pool, but its on the labile forms of carbon and nitrogen effect was ambiguous, and this was associated with the growth and death cycles of microbial biomass. Preliminary aerobic incubation of litter samples did not lead to restoration of carbon and nitrogen concentrations typical for fresh samples. The main direction of nitrogen compounds transformation was determined by the C : N ratio in labile organic matter and microbial biomass, and depended on the storage method: the processes of net mineralization predominate when drying, while the processes of net immobilization dominated during freezing and storage at low positive temperatures.

Keywords: mineralization, microbial immobilization of nitrogen, standardization of storage conditions, incubation

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INTRODUCTION

The influence of the method of sample storage on the results of carbon and nitrogen pools determination in soil is one of the debatable methodological problems. It is evident that the change of parameters versus the natural state would be minimal in the case of immediate analyzing after taking the sample from a pit [5, 19, 24]; however, it is often impossible because of great number of samples and kinds of analysis, or specificity of logistics. An additional point is that storage procedure is inevitable if repeating the analyses and confirming the results obtained earlier are required. Drying to air-dry state, freezing, and storing at low positive temperatures are most common procedures of storage of soil and litter samples until analyzing. In order to minimize the methodical errors, which can essentially skew the obtained results, it is worth to determine storage procedures, which would store securely the integrity of the sample until operator could perform the corresponding analysis.

Effects of different procedures of storage are different for different microbiological and chemical parameters and for soils with different combinations of properties. The influence of storage procedure on the result of analysis could be more pronounced for organic soils and litters than for mineral soils [16, 25]. Storing of fresh samples under the temperature +4°C [17, 22], preventing active growth of microbial biomass [13, 15] and substrate consumption, is most often recommended for studying the microbiological parameters. At the same time, it was found out that storing under +4°C during 3 and 6 months caused the decrease of microbial biomass, changed the structure of microbial community [31], and decreased potential rate of nitrification [33]. Drying and subsequent moistening could result in significant change of microbiological parameters and particularly in the decrease of microbial biomass, and changed the activities of most enzymes and soil respiration [25]. Effect of freezing caused similar results [28]. Simultaneously, drying and freezing of samples was accompanied by significant increase of concentrations of labile carbon and nitrogen [6, 8, 14, 34], supposedly connected with the damage of cells of microorganisms. Preliminary incubation of stored soil samples at temperature about +22°C during several days [1, 4] is often used in scientific research to activate microbiological processes and "revivify" preserved soil samples, but this process could be connected with intense mineralization and immobilization of carbon and nitrogen, and this could

Table 1. Main properties of studied horizons of forest litter

Zone	Horizon	pH_{H_2O}	Ignition loss	C_{tot}	N_{tot}	C : N
			%			
Northern taiga	L	4.2 ± 0.2	7.9 ± 1.2	45.5 ± 0.1	2.0 ± 0.2	27.3 ± 2.4
	H	4.8 ± 0.3	18.3 ± 2.9	44.0 ± 1.7	2.0 ± 0.1	25.3 ± 0.4
Middle taiga	L	5.0 ± 0.1	9.8 ± 2.2	43.7 ± 3.6	1.1 ± 0.2	46.3 ± 1.2
	F	4.6 ± 0.2	15.5 ± 3.2	38.2 ± 3.3	1.2 ± 0.1	37.2 ± 1.8
	H	4.9 ± 0.3	16.3 ± 2.5	36.3 ± 5.2	1.6 ± 0.2	26.5 ± 1.5
Southern taiga	L	5.7 ± 0.1	9.6 ± 0.5	46.7 ± 1.0	1.5 ± 0.1	36.2 ± 2.0
	H	5.3 ± 0.1	23.7 ± 0.7	41.2 ± 2.2	1.8 ± 0.2	26.2 ± 1.7

The data are presented in tables and figures as average values ± standard error; calculations havr been performed per dry mass (105°C, 12 h).

lead to incorrect results. Currently, there are few data suggested that restoration of biological parameters after incubation under optimal conditions depends on the method of analysis [29] and on soil properties [21].

Our work was aimed at the assessment of the effects of storing procedures and preliminary incubation of forest litter samples on the results of determination the labile and microbial pools of carbon and nitrogen.

