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1 Influence of environmental parameters on the distribution of bacterial lipids in soils
2 from the French Alps: Implications for paleo-reconstructions

3
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10
11 **Abstract**

12 Branched glycerol dialkyl glycerol tetraethers (brGDGTs) are a family of bacterial
13 lipids widely used for temperature and pH reconstructions in terrestrial settings. 3-hydroxy fatty
14 acids (3-OH FAs) with 10 to 18 carbon atoms, produced by Gram-negative bacteria, have been
15 recently proposed as independent and complementary proxies of temperature and pH in
16 terrestrial environments. Nevertheless, the correlations between mean annual air temperature
17 (MAAT)/pH and bacterial lipid (brGDGTs/3-OH FAs) distribution show a large degree of
18 scatter, as the relative abundance of these lipids is influenced by factors other than temperature
19 and pH. A full understanding of the environmental parameters influencing bacterial lipid
20 distribution in soils is required to increase the reliability of the temperature and pH proxies
21 based on these compounds in terrestrial environments. The aim of this work was to determine
22 and quantify the cumulative effect of environmental parameters on the distribution of both
23 brGDGTs and 3-OH FAs along a well-documented composite altitudinal transect in the French
24 Alps (234-2,748 m). Redundancy analysis revealed that the influence of local parameters (pH
25 and to a lesser extent soil moisture and grain size, related to vegetation and soil types) on
26 brGDGT and 3-OH FA distribution amounted to 48.1% and 26.1%, respectively, and was
27 predominant over MAAT. This likely explained the weak or lack of relationships between
28 MAAT and brGDGT-/3-OH FA-based indices in this region. The identification of lipids whose
29 fractional abundance is correlated with MAAT or pH allowed the development of local
30 calibrations with MAAT/pH applicable in the French Alps which are representative of highly
31 contrasted microenvironments, reflecting different types of soil and vegetation. The present
32 study highlighted the importance of constraining the environmental factors affecting the

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33 distribution of 3-OH FAs and brGDGTs in terrestrial settings prior to any paleoenvironmental
34 reconstruction. Such an approach should be reproduced in other sites, where local factors could
35 also strongly influence the bacterial lipid distribution.

36

37 **Keywords:** 3-hydroxy fatty acids; branched GDGTs; French Alps soils; altitudinal
38 transect; environmental parameters; local calibration

39

40 **1. Introduction**

41 Direct measurement of environmental data, such as temperature and precipitation, has
42 been possible for the last two centuries, the so-called "instrumental" period. Beyond this period,
43 it is necessary to use indirect approaches to obtain information on the different environmental
44 parameters. "Indirect" indicators of these parameters – so-called proxies – have thus been
45 developed and used regularly for last decades.

46 Organic biomarkers have been of great interest to the scientific community for the
47 reconstruction of past environments over the last decades (Eglinton and Eglinton, 2008),
48 especially microbial compounds. Microorganisms modify the lipid composition of their
49 membranes (e.g. carbon chain length, number of unsaturations, branching level) in response to
50 variations of environmental parameters (pH, temperature, osmotic pressure) (Ernst et al., 2016).
51 Such modifications are considered to maintain a functional fluidity and permeability of the
52 microbial membrane (Singer and Nicolson, 1972; Sinensky, 1974; Hazel and Williams, 1990;
53 Denich et al., 2003). These microbial lipids can be preserved in soils and sediments and be used
54 as proxies of past environmental conditions.

55 In this context, glycerol dialkyl glycerol tetraethers (GDGTs) are a family of microbial
56 lipids widely used for paleoenvironmental reconstructions. These compounds are ubiquitous in
57 terrestrial (Weijers et al., 2007; Peterse et al., 2012; De Jonge et al., 2014; Naafs et al., 2017)
58 and aquatic environments (Schouten et al., 2002, 2012; Powers et al., 2010; Peterse et al., 2015;
59 Weber et al., 2015). They are characterized by aliphatic chains connected to two glycerol units
60 via ether bonds. Two groups of GDGTs – isoprenoid and branched – can be distinguished
61 (Schouten et al., 2013 and references therein). Isoprenoid GDGTs (iGDGTs) are produced by
62 archaea. In contrast, GDGTs with branched chains but no isoprenoid alkyl chains – so-called
63 branched GDGTs (brGDGTs) – are produced by still unidentified bacteria, although some of
64 them may belong to the phylum *Acidobacteria* (Sinninghe Damsté et al., 2011, 2014, 2018).
65 They were discovered in peat (Sinninghe Damsté et al., 2000). The analysis of brGDGTs in a

66 large number of soils distributed worldwide then showed that the relative distribution of these
67 compounds is dependent on environmental parameters, mainly temperature and pH (Weijers et
68 al., 2007; De Jonge et al., 2014, 2019).

69 Despite the wide application of brGDGT indices for paleoenvironmental
70 reconstructions in terrestrial settings (Weijers et al., 2011; Wang et al., 2017; Coffinet et al.,
71 2018), the derived results must be interpreted with caution, as parameters other than
72 temperature or pH, such as humidity (Huguet et al., 2010; Menges et al., 2014), soil type
73 (Davtian et al., 2016; Mueller-Niggemann et al., 2016), vegetation composition (Weijers et al.,
74 2011; Naeher et al., 2014; Liang et al., 2019) or seasonality (Huguet et al., 2013) can also have
75 an influence on the relative abundances of brGDGTs. These lipids are the only molecular
76 proxies of temperature and pH available in the terrestrial environment to date, as most of the
77 available proxies have been developed in oceanic environments. Despite improvements in
78 brGDGT analytical methods and use of refined calibration models (De Jonge et al., 2014;
79 Hopmans et al., 2016; Dearing Crampton-Flood et al., 2020), the Root Mean Square Error
80 (RMSE) associated with Mean Annual Air Temperature (MAAT) reconstruction using the
81 global brGDGT calibrations in soils remains high ($>4^{\circ}\text{C}$), possibly due to the lack of knowledge
82 on all influencing environmental parameters. The development of new molecular proxies,
83 independent of and complementary to brGDGTs, is essential to improve the reliability of
84 environmental reconstructions in terrestrial settings.

85 Recent studies (Wang et al., 2016, 2018; Huguet et al., 2019; Yang et al., 2020) have
86 unveiled the potential of another family of lipids – 3-hydroxy fatty acids (3-OH FAs) – for
87 temperature and pH reconstructions. 3-OH FAs with 10 to 18 carbon atoms are specifically
88 produced by all Gram-negative bacteria and are bound to the lipopolysaccharide (LPS; main
89 component of the outer membrane) by ester or amide bonds. 3-OH FAs are part of the lipid A,
90 which anchors the LPS in the outer membrane of Gram-negative bacteria. Three types of 3-OH
91 FAs can be distinguished: with *normal* (i.e. straight and unbranched) or branched carbon chains,
92 either *iso* or *anteiso*. These compounds were widely used (i) to quantify Gram-negative bacteria
93 in clinical studies and detect the presence of endotoxins they release (Wollenweber and
94 Rietschel, 1990; Saraf et al., 1997; Szponar et al., 2002, 2003; Keinänen et al., 2003) and (ii) to
95 characterize and quantify Gram-negative bacteria communities in environmental samples such
96 as aerosols (Lee et al., 2004; Cheng et al., 2012), dissolved organic matter (Wakeham et al.,
97 2003), or soils (Zelles et al., 1995; Zelles, 1999).

98 3-OH FAs have been recently proposed as proxies of temperature and pH in terrestrial
99 environments after analysis of these compounds in 26 soils along Mount Shennongjia, China

100 (Wang et al., 2016). New indices were developed, with RAN_{15} and RAN_{17} (defined as the ratio
101 of C_{15} or C_{17} *anteiso* 3-OH FAs to *normal* C_{15} or C_{17} 3-OH FAs) being correlated with MAAT
102 and RIAN (defined as $-\log ([normal\ 3-OH\ FAs]/[anteiso+iso\ 3-OH\ FAs])$) being dependent
103 on soil pH.

104 Significant relationships between 3-OH FA distribution and temperature as well as pH
105 were similarly observed along two additional altitudinal transects: Mount Majella in Italy and
106 Mount Rungwe in Tanzania (Huguet et al., 2019). The RAN_{15}/RAN_{17} indices were negatively
107 correlated to air temperature along the three mountains investigated so far (Wang et al., 2016;
108 Huguet et al., 2019). This suggests that Gram-negative bacteria respond to colder temperatures
109 with an increase in *anteiso*- C_{15}/C_{17} vs. *n*- C_{15}/C_{17} 3-OH FAs in order to maintain a proper fluidity
110 and optimal state of the bacterial membrane, the so-called homeoviscous adaptation mechanism
111 (Sinensky, 1974; Hazel and Williams, 1990). Nevertheless, the relationships between RAN_{15}
112 and MAAT along Mts. Shennongjia, Rungwe and Majella showed the same slopes but different
113 intercepts (Wang et al., 2016; Huguet et al., 2019), suggesting that regional calibrations may be
114 more adapted to apply RAN_{15} as a temperature proxy in soils. In contrast, a significant ($R^2 =$
115 0.60) combined calibration between RAN_{17} and MAAT could be established using data from
116 Mts. Shennongjia, Rungwe and Majella (Wang et al., 2016; Huguet et al., 2019). Similarly,
117 RIAN was shown to be strongly negatively correlated with soil pH along the three
118 aforementioned mountains (Wang et al., 2016; Huguet et al., 2019), reflecting a general relative
119 increase in normal homologues compared to branched (*iso* and *anteiso*) ones with increasing
120 pH. This mechanism was suggested to reduce the permeability and fluidity of the membrane
121 for the cell to cope with lower pH (Watanabe and Takakuwa, 1984; Lepage et al., 1987; Russell,
122 1989; Russell et al., 1995; Denich et al., 2003; Beales, 2004). 3-OH FA indices were recently
123 applied to estimate temperature and hydrological changes over the last 10,000 years in a
124 speleothem from China (Wang et al., 2018), showing the potential of 3-OH FAs as independent
125 tools for environmental reconstruction in terrestrial settings. A very recent study based on
126 marine sediments from the North Pacific Ocean suggested that the distribution of 3-OH FAs
127 could also be used to reconstruct sea surface temperature (Yang et al., 2020).

128 Even though these results are promising, the linear regressions between pH/MAAT
129 data and 3-OH FA indices in terrestrial environments are still based on a rather small dataset
130 (ca. 70 soil samples) and show a large degree of scatter, leading to substantial errors in 3-OH
131 FA-based MAAT and pH reconstitutions ($MAAT-RMSE = 5.1^\circ C$; $pH-RMSE = 0.56$; Huguet
132 et al., 2019). One can anticipate that the distribution of 3-OH FAs in soils is impacted by
133 environmental parameters other than the temperature and pH, as observed for brGDGTs. A full

134 understanding of the environmental parameters (e.g. MAAT, pH, soil moisture, organic carbon
135 and nitrogen content, soil types and vegetation communities) influencing bacterial lipid
136 distribution (3-OH FAs and brGDGTs) in soils is required to increase the reliability of the
137 temperature and pH proxies. Nevertheless, to date, there is no report of a detailed investigation
138 of the environmental controls on 3-OH FA distribution in soils and only a limited number of
139 studies on brGDGTs address this question. The aim of this work is thus to determine and
140 quantify the cumulative effect of environmental parameters on the distribution of both
141 brGDGTs and 3-OH FAs along a composite altitudinal transect in a well-constrained sampling
142 site in the French Alps.

143 **2. Material and methods**

144 **2.1.Sites and sampling**

145 Surficial soil samples (0-10 cm depth) were collected in the French Alps in October
146 2017 along two well-documented (MAAT, soil types and vegetation composition) climate-
147 toposequences: the Bauges massif between 232 and 1,475 m a.s.l (above sea level) and the
148 Lautaret-Galibier massif between 1,540 and 2,700 m a.s.l. (Fig. 1; Supp. Fig. 1). MAAT and a
149 precise description of soil types and vegetation composition are available in this area thanks to
150 an integrated long-term observatory belonging to the Zone Atelier Alpes (Bounemoura al.,
151 1998; Carlson et al., 2017; Choler, 2018). The Lautaret-Galibier and Bauges massifs cover a
152 complementary range of altitude and make it possible to obtain a composite altitudinal transect
153 that is representative of the variations in temperature, soil characteristics and plant communities
154 in the French Alps (Table 1).

155

156 *2.1.1. Bauges massif*

157 Twenty-four soil samples were collected along the western slope of the Bauges massif
158 (Fig. 2; Table 1). This massif, located in the north-western part of the French Alps, in the French
159 National Park of the Bauges massif, is a low to medium altitude site (maximum altitude of 2,217
160 m a.s.l). To the north east, this massif is bordered by a group of mountains and the Annecy
161 Lake. It is limited to the south west by the Bourget Lake. This is a carbonated subalpine massif,
162 dominated by limestone chains oriented north-north-east / south-south-west. Their summits are
163 mainly composed of Urgonian limestones. In the lower part of the massif, at an altitude of 200
164 m, the Mean Annual Precipitation (MAP, mm/years) is 1,221 mm and the MAAT 11.4 ° C (for
165 the period 1981-2010, Chambéry meteorological station). In the upper part of the Bauges
166 massif, at an altitude of around 1,300 m, the annual average rainfall is 1,704.9 mm and the
167 MAAT 5.8 ° C (for the period 1981-2000, Feclaz weather station; Bounemoura al., 1998;
168 Carlson et al., 2017; Choler, 2018).

169 The vegetation in the lower part of the massif is characterized by mountain ash tree
170 (*Fraxinus excelsior*; around 200 m a.s.l.) and beech (*Fagus sylvatica*; between 900 and 1,000
171 m a.s.l) forest as well as meadows, dominated by *Carex foetida*. The higher part of the massif
172 is characterized by heathland patchworks (*Vaccinium myrtillus* species, between 1,200 and
173 1,350 m a.s.l.) and coniferous forest (*Pinus sylvestris* around 1350 m; Fig. 2).

174 Soils in the lower part of the Bauges massif (around 200 m) are calcosols, influenced
175 by the limestone bedrock. Within the beech forest, organosols are present on calcareous

176 substrates. Within the coniferous forest, soils are divided into patchworks of organosols –
177 resulting from the accumulation of organic litter on calcareous substrates – and brunisols,
178 mainly resulting from the accumulation of clays in topographic hollows (Table 1).

