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### ► To cite this version:

Nina Davtian, Guillemette Ménot, Edouard Bard, Jérôme Poulénard, Pascal Podwojewski. Consideration of soil types for the calibration of molecular proxies for soil pH and temperature using global soil datasets and Vietnamese soil profiles. *Organic Geochemistry*, 2016, 101, pp.140 - 153. 10.1016/j.orggeochem.2016.09.002 . hal-01683011

**HAL Id: hal-01683011**

**<https://hal.science/hal-01683011v1>**

Submitted on 2 Feb 2024

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1 Consideration of soil types for the calibration of molecular  
2 proxies for soil pH and temperature using global soil datasets  
3 and Vietnamese soil profiles

4

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22 **ABSTRACT**

23 Distribution of branched glycerol dialkyl glycerol tetraethers (brGDGTs) in  
24 soils depends on environmental parameters such as mean annual air  
25 temperature (MAAT) and soil pH. MBT'/MBT'<sub>5ME</sub> (methylation index of  
26 branched tetraethers) and CBT/CBT' (cyclization ratio of branched  
27 tetraethers) are ratios based on the relative abundances of brGDGTs. Using  
28 these ratios, global and regional/local calibrations have been established  
29 using surface soils, but generally without any preliminary study of soil  
30 types. In this study, we reconsider global MAAT–pH/brGDGT calibrations  
31 by assigning soil types to the 358 components of a composite soil dataset.  
32 Additionally, we investigate brGDGT-derived proxies in five well-described  
33 soil profiles along an altitudinal transect in the Mount Fan Si Pan National  
34 Park, northwestern Vietnam. Our results show that at the global scale,  
35 traditional MAAT–pH/brGDGT relationships per soil type are significantly  
36 different and that MAAT(pH) residuals per soil type may differ as well, for  
37 example between Alisols and Podzols. This effect persists when 5- and 6-  
38 methyl brGDGTs are quantified separately. In addition, MAAT(pH)  
39 residuals per soil type are generally not clearly reduced, including in  
40 Leptosols, in which 6-methyl brGDGTs are present. In the Fan Si Pan  
41 transect, MBT'/CBT–MAAT estimates showed no significant deviation from  
42 expected MAATs. We find, however, that soil type effect – related to  
43 vegetation changes and contrasting soil organic carbon properties – may  
44 bias MAAT–pH estimates in the Vietnamese soil transect. Additionally, soil

45 depth plays a role which differs between the different Fan Si Pan soil  
46 profiles, likely determined by soil type and history.

47

48 *Keywords:* calibration, soil, brGDGTs, temperature, pH

49

## 50 1. Introduction

51 Branched glycerol dialkyl glycerol tetraethers (brGDGTs) are  
52 membrane lipids of high molecular weight (Fig. 1) that are of bacterial  
53 origin (Weijers et al., 2006a, 2009; Sinninghe Damsté et al., 2011, 2014).  
54 They are ubiquitous in aquatic and continental environments (Schouten et  
55 al., 2013 and references therein). At the global scale, brGDGT distributions  
56 in soils mainly depend on mean annual air temperature (MAAT) and soil pH  
57 (e.g., Weijers et al., 2007; Peterse et al., 2012). Several indices have been  
58 developed to summarize brGDGT distributions, for instance the methylation  
59 index of branched tetraethers (MBT), the cyclization ratio of branched  
60 tetraethers (CBT) and the MBT' index, which is a simplified form of MBT.  
61 These indices are linked to continental temperatures and soil pH through  
62 two global soil calibrations. However, calibration errors are about 5.0 °C and  
63 about 0.8 pH unit (Weijers et al., 2007; Peterse et al., 2012). High  
64 calibration errors are due to multiple possible biases such as soil texture,  
65 humidity and oxygenation (e.g. Peterse et al., 2009a; Huguet et al., 2010;  
66 Dirghangi et al., 2013; Menges et al., 2014; Dang et al., 2016), seasonality  
67 and/or use of atmospheric rather than soil temperature (e.g., Peterse et al.,  
68 2012; Deng et al., 2016; Wang et al., 2016). Indeed, MAAT is an  
69 approximation of mean annual soil temperature that is also influenced by  
70 other climate-dependent parameters, such as snow cover, vegetation type  
71 and soil properties (Smerdon et al., 2004). Recently, new indices such as  
72 MBT'<sub>5ME</sub>, CBT'<sub>5ME</sub> and CBT' have been defined, following analytical

73 improvements that have allowed the separate quantification of 5- and 6-  
74 methyl brGDGTs (Fig. 1; De Jonge et al., 2014). Poor brGDGT separations  
75 also explain part of the scatter in the global calibrations (De Jonge et al.,  
76 2014).

77         As also seen at the global scale, substantial scatter has been observed  
78 at the regional/local scale, including for calibrations based on altitudinal  
79 transects (Sinninghe Damsté et al., 2008; Peterse et al., 2009b; Yang et al.,  
80 2010, 2015b; Ernst et al., 2013; Liu et al., 2013; Anderson et al., 2014;  
81 Coffinet et al., 2014; Zhuang et al., 2015; Deng et al., 2016; Nieto-Moreno et  
82 al., 2016). Factors other than MAAT and pH, such as vegetation and soil  
83 type, length of growing season and topography, have been suggested as  
84 causes for part of the scatter (Sinninghe Damsté et al., 2008; Peterse et al.,  
85 2009b; Liu et al., 2013; Anderson et al., 2014; Deng et al., 2016; Nieto-  
86 Moreno et al., 2016). Contrary to the classical MBT' index, MBT'<sub>5ME</sub> is well  
87 correlated with MAAT/altitude at Mt. Shennongjia, China (Yang et al.,  
88 2015a, 2015b). It is noteworthy that only a few previous brGDGT-based  
89 studies have used well-described soils and/or profiles (Huguet et al., 2010;  
90 Zech et al., 2012; Menges et al., 2014; Yamamoto et al., 2016). A better  
91 consideration of soil pedogenesis may help researchers to properly evaluate  
92 the dependency of the observed scatters on soil physico-chemical properties.

93         In the present study, we reassess a composite global soil dataset  
94 (Huguet et al., 2010; Peterse et al., 2012; Dirghangi et al., 2013; Anderson et  
95 al., 2014; De Jonge et al., 2014) by assigning a soil type to each of its 358

96 components to investigate traditional and newest MAAT–pH/brGDGT  
97 relationships as well as MAAT(pH) residuals, per soil type. We also take  
98 advantage of a well-characterized altitudinal soil transect with an elevation  
99 range of 1400 m in the Mount Fan Si Pan National Park, Vietnam  
100 (Podwojewski et al., 2011) to investigate brGDGT distributions. We focus, in  
101 particular, on the possible vegetation/soil type and depth effect and assess  
102 how well brGDGT-derived proxies reflect MAAT and soil pH in Vietnam.

103

## 104 **2. Materials and methods**

### 105 *2.1. Soil type assignment for the global soil dataset and statistical analysis*

106 A composite global soil dataset of 358 previously analyzed samples  
107 (Supplementary Table S1; Huguet et al., 2010; Peterse et al., 2012;  
108 Dirghangi et al., 2013; Anderson et al., 2014; De Jonge et al., 2014) was  
109 used for soil type assignment. For most samples taken from the  
110 International Soil Reference and Information Centre (ISRIC), soil types  
111 were obtained using the ISRIC Soil Information System (ISIS) database  
112 (<http://isis.isric.nl>, last accessed on Thursday, August 25, 2016). For the  
113 other samples, with the exception of those analyzed by Huguet et al. (2010)  
114 which were the subject of preliminary pedological studies, the most likely  
115 soil type was selected using the SoilInfo App based on SoilGrids1km (Hengl  
116 et al., 2014; <https://soilinfo-app.org>, version 0.5.0, 15 November, 2015,  
117 accessed before the update to SoilGrids250m in June 2016).



118 Statistical analysis – correlation tests, linear regressions and  
 119 homogeneity of slopes test/analysis of covariance (ANCOVA) – was  
 120 conducted using the statistical software R 3.1.2 (R Development Core Team,  
 121 2016). Prior to the investigation of each MAAT–brGDGT and each pH–  
 122 brGDGT relationship, the outliers were eliminated using the car package  
 123 (Fox and Weisberg, 2011). After conducting repeated outlier tests until no  
 124 more samples with a Bonferroni  $p$ -value  $< 0.05$  remained, all the identified  
 125 outliers were excluded from further statistical analysis. The slope  
 126 homogeneity test was then conducted to verify whether regression slopes  
 127 per soil type for a given MAAT–brGDGT or pH–brGDGT relationship were  
 128 statistically identical – the ANCOVA being conducted only when the  
 129 homogeneity of slopes test was not significant, i.e.  $p$ -value  $\geq 0.05$ . Only soil  
 130 types with at least 10 samples after exclusion of outliers and samples with  
 131 missing data were selected for statistical analysis.

132 All MAAT–pH residuals in this study were determined using the  
 133 global soil calibrations of Peterse et al. (2012) following equations (1) and  
 134 (2), and of De Jonge et al. (2014) following equations (3) and (4):

$$\text{MAAT} = 0.81 - 5.67 \times \text{CBT} + 31.00 \times \text{MBT}' \quad (1)$$

$$\text{pH} = 7.90 - 1.97 \times \text{CBT} \quad (2)$$

$$\text{MAAT} = - 8.57 + 31.45 \times \text{MBT}'_{5\text{ME}} \quad (3)$$

$$\text{pH} = 7.15 + 1.59 \times \text{CBT}' \quad (4)$$

135 With MBT', CBT, MBT'<sub>5ME</sub> and CBT' defined as follows:

$$\text{MBT}' = \frac{\text{Ia} + \text{Ib} + \text{Ic}}{\text{Ia} + \text{Ib} + \text{Ic} + \text{IIa} + \text{IIb} + \text{IIc} + \text{IIIa} + \text{IIa}' + \text{IIb}' + \text{IIc}' + \text{IIIa}'} \quad (5)$$

$$\text{CBT} = -\log\left(\frac{\text{Ib} + \text{IIb} + \text{IIb}'}{\text{Ia} + \text{IIa} + \text{IIa}'}\right) \quad (6)$$

$$\text{MBT}'_{5\text{ME}} = \frac{\text{Ia} + \text{Ib} + \text{Ic}}{\text{Ia} + \text{Ib} + \text{Ic} + \text{IIa} + \text{IIb} + \text{IIc} + \text{IIIa}} \quad (7)$$

$$\text{CBT}' = \log\left(\frac{\text{Ic} + \text{IIa}' + \text{IIb}' + \text{IIc}' + \text{IIIa}' + \text{IIIb}' + \text{IIIc}'}{\text{Ia} + \text{IIa} + \text{IIIa}}\right) \quad (8)$$

136 Roman numerals refer to the structures in Fig. 1.

137 Prior to any residual per soil type inspection, Z-scores and Tukey box  
 138 plots were used to eliminate all outliers. Once again, only soil types with at  
 139 least 10 samples after elimination of outliers and samples with missing data  
 140 were considered.

141

## 142 2.2. *Environmental setting in the Mount Fan Si Pan National Park and* 143 *MAAT modeling*

144 The environmental setting is described in detail by Podwojewski et al.  
 145 (2011). In short, the Mount Fan Si Pan National Park (22°18'13"3N,  
 146 103°46'32"5E) is located in northwestern Vietnam, close to the Chinese  
 147 border (Fig. 2A). The Fan Si Pan Mountain range is oriented northwest–  
 148 southeast and culminates at 3143 m above modern sea level, the highest  
 149 altitude in Vietnam (Podwojewski et al., 2011). The soil bedrock is  
 150 homogenous throughout the entire mountain range and the anthropic  
 151 influences are restricted to the foothills, the rest of the mountain range  
 152 being covered with primary C<sub>3</sub> vegetation that follows a pronounced  
 153 altitudinal zonation (Fig. 2B; Podwojewski et al., 2011). The Mount Fan Si  
 154 Pan National Park is subject to a typical monsoonal climate, albeit altered

155 by altitude and orientation to the winds as air moisture is high all year  
156 round with frequent drizzle and fog (Podwojewski et al., 2011).

