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Aerobic release and biodegradation of dissolved organic matter from frozen peat: Effects of temperature and heterotrophic bacteria

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ABSTRACT

Understanding the conditions of dissolved organic matter (DOM) release from thawing peat in the Arctic regions and identifying the pathways of processing DOM by soil and aquatic heterotrophic bacteria are critical in the context of rapid climate change. Until now, experimental approaches did not allow quantitative predictions of temperature and biota effects on carbon release from peat in permafrost-affected aquatic environments. In this study, we incubated frozen peat and its aqueous leachate at various temperatures (4, 25 or 45 °C), with and without culturable heterotrophic bacteria *Iodobacter* sp., extracted from thermokarst lakes, to quantify the release and the removal rate of organic carbon (OC) with time. The metabolic diversity of the native microbial community associated with the substrates involved in OC processing was also characterized. Transmission electron microscopy revealed that, after degradation, the associated bacteria are mostly located in the inner parts of plant cells, and that the degradation of organic matter around bacteria is more pronounced at 4 and 25 °C compared to 45 °C. The metabolic diversity of heterotrophic bacteria was equally high at 4 and 25 °C, but lower at 45 °C. Regardless of the microbial consortium (native community alone or with added culturable heterotrophs), both the OC release from peat and the OC removal from peat leachate by bacteria were similar at 4 and 25 °C. Very low apparent activation energies of DOM biodegradation between 4 and 25 °C ($-4.23 \pm 12.3 \text{ kJ mol}^{-1}$) suggest that the short-period of surface water warming in summer would have an insignificant effect on DOM microbial processing. Such duration (1–3 weeks) is comparable with the water residence time in peat depressions and permafrost subsidences, where peat degradation and DOM microbial processing occur. This questions the current paradigm of a drastic effect of temperature rise on organic carbon release from frozen peatlands, and should be considered for modelling short-term climate impacts in these regions.

1. Introduction

Permafrost wetlands that store a large amount of carbon (C) in Arctic regions are severely affected by the ongoing global warming, which is referred to as the “Arctic amplification” (Overland and Wang, 2013; Serreze et al., 2009). Climate scenarios in Arctic regions predict that air temperature will increase by 2 to 4.9 °C before the end of the century (Raftery et al., 2017). This will increase the active layer thickness (ALT) and enhance soil subsidences and abrupt thermokarst events (Grosse et al., 2011; Turetsky et al., 2019). As a consequence, a substantial amount of previously sequestered organic matter (OM, about 1330–1580 Pg-C) trapped in the permafrost (Hugelius et

al., 2014; Schuur et al., 2015) might be mobilised and reinjected into the global C cycle. The microbially-driven degradation of the released OM in the active layer or in thaw (thermokarst) lakes, generated by the soil surface subsidence can also be strongly affected by rising temperatures due to climate warming (Grosse et al., 2011; Kirpotin et al., 2009; Kokelj and Jorgenson, 2013; Shirokova et al., 2009).

Permafrost thaw occurs mostly in Arctic regions, mainly where soil temperature fluctuates around 0 °C (Romanovsky et al., 2010) and where the permafrost is discontinuous to sporadic. Boreal and subarctic peatlands occupy large regions with significant soil C stocks in the form of frozen and thawing peat, namely in the Western Siberia Lowland

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(WSL, 1,300,000 km²), North-Eastern European Tundra (300,000 km²), Canada (1,240,000 km²; with the Hudson Bay Lowland accounting for 372,000 km²; Packalen et al., 2014), USA (625,000 km²), Finland (96,000 km²) and Sweden (70,000 km²) (Gorham, 1991; Kuhry and Turunen, 2006; Vitt, 2006). These wetlands are subjected to highly pronounced degradation of frozen peat and massive permafrost thaw (Kokelj et al., 2017b, 2017a; Loiko et al., 2017; Overland and Wang, 2013; Turetsky et al., 2019). For example, from 2000 to 2015, the average annual soil temperature of the North-Eastern European Tundra increased from -2 ± 1 °C to $+1.5 \pm 0.5$ °C which produced sizeable increase in ALT and caused major soil subsidences (Kaverin et al., 2017). It is worth noting that the Eastern European Arctic may be among the key northern regions from which positive and negative carbon-climate feedbacks are likely to arise in the future (Ciais et al., 2019). The main mechanism that underpins such feedback is the microbial processing of DOM of peat (terrestrial) origin, as it has already been shown from various non-permafrost (Berggren et al., 2010) and permafrost (Drake et al., 2015; Mann et al., 2015) settings. Since the DOM processed by the microbial community in surface waters can be released to the atmosphere as carbon dioxide and methane, it is important to better understand the pathways of these processes to predict their impact on the climate.

The main objective of this study was to evaluate the impacts of temperature and heterotrophic bacteria on C release and DOM biodegradation, using both the frozen peat and its aqueous leachate. We hypothesised an enhanced release of DOM from peat and its biodegradation due to an acceleration of heterotrophic bacteria metabolism with increasing temperature. Usually, both aerobic and anaerobic incubations are used to assess the biodegradability of permafrost soil materials (Heslop et al., 2019b; Knoblauch et al., 2013; Schädel et al., 2016; Treat et al., 2014; Waldrop et al., 2010) and a few microbiological studies of autochthonous bacterial communities in peat demonstrated that these communities are dominated by Alphaproteobacteria and Acidobacteria (Ausec et al., 2009; Basiliko et al., 2013; Dedysh, 2011; Dedysh et al., 2006; Kraigher et al., 2006; Pankratov et al., 2011). However, in this work, we focused on aerobic incubation, because *i*) most surface waters of frozen peatlands and supra-permafrost waters interacting with soil peat are fully or partially oxygenated (Loiko et al., 2017; Manasyrov et al., 2015; Raudina et al., 2018) and *ii*) the potential C emissions under aerobic conditions are higher than under anaerobic conditions (Schädel et al., 2016). For these incubations, we selected cultivable heterotrophic species (*Iodobacter* sp.) extracted from thermokarst lakes in a discontinuous permafrost peatland area and known to occur in arctic lake sediments (Shirokova et al., 2017; Su et al., 2013). The choice of this bacterium was also made to simulate the interaction between frozen peat and surface waters, in which aquatic bacteria rather than soil bacteria may dominate the processing of organic carbon.

2. Material and methods

2.1. Bacteria and substrate: origins and use

Heterotrophic bacteria (*Iodobacter* sp., CdM7, Proteobacterian, Betaproteobacteria) were isolated from thermokarst lakes located in NE Europe permafrost-bearing peat bogs (Shirokova et al., 2017). These aerobic gram-negative bacteria, rod-shaped cells (0.7 µm wide and 1.4 to 1.7 µm length) have one or several polar and lateral flagella. The bacteria were cultured to a stationary stage in a 10% nutrient broth (NB) on a shaker for 5 days at 25 °C in darkness. Prior to the addition of peat substrate, the heterotrophic bacteria were starved in 0.01 M NaCl for 3 days to remove internally and surface adsorbed nutrient resources (Bundeleva et al., 2014, 2012; Shirokova et al., 2017, 2012). This treatment did not affect cell viability.

The frozen peat was collected at the edge of a thermokarst lake in the Bolshezemel'skaya Tundra zone of NE Europe (discontinuous permafrost area, see Shirokova et al., 2019 for details), underneath the active layer (from 30 to 40 cm depth). The oligotrophic peat is 35% degraded and composed of *Sphagnum angustifolium* (20%), cotton-grass *Eriophorum* (25%), *Scheuchzeria palustris* (15%), bog-sedge *Carex limosa* (5%), lichens (5%), dwarf shrubs (5%) and some amount of wood debris (pine) and green mosses. The studied peat essentially made of *Sphagnum* is representative of boreal peatland ecosystems (Turetsky et al., 2008).

Two types of organic substrates were used for experiments: (1) solid frozen peat (SFP), sampled using a Russian peat corer (see details of sampling and handling in Morgalev et al., 2017) and kept frozen until its use in the laboratory and (2) aqueous leachate of this frozen peat (FPL). The leachate was prepared *via* reacting the frozen peat with sterile MilliQ water (10 g_{dry} L⁻¹), in aerobic conditions at room temperature under gentle mixing on a shaker. An initial DOC concentration between 12 and 20 mg L⁻¹ was then reached, consistent with that occurring in natural thaw ponds and depressions of the permafrost zone (10–100 mg L⁻¹, Pokrovsky et al., 2016; Raudina et al., 2018). Note that, because the SFP contained its native microbial community, all incubations were run in the presence of this microbial community. After 3 days of reaction, the suspension was centrifuged at 4500g for 5 to 10 min, filtered through a 0.45 µm Nalgen sterile filter and then used as a substrate in experiments.