179

180 2.1.2. *Lautaret-Galibier massif*

181 Twenty-five soil samples were also collected in the Roche Noire high-elevation
182 watershed, between 1,540 m and 2,700 m a.s.l (Fig. 2). The site is located in the Lautaret-
183 Galibier Massif, which is part of the south-western Alps, between the passes of Lautaret (2,058
184 m a.s.l.) and Galibier (2,645 m a.s.l.). Average MAP along the Lautaret-Galibier massif is 1,300
185 mm and decreases with altitude. In the lower part of the massif, at an altitude of 1,500 m, MAAT
186 is around 6°C. In the upper part of the massif, at an altitude of 2,700m, MAAT is around 0°C.
187 Wooded vegetation, dominated by Larch forest (*Larix decidua*), occurs between 1,600 m and
188 1,900 m a.s.l. Heathlands dominated by *Vaccinium* spp. and grasslands dominated by the tall
189 festuce *Patzkea paniculata* are observed between 1,900 m and 2,300 m a.s.l. The highest part
190 of the site is dominated by a mosaic alpine meadow showing a high beta-diversity in plant
191 vascular species in relation with mesotopographical variations (Choler, 2018)

192 Calcosols are found in the lowest part of the massif, between 1,500 and 2,000 m a.s.l.,
193 whereas subalpine grasslands and alpine meadows are dominated by brunisols. Alocrisols –
194 developing on acidic rocks and intermediate between brunisols and podzolic soils – are also
195 observed between 2,200 and 2,300 m a.s.l., as well as rendisols – poorly evolved and
196 undifferentiated soils growing on calcareous substrates – at ca. 2,700 m a.s.l. and colluviosols –
197 formed by slope deposits on pre-existing soils – at ca 2,500 m a.s.l (Table 1).

198

199 2.1.3. *Sampling*

200 Vegetation along the two massifs (Bauges and Lautaret-Galibier) is distributed in
201 different biomes based on the thermal regime, but also precipitation and mesotopographical
202 parameters. Therefore, three samples representative of the different soil types and/or vegetation
203 were collected at each elevation. A total of 49 soil samples were collected (Table 1). After being
204 transported to the laboratory, they were directly stored at -20 °C and then freeze-dried, ground
205 and sieved at 2 mm.

206

2.1.4. *Temperature data*

Miniature temperature data loggers (Hobo Pendant UA, Onset Computer Corporation, Bourne, MA) were placed at 5 cm below the soil surface in 24 of the Bauges massif sites, allowing the daily measurement of the surface soil temperature. These temperatures are considered to be similar to MAAT and can be used as a first approximation as MAAT estimates (Bartlett et al., 2006; Weijers et al., 2007). The corresponding data were compiled for the period 2016-2018 and were completed by MAAT measurements from the Feclaz meteorological station (1,350 m a.s.l.). As no recorded temperature data were available for the 2016-2018 period along the Lautaret-Galibier massif, MAAT values from the Oisans massif, selected for its geographical and climatic proximity to the Lautaret-Galibier, were used instead, with 8 points distributed every 300 m between 750 and 3,550 m a.s.l. Data were provided by the SAFRAN-SURFEX/Crocus-MEPRA model chain (S2 M) developed by Météo France for the French Alps (Durand et al., 2009; Vionnet et al., 2012).

A linear regression was established between the available temperature data (Bauges and Oisans massifs as well as Feclaz station) and altitude (Supp. Fig. 2) to obtain a model allowing the reconstruction of MAAT at the different sampling sites (i.e. the whole composite transect along the Bauges and Lautaret-Galibier massifs). The MAAT values reported and discussed in the rest of the manuscript will be those obtained from this model ($R^2 = 0.97$; RMSE = 0.67 °C).

2.2. Bulk soil analyses

Soil water content (SWC) was determined by difference between the soil mass before and after freeze-drying.

The pH of the freeze-dried samples was measured in ultrapure water with a 1:2.5 soil water ratio. Typically, 10 ml of ultrapure water were added to 4 g of dry soil. The soil solution was stirred for 30 minutes, before decantation for 1 hour and pH measurement (Carter et al., 2007).

Soil samples were decarbonated before elemental (total organic carbon - C_{org} and nitrogen) and isotopic ($\delta^{13}\text{C}$ and $\delta^{15}\text{N}$) analyses following the protocol detailed by Huguet et al. (2013). Elemental and isotopic analyses were performed on the ALYSES platform (Sorbonne University / IRD) at Bondy, France. About 10 μg of decarbonated soil was typically weighed

239 in tin capsules before analyses using an Elementar Vario EL III (Elementar Analysensysteme
240 GmbH, Germany)

241 The distribution of particle sizes into five sieved and crushed soil fractions – clays (<
242 2µm); fine silts (between 2 and 20 µm); coarse silts (between 20 and 50 µm); fine sands
243 (between 50 and 200 µm) and coarse sands (between 200 and 2000 µm) – was determined at
244 the Arras Soil Laboratory, France. The cation exchange capacity (CEC) was measured in the
245 Arras Soil Laboratory using the METSON method (Metson, 1957).

246

247 **2.3.brGDGT analysis**

248 Sample preparation for brGDGT analysis was similar to that reported by Coffinet et
249 al. (2014). Briefly, ca. 10 g of soil was extracted using an accelerated solvent extractor (ASE
250 100, Dionex-ThermoScientific, USA) with a dichloromethane (DCM) / methanol (MeOH)
251 mixture (9: 1) for 3×5 minutes at 100 °C and a pressure of 100 bars in 34 ml cells. The total
252 lipid extract was rotary evaporated and separated into two fractions of increasing polarity on a
253 column of activated alumina: (i) 30 ml of heptane: DCM (9: 1, v:v) ; (ii) 30 ml of DCM: MeOH
254 (1: 1, v:v). GDGTs are contained in the second fraction, which was rotary evaporated. An
255 aliquot (300 µL) was re-dissolved in heptane and centrifuged using an Eppendorf MiniSpin
256 centrifuge (Eppendorf AG, Hamberg, Germany) at 7000 rpm for 1 min.

257 GDGTs were then analysed by high pressure liquid chromatography coupled with
258 mass spectrometry with an atmospheric pressure chemical ionisation source (HPLC-APCI-MS)
259 using a Shimadzu LCMS 2020. GDGT analysis was performed using two Hypersil Gold silica
260 columns in tandem (150 mm × 2.1 mm, 1.9 µm; Thermo Finnigan, USA) thermally-controlled
261 at 40 °C, as described by Huguet et al. (2019). This methodology enables the separation of 5-
262 and 6-methyl brGDGTs. Semi-quantification of brGDGTs was performed by comparing the
263 integrated signal of the respective compound with the signal of a C₄₆ synthesized internal
264 standard (Huguet et al., 2006) assuming their response factors to be identical.

265 The MBT'_{5Me} index, reflecting the average number of methyl groups in 5-methyl
266 isomers of GDGTs and considered as related to MAAT, was calculated according to De Jonge
267 et al. (2014; Eq. 1; Supp. Data):

268

$$269 \text{MBT}'_{5\text{Me}} = \frac{[Ia+Ib+Ic]}{[Ia+Ib+Ic]+ [IIa+IIb+IIc]+[IIIa]} \quad (1)$$

270

271 The CBT index, reflecting the average number of cyclopentyl rings in GDGTs and
272 considered as related to pH, was calculated as follows (Peterse et al., 2012; Eq. 2; Supp. Data):

$$273 \quad CBT = -\log \left(\frac{[Ib]+[IIb+IIb']}{[Ia]+[IIa+IIa']} \right) \quad (2)$$

274 The Roman numerals correspond to the different GDGT structures presented in De
275 Jonge et al. (2014). The 6-methyl brGDGTs are denoted by an accent after the Roman numerals
276 for their corresponding 5-methyl isomers. Analytical errors associated with the calculation of
277 MBT'_{5Me} and CBT indices are respectively 0.03 and 0.04 based on the analysis of 5 samples in
278 triplicates among the 49 soil samples.

279

280 **2.4. 3-OH FA analysis**

281 Sample preparation for 3-OH analysis was identical to that reported by Huguet et al.
282 (2019). 10 g of freeze-dried soil were subjected to acid hydrolysis (reflux with 30 ml of 3M
283 HCl in a 130 °C silicon oil bath for 3 hours). The suspension was then centrifuged at 15 °C and
284 3000 rpm for 10 minutes. The supernatant was collected in an Erlenmeyer flask. The residue
285 was then ultrasonically extracted with a mixture of DCM:MeOH (1:1, v/v; 2× ; 20 ml) and
286 DCM (2× ; 20 ml). Each extraction was followed by centrifugation and pooling of all extracts.
287 The DCM phase was then separated from the MeOH/H₂O layer, which was again extracted
288 with DCM (3 times; 20 ml). This DCM fraction was then rotary-evaporated, methylated in a
289 1M HCl-MeOH solution at 80 °C for 1 hour and separated into three fractions over an activated
290 silica column: (i) 30 ml of heptane/EtOAc (98: 2), (ii) 30 ml of EtOAc and (iii) 30 ml of MeOH.
291 3-OH FAs contained in the second fraction were derivatised with a solution of *N,O*-
292 bis(trimethylsilyl)trifluoroacetamide (BSTFA) – Trimethylchlorosilane (TMCS) 99:1 (Grace
293 Davison Discovery Science, USA) before gas chromatography-mass spectrometry (GC-MS)
294 analysis.

295 3-OH FAs were analysed with an Agilent Network 6980 GC-MS using a Restek RXI-
296 5 Sil MS silica column (60 m × 0.25 mm, i.d. 0.50 µm film thickness), as previously described
297 (Huguet et al., 2019). 3-OH FAs were quantified by integrating the appropriate peak (*m/z* =
298 175) on the ion chromatogram and comparing the area with an internal standard (3-
299 hydroxytetradecanoic acid, 2,2,3,4,4-d₅; Sigma-Aldrich, France). The internal standard (0.5
300 mg/ml) was added just before injection as a proportion of 3 µl of standard to 100 µl of sample,
301 as detailed by Huguet et al. (2019). The different 3-OH FAs were identified based on their
302 retention time, after extraction of the characteristic *m/z* 175 fragment (*m/z* 178 for the deuterated
303 internal standard; cf. Huguet et al., 2019).

304 The RIAN index was calculated as follows (Wang et al., 2016; Eq. 3) in the range C₁₀-
305 C₁₈ (Supp. Data):

$$306 \quad \text{RIAN} = -\log[(I + A)/ N] \quad (3)$$

307 where I, A, N represent the sum of all *iso*, *anteiso* and *normal* 3-OH FAs, respectively.

308

309 RAN₁₅ and RAN₁₇ indices are defined as follows (Wang et al., 2016; Eqs. 4 and 5; Supp. Data):

$$310 \quad \text{RAN}_{15} = [\textit{anteiso} \text{ C}_{15}] / [\textit{normal} \text{ C}_{15}] \quad (4)$$

$$311 \quad \text{RAN}_{17} = [\textit{anteiso} \text{ C}_{17}] / [\textit{normal} \text{ C}_{17}] \quad (5)$$

312 Analytical errors associated with the calculation of RIAN, RAN₁₅ and RAN₁₇ indices
313 are respectively 0.009, 0.19 and 0.06 based on the analysis of one sample injected eleven times
314 during the GC-MS analysis.

315

316 **2.5. Statistical analysis**

317 In order to investigate the correlations between the different environmental variables
318 (soil moisture, pH, MAAT, Corg and N contents) and the relative abundances of membrane
319 lipids (brGDGTs and 3-OH FAs), pairwise correlation matrices were performed in addition to
320 single or multiple linear regressions. As the majority of variables are not normally distributed
321 (except for δ¹⁵N and clay content), Spearman correlation was used with a confidence level of
322 5%. Linear regressions and correlation matrices were performed with R software, version 3.6.1
323 (R Core Team, 2014).

324 Redundancy analysis (RDA) was performed on the 49 soil samples to identify the
325 relationships between environmental variables and 3-OH FA or brGDGT distribution. RDA is
326 a "constrained" analysis, used to directly visualize the variation in the lipid data as a function
327 of the environmental variables. Redundancy analysis allows not only assessing but also
328 quantifying the influence of each explanatory variables (i.e. environmental variables) on the
329 distribution of bacterial lipids. RDA analysis yields the influence of each variable, with regards
330 to the statistical variance, on the pool of bacterial lipids, and allowed a quantification in percent
331 of the influence of each parameter. The quantification of the effect of each variable can be
332 considered in two ways. Simple (or marginal) effects are the effects of each variable considered
333 independently of the other variables. Conditional effects summarize the effects of each variable
334 taking into account the effect of variables with a greater influence (Braak and Smilauer, 2002)
335 A forward selection was performed using the Canoco software (v. 5.04) to help in the selection
336 of the best subset of the variables explaining the variation in 3-OH FA and brGDGT
337 distributions. RDA analyses were performed on centered and standardized data using the

338 CANOCO v. 5.04 software (Braak and Smilauer, 2002). The relationships between each
339 variable and the dimensions of the RDA were investigated using the corresponding r-values
340 and the percentages of variance.
341

342 3. Results

343 3.1. Bulk soil properties

344 The organic carbon content (C_{org}) shows a wide range of variation – from 1.4% for
345 poorly evolved soils (lithosol) to 49.10% for forest organosol (litter soil) – with an average of
346 9.7% and a standard deviation of 10.3% (Table 2). Only a minority of soils ($n = 4$) display high
347 C_{org} values ($> 30\%$). The $\delta^{13}C$ values show a unimodal distribution ($-26.7\text{‰} \pm 2.3\text{‰}$) and range
348 between -29.9‰ and -24.4‰ (Table 2). The N content of the soil samples is normally
349 distributed ($0.7\% \pm 0.5\%$; Table 2). Relative soil moisture is unimodally distributed and largely
350 varies between soils, ranging between 3.4 and 68.2% (mean $30.3\% \pm 15.6\%$; Table 2). With
351 regard to the texture of the sampled soils, the clay contents are on average $313.8 \pm 126.7 \text{ g/kg}$
352 of soil and largely vary between samples (minimum and maximum values of 33 and 546 g/kg
353 of soil, respectively). This large variability of the soil characteristics is also reflected in the silt
354 ($406.4 \pm 117.0 \text{ g/kg soil}$) and sand ($279.8 \pm 194.1 \text{ g/kg soil}$) contents, as well as in the CEC
355 values ($24.2 \pm 17.0 \text{ cmol } +/\text{kg}$; Table 2). Finally, the pH values show a wide range of variation
356 ($3.62\text{--}7.49$), with an average of 5.83 and a standard deviation of 1.08.