157 A linear ordinary least square regression of MAAT vs altitude was  
158 established using MAAT data as y values from 17 weather stations in  
159 Vietnam and China (Supplementary Table S2) in addition to the Hoang  
160 Lien Son Mountain MAAT value as reported in Podwojewski et al. (2011).  
161 The obtained slope and intercept were  $-4.5 \pm 0.2$  °C/km and  $23.2 \pm 0.2$  °C,  
162 the 1- $\sigma$  root mean square error (RMSE) was 0.6 °C and the *p*-value was  
163 much lower than 0.001. The established linear regression was then applied  
164 to the Mount Fan Si Pan National Park to derive MAAT at any altitude.

165

### 166 2.3. *Fan Si Pan soil profiles and samples*

167 The pedological study of Podwojewski et al. (2011) represented an  
168 opportunity to couple brGDGT distributions to soil types, as the latter had  
169 already been reliably determined. Ten soil pits had previously been  
170 excavated along the Fan Si Pan Mountain by Podwojewski et al. (2011). The  
171 soils were initially sampled for soil type characterization – soil organic  
172 matter properties and geochemical environment – with respect to  
173 environmental changes. Five of the initially investigated soil profiles were  
174 selected for this study (FAN 1, FAN 2 and FAN 4 to FAN 6; Fig. 2) as they  
175 occur along a well-defined transect (Fig. 2A) and cover the entire  
176 pedogenetic sequence (Fig. 2B; Podwojewski et al., 2011). The selected soil  
177 profiles and horizons are described in detail by Podwojewski et al. (2011). In

178 short, Podwojewski et al. (2011) described shallow (< 1 m deep) and very  
179 acidic soils (3.5–4.7 pH units, measured with a 1:2.5 soil:water ratio) with  
180 variable bulk organic carbon ( $C_{org}$ ) concentrations (0.4–44.1%) and high  
181 water content values (40–215%).  $\delta^{13}C$  analyses enabled Podwojewski et al.  
182 (2011) to confirm  $C_{org}$  inputs from dominant  $C_3$  vegetation at every altitude.  
183 Podwojewski et al. (2011) also made a floristic inventory at each soil  
184 sampling site (Fig. 2B) and distinguished three main climate/vegetation-  
185 dependent soil types:

- 186 (i) Low altitude Acrisols/Alisols – strongly weathered acidic soils with  
187 low base saturation at some depth, typical under warm and wet  
188 conditions (Podwojewski et al., 2011 and references therein; IUSS  
189 Working Group WRB, 2015) – represented by FAN 1 and FAN 2.
- 190 (ii) Mid-altitude Podzols – acidic soils with an organic layer and an  
191 ash-looking eluvial horizon directly underlain by the illuvial *spodic*  
192 or Bs horizon due to the accumulation of organo-metallic (Fe, Al)  
193 complexes leached by rainwater, typical in cold/temperate forests  
194 and in tropical mountain forests (Podwojewski et al., 2011 and  
195 references therein; IUSS Working Group WRB, 2015) –  
196 represented by FAN 4 and FAN 5.
- 197 (iii) High altitude Umbrisols – soils with dark topsoil, thick, well-  
198 humified and desaturated in the Mount Fan Si Pan National Park,  
199 typical under humid conditions with little or no moisture deficit in  
200 cool/temperate areas and in (sub)tropical mountain shrub and

201 grasslands (Podwojewski et al., 2011 and references therein; IUSS  
202 Working Group WRB, 2015) – represented by FAN 6.

203

#### 204 2.4. Sample preparation and *brGDGT* analysis

205 The samples were air dried and sieved through a 2 mm sieve before  
206 being ground and homogenized using a mortar and pestle. Between 0.8 and  
207 2.2 g of each sample, depending on  $C_{org}$  concentration, was then extracted  
208 with DCM:MeOH (9:1, v:v) using an accelerated solvent extractor ASE 350  
209 Dionex at 120 °C and  $10^7$  Pa. Total lipid extracts were separated following  
210 the automated procedure established by Sanchi et al. (2013).

211 BrGDGT analyses were performed at CEREGE by high-performance  
212 liquid chromatography–atmospheric pressure chemical ionization mass  
213 spectrometry (HPLC–APCI-MS) using positive ions on a HPLC–MS 1100  
214 Series spectrometer, following Sanchi et al. (2014).

215 The MBT' and CBT indices were calculated using equations (5) and  
216 (6). Since no local/regional calibration has yet been established for Vietnam,  
217 MAAT and pH were estimated using the extended global soil calibration  
218 developed by Peterse et al. (2012), equations (1) and (2).

219 The BIT index was calculated following Hopmans et al. (2004):

$$\text{BIT} = \frac{\text{Ia} + \text{IIa} + \text{IIIa} + \text{IIa}' + \text{IIIa}'}{\text{Cren} + \text{Ia} + \text{IIa} + \text{IIIa} + \text{IIa}' + \text{IIIa}'} \quad (9)$$

220 Roman numerals refer to the structures in Fig. 1.

221 Based on duplicate injections of the entire sample set, the mean  
222 analytical 1- $\sigma$  standard deviations (SD, mean of 1- $\sigma$  SD to individual mean

223 values) were 0.003 for MBT', 0.006 for CBT and 0.001 for BIT. These  
224 correspond to 0.1 °C and 0.01 pH unit mean analytical uncertainties.

225

### 226 **3. Results**

#### 227 *3.1. Global MAAT–pH/brGDGT relationships and MAAT–pH residuals* 228 *per soil type*

229 Twenty-seven different soil types were represented in the 358-  
230 component composite global soil dataset (Fig. 3). Visually, the scatters per  
231 soil type were not all similar and some soil types such as Luvisols and  
232 Regosols were mainly clustered into relatively small MAAT–pH/brGDGT  
233 domains and/or comprised a few outliers (Fig. 3 and Supplementary Fig.  
234 S1). The composite dataset allowed us to extend the number of soil types  
235 suitable for statistical analysis from eight to twelve – from 174 to 298 soil  
236 samples. MAAT–pH/brGDGT relationships (Tables 1 and 2) and MAAT(pH)  
237 residual distributions (Fig. 4) were thus determined for the most  
238 represented soil types only. A few examples of soil types were also selected  
239 for more detailed MAAT residual distributions (Fig. 5). The soil samples  
240 reconsidered by De Jonge et al. (2014) were used to compare the newest  
241 with traditional MAAT–pH/brGDGT relationships per soil type, as well as  
242 MAAT(pH) residual distributions per soil type using traditional and newest  
243 global soil calibrations (Figs. 4 and 5, Table 2).

244 Using the composite global soil dataset, five soil types had non-  
245 significant MAAT–MBT'/CBT relationships and seven soil types had non-

246 significant pH–CBT relationships (Table 1). For MAAT–MBT'/CBT, the  $R^2$   
247 range was 0.0–0.8 and the RMSE range was 2.2–7.0 °C. For pH–CBT, the  $R^2$   
248 range was 0.0–0.8 and the RMSE range was 0.4–1.0 pH units (Table 1).  
249 Ranges of MAAT–MBT'/CBT coefficients were 46.9 for MBT' slope, 11.7 for  
250 CBT slope and 24.7 for intercept (Table 1). Some pairs of soil types, – for  
251 example Andosols and Cambisols – had similar coefficients whereas other  
252 pairs of soil types such as Cambisols and Luvisols had at least one  
253 coefficient that was significantly different (Table 1). Ranges of pH–CBT  
254 coefficients were 2.4 for slope and 3.4 for intercept (Table 1). After the  
255 homogeneity of slopes test ( $F = 4.83, p < 0.001$ ), pH–CBT slopes per soil type  
256 were not all similar. For instance, they were similar between Cambisols and  
257 Leptosols, but not between Cambisols and Luvisols (Table 1). When  
258 compared to the global MAAT–pH/brGDGT relationships, three soil types  
259 had similar MAAT–MBT'/CBT coefficients and three soil types had similar  
260 pH–CBT coefficients (Table 1).

261         Using the global soil dataset of De Jonge et al. (2014), two or three  
262 soil types had non-significant traditional and new MAAT–pH/brGDGT  
263 relationships (Table 2). For MAAT–MBT'/CBT vs MAAT–MBT'<sub>5ME</sub>, the  $R^2$   
264 range was 0.1–0.9 vs 0.0–0.9 and the RMSE range was 1.6–6.8 °C vs 2.4–5.6  
265 °C. For pH–CBT vs pH–CBT', the  $R^2$  range was 0.1–0.7 vs 0.0–0.9 and the  
266 RMSE range was 0.5–1.1 pH units vs 0.2–0.6 pH units (Table 2). Ranges of  
267 MAAT–MBT'/CBT coefficients were 60.6 for MBT' slope, 10.9 for CBT slope  
268 and 22.2 for intercept whereas ranges of MAAT–MBT'<sub>5ME</sub> coefficients were

269 46.2 for slope and 34.2 for intercept (Table 2). Ranges of pH–CBT vs pH-  
270 CBT' coefficients were 1.9 vs 1.8 for slope and 3.0 vs 2.8 for intercept (Table  
271 2). For all bivariate traditional and newest MAAT–pH/brGDGT  
272 relationships, slopes/intercepts per soil type were not all similar after the  
273 homogeneity of slopes tests/ANCOVA (Table 2). When compared to the  
274 global MAAT–brGDGT relationships, four soil types had similar MAAT–  
275 MBT'/CBT coefficients and six soil types had similar MAAT–MBT'<sub>5ME</sub>  
276 coefficients (Table 2). When compared to the global pH–brGDGT  
277 relationships, three soil types had similar pH–CBT coefficients and two soil  
278 types had similar pH–CBT' coefficients (Table 2).

279         Using the composite global soil dataset, mean MAAT residuals per  
280 soil type based on the MAAT–MBT'/CBT global soil calibration of Peterse et  
281 al. (2012) varied between –7.0 and 3.5 °C (Figs. 4A, 5A and 5B). Mean pH  
282 residuals per soil type based on the pH–CBT global soil calibration of  
283 Peterse et al. (2012) varied between –1.3 and 0.4 pH units (data not shown).

284         Using the global soil dataset of De Jonge et al. (2014), mean MAAT  
285 residuals per soil type based on the MAAT–MBT'/CBT global soil calibration  
286 of Peterse et al. (2012) varied between –7.5 and 3.5 °C (Figs. 4B, 5C and 5E).  
287 Mean MAAT residuals per soil type based on the MAAT–MBT'<sub>5ME</sub> global soil  
288 calibration of De Jonge et al. (2014) varied between –3.5 and 2.5 °C (Figs.  
289 4C, 5D and 5F). Mean pH residuals per soil type based on the pH–CBT  
290 global soil calibration of Peterse et al. (2012) varied between –1.4 and 0.2  
291 pH units (data not shown). Mean pH residuals per soil type based on the



292 pH–CBT' global soil calibration of De Jonge et al. (2014) varied between –0.2  
293 and 0.3 pH units (data not shown).

294

### 295 3.2. *BrGDGT distributions and proxies in the Fan Si Pan National Park*

296 BrGDGTs were present in all Fan Si Pan soil samples. Compounds Ia  
297 and IIa/IIa' were the most abundant in each sample, representing together  
298 at least 86% of total brGDGTs (Supplementary Table S3). Conversely,  
299 compounds IIIb/IIIb' and IIIc/IIIc' were all reliably detected in only one-  
300 third of the samples, precluding the use of MBT and associated soil  
301 calibrations.

302 BIT, MBT' and CBT were calculated for all Fan Si Pan soil samples  
303 (Fig. 6, Table 3). The BIT range was 0.94–1.00, the MBT' range was 0.57–  
304 0.93 and the CBT range was 1.02–2.01 (Table 3). The MBT'/CBT-based  
305 MAAT estimate range was 10.0–20.5 °C using the global soil calibration of  
306 Peterse et al. (2012), corresponding to differences with modeled MAAT  
307 between –2.8 and 6.2 °C – on average  $2.3 \pm 1.7$  °C (Table 3). The CBT-based  
308 pH estimate range was 3.9–5.9 pH units using the global soil calibration of  
309 Peterse et al. (2012), corresponding to differences with measured pH  
310 between –0.1 and 2.1 pH units – on average  $0.9 \pm 0.6$  pH units (Table 3).

