

Article

The Hydration-Dependent Dynamics of Greenhouse Gas Fluxes of Epiphytic Lichens in the Permafrost-Affected Region

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Abstract: Recent studies actively debate oxalic methane (CH₄) production processes in water and terrestrial ecosystems. This previously unknown source of CH₄ on a regional and global scale has the potential to alter our understanding of climate-driving processes in vulnerable ecosystems, particularly high-latitude ecosystems. Thus, the main objective of this study is to use the incubation approach to explore possible greenhouse gas (GHG) fluxes by the most widely distributed species of epiphytic lichens (ELs; *Evernia mesomorpha* Nyl. and *Bryoria simplicior* (Vain.) Brodo et D. Hawksw.) in the permafrost zone of Central Siberia. We observed CH₄ production by hydrated (50%–400% of thallus water content) ELs during 2 h incubation under illumination. Moreover, in agreement with other studies, we found evidence that oxalic CH₄ production by ELs is linked to the CO₂ photoassimilation process, and the EL thallus water content regulates that relationship. Although the GHG fluxes presented here were obtained under a controlled environment and are probably not representative of actual emissions in the field, more research is needed to fully comprehend ELs’ function in the C cycle. This particular research provides a solid foundation for future studies into the role of ELs in the C cycle of permafrost forest ecosystems under ongoing climate change (as non-methanogenesis processes in oxic environments).

Keywords: *Bryoria simplicior*; carbon dioxide; cryptogamic cover; epiphytic lichens; *Evernia mesomorpha*; methane; oxalic methanogenesis processes; permafrost; thalli; thallus water content



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1. Introduction

Methane (CH₄) is a potent greenhouse gas (GHG), and its production is assessed worldwide as ca. 500–600 Tg per year [1,2]. Though methanogenic anaerobic production prevails in aquatic and terrestrial environments, oxalic methanogenesis processes have been actively discussed recently [1,3] due to the substantial upgrading of the knowledge on CH₄ emissions in oxic conditions by *Cyanobacteria*, which was assessed to reach 2.33 Tg per year. CH₄ production by *Cyanobacteria* might be linked to light-driven primary productivity or the demethylation of methyl phosphonates [4]. Marine and freshwater algae are also found to produce CH₄ in oxic conditions by utilizing hydrogen carbonate, dimethyl sulfide, dimethyl sulfoxide, methionine sulfoxide, or dimethylsulfoniopropionate as carbon (C) precursors [1,5,6], and light intensity is found to be positively linked to CH₄ production [7]. Nitrogenase in purple non-sulfur bacteria directly converts carbon dioxide (CO₂) to CH₄ [8,9]. CH₄ production by nitrogenase is light-dependent, and the production of CH₄ increases with higher light intensity [9]. Algae use methylated sulfur compounds as potential C precursors to produce CH₄ [1,5]. It was recently proposed that reactive

oxygen species (ROS), iron, and methyl donors interact to produce CH₄, likely sharing a mechanism with all living organisms [10]. Nowadays, due to eutrophication and climate change, resulting in *Cyanobacteria* and algae blooms, attention to non-methanogenic CH₄ production is still linked to aquatic, not terrestrial, systems [4,11,12]. As a result, compared to oxic waters, terrestrial systems remain understudied in the problem of oxic production of CH₄ by cryptogamic communities (e.g., epiphytic lichens (ELs)). As was discussed in a recent review, the EL components—fungi, *Cyanobacteria*, algae, bacteria, *Archaea*, and yeasts—can produce CH₄ in oxic conditions [3]. Fungi may generate CH₄ by degrading various substrates (e.g., methionine), and the rate of CH₄ emission is species-specific [2,13,14]. In addition to that, they prepare the essential substrates for methanogenic bacteria and *Archaea* by participating in the initial degradation of organic matter [15]. *Cyanobacteria* have been observed to produce CH₄ under aerobic conditions during both light and dark phases, with higher production rates occurring in the light phase [4]. The use of photosynthesis inhibitors also provides evidence that *Cyanobacteria*-derived CH₄ production may be light-dependent [4,16]. However, the understanding of the non-methanogenesis process is still limited, e.g., [1], including CH₄ formation pathways, CH₄ contributions, and the drivers of CH₄ production. Therefore, it is still not included in the global CH₄ budget but should be considered in future estimations.

Cryptogamic covers (including ELs) are found to produce CH₄ [17,18]. Therefore, in ELs, the production of CH₄ by algae/*Cyanobacteria* or fungi may be somehow linked to photosynthesis (both light and dark phases). We hypothesized the close link between oxic CH₄ production by ELs and their light-driven primary productivity, as it was shown for *Cyanobacteria* in aquatic systems (lakes) [4,10].

The C cycle and temperature-dependent processes in high latitudes that contribute to climate change have been the subject of much research conducted in the last few decades [19–21]. Nevertheless, not much is known about ELs, a significant segment of the cryptogamic cover community, and their contribution to maintaining the C balance of these ecosystems, particularly in Siberia. However, GHG emissions by ELs may have the potential to influence overall assessments of ecosystem GHG fluxes, thereby affecting our understanding of current processes and future projections. We discovered that ELs were producing CH₄ in the non-permafrost Siberian birch forest in our previous study [18]. Consequently, we hypothesize about the possible GHG emissions—particularly the production of CH₄—by the ELs in the permafrost area of Middle Siberia. EL CH₄ production could occur in oxic conditions; therefore, it results from non-methanogenic processes, unlike methanogenesis, which is restricted by non-oxic conditions and represented by methanogenic bacteria (e.g., *Archaea*) [22,23]. Boreal forests can be remarkably abundant in ELs [24,25]. Hence, ELs can contribute to the C balance of climate change-affected ecosystems of high latitudes due to ELs' GHG emissions. Humidity (precipitation, water availability, air humidity) is discussed as an essential driver of climate change in Siberian regions [26]. Furthermore, aside from the ecosystem's prevailing macroclimate, these organisms are also affected by the immediate microclimate they are surrounded by [27]. Therefore, our second hypothesis is that the thallus water content of ELs can seriously influence the rate and direction of ELs' GHG fluxes. Thus, our study aims (i) to assess the projecting cover and occurrence of the most widespread ELs inhabiting the larch (*Larix gmelinii* Rupr. Rupr.) forest located in the permafrost area of Middle Siberia; (ii) to evaluate the potential rates of GHG (CO₂ and CH₄) fluxes by the most widespread ELs in the study area using incubation techniques; (iii) to examine the effect of ELs' thallus water content on their GHG fluxes.

2. Materials and Methods

2.1. Study Site

The study sites are near the Tura Experimental Station (Tura settlement, Krasnoyarsk region, Russia) located in the middle reaches of the Nizhnyaya Tunguska River (64°18' N, 100°11' E) in the northern part of Middle Siberia (Figure 1). The research area encompasses

the southern half of the Syverma lava plateau as well as part of the Tunguska trap plateau in the Nizhnyaya Tunguska River basin. The Syverma Plateau, situated in the central part of the Central Siberian Plateau, is a terraced lava plateau with two surface levels, 300–400 and 400–700 m, with specific rises of up to 900 m. The region has a cold continental climate and is primarily covered in permafrost. As was assessed from 1928 to 2023, the mean monthly temperatures vary from -35.7°C in January to 16.7°C in July. For 1928–2023, the region receives an average of 363 mm of precipitation each year, of which 30%–40% falls as snow (an average of 40–50 cm) [28]. With a 55–56-day frost-free period, the growing season lasts approximately 115 days. According to long-term observations, western winds prevail in the region (Figure 2).

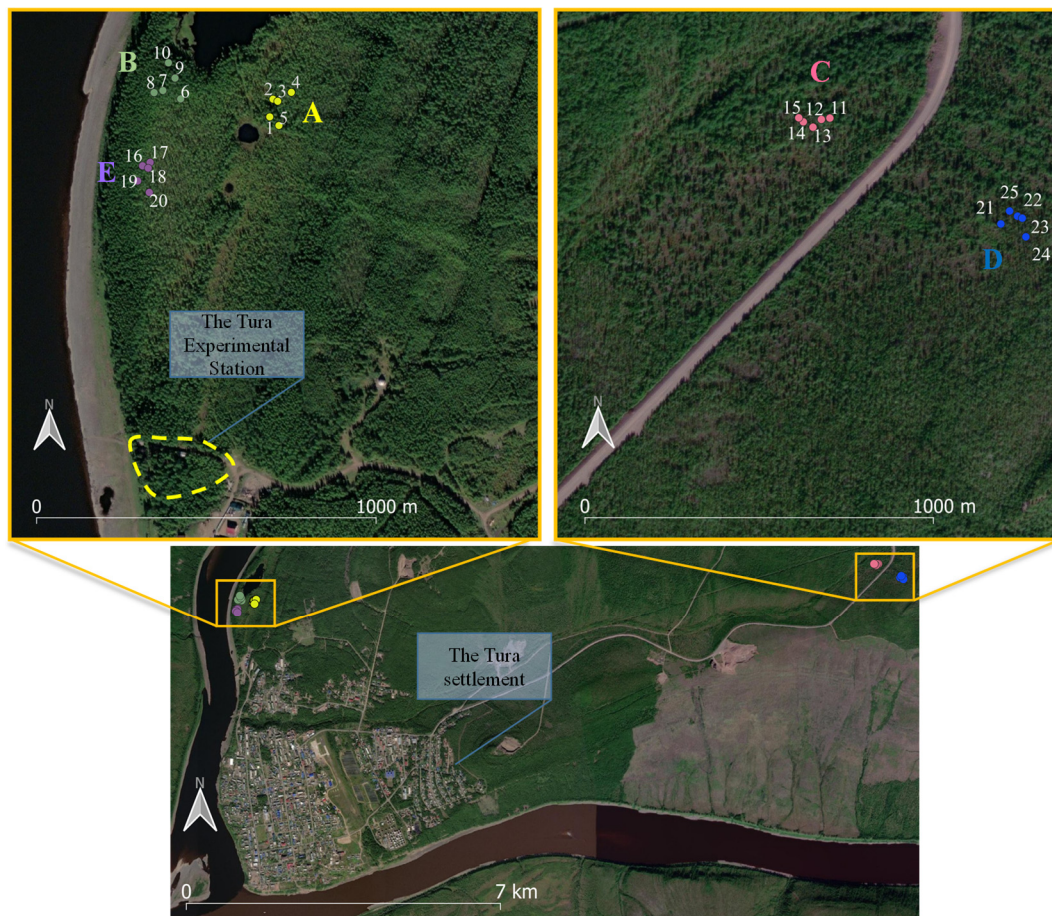


Figure 1. Map of site locations (A, B, C, D, and E) near Tura (64°N, 100°E; <https://www.esri.com/>, accessed on 31 July 2024). The numbers indicate the number of the larch tree (from the first tree to the twenty-fifth tree).