357

358 3.2. BrGDGT and 3-OH FA distribution

359 3-OH FAs and brGDGTs were detected in the 49 studied soil samples (Supp. Tables
360 1 and 2). BrGDGT distribution was dominated by acyclic compounds (Ia, Ila, Ila', IIIa, IIIa')
361 which represent in average ca. 80% of total brGDGTs (Fig. 3a). BrGDGTs Ia and Ila were the
362 most abundant homologues in all samples. The pentamethylated brGDGTs (Ila-c; mean 50%,
363 standard deviation (SD) of 6.9%) were predominant over the tetramethylated (Ia-c) and
364 hexamethylated (IIIa-c; Fig. 3a). The 5-methyl isomers were present in higher proportion (mean
365 74%, SD 15.6%) than the 6-methyl compounds (mean 26%, SD 15%) (Fig. 3a).

366 3-OH FAs with chain lengths between 8 and 26 C were identified. These compounds
367 may have various origins depending on their chain length (bacteria, plants and fungi; Zelles et
368 al., 1999; Wang et al., 2016 and reference therein). Nevertheless, most of the 3-OH FAs present
369 in our samples are homologues with 10 to 18 C atoms, generally associated with Gram-negative
370 bacteria (Saraf et al., 1997; Szponar et al., 2002, 2003; Keinänen et al., 2003) and only these
371 homologues will be considered in the following. 3-OH FAs with even carbon number and
372 *normal* chains were the most abundant compounds in all samples (about 65% of the 3-OH FAs,
373 SD 12%), with a predominance of the *n*-C₁₄ homologue (Fig. 3b). *Iso* (mean 24%, SD 4.5%)

374 and *anteiso* (mean 8%, SD 1%) isomers were also present. It must be noted that *anteiso* isomers
375 were only detected for odd 3-OH FAs (Fig. 3b).

376

377 **4. Discussion**

378 The distribution of brGDGTs (Weijers et al., 2007, Peterse et al., 2012; De Jonge et
379 al., 2014) and 3-OH FAs (Wang et al., 2016; Huguet et al., 2019) in soils was reported to be
380 influenced mainly by MAAT and soil pH. Therefore, the effect of these two environmental
381 parameters on the relative abundance of 3-OH FAs and brGDGTs in the 49 soils of the French
382 Alps was first investigated.

383

384 **4.1. BrGDGTs and 3-OH FAs as proxies of pH and temperature**

385 *4.1.1. Relationship between pH and bacterial lipid distribution*

386 BrGDGT-derived CBT index has been shown to be strongly correlated with pH in a
387 large set of globally distributed soils (De Jonge et al., 2014; Naafs et al., 2017). Consistently,
388 in the French Alpine soils, CBT displayed a significant ($p < 0.05$) and strong correlation ($R^2 =$
389 0.85 ; $RMSE = 0.42$) with soil pH (Fig. 4a), in accordance with the global CBT-pH calibration
390 proposed by De Jonge et al. (2014). This confirms the robustness of the CBT as a soil pH proxy
391 taking into account the highly contrasting environmental characteristics of these French Alps
392 soils.

393 The soils of the French Alps (Table 2) and those of Mts. Rungwe, Majella and
394 Shennongjia, previously investigated for 3-OH FA distribution (Wang et al., 2016; Huguet et
395 al., 2019), cover a similar range of pH (ca. 3 –8) and of RIAN index (0.13-0.55 in the French
396 Alps, Table 2; 0.15-0.70 for the other soils, Wang et al., 2016; Huguet et al., 2019). In the
397 French Alps, the RIAN index was observed to be moderately ($R^2 = 0.43$; $RMSE = 0.83$) but
398 significantly negatively correlated ($p < 0.001$) with soil pH (Fig. 4b), consistent with the
399 significant correlation observed along the previously investigated altitudinal transects (Wang
400 et al., 2016; Huguet et al., 2019). This confirms the general influence of pH on the relative
401 abundance of 3-OH FAs in soils.

402 Nevertheless, a stronger correlation between RIAN and pH ($R^2 = 0.62$) was obtained
403 for the dataset combining soils from Mts. Rungwe, Majella and Shennongjia (Huguet et al.,
404 2019) compared to the one from the French Alps. In addition, the RIAN-pH correlations along
405 the French Alps (Fig. 4b) and along Mts. Rungwe, Majella and Shennongjia (Huguet et al.,
406 2019) displayed significantly different slopes and intercepts (p -value = 0.049), reflecting local

407 trends between 3-OH FA distribution and pH. Finally, the scatter in the RIAN-soil pH
408 relationship along the French Alps (Fig. 4b), reflected in the average value of the prediction
409 interval (0.35), suggests that parameters other than pH also affect the distribution of 3-OH FAs
410 along this transect.

411

412 *4.1.2. Relationship between MAAT and bacterial lipid distribution*

413 In addition to pH, the relationship between bacterial membrane lipid distribution and
414 MAAT was also investigated. BrGDGT-based MBT'_{5Me} was significantly ($p < 0.001$) negatively
415 correlated with MAAT in the French Alps (Fig. 5a). Nevertheless, this correlation was
416 substantially weaker ($R^2 = 0.35$) than the one between MAAT and MBT' or MBT'_{5Me} for (i)
417 soils distributed worldwide ($R^2 = 0.66$; De Jonge et al., 2014) and (ii) along other altitudinal
418 transects: Mount Kilimanjaro, Tanzania ($R^2 = 0.75$; Sinninghe Damsté et al., 2008); Mount
419 Xiangpi, China ($R^2 = 0.64$; Liu et al., 2013); Mount Rungwe, Tanzania ($R^2 = 0.74$; Coffinet et
420 al., 2014); Andean transect, Colombia ($R^2 = 0.67$; Anderson et al., 2014), East Coast of the
421 USA ($R^2 = 0.47$; Dirghangi et al., 2013). The lower RMSE in this study (3.3 °C), in comparison
422 with the RMSE of the global calibration (4.8 °C) could be partly explained by the relatively
423 small number of samples ($n = 49$) analyzed within the French Alps, compared to the global
424 dataset ($n = 231$) presented by De Jonge et al. (2014). Even though the relationship between
425 brGDGT distribution and MAAT is generally strong, there is a substantial scatter in the
426 MBT'_{5Me}-MAAT global calibration, leading to the high RMSE mentioned above (De Jonge et
427 al., 2014), which reflects the heterogeneous and local response of brGDGT distribution to
428 temperature. Such heterogeneity is also visible at the regional level, with e.g. significant
429 differences in the relationship between brGDGT distribution and MAAT/soil pH along several
430 altitudinal transects subjected to different precipitation regimes (Anderson et al., 2014). The
431 weak relationship between MBT'_{5Me} and MAAT in the French Alps, associated with an
432 uncertainty (RMSE) of 3.3 °C, reflects the fact that local parameters other than MAAT should
433 be the primary controls of 5-methyl brGDGT relative abundance along this specific transect.

434 Regarding 3-OH FAs, the temperature proxies based on these compounds, namely
435 RAN₁₅ and RAN₁₇, did not show any correlation with MAAT in the French Alps (Fig. 5b, c).
436 In contrast, significant negative correlations between RAN₁₅ and MAAT were previously
437 observed along Mt. Shennongjia ($R^2 = 0.51$; Wang et al. 2016), Mt. Rungwe and Mt. Majella (R^2
438 $= 0.80$; $p < 0.001$ and $R^2 = 0.54$; $p = 0.01$, respectively; Huguet et al., 2019) with similar slopes
439 but different intercepts. This was interpreted as the result of local effects on RAN₁₅, despite a
440 similar general response to temperature changes (Huguet et al., 2019). As observed for RAN₁₅,

441 RAN₁₇ was significantly negatively correlated with MAAT along Mt. Shennongjia ($R^2 = 0.48$;
442 Wang et al. 2016), Rungwe and Majella ($R^2 = 0.54$ and 0.28 , respectively; Huguet et al., 2019)
443 with similar slopes and ordinates, showing a comparable response of RAN₁₇ to temperature
444 variations in these different locations. Nevertheless, there was only a weak correlation between
445 RAN₁₇ and MAAT along Mt. Majella (Huguet et al., 2019), characterized by the closest
446 altitudinal, geographical and climatic conditions to those of the French Alps in comparison with
447 other investigated mountains. This result, combined with the absence of correlation between
448 either RAN₁₅ or RAN₁₇ and the MAAT in the French Alps (Fig. 5b), suggests that locally the
449 relative abundance of C₁₅ and C₁₇ 3-OH FAs may be mainly influenced by environmental
450 parameters other than MAAT.

451 The Bauges and Lautaret-Galibier massifs, investigated in the present study, are a
452 combination of highly diverse microecosystems resulting from microtopographical and
453 geological (nature of the bedrock) differences and characterized by specific vegetation and soil
454 types, precipitation or snow cover. This multiplicity of environmental variables may therefore
455 affect the distribution of bacterial lipids in soils, as previously shown for brGDGTs (e.g. soil
456 moisture, Dirghangi et al., 2013; granulometry, Peterse and Eglinton, 2017; vegetation or soil
457 type, Davtian et al., 2016, Liang et al., 2019). This might reflect either changes in bacterial
458 communities as previously reported (Hofmann et al., 2016; Siles and Margesin, 2016; Shen et
459 al., 2019) and/or adaptation mechanisms of a constant community. Statistical analyses were
460 performed to disentangle and quantify the main drivers of changes in bacterial lipid distribution
461 along the Bauges and Lautaret-Galibier massifs and thus explain the weakness or lack of
462 correlations of the 3-OH FA-/brGDGT-derived proxies with MAAT.

463

464 **4.2. Impact of other environmental variables on bacterial lipid distribution**

465 *4.2.1. Selection of non-redundant environmental variables for statistical analyses*

466 Before constraining the influence of the different environmental factors on 3-OH FA
467 and brGDGT distribution using redundancy analyses (RDA), non-redundant environmental
468 variables, which carry significantly different information, had to be selected. To this aim, a
469 Spearman correlation matrix was first computed (Fig. 6), allowing the determination of the
470 correlations between the different environmental variables. Highly correlated variables can be
471 considered as redundant. Only correlation coefficients with red (negative correlation) or blue
472 (positive correlation) colors are statistically significant ($p < 0.05$) (Fig. 6).

473 As MAAT values were reconstructed using a linear model with altitude (cf. section
474 2.1.4.), MAAT and altitude were logically anticorrelated along the Bauges and Lautaret-

475 Galibier, reflecting the natural cooling of air with elevation (Fig. 6). Altitude (and thus MAAT)
476 was moderately correlated with soil pH ($r = -0.44$) and strongly correlated with $\delta^{13}\text{C}$ ($r = 0.77$).
477 A relationship between soil pH and altitude was similarly observed along other altitudinal
478 gradients but the sign of the correlation was shown to be strongly site-dependent (Cheng- Jim
479 et al., 2014; Bhandari et al., 2019). It can be influenced by multiple factors such as soil types,
480 source rock (e.g. calcareous or granitic) or vegetation composition (e.g. presence or absence of
481 conifers; Smith et al., 2002; Djukic et al., 2010; Gutiérrez-Girón et al., 2015). Similarly, the
482 relationship between $\delta^{13}\text{C}$ and altitude could be due to different vegetation, litter input and
483 degradation of OM with thermal regimes, as suggested along other mountains (Wei et al., 2009;
484 Du et al., 2014). Indeed at higher altitudes, the degradation of OM may be reduced, in contrast
485 with the lower altitudes and higher temperatures, where plant accumulation is higher than
486 decomposition (Davidson and Janssen, 2006; Conant et al., 2011).

487 Altitude is correlated to a set of environmental parameters, such as MAAT, carbon
488 content, pH and can be considered as an integrative variable of the changes in vegetation, soil
489 and relief types. In order to observe the sole effect of MAAT variation on brGDGT and 3-OH
490 FA distribution, MAAT rather than altitude was kept as a non-redundant parameter. The $\delta^{15}\text{N}$
491 of the different soil samples showed moderate correlations ($r < 0.7$) with six of the eleven
492 parameters investigated (Fig. 6) and was therefore considered as a confounding parameter.
493 Organic carbon content (C_{org}) of the soils was observed to be strongly correlated with the
494 nitrogen content ($r = 0.97$) and $\delta^{15}\text{N}$ ($r = -0.53$) as well as with the cation exchange capacity (r
495 $= 0.73$; Fig. 6). C_{org} was also moderately correlated with soil water content ($r = 0.64$) and soil
496 clay content ($r = 0.54$; Fig. 6) and was therefore considered as a redundant variable in the present
497 study. Similarly, based on collinearity relationships with other variables, N content, $\delta^{15}\text{N}$ and
498 CEC will not be considered in the following statistical tests. In order to confirm the choice of
499 the non-redundant variables for RDA analyses made using the Spearman correlation matrix
500 (Fig. 6) and determine the best set of variables explaining the distribution of brGDGTs/3-OH
501 FAs, a forward selection analysis was performed through the Canoco software. This highlighted
502 MAAT, pH, soil moisture, sand and clay contents (reflecting the grain size gradient of the soil)
503 as explanatory variables, in line with Spearman correlation tests (Fig. 6).

