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Environmental Factors Affect Acidobacterial Communities below the Subgroup Level in Grassland and Forest Soils

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In soil, *Acidobacteria* constitute on average 20% of all bacteria, are highly diverse, and are physiologically active *in situ*. However, their individual functions and interactions with higher taxa in soil are still unknown. Here, potential effects of land use, soil properties, plant diversity, and soil nanofauna on acidobacterial community composition were studied by cultivation-independent methods in grassland and forest soils from three different regions in Germany. The analysis of 16S rRNA gene clone libraries representing all studied soils revealed that grassland soils were dominated by subgroup Gp6 and forest soils by subgroup Gp1 *Acidobacteria*. The analysis of a large number of sites ($n = 57$) by 16S rRNA gene fingerprinting methods (terminal restriction fragment length polymorphism [T-RFLP] and denaturing gradient gel electrophoresis [DGGE]) showed that *Acidobacteria* diversities differed between grassland and forest soils but also among the three different regions. Edaphic properties, such as pH, organic carbon, total nitrogen, C/N ratio, phosphorus, nitrate, ammonium, soil moisture, soil temperature, and soil respiration, had an impact on community composition as assessed by fingerprinting. However, interrelations with environmental parameters among subgroup terminal restriction fragments (T-RFs) differed significantly, e.g., different Gp1 T-RFs correlated positively or negatively with nitrogen content. Novel significant correlations of *Acidobacteria* subpopulations (i.e., individual populations within subgroups) with soil nanofauna and vascular plant diversity were revealed only by analysis of clone sequences. Thus, for detecting novel interrelations of environmental parameters with *Acidobacteria*, individual populations within subgroups have to be considered.

Acidobacteria are considered to be ubiquitous and abundant but are rarely cultured and consequently remain a poorly studied phylum (27). In 16S rRNA gene molecular surveys, *Acidobacteria* have been observed in a wide variety of environments, including soils and sediments (5, 28, 35), hot springs (5, 27), peat bogs (15), acidic mining lakes (32), deep Mediterranean plankton (49), and caves (44, 64). Currently, 26 subgroups of *Acidobacteria* are recognized (4), and it is assumed that their phylogenetic diversity is nearly as great as that in the phylum *Proteobacteria* (27). Their phylogenetic diversity, ubiquity, and abundance, particularly in soil habitats, suggest an important role of *Acidobacteria* in biogeochemical processes and extensive metabolic versatility.

The analysis of 16S rRNA gene clone libraries showed that members of the *Acidobacteria* on average represent 20% of typical soil bacterial communities (28). Besides clone library analysis, a pyrosequencing approach of acidobacterial diversity found subgroups Gp1 to Gp4 and Gp6 to be predominant in soils (28, 29). Among the hitherto-known environmental factors that correlate with acidobacterial abundance in soils, pH is most prominent. The highest incidences of *Acidobacteria* were found in soils with the lowest pH (21, 29, 43), and phylogenetic clustering of acidobacterial communities became stronger as soil pH departed from neutrality (29). Other influential, possibly regulating factors include mean annual precipitation, soil organic carbon (OC), and soil C/N ratio (29). Carbon availability was negatively correlated with acidobacterial abundance in a large number of soils ($n = 71$) (19), suggesting that *Acidobacteria* are adapted to low substrate availabilities. The presence of high-affinity ABC transporters for sugars in subgroup Gp1 and Gp3 *Acidobacteria* (59) corroborates the idea that *Acidobacteria* are often slow-growing oligotrophs and that their overall abundance within a microbial community is

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strongly regulated by pH. *Acidobacteria* may be well adapted to resource limitation (*K*-strategists) and may be dominant in those soils where a low plant productivity causes reduced availability of plant-derived carbon sources and generally more oligotrophic niches (12).