The study area pertains to the Angara-Tunguska taiga province of the Central Siberian forest region, specifically the Nizhnyaya Tunguska district of larch and larch–dark coniferous northern taiga forests [29].

2.2. Experimental Plots, Model Trees, EL Sample Collection

For EL collection employed in the GHG flux incubation experiments, five 50×50 m experimental plots (A, B, C, D, and E) were set up in various larch forest types near the Tura Experimental Station (Tura settlement, Krasnoyarsk region) on 18–23 June 2024 (Figures 1 and S1). A brief overview of the experimental plots is given below.

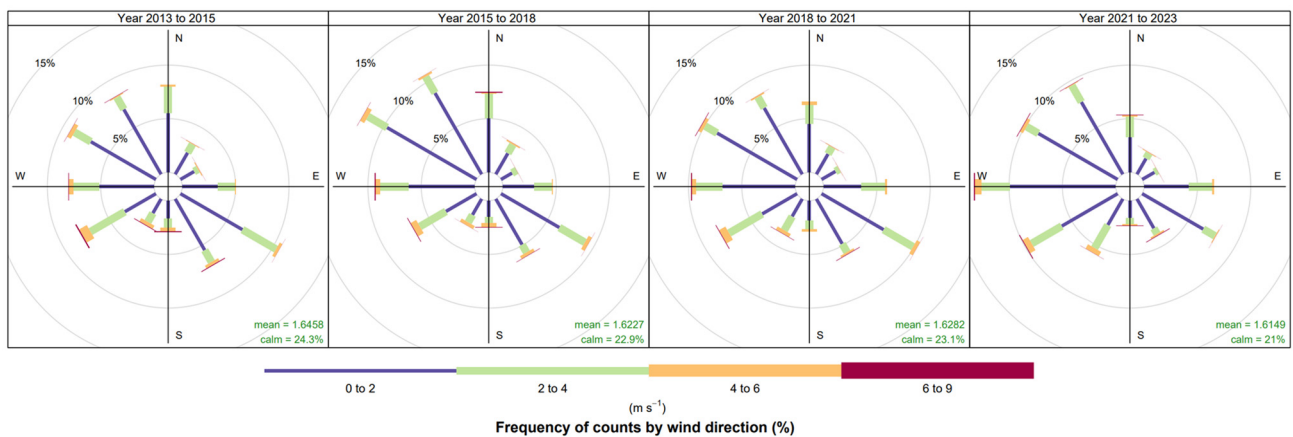


Figure 2. A wind rose from the Tura weather station, which indicates a predominantly westerly flow. Data from the entire observational period of 2013–2023 are included and were accessed at aisori.ru on 14 July 2024.

The dwarf shrub–lichen–green moss larch stand (A; Figure S1A) is dominated by larch (*Larix gmelinii*) in the tree canopy (10 L). *Betula nana* L. and various kinds of willows (*Salix boganidensis* Trautv., *S. myrtilloides* L.) are commonly found in the undergrowth. The herbaceous–dwarf shrub layer is defined by dwarf shrubs (*Chamaedaphne calyculata* (L.) Moench, *Vaccinium uliginosum* L., *V. vitis-idaea* L., *Ledum palustre* L., *Arctostaphylos uva-ursi* (L.) Spreng). The grass cover consists of *Equisetum scirpoides* Michx., *Carex globularis* L., and *C. redowskiana* C. A. Mey. *Pleurozium schreberi* (Willd. ex Brid.) Mitt. and *Aulacomnium palustre* (Hedw.) Schwägr. are co-dominant in the moss–lichen cover.

The *Pyrola*–dwarf shrub larch stand (B, Figure S1B) is dominated by larch (*Larix gmelinii*) in the tree canopy (10 L). The undergrowth is not abundant and is represented by *Rosa acicularis* Rupr. ex Nyman, *Alnus alnobetula* subsp. *fruticosa* (Rupr.) Raus, *Ribes rubrum* L., *Lonicera caerulea* subsp. *pallasii* (Ledeb.), and *Juniperus communis* var. *saxatilis* Pall.; *Sorbus sibirica* Hedl. Prain occurs sporadically. In the herbaceous–dwarf shrub layer, *Pyrola asarifolia* Michx. is dominant; *Ledum palustre*, *Chamaedaphne calyculata*, *Vaccinium vitis-idaea*, *Rubus arcticus* Card, and *Arctostaphylos uva-ursi* are co-dominant. *Equisetum scirpoides* Michx., *E. arvense* L., and *Linnaea borealis* L. are found in the grass cover. *Pleurozium schreberi* (5%) and *Hylocomium splendens* (Hedw.) Schimp. (5%) are present in the moss–lichen cover.

The *Ledum*–*Vaccinium*–green moss–lichen larch stand (C; Figure S1C) is presented by *Larix gmelinii* mixed with *Betula pendula* Roth. In the undergrowth, there are *Alnus alnobetula* subsp. *fruticosa* and *Rosa acicularis*. In the herbaceous–dwarf shrub layer, *Vaccinium uliginosum* and *Ledum palustre* are dominant; *Vaccinium vitis-idaea*, *Empetrum nigrum* L., and *Chamaedaphne calyculata* are co-dominant. In the grass cover, *Equisetum arvense* L. and *Carex globularis* L. are found. The projective cover of mosses is 80%, where *Pleurozium schreberi* and *Hylocomium splendens* are dominant. The projective cover of lichen cover is 10%, where *Cladonia stellaris* (Opiz) Pouzar and Vězda, *C. arbuscula* (Wallr.) Flot., *C. rangiferina* (L.) F. H. Wigg., *Cetraria laevigata* Rass., and *Flavocetraria cucullata* (Bellardi) Kärnefelt and A. Thell are found.

The *Ledum*–*Vaccinium*–green moss larch stand is dominated by larch (*Larix gmelinii*) in the tree canopy (10 L) (D; Figure S1D). In the underbrush, there are *L. gmelinii* and *Pinus sibirica* Du Tour. In the undergrowth, there are *Alnus alnobetula* subsp. *fruticosa* and *Rosa acicularis*. Dwarf shrubs dominate (*Ledum palustre*, *Vaccinium uliginosum*, *V. vitis-idaea*). In the herbaceous layer, *Lycopodium clavatum* L. and *Carex pediformis* var. *macroura* (Meinsh.) Kük. are found. In moss cover, *Hylocomium splendens* (40%) dominates, and *Pleurozium schreberi* (30%) co-dominates. The projective cover of lichens is 10%, where *Cladonia stellaris*, *C. arbuscula*, *C. rangiferina*, *C. uncialis* (L.) F. H. Wigg., *Cetraria islandica* L. Ach., and *Flavocetraria cucullata* (Bellardi) Kärnefelt et A. Thell are found.

The dwarf shrub–green moss larch stand is dominated by larch (*Larix gmelinii*) in the tree canopy (10 L) (E; Figure S1E). Single specimens of *Salix boganidensis* and *Alnus alnobetula* subsp. *fruticosa* compose the undergrowth. In the herbaceous–dwarf shrub layer, *Ledum palustre* dominates, and *Vaccinium vitis-idaea* co-dominates. In the herbaceous–dwarf shrub layer, *Equisetum arvense*, *Carex pediformis* var. *macroura* (Meinsh.) Kük., *Pyrola asarifolia*, *Galium boreale* L., and *Stellaria palustris* Retz. are also found. In the moss–lichen cover, *Hylocomium splendens* (70%) dominates and *Pleurozium schreberi* (20%) co-dominates.

Within these plots (A, B, C, D, or E), the five most morphologically homogeneous model larch (*Larix gmelinii*) trees were selected as average representatives of the forest stand (in height and trunk diameter), and they met the following conditions: they were upright, not oppressed, and had no signs of a pathological process (Table S1) according to the European standard method outlined by Rocha et al. [30] (see also [18]). For each tree, the height, DBH, and canopy cover have been measured (Table S1). Additionally, the photosynthetically active radiation (PAR) was measured at every tree stem exposure using an Android app Photone (Google Play, accessed on 18–23 June 2024). The active layer depth was measured with a steel probe at every stem exposure at a 1 m distance from the tree.

Then, at sites A, B, D, and E, after eye-wise assessment, the most abundant EL species (*Evernia mesomorpha* Nyl., *Bryoria simplicior* (Vain.) Brodo et D. Hawksw., and *Tuckermannopsis sepincola* (Ehrh.) Hale) were collected from the branches of various exposures (southern, northern, western, and eastern) located higher than approximately 1 m. On the trees where the crown was raised high, ELs were collected from tree stems. Thus, at site C, ELs were collected from the stems due to the scarcity of ELs on twigs and their prevalent occupation of the stems of the model trees. The epiphytic cover was recorded for the same trees where the ELs were sampled, e.g., at the branches, at the base of the stem, and at a height of 1.5 m. Lichens were collected on windless sunny days of similar air temperature conditions (19–23 °C) during the daytime for future incubation experiments in order to investigate GHG gas fluxes (CO₂ and CH₄). Additionally, as the overall light conditions varied, we took EL samples at each branch exposure (northern, eastern, southern, and western). It took seven to ten days from EL collection to the incubation experiments. Lichen samples were kept between 18 and 22 °C before the experiments.

2.3. Study Objects

This study focused on ELs: *Evernia mesomorpha*, *Bryoria simplicior*, and *Tuckermannopsis sepincola* growing in larch forests near Tura. The two most common species of ELs inhabiting *Larix gmelinii* were chosen for incubation experiments: *Evernia mesomorpha* and *Bryoria simplicior* (Figure S2). *Tuckermannopsis sepincola* only was explored regarding its biodiversity, and it was described as one of the dominant ELs in the study region (Figure S2). *Evernia mesomorpha* and *Bryoria simplicior* possess a fruticose thallus, though *Tuckermannopsis sepincola* is foliose. Vascular plants and mosses are classified according to the World Flora Online PlantList (<https://www.wfoplantlist.org>, accessed on 30 July 2024). Lichens are classified using “A checklist of the lichen flora of Russia” [31].