504 The choice of these variables for subsequent RDA analysis is not unexpected, as
505 MAAT and pH were shown to have a major influence on bacterial communities, which develop
506 adaptation mechanisms to cope with variations of such parameters (Denich et al., 2003; Beales,
507 2004; Fierer and Jackson, 2006). This is especially the case for brGDGT- and 3-OH FA-
508 producing bacteria (De Jonge et al., 2019; Huguet et al., 2019). Similarly, soil moisture may

509 affect the relative abundance of bacterial membrane lipids, as shown for brGDGTs (e.g. Peterse
510 et al., 2012; Dirghangi et al., 2013; Ding et al., 2015). Soil moisture varies, as a first
511 approximation, according to the precipitation regime, but it can also be related to other factors
512 such as evapotranspiration, grain size or vegetation cover (Crave and Gascuel-Oudou, 1997;
513 Gómez-Plaza et al., 2001). The grain size gradient is representative of the edaphic parameters
514 which may influence bacterial diversity in soils (Hemkemeyer et al., 2018) in relationship with
515 changes in soil (micro)structure and organic matter availability and which may also in turn
516 affect the distribution of 3-OH FA and brGDGT source microorganisms in soils, as proposed
517 below.

518 Selecting only non-redundant variables for RDA simplifies the analysis and makes it
519 more robust. Nevertheless, RDA analyses carried out with all the variables, confirmed that
520 MAAT, pH and soil moisture were the parameters mainly influencing 3-OH FA and lipid
521 distribution (data not shown). Therefore, in the following, we will only discuss the results of
522 the RDA analyses performed with the aforementioned non-redundant variables.

523

524 4.2.2. RDA analysis of brGDGT distribution and environmental variables

525 Regarding the RDA related to brGDGTs (Fig. 7a, Table 3), the first two axes explain
526 50.2 % of the total inertia of the dataset. The selected environmental variables are sufficient to
527 explain 89.4 % of the variance in the brGDGT relative abundance (referred to as explained
528 fitted variation in Table 3). On the first axis, the RDA analysis shows a predominant effect of
529 pH ($r = 0.96$; Table 3) and a weaker contribution of MAAT ($r = 0.71$) and soil moisture ($r = -$
530 0.26) on the distribution of brGDGTs. Axis 2, on the other hand, is influenced by MAAT ($r =$
531 0.62), relative soil moisture ($r = 0.53$) and to a lesser extent by pH ($r = -0.26$) and soil texture
532 parameters, with a positive correlation with the sand content ($r = 0.08$) and a negative one with
533 the clay content ($r = -0.20$; Fig. 7a and Table 3).

534 To go further, the influence of each environmental variable on the relative distribution
535 of brGDGTs can be quantified through RDA analysis (Table 4). When the effect of each
536 variable is considered individually, the influence of pH on brGDGT distribution appears
537 predominant (41.1 %), followed by MAAT (25.2 %) and to a lesser extent soil moisture (5.3
538 %; Table 4). This is in line with observations made from the global soil dataset by De Jonge et
539 al. (2014), where the variance of the relative abundances of the 15 individual brGDGTs in 231
540 soils was investigated using a Principal Component Analysis (PCA). When the first two
541 components of this PCA are considered, it appears that pH was positively correlated with the

542 largest component of the variance (40 %), while MAAT and MAP were correlated together
543 with the other component (17 %).

544 In the natural environment, environmental parameters do not influence bacterial lipid
545 distribution separately, but are interrelated and act concomitantly, with e.g. soil moisture
546 impacting the capacity of a soil to retain heat, thus indirectly influencing the temperature (Idso
547 et al., 1975; Davidson et al., 1998). Therefore, the combined effect of the different
548 environmental variables on brGDGT distribution was also investigated statistically. The
549 conditional influence of these variables (Table 4) on the brGDGT distribution shows once again
550 a clear predominance of the effect of pH (41.1 %), with a reduced influence of temperature (8.1
551 %), particle size (5.5 %) and soil moisture (1.5 %; Table 4). pH, grain size (clay/sand) content
552 and soil moisture can be all considered as variables related to the local environment, i.e.
553 microtopography, vegetation cover and soil type. When combined together, the influence of
554 these local parameters on brGDGT distribution amounts to 48.1 % against only 8.1 % for
555 MAAT (Table 4), which can explain the weakness of the MBT'_{5Me}-MAAT relationship in soils
556 from the French Alps and its large degree of scatter (Fig. 5a).

557 The RDA triplot also illustrates the influence of the different environmental variables
558 on the relative abundance of the individual brGDGTs (Fig. 7a). BrGDGTs comprising cyclic
559 moieties (i.e with subscript b or c) are strongly and mainly positively correlated with the first
560 axis of the RDA, and thus positively correlated with pH. As most of these cyclic compounds
561 are used in the calculation of the CBT index, this explains the strong correlation between CBT
562 and pH observed in the soils of the French Alps ($R^2 = 0.85$; Fig. 4a), consistent with the trends
563 observed in soils distributed worldwide (De Jonge et al., 2014). In contrast with cyclic
564 brGDGTs, acyclic 5-methyl brGDGTs (with subscript a) are located in the left half of the RDA
565 (Fig. 7a) and show a moderate to strong correlation with the second axis of the RDA, especially
566 brGDGT IIIa which is highly negatively influenced by MAAT. However, except compound
567 IIIa, all the brGDGTs involved in the calculation of the MBT'_{5Me} are more influenced by pH
568 than by temperature (Fig. 7a), hence a rather moderate correlation between this index and
569 MAAT ($R^2=0.35$; Fig. 5a) is observed. Therefore, in contrast with previous studies where 5-
570 methyl brGDGT distribution in soils was suggested to be mainly affected by MAAT and to a
571 lesser extent by soil pH (De Jonge et al., 2014, Naafs et al., 2017), the relative abundance of
572 the 5-methyl brGDGTs in the soils from the French Alps seems to be mainly driven by soil pH,
573 with only compound IIIa showing a strong correlation with MAAT (Fig. 7a). This may be
574 related to a recent study by De Jonge et al. (2019), which highlighted the strong effect of pH on
575 brGDGT distribution in addition to MAAT, with a different pH-dependency in soils from warm

576 and cold environments. As for the 6-methyl brGDGTs, located in the right part of the RDA
577 (Fig. 7a), their relative abundance in the soils of the French Alps appear well-correlated with
578 pH, as observed in a large set of globally distributed soils ($n = 350$) by Naafs et al. (2017).
579 BrGDGT distribution is only weakly influenced by soil moisture (especially compound Ia) and
580 soil texture in soils from the French Alps (Fig. 7a).

581

582 4.2.3. RDA analysis of 3-OH FA distribution and environmental variables

583 Similarly to brGDGTs, RDA analysis was performed to investigate the effect of the
584 different environmental parameters on 3-OH FA distribution (Fig. 7b). The first two axes of the
585 RDA explain 31.8 % of the total inertia of the dataset. The explained fitted variation represents
586 81.2 % of the total inertia of the relationship between 3-OH FAs and the environmental
587 variables (Table 3). The RDA analysis shows a predominant contribution of pH ($r = 0.95$),
588 positively correlated with axis 1, on the distribution of 3-OH FAs (Fig. 7b, Table 3). Soil
589 moisture ($r = -0.56$) MAAT ($r = 0.29$), sand content ($r = -0.21$) and clay content ($r = -0.32$)
590 show a weaker correlation with the axis 1. Axis 2 is mainly related to the MAAT ($r = -0.93$),
591 to the relative soil moisture ($r = -0.41$; Fig. 7b, Table 3) and to a lesser extent with pH ($r = 0.23$).
592 Soil structure parameters (sand and clay contents) are poorly represented on the axis 2 of the
593 RDA. (Fig. 7b; Table 3).

594 These qualitative trends are confirmed after quantification of the influence of each
595 environmental variable on 3-OH FA distribution (Table 4). The study of simple effects also
596 shows a predominant influence of pH on 3-OH FA distribution (20.0 %) followed by relative
597 soil moisture (9.9 %), temperature (11.2 %) and particle size (3.8%). The combined influence
598 of the different variables on the 3-OH FA relative abundance shows similar results, with pH
599 (20.0 %) and to a lesser extent temperature (10.9 %) and soil moisture (3.6 %) being the main
600 parameters affecting the lipid distribution (Table 4). Like brGDGTs, the influence of local
601 parameters related to soil type and vegetation (i.e. soil pH, grain size content and soil moisture)
602 on 3-OH FA distribution is predominant (26.1 %; Table 4) over MAAT, which could explain
603 the absence of correlation between 3-OH FA distribution, especially RAN₁₅, RAN₁₇ indices
604 and MAAT (Fig. 5b, c).

605 When considering the relative abundances of the individual 3-OH FAs in the RDA, it
606 appears that most 3-OH FAs are grouped in a narrow sector of the circle (Fig. 7b). They
607 encompass compounds with branched chains (*anteiso*-C₁₁, *iso*-C₁₂, *iso*-C₁₃, *anteiso*-C₁₃, *iso*-C₁₄,
608 *anteiso*-C₁₅, *iso*-C₁₆, *iso*-C₁₇, *anteiso*-C₁₇), but also *normal* ones (C₁₅, C₁₇). This sector is close
609 to the positive part of the first axis and thus these compounds are mostly influenced by pH. In

610 contrast, this sector appears orthogonal to MAAT and opposite to soil moisture. Outside this
611 sector, the *normal* C₁₄ 3-OH FA is negatively correlated with the first axis and the *iso* C₁₅ on
612 the one hand and the *normal* C₁₀ and C₁₁ on the other hand are respectively positively and
613 negatively correlated with the MAAT (Fig. 7b).

614 The predominant influence of pH on the relative abundance of all *iso*, *anteiso* and
615 *normal* 3-OH FAs except the *iso* C₁₅, *normal* C₁₈ and *normal* C₁₀ homologues (Fig. 7b) explains
616 the existence of a relationship between the RIAN index and pH (Fig. 4b). Nevertheless, this
617 correlation remains moderate due to the rather strong and opposite influence of soil moisture
618 (Table 4) and to the (positive or negative) correlation of some 3-OH FA with MAAT. The RDA
619 analysis also shows that the compounds used for the calculation of the RAN₁₅ and RAN₁₇
620 indices (*anteiso* and *normal* C₁₅/C₁₇ homologues) are located in the pH sector, orthogonal to
621 MAAT (Fig. 7b). This explains why there are no linear relations between these indices and
622 MAAT in soils from the French Alps (Fig. 5b, c).

623

624 4.2.4. Influence of vegetation and soil type on 3-OH FA and brGDGT distribution

625 As discussed above, the influence of local edaphic parameters (i.e. grain size, soil
626 moisture, pH) related to soil type and vegetation on both brGDGT and 3-OH FA distribution
627 was shown to be higher than that of MAAT. This is further confirmed by the distribution of the
628 samples in the RDAs (Fig. 7 a, b), with the samples being distributed according to their
629 affiliation to certain types of soils or vegetation communities. The fact that samples are grouped
630 in clusters may partly be due to the fact that some of them are geographically proximal. But
631 some of the samples, spatially distant, are also clustered in the RDA, for example, according to
632 soil types : samples 20 to 25 (calcosols, between 1,540 m and 1,940 m a.s.l), samples 5 to 7 and
633 17 to 19 (brunisol, between 1,920 m and 2,688 m a.s.l), or vegetation compositions : samples
634 1 to 10 which are alpine meadow (between 2,531 and 2,700 m a.s.l.) (Fig. 7, Table 1). The
635 calcosols are distributed over the entire right-hand side of the two RDAs, as they are essentially
636 differentiated based on their alkaline pH. The calcosols are also distributed along axis 2, driven
637 by altitude and relative soil moisture. Thus, lowland calcosols appear in the bottom or upper
638 right quadrants of the two RDAs respectively (Fig. 7a, b), whereas subgroups of high-altitude
639 calcosols, reflecting mountainous and subalpine vegetation covers, can be observed at the
640 bottom or upper right, respectively. This shows that calcosols can also be differentiated based
641 on the plant cover patterns and altitude. In contrast with the calcosols, brunisols are distributed
642 throughout the whole correlation circle. However, they can be subdivided into 3 distinct
643 subgroups based on the vegetation cover and altitude: a first subgroup with intermediate altitude

644 samples and mountainous vegetation, positively correlated with soil moisture; a second
645 subgroup, with high altitude samples and meadow vegetation, negatively correlated with
646 MAAT; a third subgroup of individuals, characterized by low altitude soils and lowland
647 vegetation, positively correlated with MAAT. Altogether, the RDA results show that soil
648 groups are discriminated according to their bacterial lipid distributions, which are strongly
649 dependent on vegetation cover and soil types.

650
651

652 **4.3. Development of local calibrations between the relative abundance of** 653 **bacterial lipids and MAAT/pH**

654

655 The wide diversity of microenvironments encountered the French Alps could explain
656 the absence or weakness of the relationships between MAAT and brGDGT-/3-OH FA-based
657 indices, as well as between pH and RIAN index. This stresses the need for identifying the
658 individual lipids whose fractional abundance is mainly correlated with MAAT or pH, which is
659 possible through RDA analyses (Fig. 7). The identification of these individual lipids then allow
660 the development of local relationships between environmental parameters (MAAT/pH) and the
661 relative abundances (%) of the selected compounds (Figs. 8 and 9), as detailed below.

662

663 *4.3.1. BrGDGTs*

664 Even though the MBT'_{5Me} index is not suitable for MAAT reconstruction in the French
665 Alps ($R^2 = 0.35$; Fig. 5), the RDA analysis showed that the fractional abundance of some of the
666 brGDGTs (mainly compound *IIIa* and to a lesser extent compounds *Iib'* and *Ib*) are moderately
667 to strongly correlated with MAAT. An alternate transfer function with MAAT was defined
668 based on a linear combination of the fractional abundance of these three compounds (Eq. 6;
669 Fig.8):

670

$$671 \text{ MAAT} = -0.255 \times [\textit{IIIa}] + 0.195 \times [\textit{Ib}] + 0.171 \times [\textit{Iib}'] + 6.34 \quad (6)$$

$$672 (n = 49; R^2 = 0.69; \text{RMSE} = 2.26 \text{ } ^\circ\text{C})$$

673

674 This new calibration (Eq. 6; Fig. 8) shows a much higher determination coefficient
675 and lower RMSE than the MBT'_{5Me}-MAAT relationship (Fig. 5a). The same approach was used
676 to propose a new local calibration for the brGDGTs to reconstruct pH values, but this did not
677 improve the linear relationship demonstrated between the CBT index and pH (Fig. 4a).