2.2. Experimental setup

2.2.1. Incubations

A scheme of the incubations setup is given in Fig. 1. A homogeneous suspension of the heterotrophic bacteria was transferred into 500 mL sterile bottles, with either solid peat (10 g_{dry} L⁻¹ in 300 mL of milliQ water) or aqueous peat leachate (300 mL), to obtain an initial bacterial biomass of 1 g_{wet} L⁻¹. Reactors of substrate with and without added heterotrophic bacteria were placed at 4, 25 or 45 °C under aerated conditions (the aeration was achieved *via* Biosilico® porous stoppers) in a thermostated ping-pong shaker (25 and 45 °C), or on a ping-pong shaker placed in the dark inside a refrigerator (4 °C). The three chosen temperatures correspond to currently observed conditions: (i) at the beginning of spring and end of autumn (4 °C, Manasyrov et al., 2015), (ii) in thermokarst lakes waters during summer (25 °C, Shirokova et al., 2019, 2013) and (iii) 45 °C, a temperature that can be reached on the surface of moss and lichen cover during summer heatwaves (*i.e.* Pokrovsky et al., 2013). The last case was used to reproduce possible maximum short-term summer temperatures expected in the case of most extreme scenarios of water or soil local heating. The control experiments were 0.45 µm-filtered frozen peat leachate, or frozen peat with MilliQ water, without the addition of *Iodobacter* sp. They were incubated under the same conditions as the experiments with added heterotrophic bacteria. As mentioned before, frozen peat comes with native microbial community, therefore in non-added heterotrophic bacteria experiments, the controls represented the activity of native microbial community associated with the peat substrates (SFP or FPL).

2.2.2. Sampling and chemical analyses

The SFP and FPL experiments lasted for 29 and 16 days, respectively, *i.e.* the time necessary to reach a steady-state concentration of DOC in the system. All the analyses performed on each sample during incubation are presented in Fig. 1. A homogeneous 15 mL suspension was sampled from each experimental reactor. A small non-filtered aliquot of the sample was used to measure pH (uncertainty of ± 0.01 pH units) and specific conductivity (± 0.1 µS cm⁻¹). The rest of the 15 mL sample was centrifuged at 4500g for 15 min and the supernatant

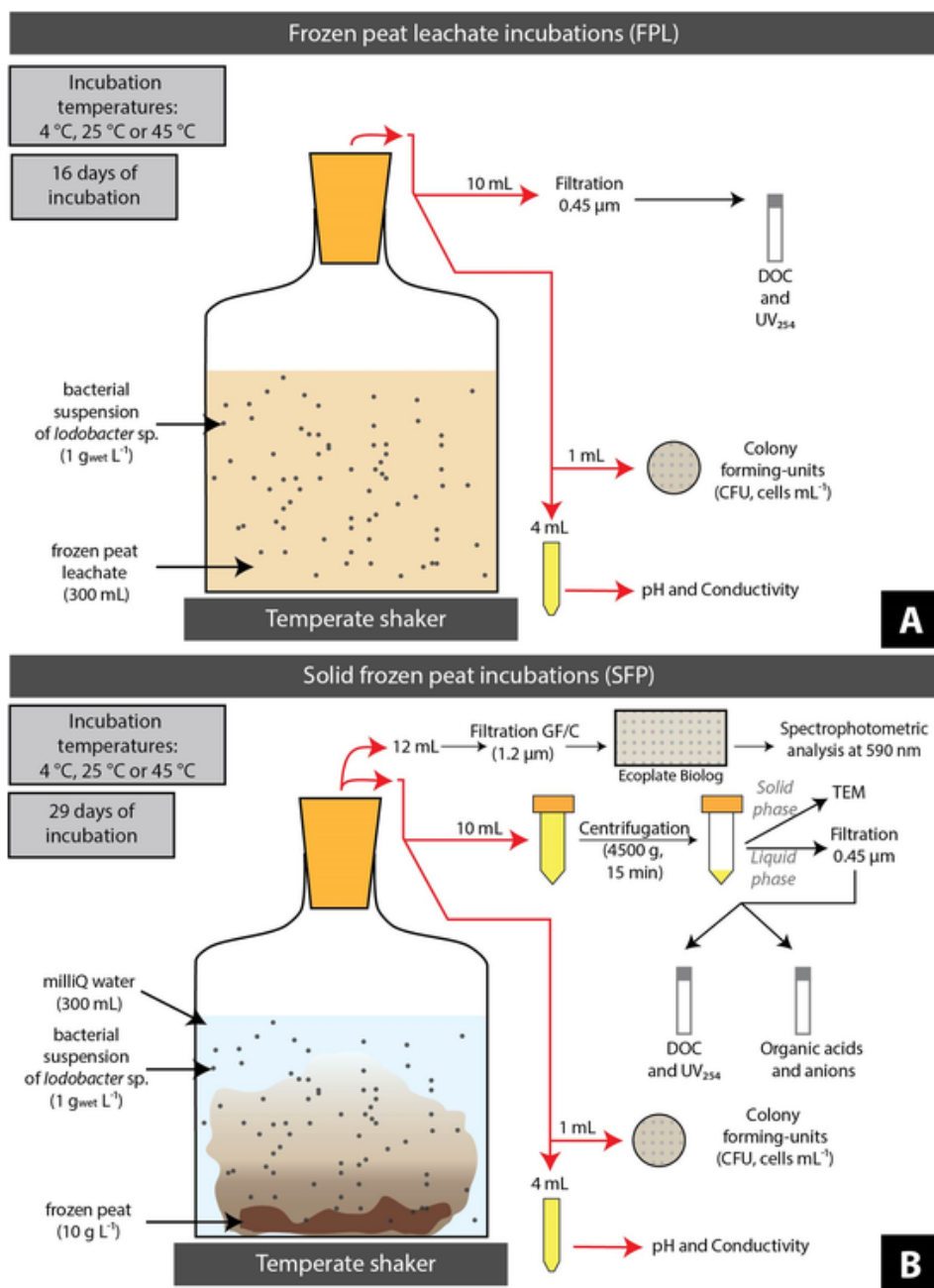


Fig. 1. Schematics of frozen peat leachate (FPL; A) and solid frozen peat (SFP; B) leachate incubations with the various analyses performed on each sample.

was then filtered through a 0.45 µm filter (Minisart® syringe filter). In the experimental filtrates, the DOC was measured with a Shimadzu TOC-VSCN with an incertitude of 3%. The UV-absorbance of water samples at 254 nm was measured using a 10 mm quartz cuvette on a SpectroFlex spectrophotometer (series 6600 – WTW), as this wavelength has been defined as the best proxy for surface water DOC (Edzward et al., 1985; Peacock et al., 2014). In addition, the specific UV absorbance (SUVA₂₅₄, L mg C⁻¹ m⁻¹) was used to assess the aromaticity of samples and the source of DOM. In the case of SFP experiment, additional analyses were conducted, such as organic acids and anions analyses, measured with a high-performance ion chromatography (Dionex ICS-5000+, analytical column: AS11-HC 4 µm).

2.2.3. Microbiological analyses

To evaluate the number of living bacteria (CFU, cells mL⁻¹) in both SFP and FPL experiments, 1 mL of non-filtrated aliquots were inoculated in nutrient agar (2.8%) after serial dilution. Inoculated agars were placed in the dark for 5 days before visual counting.