2.3.1. Species Characteristics

Evernia mesomorpha (Figure S2A) is a fruticose lichen in the family *Parmeliaceae*, and it was a study object in our previous research in the non-permafrost birch mixed forests near Krasnoyarsk (see the lichen description in [18]).

Bryoria simplicior is a fruticose lichen in the family *Parmeliaceae* (Figure S2B). The thallus is 2–4(5) cm long, sod-like, occasionally extending, reddish or dark brown or almost black, uniformly colored, and isotomically–dichotomously branched at the base, with sharp angles where dichotomy occurs. The branches are 0.2–0.4 mm in diameter, even, smooth, gradually thinning, usually straight, occasionally slightly pitted, with poor or quite frequently located (in some forms) spines, and slightly compressed at the base. No pseudocyphellae are present. The sorals are numerous, slit-like, wider than the branches on which they form, greenish–black, occasionally brownish–black or white, and usually lack

spines, but they can bear irregular, often curved, non-isidial spine-like branches. Apothecia and pycnidia are unknown. *Bryoria simplicior* typically occurs on well-lit branches and small twigs of spruce, larch, and other coniferous and deciduous trees, as well as soil, rocks, and decaying wood, in boreal forests and the subarctic zone. E.g., it is distributed in the Arctic (Polar Urals, Yamal and Taimyr Peninsulas, Yakutia, and Chukotka Peninsula), Murmansk and Arkhangelsk regions, Komi Republic, Karelia, Ivanovo region, Western and Eastern Siberia, Magadan region, Khabarovsk region, Kamchatka, Europe, Mongolia, Japan, and Northern America (USA, Canada, and Greenland).

Tuckermannopsis sepincola is a foliose lichen in the family *Parmeliaceae* (Figure S2C). The thalli are in the form of small, convex, irregular rosettes, up to 3 cm in diameter and 5–10 mm in height, tightly attached to the substrate in the center but free at the edges, composed of ascending lobes that are relatively closely assembled. The lobes are roughly 2–5 mm long and have a smooth, solid edge. The upper surface is brown, ranging in color from olive to dark brown, convex, roughly smooth, without soredia and isidia, but always with apothecia. The lower part is lighter, yellowish-brown, slightly reticulate-pitted or wrinkled, with scattered, rather long, light, simple, or branched rhizines. Apothecia are dark brown, shiny, rarely nearly matte, with a thin, smooth, or slightly jagged edge. They develop at the ends of the lobes and are almost always present in abundance. Initially flat, they later become convex discs. Pycnidia develop along the lobes' edges. *Tuckermannopsis sepincola* is found on the branches of various tree species, both deciduous and coniferous, most commonly on birch, shrubs, and less frequently on stems, processed timber, or rocky substrate. It is widely distributed in the forest-tundra and coniferous forest zones; it also moves into the tundra and, in the south areas, into the mountains. It is frequently found in wetlands. It is distributed from the Kola Peninsula, Baltic Republics, Belarus, and Ukraine to the Urals, Carpathians, Crimea, Caucasus, western and eastern Siberia, the Far East, Europe (Scandinavia, Central and Atlantic Europe, the Balkans), Asia (India and Japan), North and South America, Australia, and New Zealand.

2.4. Methods for the Evaluation of EL Diversity

Before taking the EL samples for the subsequent incubation experiments, the epiphytic cover of the same twenty-five model trees of *Larix gmelinii* was assessed at branches located within a height range of 0.4 to 3.2 m (Figure 1). The projecting cover of the ELs was assessed eye-wisely at every sample branch according to the branch exposure (northern, western, eastern, and southern) (a total of 1–6 branches per model tree). The diameter and height of the selected branches were measured. The branches that were gathered had bases that ranged in diameter from 0.2 to 2.1 cm. The species composition and projecting cover were assessed at each branch (a total of 133 branch descriptions). The occurrence of the explored EL species (%) on the larch branches was calculated as the ratio of the number of trees with lichen to the total number of trees. Branches were collected using a long-reach pruner with a telescopic handle. Also, the EL projective cover was evaluated from various exposures at the stem's base (up to 0.5 m) and a height of 1.3 m. On the stems, 200 descriptions of the epiphytic cover were made. The primary EL cover characteristics were identified as follows: the total projective EL cover on the surface of the stems and branches, the number of EL species, and the projective cover of individual EL flora. To assess the potential impact of EL species diversity on EL GHG fluxes, the Shannon index [18,32], occurrence, and species richness were calculated at branches, at the base of the stem (up to 0.5 m), and at a stem height of 1.3 m.

2.5. EL Incubation Experiments

Two incubation experiments were carried out using the EL species with the highest abundance and occurrence (*Evernia mesomorpha* and *Bryoria simplicior*, see Section 2.3.1) in order to study the peculiarities of GHG fluxes between these two EL species (see Section 2.5.1) and how varying the water content of *Evernia mesomorpha* influences its GHG fluxes (see Section 2.5.2). The incubation method allows the assessment of the important parameters

that define the growth of lichens and their capacity to adjust to fluctuating environmental factors like temperature and humidity [33]. Among them are the net CO₂ photoassimilation rates, CH₄ generation and consumption rates, and the CO₂ losses linked to dark respiration and photorespiration. Bulk samples were formed from the EL samples of each taxon (genus) harvested from the branches of model trees for certain branch exposures (northern, southern, western, and eastern). In both experiments, lichens were incubated aerobically in 125 mL flasks, SIMAX 2070/M/100 mL (Kavalierglass, Sázava, Czech Republic), for three days in a Peltier-cooled incubator with a programmable temperature-control system (Memmert, Schwabach, Germany) under an 18 h light photoperiod under a built-in illumination system, comprised of three LED lamps (Camelion LED13-A60/830/E27, Moscow, Russia) and one lamp of REPTILE UVB200 PT2341 (Hagen Deutschland GmbH & Co. KG, Holm, Germany) with a UVB spectrum of high intensity, providing a cumulative PAR range of 160–220 µmol m⁻² s⁻¹ at 18 °C, and 6 h without illumination at 14 °C [18]. The illumination levels were selected following the illumination conditions of ELs in the natural environment during collection. On each of the three days, gas samples of 22–25 mL were taken within the headspace with Luer Lock syringes through a septum in the lid of the flask's rubber seal at the starting point of incubation and 2 h after incubation of the ELs under illumination at 18 °C with the rubber seals closed. The subsequent analysis of the CO₂ and CH₄ concentrations and their δ¹³C isotopies (δ¹³CO₂, δ¹³CH₄) in gas samples was performed using the Cavity Ringdown Spectrometer Picarro G2201-*i* analyzer (Picarro, Inc., Santa Clara, CA, USA). The Picarro 2201-*i* has several modes for measuring CO₂ and CH₄; we used the mode that allows for the simultaneous measurement of two gases. Picarro's technology allows for the simultaneous measurement of δ¹³C in two gases, making it unique. In the CO₂/CH₄ mode, δ¹³C measurements are performed every few seconds, resulting in a higher measurement rate than the gas exchange rate in the cell. δ¹³C measurements in the combined mode have high reproducibility, exceeding 0.16‰ for CO₂ and 1.15‰ for CH₄. The rates of GHG fluxes were calculated using Formula (1):

$$F = \frac{(C_2 - C_1) \times V \times M}{24.5 \times m \times t} \quad (1)$$

where F is CH₄ or CO₂ flux (µg CH₄ or CO₂ g⁻¹ of the dry weight of EL h⁻¹); C_1 is the concentration of gas (CH₄ or CO₂) before 2 h incubation (ppm); C_2 is the concentration of gas (CH₄ or CO₂) after 2 h incubation (ppm); V is incubation flask volume (liter); M is the molar mass of CH₄ or CO₂ (16.043 or 44.01 g mol⁻¹); 24.5 is one mol of air (liter per mol); m is the EL sample dry weight in the incubation flask (g); and t is the incubation period of 2 h (h). The gas flux rates were then expressed per C (ng C-CH₄ g⁻¹ of the dry weight of the EL h⁻¹ or µg C-CO₂ g⁻¹ of the dry weight of the EL h⁻¹). We examined the difference in CO₂ concentration before and after the ELs' two-hour incubation in the closed flask, so the negative fluxes indicated CO₂ photoassimilation as an outcome of light physiological processes (photosynthesis and photorespiration) [34]. The negative CH₄ fluxes represented the CH₄ intake. Positive values stated CH₄ generation or respiration.

2.5.1. An Examination of CO₂ and CH₄ Fluxes in the Most Common ELs of the Studied Region

GHG fluxes in ELs (*Evernia mesomorpha* and *Bryoria simplicior*) inhabiting larch branches with varying exposures were studied (Figure S4). In particular, this study focused on *Evernia mesomorpha* of southern, northern, western, and eastern exposure, whereas regarding *Bryoria simplicior*, only southern, western, and eastern exposure was assessed (northern exposure was either absent or had too few examples). In the laboratory, air-dried EL samples were separated from the substrate (bark, mosses, etc.). Then, due to the possible osmotic stress caused by distilled water [17,35], we used artificial rainwater of the following composition: 8.8 mg L⁻¹ K₂CO₃, 4.6 mg L⁻¹ Na₂CO₃, 5.0 mg L⁻¹ CaCO₃, 4.4 mg L⁻¹ FeSO₄·7H₂O, 0.6 mg L⁻¹ MnSO₄·H₂O, pH adjusted to 7. Therefore, ELs were sprinkled with artificial rainwater in order to reach 200% EL thallus water content. We used 21 flasks for the

triplicate incubation of each variant. Before the incubation experiment, 24 h pre-incubation under an 18 h photoperiod was carried out. During this time, a weight method was used to keep the ELs' water content at roughly 200%.

2.5.2. Examination of the Effect of EL Water Content on the GHG Fluxes from the Surface of the *Evernia mesomorpha* During Incubation Under Illumination

This study utilized *Evernia mesomorpha* from southern, western, and northern exposures to examine the effects of EL thallus water content (50, 100, 200, and 400%) on GHG fluxes, with triplicate samples (36 flasks in total). A weighting method was used to control the level of EL water content that was reached. *Evernia mesomorpha* of eastern exposure was scarce, so data on GHG fluxes by ELs on the branches of eastern exposure are lacking. Before the beginning of the incubation experiment, the lichens were rewetted to the proper humidity levels (50, 100, 200, and 400%) with the artificial rainwater and went through 24 h pre-incubation conducted under an 18 h photoperiod.

