



**HAL**  
open science

## Disentangling interactions between microbial communities and roots in deep subsoil

Martina I. Gocke, Arnaud Huguet, Sylvie Derenne, Steffen Kolb, Michaela A. Dippold, Guido L.B. Wiesenberg

► **To cite this version:**

Martina I. Gocke, Arnaud Huguet, Sylvie Derenne, Steffen Kolb, Michaela A. Dippold, et al.. Disentangling interactions between microbial communities and roots in deep subsoil. *Science of the Total Environment*, 2017, 575, pp.135 - 145. 10.1016/j.scitotenv.2016.09.184 . hal-01395143

**HAL Id: hal-01395143**

<https://hal.sorbonne-universite.fr/hal-01395143v1>

Submitted on 10 Nov 2016

**HAL** is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

1 **Disentangling interactions between microbial communities and roots in deep subsoil**

2

3 Martina I. Gocke<sup>a,1,\*</sup>, Arnaud Huguet<sup>b</sup>, Sylvie Derenne<sup>b</sup>, Steffen Kolb<sup>c</sup>, Michaela A. Dippold<sup>d</sup>,

4 Guido L.B. Wiesenberg<sup>a</sup>

5 <sup>a</sup>Department of Geography, University of Zurich, Winterthurerstrasse 190, 8057 Zürich, Switzerland;

6 [mgocke@uni-bonn.de](mailto:mgocke@uni-bonn.de) (M.I. Gocke), [guido.wiesenberg@geo.uzh.ch](mailto:guido.wiesenberg@geo.uzh.ch) (G.L.B. Wiesenberg)

7 <sup>b</sup>Sorbonne Universités, UPMC Univ Paris 06, UMR 7619, METIS, 75005 Paris, France;

8 [arnaud.huguet@upmc.fr](mailto:arnaud.huguet@upmc.fr) (A. Huguet), [sylvie.derenne@upmc.fr](mailto:sylvie.derenne@upmc.fr) (S. Derenne)

9 <sup>c</sup>Leibniz Center for Agricultural Landscape Research, Landscape Biogeochemistry, Eberswalder

10 Straße 84, 15374 Münchberg, Germany; [steffen.kolb@zalf.de](mailto:steffen.kolb@zalf.de) (S. Kolb)

11 <sup>d</sup>Department of Agricultural Soil Science, University of Göttingen, Büsgenweg 2, 37077 Göttingen,

12 Germany; [dippold@gwdg.de](mailto:dippold@gwdg.de) (M.A. Dippold)

13 <sup>1</sup>Present Address: Institute of Crop Science and Resource Conservation, University of Bonn,

14 Nussallee 13, 53115 Bonn, Germany

15 \*Corresponding author, phone +49-228-7368748

16

17

18 Abstract

19 Soils, paleosols and terrestrial sediments serve as archives for studying climate change, and  
20 represent important terrestrial carbon pools. Archive functioning relies on the chronological integrity of  
21 the respective units. Incorporation of younger organic matter e.g. by plant roots and associated  
22 microorganisms into deep subsoil and underlying soil parent material may reduce reliability of  
23 paleoenvironmental records and stability of buried organic matter. Long-term effects of sedimentary  
24 characteristics and deep rooting on deep subsoil microbial communities remain largely unknown. We  
25 characterized fossil and living microbial communities based on molecular markers in a Central  
26 European Late Pleistocene loess-paleosol sequence containing recent and ancient roots with ages of  
27 up to several millenia. The molecular approach, comprising free and phospholipid fatty acids (FAs),  
28 core and intact polar glycerol dialkyl glycerol tetraethers (GDGTs), as well as 16S rRNA genes from  
29 bacterial DNA, revealed the presence of living microorganisms along the complete sequence, with a  
30 bacterial community composition comparable to that of modern topsoils. Up to 88% redundancy was  
31 found between bacterial genetic fingerprint and molecular signature of fossil microorganisms,  
32 suggesting a time-integrated signal of the respective molecular markers accumulated over a time span  
33 potentially lasting from sedimentation over one or more rooting phases until today. Free FAs, core  
34 GDGTs and DNA, considered as remains of fossil microorganisms, corresponded with both ancient  
35 and recent root quantities, whereas phospholipid FAs and intact polar GDGTs, presumably derived  
36 from living microorganisms, correlated only with living roots. The biogeochemical and ecological  
37 disequilibrium induced by postsedimentary rooting of deep subsoil may entail long-term microbial  
38 processes like organic matter mineralization, which may continue even millenia after the lifetime of the  
39 root. Deep roots and their fossil remains have been observed in various terrestrial settings, and roots  
40 as well as associated microorganisms cause both, organic matter incorporation and mineralization.  
41 Therefore, these findings are crucial for improved understanding of organic matter dynamics and  
42 carbon sequestration potential in deep subsoils.

43

44 Key words

45 biopore, microbial hotspot, molecular marker, paleoenvironmental archive, rhizosphere, terrestrial  
46 sediment

47

## 48 1. Introduction

49 The existence of microbial life in deeper parts of the unsaturated subsurface like e.g. in terrestrial  
50 sediments is well known (Holden and Fierer, 2005). Hotspots in the deep subsoil  $>> 1$  m including soil  
51 parent material stimulating microbial thriving are e.g. paleosols (Brockman et al., 1992) or places of  
52 preferential flow with accumulated dissolved organic carbon ( $C_{org}$ ; Bundt et al., 2001). In addition, deep  
53 roots entering the sediment during and after its deposition may provide such places of preferential  
54 water flow as well as  $C_{org}$  and nutrient accumulation (Kautz et al., 2013), as demonstrated by the  
55 positive correlation of  $C_{org}$  contents and root biomass distribution (Wang et al., 2010). The rhizosphere  
56 as potential microbial hotspot, however, has been largely ignored below 0.5 m depth, although the  
57 ability of roots to penetrate subsoil and underlying soil parent material to several meter depth is known  
58 for various plant groups (Canadell et al., 1996). Deep roots have been studied in various soils and  
59 unconsolidated terrestrial sediments including loess-(paleo)soil and sand-(paleo)soil sequences in  
60 Central and Southeast Europe (Gocke et al., 2014a, 2014b, 2015), where they were observed to occur  
61 with considerable abundances  $>> 2$  m below the surface. The incorporation of root- and  
62 microorganism-derived organic matter (OM) in deep sedimentary subsoil does not only potentially  
63 cause an overprint of the paleoenvironmental signals recorded in such archives, followed by  
64 insecurities for the interpretation of biomarkers like e.g. *n*-alkanes or glycerol dialkyl glycerol  
65 tetraethers (GDGTs; Huguet et al., 2013a; Gocke et al., 2014c). It might also affect terrestrial carbon  
66 (C) stocks, of which considerable amounts are stored in paleosols (Marin-Spiotta et al., 2014).

67 So far, it is unknown whether deep roots influence the microbial community in unconsolidated deep  
68 subsoil  $> 1$  m. Especially their influence on long-term maintenance of microbial life after the lifetime of  
69 the root is difficult to study, but might be important in the context of C sequestration in buried soils and  
70 sediments (Fisher et al., 1994; Johnson, 2014). Ancient lithified roots – rhizoliths – which often form  
71 through calcification of the root during or shortly after its lifetime (Klappa, 1980; Gocke et al., 2011)  
72 give a time-integrated insight into root-related processes over the lifespan of the root and thereafter.

73 Here we present a multi-proxy approach to characterize both the living and fossil microbial community  
74 in deep sedimentary subsoil based on abundance and composition of five biomarker classes. The  
75 molecular approach includes two compound classes with high potential for preservation in soils and  
76 sediments: Free-extractable fatty acids (FAs) give a broad overview over plant and microbial remains  
77 and degradation products (Harwood and Russell, 1984; Lichtfouse et al., 1995), whereas core lipids of  
78 GDGTs (CL-GDGTs), presumed to be of fossil origin, are solely produced by some microorganisms

79 (Suppl. Fig. 1). Thus, GDGTs with isoprenoid alkyl chains (*i*GDGTs) are attributed to archaea,  
80 whereas those with branched alkyl chains (*br*GDGTs) are biosynthesized by not yet identified bacteria  
81 (Schouten et al., 2013) potentially feeding on root remains (Huguet et al., 2012). Some of these  
82 bacteria might belong to the phylum *Acidobacteria* (Sinninghe Damsté et al., 2011, 2014). Further, we  
83 included the respective intact polar counterparts of these compound classes, the phospholipid FAs  
84 (PLFAs) and intact polar lipid GDGTs (IPL-GDGTs), which are attributed to living or recently deceased  
85 microorganisms due to low stability of their polar headgroups (Kindler et al., 2009; Schouten et al.,  
86 2010; Lengger et al., 2013; Suppl. Fig. 1). As third compound class, 16S ribosomal ribonucleic acid  
87 (rRNA) genes from bacterial deoxyribonucleic acid (DNA) provided an overview of the bacterial  
88 community. Depth distribution of these biomarkers was compared with that of several parameters of  
89 pedogenic, weathering and rooting processes, including C contents, alkane contents and composition,  
90 abundances of living roots and root remains, density, clay contents, color and magnetic properties of a  
91 Central European sediment-paleosol sequence.

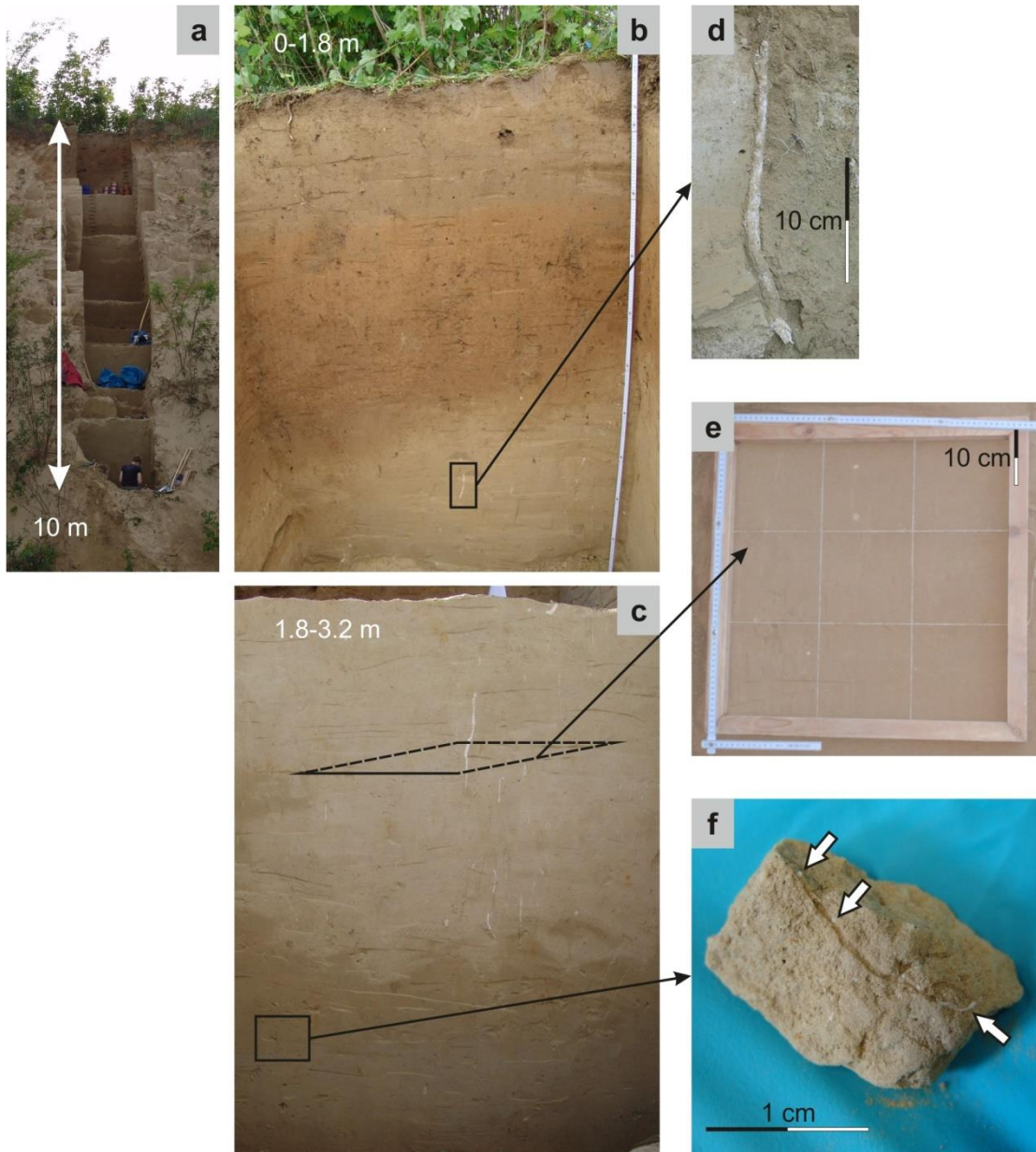
92 We aimed to elucidate i) which physical, chemical and biological factors influence microbial life in  
93 sedimentary deep subsoil, ii) if microbial community composition in deep subsoil is notably affected by  
94 root distribution, iii) if root effects on microbial community can be traced after lifetime of the root, and  
95 iv) whether roots maintain microbial life in terrestrial sediments for centuries or millenia.