In the case of SFP experiment, at the beginning and at the end of incubation (29 days), a duplicate of 12 mL aliquot per experiment at each temperature was collected to analyse the functional diversity of the bacterial community, using an EcoPlates® (Biolog Inc., Hayward, CA). The list of tested substrates (31 in total) is provided in Supplementary information (Table S1). Each well of the plate was inoculated with 130 µL of 1.2 µm-filtered (GF/C, Whatman) aliquot, and kept in the dark at 18 °C. After 5 days, the optical density of the plate at 590 nm was measured with a Synergy MX spectrophotometer (Biotek). The fol-

lowing calculated parameters were used to assess the change of microbial activity (Gryta et al., 2014; Stefanowicz, 2006; Weber and Legge, 2009; Zak et al., 1994): the average well colour development (AWCD, Eq. (1a)), the Shannon index of diversity (H, Eq. (1b)) defining the richness of the microbial community and the Shannon index of evenness (E, Eq. (1c)). In these equations, OD is the optical density of the well and p_i is the proportional colour development of the well “ i ” over the total colour development of all wells of the plate.

$$AWCD = \sum \frac{\text{mean} (OD_i - OD_{\text{blank}})}{31} \quad (1a)$$

$$H = -\sum p_i (\ln p_i) \quad (1b)$$

$$E = H/H_{\text{max}} \quad (1c)$$

2.3. Transmission Electron Microscopy (TEM)

The peat particles in the SFP experiment were observed before incubation and after 29 days of incubation (using the solid phase obtained after centrifugation of the 15 ml aliquots) with a JEOL JEM-1400 HC transmission electron microscope (TEM) equipped with a Gatan Orius SC1000B CCD camera and after negative staining. Ultrathin sections were prepared as follows: at first, the samples were fixed with a 2.5% glutaraldehyde/2% paraformaldehyde in 0.1 M cacodylate buffer at ambient temperature for 2 h. The samples were then washed three times with the same cacodylate buffer and soaked during 1 h in 1% osmium tetroxide as a post-fixation step. The soaked samples were centrifuged for 5 min at 59g, the supernatant was removed, and the samples were embedded in agarose. The consolidated samples were placed in Eppendorf cones and dehydrated in ascending ethanol series (25, 50, 70 and 90% for 15 min and three times in 100% for 30 min). They were then gradually infiltrated in epoxy resin (25, 50 and 75% for 1 h, and twice in 100% for 12 h) and polymerized at 60 °C for 48 h. 80 nm thick thin sections were cut from the resin-embedded samples with an ultramicrotome (Leica UCT), and placed on Formvar carbon-coated copper grids. Finally, the sections were stained with uranyl acetate and lead citrate.

2.4. Treatment of experimental results and statistics

In order to determine the concentration of biodegradable DOC (BDOC, Eq. (2)) over incubation time for the FPL experiments, the equation proposed by Vonk et al. (2015) was used:

$$BDOC (\%)_{t=x} = \left[\frac{(DOC_{t=0} - DOC_{t=x})}{DOC_{t=0}} \right] \times 100\% \quad (2)$$

where “ t ” is the incubation time in days. The apparent release (for SFP experiments) or removal (for FPL experiments) rate of DOC (R_{DOC} , Eq. (3)) was calculated via the equation of a linear regression slope:

$$R_{\text{DOC}} (\text{mg L}^{-1} \text{d}^{-1}) = \frac{\sum ((t_x - t_{\text{mean}}) (DOC_{t=x} - DOC_{\text{mean}}))}{\sum (t_x - t_{\text{mean}})^2} \quad (3)$$

where “ t_x ” is the day of incubation at time “ x ” and “ t_{mean} ” is the mean of incubation time in days. In addition, the apparent activation energy (E_a , Eq. (4)) was calculated from the Arrhenius equation (Arrhenius, 1889) using apparent rate of DOC release (in SFP experiments) or DOC removal (in FPL experiments) between temperatures (4 and 25 °C; 25 and 45 °C; and 4 and 45 °C) during the more active phase of DOC (i.e., during the first 2 days of incubation):

$$E_a (\text{J mol}^{-1}) = \left[\frac{(R \times T_1 \times T_2)}{(T_1 - T_2)} \right] \times \ln \left(\frac{R_{\text{DOC}(T_1)}}{R_{\text{DOC}(T_2)}} \right) \quad (4)$$

where “ R ” is the universal gas constant ($R = 8.314 \text{ J K}^{-1} \text{ mol}^{-1}$), “ T_1 ” and “ T_2 ” are the incubation temperatures in Kelvin and “ $R_{\text{DOC}(T_x)}$ ” is the apparent release or removal rates of DOC at temperatures T_1 and T_2 .

Because the data were normally distributed, a statistical treatment, including the least square method for regression and the Pearson correlation, was used. A one-way ANOVA test was used to compare the trend in DOC and BDOC concentrations, R_{DOC} rate, SUVA₂₅₄, CFU, H, E and AWCD index between *i*) two temperatures, *ii*) added heterotrophic bacteria and control run and *iii*) at the beginning and at the end of experiments and assessed via the corresponding p_{values} (Table S2). These treatments were performed under R software (version 3.5.2).

3. Results

The patterns of DOC and SUVA trend with time between 4 and 25 °C incubations in SFP and FPL experiments were insignificantly different ($p_{\text{values}} > 0.05$; except for the SUVA₂₅₄ in SFP incubation). The FPL incubation results at 25 °C were not different than those at 45 °C (excepted for the BDOC, $p_{\text{values}} = 0.00146$). However, the BDOC showed only slight differences between 4 and 25 °C (Table S2).

3.1. DOC behaviour

The changes in DOC concentrations in the SFP experiments over time exhibited similar trends at 4 and 25 °C with and without addition of *Iodobacter* sp.: after an initial increase by a factor of 2 to 5, from day 0 to day 12, the DOC concentration remained stable after 12 days (Fig. 2A and B). In contrast, at 45 °C, the DOC concentrations increased from 18.2 ± 2.4 at day 0 to $140.8 \pm 1.9 \text{ mg L}^{-1}$ at the last day of incubation in the SFP experiment with added heterotrophic bacteria, and from 11.9 ± 0.5 to $118.1 \pm 3.9 \text{ mg L}^{-1}$ in the control experiment (Fig. 2C). The apparent rate of DOC release in SFP experiments decreased with incubation time for all temperatures, with or without added heterotrophic bacteria (Table 1). For the 45 °C incubations, the R_{DOC} between day 0 and 2 decreased from 17.7 ± 1.5 to $3.6 \pm 0.12 \text{ mg L}^{-1} \text{d}^{-1}$ in the presence of *Iodobacter* sp. and from 18.4 ± 0.66 to $3.2 \pm 0.09 \text{ mg L}^{-1} \text{d}^{-1}$ in the control experiment. Between 0 and 2 days of incubation at 4 °C, the apparent release rate of DOC was lower in *Iodobacter* sp. experiment compared to the controls ($3.22 \pm 0.79 \text{ mg L}^{-1} \text{d}^{-1}$ versus $6.25 \pm 1.25 \text{ mg L}^{-1} \text{d}^{-1}$). The trend of SUVA₂₅₄ over time in SFP incubations showed an initial increase followed by a stabilization after 2 days of exposure at all temperatures (around $5.0 \pm 1.0 \text{ L mg C}^{-1} \text{m}^{-1}$), both for added heterotrophic bacteria and control experiments (Fig. 3A). The highest SUVA₂₅₄ value was observed at 25 °C at day 21 ($6.4 \pm 0.2 \text{ L mg C}^{-1} \text{m}^{-1}$).

The temporal change of DOC in the FPL experiments was characterized by an approximately 2 mg L^{-1} decrease over the first 2 days, followed by a stabilization around 12 mg L^{-1} between day 2 to 16 in both added heterotrophic bacteria and control experiments at 4 and 25 °C (Fig. 2D and E). In the case of the 45 °C FPL experiment with added *Iodobacter* sp., the DOC increased after 10 days of incubation from 13.7 ± 0.16 to $18.5 \pm 0.34 \text{ mg L}^{-1}$ (Fig. 2F). In general, the BDOC was higher for control experiments than in the presence of *Iodobacter* sp. for the 25 and 45 °C incubations (Fig. 2H and I), but the opposite trend was observed at 4 °C of incubation (Fig. 2G). The BDOC at 4 and 25 °C sharply increased between days 0 and 2 (ca. 10–18%), then slightly decreased until day 5 and remained stable and positive until the last day of incubation. The incubations at 45 °C exhibited two clearly different patterns (Fig. 2I). While BDOC of control experiment was always positive and showed a 13% increase in values until day 9,