OBJECTS AND METHODS

The samples used in the study of forest litter of *Vaccinio-Piceetum* forest were collected in ecosystems of northern (Murmansk region, Khibiny Mountains), middle (Leningrad region, Lembolovskaya Upland), and southern (Yaroslavl region, Biological Station Uleima) taiga. The samples were taken in the period from August 25, 2018 to September 5, 2018. The plot 100 m^2 was taken within each studied ecosystem, and the samples were taken in fourfold randomly all over the area. The litter samples were taken by the subhorizons: fresh litter L, fermentation layer F (was presented only in the middle taiga), and humified layer H. The identification of forest litters was performed according to [3]; all studied litters were referred to as the mulch type. General characteristics of properties of studied objects are presented in Table 1. The litters were characterized by high contents of organic carbon (36–47%) against the background, low nitrogen content (C : N = 25–46) and low ignition loss. As plant falloff was transformed in F and H horizons, carbon was gradually lost, and relative contents of nitrogen and ash constituents increased. All studied horizons of litter were regarded as acidic by the pH_{water} values, and a gradual increase of actual pH values was observed more to the south.

Fresh samples were brought to laboratory and analyzed no later than in a day after sampling, and this allowed reckoning on obtaining the results corresponding to the natural state of samples. Labile compounds of C and N were extracted by 0.05 M K_2SO_4 [8]. Extractable organic C (C_{extr}) and total extractable N (N_{extr}) were determined in automatic analyzer TOC-V CPN (Shimadzu); $N\text{-}NH_4^+$ was determined with the indophenol method; $N\text{-}NO_3^-$ was determined after reduction on cadmium column to nitrites and obtaining colored azo-compound by Griess. Colorimetric determinations were carried out on spectrophotometer GENESYS™ 10 UV (USA). Nitrogen content in extracted organic compounds (N_{org}) was calculated by the difference between N_{extr} and the sum of inorganic nitrogen compounds. Carbon and nitrogen of microbial biomass (C_{micr} and N_{micr}) were determined with fumigation-extraction method [18, 32] as the difference between concentrations C_{extr} and N_{extr} in fumigated and not fumigated weighed portions of studied samples.

In order to determine the influence of storage method on the results of microbial and labile carbon and nitrogen measurement, every analyzed fresh sample was divided to three portions. First portion was dried to air-dry state, the second was frozen at –18°C, and the third one was placed into a refrigerator at +4°C. The samples were stored during three months, and then, the above listed parameters were determined again. Dried samples were preliminary moistened to 60% of the field moisture capacity (FMC). The analyses were also carried out after 7 days of preliminary incubation at +22°C to explore the possibilities for restoration the initial properties.

Statistical analysis was carried out using the Statistica 10 program. The effects of the type of litter horizon and natural zone on concentrations of labile and microbial pools of C and N were evaluated with the help of two-way analysis of variance ANOVA (analysis was performed for L and H horizons). Factor analysis with the method of principal components was carried out for each component of carbon and nitrogen pools to determine the relationships between conservative and labile properties of litters. Pair-wise comparison of samples using *t*-test was applied in evaluating the effects of storage methods on carbon and nitrogen pools. The difference was considered to be significant at $p < 0.05$.

Table 2. Concentrations of labile and microbial forms of carbon and nitrogen in fresh samples of forest litter, mg/kg

Zone	Horizon	C_{extr}	N_{org}	$N\text{-}NH_4^+$	$N\text{-}NO_3^-$	C_{micr}	N_{micr}	$C_{micr} : N_{micr}$
Northern taiga	L	2298 ± 358	201 ± 24	29.8 ± 5.3	1.6 ± 0.1	4123 ± 695	680 ± 149	6.8 ± 0.5
	H	689 ± 45	33 ± 4	14.3 ± 2.6	1.6 ± 0.1	3494 ± 403	585 ± 111	7.3 ± 0.9
Middle taiga	L	1201 ± 112	16 ± 3	22.5 ± 3.0	3.4 ± 1.1	5059 ± 557	690 ± 85	8.6 ± 0.6
	F	501 ± 92	18 ± 4	9.0 ± 2.2	1.4 ± 0.4	2374 ± 211	316 ± 19	8.7 ± 0.6
	H	668 ± 158	23 ± 7	13.1 ± 2.0	1.1 ± 0.1	3383 ± 691	442 ± 95	8.7 ± 0.7
Southern taiga	L	727 ± 96	51 ± 6	11.5 ± 1.1	1.6 ± 0.4	4126 ± 337	532 ± 66	9.0 ± 0.7
	H	1390 ± 234	126 ± 19	20.7 ± 3.2	12.9 ± 5.1	3677 ± 257	495 ± 23	8.4 ± 0.5