96 The multi-proxy approach was applied to the Late Pleistocene loess-paleosol sequence at Nussloch,  
97 Southwest Germany, which has been investigated intensely for paleoclimate and paleovegetation (e.g.  
98 Antoine et al., 2009). The current study was performed on the uppermost 9.5 m of the profile P<sub>2011</sub>  
99 (Gocke et al., 2014b; Fig. 1a), which is defined here as associated pair of Holocene soil (0–1.1/1.5 m;  
100 Fig. 1b) and Holocene deep subsoil (1.1/1.5–9.5 m) because rhizoliths (Fig. 1b–f) of Holocene ages  
101 occur continuously between 1.5 and 9 m depth (Gocke et al., 2011; Gocke et al., 2014b). Time lags  
102 between sedimentation, lasting from 35–17 ky on the one hand, and root growth on the other hand  
103 cover a wide range of 10<sup>2</sup>–10<sup>3</sup> y. Abundant recent roots, rhizoliths between 3 and 10 ky in age, and  
104 root-derived biopores were observed along the profile, with ages of the latter likely between  
105 sedimentation and today (Gocke et al., 2014a). This provides the unique opportunity to assess short-  
106 term and long-term effects of postsedimentary rooting on microbial community composition in deep  
107 subsoil.

108

109 2. Materials and methods

110 2.1 Study site



111

112 Figure 1.

113 Overview pictures of the Nussloch profile (a–c) and detail pictures (d–f) of calcified root features (rhizoliths). a)

114 Upper Pleniglacial part of the Nussloch loess-paleosol sequence with a maximum age of 35 ky (Antoine et al.,

115 2009). b) 0–1.8 m depth interval, including the Holocene soil (HS; Calcic Luvisol Siltic). c) 1.8–3.2 m

116 interval, with *in situ*, almost vertically oriented rhizoliths. d) Rhizolith, view at profile wall. e) Rhizoliths indicated as

117 circular white spots on a horizontal level. f) Microrhizoliths (diameter < 1 mm), difficult to observe at the profile

118 wall. Picture shows one microrhizolith in longitudinal view (bottom right) and several microrhizoliths in cross

119 section (e.g. top left).

120

121 Detailed description of the loess-paleosol sequence at Nussloch, Southwest Germany, and its  
122 paleoenvironmental record was provided by e.g. Antoine et al. (2009) and Gocke et al. (2014b). The  
123 13 m thick, 17–35 ky old sequence includes 16 weakly developed and three well developed paleosols,  
124 as well as the 1.1–1.5 m thick Holocene Calcic Luvisol Siltic (IUSS, 2014). The recent vegetation,  
125 which relates to living roots and likely formed an unknown portion of root-related biopores throughout  
126 the profile, comprises natural broad-leaf forest and non-native robinia, as well as smaller shrubs and  
127 herbaceous plants (Gocke et al., 2013). Rhizoliths at Nussloch and nearby sites are of Holocene age  
128 between 3 and 10 ky. They occur with diameters of up to 5 cm but mostly 1–2 cm (reviewed by Gocke  
129 et al., 2014a), and were formed by roots of unknown trees or shrubs, likely including hazel, oak, beech  
130 and alder (Gocke et al., 2013). Living roots, rhizoliths, microrhizoliths and biopores occurred with  
131 maximum frequencies of ca. 800 m<sup>-2</sup>, 200 m<sup>-2</sup>, 12,500 m<sup>-2</sup> and 600 m<sup>-2</sup>, respectively (Suppl. Fig. 2;  
132 Gocke et al., 2014b).

## 133 2.2 Profile preparation, field methods and sampling

134 The profile was prepared by removing ≥ 1 m of material from the top and the side, respectively,  
135 followed by counting of roots, root-related biopores and rhizoliths on horizontal levels (Gocke et al.,  
136 2014b, 2014c). Afterwards, 200 g of soil, sediment or paleosol material were collected distant from  
137 visible root remains, from topsoil down to 9.5 m depth in 0.5–1.0 m increments. After sample splitting  
138 into two aliquots, one aliquot was dried at 40 °C and used for free FA and DNA analyses, whereas the  
139 other half was immediately cooled and kept frozen at -18 °C until PLFA and GDGT analyses. The  
140 sample set for DNA analyses did not include the topsoil, because microbial diversity in topsoils is very  
141 high, which would produce a bias for the other parameters. It was further reduced to seven depth  
142 intervals between 2 m and 9.5 m due to sample loss.

## 143 2.3 Analytical methods

### 144 2.3.1 Free-extractable fatty acids

145 Using an aliquot of dried material, free-extractable lipids were extracted and free FAs separated,  
146 derivatized and subsequently measured by GC-FID and GC-MS according to the protocol by  
147 Wiesenberg and Gocke (2015). Free FAs with a chain length of 12 to 32 carbons were quantified  
148 because they include microbial and plant tissues, as well as degradation products (Harwood and  
149 Russell, 1984). Dicarboxylic as well as unsaturated and branched free FAs were kept as separate  
150 groups of compounds, but not further assigned to specific sources, which was done for PLFAs, as  
151 multiple sources exist for most of these compounds.

152

153

### 154 2.3.2 Phospholipid fatty acids and glycerol dialkyl glycerol tetraethers

155 An aliquot of each frozen sample was subjected to lipid extraction following a modified protocol of the  
156 Bligh-Dyer method (Bligh and Dyer, 1959; Apostel et al., 2013) with 0.15 M citric acid, chloroform and  
157 methanol 0.8:1:2 (v/v/v). Using one half of the splitted extract, the PLFA fraction was purified over an  
158 activated silica column by elution with acetone, and derivatized for GC-MS measurement as previously  
159 described (Apostel et al., 2013). Assignment of PLFAs with chain length of 14 to 20 carbons to the  
160 respective microbial groups was performed according to literature (Zelles et al., 1999; Fernandes et  
161 al., 2013).

162 The second part of the extract was separated over an activated silica column into three fractions as  
163 described by Huguet et al. (2013b), with fraction F1 containing apolar lipids (eluted with 30 ml  
164 dichloromethane), fraction F2 containing CL-GDGTs [eluted with 30 ml dichloromethane/acetone (2:1,  
165 v/v) followed by 30 ml dichloromethane/acetone (1:1, v/v)], and fraction F3 containing IPL-GDGTs  
166 [eluted with 30 ml dichloromethane/methanol (1:1, v/v) followed by 30 ml methanol]. A small aliquot of  
167 the IPL fraction (F3) was analyzed directly using high performance liquid chromatography–  
168 atmospheric pressure chemical ionization–mass spectrometry (HPLC–APCI–MS) to determine any  
169 carryover of CLs into the IPL fraction. The analysis showed nearly complete separation of the CL- and  
170 IPL-GDGTs. The rest of the fraction F3 was subjected to acid methanolysis (24 h at 100 °C in 1 M  
171 HCl/methanol) to cleave off the polar head groups of IPL-GDGTs. CL- and IPL-derived GDGTs were  
172 analysed by HPLC-MS using a procedure described by Huguet et al. (2013b).

### 173 2.3.3 Bacterial 16S rRNA genes

174 Prior to analysis of the bacterial genetic fingerprint, a sterility test was successfully performed.  
175 Metagenomic DNA was extracted in replicates from 0.5 g aliquots of dry sample material using the  
176 FastDNA® SPIN Kit for Soil (MP Biomedicals). Bacterial 16S rRNA genes were amplified with primers  
177 27f/907mr (Schellenberger et al., 2010). Primer 27f was labeled with infrared dye 700 for t-RFLP  
178 (terminal restriction fragment length polymorphism) analyses. PCR (polymerase chain reaction)  
179 products were purified with a DNA Gel Extraction Kit (Millipore, MA) and single-stranded extensions at  
180 terminal ends were removed with mung bean endonuclease digest (Egert and Friedrich, 2003).  
181 Subsequent restriction digestion of PCR products was performed with *MspI* (Degelmann et al., 2009).  
182 DNA concentrations were determined with a DNA Quantification Kit (Invitrogen, Germany) and



183 adjusted to 2 ng  $\mu\text{l}^{-1}$ . T-RFLP analyses were performed as previously described (Hamberger et al.,  
184 2008). PCR products from all samples were pooled and used to construct one 16S rRNA gene library.  
185 Amplicons were cloned into *Escherichia coli* JM 109 competent cells using the pGEM-T Vector System  
186 II (Promega, WI). Inserted 16S rRNA gene fragments were re-amplified and sequenced (Messing,  
187 1983; MacroGen, South Korea). In total, 270 different inserts were sequenced. Identification of  
188 phylotypes was done by RDP classifier. Based on in silico digestion, terminal restriction fragments  
189 detected by software GelQuest (version 3.1.7, SequentiX GmbH, Germany) were manually affiliated to  
190 sequenced genotypes. Ribonucleic acid (RNA), representing viable microorganisms due to its  
191 presumably fast degradation, was not analysed, because i) very low DNA contents in Quaternary  
192 sediments like loess (Liu et al., 2007; current study) imply that RNA is likely too low for amplification  
193 and further analysis, and ii) rarefaction analysis (Suppl. Fig. 3) revealed that even DNA results are  
194 reliable solely at the phylum level.

