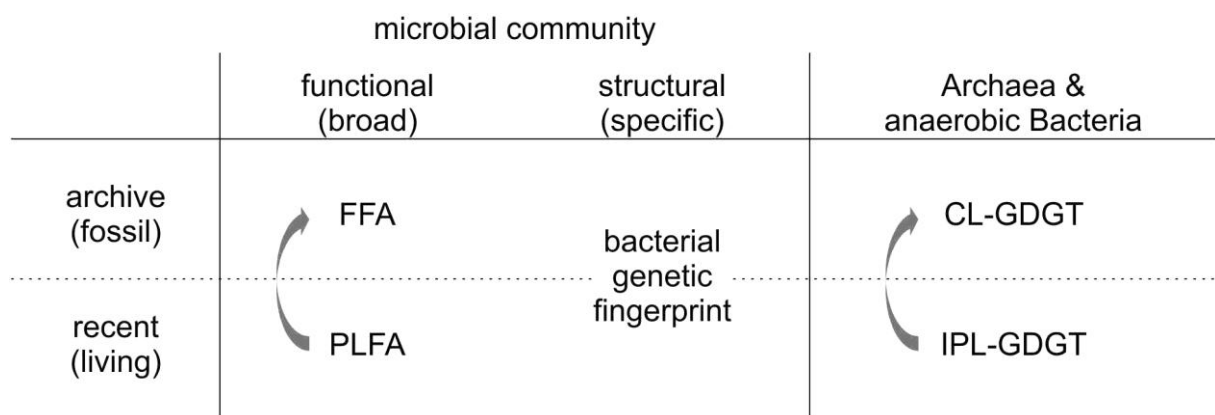


Supplement

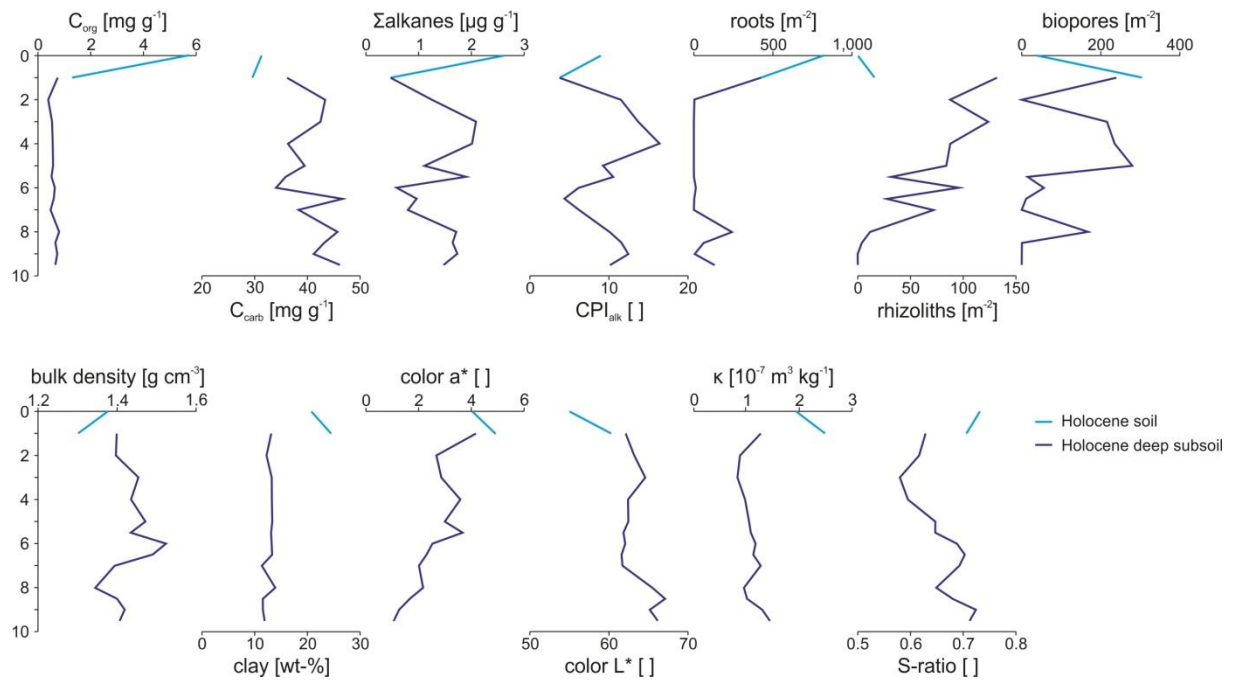
Disentangling interactions between microbial communities and roots in deep subsoil