311 BIT varied with depth by maximum 0.04 in all soil profiles (Fig. 6).  
312 MBT' decreased with depth by 0.14–0.21 in Podzols (even if FAN 5 had only  
313 three points), looked globally stable in FAN 1 (also with only three points),  
314 and was stable in the other soil profiles. CBT was stable in FAN 5,

315 fluctuated by 0.25–0.50 in FAN 6, and decreased with depth by 0.24–0.66 in  
316 the other soil profiles. CBT-based pH variations with depth were opposite  
317 and roughly twice as high as CBT variations with depth in all soil profiles.  
318 Measured pH increased with depth by 0.5–1.3 pH units in all soil profiles,  
319 except FAN 5 with only two points (Fig. 6). However, in FAN 4 and FAN 6,  
320 changes in measured pH with depth shifted from decreases in A horizons to  
321 increases in B horizons, with variations of 0.2–0.6 pH units in FAN 6 and of  
322 0.4–1.0 pH units in FAN 4. MBT/CBT-based MAAT decreased with depth  
323 by about 4–6 °C in Podzols, increased by about 2.5 °C in Acrisols/Alisols, and  
324 was stable in Umbrisols (Fig. 6).

325

## 326 4. Discussion

### 327 4.1. Global soil dataset

#### 328 4.1.1. Soil type diversity and implications for MAAT–pH reconstructions

329 The 27 soil types represented in the 358-component composite global  
330 soil dataset denote its high diversity (Fig. 3 and Supplementary Fig. S1,  
331 Supplementary Table S1). Most of the soil samples were taken from surficial  
332 and/or A horizons (Supplementary Table S1). Thus, we may assume that soil  
333 depth has no significant effect on the global brGDGT distributions  
334 considered in this study.

335 Pedogenetic processes depend on multiple factors such as climate,  
336 vegetation type, topography and parent material (e.g., IUSS Working Group  
337 WRB, 2015). These differential processes in soil formation and erosion may

338 have an impact on the production, transformation and preservation of labile  
339 substrates for brGDGT producers, including in surficial horizons. Indeed,  
340 brGDGT producers are partially attributable to *Acidobacteria* (Weijers et  
341 al., 2009; Sinninghe Damsté et al., 2011, 2014) and their metabolism is  
342 thought to be the heterotrophic assimilation of labile substrates or the  
343 chemoautotrophic consumption of respired CO<sub>2</sub> (Weijers et al., 2010).

344         Given the specificity of soil types to climatic ranges such as moisture  
345 regime/mean annual precipitation (MAP, Fig. 4, Supplementary Table S1),  
346 some soil types have small MAAT and/or pH domains that may be  
347 associated with small MBT' and/or CBT domains, which may result in non-  
348 significant MAAT–pH/brGDGT relationships (Fig. 3, Table 1 and  
349 Supplementary Table S1). For instance, Acrisols, Alisols, Ferralsols, Podzols  
350 and Umbrisols did not show a significant pH–CBT anti-correlation, likely  
351 due to their typically low pH values and high CBT values (Table 1 and  
352 Supplementary S1). Alisols and Ferralsols also had typically high MAAT  
353 and MBT' values, resulting in non-significant MAAT–MBT'/CBT  
354 relationships (Table 1 and Supplementary Table S1).

355         Regression coefficients and performances of MAAT–MBT'/CBT and  
356 pH–CBT differed at times between different soil types, whose MAAT–  
357 pH/brGDGT relationships were not always similar to global ones (Table 1).  
358 After the homogeneity of slopes test, at least one soil type had a  
359 significantly different pH–CBT relationship from the others: the wettest and  
360 driest soils – Acrisols, Alisols, Ferralsols, Podzols, Regosols and Umbrisols –

361 could be distinguished from another group comprising Cambisols, Fluvisols  
362 and Leptosols based on their pH–CBT slopes and associated standard errors  
363 (Table 1). In addition, some soil types such as Alisols, Fluvisols, Phaeozems  
364 and Regosols had peculiar MAAT–MBT'/CBT relationships when compared  
365 with most other soil types (Table 1). This suggests that high soil  
366 heterogeneity has an impact on (acido)bacterial communities involved in  
367 brGDGT production (Jones et al., 2009) and that different communities may  
368 respond in different ways to environmental changes.

369         The soil type effect on brGDGTs and their producers is also supported  
370 by the different distributions of MAAT residuals per soil type (Fig. 4A), as  
371 well as pH residuals per soil type to a lesser extent (data not shown). Six  
372 soil types had mainly negative residuals, two soil types had mainly positive  
373 residuals and four soil types had roughly equal numbers of positive as  
374 negative residuals (Fig. 4A). These three groups of soil types in terms of  
375 MAAT residuals were not distinguished by their typical moisture regimes  
376 (Fig. 4A, Supplementary Table S1). In addition, some soil types such as  
377 Alisols and Podzols had very high MAAT residuals (Figs. 4A, 5A and 5B).  
378 Thus, (almost) systematic biases on MAAT–pH reconstructions may occur  
379 for a given soil type when using global soil calibrations. This also implies  
380 that not all soil types contribute equally to global scatters (Figs. 4A, 5A, 5B  
381 and Supplementary Fig. S1).

382

383 4.1.2. *Traditional vs improved chromatographic method: is there an impact*  
384 *on the soil type effect?*

385 Using the soil samples reconsidered by De Jonge et al. (2014), we  
386 assess if 5- and 6-methyl brGDGT producers are influenced by the very high  
387 soil heterogeneity, following the hypothesis that 5- and 6-methyl brGDGTs  
388 are not produced by the same microorganisms (De Jonge et al., 2014).  
389 Regression coefficients and performances differed at times between different  
390 soil types for traditional and newest MAAT–pH/brGDGT relationships  
391 (Table 2). In addition, not all soil types had similar traditional and new  
392 MAAT–pH/brGDGT relationships to the global ones. After the homogeneity  
393 of slopes tests/ANCOVA, at least one soil type had a significantly different  
394 pH–CBT, MAAT–MBT'<sub>5ME</sub> and pH–CBT' relationship from the others (Table  
395 2). Acrisols, Ferralsols, Podzols and Regosols could be distinguished from  
396 Cambisols, Fluvisols and Leptosols by their pH–CBT slopes (Table 2). Some  
397 soil types such as Fluvisols and Regosols had peculiar MAAT–MBT'/CBT  
398 relationships when compared with most other soil types (Table 2). The main  
399 soil types generally had similar MAAT–MBT'<sub>5ME</sub> slopes, but Fluvisols had a  
400 significantly higher intercept than the other soil types (Table 2). Cambisols,  
401 Leptosols and Regosols could be distinguished from Fluvisols and Podzols by  
402 their pH–CBT' slopes (Table 2). Thus, the effect of soil type on MAAT–  
403 pH/brGDGT relationships remains significant when 5- and 6-methyl  
404 brGDGTs are quantified separately.

405           When considering MAAT–MBT'<sub>5ME</sub> instead of MAAT–MBT'/CBT per  
406 soil type,  $R^2$  changes varied between –0.4 and 0.2, and RMSEs changes  
407 varied between –1.2 and 1.1 °C (Table 2). When considering pH–CBT'  
408 instead of pH–CBT per soil type,  $R^2$  changes varied between –0.5 and 0.8,  
409 and RMSEs changes varied between –0.7 and 0.0 pH units (Table 2).  
410 Therefore, MAAT–pH/brGDGT relationships per soil type – and in  
411 particular, MAAT–brGDGT relationships – are not systematically improved  
412 when 5- and 6-methyl brGDGTs are quantified separately. In addition, the  
413 improvements or deteriorations of MAAT–pH/brGDGT relationships clearly  
414 depend on soil type (Table 2).

415           Decreases in MAAT(pH) residuals per soil type were not very clear  
416 when 5- and 6-methyl brGDGTs were quantified separately, except in  
417 Regosols (Figs. 4B and 4C). A more detailed inspection of MAAT residual  
418 distributions in Leptosols and in Regosols (Fig. 5C and E) revealed that  
419 these two soil types had high and opposite MAAT residuals using the  
420 MAAT–MBT'/CBT calibration of Peterse et al. (2012). When using the  
421 MAAT–MBT'<sub>5ME</sub> calibration of De Jonge et al. (2014), MAAT residuals of  
422 four Regosols decreased much more than the other Regosols and Luvisols  
423 (Fig. 5D and F). These four Regosols highlighted by De Jonge et al. (2014)  
424 not only had MAP values  $\leq 550$  mm, but they also had very high 6-methyl  
425 brGDGT relative abundances ( $> 60\%$  of total brGDGTs; Supplementary  
426 Table S1). Despite the more substantial MAAT residual reduction in  
427 Regosols, MAAT residual distributions per soil type were still not all

428 similar, as there remained some soil types with high and/or mainly positive  
429 or negative residuals (Fig. 4C).

430         Averaged MAP values per soil type varied between 472 and 1870 mm,  
431 which suggests that moisture regime clearly depends on soil type. Likewise,  
432 averaged soil pH values per soil type varied between 4.4 and 7.3 pH units,  
433 whereas averaged 6-methyl brGDGT relative abundances per soil type  
434 varied between 1.4 and 51.7% of total brGDGTs (Supplementary Table S1).  
435 Regosols had on average the lowest MAP values, and the highest pH values  
436 and 6-methyl brGDGT relative abundances (Supplementary Table S1),  
437 which is consistent with the greatest improvement of their pH–brGDGT  
438 relationship and the greatest decreases in MAAT(pH) residuals (Fig. 4B and  
439 C, Table 2). Conversely, soil types with averaged MAP values > 1000 mm  
440 had the lowest averaged pH values and 6-methyl brGDGT relative  
441 abundances (Supplementary Table S1), so no improvement of MAAT–  
442 pH/brGDGT relationships nor clear decrease in MAAT(pH) residuals was  
443 expected. However, pH–brGDGT relationships were improved in the wettest  
444 soils (Fig. 4B and C, Table 2). In addition, soil types with averaged MAP  
445 values between 500 and 1000 mm showed no intermediate behaviors  
446 between the wettest and the driest soils (Fig. 4B and C, Table 2). This likely  
447 explains why the separate quantification of 5- and 6-methyl brGDGTs  
448 barely reduces the soil type effect on brGDGTs.

449

450 4.2. *Fan Si Pan soil transect*

451 4.2.1. *BrGDGT-derived proxies*

452 BIT values in Fan Si Pan soils (Table 3) are close to the reported  
453 mean soil BIT value of  $0.90 \pm 0.14$  (Schouten et al., 2013) and are consistent  
454 with those observed in wet and acidic soils (e.g., Weijers et al., 2006b;  
455 Loomis et al., 2011; Dirghangi et al., 2013). For MAAT, only one Fan Si Pan  
456 soil fell outside the global MAAT–MBT'/CBT RMSE of Peterse et al. (2012)  
457 of 5 °C (Table 3). For pH, half of the Fan Si Pan soils fell outside the global  
458 pH–CBT RMSE of Peterse et al. (2012) of 0.8 pH units (Table 3). As RSME  
459 means that globally 66% of the soils will fall within this error range, Fan Si  
460 Pan soils adhere quite well to the variabilities of the global soil calibration  
461 dataset of Peterse et al. (2012), especially for MAAT–MBT'/CBT.

462 The absence of correlation between measured pH and CBT ( $r = -0.11$ ,  
463  $p = 0.67$ , Fig. 6) when all Fan Si Pan soils are considered together is likely  
464 due to their very small pH domain (3.5–4.7 pH units, Table 3) and/or to the  
465 differential relationships per soil type (Fig. 6, Table 3). Indeed, CBT and  
466 measured pH were anti-correlated in Acrisols/Alisols, positively correlated  
467 in Umbrisols, and had no clear relationship in Podzols.