678 Altogether, these results suggest that brGDGTs can be used for both pH and temperature
679 reconstructions even in highly diverse and contrasted environments such as the French Alps.
680 Nevertheless, the present study shows that a detailed investigation of the environmental controls
681 of brGDGT distribution and the development of local calibrations may be required to
682 confidently use these compounds as paleoproxies.

683

684 4.3.2. 3-OH FAs

685 The RDA results allowed the identification of the individual 3-OH FAs whose relative
686 abundance is mainly correlated with MAAT or pH (Fig. 7a). Two alternate transfer functions
687 were proposed based on a linear combination of the relative abundance of 3-OH FAs (Eqs. 7
688 and 8), using a similar approach as for brGDGTs (Eq. 6):

689

$$\begin{aligned} \text{pH} &= -0.144 \times [nC_{12}] + 0.177 \times [iC_{13}] - 0.171 \times [nC_{14}] + 0.148 \times [iC_{16}] + 10.01 \\ (n = 49; R^2 &= 0.70; \text{RMSE} = 0.6) \end{aligned} \quad (7)$$

692

$$\begin{aligned} \text{MAAT} &= -0.629 \times [nC_{10}] + 0.883 \times [iC_{15}] + 0.496 \times [iC_{17}] + 0.629 \times [nC_{18}] - 13.16 \\ (n = 49; R^2 &= 0.63; \text{RMSE} = 2.43 \text{ } ^\circ\text{C}) \end{aligned} \quad (8)$$

695

696 The proposed relationship between pH and 3-OH FA relative abundances (Eq. 7; Fig
697 9a) presents a higher determination coefficient ($R^2 = 0.70$ vs. 0.43) and accuracy ($\text{RMSE} = 0.60$
698 vs. 0.83) than the one with the RIAN index. Similarly, the identification of the individual 3-OH
699 FAs strongly influenced by MAAT allowed obtaining a new relationship with MAAT (Eq. 8;
700 Fig. 9b) which was not visible through the previously defined RAN_{15} and RAN_{17} indices. This
701 shows once again the interest and importance of constraining the environmental factors
702 affecting the relative abundance of microbial lipids in complex and highly variable
703 environments such as the French Alps, where conventional indices are not reliable for
704 temperature and pH reconstructions. Specific calibrations have to be developed instead to take
705 into account the effect of local confounding parameters on lipid relative distribution.

706

4.4. Constraints on the applicability of brGDGTs and 3-OH FAs as environmental proxies

3-OH FAs were proposed only recently as potential temperature and pH proxies in soils and were to date investigated in a limited amount of samples – ca. 70 soils, excluding the presently studied ones (Wang et al., 2016; Huguet et al., 2019). This contrasts with brGDGTs, whose applicability as environmental proxies in terrestrial (and aquatic) environments has been extensively investigated over the last 15 years (e.g. Weijers et al., 2007; Peterse et al., 2012; Coffinet et al., 2014; De Jonge et al., 2014; Huguet et al., 2014; Dang et al., 2016; Lei et al., 2016; Naafs et al., 2017; Dearing Crampton-Flood et al., 2020). Studies investigating the constraints on the applicability of 3-OH FAs as environmental proxies in terrestrial settings are thus needed. pH was shown to be the main factor influencing both brGDGT and 3-OH FA distribution in soils of the French Alps, even though RIAN shows a much more moderate correlation with a lower prediction interval (Fig. 4b; $R^2=0.43$, RMSE = 0.83) than CBT (Fig. 4a; $R^2 = 0.85$, RMSE = 0.42). The calibration between the linear combination of 3-OH FA relative abundances and pH proposed in this study (Eq. 7; Fig. 9a) is also slightly weaker ($R^2 = 0.70$) and less accurate (RMSE = 0.60) than the one with the CBT (Fig. 4a). Altogether, these results show the higher reliability of the latter index as a pH proxy in the French Alps. In contrast with pH, the MAAT was only moderately influencing the bacterial lipid distribution in soils reflected by the weak relationship between MBT'_{5Me} and MAAT and the absence of relationship of RAN₁₅ and RAN₁₇ with MAAT (Fig. 5). Nevertheless, the environmental variables investigated in the present study (including MAAT and pH) were not able to explain the total variability of the lipid distribution, the unexplained variation remaining higher for 3-OH FAs than for brGDGTs (Table 3). Seasonality might be one of the factors influencing the production of bacterial lipids in the French Alps soils, as previously suggested for brGDGTs in the French Jura Mountains (Huguet et al., 2013). Indeed, during part of the year, some of the French Alps soils are covered with snow, protecting them from extreme temperatures. Parameters such as microtopography or snowfall are known to have an important role on the thermal regime of soils and thus on the microorganisms living in them (Margesin et al., 2008). The effect of the thermal regime could therefore explain part of the dispersion remaining in the local calibrations between MAAT and bacteria lipid distribution.

The lack of information regarding the ecology of the 3-OH FA and brGDGT producers should indeed be taken into account while discussing the high uncertainty related to the lipid-based transfer functions. C₁₀ to C₁₈ 3-OH FAs, used for the calculation of the RAN₁₅ and RAN₁₇

741 pH/temperature proxies, are known to be produced by Gram-negative bacteria, which are a
742 highly diverse group of microorganisms represented by numerous genera. This non-
743 monophyletic group of bacteria does not share a common ancestor (Lecointre and Guyader,
744 2006), which explains the large genetic and biochemical differences between the various Gram-
745 negative bacteria. The numerous genera and subgenera of Gram-negative bacteria are also
746 characterized by highly diverse lipid profiles, with different relative abundances of 3-OH FAs,
747 and with the whole suite of C₁₀-C₁₈ 3-OH FAs homologues not being present in all the strains
748 (Wilkinson et al., 1988). Therefore, the 3-OH FA lipid distribution is highly dependent upon
749 what Gram-negative bacterial species are present (e.g. Parker et al., 1982; Bhat and Carlson,
750 1992; Zelles, 1999). Furthermore, the diversity of Gram-negative bacteria varies with altitude
751 (Margesin et al., 2009; Siles and Margesin, 2016). Taken together, this may partly explain the
752 lack of relationship or poor to moderate correlations observed between 3-OH FA indices
753 (RAN_{15/17}, RIAN) and MAAT/soil pH (Figs. 4b, 5b, c). Additionally, it might also not be
754 excluded that the various soil Gram-negative bacteria genera/subgenera respond differently to
755 variations of environmental parameters. In brief, the 3-OH FA profile of the various Gram-
756 negative bacteria species likely reflects the diversity of microenvironments present in the
757 French Alps, which are subjected concomitantly to large variations in several environmental
758 parameters (MAAT, soil pH, soil moisture, microtopography, vegetation cover, soil type etc...).

759 In contrast, although they appear to be ubiquitous, brGDGTs might be produced by a
760 more restricted number of bacterial species than 3-OH FAs. Indeed, so far only soil
761 *Acidobacteria* have been found to contain the building blocks of brGDGT biosynthesis and are
762 thus considered as potential brGDGT sources (Sinninghe Damsté et al., 2018). If the diversity
763 of brGDGT producers is indeed lower than that of 3-OH FA-producing microorganisms, it may
764 explain the more homogenous response and lower scatter of the relationships between
765 MAAT/pH and brGDGT-derived indices than those with 3-OH FA indices (Figs. 4 and 5). Such
766 relationships may also be affected by the different physiological responses of brGDGT and 3-
767 OH FA producers to environmental parameters. These hypotheses remain purely speculative,
768 as to date the information on brGDGT source microorganisms remains limited. This calls for
769 further studies assessing and comparing the influence of environmental parameters on brGDGT
770 and 3-OH FA producing bacteria in terrestrial environments. Such work is essential to improve
771 the reliability and accuracy of the complementary temperature and pH proxies based on the two
772 lipid families.

773

774

775 **5. Conclusions**

776 This study thoroughly investigated the environmental factors controlling the
777 distribution of brGDGTs and 3-OH FAs in soils collected along well-documented altitudinal
778 transects in the French Alps. The influence of local parameters (pH and to a lesser extent soil
779 moisture and grain size, related to vegetation and soil types) on brGDGT and 3-OH FA was
780 more important than MAAT. This likely explains the absence or weak relationships between
781 MAAT and brGDGT/3-OH FA-based indices and stresses the need for identifying the
782 individual lipids whose fractional abundance is correlated with MAAT or pH. Such work at the
783 molecular level led to the development of strong local calibrations between the individual lipids
784 identified through statistical analyses and MAAT/pH. They can be applied to soils from the
785 French Alps, representative of highly contrasted microenvironments. The present study
786 highlights the importance of constraining the environmental factors affecting the distribution of
787 3-OH FAs and brGDGTs in terrestrial settings prior to any environmental reconstruction using
788 these lipid biomarkers. Such an approach should be reproduced in other sites, where local
789 factors would also strongly influence the bacterial lipid distribution. Local effects are expected
790 to explain a large part of the scatter observed in the global calibrations between MAAT/pH and
791 3-OH FA as well as brGDGT distribution. These local confounding parameters may notably
792 explain why the calibration between 3-OH FA indices (RAN_{15/17}) and MAAT in the French
793 Alps differ from those previously obtained in other sites. As 3-OH FAs and brGDGTs may be
794 produced by different microorganisms, further work is needed to assess and compare the impact
795 of environmental parameters, especially temperature and pH, on the microbial diversity in
796 contrasted soils and the associated lipid profiles. Even though the lack of knowledge on
797 brGDGT-producing bacteria complicates such studies, the latter are essential to improve the
798 reliability and accuracy of the complementary temperature and pH proxies based on 3-OH FAs
799 and brGDGTs.

800

801

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Figure and table captions

Figure 1. Location of the two massifs of the French Alps investigated in this study. Images Google Satellites. Exact sampling locations is shown in Supp. Figure 1.

Figure 2. Altitudinal transect between the Bauges and Lautaret-Galibier massifs showing the dominant plant species and the different alpine stages.

Figure 3. Average distribution of (a) brGDGTs and (b) 3-OH FAs in the 49 soil samples of the French Alps.

Figure 4. (a) Linear regression between CBT and pH along the two massifs in the French Alps. The black dotted line corresponds to the global linear regression between CBT and pH from De Jonge et al. (2014). (b) Linear regression between RIAN and pH along the two massifs in the French Alps. The black dotted line corresponds to the linear regression between RIAN and pH from Huguet et al. (2019). Dotted and colored lines represent the 95% prediction interval for each regression and colored areas represent the 95% confidence interval for each regression.

Figure 5. (a) Linear regression between MAAT and MBT'_{5Me} along the two massifs in the French Alps. The black dotted line corresponds to global the linear regression between MBT'_{5Me} and MAAT from De Jonge et al. (2014). Linear regressions between (b) RAN_{15} and (c) RAN_{17} along the two massifs in the French Alps. Dotted and colored lines represent the 95% prediction interval for each linear regression and colored areas represent the 95% confidence interval for each regression.

Figure 6. Correlation matrix between the different environmental variables and soil properties. Correlation coefficients in red (negative correlation) or blue (positive correlation) are statistically significant. Red variables have been chosen as explanatory variables for statistical tests.

Figure 7. Triplot presenting the results of the RDA carried out on the relative abundances of (a) brGDGTs and (b) 3-OH FAs and the non-redundant physicochemical parameters: pH, MAAT, soil moisture, sand and clay contents.

Figure 8. MAAT estimated using the new calibration based on a linear combination of brGDGT relative abundances (Eq. 6) vs. MAAT observed along the the Bauges and Lautaret-Galibier massifs. The roman numerals correspond to the different GDGT structures presented in De Jonge et al. (2014).

Figure 9. (a) pH estimated using the new calibration based on a linear combination of 3-OH FA relative abundances (Eq. 7) vs. pH observed along the Bauges and Lautaret-Galibier massifs. (b) MAAT estimated using the new calibration based on a linear combination of 3-OH relative abundances (Eq. 8) vs. MAAT observed along the Bauges and Lautaret-Galibier massifs. The roman numerals correspond to the different GDGT structures presented in De Jonge et al. (2014).

Table 1. List of the soil samples associated with corresponding soil type and vegetation collected along the Bauges and Lautaret-Galibier massifs.

Table 2. Physicochemical characteristics of the 49 samples collected along the Bauges and Lautaret-Galibier massifs. The numbering of the samples corresponds to that used in Table 1. Samples with stars represent sites instrumented with temperature sensors.

Table 3. RDA correlation coefficients of the environmental variables selected along axes 1 and 2.

Table 4. Quantification of the influence of the different environmental variables on brGDGTs and 3-OH FAs. Statistical significancies are shown as follows: *** ($0 < p < 0.001$); ** ($0.001 < p < 0.01$); * ($0.01 < p < 0.05$); ° ($0.05 < p < 0.1$).

Supplementary Figure 1. Location of the sampling sites along the a) Bauges and b) Lautaret-Galibier massifs.

Supplementary Figure 2. Linear model used to reconstruct MAAT along the Bauges and Lautaret-Galibier massifs. Dotted lines represent the 95% prediction interval for the linear regression and colored area represent the 95% confidence interval.

Supplementary Table 1. Relative abundances (%) of the *normal*, *iso* and *anteiso* 3-OH FAs in soils from the French Alps and corresponding indices.

Supplementary Table 2. Relative abundances (%) of the brGDGTs in soils from the French Alps and corresponding indices.

