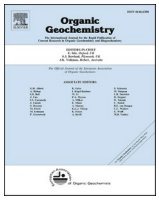
Organic Geochemistry 208 (2025) 105026

Contents lists available at ScienceDirect

Organic Geochemistry

journal homepage: www.elsevier.com/locate/orggeochem



Occurrence of nucleoside-bacteriohopanepolyol in high latitude soils: evidence of environmental controls on bacterial lipid membrane distributions

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ARTICLE INFO

Associate Editor – Rienk Smittenberg

Keywords:
Alaska
Soil
Bacteriohopanepolyols
Nucleoside BHP
Soil temperature proxy

ABSTRACT

Recently, the analysis of non-derivatised bacteriohopanepolyols (BHPs) by ultra-high performance liquid chromatography-high resolution mass spectrometry (UHPLC-HRMS^2) revealed a broad structural diversity in this lipid class. Multiple unique BHPs with nucleoside-type polar head groups (Nu-BHPs) were identified in soils. Nu-BHPs had previously been identified in high abundances in soil organic matter, but only by analysing acetylated BHPs, which hindered their structural elucidation. In this study, we apply the UHPLC-HRMS^2 analysis method for the first time to a soil transect to re-examine the distribution of Nu-BHPs, their environmental dependencies, and their proxy potential. The presence and distribution of Nu-BHPs was examined in 17 surface soils along a ~800 km transect in northern Alaska. Our results indicate that certain Nu-BHPs show significant correlation with environmental parameters, such as temperature and soil pH. The variation in 9 Nu-BHPs is captured using a novel ratio, and a regional calibration for warmest quarter soil temperature (WQST) was developed using a linear regression approach ($R^2 = 0.72$). Other calibrations developed for summer air and mean annual temperatures also show strong positive correlations. As BHPs are ubiquitous in soils globally, this study highlights the potential benefit of complementing established organic proxies for soil pH and temperature (e.g., branched tetraether lipids) with calibrations based on Nu-BHPs. Nevertheless, the mechanism behind the environmental dependencies of these BHPs remains unknown. Further work to explore the proxy potential as well as the bacterial sources of these lipids should be undertaken, for instance by sampling soils along relevant (soil pH and temperature) gradients.

1. Introduction

Bacteriohopanepolyols (BHPs) are pentacyclic triterpenoid lipids found in the membranes of bacteria belonging to several different phyla (Kusch and Rush, 2022). They are the precursors of hopanoids, a class of compounds found ubiquitously in the geological record (Ourisson and Albrecht, 1992). BHPs are found in many modern ecosystems, including soils (Cooke et al., 2008a; Xu et al., 2009; Spencer-Jones et al., 2015) and low temperature environments (Rethemeyer et al., 2010; Höfle et al., 2015; Saleem et al., 2019). BHPs contain pentacyclic triterpenoid ring systems with extended side chains that can be made up of highly functionalized groups such as amines, hydroxyls, amino sugars, and nucleosides (Rohmer et al., 1984; Talbot et al., 2007a, b; Hopmans et al., 2021). Specific BHPs have been used as biomarker lipids for biogeochemical processes (e.g., anammox; Rush et al., 2014) and bacterial assemblages (e.g., methanotrophs; Cvejic et al., 2000).

Recently, analytical advances in the direct analysis of non-derivatised BHPs using ultra high pressure liquid chromatography (UHPLC) coupled to an electrospray ionization (ESI)-high resolution dual-stage mass spectrometer (HRMS^2) have further revealed the breadth of structural diversity in these lipids (Hopmans et al., 2021). In particular, an extensive set of BHPs with nucleoside-type polar head

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https://doi.org/10.1016/j.orggeochem.2025.105026
Received 14 January 2025; Received in revised form 10 April 2025; Accepted 22 May 2025
Available online 27 May 2025

groups (Nu-BHPs, previously known as adenosylhopanes) were tentatively identified in soils (Hopmans et al., 2021) and lakes (Richter et al., 2023). The side chain structures of these Nu-BHP contain a nucleobase of either adenosyl or inosyl, and they have been shown to contain up to 4 additional methyl groups in the functionalised head group (HG) and/or at the C-2 and C-3 position on the core ring structure (Fig. 1). Using the improved methodology, so far 25 unique Nu-BHPs have been identified in soil and lacustrine environments (Richter et al., 2023, Hopmans et al., 2021), whereas only 6 "adenosylhopanes" were identified through the HPLC analysis of derivatised (acetylated) BHPs. This offset is likely due to a combination of improved chromatographic resolution and accurate mass diagnostics. The original "adenosylhopanes" were proposed as biomarkers of soil organic carbon (Cooke et al., 2008b; Cooke et al., 2009; Zhu et al., 2011; Doğrul Selver et al., 2012; Doğrul Selver et al., 2015), and proxies were developed to trace soil organic matter transport into the marine environment (i.e., R_{soil}, R'_{soil}; Doğrul Selver et al., 2012; Zhu et al., 2011). Given the methodological advances, it is imperative to re-evaluate the occurrence and distribution of Nu-BHPs in soils in order to better understand their applicability as lipid biomarkers.

Despite the ubiquitous presence of Nu-BHPs in global soil and lake environments, the diversity of their producers is poorly constrained, as only three Nu-BHPs have been reported in very few cultivated bacteria (i.e., adenosylhopane and 2Me-adenosylhopane (Fig. 1) have been reported in *Bradyrhizobium japonicum*, *Rhodoblastus acidophilus*, *Rhodopseudomonas palustris*, *Rhodomicrobium vannielii*, *Nitrosomonas europaea*, *Hymenobacter roseus*, *Salinimicrobium sediminis*, and five nitrite-oxidising bacterial isolates belonging to the phyla *Nitrococcus*, *Nitrospira*, and *Nitrobacter*; Neunlist and Rohmer, 1985a; Seemann et al., 1999; Bravo et al., 2001; Talbot et al., 2007a; Subhash et al., 2014; Elling et al., 2022), and N1-methyl-inosylhopane was detected but not fully identified in *Rhodomicrobium vannielii* (Talbot et al., 2007a). The specific function of adenosylhopane and other Nu-BHPs is also unknown. The poor constraint of Nu-BHPs producers is especially striking as adenosylhopane is the universal intermediate molecule in the synthesis of all C_{35} BHPs (Bradley et al., 2010). Thus, all BHP-producing bacteria have the potential to synthesize adenosylhopane, yet of the bacteria that have been investigated for BHP content, very few seem to accumulate it in their cells. Furthermore, the *hpnH* gene encoding the enzyme responsible for the addition of the adenosyl group to the side chain is prevalently found in bacteria across many phyla, including soil bacteria such as *Acidobacteria* and *Burkholderia cepacia* complex (Schmerk et al., 2015; Sinninghe Damsté et al., 2017). Nevertheless, the bacteria responsible for producing the other Nu-BHPs found in the environment remain unidentified. While environmental profiling of bacterial communities in relevant environmental samples can be used to identify potential producers, the environmental drivers of Nu-BHP variation need to be constrained before relevant samples can be collected.