468 MAAT–pH reconstructions along the Fan Si Pan transect may be  
469 substantially biased by the co-elution of 5- and 6-methyl brGDGTs due to  
470 our chromatographic method. Indeed, Yang et al. (2015a) have shown that  
471 6-methyl brGDGTs significantly deteriorate the performance of MBT' as a  
472 MAAT/altitude proxy along Mt. Shennongjia, China. Additionally, MBT' is  
473 not (well) correlated with MAAT/altitude along most other altitudinal



474 transects studied so far in China, India and Tanzania (Peterse et al., 2009b;  
475 Yang et al., 2010; Ernst et al., 2013; Coffinet et al., 2014; Deng et al., 2016),  
476 which may be due to 6-methyl brGDGTs as well. Conversely, soil depth  
477 effect is not a plausible explanation as the soil samples considered for  
478 altitudinal lapse rate studies were taken from surficial horizons.

479

#### 480 *4.2.2. Soil type effect on brGDGTs: a focus on contrasting soil organic matter* 481 *properties*

482 For the Fan Si Pan surficial soils – sampled from horizons with a  
483 mean soil depth < 10 cm – MBT' and MBT'/CBT-based MAAT mainly  
484 decreased between the lowest and highest altitude soils, in particular  
485 between the Podzols FAN4.1/FAN5.1 and the Umbrisols FAN6.1/FAN6.2  
486 (Fig. 6, Table 3). Conversely, MAAT estimates did not decrease with altitude  
487 between the Acrisol FAN1.1, the Alisols FAN2.A0/FAN2.1 and the Podzol  
488 FAN4.1. Despite our difficulties in separating the effects of vegetation/soil  
489 type and adiabatic cooling on brGDGT distributions along the Fan Si Pan  
490 transect, the absence of a decrease in MAAT estimates between the four  
491 lowest altitude soils suggests the importance of the diversity of soils  
492 encountered along this transect.

493 The different main soil types present along the Fan Si Pan transect  
494 have contrasting  $C_{org}$  sources, stocks and mineralization rates (Podwojewski  
495 et al., 2011). In the Fan Si Pan Acrisols,  $C_{org}$  has a rapid turnover due to  
496 relatively high temperatures and constant inputs from leaves, resulting in a

497 thin litter layer and rapid  $C_{org}$  mineralization. In the Fan Si Pan Podzols,  
498  $C_{org}$  decomposition rate is lower, partially due to cooler temperatures and  
499 seasonal inputs from leaves (Podwojewski et al., 2011). The Fan Si Pan  
500 Podzols thus have a thicker litter layer, resulting in very high topsoil  $C_{org}$   
501 stocks. In addition, the second major change in vegetation type along the  
502 Fan Si Pan transect has induced the transition from Podzols to Umbrisols  
503 with a thinning of the litter layer, resulting in lower topsoil  $C_{org}$  stocks and a  
504 different  $C_{org}$  source (Fig. 2B; Podwojewski et al., 2011). Indeed,  $C_{org}$  inputs  
505 for Podzols are exogenic – mainly from the thick litter layer – whereas  $C_{org}$   
506 inputs for Umbrisols are endogenic, mainly from bamboo roots and thus  
507 more homogenous.

508       Substantial changes in  $C_{org}$  sources, stocks and/or mineralization  
509 rates in soils may induce changes in substrate nature and/or nutrient  
510 availability for brGDGT producers, which may modify brGDGT distributions  
511 and so bias MAAT–pH reconstructions, as suggested by previous workers  
512 such as Anderson et al. (2014). Thus, biases due to vegetation and soil types  
513 should be jointly investigated, preferentially along lateral and local soil  
514 transects with as many constant environmental parameters as possible,  
515 such as MAAT, soil temperature, vegetation type and parent material.

516

#### 517 *4.2.3. Effects of soil depth on brGDGT-derived proxies*

518       The soil collection of the Fan Si Pan transect allows to investigate five  
519 well-studied soil profiles. Substantially lower brGDGT-based MAATs in

520 deep soils as opposed to topsoils were reported in two Podzol profiles under  
521 different climates (Huguet et al., 2010), a trend that also occurred in the  
522 Fan Si Pan Podzol profiles, but not in the other Fan Si Pan soil profiles (Fig.  
523 6). Likewise, changes in CBT-based pH with depth were not similar between  
524 the different Fan Si Pan soil profiles (Fig. 6). We do not know to what extent  
525 (acido)bacterial communities change with depth in the different Fan Si Pan  
526 soil profiles nor to what extent such changes impact brGDGT distributions  
527 in soils. For this reason, we put forward various explanations for the  
528 observed changes in brGDGT distributions with soil depth.

529       Every Fan Si Pan sample was 2-mm sieved (Podwojewski et al.,  
530 2011), meaning that root-associated brGDGTs in subsoils (Huguet et al.,  
531 2013b) cannot explain the decreased MAAT estimates in the deep Fan Si  
532 Pan Podzols. Nevertheless, given that bamboo roots (abundant in the  
533 Umbrisol profile FAN 6) are more mineralizable than tree roots (abundant  
534 in the Podzol profiles FAN 4 and FAN 5), brGDGT producing communities  
535 may change differently with depth between the main Fan Si Pan soil types,  
536 resulting in differential associated biases on MAAT–pH estimates, following  
537 the hypothesis that different (acido)bacterial species do not all produce the  
538 same brGDGTs (Sinninghe Damsté et al., 2011, 2014; De Jonge et al., 2014).  
539 Alternatively, Podzols have the peculiarity of having a subsoil enriched in  
540 Fe, Al and organic matter – including lipids – due to rainwater percolation  
541 (e.g., Huguet et al., 2010; Podwojewski et al., 2011). Therefore, substantial  
542 changes with depth in biotic and abiotic processes not related to brGDGT

543 producers – for instance, lipid transfer to a non-extractable pool via organo-  
544 metallic complexation – may occur in podzol soils (e.g., Huguet et al., 2010).  
545 Nevertheless, such processes – which also include soil bioturbation by soil  
546 fauna and soil reworking by rooted trees – are unlikely to be restricted to  
547 Podzols.

548         BrGDGT distributions may also be influenced by the history of the  
549 soils. Former agricultural activities – mainly maize crops (Fig. 2B;  
550 Podwojewski et al., 2011) – may have impacted brGDGT distributions in the  
551 FAN 1 soil profile. Additionally, the transition from primary broad-leaved  
552 forests to alpine vegetation (Fig. 2B; Podwojewski et al., 2011) has shifted  
553 upwards in altitude under the effect of the present global warming (IPCC,  
554 2013). This transition is now close to the FAN 6 site, which may thus have  
555 experienced a major temporal change in vegetation type. However, the  
556 brGDGT turnover times reported so far – from a few months to less than 45  
557 years (Peterse et al., 2010; Weijers et al., 2010; Huguet et al., 2013a) – may  
558 be true for surficial soils only. Indeed, brGDGTs may be associated with the  
559 mineral phase and/or be incorporated within the macromolecular matrix in  
560 deeper soil horizons (e.g., Huguet et al., 2010), whereas microbial activity  
561 may decrease with depth, resulting in substantially longer brGDGT  
562 turnover times in deep soils. The latter is suggested by decreases in  $C_{org}$ -  
563 normalized brGDGT absolute abundances with depth in FAN 1, FAN 2 and  
564 FAN 4 (data not shown). Additionally, imprints of former communities may  
565 be muted by the post-depositional effect (e.g., Zech et al., 2012; Yamamoto et

566 al., 2016), and such an effect is indeed suggested by an increase in  $C_{org}$ -  
567 normalized brGDGT absolute abundances with depth in FAN 6 (data not  
568 shown).

569

## 570 **5. Conclusions and perspectives**

571 Soil types were assigned to a composite global dataset of 358 soils to  
572 investigate MAAT–pH/brGDGT relationships and MAAT(pH) residuals per  
573 soil type. Distributions of brGDGTs were also investigated in five soil  
574 profiles excavated along an altitudinal transect in the Mount Fan Si Pan  
575 National Park. Surrounding vegetation, geological setting, as well as soil  
576 sequence had previously been intensively characterized, providing an  
577 opportunity to investigate their impact on proxies intensively used in paleo-  
578 studies.

579 The soil type diversity of the global dataset was high, traditional  
580 MAAT–pH/relationships per soil type were not all similar and different soil  
581 types (e.g., Alisols and Podzols) did not all provide the same contribution to  
582 global MAAT(pH)/brGDGT scatters. Additionally, the soil type effect on  
583 MAAT–pH relationships remained significant when 5- and 6-methyl  
584 brGDGTs were quantified separately and no obvious reduction in  
585 MAAT(pH) residuals per soil type occurred in general (e.g., in Leptosols,  
586 which contained 6-methyl brGDGTs). In the Mount Fan Si Pan National  
587 Park, MBT/CBT-based MAATs were not significantly different to modeled  
588 MAATs when the global calibration error of 5 °C was taken into account.

589 However, the decrease in MAAT estimates along the altitudinal transect  
590 lacked clarity when the focus was on surficial soils, and the pH–CBT  
591 relationship was absent when all Fan Si Pan soils were considered. On the  
592 one hand, substantial changes in soil/vegetation type along the Fan Si Pan  
593 transect may induce biases on MAAT–pH reconstructions in surficial soils.  
594 On the other hand, MBT'/CBT indices varied slightly or substantially with  
595 depth in the Fan Si Pan soil profiles, possibly as a function of soil type and  
596 history.

597         To better understand the biases on brGDGT-based estimates, more  
598 soil profiles should be investigated along topo-sequences, as attempted in  
599 our work. Indeed, this study reveals the importance of direct collaborations  
600 between organic geochemists and pedologists, ensuring that specific organic  
601 compounds and physico-chemical soil properties are jointly investigated. To  
602 consider the evolution of soil properties through time, as well as the transfer  
603 time of soil C<sub>org</sub>, radiocarbon analyses on bulk soil C<sub>org</sub> and on specific  
604 organic compounds should be conducted. Additionally, future  
605 global/regional/local soil MAAT–pH/brGDGT calibrations should consider  
606 sample sizes of a few dozen to a few hundred per soil type to ensure  
607 adequate specificity for all given soil types.

608

## 609 **Acknowledgements**

610         G.M. thanks Anneke de Rouw and the HYDRARIDE consortium for  
611 making this study and the connection with pedologists possible through

612 discussions in the field in Cameroon. Work at CEREGE is supported by  
613 ECCOREV FR3098 (project ECCOTEMP) and the Collège de France. N.D.  
614 thanks Ecole Normale Supérieure de Lyon for providing salary support. We  
615 are grateful to two anonymous reviewers for their constructive suggestions  
616 that greatly helped us to considerably improve this manuscript.

617

## 618 **Appendix A. Supplementary material.**

619

620 *Associate Editor*—**Klaas Nierop**

621

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804

805 **Figure captions**

806 **Fig. 1.** Structures of crenarchaeol (Cren), 5-methyl (Roman numbers with  
807 no prime symbol) and 6-methyl (Roman numbers with a prime symbol)  
808 branched glycerol dialkyl glycerol tetraethers (brGDGTs, I-II-III) with the  
809 different  $\alpha 5$  and/or  $\omega 5$  positions and the different  $\alpha 6$  and/or  $\omega 6$  positions of  
810 the methyl groups.

811

812 **Fig. 2.** (A) Maps showing the locations of the study site (left-hand map) and  
813 the studied soil profiles (right-hand map). Contour interval is 200 m and  
814 contour lines indicate altitudes between 1000 and 3000 m on the right-hand  
815 map, with darker tones for higher altitudes. Modified from maps generated  
816 from GeoMapApp (<http://www.geomapapp.org>). (B) Profile showing the  
817 studied soil pits. Altitudinal zonation of vegetation types is indicated in  
818 green. The profile does not pass exactly through the soil pits, which explains  
819 why their altitudes in the profile do not correspond to their actual altitudes.  
820 Horizons are delimited and color-coded as per the samples taken by  
821 Podwojewski et al. (2011): orange-yellow tones for A horizons, blue tones for  
822 Bw horizons and green tones for Bs horizons. Like tones correspond to like  
823 horizons. The horizons considered for soil brGDGT distributions are  
824 indicated in red. Adapted from Podwojewski et al. (2011).