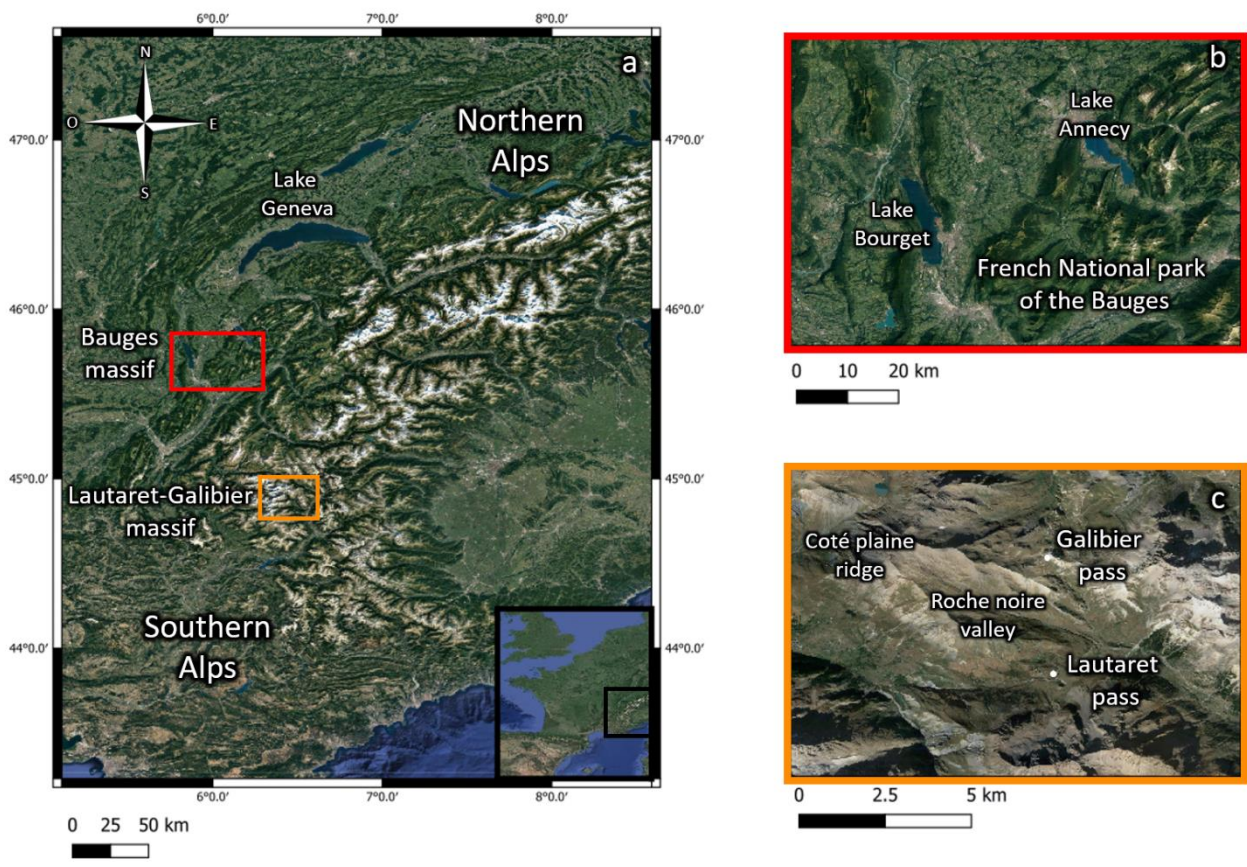


Figure 1

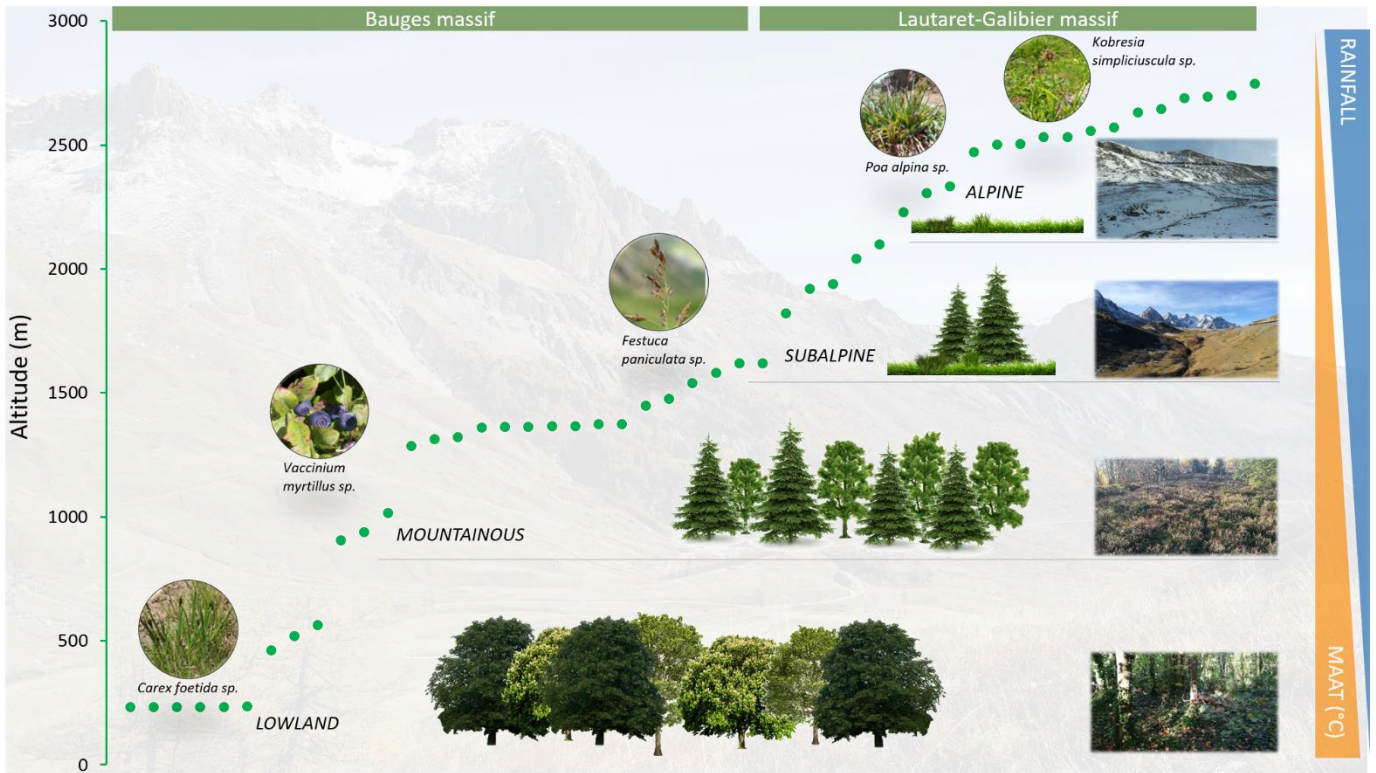


Figure 2

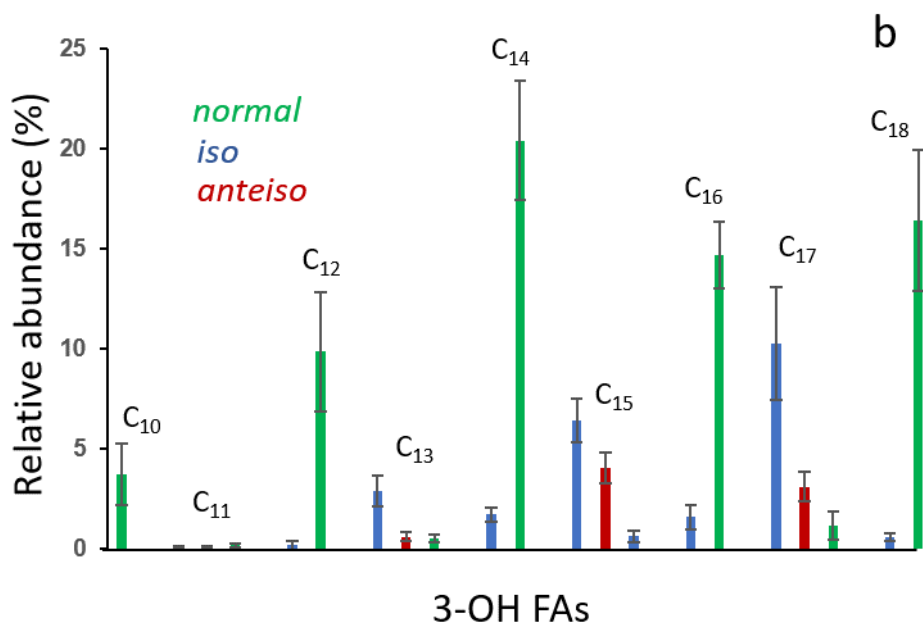
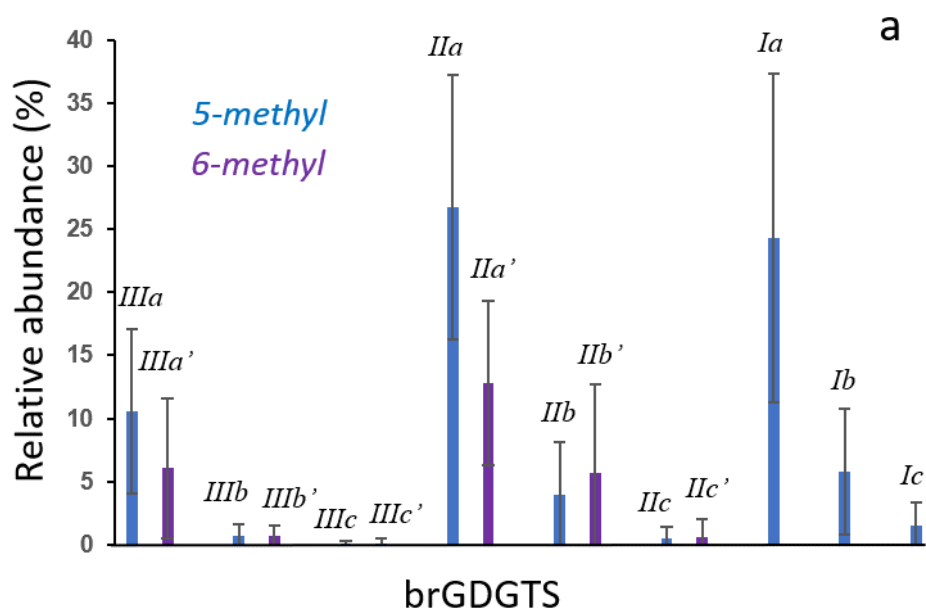


Figure 3

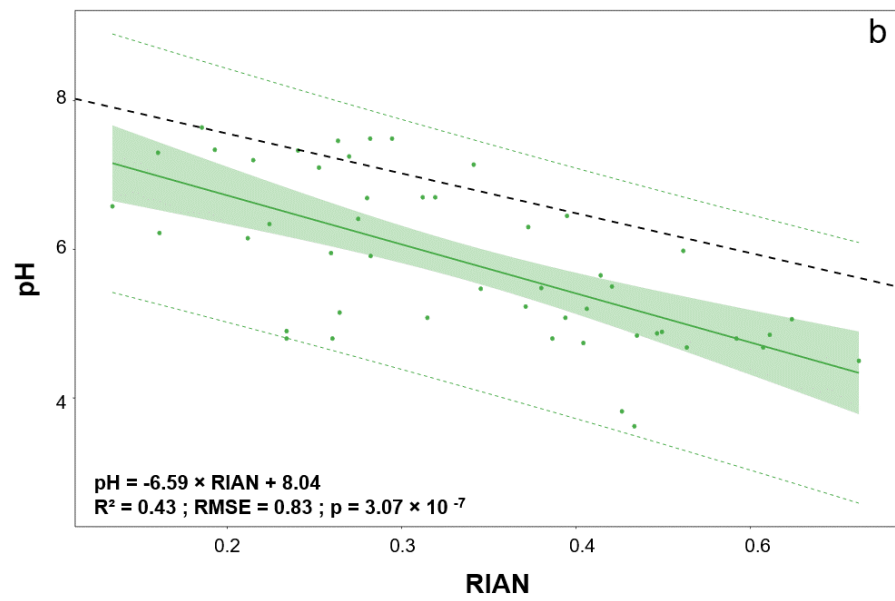
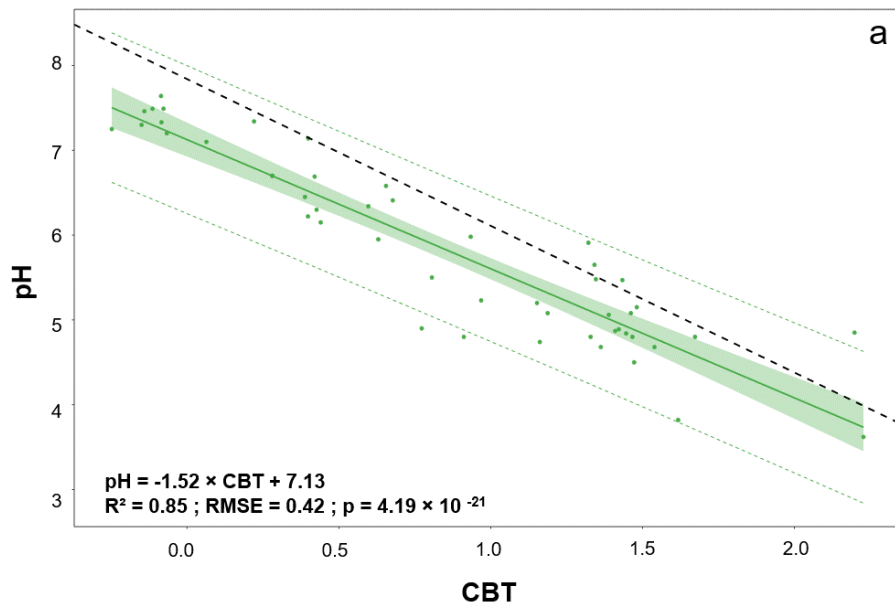