RESULTS AND DISCUSSION

Concentrations of carbon and nitrogen of labile and microbial pools in fresh samples. Forest litter was characterized by high concentration of C_{extr} (500–2300 mg/kg, Table 2), and this was essentially higher than in humus horizons of mineral soils in European territory of Russia [7]. The fraction of extractable carbon in total pool ranged from 0.1 to 0.5%. Organic compounds were the predominating form of extractable nitrogen fraction, and ammonium form dominated the mineral pool of nitrogen. Forest litter was characterized by high contents of carbon and nitrogen of microbial biomass; the portions of these fractions averaged 0.6–1.2% C_{tot} and 2.6–3.5% N_{tot}. Enrichment of microbial biomass with nitrogen was recorded (the C : N ratio) ranged within 7–9.

Variations in concentrations of labile and microbial pools of carbon and nitrogen in litter were determined to different extents by the effects of two factors: natural zone and subhorizon of litter. Statistically significant ($p < 0.05$) influences of both factors and the interaction between them were found for all parameters of labile pool. At the same time, natural zone had not effects on the concentrations of microbial pools of carbon and nitrogen, and they depended statistically significantly only on subhorizon type. Hence, we can make a conclusion that the influence of edificatory species determined in forest ecosystems to a far greater degree the sizes of microbial pools of carbon and nitrogen in comparison with geographical zone. The importance of the tree species factor was demonstrated earlier for the processes of microbiological transformation of nitrogen [10] and for biomass of heterotrophic and denitrifying microorganisms [9].

Factor analysis allowed establishing that more than 70% of variations of labile and microbial pools of carbon and nitrogen in L and H horizons of litter could be explained by effects of two groups of factors (Table 3). Of all parameters, the first group of factors, explained 41–58% of variation, including the content of total carbon, C/N ratio in organic matter of litter, and ash content, i.e. the properties, which could be combined into the group "organic matter character". Varying of microbial pool parameters in litter was explained by the influence of factors connected with organic matter more than that of concentrations of labile forms. The second group of factors, responsible for about 30% of variation, was different for different parameters. For example, this group included the C_{micr} and $N\text{-}NH_4^+$, parameters for extractable carbon content, and this was in our opinion connected with consumption of organic substrate by microorganisms depending on their supply with the sources of nitrogen. Contents of total and extractable nitrogen are the second factor regulated nitrogen concentration in microbial biomass.

Effects of the method of storage the samples of forest litter on the results of determination of labile and microbial carbon and nitrogen. Storing litter samples during three months affected significantly the results of determination of carbon and nitrogen in labile and microbial pools (Figs. 1–3). Despite the trend towards the similarity of the main relative parameters, the method of sample storage caused in most cases significant changes in carbon and nitrogen pools in absolute values. The change of concentrations of labile and microbial pools of carbon and nitrogen after storage did not depend on genetic features of litter horizons: the two-way ANOVA did not reveal significant interactions between the factors horizon–storage method. Hence, we can say that the changes in litter properties observed after storage did not depend on the degree of decomposition of plant material, but they were determined by the response of microorganisms.

Drying to air-dry state and subsequent moistening to 60% of FMC resulted in the most significant change in concentrations of labile and microbial carbon and nitrogen in comparison with the data obtained for

Table 3. Variability of concentrations of labile and microbial forms of carbon and nitrogen attributed to total factors (%)

Parameter	L horizon		H horizon	
	F1	F2	F1	F2
C_{extr}	52.7	33.8	42.9	33.0
N_{org}	49.2	35.6	41.4	33.2
$N\text{-}NH_4^+$	56.2	29.1	45.7	32.9
$N\text{-}NO_3^-$	55.9	30.4	42.0	33.1
C_{micr}	58.1	30.4	50.3	32.5
N_{micr}	58.6	30.4	50.4	30.1