Research into the origin of the hopanoid ring methylations has identified the genes encoding for the methylations at the C-2 and C-3 positions (*hpnP* and *hpnR*, respectively) (Welander et al., 2010; Welander and Summons, 2012), and the occurrence of 2,3-methylated BHPs has been proposed to be the result of the sequential use of both of these genes (Sinninghe Damsté et al., 2017). C-2 methylated BHPs were originally associated with cyanobacteria (Summons et al., 1999) whereas C-3 methylations were typically associated with methanotrophs (Neunlist and Rohmer, 1985b). However, the exclusivity of both of these methylation positions to specific bacterial groups has since been contested: C-2 methylations are widespread in Acidobacteria and Alphaproteobacteria (Welander et al., 2010; Ricci et al., 2014; Ricci et al., 2015), and C-3 methylation has been shown to play a role in stress regulation across many bacteria phyla (Welander and Summons, 2012; Mayer et al., 2021). It remains unclear what biosynthetic mechanisms or phylogeny are responsible for the methylations observed on the nucleoside structures. Metagenomic profiling of relevant environmental samples can aid in answering these questions, but relies on our understanding of the environmental variability of methylated Nu-BHPs in soils.

Although the producers and biological mechanisms of Nu-BHP production are poorly constrained, their distribution in soils and response to environmental parameters can be used to derive information on their

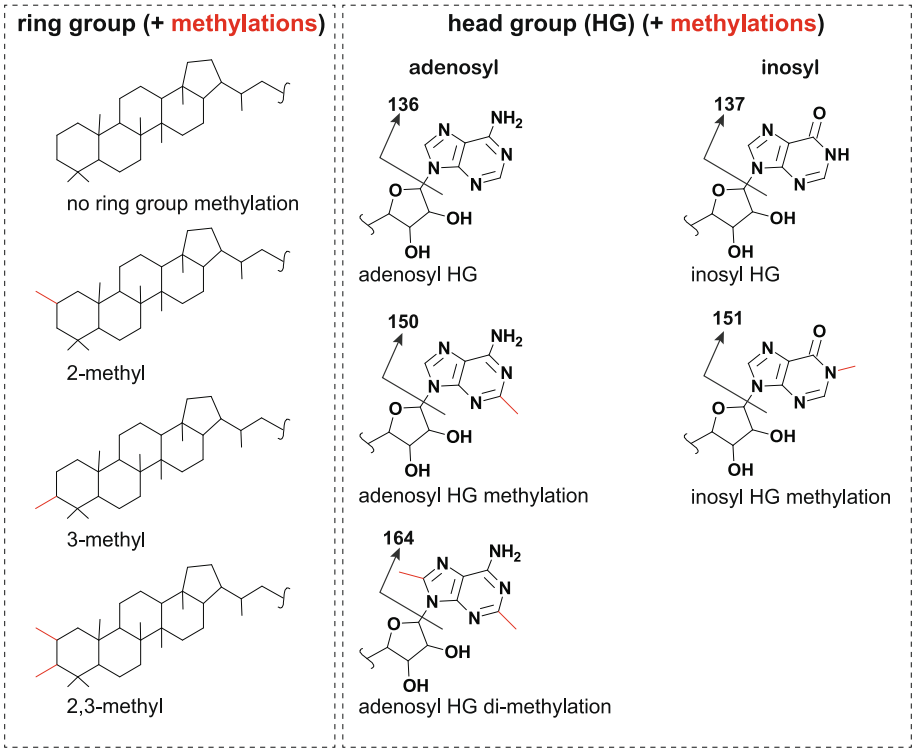

Fig. 1. Tentative chemical structures of nucleoside BHPs. Characteristic fragment ions indicative of the headgroup are shown, and proposed methylation positions are indicated in red. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

function. In this study, we assess the distribution of Nu-BHPs in a terrestrial transect to obtain information on the environmental parameters potentially controlling their occurrence in soils.

2. Materials and methods

2.1. Study area

Seventeen tundra and boreal surface soil samples were collected at thirteen sites along a north-south transect in Alaska, U.S.A. (Fig. 2). Soils were collected from Deadhorse (70.03°N, 148.65°W) to Fairbanks (64.86°N, 147.72°W) in late July to early August 2016 (O'Connor et al., 2020). All samples were sealed with polytetrafluoroethylene (PTFE) tape in glass jars that were previously ashed for four hours at 450 °C. Samples were stored in a −80 °C freezer prior to analysis. Climate variables (i.e., mean annual air temperature (MAAT), mean annual soil temperature (MAST), warmest quarter air temperature (WQAT), warmest quarter soil temperature (WQST), growth season temperature (GST)) at the sampling sites were retrieved from CHELSA V2.1 (Karger et al., 2017; Karger et al., 2018).

2.2. Bulk properties

Surface soils (upper 1 cm under the litter layer) were split and a subset was freeze-dried to determine percent moisture content by weight (Table 1). pH was determined using 1 g of freeze-dried soil, to which 30 mL of deionised water was added, shaken for 20 min, allowed to settle to the bottom, and then measured using a calibrated Hanna HI 99121 pH meter. The pH meter was checked using a pH standard (4.01) every two samples to ensure reproducibility of results with an average standard deviation less than 0.02 across all measurements.

Samples (~1 to 4.5 mg) were analyzed for total organic carbon (TOC) and total nitrogen (TN) using a Costech Elemental Analyzer (EA) coupled to a Delta V Advantage Isotope Ratio Mass Spectrometer (IRMS). TOC was determined following soil pre-treatment with 5 % HCl to remove carbonates. The combustion reactor was run at 1000 °C, with the column at 65 °C. Acetanilide (Costech) of known composition (C = 71.09 % and N = 10.36 %) was used to calculate TOC and TN, with average standard deviation of 0.3 % and 0.4 %, respectively. All samples were run in duplicate, with average values used for analyses.

2.3. Lipid extraction

All soils were freeze-dried and extracted following a modified Bligh Dyer extraction (Bligh and Dyer, 1959; Bale et al., 2021). Briefly, ca. 2 g of freeze-dried soil was ultrasonically extracted twice in methanol (MeOH):dichloromethane (DCM): phosphate buffer (2:1:0.8; *v:v:v*). The solvent was collected in a separate flask after each extraction, and DCM and phosphate buffer were added to obtain a new volume ratio of 1:1:0.9 (*v:v:v*), which results in a biphasic solution. The DCM layer was collected, and the aqueous layer was washed twice more with DCM. The residue was then extracted again following the same procedure in 2:1:0.8 (*v:v:v*) of MeOH:DCM:aqueous trichloroacetic acid solution. For phase separation, the phosphate buffer was used. The combined DCM layers (BDE) were dried under N_2 gas and stored at −20 °C until analysis. A known amount (0.04 ng/μL) of deuterated diacylglyceryltrimethylhomoserine (DGTS D-9; Avanti® Polar Lipids, USA) was added as internal standard to aliquots of the BDEs. BDEs were dissolved in MeOH:DCM (9:1; *v:v*) and filtered through a 0.45 μm regenerated cellulose filter (4 mm diameter; Grace Alltech, Deerfield, IL).