Figure 4

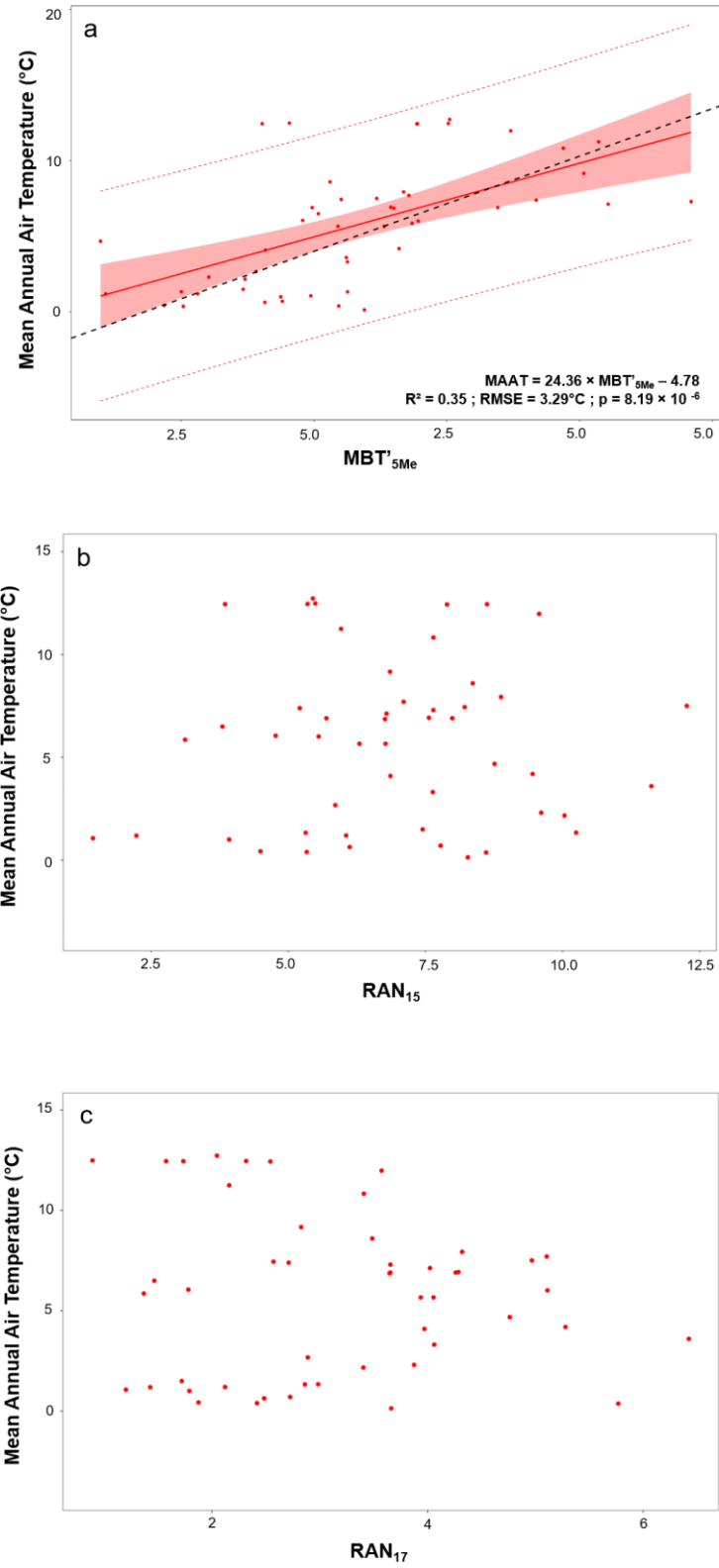


Figure 5

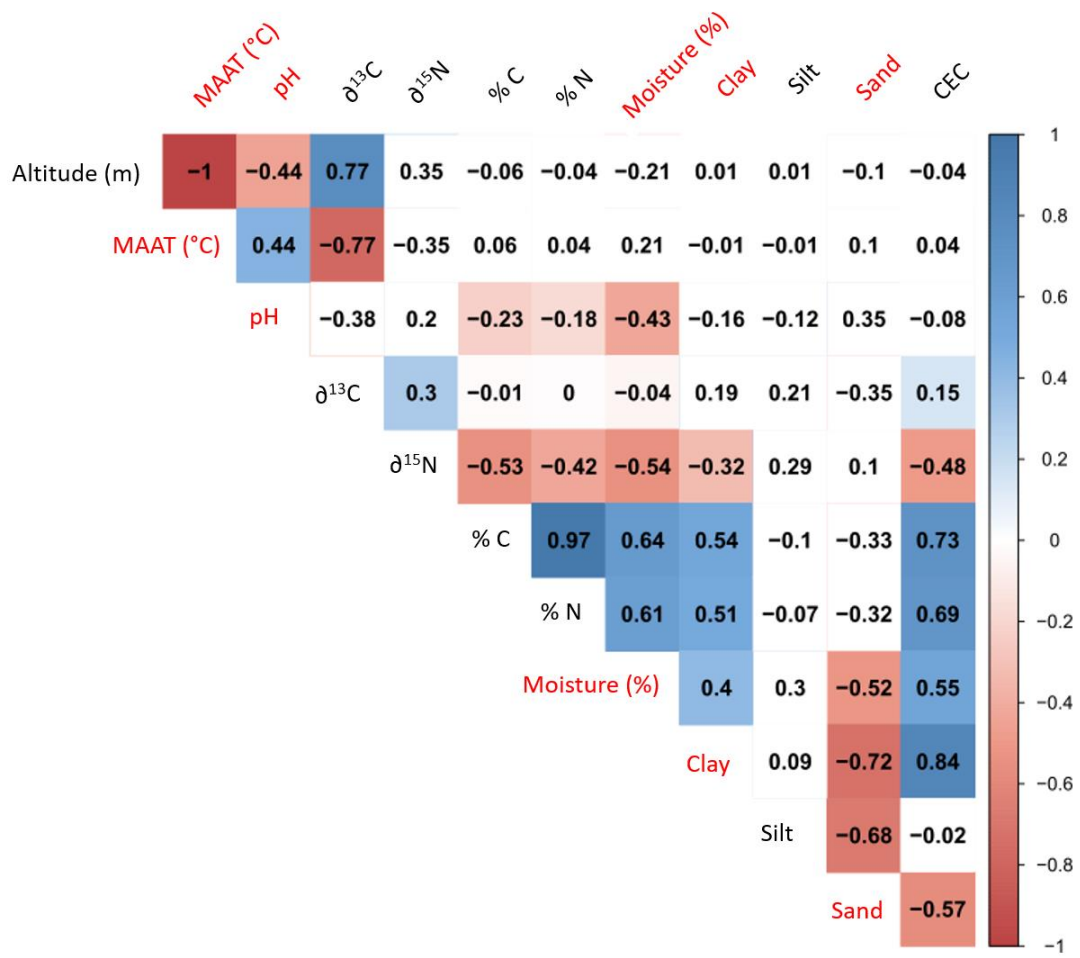
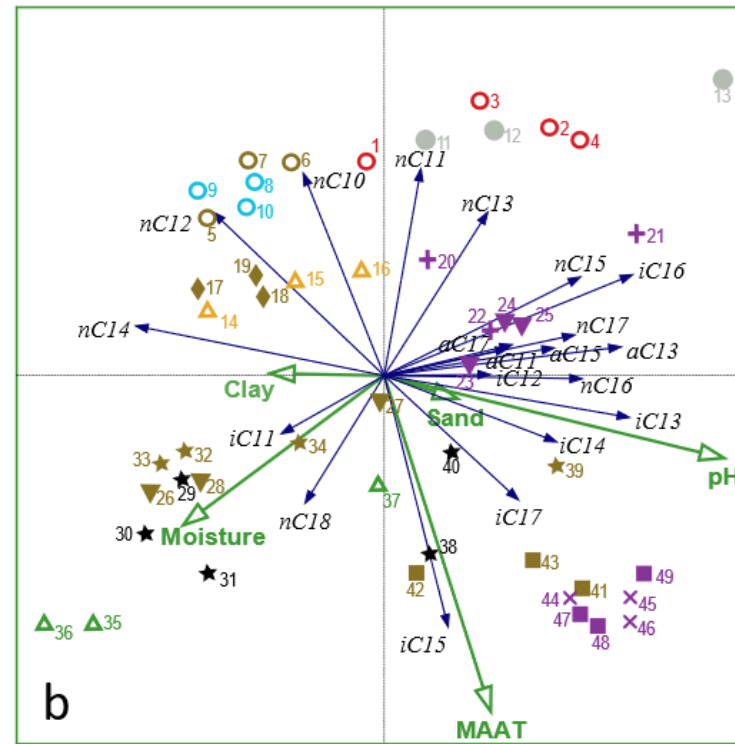
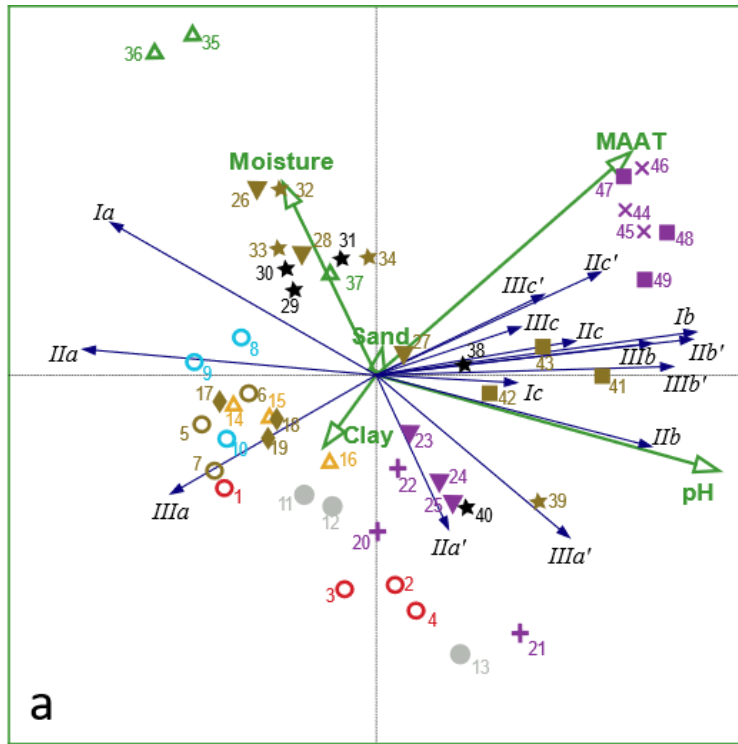


Figure 6



- Alpine meadow
- Rocks
- ▲ Heathlands
- ◆ Subalpine meadow
- ⊕ Subalpine forest
- ▼ Mountainous meadow
- ★ Mountainous forest
- Lowland meadow
- × Lowland forest
- Rendisol
- Brunisol
- Colluviosol
- Lithosol
- Alocrisol
- Calcosol
- Organosol
- Podzosol

Figure 7

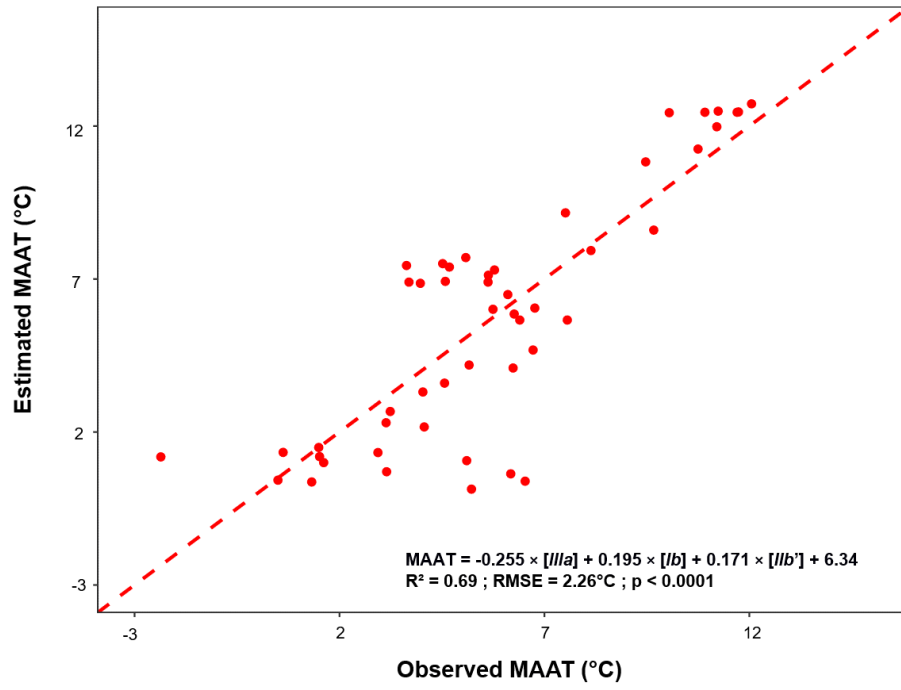


Figure 8

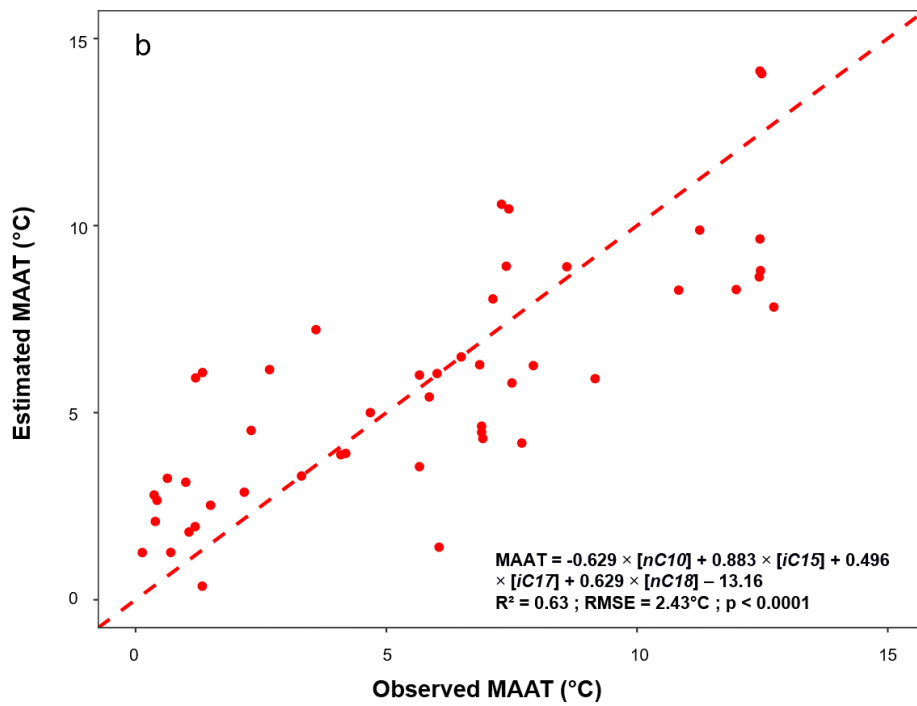
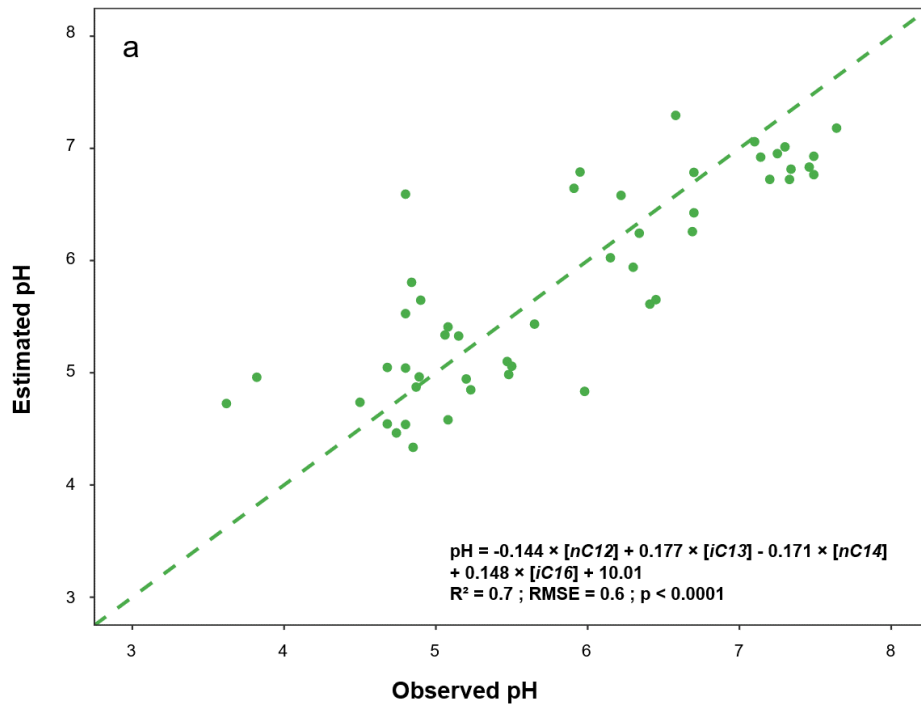