So far, studies on influences of plant cover and diversity on soil bacterial communities and especially *Acidobacteria* showed contrasting results. Plant diversity did not affect bacterial community composition (65), led to minor changes in microbial communities (30), or had a significant effect on bacterial composition but no influence on richness (22). In another study, vegetation cover had a higher impact on soil bacterial community composition than climate and soil chemical properties; *Acidobacteria* dominated in broad-leaved forest soils but were less frequent in shrub and pasture soils (13).

Furthermore, the response of soil bacterial communities to changes in land use is poorly understood. When the relative abundance of rRNA from *Eukarya*, *Bacteria*, *Actinobacteria*, *Alphaproteobacteria*, *Betaproteobacteria*, and *Gammaproteobacteria* in soil was monitored, community composition showed similarities among plots that shared a long-term history of agricultural management despite differences in plant community composition and land management (10). However, bacterial communities differed significantly between sites that had never been cultivated and those having a long-term history of cultivation (10). Sun et al. (57) demonstrated that bacterial community structure is closely related to agro-ecosystem management practices. In a study of bacterial communities of four land-use types (hardwood, pine forest, cultivated, and livestock pasture lands), relative abundances of *Acidobacteria* were significantly higher in forest than in agricultural soils (37). In the light of these often contradictory findings, the functional implication of *Acidobacteria* diversity and its link to plant diversity and land use remain obscure.

To elucidate the potential interrelation of acidobacterial community composition and different management practices, we studied *Acidobacteria* diversity in 27 grassland and 30 forest soils subjected to a broad range of different management types ranging from virtually unused to intensely managed sites of the German Biodiversity Exploratories project (20). The sites selected were used to assess potential correlations of land use, soil chemical parameters, plant diversity, and soil nanofauna with *Acidobacteria* diversity as analyzed by terminal restriction fragment length polymorphism (T-RFLP), denaturing gradient gel electrophoresis (DGGE), and 16S rRNA gene clone library analysis.

MATERIALS AND METHODS

Study sites and soil sampling. Our study is part of the Biodiversity Exploratories project (http://www.biodiversity-exploratories.de) (20). This study was carried out on 27 grassland and 30 forest plots of the three German biodiversity exploratories: Schorfheide-Chorin in Brandenburg, Hainich with surrounding area in Thüringen, and Schwäbische Alb in Baden-Württemberg (see Table S1 in the supplemental material). The three study regions, Alb, Hainich, and Schorfheide, vary in location (southwest, middle, and northeast Germany), altitude (10 to 860 m above sea level), mean annual temperature (6.0 to 8.4°C), and precipitation (520 to 960 mm; Table S1). The 57 sites represent differences in management ranging from near-natural and protected to intensely used plots. Grassland plots can be divided into pastures, mown pastures, and meadows differing in fertilization, grazing, and number of cuts per year. Forest plots range from coniferous over beech age class to natural beech forests. Each management type is represented by three plots per region. In Hainich, beech selection cutting forest represents an additional management category; therefore, 12 instead of 9 forest sites were studied in this region. Sites are denominated as follows: the first letter stands for the region (A, Alb; H, Hainich; S, Schorfheide), followed by G for grassland or W for woodland (forest). A composite soil sample of the A horizon from 9 locations per plot (5 cores of 8.3-cm diameter in the corners and the center and 4 cuts with a spade in between) was taken in spring 2008. Plant debris, large roots, and stones were removed, and soils were sieved to 2 mm and stored at −80°C for nucleic acid extraction or dried at room temperature.