2.4. BHP analysis

Samples were analysed according to Hopmans et al. (2021) using an Agilent 1290 Infinity I UHPLC coupled to a Quadrupole-Orbitrap HRMS (Q Exactive, Thermo Fisher Scientific, Waltham, MA) equipped with an Ion Max source and heated electrospray ionization (HESI) probe (Thermo Fischer Scientific, Waltham, MA). Separation was achieved using an ACQUITY BEH C18 column (1.7 μm, 2.1 x 150 mm; Waters) fitted with a precolumn and maintained at 30 °C. Solvents used were solution A (MeOH:H_2O; 85:15) and solution B (MeOH:isopropanol; 1:1) and both contained 0.12 % (v/v) formic acid and 0.04 % (v/v) aqueous ammonia. The flow rate was 0.2 mL min^{-1}, and the elution program was: 5 % solution B for 3 min, followed by a linear gradient to 40 %

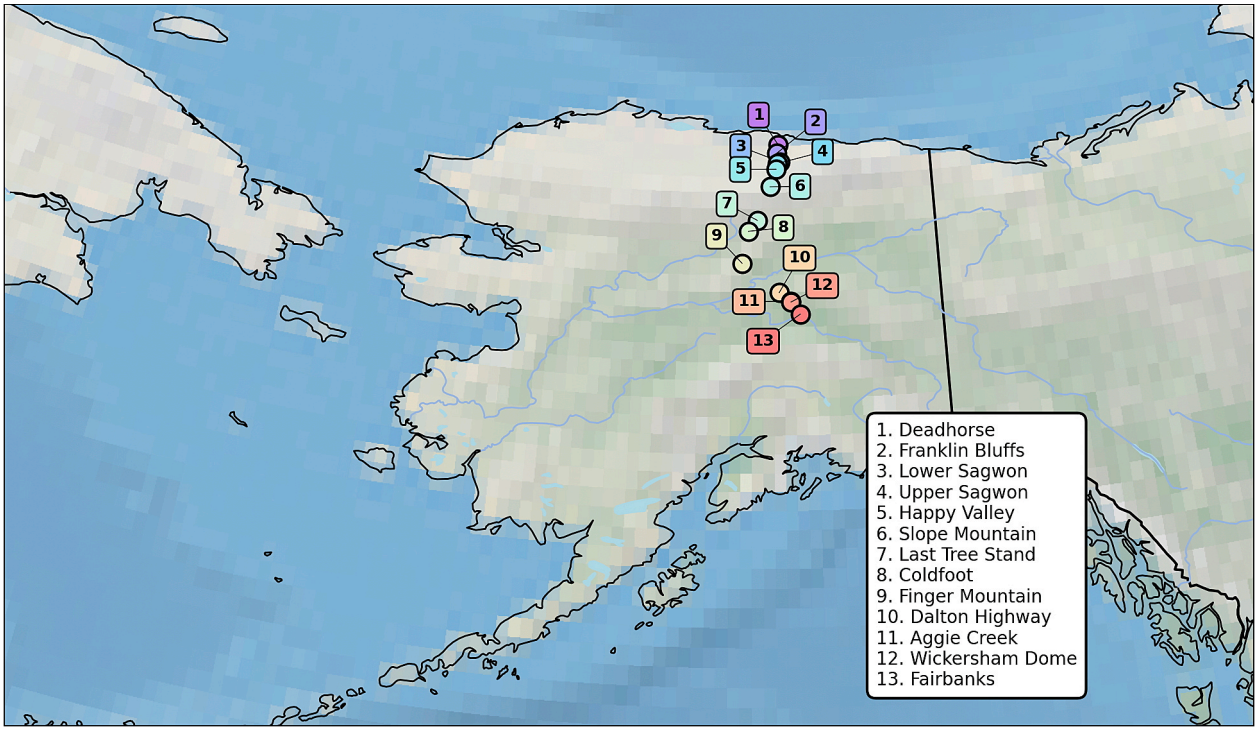

Fig. 2. Map of Alaska, (U.S.A.) showing the location of the surface soils used in this study.

Table 1
Diagnostic $[M+H]^+$ ions and diagnostic MS^2 fragment ions of the nucleoside head group used to report the nucleoside-BHPs in this study. Information in paratheses indicate the proposed location of extra methylations (core and/or head group [HG]).

Nu-BHP type	$[M+H]^+$ (*m/z*)	Diagnostic fragment ion (*m/z*)
adenosylhopane	662.500	136.062 (no Me)
adenosylhopane + 1 methylation	676.516	136.062 (Me core) 150.077 (HG-Me)
adenosylhopane + 2 methylation	690.532	136.062 (diMe core) 150.077 (Me core and HG-Me) 164.093 (HG-diMe)
adenosylhopane + 3 methylation	704.547	150.077 (diMe core and HG-Me) 164.093 (Me core and HG-diMe)
adenosylhopane + 4 methylation	718.563	164.093 (diMe core and HG-diMe)
N1-methylinosylhopane	677.500	151.061 (HG-Me)
N1-methylinosylhopane + 1 methylation	691.516	151.061 (Me core and HG-Me)
inosylhopane	663.484	137.046 (no Me)

solution B at 12 min, then 100 % solution B at 50 min, which was held for 30 min. The positive HESI settings were: sheath gas (N_2) pressure, 40 arbitrary units (AU); auxiliary gas (N_2) pressure, 10 AU; spray voltage, 4.5 kV; probe heater temperature, 50 °C; and the S-lens, 70 V. Lipid detection was achieved using positive ion monitoring of mass range *m/z* 350–2000 (resolving power 70,000 ppm at *m/z* 200). Data-dependent MS^2 (tandem mass spectrometry) with an isolation window of 1 *m/z* and a resolution of 17,500 ppm at *m/z* 200 was used for the 10 most abundant ions for a total cycle of ca. 1.2 s and dynamic exclusion (6 s) with a 3 ppm mass tolerance, with an inclusion list of calculated exact masses of BHPs (Richter et al., 2023). Mass chromatograms for Nu-BHPs were generated based on their protonated adducts (Table 1). As there is no commercially available standard for non-derivatized BHPs, this analysis is semi-quantitative, with potential injection loss and matrix effects determined using the betaine standard. Peak areas were corrected accordingly. We report Nu-BHPs as response units normalised to g organic carbon (RU/g OC). The fractional abundances of individual Nu-BHPs relative to the total suite of Nu-BHPs quantified were used further to assess correlations with environmental variables.

2.5. Statistical analyses

All statistical analyses were performed in R version 4.2.2 (R Core Team, 2018) using the vegan package (Oksanen et al., 2022), with CHELSA values accessed using the raster and rgdal packages (Bivand et al., 2023; Hijmans and Van Etten, 2023). Correlations of individual compounds to environmental and geographic variables were done using relative abundances. A principal component analysis (PCA) was performed on the scaled fractional abundances of all 24 BHP compounds. Determining the proposed Nu-BHP index was done after Kim et al. (2010) and Peterse et al. (2012), using the archived package prob and custom-made code. Here, a calibration equation was calculated with a BHP index of the form:

$$BHP\ \ index = \frac{\sum k_1 Y_j}{\sum k_2 Y_j}$$

The following compounds were not included in the ratio calculation because of low fractional abundance: peaks b, c, and m. Peak k was excluded from the ratio as the PCA illustrates that it shows little variability. In the index, Y_j thus refers to any of the 20 selected BHP components. The summation in the numerator and the denominator is over the sets k1 and k2, respectively, where a set can be any possible combination of BHPs. For 20 BHP components, 1,048,575 combinations are possible. The sets k1 and k2 were selected based on the largest r value between the ratio values and selected environmental parameters (calibration correlation). After obtaining the statistically best ratios, minor BHP compounds whose removal did not influence the correlation r, were removed from the ratio.

3. Results and discussion

Seventeen soils from 13 sites across a transect in Alaska were analysed for their Nu-BHP content (Table 2; Table S1). Within this high-latitude north-to-south transect, there are relatively large ranges in climate and soil chemistry variables such as temperature (mean annual air temperature: −10.7 to −1.2 °C; warmest quarter soil temperature: 5.6–13.2 °C), soil pH (5.5–8.3), soil moisture content (12.7–78.3 %), TOC (2.1–43.0 %), and $\delta^{13}C$ (−29.2 to −13.4 ‰). Soil moisture and temperature showed correlations with latitude.