2.6. Data Analysis

The homoscedasticity of the residuals' distribution is confirmed for both CO₂ and CH₄ flux data regarding all studied factors (exposures, species, and EL thallus water contents). However, the distribution of CH₄ fluxes in both experiments is normal, while the distribution of CO₂ fluxes deviates from the normal distribution. Consequently, the pairwise Wilcoxon test was employed to evaluate the variations among exposures, species, and EL thallus water contents. The EL GHG (CO₂ and CH₄) fluxes were assessed regarding the effects of the EL species, the photoperiod conditions, the exposure of the branch occupied by the ELs, and the day of the incubation using a Kruskal–Wallis rank sum test due to the deviation of CO₂ fluxes data from the normal distribution. In our study, variations in C sources and CO₂ and CH₄ fluxes caused by the simultaneous occurrence of various processes in different EL components were assessed by $\delta^{13}\text{C}$ in CO₂ and CH₄ during incubation. For example, when accompanied by isotopic fractionation, the processes of photorespiration of the lichen photobiont and respiration of the mycobiont impart different isotopic signatures into the air. To identify sources of $\delta^{13}\text{C}$ variations in atmospheric C fluxes, we applied the widely used method of Keeling plots. For that, we utilized the simultaneous Picarro measurements of $\delta^{13}\text{CO}_2$ and $\delta^{13}\text{CH}_4$, as well as inverse concentrations of CO₂ and CH₄, which were assessed during incubation, and we constructed a linear regression line that yields a y-intercept value indicating the isotopic composition of the emitting gas source [36]. Due to the distribution of CO₂ fluxes deviating from the normal distribution, we used Spearman's rank correlation coefficients to assess the relationships between the CO₂ and CH₄ fluxes by the ELs at 1st (A), 2nd (B), and 3rd (C) day of the first incubation experiment. Principal component analysis (PCA) was employed for the analysis of GHG fluxes by the ELs that colonized the larch branches of various exposures depending on the thallus water content, and this was visualized using R-package "factoextra". Statistical analyses and visualizations were performed using Excel 2013 and R software (ver. 4.2.3, 15 March 2023—"Shortstop Beagle") and the following R packages: "ggplot2", "ggpubr", "tidyverse", "FactoMineR", "factoextra", "windRose", etc.

3. Results

3.1. Survey of EL Diversity in Larch Forests in the Permafrost Area

In permafrost habitats, 20 lichen species representing 12 genera and 3 families, with fruticose and foliose thalli, were identified on Gmelin's larch (*Larix gmelinii*). Most species (79%) belong to the family *Parmeliaceae*.

At a stem height of up to 0.5 m, 17 lichen species were found: *Bryoria simplicior*, *Cladonia chlorophaea* Flörke ex Sommerf. Spreng., *C. fimbriata* (L.) Fr., *C. ochrochlora* Flörke, *Evernia mesomorpha*, *Hypocenomyce scalaris* (Ach.) M. Choisy, *Hypogymnia bitteri* (Lyngé) Ahti, *H. physodes* (L.) Nyl., *H. tubulosa* (Schaer.) Hav., *H. vittata* (Ach.) Parrique, *Imshaugia aleurites* (Ach.) S. L. F. Mey., *Melanohalea olivacea* (L.) O. Blanco et al., *Parmelia omphalodes* (L.)

Ach., *P. saxatilis* (L.) Ach., *P. sulcata* Taylor, *Parmeliopsis ambigua* (Hoffm.) Nyl., and *Vulpicida pinastri* (Scop.) J.-E. Mattsson et M. J. Lai. Beyond the epiphytes, facultative epiphytes of the genus *Cladonia* frequently actively populated the base of *Larix gmelinii* tree stems. The bases of the tree stems were dominated by *Hypogymnia physodes* (12%), *Imshaugia aleurites* (7%), *Hypogymnia bitteri* (6%), *H. tubulosa* (5%), *Cladonia fimbriata* (4%), and *Vulpicida pinastri* (4%). The most common species (high occurrence $\geq 50\%$ or constant) were *Hypogymnia physodes*, *Vulpicida pinastri*, and *Hypocenomyce scalaris*.

At a stem height of 1.3 m, five EL species were identified: *Bryoria simplicior*, *Evernia mesomorpha*, *Hypogymnia bitteri*, *H. physodes*, and *Vulpicida pinastri*. The following species were found to have the largest projective cover: *Bryoria simplicior* (13%), *Evernia mesomorpha* (5%), and *Hypogymnia physodes* (4%). Two species had a high occurrence rate ($\geq 50\%$): *Bryoria simplicior* and *Evernia mesomorpha*.

At *Larix* branches, 11 EL species were identified: *Bryoria implexa* (Hoffm.) Brodo et D. Hawksw., *B. simplicior*, *Evernia mesomorpha*, *Hypogymnia bitteri*, *H. physodes*, *H. tubulosa*, *Melanelia olivacea*, *Parmelia sulcata*, *Tuckermannopsis sepincola*, *Usnea glabrescens* (Nyl. ex Vain.) Vain., *Vulpicida pinastri*, *Bryoria simplicior*, *Evernia mesomorpha*, *Melanelia olivacea*, *Parmelia sulcata*, and *Tuckermannopsis sepincola* were characterized by high occurrence ($\geq 50\%$). *Evernia mesomorpha* (35%), *Tuckermannopsis sepincola* (12%), and *Melanelia olivacea* (11%) are the EL species found to have the largest projective cover on larch branches.

Based on the primary survey, to carry out the incubation experiments to assess the GHG fluxes by the ELs, we collected the most common ELs inhabiting model larch trees in the permafrost zone. For that, we assessed the projecting cover and occurrence of the EL species on branches on the model trees. It was shown that *Tuckermannopsis sepincola* was only found growing on branches, where its projecting cover ranged from 1 to 18%, rather than on stems (at a height of 1.3 m) (Figure 3). The projecting cover of *Bryoria simplicior* on stems varied widely from 0 to 35%, and on branches, it was within 0%–22% (Figure 3). The projecting cover of *Evernia mesomorpha* was significantly lower on stems (0%–12.5%) as compared to branches (10%–70%) (Figure 3). The occurrence of *Evernia mesomorpha* and *Bryoria simplicior* on stems and branches widely varied from 0 to 100%.

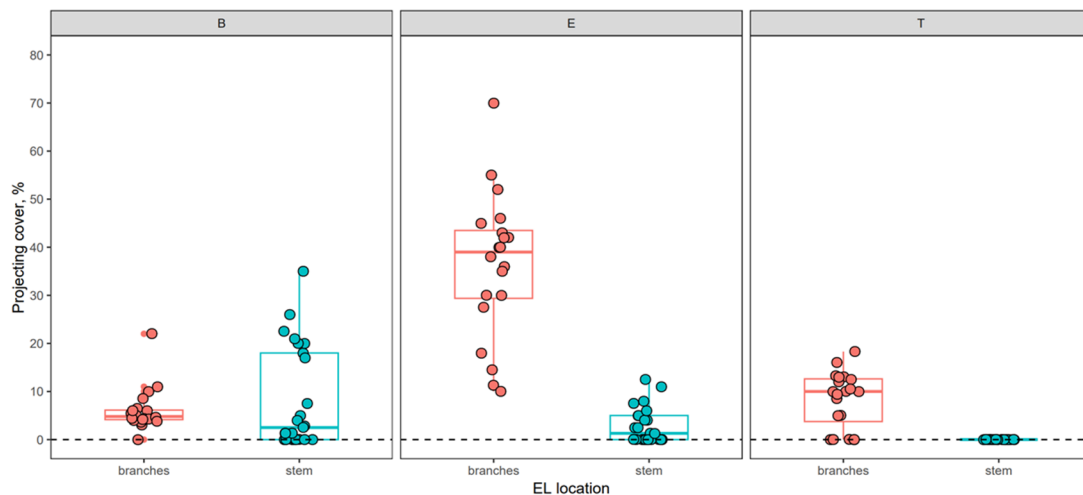


Figure 3. Projecting cover (boxplots) of the ELs on the branches and stems of larches in the permafrost area. B, *Bryoria simplicior*; E, *Evernia mesomorpha*; T, *Tuckermannopsis sepincola*. The horizontal line within the box indicates median, box boundaries indicate 25th and 75th percentiles, and whiskers indicate highest and lowest values.

Bryoria simplicior was found to have more projecting cover ($>18\%$) on the eastern and northern stem exposures (Table 1). The projecting cover of *Bryoria simplicior* on branches was not impacted by the branches' exposure, where it was within the range of 5.3% to 6.9% (Figure 4A). *Evernia mesomorpha* had a higher projecting cover on the north-exposed stem

sides (Figure 4A), where it has been frequently found (Figure 5A). Meanwhile, regardless of branch exposure, *Evernia mesomorpha* exhibited high projecting cover on branches. *Tuckermannopsis sepincola* was found only on branches where its projecting cover did not depend on the exposure of the branch and reached 10.5%–12.9% (Figures 4 and 5).

Table 1. The results of a Kruskal–Wallis rank sum test for the evaluation of the effects of EL species, the exposure of ELs’ occupied larch branches, and the day of incubation on the CO₂ and CH₄ fluxes by ELs in the first incubation experiment.