825

826 **Fig. 3.** BrGDGT-derived proxy data from the composite global soil dataset  
827 (Huguet et al., 2010; Peterse et al., 2012; Dirghangi et al., 2013; Anderson et



828 al., 2014; De Jonge et al., 2014), with 27 soil types being distinguished. (A)  
829 Instrumental/modeled mean annual air temperature (MAAT) vs MBT'/CBT.  
830 (B) Instrumental/modeled MAAT vs MBT'<sub>5ME</sub>. (C) Measured pH vs CBT. (D)  
831 Measured pH vs CBT'. The outliers are also shown in each plot. The red  
832 lines in plots (B), (C) and (D) correspond to the global linear regressions on  
833 the composite global soil dataset after outlier removal.

834

835 **Fig. 4.** Box-whisker plots of mean annual air temperature (MAAT) residuals  
836 per soil type showing minima and maxima with whiskers, medians with  
837 horizontal bars, 1<sup>st</sup> and 3<sup>rd</sup> quartiles with boxes, and means with pluses.  
838 Plot (A) is based on the composite global soil dataset (Huguet et al., 2010;  
839 Peterse et al., 2012; Dirghangi et al., 2013; Anderson et al., 2014; De Jonge  
840 et al., 2014). Plots (B) and (C) are based on the soils reconsidered by De  
841 Jonge et al. (2014). In plots (A) and (B), residuals are based on the MAAT–  
842 MBT'/CBT global soil calibration of Peterse et al. (2012). In plot (C),  
843 residuals are based on the MAAT–MBT'<sub>5ME</sub> global soil calibration of De  
844 Jonge et al. (2014). Only soil types comprising at least 10 soils from the  
845 global datasets are considered. When outliers are identified for a given soil  
846 type, their number is indicated as (initial number of samples – number of  
847 outliers) below the number of samples actually considered. Moisture  
848 regimes based on averaged mean annual precipitation (MAP) values per soil  
849 type are specified with the following color-coding of the numbers of samples

850 per soil type: blue if MAP > 1000 mm, orange if MAP 500–1000 mm and red  
851 if MAP < 500 mm.

852

853 **Fig. 5.** Mean annual air temperature (MAAT) residual distributions for (A)  
854 Podzols, (B) Alisols, (C) and (D) Leptosols, and (E) and (F) Regosols. Plots  
855 (A) and (B) are based on the composite global soil dataset (Huguet et al.,  
856 2010; Peterse et al., 2012; Dirghangi et al., 2013; Anderson et al., 2014; De  
857 Jonge et al., 2014). Plots (C), (D), (E) and (F) are based on the soils  
858 reconsidered by De Jonge et al. (2014). In plots (A), (B), (C) and (E),  
859 residuals are based on the MAAT–MBT'/CBT global soil calibration of  
860 Peterse et al. (2012). In plots (D) and (F), residuals are based on the MAAT–  
861 MBT'<sub>5ME</sub> global soil calibration of De Jonge et al. (2014). Gaussians indicate  
862 the mean values with their standard deviations. Filled circles represent the  
863 medians and filled diamonds represent the extreme values and the 1<sup>st</sup> and  
864 3<sup>rd</sup> quartiles. When outliers are identified for a given soil type, their number  
865 is indicated as (initial number of samples – number of outliers) below the  
866 number of samples actually considered.

867

868 **Fig. 6.** BIT (Hopmans et al., 2004), MBT' (Peterse et al., 2012), CBT  
869 (Weijers et al., 2007), and MBT'/CBT-derived mean annual air temperature  
870 (MAAT) and pH based on the extended soil calibration (Peterse et al., 2012)  
871 in the studied Fan Si Pan soil profiles. Modeled MAAT and measured pH  
872 (Podwojewski et al., 2011 for the latter) are shown for comparison. Horizons

873 are delimited and color-coded as described in the caption for Fig. 2B.  
874 Symbols with error bars next to axis titles represent mean 1- $\sigma$  standard  
875 deviations to mean values of duplicates. Surficial horizons, with a mean  
876 depth < 10 cm, are indicated in red.

877

## 878 **Table captions**

### 879 **Table 1**

880 Number of samples ( $n$ ), coefficients ( $a$ ,  $b$ ,  $c$ ,  $S$ ,  $I$  and  $R^2$ ), and root mean  
881 square errors ( $E$ ) of traditional mean annual air temperature (MAAT)–  
882 pH/brGDGT linear regressions per soil type using the composite global soil  
883 dataset (Huguet et al., 2010; Peterse et al., 2012; Dirghangi et al., 2013;  
884 Anderson et al., 2014; De Jonge et al., 2014). Only soil types comprising at  
885 least 10 soils from the global dataset are considered for the linear  
886 regressions. When outliers are identified for a given MAAT–pH/brGDGT  
887 relationship per soil type, their number is indicated as (initial number of  
888 samples – number of outliers) next to the number of samples actually  
889 considered. Statistical significance is reached for  $p$ -values < 0.05.

890

### 891 **Table 2**

892 Number of samples ( $n$ ), coefficients ( $a$ ,  $b$ ,  $c$ ,  $S$ ,  $I$  and  $R^2$ ), and root mean  
893 square errors ( $E$ ) of traditional and newest mean annual air temperature  
894 (MAAT)–pH/brGDGT linear regressions per soil type using the soils of the  
895 composite global soil dataset (Huguet et al., 2010; Peterse et al., 2012;

896 Dirghangi et al., 2013; Anderson et al., 2014; De Jonge et al., 2014)  
897 reconsidered by De Jonge et al. (2014). Only soil types comprising at least 10  
898 soils from the global dataset are considered for the linear regressions. When  
899 outliers are identified for a given MAAT–pH/brGDGT relationship per soil  
900 type, their number is indicated as (initial number of samples – number of  
901 outliers) next to the number of samples actually considered. Statistical  
902 significance is reached for  $p$ -values  $< 0.05$ .

903

904 **Table 3**

905 BrGDGT-derived proxies and MBT'/CBT-derived mean annual air  
906 temperature (MAAT) and pH based on the extended soil calibration (Peterse  
907 et al., 2012) along the Fan Si Pan Mountain transect. Altitude, coordinates,  
908 horizon, mean depth, organic carbon ( $C_{org}$ ) concentration, water content  
909 ( $H_2O$ ), and measured pH are from Podwojewski et al. (2011). nd = not  
910 determined.

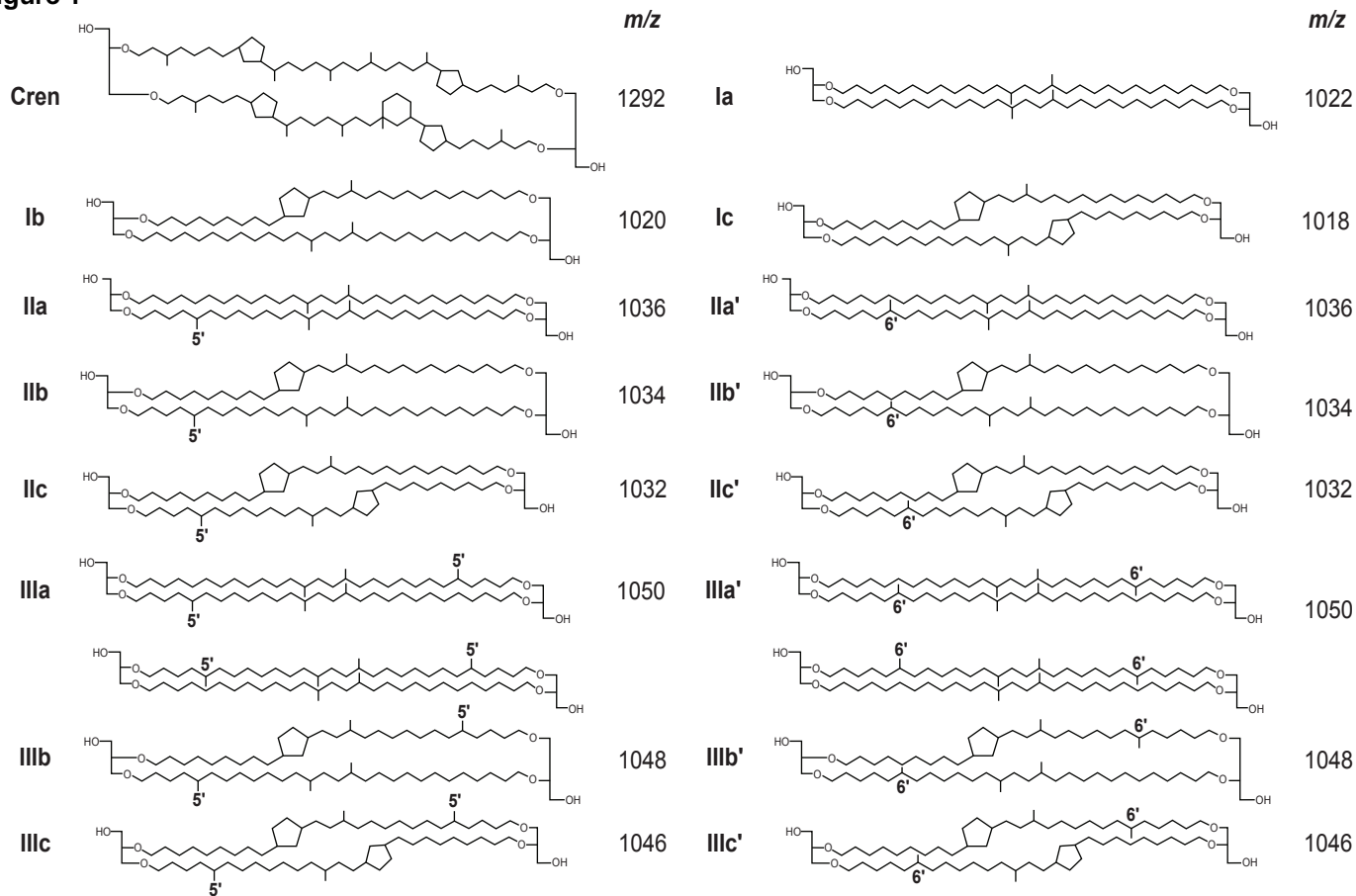
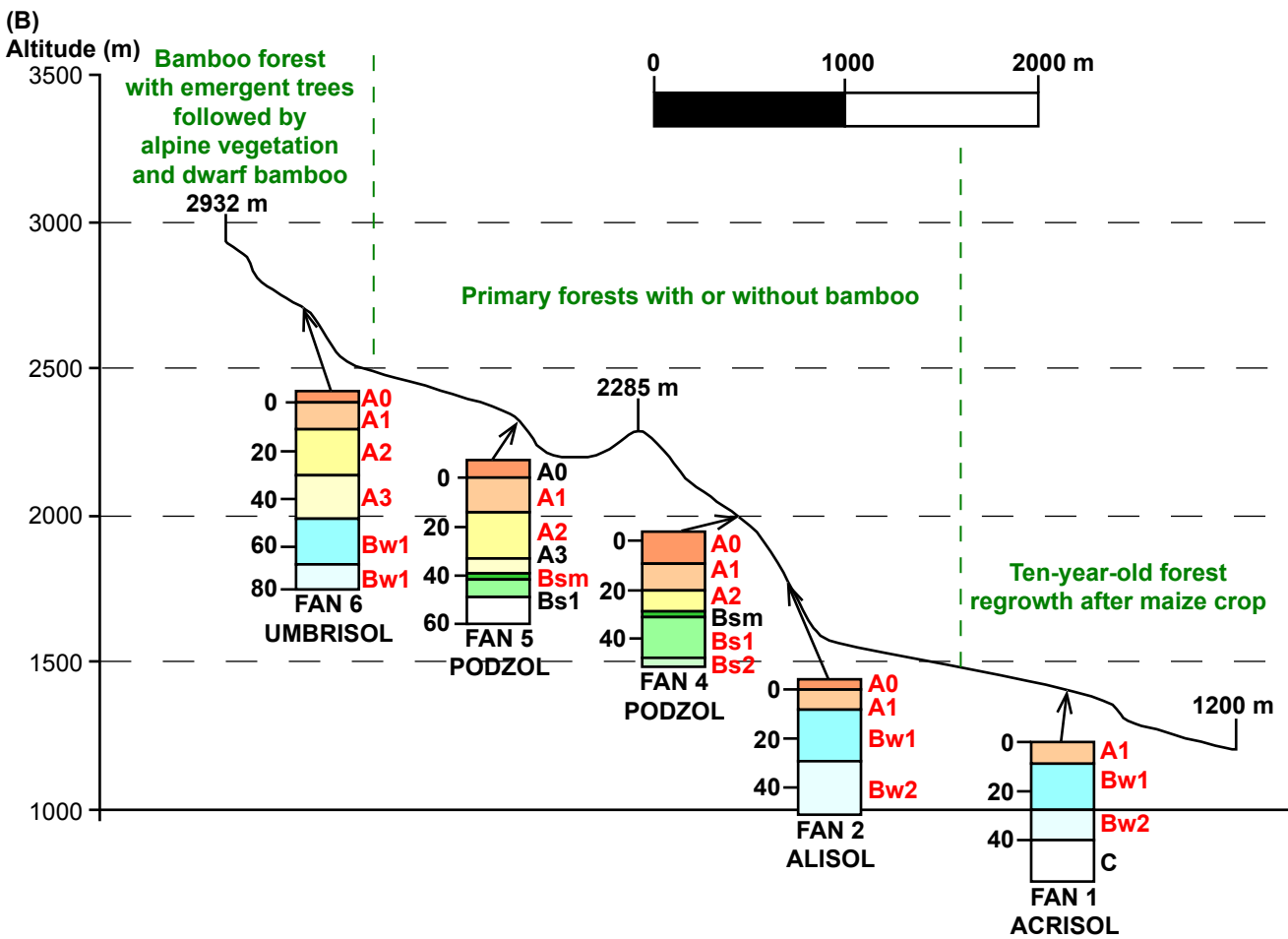
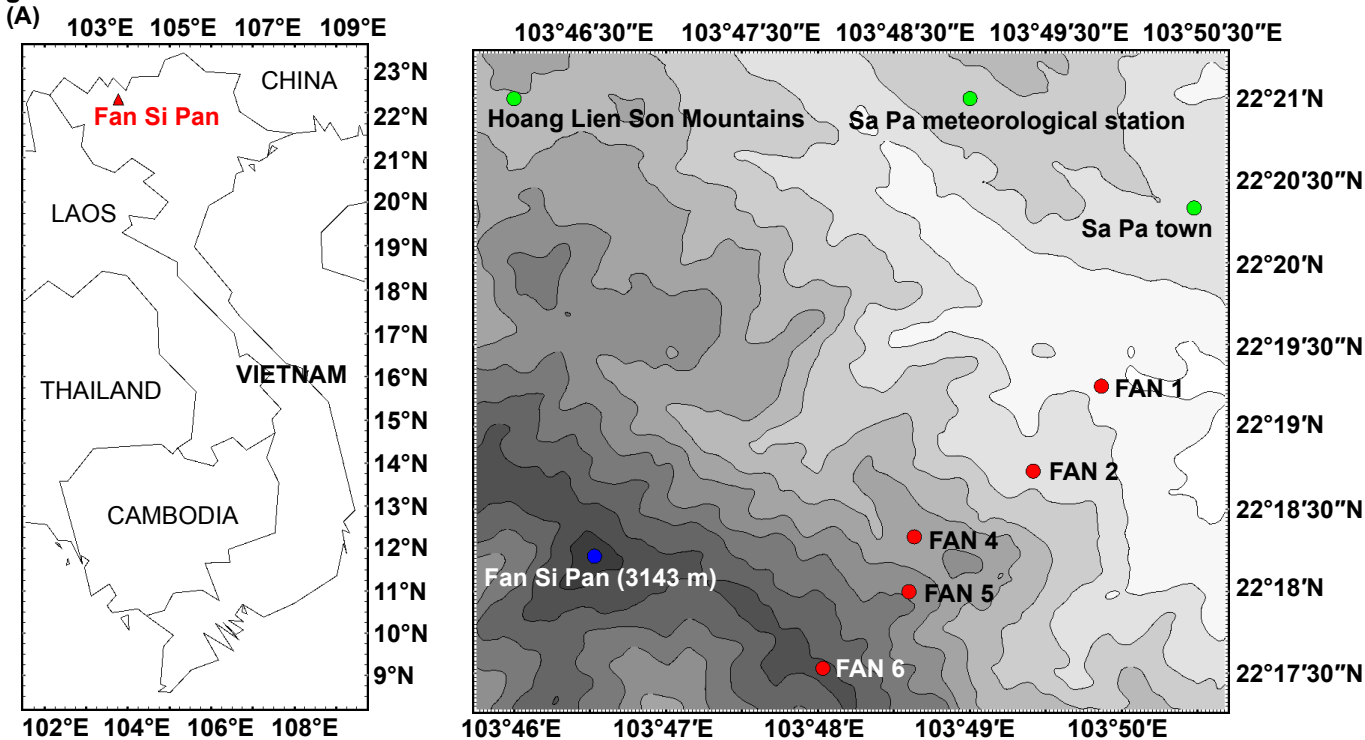
**Figure 1**