Soil parameters and plant diversity. Land use and disturbance intensity of each forest plot were taken from reference 42, and those data for grassland plots were taken from reference 7. Soil pH was measured in distilled water and in a 10 mM $CaCl_2$ solution (ratio of soil to liquid, 1:2.5). Ground soil samples were taken for total carbon and total nitrogen (N [g kg^{-1}]) analysis by dry combustion (Vario Max; Elementar Analysensysteme GmbH, Hanau, Germany). After removal of organic carbon by ignition at 450°C for 16 h, inorganic carbon was determined with the same elemental analyzer. Organic carbon concentrations (C [g kg^{-1}]) were calculated as the difference between total carbon and inorganic carbon. Total phosphorus (P [mg kg^{-1}]) was determined according to methods in references 24 and 34, and P concentrations in the extracts were measured colorimetrically with a continuous flow analyzer (Seal, Norderstedt, Germany) according to the methods in references 2 and 45. To determine soluble ammonium(μmol kg^{-1} dry soil) and nitrate (μmol kg^{-1} dry soil), soil was shaken in 1 mM $CaCl_2$, filter sterilized, freeze-dried, and resuspended in water for high-pressure liquid chromatography (HPLC) analysis. Soil temperature (°C; 5- to 10-cm depths), soil moisture (percent volume; at 0- to 8-cm depths), and soil respiration were measured during sampling. Soil respiration (μmol CO_2 m^{-2} s^{-1}) was measured (after aboveground vegetation removal) with a Licor 6400 soil respiration chamber calibrated against reference CO_2 concentrations. The soil nanofauna abundance, e.g., that of amoebae, flagellates, and ciliates, was determined as number of individuals according to the method in reference 9. The number of vascular plant species was recorded in 2008 for all grassland plots and in 2009 for all forest plots.

Cloning, sequencing, and phylogenetic analysis. DNA of soil samples was extracted using the PowerSoil DNA isolation kit (MoBio Laboratories, Solana Beach, CA) according to the protocol provided by the manufacturer. Almost-full-length 16S rRNA genes were amplified from each of the 57 samples using the *Acidobacteria*-specific forward primer 31F (5) and the universal reverse primer 1492R (36). Primer 31F is highly specific for and covers the most abundant *Acidobacteria* (subgroups Gp1, Gp3, Gp4, Gp5, and Gp6) but excludes some subgroups also present in soils (4, 31, 38). For all samples from AEG, AEW, HEG, HEW, and SEW, the 50-μl reaction mixture contained 1× PCR buffer including 1.5 mM $MgCl_2$ (Qiagen, Hilden, Germany), 200 μM deoxynucleoside triphosphate (dNTP) mix, 20 μg of bovine serum albumin (BSA; Sigma-Aldrich, Steinheim, Germany), 0.2 μM (each) primer, 1 unit of *Taq* polymerase (Qiagen), and 1 to 2 μl DNA template (20 to 100 ng). For some samples, amplification was successful only upon increasing the final $MgCl_2$ concentration up to 3.5 mM. Thermal cycling included an initial denaturation step at 95°C for 2 min and 8 cycles of denaturation at 95°C for 30 s, annealing for 30 s at an initial temperature of 60°C (which was lowered by 0.5°C per cycle), and extension at 72°C for 60 s followed by 27 or 32 further cycles with an annealing temperature of 56°C; cycling was completed by a final elongation step at 72°C for 7 min. For 16S rRNA gene amplification of all 9 SEG samples, the PCR setup and the DNA template were the same as those used for T-RFLP analysis (see below). Equal quantities of purified PCR products originating from the same region and from either grassland (9 samples) or forest (9 or, in one case, 12 samples) soils were pooled, yielding 6 different combined samples, one grassland and one forest sample for each of the three exploratories. Fragments were cloned using the pGEM-T vector system (Promega, Mannheim, Germany) according to the manufacturer's instructions. Randomly selected clones were checked for correct insert size by vector-targeted PCR, and

sequences of purified PCR products were determined by Sanger sequencing. Clone libraries were screened for chimeras with Mallard software (3) and the Bellerophon server (26). Putative chimeras were verified by fractional treeing (39) and excluded from further analysis. The number of operational taxonomic units (OTUs), diversity indices, and coverage of each clone library were determined with the DOTUR software using the furthest neighbor sequence assignment (54) and PHYLOCOM 4.1 (60). Phylogenetic analysis was performed using the ARB software package (http://www.arb-home.de) (40). The 16S rRNA gene sequences were aligned with the SINA Webaligner (http://www.arb-silva.de/aligner) (48) and added to the database (SSUref_104), sequence alignment was manually refined, and phylogenetic trees were constructed by the neighbor joining method. Shorter sequences obtained from DGGE bands (see the supplemental material) were added without changing the overall tree topology using the Quick Add parsimony tool in ARB. To assign clones to T-RFs, the T-RF-cut tool was used (53). The 16S rRNA gene sequences have been deposited in GenBank under the accession numbers given below.