Nu-BHPs were found in every soil, verifying previous reports of the ubiquitous presence of these BHPs in terrestrial organic matter (Cooke et al., 2008a; Spencer-Jones et al., 2015), even at high latitudes (Cooke et al., 2009; Rethemeyer et al., 2010; Doğrul Selver et al., 2012; Höfle et al., 2015; De Jonge et al., 2016; Saleem et al., 2019). A notable diversity of Nu-BHPs was observed using the improved analytical method employed, with 24 distinct Nu-BHPs detected in the soil transect (Fig. 3; Table S1), including adenosylhopanes with 0–4 methylations and inosylhopanes with 0–2 methylations. Three of these were novel Nu-BHPs: early-eluting adenosylhopanes (Fig. 3, peaks a–c; Supplemental Fig. 1). Our results confirm recent suggestions from studies of soils (Hopmans et al., 2021) and lakes (Richter et al., 2023) that the $HRMS^2$ method reveals a diversity in Nu-BHP structures that had previously been overlooked by the lower chromatographic and mass resolutions, as well as by the need for compound derivatization, of other analytical methods. Here, we examined the potential environmental controls on the distribution of Nu-BHPs in soils.

3.1. Distribution of nucleoside-BHPs in Alaskan soils

Although Nu-BHPs were detected in all Alaskan soils, there are notable differences between soils from the north and the south. Using Upper Sagwon 1 as representative of northern soil (Fig. 3A) and

Table 2
Overview of locations, total Nu-BHPs, soil characteristics and climate data for the 17 soils analysed in this study. Site information includes elevation (m), mean annual air temperature (°C), growth season temperature (summer, °C), soil temperature in the warmest quarter (°C), soil pH, soil moisture content, and the total content and isotopic compositions of carbon and nitrogen.

	Latitude (°N)	Longitude (°W)	Elevation (m)	Mean annual air temperature (°C)	Growth season temperature (°C)	Soil temperature warmest quarter (°C)	Soil pH	Soil moisture content (%)	TOC (%)	C:N	TON (%)	$\delta^{13}C$	$\delta^{15}N$
Deadhorse	70.034	148.647	41	−10.7	7.3	5.6	8.3	47.9	13.7	17.9	0.8	−23.3	1.7
Franklin Bluffs 1	69.768	148.708	92	−10.7	8.1	6.0	7.9	n.a.	5.6	40.7	0.1	−13.4	1.1
Franklin Bluffs 2	69.768	148.708	92	−10.7	8.1	6.0	7.9	25.4	5.6	40.7	0.1	−13.4	1.1
Franklin Bluffs 3	69.768	148.708	92	−10.7	8.1	6.0	7.9	14.8	5.6	40.7	0.1	−13.4	1.1
Lower Sagwon	69.479	148.559	179	−10.7	8.9	6.4	6.1	78.3	23.4	11	2.1	−29.2	4.1
Upper Sagwon 1	69.424	148.694	304	−10.6	8.7	5.9	6.5	12.7	3.1	10.6	0.4	−26.0	2.6
Upper Sagwon 2	69.424	148.694	304	−10.6	8.7	5.9	6.5	16.1	3.1	10.6	0.4	−26.0	2.6
Happy Valley 1	69.240	148.774	233	−10.5	9.1	6.6	7.4	n.a.	33.7	22.9	1.5	−27.3	0.3
Happy Valley 2	69.240	148.774	233	−10.5	9.1	6.6	7.4	34.7	33.7	22.9	1.5	−27.3	0.3
Slope Mountain	68.687	149.080	717	−10.1	8.2	5.2	5.6	35.5	7.2	14.8	0.5	−24.6	4
Last Tree Stand	67.650	149.723	778	−7.0	11.3	9.4	4.7	19.6	15.3	15.8	1	−27.0	−0.4
Coldfoot	67.317	150.155	347	−6.8	11.8	10.4	5.7	19.9	2.1	23.5	0.1	−24.5	2.1
Finger Mountain	66.359	150.460	642	−5.7	10.0	8.5	5.5	70.9	43.9	25.4	1.7	−26.6	1.6
Dalton Highway	65.498	148.687	343	−4.4	12.4	10.4	7.5	71.9	21.2	27.1	0.8	−25.3	2.4
Aggie Creek	65.238	148.137	357	−3.2	12.2	10.5	6.6	39.7	36.7	30.9	1.2	−25.9	1.6
Wickersham Dome	65.217	148.137	507	−3.5	11.3	9.7	6.5	65.8	13.3	43	0.3	−26.7	−1.2
Fairbanks	64.863	147.717	156	−1.2	13.5	13.2	5.5	71.2	33.8	21.7	1.6	−27.3	−0.1

n.a. not analysed.