Figure 2 - revised



**Figure 3 - revised**

- |   |           |   |           |   |          |   |             |   |            |   |          |   |            |
|---|-----------|---|-----------|---|----------|---|-------------|---|------------|---|----------|---|------------|
| + | Luvisol   | ▲ | Lixisol   | ✦ | Nitisol  | ◆ | Cambisol    | ○ | Cryosol    | ★ | Histosol | ◇ | Umbrisol   |
| ◆ | Acrisol   | ✱ | Anthrosol | ✕ | Podzol   | + | Phaeozem    | △ | Andosol    | ▣ | Planosol | ■ | Kastanozem |
| ■ | Vertisol  | ▼ | Ferralsol | ✱ | Fluvisol | ◇ | Albeluvisol | ⊠ | Arenosol   | ▽ | Calcisol | ✱ | Chernozem  |
| ● | Solonchak | ▲ | Gleysol   | ★ | Alisol   | □ | Leptosol    | × | Plinthosol | □ | Regosol  |   |            |

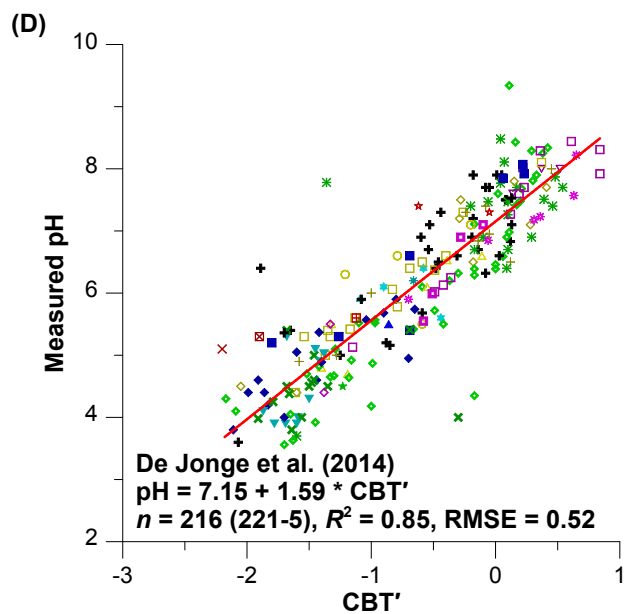
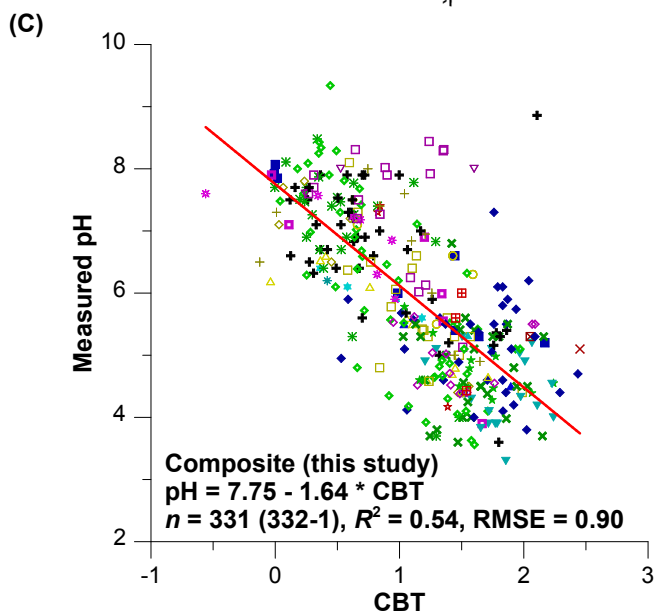
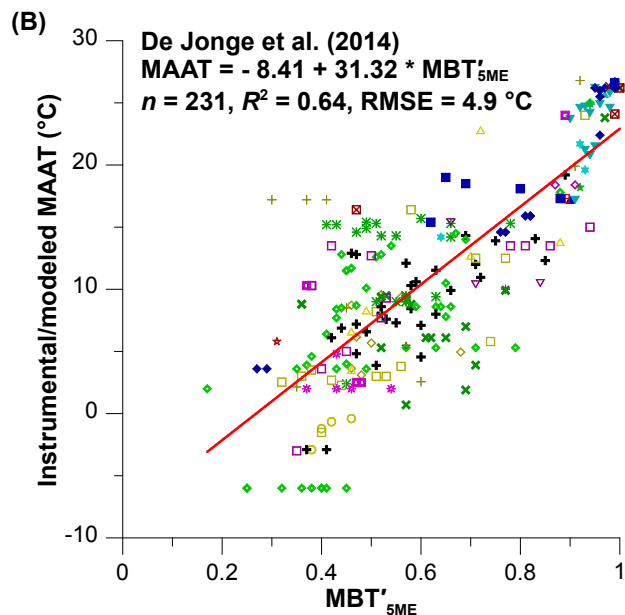
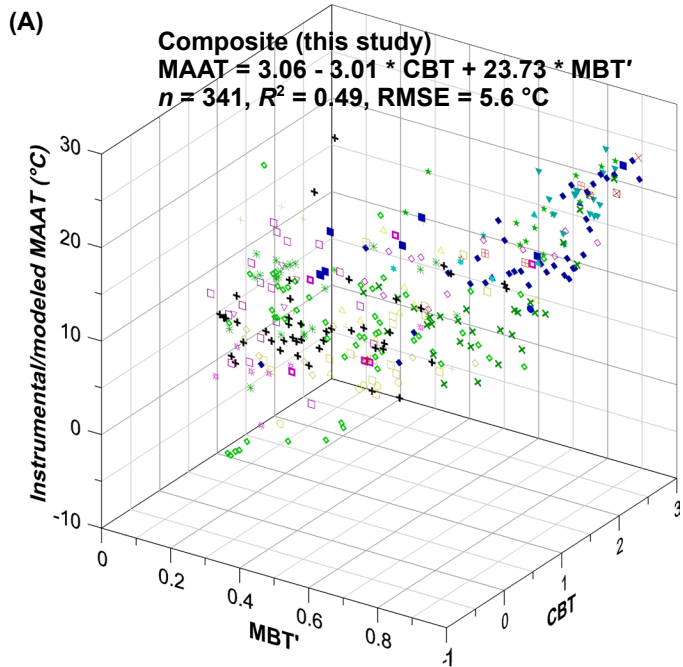
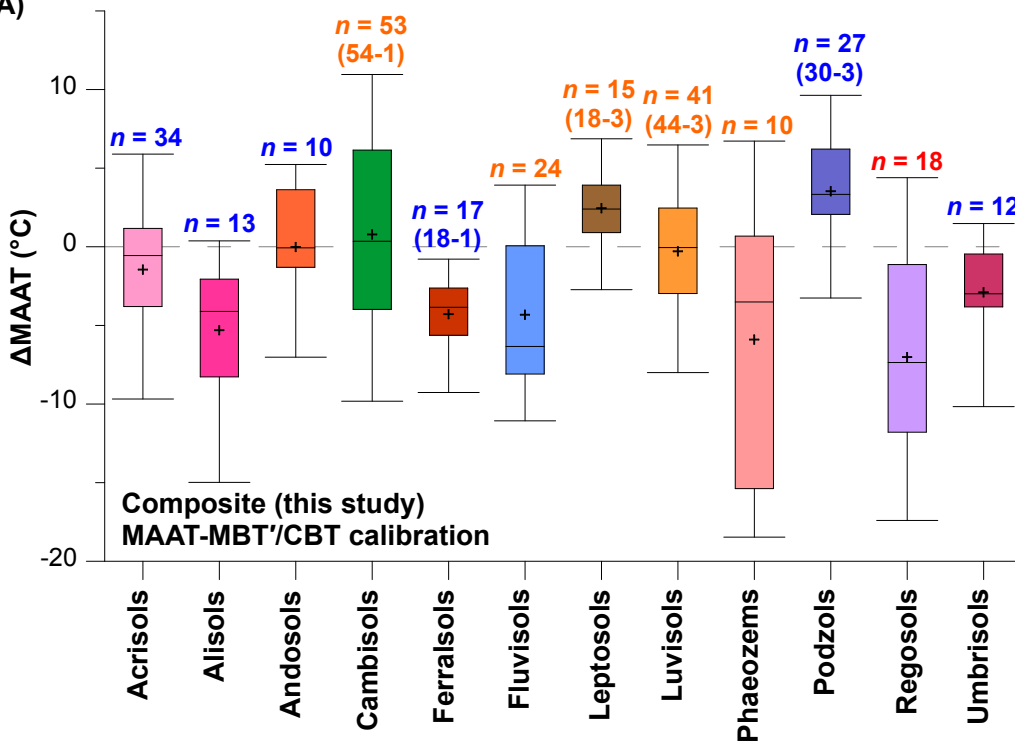
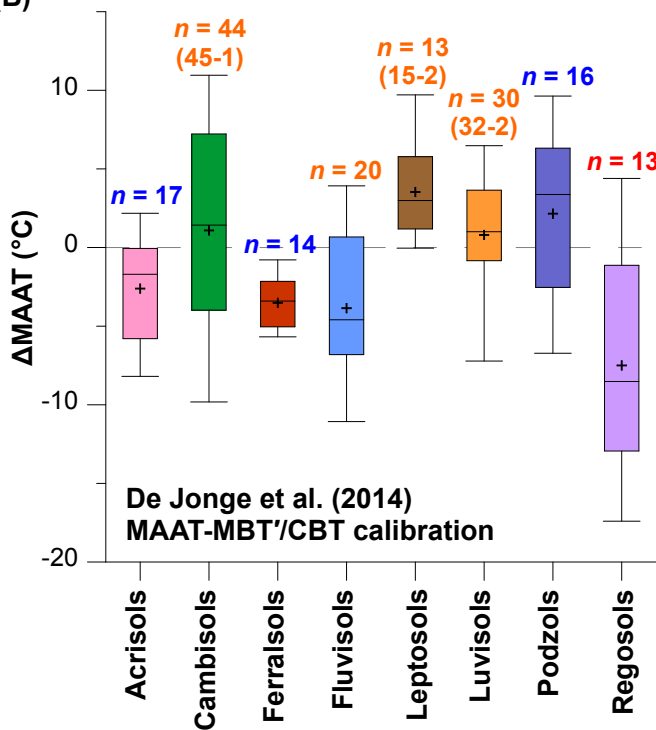