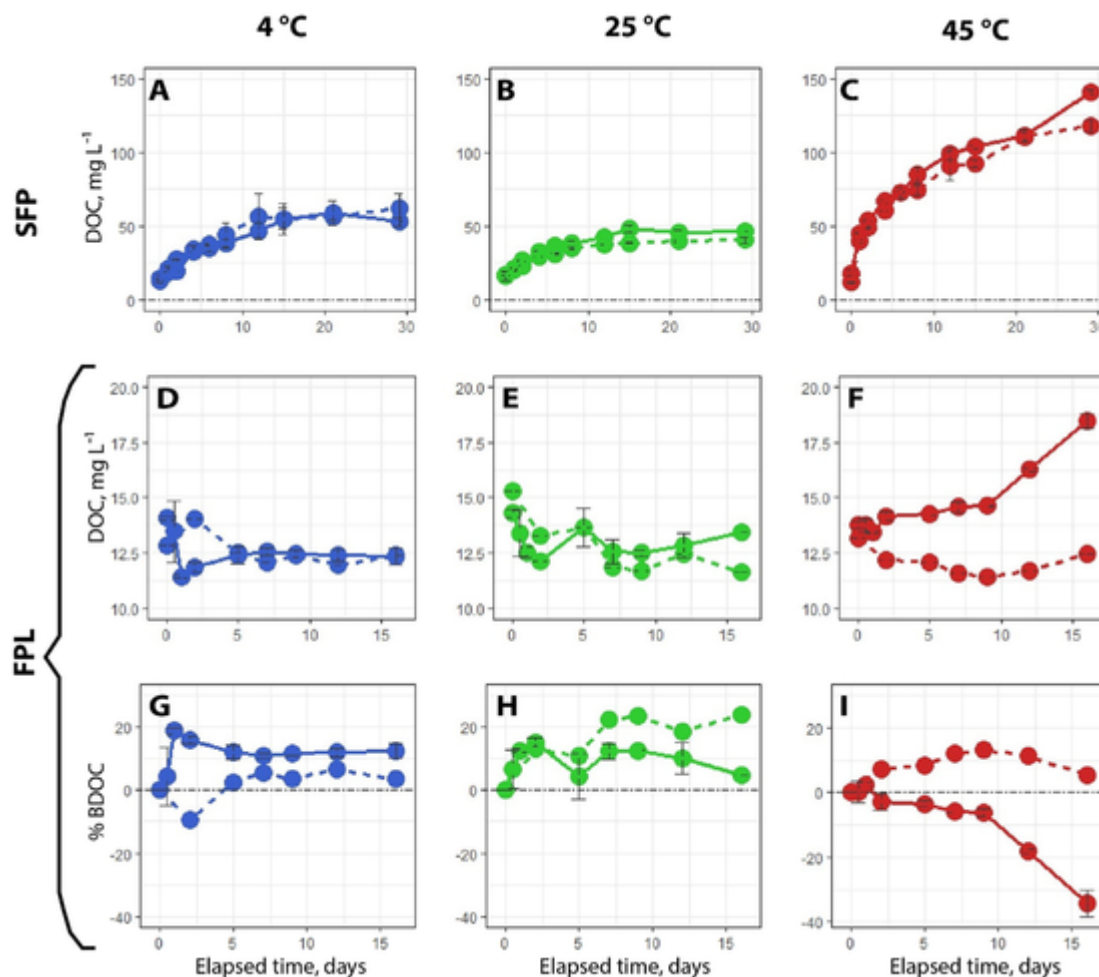


Fig. 2. Dissolved Organic Carbon (DOC) of solid frozen peat (SFP, A, B and C) and frozen peat leachate (FPL, D, E and F), Biodegradable Dissolved Organic Carbon (% BDOC) of FPL (G, H and I) over incubation time at 4 °C (A, D and G), 25 °C (B, E and H) and 45 °C (C, F and I). These figures show the evolutionary trend of control incubations (dotted lines) and added heterotrophic bacteria incubations (solid lines). Note that the SFP and FPL experiments did not last the same duration. Most of the error bars are smaller than the symbol size. The negative BDOC in FPL experiments at 45 °C might be due to bacterial cell lysis and DOM production (see Section 4.1.2).

Table 1

R_{DOC} : apparent release rate of DOC ($\text{mg L}^{-1} \text{d}^{-1}$) for solid frozen peat (SFP) and apparent removal rate of DOC for frozen peat leachate (FPL) incubations, with and without added heterotrophic bacteria (control) at 4, 25 and 45 °C.

	4 °C		25 °C		45 °C	
	Added bacteria	Control	Added bacteria	Control	Added bacteria	Control
SFP incubations						
Day 0 to 2	3.22 ± 0.79	6.25 ± 1.25	5.28 ± 1.87	3.01 ± 0.56	17.74 ± 1.53	18.41 ± 0.66
Day 0 to 6	3.82 ± 0.56	3.78 ± 0.33	3.47 ± 0.38	2.47 ± 0.1	8.11 ± 0.25	9.05 ± 0.1
Day 0 to 12	2.78 ± 0.49	3.26 ± 1.29	2.14 ± 0.2	1.71 ± 0.03	5.88 ± 0.4	5.68 ± 0.53
Day 0 to 29	1.51 ± 0.23	1.56 ± 0.33	0.98 ± 0.07	0.78 ± 0.06	3.57 ± 0.12	3.18 ± 0.09
FPL incubations						
Day 0 to 2	-1.18 ± 0.16	0.6	-1.06 ± 0.24	-1.01	0.19 ± 0.1	-0.48
Day 0 to 5	-0.22 ± 0.02	-0.08	-0.25 ± 0.29	-0.29	0.12 ± 0.01	-0.2
Day 0 to 12	-0.04 ± 0.02	-0.12	-0.05 ± 0.06	-0.23	0.17 ± 0.01	-0.12
Day 0 to 16	-0.03 ± 0.01	-0.07	-0.04 ± 0.07	-0.19	-0.26 ± 0.01	-0.04

followed by a 5.5% decrease between day 9 to 16, the added heterotrophic bacteria experiment showed negatives values (corresponding to DOC production/release) from day 0 to 16 with a 5-fold drop after day 9. The apparent rate of DOC removal in FPL incubations (Table 1) increased with incubation time at 4 and 25 °C without any significant

difference between the two temperatures ($p_{\text{values}} = 0.497$, Table S2). However, at 45 °C with added *Iodobacter* sp. there was a release rather than a removal of DOC in contrast with the control experiment at 45 °C, which revealed a net removal of DOC. The SUVA_{254} trend in FPL experiments did not exhibit any clear trend with time, and the differ-

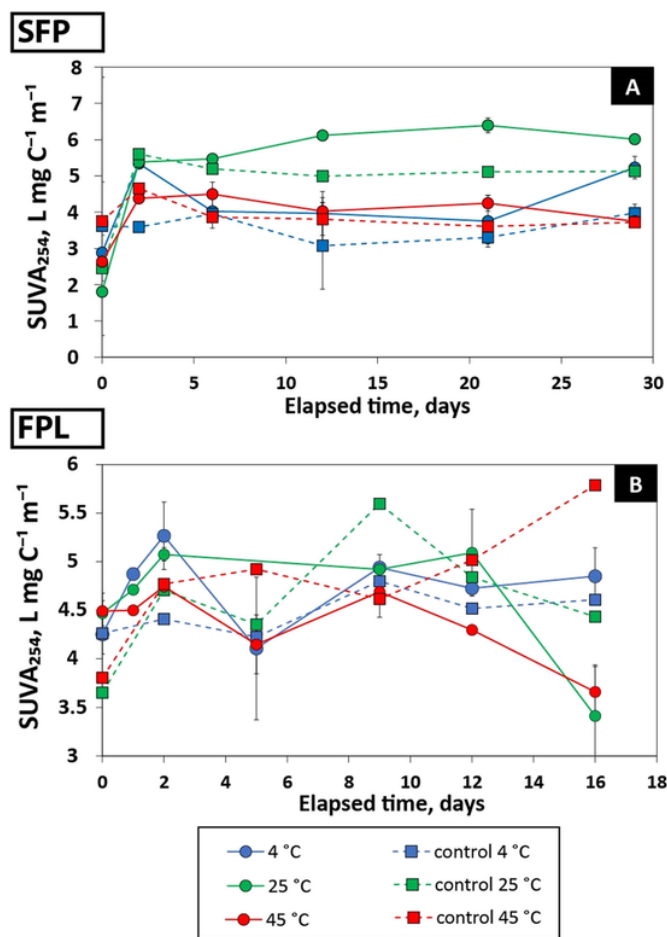


Fig. 3. Temporal evolution of SUVA₂₅₄ during incubation of solid frozen peat (SFP; A) and frozen peat leachate (FPL; B). These figures show the evolutionary trend of control incubations (dotted lines) and added heterotrophic bacteria incubations (solid lines).

ences between added heterotrophic bacteria and control experiments, and between different temperatures, were not significant (Fig. 3B).

Apparent activation energies (E_a) of DOC release in the SFP incubations (R_{DOC} ; Table S3) between 4 and 25 °C for the first two days of exposure showed a positive value with added heterotrophic bacteria ($E_{a_{added\ bacteria(4-25)}} = 15.17 \pm 3.9 \text{ kJ mol}^{-1}$) and a negative value in the control ($E_{a_{control(4-25)}} = -23.98 \pm 0.5 \text{ kJ mol}^{-1}$). The E_a was positive between 25 and 45 °C ($E_{a_{added\ bacteria(25-45)}} = 50.56 \pm 18.1 \text{ kJ mol}^{-1}$) but higher for the control $E_{a_{control(25-45)}} = 72.56 \pm 6.1 \text{ kJ mol}^{-1}$). In the case of FPL incubations, the activation energy of DOC removal between 4 and 25 °C was low and negative in the added heterotrophic bacteria incubation ($E_{a_{added\ bacteria(4-25)}} = -4.23 \pm 12.3 \text{ kJ mol}^{-1}$).