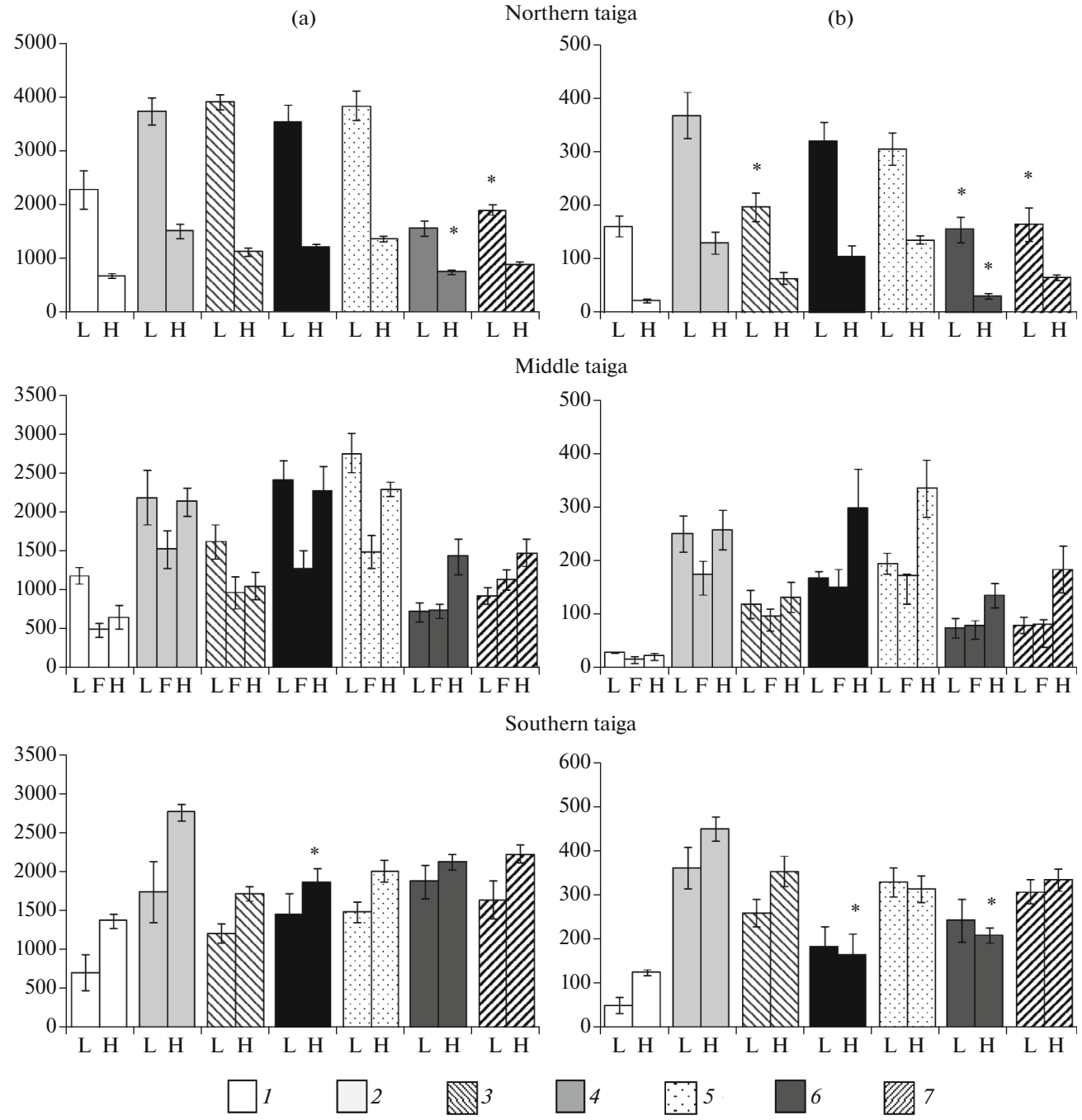

Fig. 1. Concentrations (mg/kg) of extractable (a) organic carbon and (b) organic nitrogen in litter horizons of bilberry spruce forest.Hereinafter: (*1*) fresh sample, (*2*) dried sample, (*3*) dried sample incubated for 7 days, (*4*) frozen sample, (*5*) frozen sample incubated for 7 days, (*6*) sample stored under low above-zero temperature, (*7*) sample stored under low above-zero temperature and incubated for 7 days. Asterisk (*) designates litter horizons, for which no statistically significant difference ($p < 0.05$) between the results obtained for fresh and stored samples has been found.

fresh samples. An abrupt decrease of concentrations of microbial components against the background of increase of labile forms of carbon and nitrogen contents (including mineral nitrogen) was the main trend for all studied litter samples. Significant enrichment of labile organic matter with nitrogen (C : N = 6–10, in average about 8) was observed after drying. The increase of concentration of labile components was relevant to decrease of carbon and nitrogen contents in microbial biomass (Fig. 3), and this allowed assuming that this biomass was the source of additional amounts of labile carbon and nitrogen. The mechanism of this phenomenon was connected with denaturation of cytoplasmic membranes of microorganisms during