#### 195 2.4 Statistic evaluation

196 Variables within the individual datasets (free FAs, PLFAs, core GDGTs, intact polar GDGTs and DNA)  
197 were reduced by factor analysis. Factor values of most significant factors were compared between the  
198 datasets by canonical correlation analysis, and redundancy values for the datasets were computed.  
199 Further, environmental and profile parameters (EPP) significantly affecting the depth distribution of the  
200 biomarker groups were identified by regression analysis. The bulk elemental composition, measured  
201 via X-ray fluorescence analysis (Gocke et al., 2014b), did not reveal significant correlations with any of  
202 the biomarker sets and is therefore not shown. A significance level = 0.1 was chosen, because of i)  
203 small size of the sample set, ii) investigation of natural samples in contrast to samples from laboratory  
204 experiments performed under controlled conditions, and iii) heterogeneous nature of the sample set in  
205 terms of age and material. All statistical analyses were performed with Statistica 6.0 (StatSoft, Tulsa,  
206 USA).

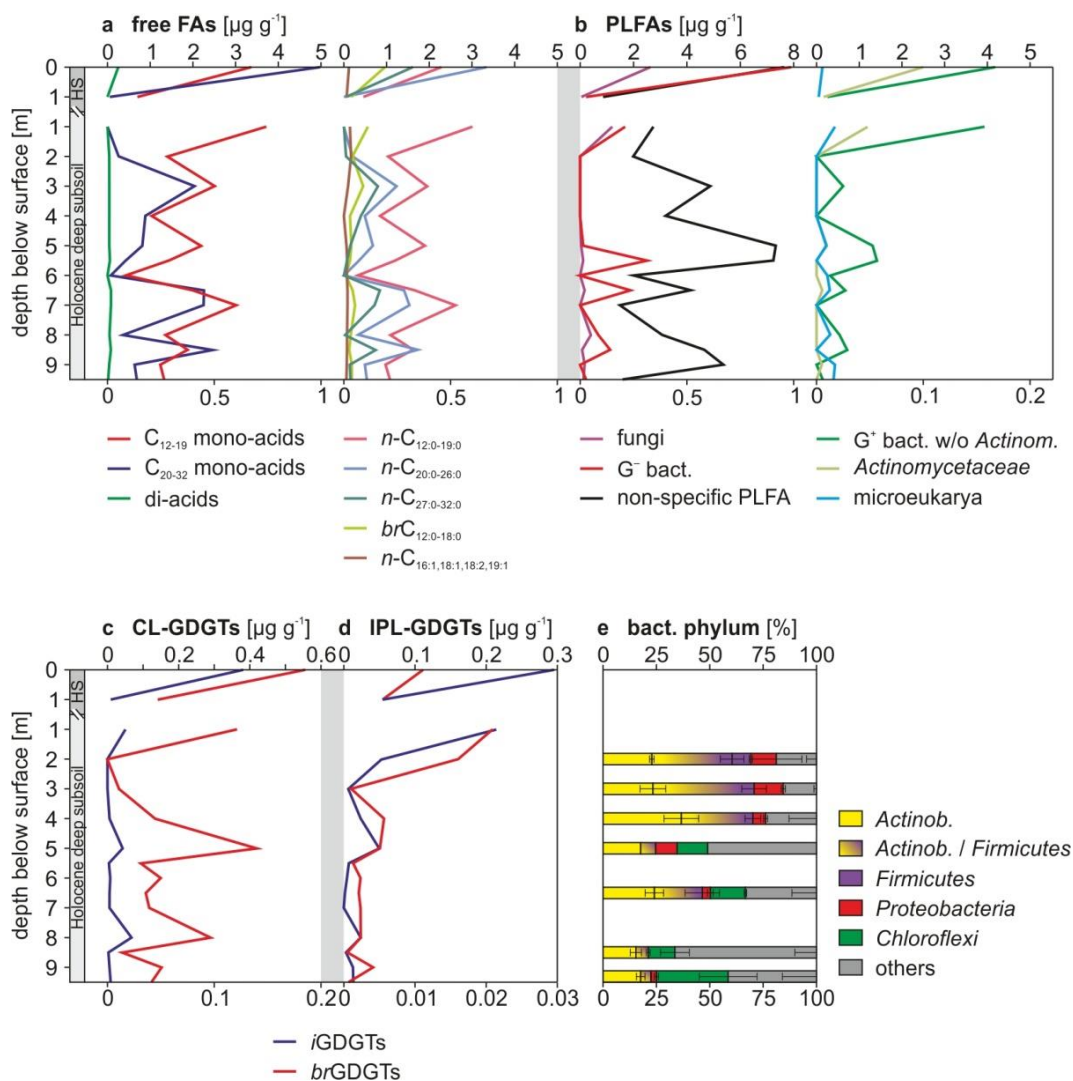
### 207 3. Results and Discussion

#### 208 3.1 Lipid distribution

209 Lipids were more abundant in the topsoil than in subsoil collected at 1 m depth (Fig. 2a-d), and  
210 concentrations in deep subsoil reached max. 28% of the respective topsoil value.

##### 211 3.1.1 Distribution patterns of free-extractable and phospholipid fatty acids

212 Free FA distribution patterns were mostly dominated by short-chain homologues (Gocke et al., 2014c;  
 213 C<sub>12-19</sub>; Fig. 2a). The ratio of short- vs. long-chain (C<sub>20-32</sub>) free FAs (R<sub>S:L</sub>) was mostly > 1.2 (Suppl. Tab.  
 214 1), indicating the dominance of belowground OM including microbial remains, rhizodeposits and  
 215 degradation products over higher plant aboveground biomass inputs (Harwood and Russell, 1984).  
 216 Contents of unsaturated free FAs (C<sub>16,18,19</sub>) were low due to their high degradability. Branched free  
 217 FAs (*br*C<sub>12-18</sub>), most likely originating from gram positive (Gram<sup>+</sup>) bacteria, and dicarboxylic acids (C<sub>9-</sub>  
 218 <sub>11,18,20</sub>), deriving mainly from plant above- and belowground tissues, contributed only to a minor extent  
 219 to free FAs.



220  
 221 Figure 2.

222 Biomarker classes representing fossil microorganisms (free fatty acids [FAs], core lipid glycerol dialkyl glycerol  
 223 tetraethers [CL-GDGTs] and bacterial genetic fingerprint) and living microorganism communities (phospholipid  
 224 fatty acids [PLFAs] and intact polar lipid glycerol dialkyl glycerol tetraethers [IPL-GDGTs]). Diagrams a–d show  
 225 concentrations of lipids, where in each diagram the upper x-axis refers to lipid contents in the Holocene soil (HS,  
 226 0–1 m), and the lower x-axis refers to lipid contents in the deep subsoil 1–9.5 m. a) Free FA contents. The left

227 diagram contains contents of total (saturated, unsaturated and branched) short-chain (C<sub>12-19</sub>) and long-chain (C<sub>20-</sub>  
228 <sub>32</sub>) monocarboxylic acids as well as dicarboxylic acids (C<sub>9-11,18,20</sub>) deriving from cutin and suberin biopolymers  
229 (Kolattukudy, 1984), whereas the right diagram displays contents of mainly microorganism-derived saturated  
230 short-chain free FAs (C<sub>12:0-19:0</sub>), plant-derived saturated long-chain (C<sub>20:0-26:0</sub>) and very long-chain free FAs (C<sub>27:0-</sub>  
231 <sub>32:0</sub>; Kolattukudy et al., 1976), branched free FAs (*br*C<sub>12:0-18:0</sub>) from total Gram<sup>+</sup> bacteria (Zelles et al., 1999;  
232 Fernandes et al., 2013), as well as microorganism- and plant-derived unsaturated free FAs (C<sub>16:1,18:1,18:2,19:1</sub>;  
233 Kolattukudy et al., 1976; Harwood and Russell 1984). Please note that branched free FAs did not enable  
234 distinction between *Actinomycetaceae* and other Gram<sup>+</sup> bacteria. b) Concentrations of specific PLFAs attributed  
235 to Gram<sup>+</sup> bacteria (excluding Gram<sup>+</sup> family *Actinomycetaceae* [*i*- and *ai*-C<sub>15-17</sub>]), *Actinomycetaceae* (10Me-C<sub>16,18</sub>),  
236 Gram<sup>-</sup> bacteria (C<sub>16:1 $\omega$ 7,18:1 $\omega$ 9,18:1 $\omega$ 7</sub>, *cy*-C<sub>17,19</sub>), fungi (C<sub>16:1 $\omega$ 5</sub>, C<sub>18:2 $\omega$ 6,9</sub>) and microeukaryotes (formerly called  
237 protozoa [C<sub>20:4 $\omega$ 6</sub>]; Zelles et al., 1999; Fernandes et al., 2013), as well as of non-specific PLFAs (saturated C<sub>14:0-</sub>  
238 <sub>18:0</sub>). c) Concentrations of CL-GDGTs, divided into archaea-derived isoprenoid GDGTs (*i*GDGTs; m/z 1292–1302)  
239 and bacteria-derived branched GDGTs (*br*GDGTs; m/z 1018–1050; Schouten et al., 2013). d) Concentrations of  
240 IPL-GDGTs, divided into *i*GDGTs and *br*GDGTs. e) Distribution of phylogenetic groups, based on 16S rRNA  
241 genes from DNA. Phylogenetic groups accounting for  $\leq 3\%$  as well as portions of unclassified sequences and  
242 phylogenetic groups occurring solely in one depth are summarized in the artificial group ‘others’.

243  
244 Specific PLFAs suggested a dominance of bacteria (methylated, cyclo- and most mono-unsaturated  
245 FAs) over fungi (C<sub>16:1 $\omega$ 5,18:2</sub>; Fig. 2b) in all depth intervals except for 9 m (Suppl. Tab. 1). At four  
246 sampling depths between 5.0 and 8.5 m, ratio of bacterial to fungal PLFAs ( $R_{B:F}$ ) exceeded the value  
247 of the topsoil by a factor ranging between 2 and 4. Higher relative portions of fungi occurred solely in  
248 the lowermost part of the profile (8.0, 9.0 and 9.5 m) and might be attributed either to pedogenic  
249 conditions in well-developed paleosols or to postsedimentary penetration by fossil and/or living roots,  
250 both of which coincide in these depths (Gocke et al., 2014b). The ratio of Gram<sup>+</sup> (methylated FAs) to  
251 gram negative (Gram<sup>-</sup>) bacteria (cyclo- and most mono-unsaturated FAs)  $R_{P:N}$  was approximately  
252 twice as high in deeper parts of the Holocene soil (1 m depth) compared to surface soil (Suppl. Tab.  
253 1). Between 5.5 and 9.5 m, very low  $R_{P:N}$  resulted from high contents of Gram<sup>-</sup> bacterial PFLAs.

254 3.1.2 Distribution patterns of core and intact polar glycerol dialkyl glycerol tetraethers

255 Bacterial and archaeal GDGTs were present in all samples (Fig. 2c, d), with the ratio of isoprenoid to  
256 branched GDGTs ( $R_{i:b}$ ; Yang et al., 2014) ranging between 0.03 and 0.68 for core GDGTs and  
257 between 0.19 and 2.65 for intact polar GDGTs (Suppl. Tab. 1). Archaea predominated over bacteria  
258 only in topsoil and at the bottom of the sequence according to IPL-GDGT analyses. During recent  
259 years, studies emphasized the ubiquitous occurrence of archaea and especially one phylum,

260 *Thaumarchaeota*, in surface soils (uppermost 0.2 m; Harvey et al., 1986; Buckley et al., 1998). The  
261 current work thus provides extended knowledge on the occurrence of archaea in deep subsoil. The  
262 relative amounts of *i*GDGTs vs. *br*GDGTs decreased for both core and intact polar GDGTs from 0 to 1  
263 m and from 0 to 2 m, respectively (Suppl. Tab. 1). Below these depths,  $R_{i:b}$  was mostly higher for intact  
264 polar (25–65%) than for core GDGTs (3–33%; Suppl. Tab. 1), which might be due to the more labile  
265 nature of the predominantly phospho-headgroup of *br*GDGTs compared to the usually glyco-  
266 headgroup of *i*GDGTs (Harvey et al., 1986).