T-RFLP analysis. DNA was isolated from 1.2 g of soil by bead beating in the presence of sodium phosphate and sodium dodecyl sulfate (25), purified by consecutive steps of phenol-chloroform-isoamyl alcohol extraction, and precipitated with polyethylene glycol. 16S rRNA genes were specifically amplified using the AmpliTaq DNA polymerase (Applied Biosystems, Carlsbad, CA) with primers 31F-FAM (6-carboxyfluorescein labeled) and 907R (46) from 1 to 2 μl DNA template (20 to 100 ng) as described previously (41). To avoid inhibitory effects of coextracted humic acids, 0.2 mg ml^{-1} bovine serum albumin (BSA; Roche, Risch, Switzerland) was added to PCR mixtures. The PCR thermal profile included an initial denaturation step at 94°C for 3 min and 32 cycles of 30 s of denaturation at 94°C, 45 s of annealing at 52°C, and 60 s of extension at 72°C. The final extension step at 72°C was carried out for 7 min. The acidobacterial community composition was analyzed by T-RFLP profiling. Briefly, 6-carboxyfluorescein-labeled PCR product (120 ng) was digested with restriction enzyme MspI (Promega) as previously described (16). Fluorescently labeled terminal restriction fragments (T-RFs) were size separated on an ABI 3130 genetic analyzer (Applied Biosystems) in GeneScan mode, and T-RFLP electropherograms were analyzed using the GeneScan 4.0 software (Applied Biosystems). T-RFs of 50 to 600 bp in length were included in the analysis. T-RFLP data were normalized and standardized with T-REX software for the processing and analysis of T-RFLP data (14) (http://trex.biohpc.org/). T-REX uses an approach outlined by reference 1 to identify true peaks and eliminate background noise. True peaks were defined as those whose area exceeds the standard deviation (SD) computed over all peaks. T-REX bins peaks across all samples by the approach of the software program T-Align (56). After normalization, the relative abundance of each T-RF was calculated as the percentage of the peak area from the normalized total peak areas of each sample. The method used for DGGE analysis is described in the supplemental material.

Statistical analysis. The choice of linear or unimodal species response models depends on the underlying gradient length, which is measured in standard deviation (SD) units along the first ordination axis and can be estimated by detrended correspondence analysis (DCA). It is recommended to use linear methods when the gradient length is <3 SD units, unimodal methods when it is >4 SD units, and any method for intermediate gradient lengths (58). The DCA gradient length for T-RFLP patterns was 2.69 SD units, and that for DGGE patterns (see the supplemental material) was 3.02 SD units. Hence, linear species response models such as principal component analysis (PCA), partial least-squares regression (PLSR), and redundancy analysis (RDA) were used for multivariate statistical analysis. We first compared the Bray-Curtis distances of the two fingerprinting methods by analysis of similarities (ANOSIM) and PCA implemented in the PAST statistical package (http://folk.uio.no/ohammer/past/) (23). ANOSIM is a test of significant difference between two or more multivariate groups based on any distance measure. Large positive *R* values (up to 1) signify dissimilarity between groups, and the significance is computed by permutation of group membership with 10,000 replicates. To assess the potential effect of soil chemical parameters, plant diversity, soil fauna, and land use on *Acidobacteria* community composition as determined by 16S rRNA gene fingerprinting methods, we used partial least-squares regression analysis (62). PLSR is an extension of multiple regression analysis in which the effects of linear combinations of several predictors on a response variable (or multiple response variables) are analyzed. PLSR is especially useful when the number of predictor variables is similar to or higher than the number of observations and/or predictors are highly correlated (11). Additionally, RDA was performed on 16S rRNA gene T-RFLP and DGGE patterns. RDA can be considered an extension of PCA in which the main components are constrained to be linear combinations of the environmental variables. RDA does not only represent the main patterns of species variation as much as they can be explained by the measured environmental variables but also displays correlations between each species and each environmental variable in the data (50). For a more detailed analysis of *Acidobacteria* subgroups, relative abundances of single T-RFs, DGGE bands, and OTUs were correlated with soil and site parameters by Spearman's rank correlation; the false discovery rate (FDR) was used to adjust the *P* value for multiple comparisons (6). All analyses were performed with R 2.8.0 (52).