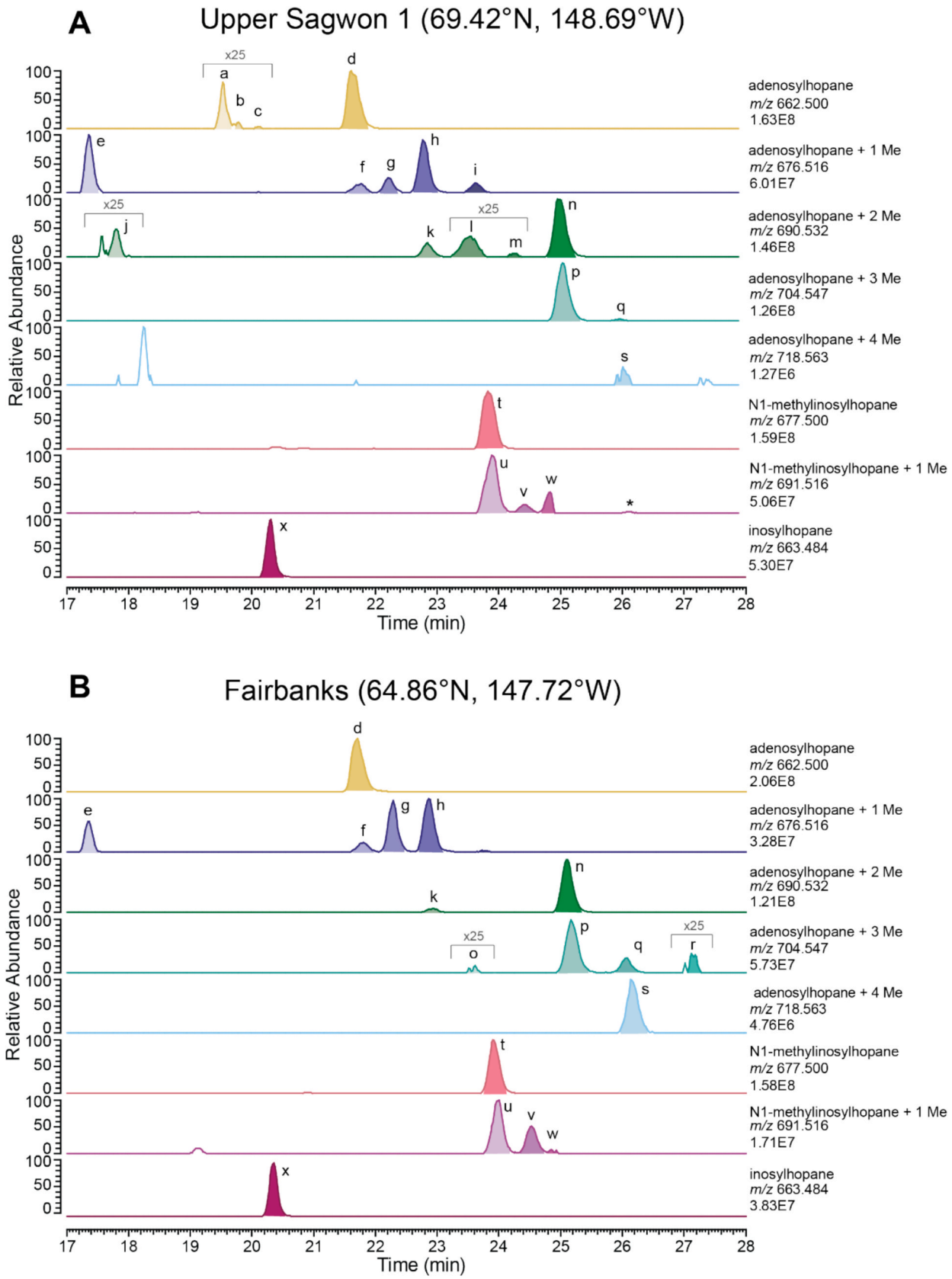

Fig. 3. Partial mass chromatograms of adenosylhopane, with 0–4 additional methylations, and inosylhopane with 0–2 additional methylations, in soils from A) Upper Sagwon (69.42°N, 148.69°W) and B) Fairbanks (64.86°N, 147.72°W). Each trace is labeled with the exact mass used to generate the chromatogram, and the intensity of the highest peak in arbitrary units (AU). Peak labeled with '*' is potential 3Me-N1-methyl-inosylhopane, with no MS^2 associated.

Fairbanks (Fig. 3B) as representative of southern soil, we compare their Nu-BHP distributions. In the northern soil, there are 4 isomers of adenosylhopane (peaks a – d) and 5 isomers of adenosylhopane + 2 Me (peaks j – n), whereas in the southern soil there is only one dominant compound of each (peaks d and n, respectively). Conversely, the number of isomers of adenosylhopane + 3 Me is higher in the southern soil. Moreover, some Nu-BHPs are observed to make up a significant proportion of total Nu-BHPs in all soils of this Alaskan transect (e.g., N1-methyl-inosylhopane; Fig. 4, peak t), whereas the relative abundances of others (e.g., early-eluting isomers of adenosylhopane, peaks a – c) are always low (Fig. 5). Additionally, the distribution of isomers of these Nu-BHPs varies between soils. For example, of the N-1-methylinosylhopane + 1 Me isomers, the abundance of peak v increases with decreasing latitude, whereas peak w decreases (Fig. 4). For the isomers of adenosylhopane + 1 Me, peak g increases with decreasing latitude.

Present in all Alaskan soils are (1) C-2 methylated Nu-BHPs (peaks f, k, p, and u), (2) C-3 methylated Nu-BHPs (peaks i and r), (3) Nu-BHPs that are methylated at an unknown position, possibly at C-31 (peaks g, q, v and w; Hopmans et al. 2021); and (4) di-methylated Nu-BHPs, with a methylation at the C-2 and C-3 positions (peaks l, m, o, and s) (Table S1). A surface sediment transect from Kalix River–Bothnian Bay, in northern Sweden, found C-2 methylated homologs of "soil-marker BHPs" (i.e., Nu-BHPs) discontinuously present (Doğrul Selver et al., 2012), and were subsequently removed from the R_{soil} index in favour of a new index (R'_{soil}) to be used in (sub)arctic regions. Their presence in this dataset indicates that C-2 methylation of Nu-BHPs is not absent in all cold regions. *Acidobacteria* have been shown to synthesize C-2 methylated Nu-BHPs (Sinninghe Damsté et al., 2017), however, there are no obvious indications that this bacterial phylum might be responsible for their presence in this soil transect (e.g., no correlation of C-2 Nu-BHP fractional abundances with soil pH; Fig. 6, Table S2). The presence of the other core methylated Nu-BHPs has not been reported previously in soils, however, they are present here in relatively high abundance (peaks g, q, and w) with some showing strong correlations between their fractional abundance and pH (peaks q, w) and temperature (peak g) (Fig. 6).

3.2. Climatic controls on Nu-BHP distributions

Based on these observations, it is clear that environmental factors across the north-south transect affect the distributions of several Nu-BHPs in this soil transect. Previously, changes in the fractional abundance of Nu-BHPs in soils have been attributed to soil physiochemical controls (Höfle et al., 2015). In this dataset, significant correlations are also observed between compounds f and p and C/N ratios, and between compounds l, q, and w and bulk $\delta^{13}C$ isotopic compositions (Fig. 6). Höfle et al. (2015) found a positive correlation between Nu-BHPs and both TOC and TN in Russian soils. However, in the Alaskan soils presented here we find no significant correlation between bulk TOC and Nu-BHP fractional abundances.

The fractional abundance of 9 Nu-BHPs varies significantly with soil pH and 8 with air and soil temperature (Fig. 6; Table S2). To investigate potential climatic controls on the microbial production of BHPs in surface soils, changes in fractional abundance with mean and (growth) seasonal temperatures (MAAT, MAST, WQAT, WQST and GST) are evaluated. A clear temperature dependency of a subset of BHPs is observed, with selected adenosylhopane and adenosylhopane + 1Me compounds showing a relative increase, and adenosylhopanes with higher number of methylations (+1 to +4 Me) generally decreasing (Fig. 6). As both soil chemistry and climatic parameters correlate with