3.2. Organic acids and inorganic anions behaviour in SFP incubations

Among the measured anion (phosphate) and organic acids (acetate, formate, malate, oxalate and tartrate), only the phosphate concentration in the experiment with added *Iodobacter* sp. (Table 2) showed a significant increase in concentration over the time of incubation, following the order $45^\circ\text{C} \geq 25^\circ\text{C} > 4^\circ\text{C}$. The increase of PO_4^{3-} concentration from 0 to the 29th day of incubation was equal to 2.38×10^{-2} , 2.24×10^{-2} and $8.65 \times 10^{-3} \text{ mg L}^{-1} \text{ d}^{-1}$ at 45, 25 and 4 °C, respectively. In contrast, the control experiment only revealed a slight decrease of phosphate concentration over incubation time at all temperatures (-1.27×10^{-3} , -1.32×10^{-4} and $-7.04 \times 10^{-4} \text{ mg L}^{-1} \text{ d}^{-1}$ at 4 °C, 25 °C and 45 °C, respectively).

Among the organic acids, only acetate exhibited a significant decrease in the control experiment ($-4.89 \times 10^{-3} \text{ mg L}^{-1} \text{ d}^{-1}$ over 29 days of incubation at 4 °C), and a slight increase in the added *Iodobacter* sp. experiment (2.39×10^{-4} , 3.19×10^{-3} and $1.42 \times 10^{-3} \text{ mg L}^{-1} \text{ d}^{-1}$ at 4, 25 and 45 °C). The oxalate increased by $1.22 \times 10^{-2} \text{ mg L}^{-1} \text{ d}^{-1}$ at 45 °C in the added heterotrophic bacteria experiment. Formate, malate and tartrate did not exhibit any significant variation over the 29 days of incubations at all temperatures, and the changes of their concentrations did not generally exceed $10^{-3} \text{ mg L}^{-1} \text{ d}^{-1}$ (0.05 mg L^{-1} from day 0 to day 29) which was $< 0.1\%$ of the overall DOC level.

3.3. Bacterial cell number and Ecoplate analyses of microbial functional diversity in the SFP incubations

The live cell number in SFP (Fig. 4A) and FPL (Fig. 4B) experiments remained stable during incubations (around 10^9 CFU mL^{-1} with added *Iodobacter* sp. and 10^5 CFU mL^{-1} in the control). For experiments at 45 °C, the bacterial number drastically decreased between 0 and 2 days in SFP and FPL incubations, losing almost half of the initial cell number in both types of incubations. The incubation at 45 °C with SFP had CFU values comparable with those at 4 and 25 °C from day 20 to day 29. In contrast, the CFU values of FPL incubations at 45 °C were always an order of magnitude lower than those at 4 and 25 °C.

The AWCD parameter, reflecting the heterotrophic microbial diversity, was three times higher at the beginning of incubation in control experiments compared with added *Iodobacter* sp. experiments for all incubation temperatures ($p_{\text{value}} < 0.001$, Tables 2 and S2). At the end of incubations (day 29), the AWCD with added *Iodobacter* sp. and control experiments were similar ($p = 0.632$, Table S2) for the three temperatures. A net decrease in richness (H) and evenness (E) of metabolic diversity was also detected for both control and added *Iodobacter* sp. experiments at all temperatures, except for the E index with added *Iodobacter* sp. at 4 and 25 °C (Table 2).

Ecoplate analyses also revealed a drastic decrease (*i.e.* $\geq 70\%$) of community richness, over 29 days of incubation for each type of consumed substrate (polymers, carbohydrates, amino acids, amines/amides and carboxylic acids, Fig. 5). In control experiments, the decrease of each substrate richness was $> 90\%$ (hatched columns in Fig. 5), while for added *Iodobacter* sp. experiments, the decrease was between 70 and 80% at 4 and 25 °C and over 90% at 45 °C. Furthermore, for all types of incubations and at all temperatures, the polymers, carbohydrates, amino acids, and carboxylic acids were mostly consumed at the beginning of experiments, while at the end of experiments, only polymers and carbohydrates were used by the microbial community.

3.4. TEM observations of incubated peat

The TEM observations of peat at the beginning of the SFP experiments revealed a compact and complex material in which the original structures of the flora composing the peat are partially erased (Fig. 6A). However, some parts of the peat material still contain recognizable organelles such as the primary and secondary walls of vegetative cells, medium lamella, plasma membrane, inner cells residues and plasmodesmata, as illustrated in Fig. 6B.1, C.1 and D.1 and schematically outlined in Fig. 6B.2, C.2 and D.2. Cell walls seem to be well preserved figuring the double-wall configuration and sometimes even the plasma membrane (Fig. 6B, C and D), which appears as light matter under TEM. The cells of microorganisms were essentially observed near the inner part of the plant cells (Fig. 6B, D and C).

The TEM observations of peat incubated during 29 days showed that the inner part of the cells at 4 and 25 °C were substantially degraded compared with those after a 45 °C incubation (Fig. S1). Such change between different temperatures was detected for both added

Table 2

Ecoplate (AWCD, H and E), Anions and Organic acids analysis contents in solid frozen peat (SFP) incubations (control and with added heterotrophic bacteria) at the beginning and at the end of the experiments (day 0 and 29) and evolution rate of anions and organic acids concentration between day 0 and day 29.

Control		Incubation time (days)		0			29				
		Temperature (°C)		4	25	45	4	25	45		
Ecoplate	AWCD			1.044	1.044	1.044	0.061	0.085	0.093		
	Shannon richness index (H)			268.5 ± 35.2	268.5 ± 35.2	268.5 ± 35.2	14.4 ± 3	19.6 ± 7.1	18.9 ± 4.1		
	Shannon evenness index (E)			20.7 ± 1.3	20.7 ± 1.3	20.7 ± 1.3	14.3 ± 1.6	11.1 ± 2	9 ± 3.4		
Anions and Organic acids	Concentration (mg L ⁻¹)	Phosphate (PO ₄ ³⁻)		0.095	0.016	0.11	0.032	0.009	0.029		
		Acetate		0.52	0.014	0.027	0.092	0.055	0.09		
		Formate		0.035	0.034	0.008	0.048	0.009	0.018		
		Malate		0.035	0.011	0.015	0.028	0.006	0.009		
		Tartrate		0.018	0.017	0.018	0.072	0.017	0.019		
		Oxalate		0.045	0.015	0.016	0.096	0.017	0.035		
	Rate between day 0 and day 29 (mg L ⁻¹ d ⁻¹)	Temperature (°C)			4		25		45		
			Phosphate (PO ₄ ³⁻)			-1.27 × 10 ⁻⁰³		-1.32 × 10 ⁻⁰⁴		-7.04 × 10 ⁻⁰⁴	
			Acetate			-4.89 × 10 ⁻⁰³		-1.29 × 10 ⁻⁰²		8.93 × 10 ⁻⁰⁴	
			Formate			6.28 × 10 ⁻⁰⁴		-4.55 × 10 ⁻⁰⁴		2.43 × 10 ⁻⁰⁴	
Expand	Added heterotrophic bacteria	Anions and Organic acids	Concentration (mg L ⁻¹)	Phosphate (PO ₄ ³⁻)		0.061	0.066	0.11	0.334	0.643	1.302
				Acetate		0.017	0.012	0.027	0.05	0.082	0.099
				Formate		0.057	0.007	0.008	0.011	0.01	0.013
				Malate		0.012	0.008	0.015	0.008	0.006	0.009
Rate between day 0 and day 29 (mg L ⁻¹ d ⁻¹)	Temperature (°C)			4		25		45			
		Phosphate (PO ₄ ³⁻)			8.65 × 10 ⁻⁰³		2.24 × 10 ⁻⁰²		2.38 × 10 ⁻⁰²		
		Acetate			2.39 × 10 ⁻⁰⁴		3.19 × 10 ⁻⁰³		1.42 × 10 ⁻⁰³		
		Oxalate			0.018	0.017	0.018	0.017	0.017	0.021	

Table 2 (Continued)

Added heterotrophic bacteria							
	Incubation time (days)			Temperature (°C)			
	0	25	29	4	25	45	
Formate	-7.70×10^{-04}		9.37×10^{-04}			4.01×10^{-04}	
Malate	-5.47×10^{-05}		1.90×10^{-04}			-5.49×10^{-05}	
Tartrate	-3.81×10^{-06}		9.21×10^{-04}			1.25×10^{-04}	
Oxalate	8.99×10^{-05}		7.13×10^{-04}			1.22×10^{-02}	