Nucleotide sequence accession numbers. The 16S rRNA gene sequences have been deposited in GenBank under the indicated accession numbers: AEG, HQ597043 to HQ597396; HEG, HQ597397 to HQ597542 and HQ597544 to HQ597731; SEG, HQ597733 to HQ597739, HQ597741, HQ597743 to HQ597754, HQ597756 to HQ597759, HQ597761, HQ597762, HQ597764 to HQ597768, HQ597771 to HQ597774, HQ597776 to HQ597786, HQ597788 to HQ597893, HQ597796 to HQ597817, HQ597819 to HQ597823, HQ597825 to HQ597826, HQ597829 to HQ597835, HQ597837 to HQ597846, HQ597848 to HQ597850, HQ597852 to HQ597859, HQ597864 to HQ597868, HQ597870, HQ597871, HQ597873, HQ597874, HQ597876 to HQ597880, HQ597882, HQ597884 to HQ597886, HQ597888, HQ597889, HQ597893 to HQ597900, HQ597902 to HQ597904, HQ597906 to HQ597917, HQ597920 to HQ597923, HQ597925, HQ597926, HQ597930, HQ597932, HQ597934 to HQ597936, HQ597939 to HQ597942, HQ597944 to HQ597950, HQ597952 to HQ597964, HQ597967, HQ597969, HQ597972, HQ597974, HQ597977, HQ597979, HQ597980, HQ597982, HQ597984, HQ597986, HQ597988, HQ597990, HQ597991, HQ597993, HQ597996, HQ597997, HQ729774 to HQ729915; AEW, HQ598095 to HQ598414; HEW, HQ598415 to HQ598744; SEW, HQ598745 to HQ598871 and HQ598873 to HQ599092; DGGE, HQ597998 to HQ598094.

RESULTS

We studied the diversity of *Acidobacteria* in 27 grassland and 30 forest samples using T-RFLP and DGGE fingerprinting and an analysis of 16S rRNA gene clone libraries with the aim of assessing the prospective effect of land use, soil chemical parameters, plant diversity, and soil nanofauna on *Acidobacteria* community composition. The grassland and forest soils of the three study areas differed to a great extent in soil parameters. Grassland soils showed higher soil pH, soil temperature, soil respiration rate, amoeba abundance, nitrate concentrations, and P content (2) but lower C/N ratio and ammonium contents than forest soils (see Table S1 in the supplemental material). Schorfheide forest soils differed the most from all other soils with respect to nearly all examined soil parameters. Because of largely differing soil and site parameters, we expected to observe differing acidobacterial communities across the 57 samples, potentially correlated with different lifestyles and roles in soil biogeochemical processes.

Relative abundance and diversity of acidobacterial subgroups. The phylogenetic diversity of *Acidobacteria* was assessed by analysis of the almost-complete 16S rRNA gene sequences. Six clone libraries were constructed from all soil samples studied,