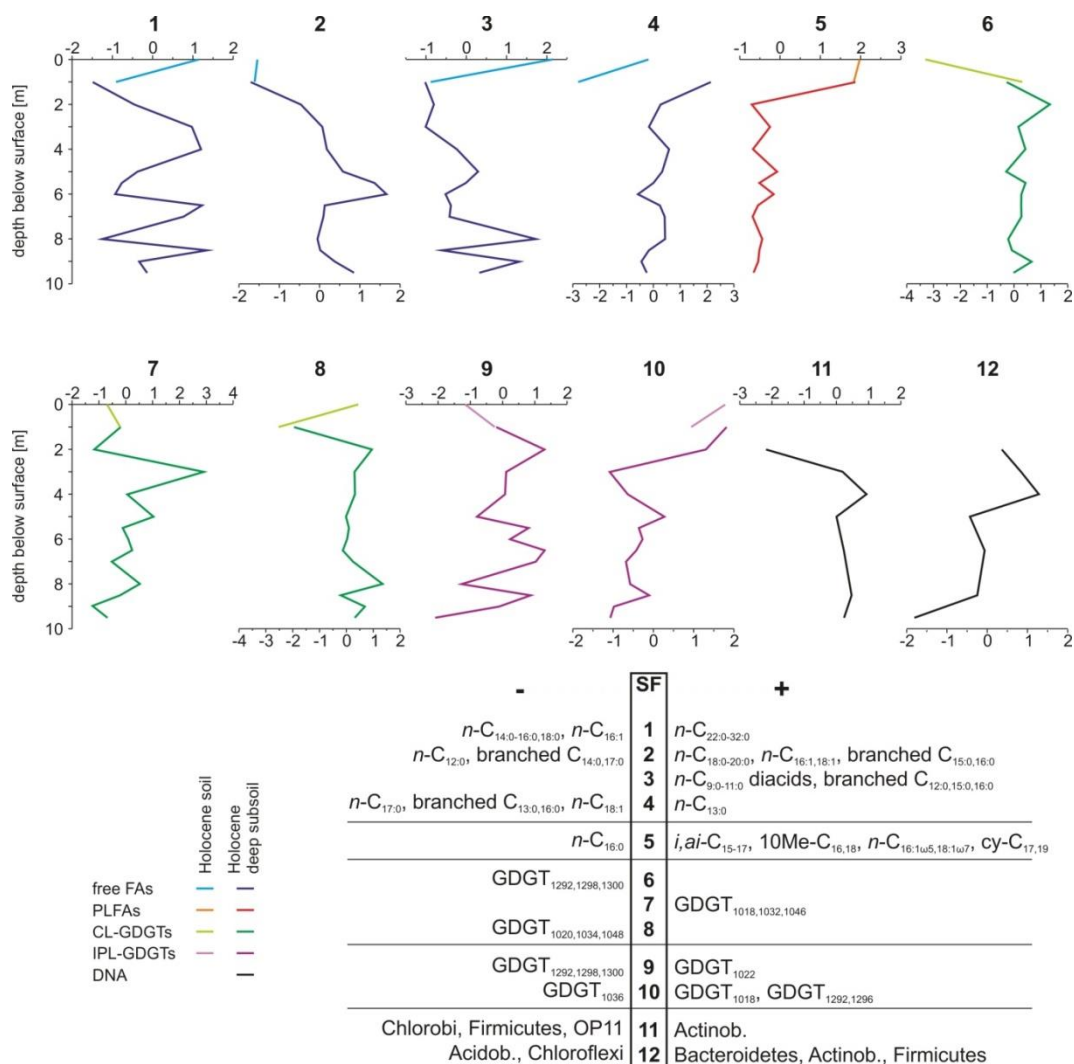
### 267 3.2 Bacterial genetic fingerprint

268 Phylotypic composition of bacterial DNA in deep subsoil was dominated by *Actinobacteria*, *Firmicutes*,  
269 *Chloroflexi* and *Proteobacteria*, besides minor abundance of *Acidobacteria*, *Bacteroidetes* and  
270 candidate division OP11 (Fig. 2e). Throughout identified phylogenetic groups with abundances > 3%,  
271 the predominance of Gram<sup>+</sup> bacterial phyla (*Actinobacteria* and *Firmicutes*) at 2, 3, 4 and 6.5 m  
272 matched well the absence of Gram<sup>-</sup> bacterial PLFAs at 3 and 6 m, but not at 5 m (Suppl. Tab. 1).  
273 Further, the much lower  $R_{P:N}$  in lower parts of the loess-paleosol sequence, excluding 9 m, agreed  
274 with higher portions of Gram<sup>-</sup> bacteria (*Chloroflexi* and *Proteobacteria*).

### 275 3.3 Fossil and living microbial communities in Holocene soil and deep subsoil

276 The multi-proxy approach indicated the dominance of bacterial signatures over fungal, archaeal and  
277 microeukaryotic ones throughout the Holocene soil and deep subsoil, suggesting a microbial  
278 community typical for the vadose subsurface (Fierer et al., 2003). Also, bacterial DNA composition  
279 resembled that in modern soils (Janssen, 2006). However, as bacterial genes were already close to  
280 detection limit, the presumably less abundant archaeal or fungal genes were not investigated. The  
281 deep subsoil properties of the loess-paleosol sequence were strengthened by its increased ratio of  
282 bacterial to fungal PLFAs compared to topsoil (Suppl. Tab. 1), which matches previous studies  
283 showing an increase of the latter with soil depth (Holden and Fierer, 2005; Stone et al., 2014). Further,  
284 the high ratio of Gram<sup>+</sup> to Gram<sup>-</sup> PFLAs below the topsoil (Suppl. Tab. 1) was already described for  
285 the uppermost 2 m of agricultural and grassland soils (Blume et al., 2002; Fierer et al., 2003).  
286 Presence of PLFAs and intact polar GDGTs, though in amounts up to one order of magnitude lower  
287 compared to their free/core counterparts, revealed the presence of living microorganisms at any depth  
288 throughout the profile, including the deep subsoil of Late Pleistocene age. This contradicts the  
289 traditional assumption of organic compounds being incorporated solely during deposition of terrestrial  
290 sediments (Conte et al., 2003). Intact polar GDGTs contributed less than 15% to total GDGTs below 2

291 m (Suppl. Fig. 4b), similarly to *br*GDGT data from topsoils (Peterse et al., 2010), whereas the  
 292 percentages of PLFAs from total FAs scattered between 24% and 88% (Suppl. Fig. 4a).



293  
 294 Figure 3.

295 Statistical factors (SF) of the biomarker groups obtained by factor analysis. Compounds with strong positive and  
 296 negative loadings (min. > 0.6 or < -0.6, but mostly > 0.9 or < -0.9) on these factors are listed for free FAs (SF 1–  
 297 4), PLFAs (SF 5), CL-GDGTs (SF 6–8), IPL-GDGTs (SF 9, 10) and DNA (SF 11, 12), respectively.

298  
 299 FAs, GDGTs and bacterial DNA are produced independently from each other and partially by different  
 300 source organisms. To elucidate the degree of overlap between individual biomarker data sets, and  
 301 consequently disentangle the time range represented by free/core lipids, redundancy values between  
 302 the five biomarker classes (Tab. 1) were calculated after reduction of the respective data sets by factor  
 303 analysis. Free/core lipids of FAs and GDGTs could be well explained by the respective other free/core  
 304 lipid group with 65% and 52% conformity, whereas they were less well predictable by their intact polar  
 305 counterpart and the respective intact polar lipid group of the other lipid category. Best predictor of free  
 306 FAs and core GDGTs was DNA with 88% and 79% redundancy. Similarly, DNA was better explained

307 by free FAs and core GDGTs than by intact polar lipids. The high conformity of free FAs and core  
 308 GDGTs with DNA, together with the potential of DNA to be preserved outside a cell in soils for  
 309 centuries to millenia (Agnelli et al., 2007), support the assumption that these lipids represent a time-  
 310 integrated signal of microorganism remains accumulated since sedimentation until today.

311  
 312 Table 1.

313 Redundancies given in %, describing the portion, to which extent a predictor data set can explain the outcome  
 314 data set. The data derives from canonical correlation analysis of the five microbial biomarker classes investigated.

Outcome data set \ Predictor data set	Free FAs	PLFAs	CL-GDGTs	IPL-GDGTs	Bacterial phylotypes
Free FAs		31	52	31	80
PLFAs	38		38	42	35
CL-GDGTs	65	38		31	70
IPL-GDGTs	51	56	41		31
Bacterial phylotypes	88	45	79	65	

315  
 316 3.4 Influence of environmental setting on deep subsoil microorganisms

317 We aimed to determine the linkage of living and fossil microorganisms to biological, physical or  
 318 chemical conditions that prevailed during phases of sedimentation, pedogenesis, rooting or inbetween.  
 319 Therefore, the twelve statistical factors (SF; Fig. 3), obtained after reduction of the individual  
 320 compounds of each biomarker class by factor analysis, were tested for correlation with the following  
 321 13 environmental and profile parameters (EPP; Tab. 2, Suppl. Fig. 2): (1) organic carbon ( $C_{org}$ ) and (2)  
 322 carbonatic carbon ( $C_{carb}$ ) contents; (3) contents of *n*-alkanes ( $n-C_{15-37}$ ), deriving from microorganisms  
 323 and higher plants; (4) alkane carbon preference index ( $CPI_{alk}$ ) enabling the distinction between fresh  
 324 plant aboveground biomass and microbial and degraded OM; frequencies of (5) living roots, (6)  
 325 rhizoliths and (7) root-derived biopores; (8) dry bulk density; (9) clay contents; (10) color indices  $a^*$  and  
 326 (11)  $L^*$ ; (12) magnetic susceptibility measured at 0.3 kHz ( $\kappa$ ), and (13) S-ratio of soil or sediment. All  
 327 data except for alkane content and  $CPI_{alk}$  (Gocke et al., unpublished data) were adopted from Gocke  
 328 et al. (2014b).