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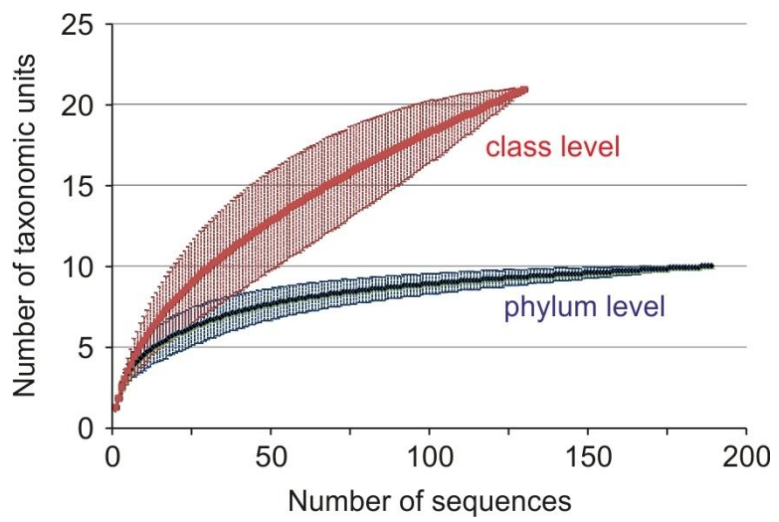
Supplementary Figure 1.

Scheme of the five biomarker classes investigated in the current study. Arrows show the removal of the polar headgroup of PLFAs and IPL-GDGTs after senescence of the respective source organisms followed by release of the compounds into the soil or sediment as free FAs and CL-GDGTs.



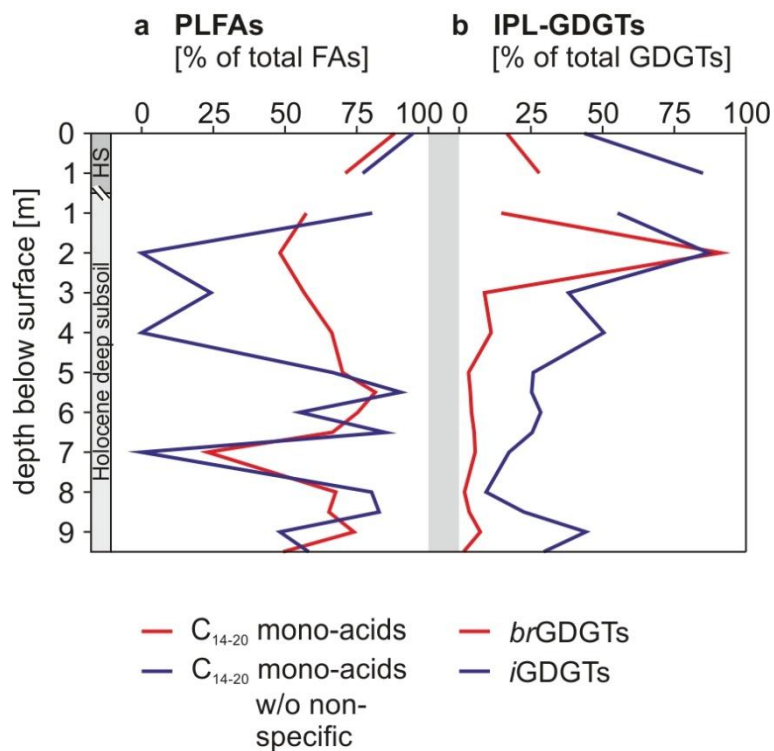
SupplementaryFigure2.

Physical, chemical and biological profile characteristics, summarized as environmental and profile parameters (EPP). All data except for alkane content and CPI_{alk} (Gocke et al., unpublished data) were adopted from Gocke et al. (2014). The reason for the occurrence of abundant living roots around 8 m depth is the preparation of the profile at a slope which was vegetated at that position by young trees and shrubs (Gocke et al. (2014). CPI = carbon preference index.



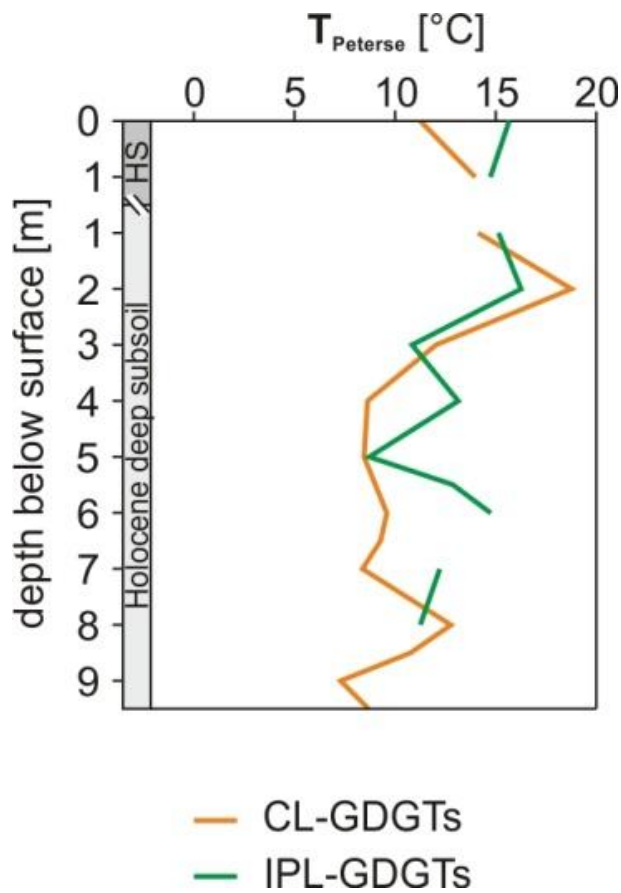
SupplementaryFigure3.