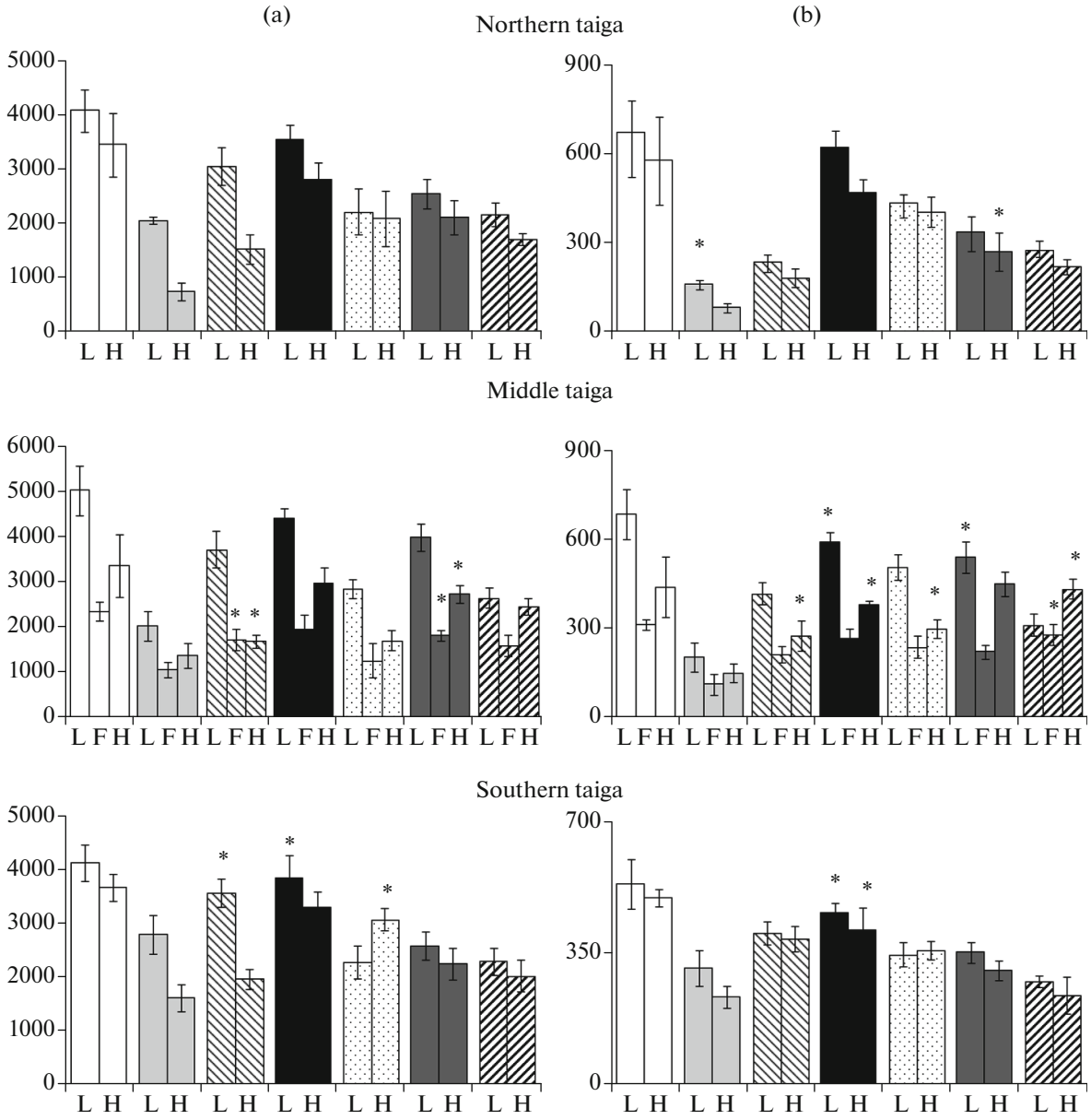

Fig. 2. Concentrations (mg/kg) of (a) ammonium and (b) nitrate nitrogen.

drying [11] and passing into solution of cytoplasm during subsequent moistening [2]. Similar pattern of changes in proportions between labile and microbial pools of carbon and nitrogen was demonstrated earlier for other soils including organic ones [7, 26, 27].

Drying of forest litter resulted in sharp increase of concentrations of mineral nitrogen and primarily of ammonium nitrogen (Fig. 2). After drying, the active mineralization of organic compounds of nitrogen labile pool was performed by the preserved part of resistant to drying microbial community, which gained an access to easily available substrate after subsequent moistening [20, 27, 34]. Drying of forest litter had not statistically significant effect on nitrate concentration, which was in general low, because nitrification was weak in acid medium under natural conditions.