Figure 4 - revised

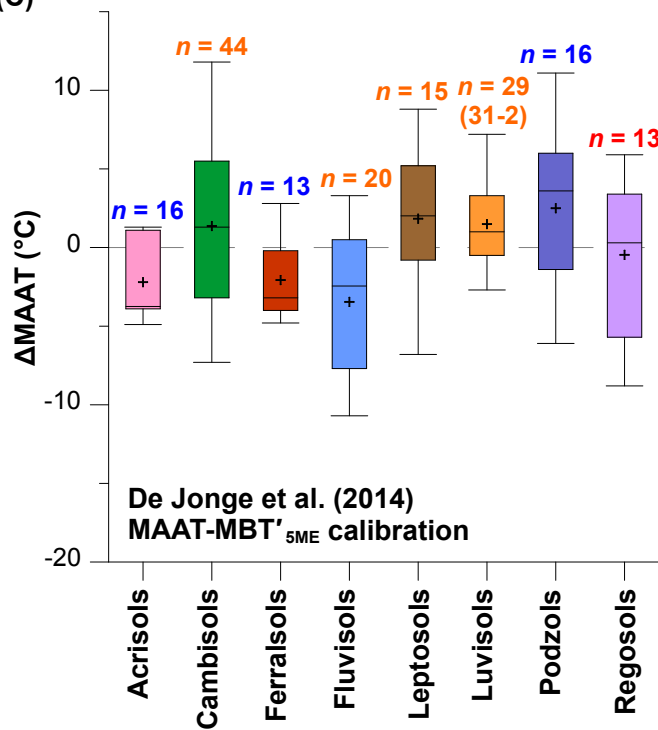
(A)



(B)



(C)