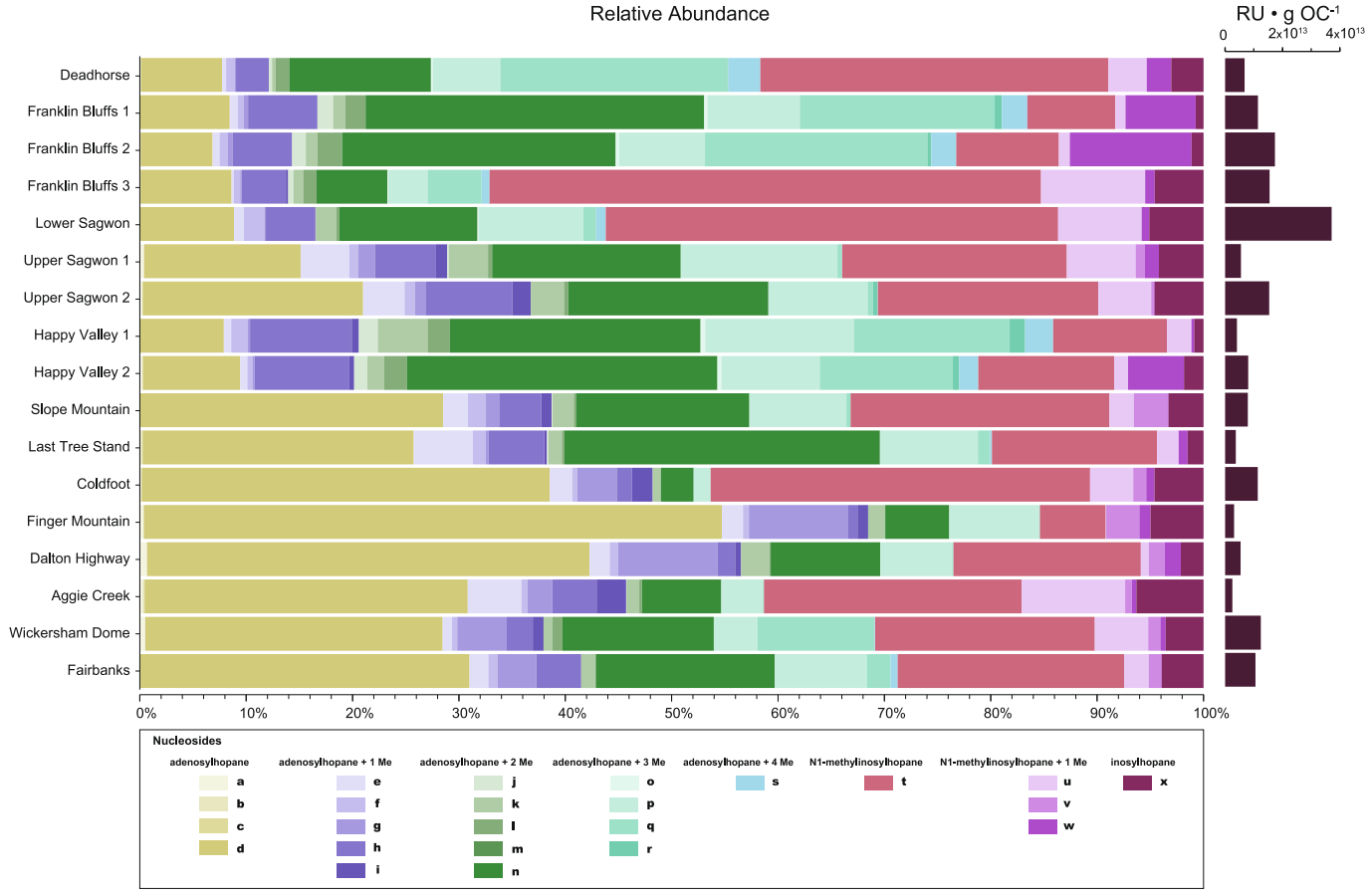

Fig. 4. Relative abundances of Nu-BHPs identified in the Alaskan soil transect and the response units (RUs) of all Nu-BHPs in the soils normalized to gram organic carbon (g OC). Note the replicate samples for Franklin Bluffs, Upper Sagwon, and Happy Valley.

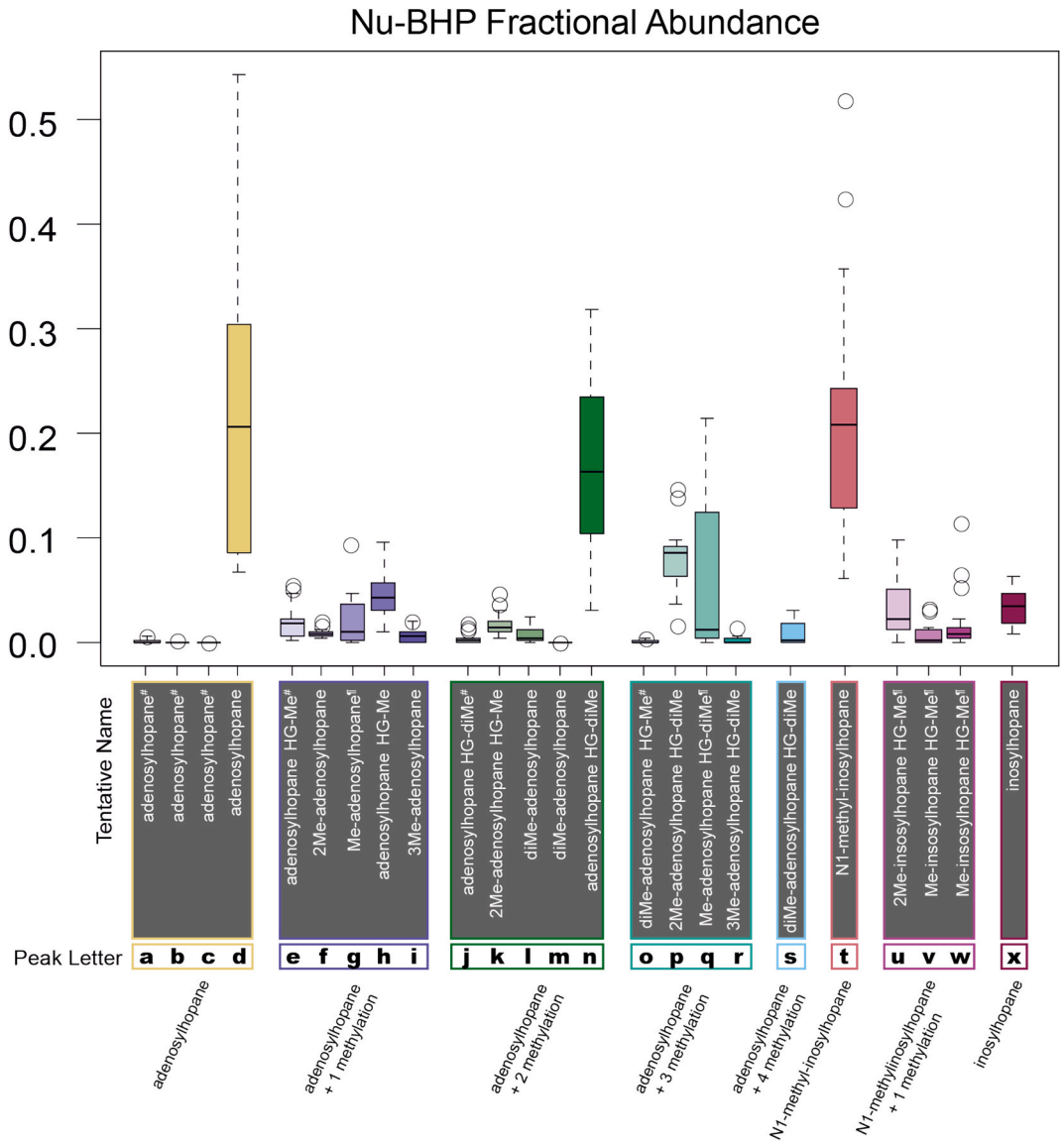

Fig. 5. Boxplots of Nu-BHP fractional abundances. Colours designate the type of BHP, with peak letters and tentative names from Fig. 2 indicated. The average value is shown as a horizontal line, with the box boundaries reflecting the first and second quartile, and the whiskers extending to the 95 % confidence interval. Outliers are plotted as circles. #early eluting isomer ¶methylation on core, unknown position.

the fractional abundance of BHPs, a principal component analysis is used to visualize the existing variations in BHPs (PCA; Fig. 7). Selected edaphic and climate variables are plotted in the ordinance space *a posteriori*. The first principal component (PC1) accounted for 41 % of the Nu-BHP variance of all surface soil samples, and the second principal component (PC2) accounted for 15 %. Some Nu-BHPs with a high loading on the 1st principal component (PC1 > 0.25; compounds j, l, o, q, s), show a significantly positive correlation with pH, and generally a negative correlation with the temperature parameters (Fig. 6, Table S2). As pH is anti-correlated significantly with temperature ($r = -0.48$, $p < 0.01$), the unique effects of pH and temperature cannot be distinguished. However, not all temperature-dependent Nu-BHPs show a strong correlation with pH. Compounds d and g have a negative loading on PC1 and PC2, indicating that either temperature directly, latitude, or an associated soil parameter such as soil moisture, impacts the BHP distribution. However, as PC2 only explains a minor amount of BHP variation, this only results in a significant correlation between soil moisture and Nu-BHP fractional abundance for compound g. Temperature and pH are thus proposed as environmental parameters that explain the most variation in soil Nu-BHPs.

3.2.1. *Nu-BHPs and pH and temperature*

Soil bacteria have been shown to modify their lipid membranes as an adaptation to their environment. For example, the distribution of lipid membrane-spanning branched glycerol dialkyl glcerol tetraethers (brGDGTs), of mostly unknown bacterial origin(s), is widely used to assess temperature and soil pH using the methylation and cyclization indices MBT and CBT, respectively (Weijers et al., 2007; Peterse et al., 2012; De Jonge et al., 2014). Incorporating BHPs in the cell membrane is believed to facilitate membrane adaptation to physicochemical stress such as pH and temperature (Kulkarni et al., 2013; Osborne et al., 2017; Sáenz et al., 2015; Tookmanian et al., 2021, 2022; Welander and Summons, 2012). Our observations from Alaska appear to show that the distribution of Nu-BHPs in soils are also affected by these environmental factors.