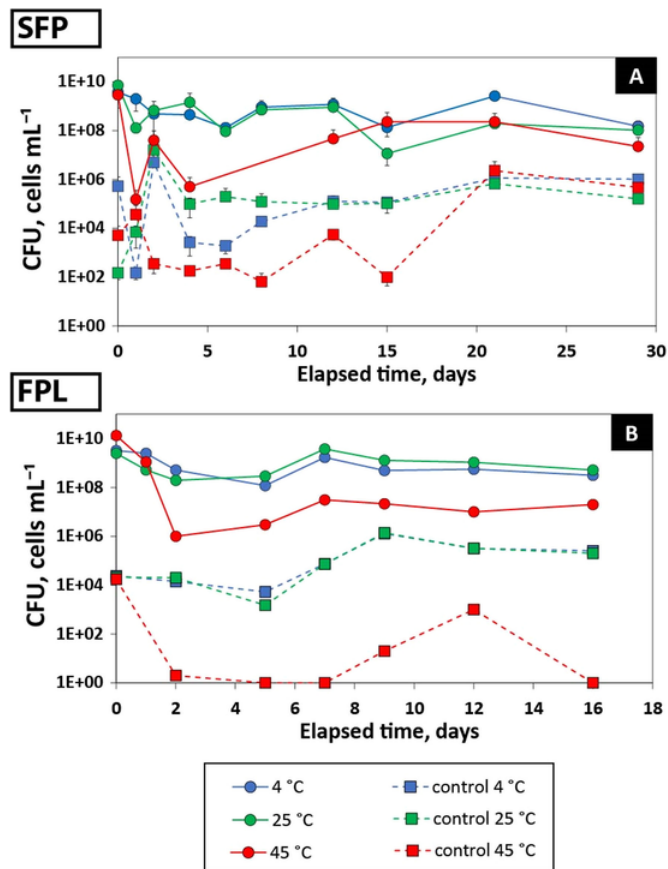


Fig. 4. Temporal evolution of CFU (log scale) during incubation of solid frozen peat (SFP; A) and frozen peat leachate (FPL; B). These figures show the evolutionary trend of control incubations (dotted lines) and added heterotrophic bacteria incubations (solid lines).

Iodobacter sp. experiments and their respective controls. The secondary walls seem to have been partially biodegraded, as it is demonstrated by lysis features of the walls in experiments with added *Iodobacter* sp. at 4 and 25 °C (Fig. 6D). It is worth noting that the number of living bacterial cells (CFU, cells mL⁻¹) was lower in experiments at 45 °C compared with those carried out at 4 and 25 °C (Fig. 4A). Unfortunately, comparisons between incubations at different temperatures (*i.e.*, assessment of the degree of the inner cell degradation and of the cell abundance) could not be statistically confirmed, first, because of an insufficient number of observations, and then, because spots of observation were too small to provide an appropriate representation of the intrinsic heterogeneity of the frozen peat.

4. Discussion

4.1. DOC trend during peat leaching and biodegradation

We hypothesised that the change of DOM through time in SFP incubation will be driven by (1) its release in solution from the peat and (2) its consumption by the microbial community. The release and the consumption of dissolved organic matter can be followed by the DOC trend in SFP incubations (Fig. 2A, B and C). In contrast, in FPL incubation (filtered substrates), we hypothesised that in the absence of particulate OM (POM), only biodegradation occurs and no DOM could be released (at the exception of lysis of microbial cells). In FPL experiment, the consumption of DOC (Fig. 2D, E and F) can be followed by the BDOC trend (Fig. 2G, H and I).

4.1.1. DOC release from frozen peat at different temperatures

The impact of temperature on the permafrost soil OM degradation has been intensively investigated, both in field soil heating experiments (Eliasson et al., 2005; Hartley et al., 2007; Melillo et al., 2002) and in laboratory incubations (Bracho et al., 2016; McKenzie et al., 1998). However, no strict consensus has been reached about the temperature control on soil OM degradation and significant controversies persist as discussed below.

In this work, no significant difference could be detected between 4 and 25 °C experiments in DOC concentration over time (Fig. 2A and B, P_{value} : 0.199) and the apparent release rate of DOC (R_{DOC}) (Table 1, P_{value} : 0.302). We then conclude, that under experimental conditions of this work, the temperature rise from 4 to 25 °C was not a key factor controlling OM release from peat of North-Eastern European Tundra. This is in agreement with results of Alaskan soil incubation in water (139 g soil + 500 mL water) by Reyes and Lougheed (2015), who demonstrated that the release of DOC and macronutrients (such as P, NO₃⁻) were not affected by temperature changes from 7 to 17 °C for the active soil layer but were affected in the case of permafrost soils. The same observations were made using anaerobic incubations, by Heslop et al. (2019a) who demonstrated that the permafrost thaw affects the methanogen community composition and the temperature sensitivity of CH₄ production thus highlighting the higher temperature sensitivity of recently-thawed permafrost and seasonally-thawed active layer. Our observations also corroborate those of Ernakovich and Wallenstein (2015) who conducted experiments at 1, 10 and 20 °C and observed that the amount of OM used by microbes at these 3 temperatures were the same. Moreover, some studies even noticed an increase in the decomposition rate of OM at lower temperatures (Del Grosso et al., 2005; Kirschbaum, 1995; Lloyd and Taylor, 1994). At the same time, a number of studies on permafrost peat biodegradation demonstrated an increase of OM decomposition with increasing temperature (Davidson and Janssens, 2006; Dioumaeva et al., 2002; Eliasson et al., 2005; Friedlingstein et al., 2006; Hartley et al., 2007; Melillo et al., 2002; O'Donnell et al., 2016). For ex-

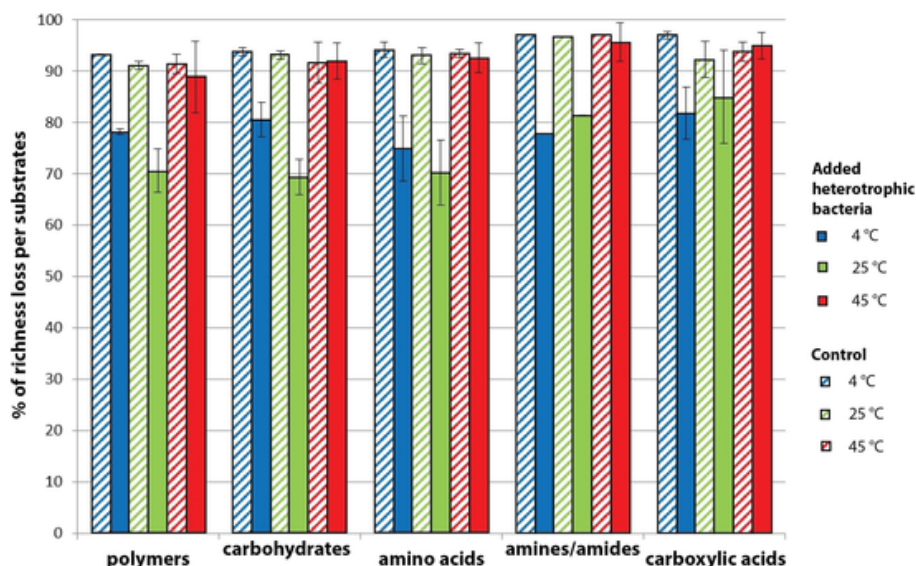


Fig. 5. Percentage of richness (H) loss in solid frozen peat (SFP) experiment between day 0 and day 29 of incubation for each type of consumed substrates (hatched columns = controls, fill columns = with added heterotrophic bacteria, blue columns at 4 °C, green at 25 °C and red at 45 °C incubations). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

ample, a 30 days incubation of moss and peat have shown that the increase of temperature from 5 to 20 °C increased the C release (DOC production) by 20 to 135% (O'Donnell et al., 2016). A similar pattern was observed in our incubations (*i.e.*, a 3-fold increase in DOC concentration (Fig. 1C) and R_{DOC} values (Table 1) at 45 °C compared to those at 4 and 25 °C). However, rare incubations studies were conducted at such high temperatures (Shirokova et al., 2017), limiting a possible comparison between the data. Note that the decomposition of recalcitrant soil organic matter exhibits a higher temperature sensitivity (Conant et al., 2008), which could explain the difference between the observed release rate of DOC at 4 and 25 °C compared with those at 45 °C. It is thus possible that the frozen peat used in our study releases only labile OC at 4 and 25 °C and that the excess of DOC observed at 45 °C corresponds to the recalcitrant OM of peat. It should be further noted that in our study, the substrate-normalized DOC yields of frozen peat ($\sim 5 \text{ mg g}^{-1}$ peat) were substantially higher than the values obtained for organic soil samples of Alaska ($0.14\text{--}2.2 \text{ mg g}^{-1}$ of soil, Gao et al., 2018).