329 3.4.1 Free-extractable fatty acids

330 Positive correlation of long-chain ( $C_{22-32}$ ; SF 1) and dicarboxylic free FAs ( $C_{9-11}$ ; SF 3) with alkane  
 331 contents (Fig. 2, Tab. 2) confirmed the higher plant origin of the former and suggested the same for  
 332 the latter, because average chain length of long-chain alkanes ( $C_{25-37}$ ), which was mostly  $> 29$  at the  
 333 Nussloch deep subsoil (Gocke et al., 2013), indicated major contribution of higher plant leaf waxes  
 334 (Eglinton et al., 1962). Gram<sup>+</sup> bacteria-derived branched free FAs ( $C_{14,17}$ ; SF 2;  $C_{12,15,16}$ ; SF 3;  $C_{13,16}$ ;  
 335 SF 4) positively correlated with indicators of advanced pedogenesis (high  $C_{org}$  and clay contents) and  
 336 weathering (high  $a^*$  and  $\kappa$ ), low density, abundant living roots and high alkane contents. They  
 337 negatively correlated with rhizolith abundances (SF 3, 4), suggesting that Gram<sup>+</sup> bacteria in subsoil  
 338 feed mainly on old bulk OM and not younger plant-derived OM (Kramer and Gleixner, 2006).  
 339 Composition of higher plant-derived long-chain free FAs was probably hardly affected by microbial  
 340 overprint, as implied by the absence of correlation between long-chain and dicarboxylic free FAs, and  
 341 recent roots. Plant-derived free FAs can thus be used as (paleo)environmental tracer (Reiffarth et al.,  
 342 2016), as long as material free of root remains is collected.

343  
 344 Table 2.

345 Significance levels of the correlations between twelve statistical factors (SF) derived from the five investigated  
 346 biomarker groups, and 13 environmental and profile parameters (EPP). All EPP were adapted from Gocke et al.  
 347 (2014b) except for alkane contents and  $CPI_{alk}$ . For depth diagrams see Suppl. Fig. 2.

	Free FAs				PLFAs	CL-GDGTs			IPL- GDGTs		DNA	
	1	2	3	4	5	6	7	8	9	10	11	12
$C_{org}$ [ $mg\ g^{-1}$ ]		-	+		+	---				+		+
$C_{carb}$ [ $mg\ g^{-1}$ ]					--			+		-		
alkanes [ $\mu g\ g^{-1}$ ] <sup>a</sup>	+		+					+				
$CPI_{alk}$ [ ] <sup>b</sup>					-			+				
roots [ $m^{-2}$ ] <sup>c</sup>		--			+++	---			-	++		-
rhizoliths [ $m^{-2}$ ] <sup>c</sup>			-	+			+					+
biopores [ $m^{-2}$ ] <sup>c</sup>					+		+	-				
dry bulk density [ $g\ cm^{-3}$ ]		++										
clay [wt-%]		-		-	+++	-		-		+		
color $a^*$ [ ] <sup>d</sup>		-			++			-		++		+

color L* [ ] <sup>e</sup>			--	+		-	
$\kappa$ [m <sup>3</sup> kg <sup>-1</sup> ] <sup>f</sup>		-	--	++		-	-
S-ratio [ ] <sup>g</sup>						-	-

348 +/- significant positive/negative correlation (.01 < p < .1); ++/-- very significant positive/negative correlation (.001 <  
349 p < .01); +++/--- highly significant positive/negative correlation (p < .001).

350 <sup>a</sup> Alkanes in terrestrial sediments like loess are traditionally assumed to originate from aboveground biomass of  
351 synsedimentary vegetation, incorporated via litterfall and abraded particulate organic matter (Conte et al., 2003).

352 <sup>b</sup>  $CPI_{alk} = [(\sum n-C_{17-35 \text{ odd}} / \sum n-C_{16-34 \text{ even}}) + (\sum n-C_{17-35 \text{ odd}} / \sum n-C_{18-36 \text{ even}})] / 2$

353 Carbon preference index of odd over even *n*-alkane homologues. High  $CPI_{alk}$  indicates dominance of fresh plant  
354 aboveground biomass, whereas low values suggest predominantly microorganism-derived and degraded organic  
355 matter (Cranwell, 1981).

356 <sup>c</sup> Frequencies of roots, rhizoliths and biopores were determined on horizontal areas using a grid with dimension  
357 0.5x0.5 m (Gocke et al., 2014b).

358 <sup>d</sup> High  $a^*$  values indicate reddish color and low values green color (CIE, 1931). Throughout the investigated  
359 profile, highest  $a^*$  in the Holocene soil indicates pedogenic processes like formation of iron oxides, whereas in  
360 sedimentary deep subsoil, high  $a^*$  (mostly loess > paleosol) likely result from hydromorphic bleaching.

361 <sup>e</sup> High  $L^*$  values characterize light color and low values dark color (CIE, 1931). Although organic matter content  
362 can strongly influence  $L^*$ ,  $C_{org}$  and  $L^*$  do not correlate with each other at Nussloch. Rather, high  $L^*$  may result  
363 from hydromorphic bleaching of paleosols (Gocke et al., 2014b).

364 <sup>f</sup> Measure for concentration and magnetic grain size variation of ferrimagnetic minerals (Maher, 2011). Strong  
365 pedogenesis is represented by very high  $\kappa$  in the Holocene soil, but contrary to Eurasian loess deposits, high  $\kappa$  of  
366 the periglacial Nussloch loess-paleosol sequence represents seasonal waterlogging and subsequent *in situ*  
367 weathering rather than pedogenesis (Gocke et al., 2014b).

368 <sup>g</sup> Parameter for the relative portions of ferrimagnetic vs. antiferromagnetic minerals (Wang et al., 2006). More  
369 intense pedogenesis caused lower S-ratio and weak pedogenesis higher S-ratio in the loess-paleosol sequence,  
370 whereas the opposite holds true for the Holocene soil.

### 371 3.4.2 Phospholipid fatty acids

372 The SF created from PLFA composition (SF 5) yielded factor loadings similarly high for all of the  
373 compounds with positive loading on this SF, i.e. Gram<sup>+</sup> bacteria, *Actinomycetaceae*, Gram<sup>-</sup> bacteria  
374 and fungi, and showed significant to highly significant correlations with nine EPP. Living bacteria and  
375 fungi were associated with depths characterized by strong pedogenesis and weathering. For eight out  
376 of nine EPP, the chronological context is ambiguous, as they might be of synsedimentary /  
377 synpedogenic nature or could partially be related to postsedimentary waterlogging effects (high  $a^*$  and



378  $\kappa$ ) or later pedogenic (high  $C_{org}$  and low  $L^*$  due to OM accumulation, low  $C_{carb}$  due to carbonate  
379 dissolution, low  $CPI_{alk}$  from degradation, high clay) and rooting phases (abundant biopores from  
380 recent/former roots). Root abundances, showing one of the highest significances throughout the  
381 sample set ( $p = .00001$ ) with SF 5, emphasized the role of the recent vegetation for living microbial  
382 community in the deep subsoil. Large similarities of short-chain free FAs and PLFAs were found: Both  
383 showed that Gram<sup>+</sup> bacteria and *Actinomycetaceae* prefer depths characterized by stronger  
384 pedogenesis and weathering as well as recent rooting (SF 2–5). This implies that notable parts of  
385 short-chain free FAs in terrestrial sediments might result from rhizodeposits and microorganisms  
386 stimulated by plant roots, and thus represent a mixed paleoenvironmental signal from a broad  
387 timespan.

### 388 3.4.3 Core lipid glycerol dialkyl glycerol tetraethers

389 In terms of core GDGTs, archaeal GDGT<sub>1292,1298,1300</sub> (SF 6) were more abundant in depths that are  
390 influenced by both ancient/long-term pedogenic processes (high  $C_{org}$  and clay contents, low  $L^*$ ), and  
391 recent rooting. The correlation of archaeal *i*GDGTs and recent roots points to a similar direction as  
392 previous studies, which demonstrated the increased occurrence of bacterial *br*GDGTs in vicinity of  
393 roots (Huguet et al., 2013a). Analogously to *br*GDGT source organisms (Weijers et al., 2010; Ayari et  
394 al., 2013), our findings thus suggest that *i*GDGT source organisms might have a heterotrophic  
395 metabolism as well. However, the high significance of this relation as well as the correlation between  
396 core GDGTs, considered as fossil markers, and recent roots was unexpected. The latter might indicate  
397 low stability of archaeal intact polar GDGTs in soil, leading to a fast release of the respective core  
398 GDGTs. The assumed link of archaea mainly to recent and less to ancient rooting is enforced by the  
399 absence of correlations between any archaeal core and intact polar GDGTs (SF 6, 9, 10) and  
400 rhizoliths or biopores. Short residence times of archaeal intact polar GDGTs of few days to weeks  
401 were shown for marine sediments (Ingalls et al., 2012), and might be similar in soils. Among bacterial  
402 compounds, homologues with two cyclopentyl moieties (GDGT<sub>1018,1032,1046</sub>; SF 7) occurred mainly in  
403 depths strongly affected by ancient rooting (abundant rhizoliths, biopores) or by weathering (low S-  
404 ratio). Homologues with one cyclopentyl moiety (GDGT<sub>1020,1034,1048</sub>; SF 8) were also more abundant in  
405 weathered depths (low  $C_{carb}$  and alkane content, low  $CPI_{alk}$ , abundant biopores, high clay content,  $a^*$   
406 and  $\kappa$ ).

### 407 3.4.4 Intact polar glycerol dialkyl glycerol tetraethers

408 Archaeal intact polar GDGTs (GDGT<sub>1292,1298,1300</sub>; SF 9; GDGT<sub>1292,1296</sub>; SF 10) were most abundant in  
409 depths affected by recent rooting, similar to their CL counterparts (SF 6), whereas the significance  
410 level was distinctly lower than for SF 6. In a Central Chinese soil profile, Ayari et al. (2013) observed  
411 enriched intact polar GDGTs in close vicinity of living roots. This suggests that the respective source  
412 organisms were strongly stimulated by postsedimentary penetrating roots, which is in agreement with  
413 our findings. The two bacterial intact polar GDGTs with commonly highest abundance in soil,  
414 GDGT<sub>1022</sub> and GDGT<sub>1036</sub>, generally loaded opposite of archaeal GDGTs within SF 9 and SF 10, and  
415 thus also behaved contrary to homologues with one (GDGT<sub>1020,1034,1048</sub>) and two cyclopentyl moieties  
416 (GDGT<sub>1018,1032,1046</sub>; SF 7, 8). In summary, core and intact polar GDGTs revealed that source organisms  
417 prefer places of stronger reworking, except for GDGT<sub>1022</sub> and GDGT<sub>1036</sub>, potentially deriving from a  
418 different group of microorganisms than other GDGTs.