Rarefaction analysis demonstrated that results from DNA analysis are reliable on the phylum level.



SupplementaryFigure4.

Relative portions of PLFAs from total FAs (PLFAs + free FAs), and of intact polar GDGTs from total GDGTs (intact polar + core GDGTs). a) For comparability of FAs, solely free FAs with a chain length of 14–20 carbons were included, which is the range commonly detected for PLFAs. Further, plant-derived dicarboxylic free FAs were excluded to enable comparison of compounds originating only or mainly from microorganisms (red line). Additionally, the blue line shows portions of PLFAs, if non-specific homologues from both PLFAs and free FAs are excluded, i.e. saturated unbranched compounds. b) Relative portions of intact polar GDGTs, shown separately for archaeal *i*GDGTs and for bacterial *br*GDGTs. HS = Holocene soil.



SupplementaryFigure5.

Reconstructed annual mean air temperature (MAT), calculated based on CL- and IPL-*br*GDGTs composition. For the calculation, the equation introduced by Peterse et al. (2012) was used:

$$\text{MBT}' = (\text{GDGT}_{1022,1020,1018} / \text{GDGT}_{1022,1020,1018,1036,1034,1032,1050}) \quad (\text{Eq. 1})$$

$$\text{CBT} = -\log(\text{GDGT}_{1020,1034} / \text{GDGT}_{1022,1036}) \quad (\text{Eq. 2})$$

$$\text{MAT} = 0.81 - (5.67 * \text{CBT}) + (31 * \text{MBT}') \quad (\text{Eq. 3})$$

with MBT' = Methylation index of Branched Tetraethers, modified by Peterse et al. (2012) from a previous version, and CBT = Cyclisation index of Branched Tetraethers.

SupplementaryTable 1.

FA and GDGT parameters calculated based on composition of FAs and GDGTs: ratio of short- vs. long-chain free FAs ($R_{S:L}$), ratio of bacterial vs. fungal compounds ($R_{B:F}$), ratio of Gram⁺ vs. Gram⁻ bacterial compounds ($R_{P:N}$), ratios of isoprenoid vs. branched GDGTs ($R_{i:b}$) in both CL and IPL pools, and portions of *i*GDGTs from total core GDGTs as well as from total intact polar GDGTs. At 1.0 m depth, both the Holocene soil (HS) and the Holocene deep subsoil (HDS) were analysed.

Parameter	FAs			GDGTs			
	$R_{S:L}$	$R_{B:F}$	$R_{P:N}$	$R_{i:b}$	$R_{i:b}$	%	%
	free FAs	PLFAs	PLFAs	CL-	IPL-	<i>i</i> GDGTs	<i>i</i> GDGTs
				GDGTs	GDGTs	GDGTs	GDGTs
0.0	0.7	5.5	0.8	0.68	2.65	40.6	72.6
1.0 HS	11.6	12.5	1.8	0.07	1.00	6.3	50.0
1.0 HDS	n.d. ^a	2.7	1.0	0.14	1.03	12.6	50.6
2.0	4.6	n.d. ^b	n.d. ^b	0.49	0.33	33.1	24.9
3.0	1.2	n.d. ^c	n.d. ^d	0.09	0.58	8.5	36.9
4.0	1.2	n.d. ^b	n.d. ^b	0.05	0.42	5.0	29.7
5.0	2.7	13.7	4.3	0.10	0.97	9.3	49.2
5.5	3.3	24.1	0.2	0.05	0.40	5.0	28.6
6.0	5.6	3.3	n.d. ^d	0.06	0.48	5.3	32.3
6.5	0.9	12.1	0.1	0.06	0.36	5.7	26.3
7.0	1.3	n.d. ^b	n.d. ^b	0.06	0.19	5.3	16.2
8.0	3.6	2.1	0.3	0.24	1.02	19.0	50.6
8.5	0.8	16.2	0.2	0.09	0.64	8.0	39.0
9.0	2.0	0.2	n.d. ^d	0.03	0.32	3.1	24.1
9.5	2.0	1.9	0.2	0.08	1.89	7.2	65.4

^ano long-chain free FAs present.

^bno specific PLFAs present.

^cno fungal PLFAs present.

^dno Gram⁻ bacterial PLFAs present.

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