4.1.2. DOC removal during biodegradation of peat leachate

A former study of frozen peat leachate with the addition of *Iodobacter* sp. revealed no significant decrease of DOC at 4 °C but a slight decrease at 25 °C (Shirokova et al., 2017). Oleinikova et al. (2018) reported a decrease in DOC concentration by $5 \pm 3\%$ over the first 96 h of incubation at 25 °C in a peat leachate. In contrast, the BDOC obtained in this study ranged between 15 and 20% after 2 days of incubation at 4 and 25 °C. These values are somewhat higher than the field incubation values of BDOC along the hydrological continuum of frozen peatlands (0 to 10%, Shirokova et al., 2019) but in agreement with those obtained for Arctic riverine BDOC (10 to 40%, Holmes et al., 2008; Mann et al., 2012; Wickland et al., 2012). They are also consistent with the 10–40% range obtained for BDOC from the thawing and collapsing permafrost experiment of Abbott et al. (2014), as well as with values of the microbial utilisation of DOC in streams (3–11%) and in vegetation leachates (66%) of a 28 days incubations conducted at 20 °C (Textor et al., 2019). In the case of incubation at 45 °C with added heterotrophic bacteria, we obtained negative BDOC values (Fig. 2I) which was not expected considering that the biodegradation was run in previously filtered solutions containing no particulate organic carbon. We hypothesize that some lysis of bacterial cells at these condi-

tions may be responsible for additional DOM production and apparently 'negative' BDOC values.

In our study, the biodegradation was followed for 16 days and showed stabilization of both DOC concentration (Fig. 2) and DOC removal (Table 1) between days 5 and 12 for the 4 and 25 °C experiments. This relatively short period appeared to be sufficient for the heterotrophic bacteria to remove the labile C by respiration (Moran and Hodson, 1990; Søndergaard and Middelboe, 1995). Consequently, the remaining C in FPL incubations (approximately 12 mg L^{-1}) represents the refractory C pool of the DOC. Although we hypothesised no release of DOC in FPL experiments, some studies have shown a bacterial contribution to DOC concentration in waters (30–50% in $0.7 \mu\text{m}$ filtered freshwaters incubated at 20 °C) and have highlighted the resistance of this bacterially-derived organic matter to the microbial degradation (Kawasaki et al., 2013). To a lesser extent, because of a smaller filtration pore size used in our study (*i.e.* $0.45 \mu\text{m}$), the 12 mg L^{-1} of C remaining can also be partially composed of this bacterially-derived organic matter. The R_{DOC} measured in this study in FPL experiments at 4 °C during the first two days of incubations ($-1.18 \pm 0.16 \text{ mg L}^{-1} \text{ d}^{-1}$; Table 1) is comparable with the values assessed in subarctic permafrost mire complex for the same incubation time (2 days) at 3 °C for the active layer where the consumption of DOC is about $1.75 \pm 0.79 \text{ mg L}^{-1} \text{ d}^{-1}$ (Roehm et al., 2009). Furthermore, we did not detect any change in the apparent rate of DOC removal between 4 and 25 °C over this short incubation period which corroborates the former finding on very weak impact of temperature on labile C pool (Biasi et al., 2005; Bracho et al., 2016; Conant et al., 2008; Hartley and Ineson, 2008).

4.2. Heterotrophic bacteria functional diversity, microbial cell number and organic acids in the presence of frozen peat and frozen peat leachate

Consistent with the negligible impact of temperature on both DOC release from peat and DOC removal in peat leachate, the number of viable bacteria was similar at 4 and 25 °C ($(10 \pm 1) \times 10^8 \text{ CFU mL}^{-1}$). This refutes the possibility of variance in the biodegradation rate as a function of temperature due to a difference in microbial cell numbers. In addition, the non-significant variations of richness (H), evenness (E) and AWCD indices between 4 and 25 °C incubations with SFP suggest a comparable metabolic activity of the microbial community at low and

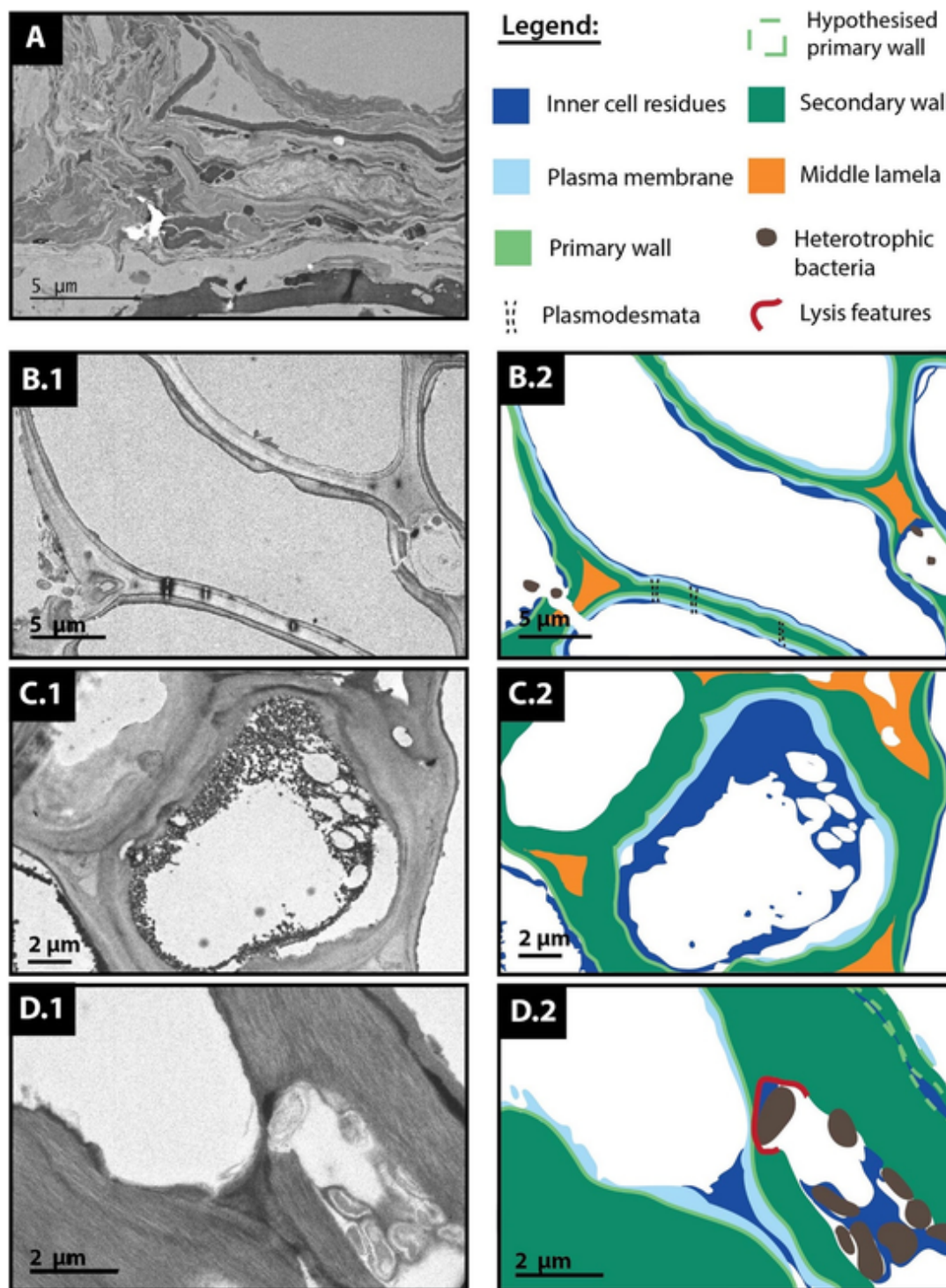


Fig. 6. TEM images of solid frozen peat (SFP) before incubation (A) and TEM images (B.1, C.1 and D.1) of SFP after 29 days of incubation at 25 °C (left panels). The right panels (B.2, C.2 and D.2) represent schematic interpretations of TEM images.

medium temperature. It indicates a quite high degradation rate of frozen peat from freshly thawed permafrost at 4 °C. This is contradictory to the hypothesis that the active microbial community at low temperature needs a few growing seasons to reach the degradation level of a warmer community after the first thaw of permafrost (Ernakovich and Wallenstein, 2015). Furthermore, the lower values of AWCD, H and E indexes at 45 °C incubation compared with those at 4 and 25 °C are in agreement with observations made by Steinweg et al. (2008)

showing that the microbial carbon use efficiency declined at the warmest temperatures.