#### 419 3.4.5 Bacterial genetic fingerprint

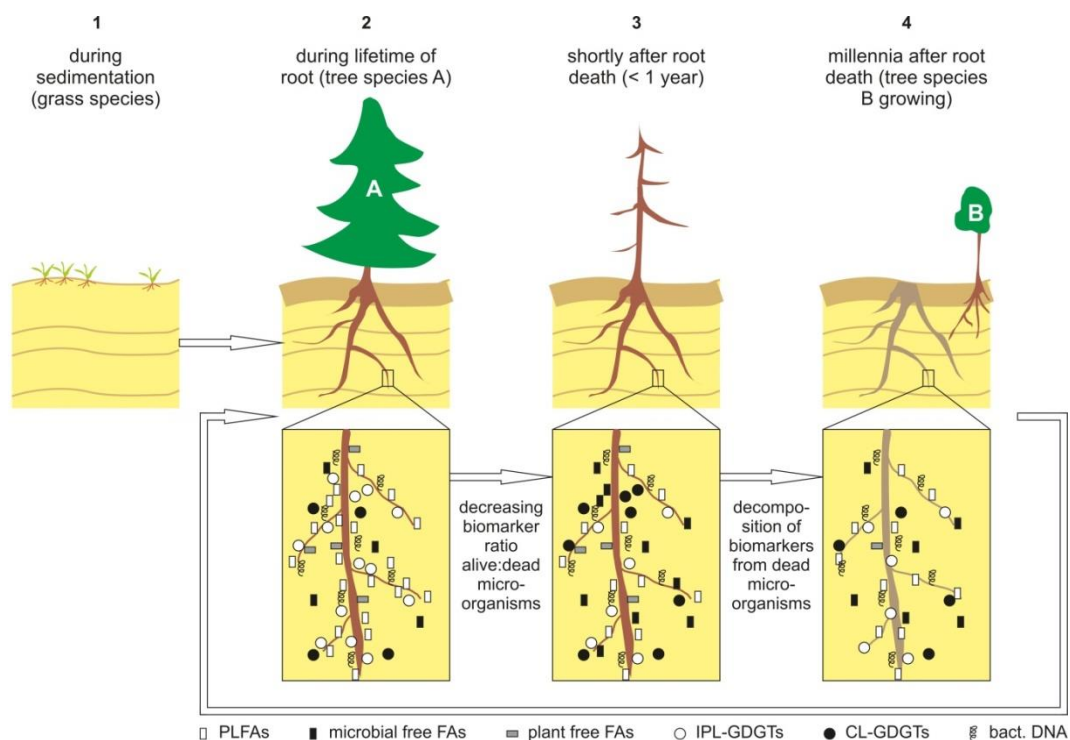
420 Based on the 16S rRNA genetic fingerprint, positive correlation of *Actinobacteria* with high C<sub>org</sub>  
421 contents (SF 11) supported free FA and PLFA results (SF 2, 3, 5). However, a direct comparison of  
422 DNA and PLFA-based community composition needs to be regarded with caution as both datasets  
423 have deviating taxonomic accuracy. *Actinobacteria* and *Firmicutes* (SF 12) preferred depths strongly  
424 affected by seasonal waterlogging (high a\*, low κ), and were abundant in depths with rhizoliths, but  
425 not in recently rooted depths, which contradicts free FA and PLFA results (SF 2–5). This discrepancy  
426 may result either from the smaller size of the DNA sample set excluding the peak root abundances in  
427 the Holocene soil, or may demonstrate the time-integrated character of the DNA signal in terrestrial  
428 sediments, covering a wide range from potentially fossil to recent microbial communities. The latter  
429 hypothesis is supported by the DNA-based SF 12 which is the only statistical factor that correlates with  
430 both recent roots and rhizoliths (Tab. 2), corresponding to ancient and recent C input, whereas other  
431 statistical factors correlated solely with one of them.

#### 432 3.5 Root-related long-term alteration of deep subsoil microbial communities – consequences and open 433 questions

434 Generally, the microbial community composition in deep subsoil was affected by pedogenic and  
435 weathering factors, as shown by correlations with clay, a\* and κ. Additionally, our results emphasize  
436 C<sub>org</sub> as main driving factor. This supports the assumption that C<sub>org</sub> is crucial for microbial abundance  
437 (Helgason et al., 2014), community (Hansel et al., 2008) or long-term survival of microorganisms in  
438 terrestrial sediments (Breuker et al., 2001), presumably due to close spatial association of

439 microorganisms and  $C_{org}$  (Holden and Fierer, 2005). As roots represent an important source of  
 440 available  $C_{org}$  in  $C_{org}$ -poor eolian sediments, root distribution considerably affected microbial  
 441 communities. At Nussloch, the main root effect was that intact polar lipids representing living  
 442 microorganisms were affected solely by recent root features, which includes partially also biopores. In  
 443 contrast, microbial remains reflecting an ancient or time-integrated signal, i.e. free/core lipids and  
 444 DNA, correlated predominantly with ancient root features including rhizoliths and biopores but  
 445 simultaneously also with living roots.

446 These findings strongly suggest that microbial life in sedimentary deep subsoil is not only altered by  
 447 postsedimentary rooting, but may thrive more or less continuously long after the lifetime of the root in  
 448 remaining hotspots. This is potentially stimulated by successive generations of roots partially re-  
 449 utilizing existing root-derived biopores (Fig. 4), a mechanism well known for crops in shallow subsoil  
 450 (Kautz et al., 2013). Analogously to deep sea sediments (Takano et al., 2010) or topsoils (Dippold and  
 451 Kuzyakov, 2015), in ancient terrestrial sediments and root systems like at the Nussloch loess-paleosol  
 452 sequence, microorganisms may thus feed on microbial degradation products and, in case of terrestrial  
 453 settings, rhizodeposits even millenia after decay of the initial microorganisms and/or root. Likely,  
 454 ongoing microbial dynamics in sedimentary deep subsoil is a widespread phenomenon. As shown by  
 455 a survey of root and root feature distribution in sedimentary deep subsoils of various age and texture  
 456 throughout Central and Southeast Europe (Gocke et al., 2014a), deep roots entering soil parent  
 457 material are not an exception but likely abundant at many sites globally.



458

459 Figure 4.  
460 Conceptual figure of root-related microorganism distribution and associated processes over time for vegetation  
461 growing on terrestrial sediments like e.g. loess or dune sands. 1) Syndimentary situation with scarce vegetation  
462 cover consisting of grasses and herbaceous plants, with shallow roots. 2) Roots of tree vegetation A enter the  
463 deep subsoil, stimulating microbial life in the rhizosphere adjacent to roots. 3) After death of the root, the ratio  
464 living : dead microorganisms and thus the ratio intact polar : free/core lipids decreases. 4) Millenia after root  
465 death, biopores and/or rhizoliths remain. Quantities of signature molecules of dead microorganisms are reduced  
466 by degradation. Process rates decrease with time, but proceed continuously over long time spans. Living  
467 microorganisms feed on dead microbial remains as well as on plant remains and sedimentary organic matter. In  
468 the figure, roots of the next tree vegetation (B) approach the ancient root systems, because the latter provide  
469 plant-available nutrients and old microorganism hotspots can be re-activated subsequently. New root effects may  
470 overprint old ones, e.g. if subsequent roots use old biopores. After phase 3, the cycle may thus continue with  
471 phase 2 and tree species B.  
472  
473 Our results disprove the traditional assumption of organic remains being solely incorporated during  
474 sediment deposition (Conte et al., 2003). This implies uncertainties for paleoenvironmental records in  
475 Quaternary terrestrial archives with allegedly high chronological resolution like loess-paleosol  
476 sequences. Horizontal transects from rhizoliths via rhizosphere towards root-free sediment at various  
477 study sites revealed the postsedimentary overprint of free FAs and core GDGTs (Huguet et al., 2012,  
478 2013a; Gocke et al., 2014c), and similarly, PLFAs and intact polar GDGTs from microorganisms  
479 associated with living roots might have been incorporated (Fig. 4). The overprint of syndimentary  
480 records and time-integrated character of free/core lipids are further enforced by the suspiciously  
481 similar depth diagrams of paleotemperature, reconstructed based on core and intact polar *br*GDGTs at  
482 Nussloch, which strongly disagree with Glacial conditions (Suppl. Fig. 5). This urges us to recommend  
483 the following cautions prior to sampling, i) at least 0.5 m of sediment should be removed from each  
484 side of the profile to avoid recent root effects, ii) the archive should be carefully regarded with respect  
485 to deep roots and iii) samples should be collected distant from visible root remains.  
486 Further, ongoing OM incorporation and turnover by root-related microorganisms in deep subsoil will  
487 not necessarily entail the sequestration of additional C as recently postulated (Kell, 2012), but might  
488 rather cause net loss of considerable amounts of C stored in paleosols (Marin-Spiotta et al., 2014) due  
489 to priming effects. Thus, the biogeochemical and ecological disequilibrium induced by fresh younger  
490 root and microbial biomass might even stimulate degradation of stabilized sedimentary OM (Fontaine  
491 et al., 2007). To assess this question, carbon stocks in the deep rhizosphere and in respective bulk

492 soil/sediment have to be determined and opposed to each other. Generally, studies on the terrestrial C  
493 cycle as well as soil microbial studies should be extended to greater depths, including the soil parent  
494 material which has been largely neglected so far (Richter and Markewitz, 1995; Harper and Tibbett,  
495 2013).

#### 496 4. Conclusions

497 Signature molecules for both living and fossil microorganisms were found throughout a Central  
498 European 9.5 m thick soil-sediment profile, i.e. in the Holocene soil and in associated sedimentary  
499 deep subsoil of Pleistocene age. For the first time, we demonstrated significant correlations of  
500 microbial communities in sedimentary deep subsoil with frequencies of recent roots as well as fossil  
501 calcified roots, so-called rhizoliths, and root-derived biopores. Our unique multi-proxy approach  
502 revealed significant interrelationships of living microorganisms with recent root quantities, and of fossil  
503 microbial communities with frequencies of recent roots and ancient root remains. This indicated that i)  
504 the paleoenvironmental signal recorded in terrestrial sediments can be altered by postsedimentary  
505 rooting, and ii) roots may entail long-term maintenance of microbial life in deep subsoil. Plant roots  
506 entering deeper parts of the soil including the parent material up to several millenia after its deposition  
507 are thus an important source of carbon and nutrients, stimulating microbial life far beyond the lifetime  
508 of the root. The widespread occurrence of deep roots up to several meters below the topsoil not only  
509 at the current study site, but at several sites around the world, suggests that these root effects on deep  
510 subsoil microbial communities are not an exception.

#### 511 References

- 512 Agnelli, A., Ascher, J., Corti, G., Ceccherini, M.T., Pietramellara, G., Nannipieri, P., 2007. Purification  
513 and isotopic signatures ( $\delta^{13}\text{C}$ ,  $\delta^{15}\text{N}$ ,  $\Delta^{14}\text{C}$ ) of soil extracellular DNA. *Biology and Fertility of Soils* 44,  
514 353-361, doi: 10.1007/s00374-007-0213-y.
- 515 Antoine, P., Rousseau, D.D., Moine, O., Kunesch, S., Hatté, C., Lang, A., Tissoux, H., Zöller, L., 2009.  
516 Rapid and cyclic aeolian deposition during the Last Glacial in European loess: a high-resolution  
517 record from Nussloch, Germany. *Quaternary Science Reviews* 28, 2955-2973, doi:  
518 10.1016/j.quascirev.2009.08.001.
- 519 Apostel, C., Dippold, M., Glaser, B., Kuzyakov, Y., 2013. Biochemical pathways of amino acids in soil:  
520 Assessment by position-specific labeling and  $^{13}\text{C}$ -PLFA analysis. *Soil Biology & Biochemistry* 67,  
521 31-40, doi: 10.1016/j.soilbio.2013.08.005.

522 Ayari, A., Yang, H., Wiesenberg, G.L.B., Xie, S., 2013. Distribution of archaeal and bacterial tetraether  
523 membrane lipids in rhizosphere-root systems in soils and their implication for paleoclimate  
524 assessment. *Geochemical Journal* 47, 337-347, doi: 10.2343/geochemj.2.0249.

525 Bligh, E.G., Dyer, W.J., 1959. A rapid method of total lipid extraction and purification. *Canadian Journal*  
526 *of Biochemistry and Physiology* 37, 911-917, doi: 10.1139/o59-099.

527 Blume, E., Bischoff, M., Reichert, J.M., Moorman, T., Konopka, A., Turco, R.F., 2002. Surface and  
528 subsurface microbial biomass, community structure and metabolic activity as a function of soil  
529 depth and season. *Applied Soil Ecology* 20, 171-181, doi: 10.1016/S0929-1393(02)00025-2.