Many of the Nu-BHPs which correlate positively with pH and negatively with temperature (Fig. 6; Table S2) are adenosylhopanes with 2–4 additional methylation groups (i.e., peaks j, l, n, q, and s). Conversely, Nu-BHPs that show a relative increase with temperature, and either do not correlate or have a negative correlation with pH are adenosylhopanes with a single additional methylation, or without additional

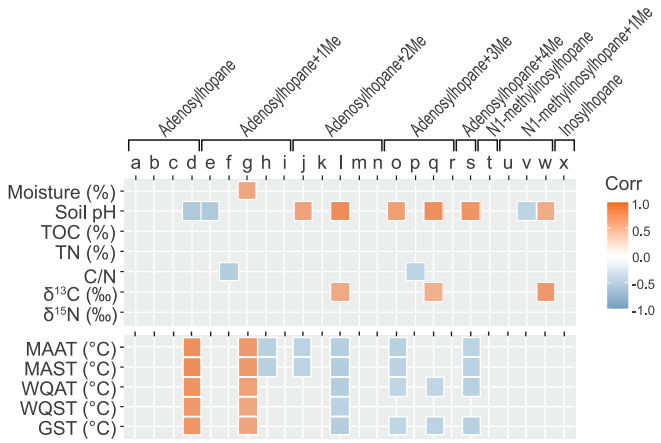

Fig. 6. Heatmap showing linear dependencies between Nu-BHP fractional abundance, soil and climate parameters. MAAT = Mean Air Temperature, MAST = Mean Soil Temperature, WQAT = Warmest Quarter Air Temperature, WQST = Warmest Quarter Soil Temperature, GST = Growth Season Temperature. Significant (red = positive; blue = negative) correlations are delineated by a coloured box. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

methylation (peaks d, g). This might suggest that methyl groups play a role in regulating proton permeability in the membranes of Nu-BHP-producing bacteria. The fact that the distributions of some Nu-BHPs vary significantly with temperature and pH may demonstrate that these lipids, which had previously been used to trace the input of soil organic matter into marine systems (i.e. R_{soil}), can possibly also be used to develop indices that relate with soil environmental parameters. However, it is not possible to determine where the position of the methylation is located in the nucleoside HG using mass spectral analysis, and further insights in the role (methylated) Nu-BHPs play in membrane stability are needed. Considering the ubiquity of Nu-BHPs in soils and their preservation potential, Nu-BHPs proxies should be developed as tools to trace past soil environments, thus complementing existing proxies to generate more robust paleo-reconstructions.

3.3. Temperature calibration based on Alaskan Nu-BHP distributions

Based on the observed environmental dependencies of Nu-BHPs, ratios can be developed that correlate with environmental parameters of interest. This allows to identify soils with contrasting BHP distribution and can eventually be applied for paleo-environmental reconstructions. Our data set is relatively constrained geographically and does not reflect global scale variability; this first step towards developing a temperature calibration needs to be complemented with an analysis across larger geographical scales. We determined ratios using the relative abundances of the Nu-BHPs with the best correlation for five temperature parameters (MAAT, MAST, WQAT, WQST and GST) using the linear regression approach (Kim et al., 2010; Peterse et al., 2012) (Supplemental Information). All temperature parameters are closely correlated (see PCA, Fig. 7), and calibrations were generated for all of them (Supplemental Information). Nevertheless, as we do not know the growth season of bacteria synthesizing Nu-BHPs, we chose to focus on the calibration for the warmest quarter soil temperature (WQST). It is likely that the highest activity of the soil bacteria responsible for Nu-BHP synthesis occurs during the summer growth season, especially considering the mean annual temperatures in this transect are below freezing.

Using the custom-made calibration code, a calibration equation was calculated.

$$Ratio_{Nu-BHP}WQST = \frac{(e+g+h+p+s+w)}{(i+j+p+r+w)}$$

This ratio includes nine Nu-BHPs, of which four adenosylhopane + 1 Me, one adenosylhopane + 2 Me, two adenosylhopane + 3 Me, one adenosylhopane + 4 Me, and one inosylhopane + 2 Me. Not all of the Nu-BHPs compounds selected in this ratio show significant correlation with temperature.

Plotting this ratio versus observed WQST, resulted in the following

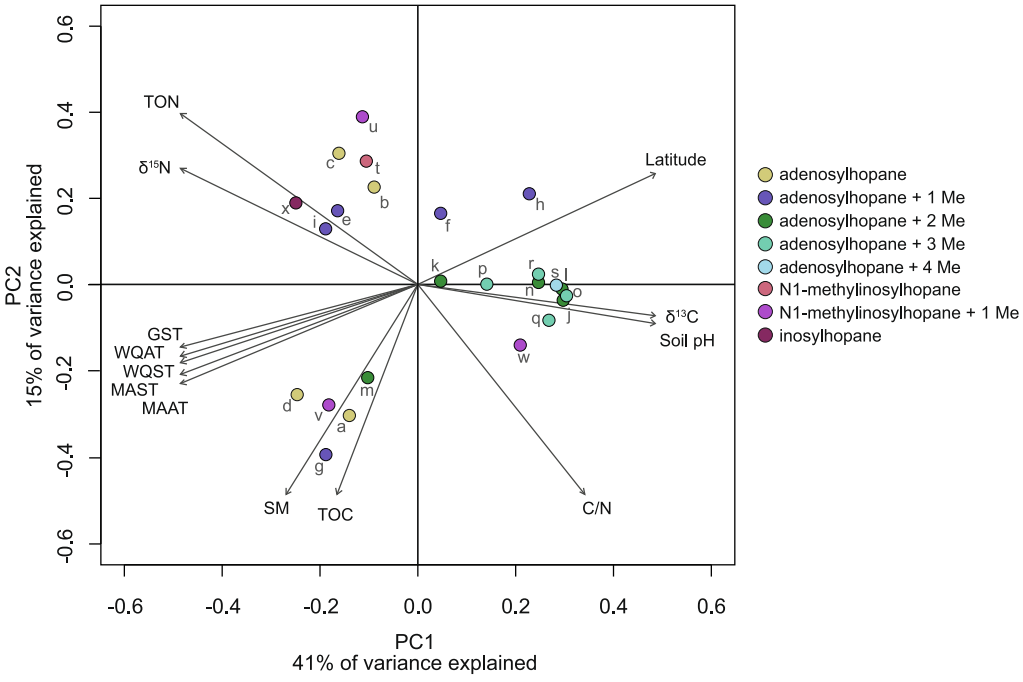

Fig. 7. Principal component analysis based on standardized fractional abundances of all 24 BHP compounds identified in this study. The loadings of the individual BHPs are plotted as colored symbols with peak letter indicated, where colors refer to the structural variability, as summarized in the legend. Environmental parameters are plotted *a posteriori* in the ordination space. Compounds with high loading on PC1 (A) and PC2 (B) were further plotted against temperature parameters (growth season temperature (GST), soil temperature of the warmest quarter), soil pH values and soil moisture (SM) values (Fig. 5).

linear correlation (Fig. 8):

$$Ratio_{Nu-BHP}WQST = 0.861 + 0.119 \times WQST (n = 17; R^2 = 0.72; p < 0.01)$$

There is a clear correlation ($R^2 = 0.72$) between certain Nu-BHPs and temperature, and this first temperature calibration is promising.