Factors	Kruskal–Wallis Chi-Squared	<i>p</i> -Value
CO ₂ fluxes		
Day of the incubation	1.3757	0.5027
EL species	2.2458	0.134
Exposure of the ELs	6.0341	0.11
CH ₄ fluxes		
Day of the incubation	2.3341	0.3113
EL species	2.3674	0.1239
Exposure of the ELs	5.5712	0.1344

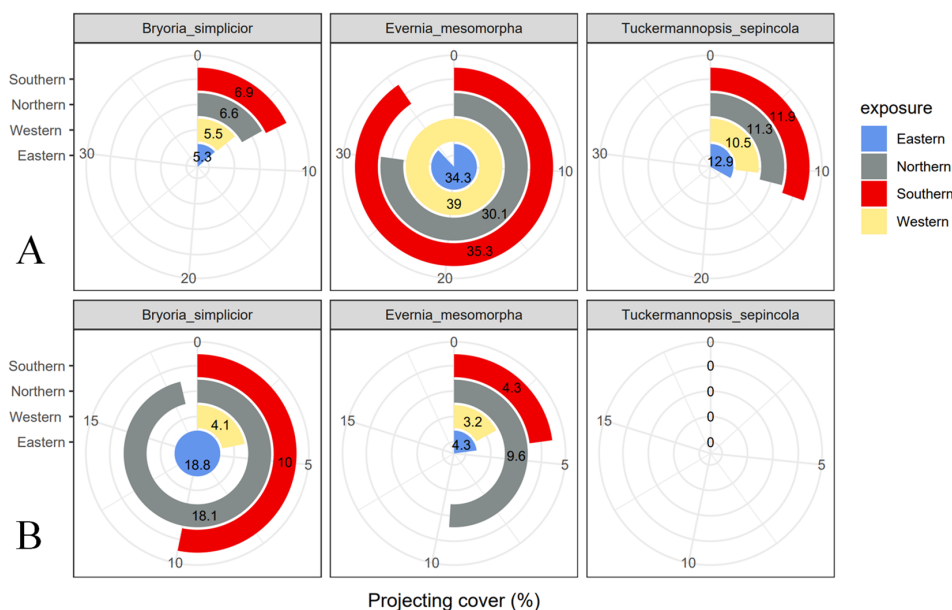


Figure 4. Projecting cover (%) of ELs regarding the branch (A) or stem (B) exposure.

At a stem height of up to 0.5 m, species richness varied from 3 to 8 EL species, though at a stem height of 1.3 m, the number of EL species was 1–4 (Figure S3). On larch branches, the number of species varied from 3 to 9 (Figure S3). The Shannon index widely varied from 1.27 to 2.24 at individual trees (Figure S3).

3.2. GHG Fluxes by the EL Species with the Highest Abundance and Occurrence

The first incubation experiment aimed to reveal the peculiarities in the GHG fluxes by the various ELs of the same 200% thallus water content and various larch branches’ exposures. The Kruskal–Wallis rank sum test did not reveal the overall significant influence of the branch exposure that was inhabited by the ELs on the CO₂ fluxes by ELs (Table 1). However, *Bryoria simplicior* of southern and western (SW) exposures and eastern (E) exposure had significant differences in the CO₂ fluxes (Figure 6). Specifically, the SW-exposed *Bryoria simplicior* demonstrated mixed dynamics of CO₂ fluxes during 2 h incubation under illumination, though the E-exposed *Bryoria simplicior* predominantly showed the photoas-

simulation of CO₂ (Figure 6). On the contrary, *Evernia mesomorpha* showed no significant differences regarding branch exposure and mostly emitted CO₂ during 2 h incubation under illumination. Interestingly, on north- and east-exposed branches, *Bryoria simplicior* and *Evernia mesomorpha* showed contrasting results regarding CO₂ fluxes, i.e., *Bryoria simplicior* mostly showed CO₂ photoassimilation, though *Evernia mesomorpha* produced CO₂ under illumination (Figure 6).



Figure 5. Occurrence of ELs regarding the branch (A) or stem (B) exposure.

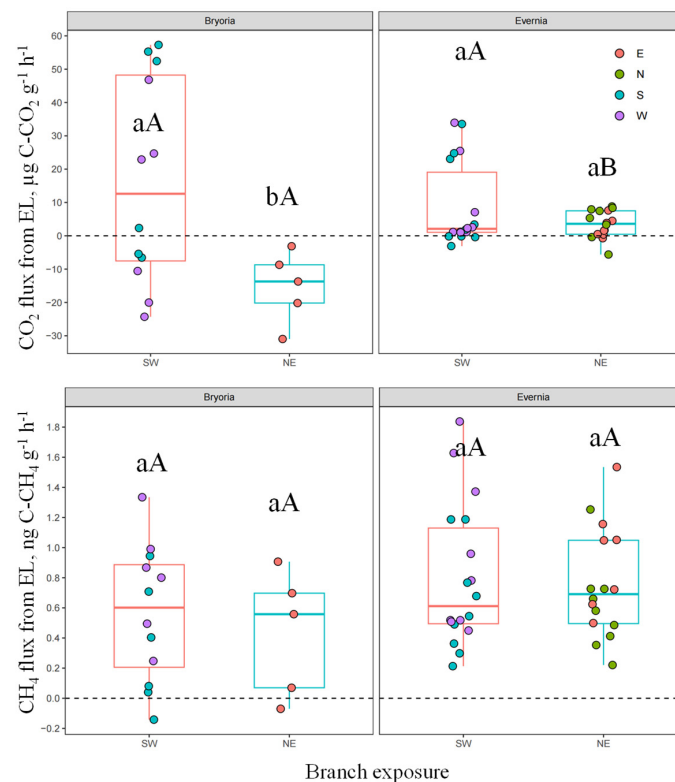


Figure 6. Boxplots of day CO₂ and CH₄ fluxes by EL species (*Bryoria simplicior* and *Evernia mesomorpha*) occupying larch branches of various exposures and which were incubated in controlled photoperiod

and thermal regimes. The horizontal line within the box indicates median, box boundaries indicate 25th and 75th percentiles, and whiskers indicate highest and lowest values. Negative values reflect CO₂ photoassimilation (or CH₄ consumption), and positive values reflect respiration (or CH₄ production). E, eastern exposure of the branches occupied by the ELs; N, northern exposure of the branches occupied by the ELs; S, southern exposure of the branches occupied by the ELs; W, western exposure of the branches occupied by the ELs. Small letters designate the differences between the SW and NE exposures within the same EL species according to the pairwise Wilcoxon test. Capital letters describe the differences between the EL species within the same branch exposure (SW or NE) according to the pairwise Wilcoxon test.

Regarding the CH₄ fluxes by ELs, none of the factors significantly affected their rates (Table 1). During 2 h incubation under illumination, both species, regardless of branch exposure, emitted CH₄ with median values of ca. 0.6 ng C-CH₄ g⁻¹ h⁻¹, though the range of the CH₄ fluxes was within the values from −0.15 to 1.8 ng C-CH₄ g⁻¹ h⁻¹ (Figure 6).

The Spearman's rank correlation shows an increase in the negative correlation between the CO₂ and CH₄ fluxes by the 3rd day of the first incubation experiment (Figure 7). Interestingly, this negative correlation is higher for ELs inhabiting south- and west-exposed larch branches compared to ELs occupying north- or east-exposed branches (Figure S6).

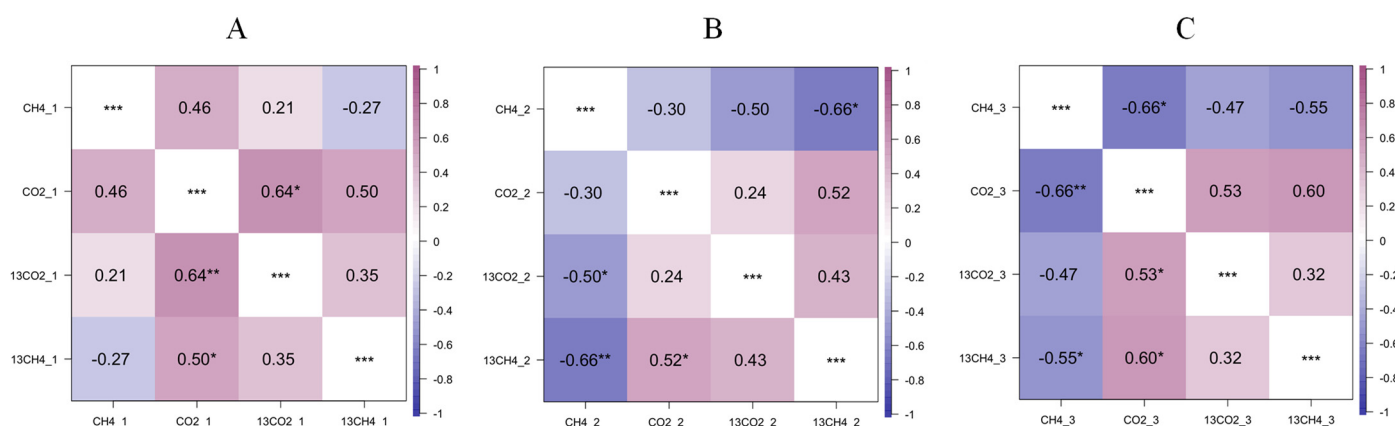


Figure 7. Correlation (Spearman's rank correlation coefficients) heat maps of the studied parameters of the ELs on the 1st (A), 2nd (B), and 3rd (C) days of the first incubation experiment. ***, $p < 0.001$; **, $p < 0.05$; *, $p < 0.5$.

3.3. Impact of the EL Thallus Water Contents on the GHG Fluxes in *Evernia mesomorpha*, One of the Most Widespread EL Species in the Permafrost Zone

The second incubation experiment was aimed at understanding the impact of EL water content on the GHG fluxes of *Evernia mesomorpha*. *Evernia mesomorpha* was presumably the emitter of studied GHG across a range of EL thallus water contents from the lowest (50%) to the highest (400%). Regarding CO₂ fluxes, a Kruskal–Wallis rank sum test showed a significant influence of lichen thallus water content within the variety showing 50%–400% water content (Table 2). Specifically, at 400% of *Evernia mesomorpha* thallus water content, respiration was significantly four times higher in the northerly exposed ELs ($p = 0.036$) as compared to southern and western exposures (Figure 8). A rise in the thallus's water content accelerated the release of CO₂ by EL and slowed down its photoassimilation of CO₂. As shown, when hydrated to 400%, *Evernia mesomorpha* lacked the process of CO₂ photoassimilation after 2 h of incubation under illumination (Figure 8).

Table 2. The results of a Kruskal–Wallis rank sum test for the evaluation of the effects of *Evernia mesomorpha* thallus water content, the day of incubation, and the exposure of EL-occupied branches on the CO₂ and CH₄ fluxes by ELs in the second incubation experiment.