530 Breuker, A., Köweker, G., Blazejak, A., Schippers, A., 2001. The deep biosphere in terrestrial  
531 sediments in the Chesapeake Bay area, Virginia, USA. *Frontiers in Microbiology* 2, 156, doi:  
532 10.3389/fmicb.2011.00156.

533 Brockman, F.J., Kieft, T.L., Fredrickson, J.K., Bjornstad, B.N., Li, S.W., Spangenburg, W., Long, P.E.,  
534 1992. Microbiology of Vadose Zone Paleosols in South-Central Washington State. *Microbial*  
535 *Ecology* 23, 279-301, doi: 10.1007/BF00164101.

536 Buckley, D.H., Graber, J.R., Schmidt, T.M., 1998. Phylogenetic analysis of nonthermophilic members  
537 of the kingdom Crenarchaeota and their diversity and abundance in soils. *Applied and*  
538 *Environmental Microbiology* 64, 4333-4339.

539 Bundt, M., Widmer, F., Pesaro, M., Zeyer, J., Blaser, P., 2001. Preferential flow paths: biological 'hot  
540 spots' in soils. *Soil Biology & Biochemistry* 33, 729-738, doi: 10.1016/S0038-0717(00)00218-2.

541 Canadell, J., Jackson, R.B., Ehleringer, J.R., Mooney, H.A., Sala, O.E., Schulze, E.D., 1996.  
542 Maximum rooting depth of vegetation types at the global scale. *Oecologia* 108, 583-595, doi:  
543 10.1007/BF00329030.

544 Commission Internationale de l'Eclairage (CIE), 1931. Proceedings of the 8<sup>th</sup> session of CIE.  
545 Cambridge University Press, UK.

546 Conte, M.H., Weber, J.C., Carlson, P.J., Flanagan, L.B., 2003. Molecular and carbon isotopic  
547 composition of leaf wax in vegetation and aerosols in a northern prairie ecosystem. *Oecologia* 135,  
548 67-77, doi: 10.1007/s00442-002-1157-4.

549 Cranwell, P.A., 1981. Diagenesis of free and bound lipids in terrestrial detritus deposited in a  
550 lacustrine sediment. *Organic Geochemistry* 3, 79-89, doi: 10.1016/0146-6380(81)90002-4.

551 Degelmann, D., Kolb, S., Dumont, M., Murrell, J.C., Drake, H.L., 2009. *Enterobacteriaceae* facilitate  
552 the anaerobic degradation of glucose by a forest soil. *FEMS Microbiological Ecology* 68, 312-319,  
553 doi: 10.1111/j.1574-6941.2009.00681.x.

554 Dippold, M.A., Kuzyakov, Y., 2015. Direct incorporation of fatty acids into microbial phospholipids in  
555 soils: Position-specific labeling tells the story. *Geochimica et Cosmochimica Acta* 174, 211-221,  
556 doi: 10.1016/j.gca.2015.10.032.

557 Egert, M., Friedrich, M.W., 2003. Formation of pseudoterminal restriction fragments, a PCR-related  
558 bias affecting terminal restriction fragment length polymorphism analysis of microbial community  
559 structure. *Applied and Environmental Microbiology* 69, 2555-2562, doi: 10.1128/AEM.69.5.2555-  
560 2562.2003.

561 Eglinton, G., Gonzalez, A.G., Hamilton, R.J., Raphael, R.A., 1962. Hydrocarbon constituents of the  
562 wax coatings of plant leaves: a taxonomic survey. *Phytochemistry* 1, 89-102, doi: 10.1016/S0031-  
563 9422(00)88006-1.

564 Fernandes, M.F., Saxena, J., Dick, R.P., 2013. Comparison of Whole-Cell Fatty Acid (MIDI) or  
565 Phospholipid Fatty Acid (PLFA) Extractants as Biomarkers to Profile Soil Microbial Communities.  
566 *Microbial Ecology* 66, 145-157, doi: 10.1007/s00248-013-0195-2.

567 Fierer, N., Schimel, J.P., Holden, P.A., 2003. Variations in microbial community composition through  
568 two soil depth profiles. *Soil Biology & Biochemistry* 35, 167-176, doi: 10.1016/S0038-  
569 0717(02)00251-1.

570 Fisher, M.J., Rao, I.M., Ayarza, M.A., Lascano, C.E., Sanz, J.I., Thomas, R.J., Vera, R.R., 1994.  
571 Carbon storage by introduced deep-rooted grasses in the South American savannas. *Nature* 371,  
572 236-238, doi: 10.1038/371236a0.

573 Fontaine, S., Barot, S., Barré, P., Bdioui, N., Mary, B., Rumpel, C., 2007. Stability of organic carbon in  
574 deep soil layers controlled by fresh carbon supply. *Nature* 450, 277-280, doi: 10.1038/nature06275.

575 Gocke, M., Pustovoytov, K., Kühn, P., Wiesenberg, G.L.B., Löscher, M., Kuzyakov, Y., 2011.  
576 Carbonate rhizoliths in loess and their implications for paleoenvironmental reconstruction revealed  
577 by isotopic composition:  $\delta^{13}\text{C}$ ,  $^{14}\text{C}$ . *Chemical Geology* 283, 251-260, doi:  
578 10.1016/j.chemgeo.2011.01.022.

579 Gocke, M., Kuzyakov, Y., Wiesenberg, G.L.B., 2013. Differentiation of plant derived organic matter in  
580 soil, loess and rhizoliths based on *n*-alkane molecular proxies. *Biogeochemistry* 112, 23-40, doi:  
581 10.1007/s10533-011-9659-y.

582 Gocke, M., Gulyás, S., Hambach, U., Jovanović, M., Kovács, G., Marković, S.B., Wiesenberg, G.L.B.,  
583 2014a. Biopores and root features as new tools for improving paleoecological understanding of  
584 terrestrial sediment-paleosol sequences. *Palaeogeography Palaeoclimatology Palaeoecology* 394,  
585 42-58, doi: 10.1016/j.palaeo.2013.11.010.

586 Gocke, M., Hambach, U., Eckmeier, E., Schwark, L., Zöller, L., Fuchs, M., Wiesenberg, G.L.B., 2014b.  
587 Introducing an improved multi-proxy approach for paleoenvironmental reconstruction of loess-  
588 paleosol archives applied on the Late Pleistocene Nussloch sequence (SW Germany).  
589 *Palaeogeography Palaeoclimatology Palaeoecology* 410, 300-315, doi:  
590 10.1016/j.palaeo.2014.06.006.

591 Gocke, M., Peth, S., Wiesenberg, G.L.B., 2014c. Lateral and depth variation of loess organic matter  
592 overprint related to rhizoliths – Revealed by lipid molecular proxies and X-ray tomography. *Catena*  
593 112, 72-85, doi: 10.1016/j.catena.2012.11.011.

594 Gocke, M.I., Kessler, F., van Mourik, J.M., Jansen, B., Wiesenberg, G.L.B., 2015. Paleosols can  
595 promote root growth of the recent vegetation – a case study from the sandy soil-sediment  
596 sequence Rakt, the Netherlands. *SOILD* 2, 1273-1308, doi: 10.5194/soild-2-1273-2015.

597 Hamberger, A., Horn, M.A., Dumont, M.G., Murrell, J.C., Drake, H.L., 2008. Anaerobic consumers of  
598 monosaccharides in a moderately acidic fen. *Applied and Environmental Microbiology* 74, 3112-  
599 3120, doi: 10.1128/AEM.00193-08.

600 Hansel, C.M., Fendorf, S., Jardine, P.M., Francis, C.A., 2008. Changes in Bacterial and Archaeal  
601 Community Structure and Functional Diversity along a Geochemically Variable Soil Profile. *Applied*  
602 *and Environmental Microbiology* 74, 1620-1633, doi: 10.1128/AEM.01787-07.

603 Harper, R.J., Tibbett, M., 2013. The hidden organic carbon in deep mineral soils. *Plant and Soil* 368,  
604 641-648, doi: 10.1007/s11104-013-1600-9.

605 Harvey, H.R., Fallon, R.D., Patton, J.S., 1986. The effect of organic matter and oxygen on the  
606 degradation of bacterial membrane lipids in marine sediments. *Geochimica et Cosmochimica Acta*  
607 50, 795-804, doi: 10.1016/0016-7037(86)90355-8.

608 Harwood, J L., Russell, N.J., 1984. *Lipids in plants and microbes*. George Allen & Unwin, London.

609 Helgason, B.L., Konschuh, H.J., Bedard-Haughn, A., VandenBygaart, A.J., 2014. Microbial distribution  
610 in an eroded landscape: Buried A horizons support abundant and unique communities. *Agriculture,*  
611 *Ecosystems & Environment* 196, 94-102, doi: 10.1016/j.agee.2014.06.029.



612 Holden, P.A., Fierer, N., 2005. Microbial Processes in the Vadose Zone. *Vadose Zone Journal* 4, 1-21,  
613 doi: 10.2113/4.1.1.

614 Huguet, A., Wiesenberg, G.L.B., Gocke, M., Fosse, C., Derenne, S., 2012. Branched tetraether  
615 membrane lipids associated with rhizoliths in loess: Rhizomicrobial overprinting of initial biomarker  
616 record. *Organic Geochemistry* 43, 12-19, doi: 10.1016/j.orggeochem.2011.11.006.

617 Huguet, A., Gocke, M., Derenne, S., Fosse, C., Wiesenberg, G.L.B., 2013a. Root-associated  
618 branched tetraether source microorganisms may reduce estimated paleotemperatures in subsoil.  
619 *Chemical Geology* 356, 1-10, doi: 10.1016/j.chemgeo.2013.07.017.

620 Huguet, A., Fosse, C., Laggoun-Défarge, F., Delarue, F., Derenne, S., 2013b. Effects of a short-term  
621 experimental microclimate warming on the abundance and distribution of branched GDGTs in a  
622 French peatland. *Geochimica et Cosmochimica Acta* 105, 294-315, doi:  
623 10.1016/j.gca.2012.11.037.

624 Ingalls, A.E., Huguet, C., Truxal, L.T., 2012. Distribution of Intact and Core Membrane Lipids of  
625 Archaeal Glycerol Dialkyl Glycerol Tetraethers among Size-Fractionated Particulate Organic Matter  
626 in Hood Canal, Puget Sound. *Applied and Environmental Microbiology* 78, 1480-1490, doi:  
627 10.1128/AEM.07016-11.

628 IUSS Working Group WRB, 2014. World Reference Base for Soil Resources 2014. International soil  
629 classification system for naming soils and creating legends for soil maps. *World Soil Resources*  
630 *Reports No. 106*. FAO, Rome.

631 Janssen, P.H., 2006. Identifying the Dominant Soil Bacterial Taxa in Libraries of 16S rRNA and 16S  
632 rRNA Genes. *Applied and Environmental Microbiology* 72, 1719-1728, doi:  
633 10.1128/AEM.72.3.1719-1728.2006.

634 Johnson, W.C., 2014. Carbon cycle: Sequestration in buried soils. *Nature Geoscience* 7, 398-399, doi:  
635 10.1038/ngeo2172.