Freezing of samples of forest litter and storing of these samples at −18°C skewed the results of determination of carbon and nitrogen in microbial biomass least of all: the pair-wise comparison with the data for fresh samples using *t*-test did not demonstrate significant ($p < 0.05$) difference. The stability of microbial pools of carbon and nitrogen could be connected with adaptation of microorganisms of northern territories to the influence of negative temperatures, because they function under the conditions of periodical and long cycles of thawing–freezing. Such feature was reflected in the method of soil samples storage accepted in Swe-

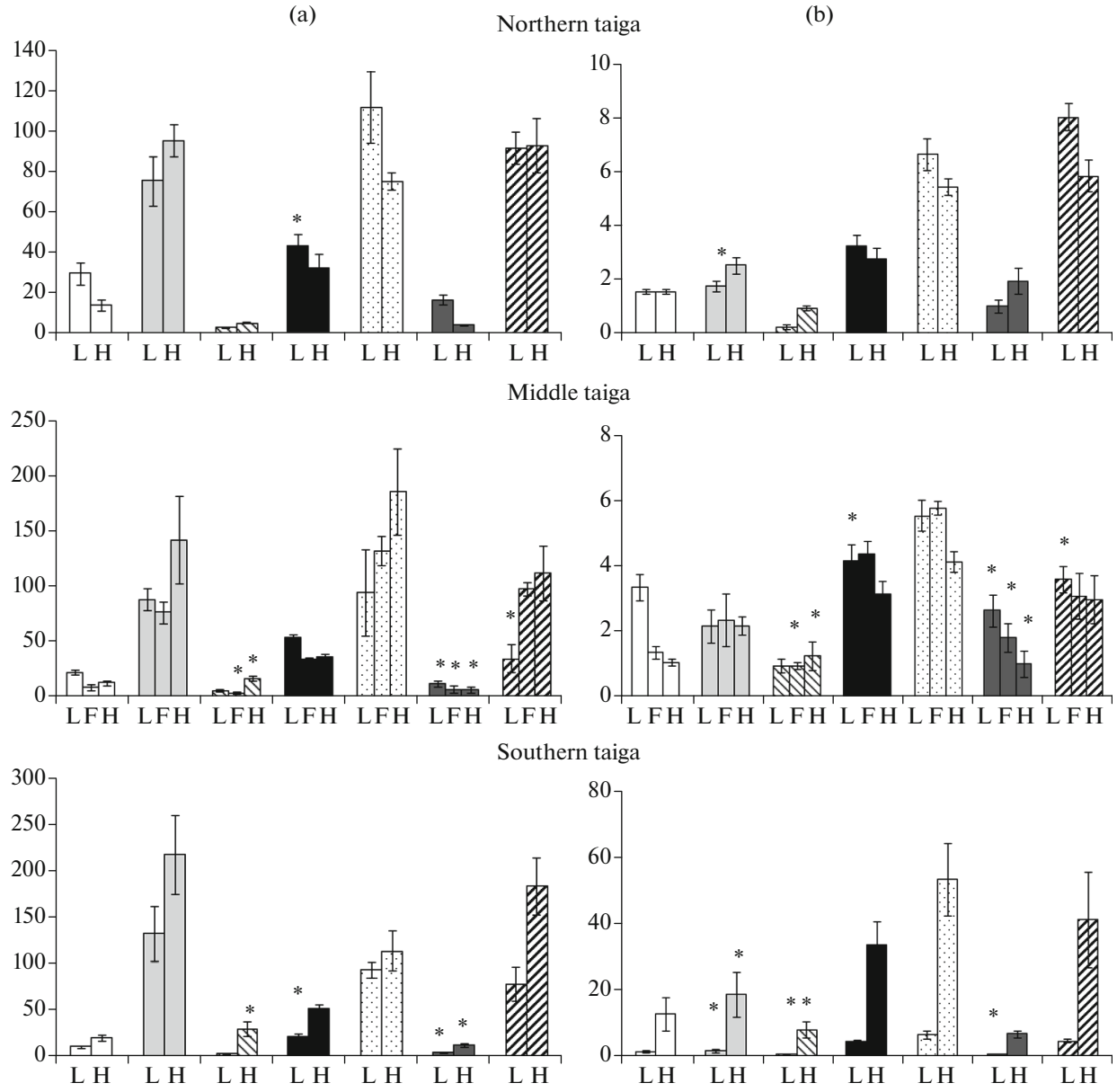

Fig. 3. Concentrations (mg/kg) of (a) carbon and (b) nitrogen of microbial biomass.