Factors	Kruskal–Wallis Chi-Squared	<i>p</i> -Value
CO ₂ fluxes		
Day of the incubation	1.5437	0.4621
Water content	7.832	0.04961 **
Exposure of the ELs	2.2122	0.5295
CH ₄ fluxes		
Day of the incubation	14.695	0.0006 ***
Water content	4.9773	0.1735
Exposure of the ELs	2.993	0.3927

***, *p* < 0.01; **, *p* < 0.05.

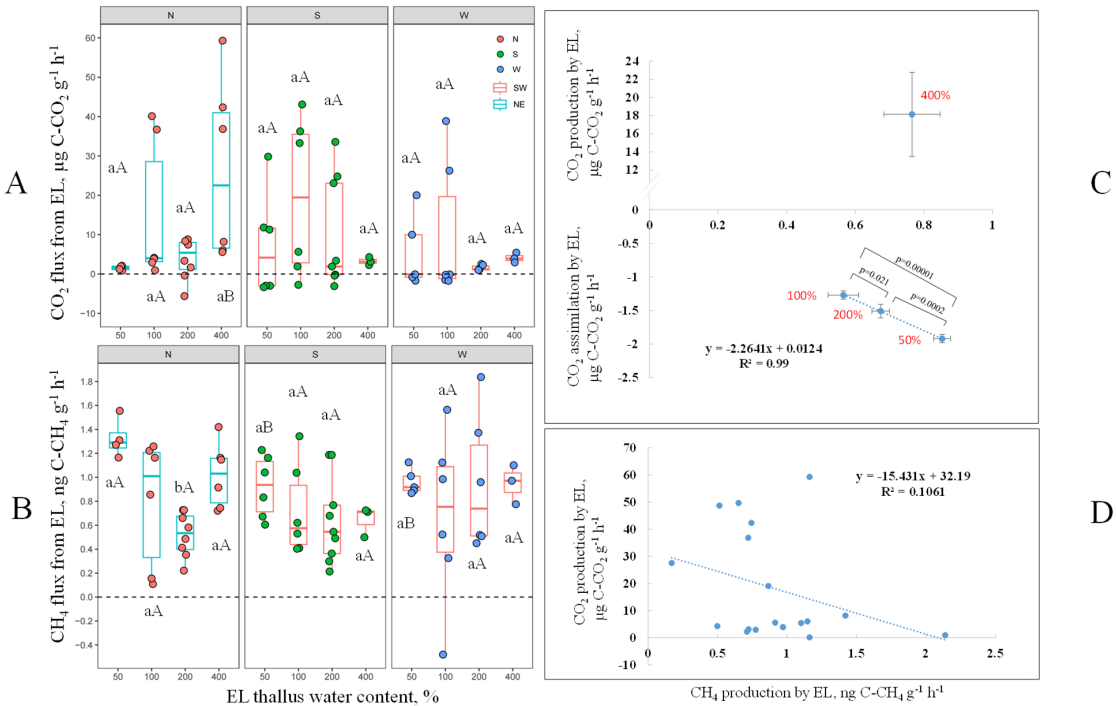


Figure 8. Boxplots of the day CO₂ (A) and CH₄ (B) fluxes by hydrated (50%–400% of thallus water content) *Evernia mesomorpha* sampled from various larch branch exposures and incubated under controlled photoperiod and thermal regimes. The horizontal line within the box indicates median, box boundaries indicate 25th and 75th percentiles, and whiskers indicate highest and lowest values. Negative values reflect CO₂ photoassimilation (or CH₄ consumption), and positive values reflect respiration (or CH₄ production). The (C,D) panels display the relationships (mean values and standard errors) between the CO₂ and CH₄ production and accumulation processes in the ELs of various (50%–400%) thallus water contents as a summary of the (A,B) panels. The (D) panel represents the relationship between the CO₂ and CH₄ production processes in the EL of 400% thallus water content. N, *Evernia mesomorpha* occupying larch branches of northern exposure; S, *Evernia mesomorpha* occupying larch branches of southern exposure; W, *Evernia mesomorpha* occupying larch branches of western exposure. Because of the extremely low abundance of ELs on east-exposed larch branches, the GHG fluxes are not shown for the eastern exposure. Small letters designate the differences between the fluxes by ELs of various thallus water content but of the same branch exposure (N, S, W) according to the pairwise Wilcoxon test. Capital letters describe the differences between the branch exposures within the same thallus water content of the *Evernia mesomorpha* according to the pairwise Wilcoxon test.

A Kruskal–Wallis rank sum test did not show a significant influence of different EL thallus water contents on EL CH₄ fluxes (Table 2). CH₄ generation by north-exposed *Evernia mesomorpha* of 200% water content was significantly lower ($p = 0.012$) than by those with other water contents (Figure 8). Despite a generally high degree of variation, lichen water content had no apparent effect on the CH₄ fluxes by the ELs collected from the south- and west-exposed branches, nor did they differ significantly from one to another. Interestingly, north-exposed *Evernia mesomorpha* with minimal thallus water content (50%) produced noticeably more CH₄ ($p = 0.029$) than those that occupied the south- and west-exposed larch branches (Figure 8). We observed CH₄ emission at all levels of thallus water contents (Figure 8B). At the same time, CO₂ emission was in antiphase with CH₄ emission; this phenomenon was especially pronounced at 400% (Figure 8D). CO₂ photoassimilation and CH₄ production were directly correlated at lower thallus water contents (50%–200%), with the highest levels of CH₄ production and CO₂ photoassimilation occurring at the lowest thallus water content (50%) (Figure 8C).

Since ELs are complex multi-component organisms consisting of phycobionts, mycobionts, *Archaea*, yeasts, etc., PCA was employed to understand the relationships within the processes gathered in the CO₂ and CH₄ fluxes from the thallus of ELs (Figure 9). PCA showed that about 52% of the variation was captured by the two components (PC1 and PC2) regarding CO₂ fluxes (Figure 9A) and about 63% regarding CH₄ fluxes (Figure 9B) from the surface of *Evernia mesomorpha* inhabiting the larch branches of different exposures and having various thallus water content (50, 100, 200, and 400%). The main contributor to PC1 was CH₄ flux, whereas the main contributor to PC2 was $\delta^{13}\text{CH}_4$ (Figure 9B). As indicated by PCA, CH₄ fluxes and $\delta^{13}\text{CH}_4$ are negatively correlated (Figure 9B), demonstrating the clear dominance of certain processes (groups of organisms) responsible for CH₄ generation.

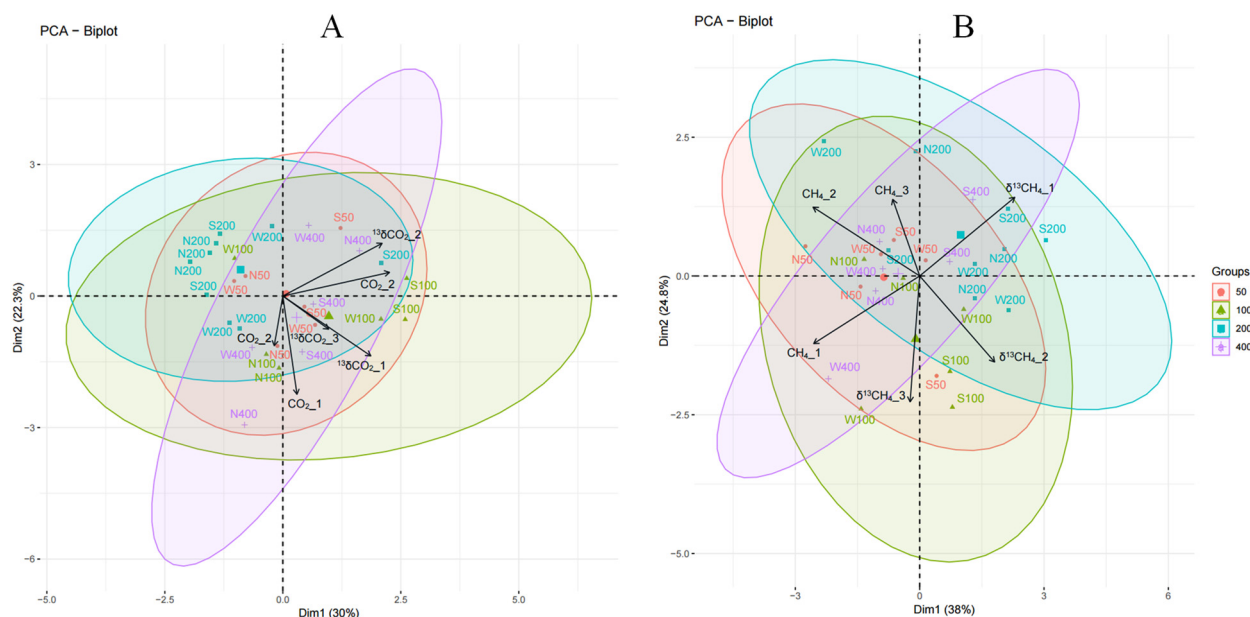


Figure 9. PCA of the relationships within the processes gathered in the CO₂ (A) and CH₄ (B) fluxes from the thallus of *Evernia mesomorpha* inhabiting the larch branches of various exposures and different thallus water contents. S50, south-exposed EL of 50% of water content; S100, south-exposed EL of 100% of water content; S200, south-exposed EL of 200% of water content; S400, south-exposed EL of 400% of water content; N50, north-exposed EL of 50% of water content; N100, north-exposed EL of 100% of water content; N200, north-exposed EL of 200% of water content; N400, north-exposed EL of 400% of water content; W50, west-exposed EL of 50% of water content; W100, west-exposed EL of 100% of water content; W200, west-exposed EL of 200% of water content; W400, west-exposed EL of 400% of water content.

The studied ELs exhibited a natural abundance of $\delta^{13}\text{C}$ of ca. -26‰ . The CO_2 of air samples taken after 2 h incubation of the ELs under illumination was enriched in ^{13}C . Keeling plots (Figure 10) showed the change in the ^{13}C source in CO_2 in the air samples collected after 2 h incubation of *Evernia mesomorpha* collected from south- and west-exposed larch branches under illumination from -25‰ to ca. -5‰ , whereas there were no changes in the ^{13}C source observed after 2 h incubation among north- and east-exposed ELs. The air sampled in the flasks after 2 h incubation of ELs was depleted in ^{13}C (from -57‰ to -70‰) in CH_4 (Figure S5). That supports the dominant process of CH_4 emission during 2 h ELs incubation under illumination (Figures 6 and 8).