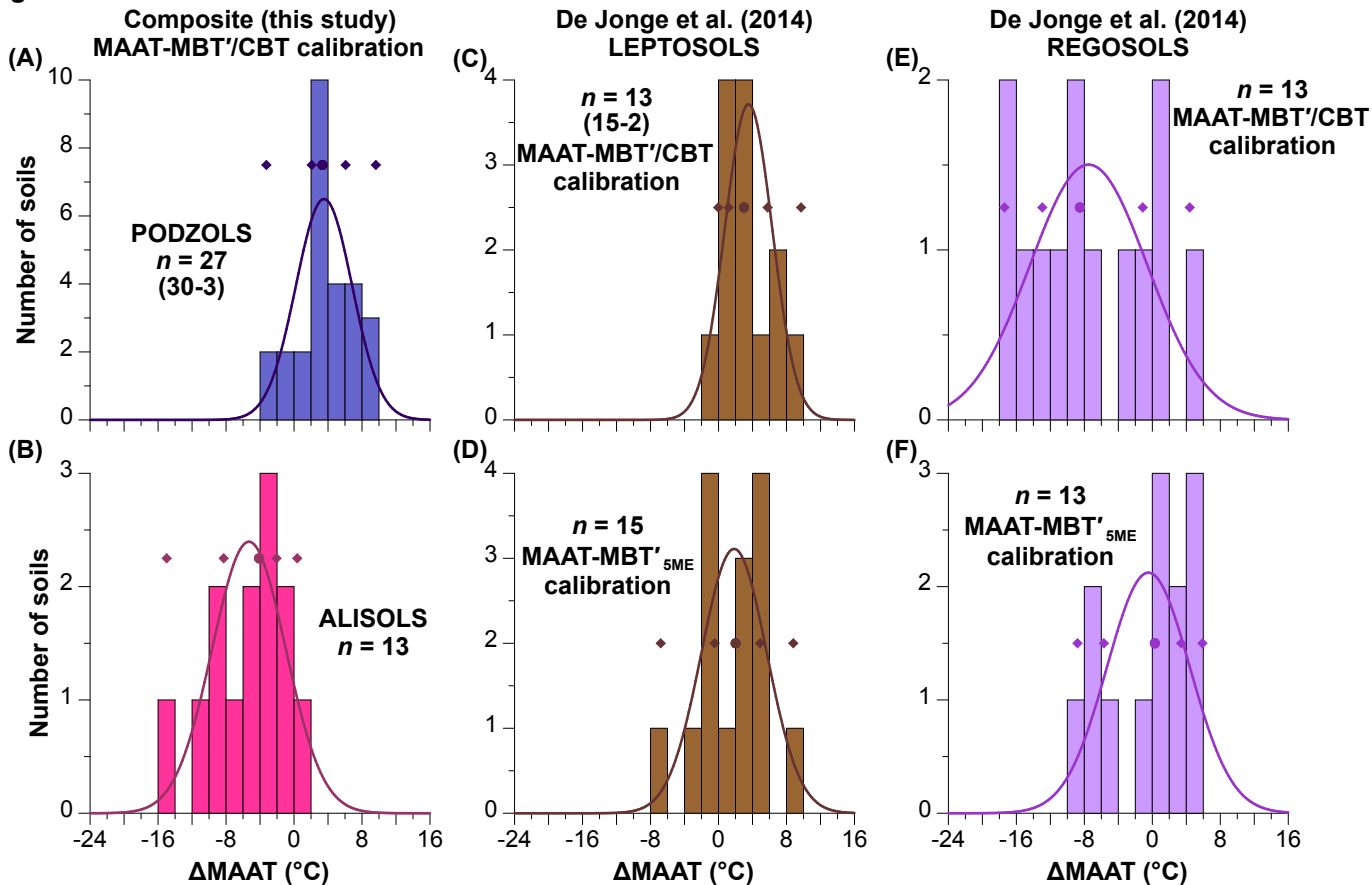
**Figure 5 - revised**

Figure 6

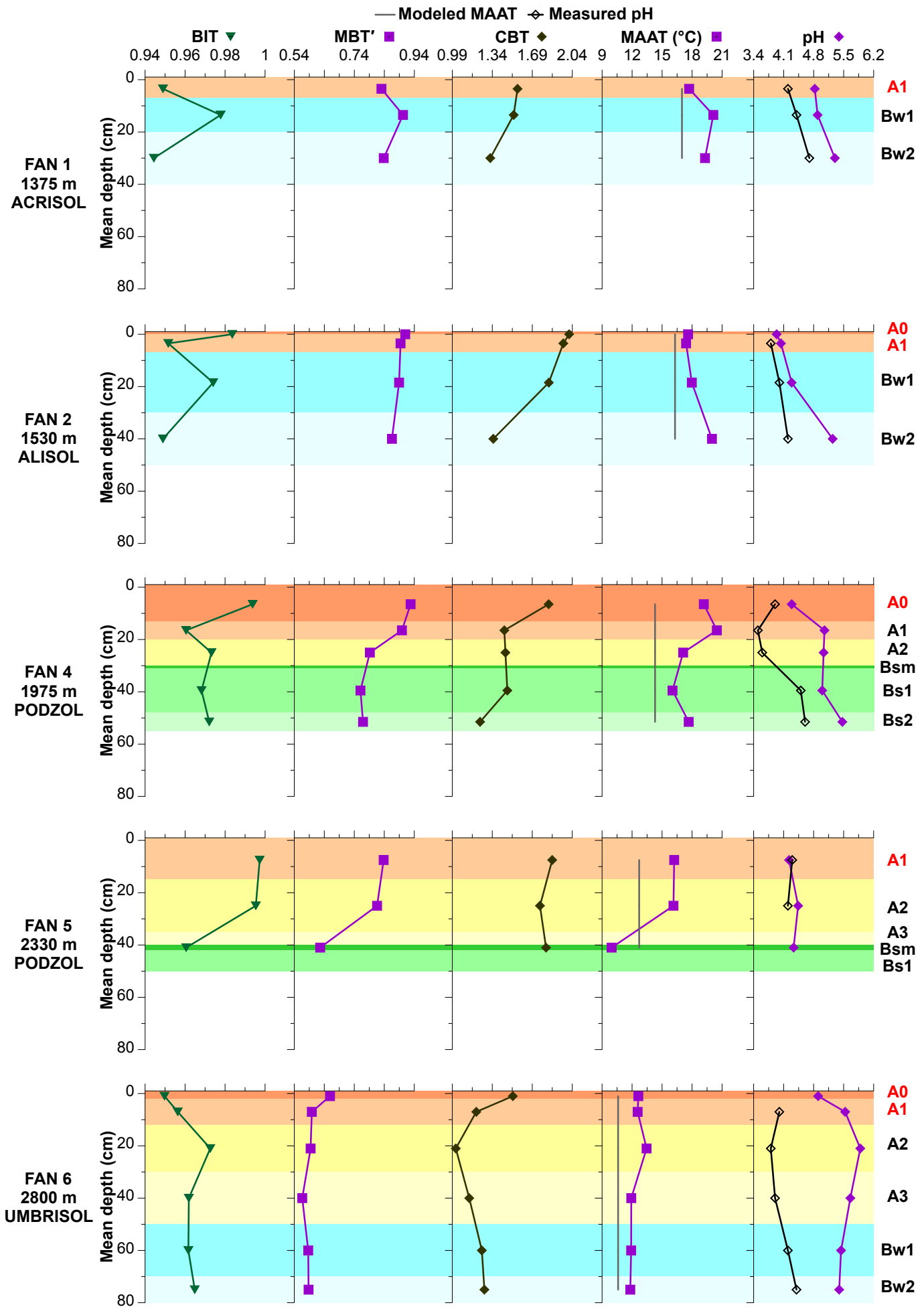


Table 1

	MAAT = a * MBT <sup>v</sup> + b * CBT + c						pH = S * CBT + I						
	<i>n</i>	a	b	c	<i>R</i> <sup>2</sup>	<i>p</i>	E	<i>n</i>	S	I	<i>R</i> <sup>2</sup>	<i>p</i>	E
All soils	341	23.73 ± 1.43	-3.01 ± 0.65	3.06 ± 0.72	0.5	< 0.001	5.6	331 (332-1)	-1.64 ± 0.08	7.75 ± 0.11	0.5	< 0.001	0.9
Acrisols	33 (34-1)	38.23 ± 3.71	-4.20 ± 1.48	-6.32 ± 2.72	0.8	< 0.001	3.1	33	-0.16 ± 0.32	5.45 ± 0.53	0.0	0.61	0.8
Alisols	13	0.46 ± 5.49	4.49 ± 2.17	15.37 ± 4.17	0.4	0.10	2.3	13	-0.66 ± 0.30	5.79 ± 0.49	0.3	0.052	0.4
Andosols	10	24.61 ± 9.77	-4.22 ± 2.61	2.89 ± 6.10	0.6	0.049	4.4	10	-1.28 ± 0.23	6.74 ± 0.25	0.8	< 0.001	0.4
Cambisols	54	23.64 ± 5.27	-7.20 ± 1.91	4.72 ± 2.18	0.3	< 0.001	6.3	53	-2.32 ± 0.25	8.25 ± 0.27	0.6	< 0.001	0.9
Ferralsols	18	24.07 ± 15.22	-1.90 ± 2.12	5.05 ± 14.80	0.2	0.21	2.5	18	-0.42 ± 0.50	5.19 ± 0.91	0.0	0.41	0.6
Fluvisols	24	5.16 ± 7.84	-0.66 ± 2.72	11.45 ± 2.23	0.0	0.78	3.8	24	-1.91 ± 0.49	8.06 ± 0.33	0.4	0.001	0.9
Leptosols	18	30.55 ± 6.70	-2.19 ± 3.34	-4.63 ± 3.95	0.6	0.001	4.6	18	-1.89 ± 0.44	7.92 ± 0.53	0.5	0.001	0.7
Luvisols	42 (44-2)	15.02 ± 2.78	-4.09 ± 1.02	5.83 ± 1.21	0.5	< 0.001	3.1	40 (41-1)	-1.46 ± 0.22	7.81 ± 0.20	0.5	< 0.001	0.7
Phaeozems	10	4.79 ± 8.88	-7.19 ± 4.19	18.33 ± 6.17	0.4	0.21	7.0	10	-0.95 ± 0.55	7.52 ± 0.59	0.3	0.12	1.0
Podzols	30	23.24 ± 5.53	-2.48 ± 2.98	-1.74 ± 4.75	0.4	0.001	4.3	29	-0.32 ± 0.48	5.27 ± 0.77	0.0	0.51	0.8
Regosols	17 (18-1)	-8.65 ± 8.55	4.27 ± 2.12	7.69 ± 2.35	0.2	0.15	3.4	17	-0.73 ± 0.60	8.07 ± 0.60	0.1	0.25	1.0
Umbrisols	12	15.34 ± 3.92	-0.89 ± 2.15	6.71 ± 3.05	0.7	0.01	2.2	12	0.09 ± 0.43	4.81 ± 0.65	0.0	0.84	0.5

Table 2

		All soils	Acrisols	Cambisols	Ferralsols	Fluvisols	Leptosols	Luvisols	Podzols	Regosols
MAAT = a × MBT' + b × CBT + c	<i>n</i>	234 (235–1)	17	45	14	19 (20–1)	15	30 (32–2)	16	12 (13–1)
	<i>a</i>	27.47 ± 1.79	37.02 ± 2.99	23.61 ± 6.27	37.78 ± 14.23	–10.47 ± 9.61	28.52 ± 8.02	22.14 ± 4.70	24.32 ± 9.20	–22.85 ± 9.57
	<i>b</i>	–4.53 ± 0.78	–2.53 ± 1.23	–7.05 ± 2.29	–3.92 ± 1.69	1.33 ± 2.94	–1.84 ± 3.87	–5.28 ± 1.16	–3.05 ± 6.42	3.81 ± 2.24
	<i>c</i>	2.35 ± 0.88	–6.48 ± 2.33	4.28 ± 2.66	–4.99 ± 14.52	15.77 ± 2.37	–4.34 ± 4.48	3.42 ± 2.02	–1.43 ± 9.82	12.02 ± 2.96
	<i>R</i> <sup>2</sup>	0.5	0.9	0.3	0.6	0.1	0.5	0.6	0.4	0.5
	<i>p</i>	< 0.001	< 0.001	0.001	0.003	0.44	0.01	< 0.001	0.053	0.07
	<i>E</i>	5.8	2.3	6.8	1.6	2.9	5.0	3.2	5.9	3.0
MAAT = S × MBT' <sub>5ME</sub> + I	<i>n</i>	231	16	44	13	20	15	31	16	13
Homogeneity of slopes test:	<i>S</i>	31.32 ± 1.55	32.96 ± 2.79	31.21 ± 5.31	50.07 ± 28.84	3.91 ± 9.70	31.51 ± 6.27	25.80 ± 4.40	24.41 ± 7.99	15.98 ± 5.75
<i>F</i> = 1.59, <i>p</i> = 0.14	<i>I</i>	–8.41 ± 1.01	–7.65 ± 2.40	–9.74 ± 2.80	–24.01 ± 27.14	10.17 ± 5.39	–10.36 ± 3.57	–6.18 ± 2.62	–6.59 ± 5.25	0.61 ± 3.43
ANCOVA:	<i>R</i> <sup>2</sup>	0.6	0.9	0.5	0.2	0.0	0.7	0.5	0.4	0.4
<i>F</i> = 5.34, <i>p</i> < 0.001	<i>p</i>	< 0.001	< 0.001	< 0.001	0.11	0.69	< 0.001	< 0.001	0.01	0.02
	<i>E</i>	4.9	2.5	5.6	2.4	3.7	4.1	3.2	5.5	4.1
pH = S × CBT + I	<i>n</i>	231 (232–1)	16	44	14	20	15	31 (32–1)	16	13
Homogeneity of slopes test:	<i>S</i>	–1.75 ± 0.10	–0.63 ± 0.27	–2.44 ± 0.29	–0.56 ± 0.63	–2.20 ± 0.51	–2.11 ± 0.36	–1.55 ± 0.23	–0.54 ± 0.50	–0.77 ± 0.80
<i>F</i> = 3.83, <i>p</i> = 0.001	<i>I</i>	7.90 ± 0.12	5.86 ± 0.44	8.35 ± 0.31	5.47 ± 1.12	8.18 ± 0.32	8.31 ± 0.42	7.84 ± 0.22	5.32 ± 0.83	8.11 ± 0.86
	<i>R</i> <sup>2</sup>	0.6	0.3	0.6	0.1	0.5	0.7	0.6	0.1	0.1
	<i>p</i>	< 0.001	0.03	< 0.001	0.39	< 0.001	< 0.001	< 0.001	0.30	0.36
	<i>E</i>	0.9	0.6	0.9	0.7	0.9	0.5	0.7	0.5	1.1
pH = S × CBT' + I	<i>n</i>	216 (221–5)	15	42 (44–2)	10	17 (19–2)	15	31	14	12
Homogeneity of slopes test:	<i>S</i>	1.59 ± 0.05	1.14 ± 0.20	1.78 ± 0.10	1.97 ± 1.03	0.56 ± 0.61	1.66 ± 0.11	1.29 ± 0.18	0.18 ± 0.33	1.76 ± 0.19
<i>F</i> = 4.64, <i>p</i> < 0.001	<i>I</i>	7.15 ± 0.05	6.49 ± 0.30	7.04 ± 0.10	7.59 ± 1.65	7.32 ± 0.16	7.43 ± 0.12	7.25 ± 0.14	4.75 ± 0.49	7.11 ± 0.11
	<i>R</i> <sup>2</sup>	0.8	0.7	0.9	0.3	0.1	0.9	0.6	0.0	0.9
	<i>p</i>	< 0.001	< 0.001	< 0.001	0.09	0.37	< 0.001	< 0.001	0.59	< 0.001
	<i>E</i>	0.5	0.4	0.5	0.5	0.5	0.2	0.6	0.5	0.4

Table 3

Sample	Altitude (m)	Coordinates	Horizon	Mean depth (cm)	C <sub>org</sub> (%)	H <sub>2</sub> O (%)	Measured pH	Modeled MAAT (°C)	BIT	MBT'	CBT	Calculated MAAT (°C)	Calculated pH
FAN1.1	1375	22°19'15"N 103°49'52"E	A1	3.5	8.27	76	4.2	17.0	0.95	0.83	1.56	17.7	4.8
FAN1.2			Bw1	13.5	2.72	nd	4.4		0.98	0.90	1.53	20.1	4.9
FAN1.3			Bw2	30	0.62	60	4.7		0.94	0.84	1.32	19.3	5.3
FAN2.A0	1530	22°18'44"N 103°49'25"E	A0	0	nd	nd	nd	16.3	0.98	0.91	2.01	17.6	3.9
FAN2.1			A1	3.5	4.30	80	3.8		0.95	0.89	1.96	17.4	4.0
FAN2.2			Bw1	18.5	2.54	nd	4.0		0.97	0.89	1.83	18.0	4.3
FAN2.3			Bw2	40	0.66	50	4.2		0.95	0.87	1.35	20.0	5.2
FAN4.1	1975	22°18'20"N 103°48'38"E	A0	6.5	44.13	nd	3.9	14.3	0.99	0.93	1.83	19.2	4.3
FAN4.2			A1	16.5	8.11	215	3.5		0.96	0.90	1.45	20.5	5.1
FAN4.3			A2	25	3.59	66	3.6		0.97	0.79	1.46	17.1	5.0
FAN4.5			Bs1	39.5	1.22	40	4.5		0.97	0.76	1.47	16.0	5.0
FAN4.6			Bs2	51.5	1.16	nd	4.6		0.97	0.77	1.23	17.7	5.5
FAN5.1			2330	22°18'00"N 103°48'36"E	A1	7.5	5.99		114	4.3	12.7	1.00	0.84
FAN5.2	A2	25			4.38	nd	4.2	1.00	0.82	1.76		16.1	4.4
FAN5.4	Bsm	41			nd	nd	nd	0.96	0.63	1.81		10.0	4.3
FAN6.1	2800	22°17'32"N 103°48'02"E	A0	1	6.14	nd	nd	10.6	0.95	0.66	1.52	12.6	4.9
FAN6.2			A1	7	3.89	106	4.0		0.96	0.60	1.20	12.6	5.5
FAN6.3			A2	21	2.95	nd	3.8		0.97	0.59	1.02	13.5	5.9
FAN6.4			A3	40	2.07	53	3.9		0.96	0.57	1.14	11.9	5.7
FAN6.5			Bw1	60	0.77	nd	4.2		0.96	0.59	1.25	11.9	5.4
FAN6.6			Bw2	75	0.39	nd	4.4		0.96	0.59	1.27	11.8	5.4