den and Finland, where freezing and storing at −20°C during time interval up to one year is considered not to have any effect on the results of determination of soil biological properties [26]. At the same time, concentrations of carbon and nitrogen in labile pool increased after storage under refrigeration, including 2–2.5 time increase of concentration of ammonium and nitrate nitrogen. The most probable mechanism of this phenomenon was similar to mechanisms of the change of proportions between labile and microbial pools in the result of drying and was connected with the disruption of continuity of cytoplasmic membranes in microorganisms owing to freezing and formation of ice crystals. Activation of nitrogen mineralization after freezing was demonstrated earlier for organic horizon of soil in subarctic waste [23], mountain waste [14], and representative set of humus horizons in soil of European part of Russia [7].

Storage of fresh samples of forest litter under low positive temperature (+4°C) had not such unequivocal effect on the analytical results as drying and freezing. The decrease of C_{micr} and N_{micr} contents (significant difference from fresh samples for most objects) was observed in litter samples (Fig. 3). Concentrations of extractable carbon and organic and mineral nitrogen demonstrated as a rule statistically significant, but various-directional changes (concentrations of mineral nitrogen and extractable carbon decreased in most cases). It is known that most microbiological processes in soil have fluctuations connected with alter-

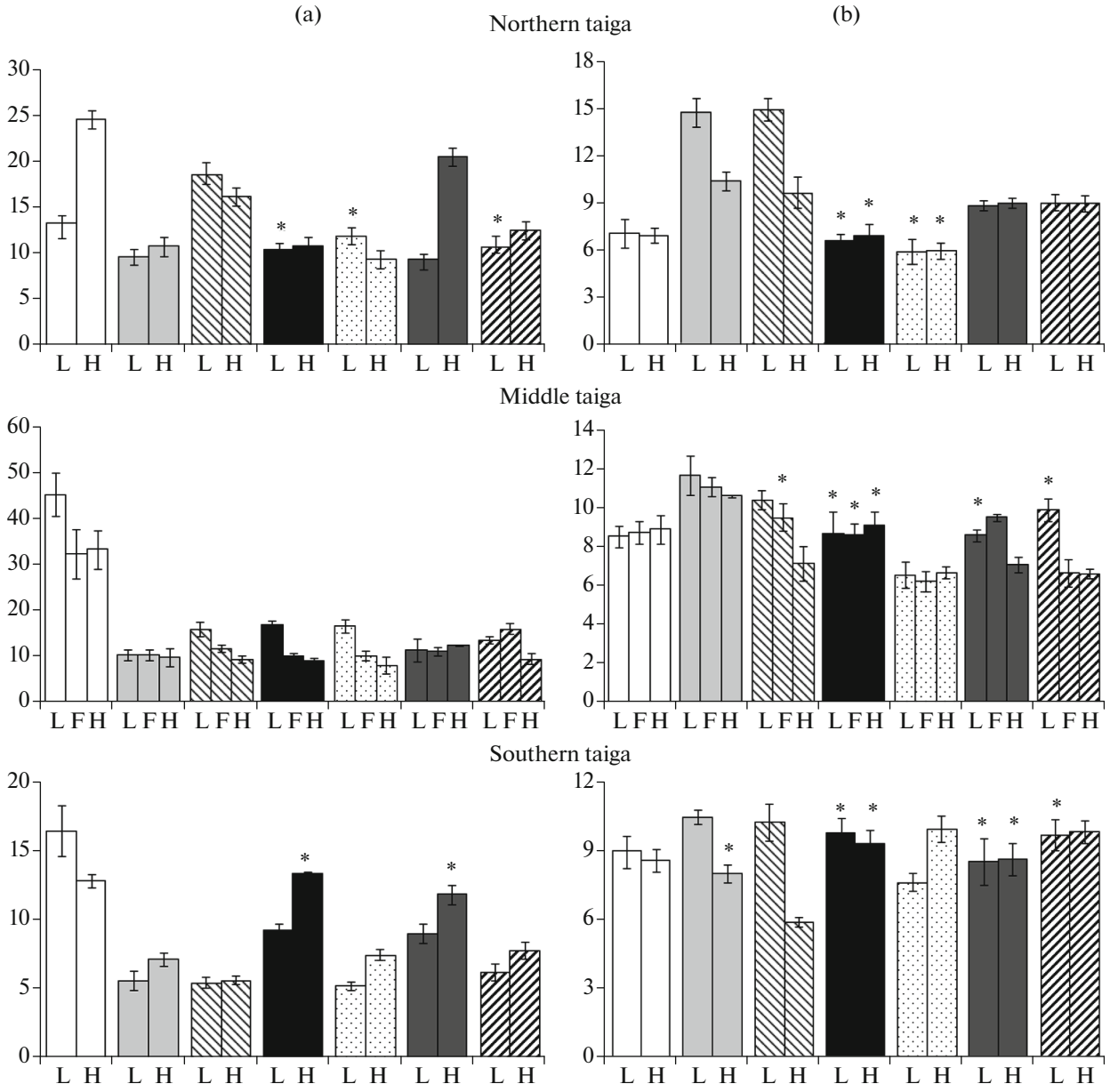

Fig. 4. The C : N ratios in the extracted (a) organic matter and (b) microbial biomass.

nating growth and die-away cycles of a part of microbial population. Such dynamics was demonstrated for population density of microorganisms [30, 35], concentration of mineral nitrogen [35], and nitrogen fixation [12]. The absence of uniform pattern in changes of evaluated parameters was connected with the fact that storage under low temperatures did not stop the biological processes, but only decreased their rates.

Effect of preliminary incubation of stored samples on the results of labile and microbial carbon and nitrogen determination. Preliminary aerobic incubation did not result in most cases in restoration of initial (typical for fresh samples) concentrations of carbon and nitrogen pools. Contents of microbial and labile organic and mineral nitrogen were most sensitive to incubation after storage. Different trends of nitrogen transformation of labile and microbial pools were often observed for the samples of the same litters stored by different methods.

When dried samples were incubated during seven days after moistening to 60% of FMC, significant net immobilization and decrease of concentrations of organic and mineral nitrogen compounds was observed. Preliminary incubation of frozen samples and samples stored under low positive temperature resulted in an increase of extractable carbon and organic nitrogen concentrations (Fig. 1) and decrease of those of C_{micr} and N_{micr} (Fig. 3). Net mineralization (predominately ammonification) was the predominating process. The changes of microbial and labile pools