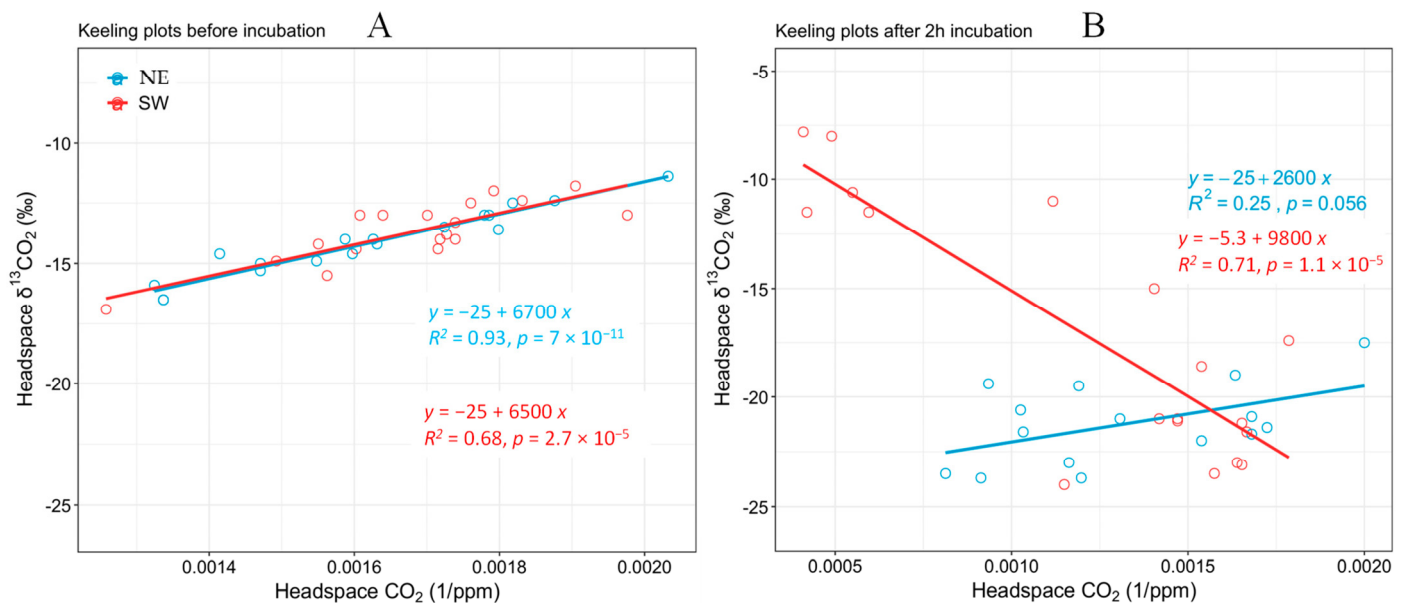


Figure 10. Keeling plots of $\delta^{13}\text{C}$ in CO_2 samples collected before (A) and after 2 h (B) of incubation of *Evernia mesomorpha* (at 200% of thallus water content) during a course of three-day incubation under illumination. SW (red), larch branches of southern and western exposures; NE (blue), larch branches of northern and eastern exposures.

4. Discussion

4.1. EL Diversity in Larch Forest in Permafrost Area

By undertaking a botanical survey within the study area, we identified 20 EL species with fruticose and foliose life forms, inhabiting larch (*Larix gmelinii*) trees and belonging to 12 genera and 3 families. The majority of species (79%) are found in the *Parmeliaceae* family and spread on tree branches (11 species) and at the base of tree stems (17 species). Additionally, compared to tree stems, the EL projective cover is substantially higher on branches. The Shannon index varied widely from 1.27 to 2.24. Two of the most common ELs with the highest projective cover and occurrence (*Bryoria simplicior* and *Evernia mesomorpha*; Figures 3–5) were selected to be employed in the incubation experiments to study potential GHG fluxes by ELs in permafrost areas.

Colonization by the ELs of various substrates occurs slowly (0.5–5 mm per year) [37]. Lichens colonize tree stems and branches unevenly, depending on a variety of factors such as light, water, temperature, and CO_2 concentration, which create specific microhabitats over a long period, particularly at various exposures of stems or branches [37,38]. Thus, epiphytic *Cladonia* was found to change the ratio of photobiont and mycobiont in a lichen community depending on the ambient temperature [39]. The lichens' colonization of the tree surface is facilitated by the larch trees' adaptations. Thus, a fascinating trait of larch (*Larix gmelinii*) in the north is the peeling of the bark, which is actively colonized by ELs, such as *Bryoria*. The angle at which a tree's bark is positioned is the most important factor influencing epiphytic organisms' expansion [40,41]. However, perhaps the predominant

colonization of larch branches, rather than larch stems, by ELs is more related to the prevailing wind rose (westerly direction) in the last decade (Figure 2), since, as shown by the geobotanical description, the greatest projective cover and occurrence of various ELs is confined to areas of the stem of northern and eastern exposure (for example, *Bryoria simplicior* and *Evernia mesomorpha*). This is supported by the observation that the highest projective cover of *Bryoria simplicior* and *Evernia mesomorpha* on larch branches corresponds to branches exposed to the south and west and that these species are 100% more frequently found there (Figures 4 and 5).

4.2. Potential GHG (CH₄ and CO₂) Fluxes by EL Species in Permafrost Areas

Our studies showed that during 2 h incubation under illumination, hydrated EL species (at 50%–400% of EL thallus moisture content) predominantly produce CH₄ (up to 1.8 ng C-CH₄ g^{−1} h^{−1}; Figures 6 and 8). Additionally, no factors were identified that significantly affected EL CH₄ rates (Table 1). As opposed to our earlier study evaluating potential GHG fluxes generated by lichens inhabiting birch trees in the non-permafrost region, where *Evernia mesomorpha* demonstrated CH₄ emissions up to 4.3 ng C-CH₄ g^{−1} of the dry weight of ELs per hour [18], in our current study, the same species (inhabiting larch) in the permafrost zone showed much lower CH₄ emissions (up to 1.8 ng C-CH₄ g^{−1} of the dry weight of ELs per hour). It corresponds to the lower limit of the range of CH₄ emission rates presented so far for terrestrial plants and lichens, i.e., 0.3–370 ng CH₄ g^{−1} DW (dry weight) h^{−1} [17,18,42–45]. CO₂ emissions by ELs in the permafrost zone were found to be almost 10 times lower (up to 60 µg C-CH₄ g^{−1} of the dry weight of ELs per hour) compared to the non-permafrost zone (up to 511 µg C-CH₄ g^{−1} of the dry weight of ELs per hour). CO₂ photoassimilation by *Evernia mesomorpha* in the permafrost zone was in the same range as that by *Evernia prunastri* (up to 4.8 nmol CO₂ g^{−1} DW s^{−1}, which is equivalent to ca. 1.3 µg C-CO₂ g^{−1} h^{−1}; [46]) at the same EL water content collected in a deciduous sub-Mediterranean forest in Portugal.

Our study shows that, unlike CH₄ fluxes, CO₂ fluxes vary depending on the type of EL, its thallus water content, and the exposure of the branch to which it is attached. For example, south- and west-exposed *Bryoria simplicior* demonstrated mixed dynamics of CO₂ fluxes during 2 h incubation under illumination, though north- or east-exposed *Bryoria simplicior* predominantly showed the photoassimilation of CO₂ (Figure 6). Such variation can be associated with the variation in the PAR and ALT regarding branch exposure in this particular species. On the contrary, *Evernia mesomorpha* showed no significant differences regarding branch exposure and mostly emitted CO₂ during 2 h incubation under illumination. Interestingly, *Bryoria simplicior* and *Evernia mesomorpha* inhabiting branches of northern and eastern exposure showed contrasting results regarding CO₂ fluxes, i.e., *Bryoria simplicior* mostly showed CO₂ photoassimilation, though *Evernia mesomorpha* demonstrated CO₂ emission under illumination (Figure 6). These specific responses of various EL species toward different factors broaden the uncertainty regarding GHG fluxes by EL communities of various compositions, which complicates the assessment of the EL contribution to the C cycle of forest ecosystems.

Els, like other lichens, employ a poikilohydric strategy for water and nutrient utilization [47]. Because they cannot control the amount of water in their cells through stomata, lichens engage in frequent cycles of wetting and drying (poikilohydry), which are contingent upon the presence of dew, rain, snow, or occasionally even high humidity [33]. Furthermore, ELs are capable of substantial changes in their thallus water content; they can dry out almost completely while remaining viable and maintaining a minimal metabolism, or, they can humidify in a very short time, which allows them to carry out the same metabolic processes as during periods of sufficient moisture [48–51]. In this context, evaluating the effects of varying lichen thallus hydration on GHG fluxes allows the assessment of the ranges of EL GHG fluxes depending on the environmental conditions. In this study, we assessed the effect of various EL water contents on GHG fluxes by *Evernia mesomorpha* during a 2 h incubation experiment under illumination. We found that *Evernia*