Figure 9

Samples	Massif	Coordinates	Altitude (m)	Soil Type	Vegetation
1		45° 3'35.24"N 6°23'30.96"E	2700	Rendisol	Alpine meadow (<i>Kobresia simpliciuscula</i>)
2		45° 3'35.24"N 6°23'30.96"E	2646	Rendisol	Alpine meadow (<i>Kobresia simpliciuscula</i>)
3		45° 3'36.04"N 6°21'54.02"E	2748	Rendisol	Alpine meadow (<i>Kobresia simpliciuscula</i>)
4		45° 3'32.98"N 6°22'4.63"E	2695	Rendisol	Alpine meadow (<i>Kobresia simpliciuscula</i>)
5		45° 3'15.48"N 6°24'3.11"E	2470	Brunisol	Alpine meadow (<i>Poa alpina</i>)
6		45° 3'29.19"N 6°22'22.86"E	2632	Brunisol	Alpine meadow (<i>Poa alpina</i>)
7		45° 3'33.77"N 6°22'9.07"E	2688	Brunisol	Alpine meadow (<i>Poa alpina</i>)
8		45° 3'0.43"N 6°23'6.15"E	2503	Colluviosol	Alpine meadow (<i>Carex foetida</i>)
9		45° 3'15.75"N 6°22'30.15"E	2571	Colluviosol	Alpine meadow (<i>Carex foetida</i>)
10		45° 3'13.32"N 6°22'41.51"E	2531	Colluviosol	Alpine meadow (<i>Carex foetida</i>)
11		45° 2'55.20"N 6°23'14.02"E	2504	Lithosol	Rocks
12		45° 3'1.99"N 6°22'54.88"E	2533	Lithosol	Rocks
13	Lautaret	45° 3'10.66"N 6°22'39.90"E	2558	Lithosol	Rocks
14		45° 2'38.94"N 6°24'8.20"E	2230	Alocrisol	Heathland (<i>Vaccinium myrtillus</i>)
15		45° 2'53.79"N 6°23'45.81"E	2305	Alocrisol	Heathland (<i>Vaccinium myrtillus</i>)
16		45° 2'59.32"N 6°23'32.53"E	2333	Alocrisol	Heathland (<i>Vaccinium myrtillus</i>)
17		45° 2'42.11"N 6°24'25.73"E	2100	Brunisol	Subalpine meadow(<i>Festuca paniculata</i>)
18		45° 2'26.05"N 6°25'8.51"E	1920	Brunisol	Subalpine meadow(<i>Festuca paniculata</i>)
19		45° 2'25.32"N 6°24'30.39"E	2041	Brunisol	Subalpine meadow(<i>Festuca paniculata</i>)
20		45° 2'22.35"N 6°26'6.73"E	1940	Calcosol	Subalpine forest (<i>Larix decidua</i>)
21		45° 1'33.34"N 6°27'8.63"E	1820	Calcosol	Subalpine forest (<i>Larix decidua</i>)
22		44°59'47.61"N 6°28'50.40"E	1620	Calcosol	Subalpine forest (<i>Larix decidua</i>)
23		45° 0'49.54"N 6°28'0.44"E	1620	Calcosol	Mountainous meadow
24		45° 0'4.78"N 6°28'27.34"E	1580	Calcosol	Mountainous meadow
25		44°59'39.52"N 6°28'43.30"E	1540	Calcosol	Mountainous meadow
26		45°40'2.47"N 5°58'17.07"E	1475	Brunisol	Mountainous meadow
27		45°40'14.96"N 5°58'25.39"E	1450	Brunisol	Mountainous meadow
28		45°40'29.61"N 5°59'53.47"E	1360	Brunisol	Mountainous meadow
29		45°40'31.96"N 5°59'46.20"E	1375	Organosol	Mountainous forest
30		45°40'33.31"N 5°59'51.52"E	1367	Organosol	Mountainous forest
31		45°40'27.77"N 5°59'47.87"E	1362	Organosol	Mountainous forest
32		45°40'31.96"N 5°59'46.20"E	1375	Brunisol	Mountainous forest
33		45°40'33.31"N 5°59'51.52"E	1367	Brunisol	Mountainous forest
34		45°40'27.77"N 5°59'47.87"E	1362	Brunisol	Mountainous forest
35		45°38'20.27"N 5°59'36.04"E	1286	Podzosol	Heathland (<i>Vaccinium myrtillus</i>)
36		45°38'43.73"N 5°59'26.42"E	1314	Podzosol	Heathland (<i>Vaccinium myrtillus</i>)
37	Bauges	45°38'47.71"N 5°59'24.09"E	1321	Podzosol	Heathland (<i>Vaccinium myrtillus</i>)
38		45°40'22.12"N 5°57'43.67"E	905	Organosol	Mountainous forest (<i>Fagus sylvatica</i>)
39		45°40'17.69"N 5°57'44.82"E	938	Brunisol	Mountainous forest (<i>Fagus sylvatica</i>)
40		45°40'6.46"N 5°57'41.22"E	1017	Organosol	Mountainous forest (<i>Fagus sylvatica</i>)
41		45°40'48.07"N 5°56'30.30"E	462	Brunisol	Lowland meadow
42		45°40'47.24"N 5°57'1.91"E	565	Brunisol	Lowland meadow
43		45°40'52.82"N 5°56'52.87"E	520	Brunisol	Lowland meadow
44		45°38'53.89"N 5°52'3.84"E	232	Calcosol	Lowland forest (<i>Fraxinus excelsior</i>)
45		45°38'51.44"N 5°52'3.85"E	234	Calcosol	Lowland forest (<i>Fraxinus excelsior</i>)
46		45°38'9.90"N 5°52'9.58"E	237	Calcosol	Lowland forest (<i>Fraxinus excelsior</i>)
47		45°38'54.08"N 5°51'58.44"E	232	Calcosol	Lowland meadow (<i>Carex foetida</i>)
48		45°38'59.87"N 5°52'0.01"E	232	Calcosol	Lowland meadow (<i>Carex foetida</i>)
49		45°38'22.21"N 5°52'12.53"E	234	Calcosol	Lowland meadow (<i>Carex foetida</i>)

Table 1

Samples	MAAT (°C)	pH	C org (%)	N (%)	$\delta^{13}\text{C}$	$\delta^{15}\text{N}$	Moisture (%)	Clay (g/kg)	Silt (g/kg)	Sand (g/kg)	CEC (cmol +/-kg)
1	0.4	5.1	3.8	0.31	-25.9	2.8	12.4	112	207	681	49.1
2	0.6	6.3	6.2	0.59	-25.4	3.0	27.5	273	411	316	22.6
3	0.1	6.0	5.9	0.57	-24.4	6.7	22.7	312	438	250	21.4
4	0.4	6.5	12.7	1.22	-24.9	2.8	39.4	323	407	270	38.3
5	1.5	4.7	7.0	0.62	-25.4	3.6	29.5	380	457	163	20.8
6	0.7	4.9	5.0	0.46	-25.1	4.3	30.3	290	579	131	16.1
7	0.4	4.9	7.7	0.59	-25.6	2.1	28.9	385	467	148	21
8	1.3	4.8	2.0	0.21	-25.0	4.0	26.0	248	623	129	10.3
9	1.0	4.5	4.1	0.37	-25.2	3.7	31.4	321	557	122	13.4
10	1.2	4.8	7.3	0.56	-25.7	8.5	34.1	378	461	161	19.2
11	1.3	5.7	2.0	0.18	-26.3	4.2	8.6	197	423	380	9.48
12	1.2	5.9	1.4	0.15	-25.7	4.2	9.1	136	381	483	8.27
13	1.1	7.1	1.5	0.16	-25.4	1.9	3.4	115	310	575	5.98
14	2.7	4.7	17.8	1.10	-25.8	-0.2	45.8	448	406	146	31.9
15	2.3	5.1	13.6	0.81	-25.3	0.0	44.8	368	405	227	28.6
16	2.2	5.5	17.7	1.22	-25.0	2.0	52.9	432	444	124	47.5
17	3.3	4.8	9.1	0.71	-25.5	2.9	26.3	416	424	160	23.5
18	4.2	5.2	5.9	0.50	-26.1	2.5	16.6	377	420	203	20.4
19	3.6	5.2	6.6	0.58	-25.4	3.3	22.9	412	428	160	23.1
20	4.1	6.2	6.5	0.57	-25.7	1.7	16.0	395	392	213	24.6
21	4.7	7.3	6.3	0.54	-25.7	2.6	12.2	385	399	216	19.4
22	5.7	6.3	5.1	0.34	-27.4	1.7	5.8	194	232	574	13.2
23	5.7	6.2	9.4	0.78	-27.0	1.2	23.9	254	299	447	24.6
24	5.9	6.6	7.5	0.64	-26.9	3.2	11.7	277	341	382	23.3
25	6.1	6.7	5.8	0.56	-26.4	4.2	13.6	350	381	269	20.2
26*	7.5	4.7	3.1	0.28	-26.7	1.1	31.8	321	443	236	15.8
27	6.5	6.0	3.8	0.40	-26.9	5.1	33.1	344	457	199	21
28*	7.4	5.1	5.9	0.53	-26.2	1.0	35.6	396	434	170	23.1
29*	6.0	4.9	32.9	1.89	-26.7	-5.0	61.7	513	458	29	74.7
30	6.9	4.8	49.1	2.10	-25.1	-2.50	64.7	540	428	32	64.3
31*	7.7	5.2	41.0	2.03	-39.9	-3.8	68.2	546	452	2	96
32	6.9	4.8	4.7	0.33	-25.6	-1.0	33.7	305	525	170	21.5
33	6.9	4.9	6.4	0.49	-26.5	0.0	36.4	398	458	144	26.9
34	6.9	5.5	6.8	0.42	-25.2	-0.8	39.9	345	541	114	30.5
35	7.3	3.8	29.0	1.37	-27.7	-1.8	62.7	114	191	695	11.5
36*	7.4	3.6	31.0	1.74	-28.8	-1.5	53.4	98	100	802	13.2
37	7.1	5.5	2.4	0.12	-27.9	-0.3	21.0	33	98	869	8.05
38	9.2	6.4	19.8	1.05	-27.9	-2.7	46.4	411	285	304	32.9
39*	8.6	7.2	11.6	0.88	-26.5	-1.0	28.0	432	304	264	34.7
40*	7.9	6.7	5.8	0.39	-27.1	-0.6	30.4	516	294	190	35.8
41*	12.0	7.5	8.7	0.86	-27.1	2.3	23.3	357	329	314	24.7
42	10.8	6.7	4.3	0.35	-28.4	-0.2	26.1	477	314	209	28.4
43*	11.2	7.1	1.7	0.18	-25.9	7.0	17.5	276	330	394	17.8
44*	12.5	7.3	5.2	0.53	-27.9	2.9	26.8	227	577	196	11.8
45	12.5	7.5	7.3	0.69	-28.0	1.9	26.5	181	510	309	10.8
46	12.4	7.3	2.7	0.24	-29.4	1.3	31.6	95	501	404	9.65
47*	12.7	7.3	2.2	0.23	-28.3	5.0	27.2	171	559	270	9.02
48	12.5	7.5	9.8	0.90	-28.7	2.2	47.2	327	599	74	26.9
49	12.5	7.6	2.4	0.21	-27.7	6.2	16.0	175	434	391	7.32

Table 2

Variables	brGDGTs		3-OH FAs	
	Axis 1	Axis 2	Axis 1	Axis 2
pH	0.96	-0.26	0.95	0.23
MAAT (°C)	0.71	0.62	0.29	-0.93
Moisture (%)	-0.26	0.53	-0.56	-0.41
Clay (g/kg)	-0.14	-0.2	-0.32	0.006
Sand (g/kg)	0.02	0.08	-0.21	-0.06
Expl. variation (%)	44.09	6.13	21.27	10.51
Expl. fitted variation (%)	78.47	10.91	54.39	26.84

Table 3

Variables	brGDGTs		3-OH FAs	
	Simple effects (%)	Conditional effects (%)	Simple effects (%)	Conditional effects (%)
pH	41.1 ***	41.1***	20.0 ***	20.0 ***
MAAT (°C)	25.2 ***	8.1 ***	11.2 **	10.9 ***
Moisture (%)	5.3 *	1.5 °	9.9 ***	3.6*
Clay (g/kg)	2.8	2.0 *	3.8°	2.5 °
Sand (g/kg)	1.4	3.5 **	2.1	2.2

Table 4