636 Kautz, T., Amelung, W., Ewert, F., Gaiser, T., Horn, R., Jahn, R., Javaux, M., Kemna, A., Kuzyakov,  
637 Y., Munch, J.C., Pätzold, S., Peth, S., Scherer, H.W., Schloter, M., Schneider, H., Vanderborght, J.,  
638 Vetterlein, D., Walter, A., Wiesenberg, G.L.B., Köpke, U., 2013. Nutrient acquisition from arable  
639 subsoils in temperate climates: A review. *Soil Biology & Biochemistry* 57, 1003-1022, doi:  
640 10.1016/j.soilbio.2012.09.014.

641 Kell, D.B., 2012. Large-scale sequestration of atmospheric carbon via plant roots in natural and  
642 agricultural ecosystems: why and how. *Philosophical Transactions of The Royal Society B*  
643 *Biological Sciences* 367, 1589-1597, doi: 10.1098/rstb.2011.0244.

644 Kindler, R., Miltner, A., Thullner, M., Richnow, H.H., Kästner, M., 2009. Fate of bacterial biomass  
645 derived fatty acids in soil and their contribution to soil organic matter. *Organic Geochemistry* 40,  
646 29-37, doi: 10.1016/j.orggeochem.2008.09.005.

647 Klappa, C.F., 1980. Rhizoliths in terrestrial carbonates: classification, recognition, genesis and  
648 significance. *Sedimentology* 27, 613-629, doi: 10.1111/j.1365-3091.1980.tb01651.x.

649 Kolattukudy, P.E., 1984. Biochemistry and function of cutin and suberin. *Canadian Journal of Botany*  
650 62, 2918-2933, doi: 10.1139/b84-391.

651 Kolattukudy, P.E., Croteau, R., Buckner, J.S., 1976. Biochemistry of plant waxes. In: Kollatukudy, P.E.  
652 (Ed.), *Chemistry and Biochemistry of Natural Waxes*. Elsevier, Amsterdam, pp. 290-347.

653 Kramer, C., Gleixner, G., 2006. Variable use of plant- and soil-derived carbon by microorganisms in  
654 agricultural soils. *Soil Biology & Biochemistry* 38, 3267-3278, doi: 10.1016/j.soilbio.2006.04.006.

655 Lengger, S.K., Kraaij, M., Tjallingii, R., Baas, M., Stuut, J.B., Hopmans, E.C., Sinninghe Damsté, J.S.,  
656 Schouten, S., 2013. Differential degradation of intact polar and core glycerol dialkyl glycerol  
657 tetraether lipids upon post-depositional oxidation. *Organic Geochemistry* 65, 83-93, doi:  
658 10.1016/j.orggeochem.2013.10.004.

659 Lichtfouse, É., Berthier, G., Houot, S., Barriuso, E., Bergheaud, V., Vallaey, T., 1995. Stable carbon  
660 isotope evidence for the microbial origin of C<sub>14</sub>-C<sub>18</sub> n-alkanoic acids in soils. *Organic Geochemistry*  
661 23, 849-852, doi: 10.1016/0146-6380(95)80006-D.

662 Liu, W., Yang, H., Ning, Y., An, Z., 2007. Contribution of inherent organic carbon to the bulk  $\delta^{13}\text{C}$   
663 signal in loess deposits from the arid western Chinese Loess Plateau. *Organic Geochemistry* 38,  
664 1571-1579, doi: 10.1016/j.orggeochem.2007.05.004.

665 Maher, B.A., 2011. The magnetic properties of Quaternary aeolian dusts and sediments, and their  
666 palaeoclimatic significance. *Aeolian Research* 3, 87-144, doi: 10.1016/j.aeolia.2011.01.005.

667 Marin-Spiotta, E., Chaopricha, N.T., Plante, A.F., Diefendorf, A.F., Mueller, C.W., Grandy, A.S.,  
668 Mason, J.A., 2014. Long-term stabilization of deep soil carbon by fire and burial during early  
669 Holocene climate change. *Nature Geoscience* 7, 428-432, doi: 10.1038/ngeo2169.

670 Messing, J., 1983. New M13 vectors for cloning. *Methods in Enzymology* 101, 20-78, doi:  
671 10.1016/0076-6879(83)01005-8.

672 Peterse, F., Graeme, W.N., Schouten, S., Sinninghe Damsté, J.S., 2010. Influence of soil pH on the  
673 abundance and distribution of core and intact polar lipid-derived branched GDGTs in soil. *Organic*  
674 *Geochemistry* 41, 1171-1175, doi: 10.1016/j.orggeochem.2010.07.004.

675 Reiffarth, D.G., Peticrew, E.L., Owens, P.N., Lobb, D.A., 2016. Sources of variability in fatty acid (FA)  
676 biomarkers in the application of compound-specific stable isotopes (CSSIs) to soil and sediment  
677 fingerprinting and tracing: A review. *Science of the Total Environment* 565, 8-27, doi:  
678 10.1016/j.scitotenv.2016.04.137.

679 Richter, D D., Markewitz, D., 1995. How deep is soil? *BioScience* 45, 600-609, doi: 10.2307/1312764.

680 Schellenberger, S., Kolb, S., Drake, H.L., 2010. Metabolic responses of novel cellulolytic and  
681 saccharolytic agricultural soil Bacteria to oxygen. *Environmental Microbiology* 12, 845-861, doi:  
682 10.1111/j.1462-2920.2009.02128.x.

683 Schouten, S., Middelburg, J.J., Hopmans, E.C., Sinninghe Damsté, J.S., 2010. Fossilization and  
684 degradation of intact polar lipids in deep subsurface sediments: a theoretical approach.  
685 *Geochimica et Cosmochimica Acta* 74, 3806-3814, doi: 10.1016/j.gca.2010.03.029.

686 Schouten, S., Hopmans, E.C., Sinninghe Damsté, J.S., 2013. The organic geochemistry of glycerol  
687 dialkyl glycerol tetraether lipids: A review. *Organic Geochemistry* 54, 19-61, doi:  
688 10.1016/j.orggeochem.2012.09.006.

689 Sinninghe Damsté, J.S., Rijpstra, W.I.C., Hopmans, E.C., Weijers, J.W.H., Foesel, B.U., Overmann,  
690 J., Dedysh, S.N., 2011. 13,16-Dimethyl Octacosanedioic Acid (iso-Diabolic Acid), a Common  
691 Membrane-Spanning Lipid of Acidobacteria Subdivisions 1 and 3. *Applied and Environmental*  
692 *Microbiology* 77, 4147-4154, doi: 10.1128/AEM.00466-11.

693 Sinninghe Damsté, J.S., Rijpstra, W.I.C., Hopmans, E.C., Foesel, B.U., Wüst, P.K., Overmann, J.,  
694 Tank, M., Bryant, D.A., Dunfield, P.F., Houghton, K., Stott, M.B., 2014. Ether- and Ester-Bound iso-  
695 Diabolic Acid and Other Lipids in Members of Acidobacteria Subdivision 4. *Applied and*  
696 *Environmental Microbiology* 80, 5207-5218, doi: 10.1128/AEM.01066-14.

697 Stone, M.M., DeForest, J.L., Plante, A.F., 2014. Changes in extracellular enzyme activity and  
698 microbial community structure with soil depth at the Luquillo Critical Zone Observatory. *Soil Biology*  
699 *& Biochemistry* 75, 237-247, doi: 10.1016/j.soilbio.2014.04.017.

700 Takano, Y., Chikaraishi, Y., Ogawa, N.O., Nomaki, H., Morono, Y., Inagaki, F., Kitazato, H., Hinrichs,  
701 K.-U., Ohkouchi, N., 2010. Sedimentary membrane lipids recycled by deep-sea benthic archaea.  
702 *Nature Geoscience* 3, 858-861, doi: 10.1038/ngeo983.

- 703 Wang, Y., Li, Y., Ye, X., Chu, Y., Wang, X., 2010. Profile storage of organic/inorganic carbon in soil:  
704 From forest to desert. *Science of the Total Environment* 408, 1925-1931, doi:  
705 10.1016/j.scitotenv.2010.01.015.
- 706 Wang, X., Lu, H., Xu, H., Deng, C., Cheng, T., Wang, X., 2006. Magnetic properties of loess deposits  
707 on the northeastern Qinghai–Tibetan Plateau: palaeoclimatic implications for the Late Pleistocene.  
708 *Geophysical Journal International* 167, 1138-1147, doi: 10.1111/j.1365-246X.2006.03007.x.
- 709 Weijers, J.W.H., Wiesenberg, G.L.B., Bol, R., Hopmans, E.C., Pancost, R.D., 2010. Carbon isotopic  
710 composition of branched tetraether membrane lipids in soils suggest a rapid turnover and a  
711 heterotrophic life style of their source organism(s). *Biogeosciences* 7, 2959-2973, doi: 10.5194/bg-  
712 7-2959-2010.
- 713 Wiesenberg, G.L.B., Gocke, M.I., 2015. Analysis of lipids and polycyclic aromatic hydrocarbons as  
714 indicators of past and present (micro)biological activity. In: McGenity, T.J., Timmis, K.N., Nogales,  
715 B. (Eds.), *Hydrocarbon and Lipid Microbiology: Cultivation*. Springer Protocol Handbooks. Springer,  
716 Berlin Heidelberg, pp. 1-31, doi: 10.1007/8623\_2015\_157.
- 717 Yang, H., Pancost, R.D., Dang, X., Zhou, X., Evershed, R.P., Xiao, G., Tang, C., Gao, L., Guo, Z., Xie,  
718 S., 2014. Correlations between microbial tetraether lipids and environmental variables in Chinese  
719 soils: Optimizing the paleo-reconstructions in semid-arid and arid regions. *Geochimica et*  
720 *Cosmochimica Acta* 126, 46-69, doi: 10.1016/j.gca.2013.10.041.
- 721 Zelles, L., 1999. Fatty acid patterns of phospholipids and lipopolysaccharides in the characterisation of  
722 microbial communities in soil: a review. *Biology and Fertility of Soils* 29, 111-129, doi:  
723 10.1007/s003740050533.

#### 724 Acknowledgments

725 The study was funded by the German Research Foundation (grant number DFG-WI 2810/10-1) and  
726 by the Swiss National Science Foundation (grant number 153631). The authors further thank LabEx  
727 Matisse for funding, and acknowledge financial support from the French–German PHC/ DAAD  
728 PROCOPE program. HeidelbergCement AG (Heidelberg, Germany) enabled sampling in their  
729 quarries, assisted by M. Löscher (Leimen, Germany). Several MSc and PhD students as well as  
730 technicians supported DNA (S. Hetz, R. Mertel, O. Schmidt, A. Stacheter, A. Wieczorek [University of  
731 Bayreuth, Germany]) and GDGT analyses (C. Anquetil [UPMC Paris, France], C. Fosse [Chimie Paris

732 Tech, France], C.-L. Müller [University of Bayreuth, Germany]). Access to microbiology laboratory was  
733 provided by H. Drake (University of Bayreuth, Germany).

734 Author contributions

735 M.I.G. and G.L.B.W. initiated the study, conducted field work and data compilation as well as  
736 interpretation. M.I.G. prepared the first draft of the manuscript and performed analyses and evaluation  
737 of free FAs, PLFAs and DNA. A.H. and S.D. conducted GDGT analyses and evaluation. S.K. provided  
738 equipment for DNA analyses and introduced M.I.G. to the respective methods. M.A.D. contributed to  
739 PLFA analysis and conducted statistical evaluation of the data set. All authors actively participated in  
740 the discussion and manuscript improvement.

741