of carbon and nitrogen were not always quantitatively consistent in the case of preliminary incubation of stored samples, and this was related, on one hand, with gaseous losses first of all by microorganisms respiration, and, on the other hand, with mobilization of a part of carbon and nitrogen of conservative pool.

Predominating process of labile nitrogen compounds transformation during aerobic incubation was determined in large part by the C : N ratio in microbial biomass and labile organic matter (Fig. 4). After freezing and storage under low positive temperatures, microbial biomass was enriched with nitrogen more than labile organic matter, so microorganisms were limited by C availability, and their further growth was impossible without death of a part of community and release of additional amount of carbon. Released in the result of the death of microorganisms organic nitrogen was included into the pool of labile organic and mineral nitrogen. After drying, labile organic matter was as a rule enriched with nitrogen in a greater degree than the microbial biomass. In this case, microbial immobilization of nitrogen was observed during the process if the forest litter incubation affected first of all mineral forms of N, concentration of which could decrease essentially (Fig. 2). Hence, predomination of processes of net mineralization or net immobilization in litter depended largely on the storage method. Net mineralization became the predominating process of transformation of nitrogen compounds after drying, but this was not typical for natural state of forest litter.

CONCLUSIONS

Realistic data on carbon and nitrogen concentrations in microbial biomass, mineral forms of nitrogen, and process of their transformation, when studying the aboveground detritus, can be obtained only with the fresh samples. Any methods of sample storage cause some skewing of absolute values of carbon and nitrogen pools. Drying of samples and their storage in air-dry state affected maximally the results obtained. Storage of frozen litter is possible in most cases for evaluating concentrations of carbon and nitrogen in microbial biomass, but this method is not sufficient for subsequent determination of extracted carbon and nitrogen contents, including mineral forms of nitrogen. Found regularities of changes in concentrations of labile and microbial pools of carbon and nitrogen after storage under different conditions were similar for litter horizons with different degree of plant material decomposition.

Preliminary incubation of stored samples did not allow restoration of their initial properties. The intensity of processes of net mineralization and microbial net immobilization of nitrogen compounds during preliminary incubation of forest litter samples depended on the degree of enrichment of microbial biomass and labile organic matter with nitrogen and was determined by storage method for the samples of the same horizon.

Reasonable and attentive approaches are required, when integrating and interpreting earlier results obtained using long stored dried or preliminary incubated samples.

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