The exact reasons of this unusual temperature impact on microbial processing of DOC are unknown. Although the functional diversity of the microbial community was not affected by temperature, it is still possible that the community structure changed with temperature. Indeed, as it was shown for plant communities of peatlands, the taxonomic adaptation to climate change is not coupled with the functional diversity of community (Robroek et al., 2017). Further, Vigneron et

al. (2019) have shown a drastic difference in the microbial community of thermokarst lake between summer and winter driven by the change in redox environment. It follows that further metagenomic analyses of the microbial community structure are necessary to elucidate the mechanisms driving the temperature impact on biodegradation of DOM.

Ecoplate analyses indicate that the AWCD as well as the Shannon index of richness and evenness (H and E) decrease over incubation time (except for E with added *Iodobacter* sp. experiment), reflecting a decrease of heterotrophic metabolic diversity, i.e., consumption of various nutritive resources by microorganisms. Soil DOM released from aged permafrost is known to contain a high proportion of biologically-labile protein-like substances (Gao et al., 2018). At the end of our experiment, carbohydrates and polymers were essentially consumed by microbial community (approximately 25 to 30%, respectively, of the total C used), which in the case of carbohydrates is slightly higher than the rate obtain by Boddy et al. (2008) on the respiration of glucose and amino acids (<25% of the total respiration). This difference can be attributed to a larger microbial diversity of the frozen peat substrate used in the present study. Our peat sample originated just below the active layer, adjacent to the high bacterial metabolism zone (Morgalev et al., 2017), whereas the one used by Boddy et al. (2008) was a topsoil sample (10 cm depth). This result suggests that the downward migration of the thawing front will intensify and diversify, on a short-term scale, the microbial activity of frozen peatlands. The higher metabolic capacity of this interface thus reflects a higher C mineralization potential by bacteria.

The frozen peat from the Bolshezemelskaya Tundra (BZT) released a relatively low amount of carboxylic acids and the DOC was generally refractory. This contrasted with the behaviour of ancient organic carbon from the Yedoma soils, in which about 53% of DOC was consumed over 200 h of incubation, with 87% of microbially consumed DOC being derived from acetate and butyrate respiration (Drake et al., 2015). Low concentrations of acetate (<2 μ M) were reached in aqueous fluids that interacted with peat in the SFP experiments of the present study. These values are much lower than those reported in Yedoma permafrost cores from central Alaska, where acetate C accounted for 24% of DOC and ranged from 0.38 to 30 mM (Ewing et al., 2015). This difference can be explained by the enrichment in acetate of the Yedoma samples under anoxic conditions (acetogenesis) in contrast to already partially processed peat used in this study (that still contained some labile C but very low acetate concentration) and that was incubated under oxygenated conditions.

4.3. Microscopic morphological features of reacted peat

4.3.1. General structure of peat

Until now, the TEM observations of frozen peat biodegradation have remained very limited and the majority of microscopic studies have been devoted to the characterisation of moss degradation (Tsuneda et al., 2001). Former scanning electron microscopy (SEM) observations reported similar well-defined features (plant cell walls and membranes) for the south Australian peat (Dart et al., 1969), although we observed a slightly more compact aspect of the frozen peat from Bolshezemelskaya Tundra (Fig. 6A). Such a compact structure of frozen peat from BZT is consistent with a general trend of decreasing the peat porosity and pore size with increasing depth (Price and Schlotzhauer, 1999; Rezanezhad et al., 2016).

The cell wall like fragments, identified in a previous study (Dart et al., 1969) can be compared to primary and secondary cell walls evidenced in this work (Fig. 6B, C and D). The inner cell residues which appear dark under TEM because of strong heavy metal staining are identified as the inner part cells residues (Fig. 6C, B and D). Most of those inner cell residues seem to have undergone intense degradation

that might be linked to the activity of heterotrophic bacteria as described below.

4.3.2. Peat degradation features revealed by TEM

The peat used in this study was composed of plant cells components such as mosses (*Sphagnum*), lichens, dwarf shrubs and wood (Manasy-pov et al., 2017; Shirokova et al., 2017). The TEM can be a useful tool in the investigation of peat biodegradation as it was used to monitor the degradation of wood (Daniel, 1994; Daniel et al., 1987) and peat-forming sphagnum and other mosses structures (Kettridge and Binley, 2011; Rezanezhad et al., 2016).

The poorly decomposed peat, like the one used in this study, is mostly represented by refractory compounds such as *Sphagnum* cell walls made of essentially cellulose but also of some non-cellulosic polysaccharides, including pectic polysaccharides as well as wood debris represented by lignitic components of cell walls of vascular plants (Kremer et al., 2004; Steinen, 1982; Theander et al., 1954). The hyaline cells of *Sphagnum*, whose major cell wall constituents are cellulose, phenolic compounds, waxes, and polymerized lipids are very decay-resistant (Aerts et al., 1999; Tsuneda et al., 2001; van Breemen, 1995). They prevent the peat from microbial degradation as they form a barrier for microorganisms and their enzymes to gain access to cellulose (Dobrovol'skaya et al., 2014; Tsuneda et al., 2001). For these reasons, only minimal alterations of peat structure and vegetative cells upon *Iodobacter* sp. interaction with frozen peat were detected in this study.

Osmium tetroxide used for the preparation of TEM sample is a heavy metal stain that provides contrasts in TEM images and reacts/cross-links with proteins and lipids, particularly phospholipids containing double bonds. In our study, the darker area under TEM appeared to be the inner part of cells, as it is strongly linked to the added metal stain (Hayat, 2012). We can also see that, over the incubation time, the concentration of dissolved phosphate increased by a factor of 5, 10 and 12, respectively at 4, 25 and 45 °C in all SFP experiments with added *Iodobacter* sp. (Table. 2). This can be explained by the degradation of these phosphate-rich areas that lead to the release of phosphate ions in the solution. Moreover, the lysis features of secondary walls observed at 4 and 25 °C may reflect the efficient degradation of complex components such as cellulose by Gram-negative bacteria (Blanchette et al., 1990; Daniel, 1994; Eriksson et al., 2012), occurring in the environment which is deficient in labile C compounds. Overall, the direct visualization of peat interaction with aqueous solutions and microbial cells complements the results of water chemistry monitoring thus providing new insights into the mechanisms of interaction between bacteria and organic matter.

5. Conclusion

The laboratory incubations performed in this study were aimed at quantifying several controlled parameters (primarily the temperature and one heterotrophic bacterium) which certainly cannot represent the wide heterogeneity of natural aquatic environments. In particular, this study does not take into account vertical and lateral water flow in soils, fresh input of OM, the use of POM by the microbial community, the diel change of temperature and respiration/photosynthesis cycle. However, the incubation of frozen peat and its aqueous leachate with and without culturable heterotrophic bacteria in a wide range of temperatures provided novel observations that might be necessary to consider in biogeochemical modelling of C cycle during permafrost thaw and surface water warming in permafrost zones of frozen peatlands.

- (i) Peat lixiviation and peat leachate aerobic biodegradation under early spring and late autumn temperatures (surface water and soil fluids temperatures are 0 to 4 °C) release and consume amounts of organic carbon (OC) similar to those measured at 25 °C, i.e. a typi-

cal summertime temperature in surface waters of frozen peatlands. The unexpectedly high release of bio-available carbon from peat into lakes and fluvial systems, at the beginning and at the end of the active season (unfrozen), requires the consideration of the full active period for the estimate of C release into the environment. An important conclusion is that the major input of newly available OM from frozen peat to the aquatic ecosystems is likely to occur at the beginning of thermokarst lake formation in peat soil depressions, and this does not require particularly high summertime temperatures.

- (ii) The temporal change of functional diversity over the 4 weeks of incubation suggests that the downward migration of the thawing front (the interface between the active layer and the frozen peat) will intensify and diversify the microbial activity of frozen peatlands on a short-term scale. Overall, the high metabolic diversity observed in the native microbial community (originated from the frozen peat) incubations reflects a higher potential of C mineralization by the natural consortium, as compared to the added culturable heterotrophic bacteria.
- (iii) The morphological microscopic features of the association between bacteria and the peat particles were examined using TEM. Although the low number of observations and the high heterogeneity of the frozen peat used for the incubations do not allow any quantitative analysis, our study points out the preferential degradation of the inner plant cell by heterotrophic bacteria and the degradation of phospholipids materials leading to a release of phosphate.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.chemgeo.2019.119448>.

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