3.4. Future research and considerations for applications of Nu-BHPs as proxies for soil pH and temperature

Until the sample range can be extended, it would be inappropriate to apply this regional proxy to other locations. Future investigations should extend beyond high latitude regions. Furthermore, a much larger dataset is necessary to determine whether the type and location of soil influences the distribution of Nu-BHPs and if Nu-BHP proxies can be applied globally to various sedimentary archives. Currently, BHPs have mainly been described from marine records. Recent observations show that Nu-BHPs are in situ produced in low oxygen marine environments (Kusch et al., 2021). A marine source of Nu-BHPs could potentially introduce a mixed signal of Nu-BHPs alongside soil-produced Nu-BHPs in coastal sediments, which could complicate the interpretations of proxies based on soil BHPs (cf. proxies based on brGDGTs; Peterse et al., 2009). However, it is also possible that the distribution of marine-produced Nu-BHPs has a distinct fingerprint compared to soil-produced Nu-BHPs and, once disentangled, marine sedimentary archives may have the potential to record marine environmental conditions (e.g., temperature, pH, oxygen availability).

Lipids undergo structural alterations by microbial and chemical degradation processes after deposition in the sediment record (e.g., Johnson and Calder, 1973; Sun and Wakeham, 1994; Hoefs et al., 2002; Rontani et al., 2005). Some BHPs are relatively resistant to degradation, being found in Eocene and Paleocene sediments (van Dongen et al., 2006; Talbot et al., 2016). However, the preservation potential of these newly described Nu-BHPs is unknown. To consider diagenesis when applying proxies to sediment records, analysing sediments for Nu-BHPs across (thermal) maturation gradients or by simulating degradation in laboratory experiments (Koopmans et al., 1996; Schouten et al., 2004; Jaeschke et al., 2008; Rush et al., 2011) is important. Clearly, a wider variety of soils representing different environments and sample maturities need to be investigated using the advanced analytical method of UHPLC-HRMS^2. This is vital for the use of Nu-BHP based proxies in conjunction with other proxies to provide a more robust interpretation of paleoclimate records.

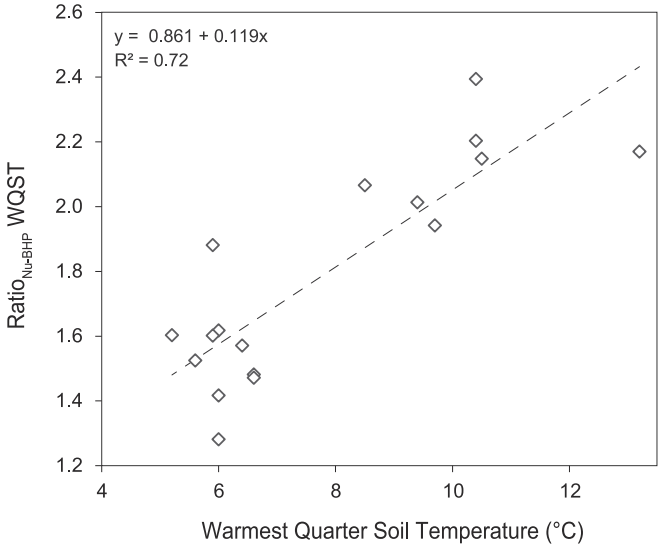

Fig. 8. Calibration plot of the statistically-calculated ratio of Nu-BHPs ($Ratio_{Nu-BHP}WQST$) in soils vs. observed warmest quarter soil temperature (WQST).

To better constrain future proxy application, understanding the biological mechanisms responsible for the observed temperature- and pH-dependencies in Nu-BHP-producing soil bacteria is crucial. Only five bacteria isolated from soil environments have been shown to accumulate adenosylhopane and/or 2Me-adenosylhopane in their cells. Furthermore, none of these strains have been analysed using the direct analysis method. Thus, the bacterial source(s) of most of the Nu-BHPs found in soil environments are currently still unknown. Future work should include molecular biological approaches, such as metagenomic analysis of soil communities to reveal the bacterial source(s) of environmental *hpnH* gene sequences, targeting soils with contrasting temperature and pH conditions. To isolate specific parameters, experiments where soils with a large diversity of Nu-BHPs are incubated under different (e.g., pH and temperature) conditions should be set up. Changes in Nu-BHP production in these incubation experiments together with shifts in microbial diversity could allow us to connect the novel Nu-BHPs with their potential producers. Further microbiology work should also include incubating model soil bacteria known to synthesize Nu-BHPs (e.g., *Bradyrhizobium japonicum*) to investigate whether these bacteria modify their lipid content with changing environmental conditions (cf. studies by Eickhoff et al., 2013; Elling et al., 2022), or whether the Nu-BHP distribution changes observed in soils are due rather to shifts in the dominant Nu-BHP-producing soil bacteria of the community. These experiments will also help determine which potential genes are involved in Nu-BHP structural modifications. Assuming the membrane modifications seen in this study are due to biophysical reasons, more work investigating the bacterial sources of these orphaned lipids is obviously needed.

4. Conclusions

In this study, we found strong edaphic and climatic controls on the distribution of microbially produced nucleoside BHPs (Nu-BHPs) in arctic soils. Across a latitudinal gradient of soils in Alaska, the distribution of Nu-BHPs was dependent on the environmental parameters of pH, growth season and/or warmest quarter temperatures. Regional calibrations using ratios of Nu-BHP fractional abundance showed strong positive correlations ($R^2 > 0.72$) with temperature variables. Future proxies developed to exploit these adaptations of the soil bacterial community to temperature and pH could complement existing organic geochemical proxies such as branched GDGT-based MBT'_{5ME}/CBT'. This would allow more robust reconstructions of terrestrial paleoclimate. To provide a useful set of paleoclimate proxies based on Nu-BHPs, further work should include: (i) extending the range of soils in order to determine if these correlation trends with climate parameters are widespread, (ii) determining the preservation potential of Nu-BHPs to establish the appropriate sample maturity where Nu-BHP proxies can be applied confidently; and (iii) investigating the bacterial sources of Nu-BHPs in soils and sediments to better understand the mechanisms underpinning these lipid distributions.

CRediT authorship contribution statement

Keith F. O'Connor: Writing – original draft, Formal analysis, Conceptualization. **Melissa A. Berke:** Writing – review & editing, Supervision, Formal analysis, Conceptualization. **Cindy De Jonge:** Writing – review & editing, Visualization, Validation, Formal analysis, Conceptualization. **Ellen C. Hopmans:** Methodology, Formal analysis. **Lori A. Ziolkowski:** Conceptualization. **Darci Rush:** Writing – original draft, Supervision, Methodology, Formal analysis.

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.

Acknowledgements

This work was funded by the Clare Boothe Luce Foundation (to MB), Geological Society of America (GSA), the University of Notre Dame's Center for Environmental Science and Technology (CEST) Predoctoral Fellowship, and a European Association of Organic Geochemists (EAOG) Research Award (awarded to KFO), and NSF award 1417711 (awarded to LZ). This publication is part of the project Nu-BHPs (project number 213.176 of the Vidi research programme, which is financed by the Dutch Research Council (NWO) and awarded to DR). CDJ acknowledges an SNSF PRIMA fellowship GN 179783. We would like to thank Denise Dorhout, Michel Koenen, Anchelique Mets, Jessica Riekenberg, and Monique Verweij for aiding in sample preparation and analyses.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.orggeochem.2025.105026.

Data availability

Data used in this study are publicly available at the NIOZ data repository, which can be accessed 1027 using the following link: https://dataportal.nioz.nl/doi/10.25850/nioz/7b.b.jj.

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