mesomorpha was presumably the emitter of studied GHG at all levels of EL water content, from the lowest (50%) to the highest (400%). The studied levels of thallus hydration of the EL and the branch exposure had, with one exception, negligible impact on the rate of CO₂ fluxes. Lichens limited to branches facing northward emitted four times more CO₂ at the maximum lichen water content (400%) than lichens gathered from branches facing south or west (Figure 8). This means that ELs, which face north and have sufficient thallus hydration, like *Evernia mesomorpha* in this particular case, could exhibit higher CO₂ emissions. At the same time, the direction of CO₂ processes was still affected by the lichen thallus hydration level; thus, with a thallus water content of 400%, *Evernia mesomorpha* demonstrated exclusively the process of CO₂ release and the absence of CO₂ photoassimilation during 2 h of incubation under illumination conditions (Figure 8). Lange et al. [33] report that lichens can continue photosynthesis despite drastic variations in the thallus water content; however, at some water content levels, the thallus becomes supersaturated, which decreases net photosynthesis due to constraints on CO₂ diffusion in overhydrated EL thalli. Morphological features of the lichens, such as the conglutinate cortical layer, the thickness and density of the thallus, and concurrent structural changes during water absorption, may also increase CO₂ diffusion resistances [37]. According to Snelgar et al. [52], lichens are divided into groups with different abilities to increase their resistance to CO₂ diffusion with increasing water content. Certain photobionts may accumulate inorganic C, which raises the CO₂ concentration close to Rubisco's sites and reduces photorespiration in order to mitigate CO₂ limitation. This mechanism is known from 1950 to 1980 and is called the CO₂-concentrating mechanism (CCM), and it is related to the pyrenoids found in green algae as well as the carboxysomes found in *Cyanobacteria* [46,53–56]. Although the mycobiont dominates the thallus in terms of biomass, its C is obtained through photosynthesis in the photobiont and exported from the photobiont to the mycobiont [37]. The CCM provides algae with an additional ecological advantage as it allows both CO₂ and HCO₃[−] to be efficiently exploited by carbonic anhydrases (CAs), which significantly contribute to the transformation of CO₂ [57]. CAs are found in the archaea *Methanosarcina* [58,59], yeasts [60], microalgae and *Cyanobacteria* [61–67], diatom algae [68], and fungi [69–71]. Chloroplast stroma, mitochondria, periplasmic space, and chloroplast thylakoid lumens of eukaryotic algae have all been found to contain CAs [55]. Therefore, the efficiency of CO₂ photoassimilation of algae allows for the full supply of organic matter to the fungal component and other heterotrophs of the lichen at very unequal ratios of photobiont to mycobiont in lichen, sometimes less than 10% [27,72]. Microalgae and cyanobacteria metabolize C in the same way as C3 plants and, as an add-on to the reductive pentose phosphate cycle, use “biophysical” mechanisms for CO₂ accumulation, which represent direct C “pumping” into the cell (generally as HCO₃[−]), activated by the load of CO₂ molecules at carboxylation sites. In addition to being able to withstand variations in temperature and humidity, the algae and *Cyanobacteria* that comprise lichens are also capable of conducting photosynthesis in a broad range of CO₂ concentrations, from low to high. The CCM enables the buildup of inorganic C in the form of bicarbonate ions and the concentration of CO₂ molecules close to the Rubisco sites. The CCM is activated only under light, and the accumulation of CO₂ and bicarbonate pools occurs in special microcompartments of the cell: carboxysomes in *Cyanobacteria* [73] and pyrenoids in eukaryotic algae [74].

In our study, EL CH₄ fluxes were partially impacted by varying EL hydration levels. For example, north-exposed *Evernia* with 200% thallus water content showed significantly lower CH₄ emissions compared to other water content levels (Figure 8). Furthermore, the lichen from branches exposed to the north released more CH₄ than the lichen from branches exposed to the south and west at the minimum thallus water content of 50%.

A negative correlation between ELs' CO₂ and CH₄ fluxes (Figure 7) is thought to be explained by the controversial relations between production and consumption processes. For example, there was a significant trend of CH₄ production increasing with the rise in CO₂ photoassimilation at lower thallus water contents (50%–200%) (Figure 8C); however, at a high thallus water content (400%), lower values of CO₂ production visually aligned

with higher values of CH₄ production, and CO₂ photoassimilation was lacking (Figure 8D). Moreover, respired CO₂ can also be refixed during incubation under illumination as it occurs in natural ecosystems [75]. As a result, under illumination, the same EL species may exhibit significantly different life activities because of the numerous GHG exchange processes involved and various regulatory factors (thallus water content, branch exposure, etc.). Therefore, under a changing climate, ELs as multicomponent systems are thought to demonstrate large uncertainties regarding their contribution to GHG exchange in climate-affected ecosystems.

CO₂ samples taken after 2 h incubation under illumination were enriched in ¹³C by 2–19‰ relative to EL thallus δ¹³C (ca. −26‰, Figure 10), possibly indicating specific C fractionation by photosynthetic processes. Calculated Keeling plot intercepts (Figure 10) showed the change of the ¹³C source in the CO₂ samples after 2 h incubation of hydrated *Evernia mesomorpha* (200% of thallus water content), and it was sensitive to the exposure of the branch the ELs inhabited. Thus, there was no C source change observed after 2 h incubation under illumination among north- and east-exposed ELs, whereas ELs collected from south- and west-exposed larch branches showed a change in the ¹³C source in the CO₂ from −25‰ to ca. −5‰. In the multicomponent system of ELs under illumination, the inward CO₂ fluxes (CO₂ assimilation and production processes (photorespiration, respiration)), the CO₂ source signature from the substrate, and the CO₂ fixation mechanism of their photobiont influence both CO₂ and δ¹³C [27]. Therefore, ELs accustomed to specific microenvironments shaped by long-term (decadal) various branch exposures, like substrate (e.g., bark) qualities (e.g., pH, conductivity), hydrothermal conditions, etc., may have inherited differences in the ratio of algal/fungal components and therefore their functional activities. Máguas et al. [27] also suggested that specific microenvironments (the place of EL attachment to stem or branch and their exposure, the height of the tree, the vertical CO₂ profile, the light intensity, etc.) may influence δ¹³C and its photosynthetic fractionations in ELs. The mechanisms of capture, diffusion, and sources of CO₂ can be related to ¹³C discrimination processes [37]. These processes are contingent upon the thallus moisture content, light intensity, and algae CO₂ fixation. Regarding δ¹³CH₄ values in the air sampled from the flasks following the 2 h incubation in this study, since CH₄ can both be emitted (for still unknown reasons) and be consumed by methanotrophs concurrently in aerobic conditions, we are unable to identify the source of the ¹³C in CH₄. We cannot appropriately compare the δ¹³CH₄ with other EL studies due to the absence of the latter. Only a preliminary comparison between our δ¹³CH₄ data and ¹³CH₄ studies on ELs' constituent organisms could be made. As an example, δ¹³CH₄ in *Cyanobacteria* species ranged from −36‰ to −49‰ [76]. In contrast, Figure S5 shows that the range of δ¹³CH₄ in the air sampled in the flasks following 2 h EL incubation was −57‰ to −70‰ in our study. Thus, more research on that topic is required.

Thus, based on our incubation experiments, we can conclude that the EL *Evernia mesomorpha* increases CO₂ release when over-moistened and increases CH₄ production and CO₂ photoassimilation when the thallus' water content is lowered. However, it is necessary to account for the significant variation in GHG fluxes from the EL's surface, which is caused by species specificity as well as the exposure of the branch to which the lichen is confined. EL contribution to GHG (CO₂ and CH₄) exchange and the C cycle of climate-affected ecosystems is poorly understood.

Because of the risk of climate change-induced algal blooms, CH₄ production in oxic water bodies is being more or less studied; however, terrestrial ecosystems, particularly permafrost ecosystems, are still understudied regarding non-methanogenic CH₄ production. In particular, not enough research has been carried out on the involvement of EL in oxic CH₄ production. Even though numerous valuable studies over the years have greatly advanced our understanding of non-methanogenesis oxic processes, the exact underlying pathways of the production of CH₄ remain unknown. The omics approach is a promising method for further studying the various pathways of production of CH₄ in the non-methanogenesis process. Furthermore, because of the potential links to light-dependent physiological

processes in the photobiont of an EL, our analysis is restricted to the measurement of GHG fluxes under illumination conditions. Therefore, to understand the mechanisms underlying oxic CH₄ emissions, the next step will be the investigation of the dark-related processes that lead to GHG fluxes by ELs. Overall, these results on oxic CH₄ production by the studied EL species may contribute significantly to regional and global CH₄ emissions due to their widespread distribution in permafrost larch stands, and permanent GHG monitoring is needed in this highly susceptible climate change area.

5. Conclusions

Here, we expand current knowledge on non-methanogenesis CH₄ emissions in oxic conditions by including some ELs inhabiting permafrost areas. In our study, using incubation experiments with illumination, we found that permafrost ELs primarily emit GHGs like CO₂ and CH₄. Using the most common epiphytic species in the study region, *Bryoria simplicior* and *Evernia mesomorpha*, which reside on larches with a projecting cover of up to 70% on larch branches, it has been shown that the GHG fluxes from the surface of these lichens are highly species-specific. Overall, among the studied EL species, CH₄ fluxes varied from -0.15 to $1.8 \text{ ng C-CH}_4 \text{ g}^{-1} \text{ h}^{-1}$ and CO₂ fluxes varied from -31 to $59.3 \text{ } \mu\text{g C-CO}_2 \text{ g}^{-1} \text{ h}^{-1}$. Moreover, the lichen-inhabited branch exposure and the water content of the EL thallus determine the magnitude and direction of CO₂ and CH₄ fluxes. It can be preliminary said that due to ELs' lower C emission rates in the permafrost zone, their contribution to permafrost area GHG emissions is expected to be less than those in the non-permafrost zone of Siberia. It should be noted that the GHG fluxes presented here were obtained under a controlled environment and are not likely to be indicative of real emissions in field conditions. Therefore, to fully comprehend the function of ELs in the C cycle, more research is necessary. Additionally, it is crucial to investigate the impact of ecological and environmental condition fluctuations caused by ongoing climate change on various EL species, taking into account the revealed differences in GHG fluxes by ELs in permafrost and non-permafrost habitats. New data on EL GHG fluxes, along with related parameters like branch or stem exposure, EL thallus water content, etc., are suggested to be incorporated into models on a long-term basis.

Supplementary Materials: The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/f15111962/s1>, Figure S1: Study sites. A, dwarf shrub-lichen-green mosses larch stand; B, *Pyrola*-dwarf shrub larch stand; C, *Ledum-Vaccinium*-green moss-lichen larch stand; D, *Ledum-Vaccinium*-green mosses larch stand; E, dwarf shrub-green mosses larch stand; Figure S2: Epiphytic lichens in studied larch stands. A, *Evernia mesomorpha*; B, *Bryoria simplicior*; C, *Tuckermannopsis sepincola*; Figure S3: The Shannon index and species richness of ELs at model larch trees (n = 25); Figure S4: The setup of the first incubation experiment; Figure S5. Keeling plots of $\delta^{13}\text{C}$ in CH₄ samples collected before (A) and after 2 h (B) of incubation of *Evernia mesomorpha* (at 200% of thallus water content) during a course of three-day incubations under illumination. SW (blue), larch branches of southern and western exposures; NE (red), larch branches of northern and eastern exposures; Figure S6: Correlation (Spearman's rank correlation coefficients) heat maps of the studied parameters depending on the branch exposure of the ELs at the 1st (A), 2nd (B), and 3rd (C) days of the first incubation experiment. ***, $p < 0.001$; **, $p < 0.05$; *, $p < 0.5$; Table S1: Description of model trees.

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