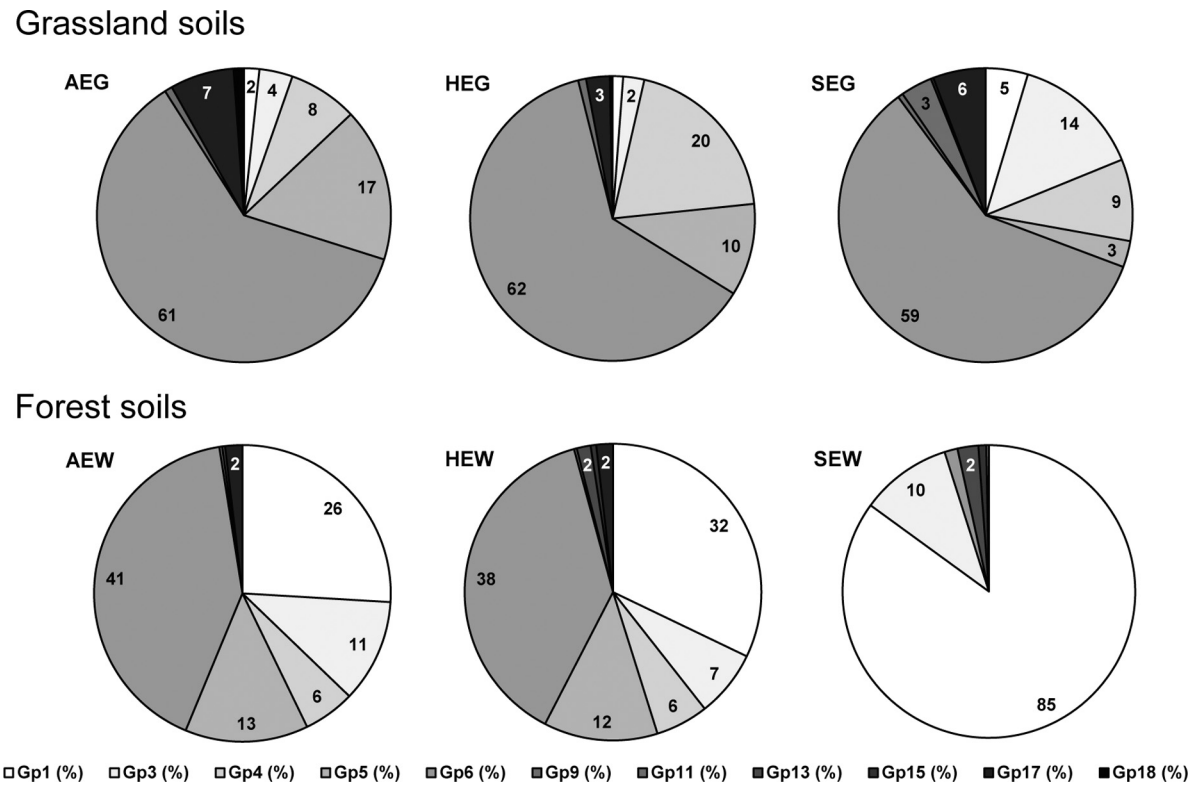

FIG 1 Affiliations of 16S rRNA gene sequences of clone libraries to the different subgroups of *Acidobacteria*, shown as percentages. Pooled samples of all grassland plots of Schwäbische Alb (AEG), Hainich (HEG), and Schorfheide-Chorin (SEG) or all forest plots of Schwäbische Alb (AEW), Hainich (HEW), and Schorfheide-Chorin (SEW) have been used to generate clone libraries.

yielding a total of 2,031 clones (see Table S3 in the supplemental material). Of the 26 different acidobacterial subgroups that are currently recognized (4), 11 were detected (Gp1, Gp3, Gp4, Gp5, Gp6, Gp9, Gp11, Gp13, Gp15, Gp17, and Gp18). The distribution and relative abundance of members of the subgroups detected differed largely between grassland and forest sites (Fig. 1; see also Table S3). In grassland soils, subgroup Gp6 was the dominant clone group (59 to 62%) followed by subgroups Gp4 (8 to 20%), Gp5 (3 to 17%), Gp17 (6 to 7%), and Gp3 (SEG, 14%). All other subgroups ranged between 0 and 5% in grassland soils (Fig. 1). In forest soils, subgroups Gp1 (26 to 85%) and Gp6 (1 to 41%) dominated the communities, but subgroups Gp3 (7 to 11%), Gp4 (6%), and Gp5 (12 to 13%) were abundant as well. All other subgroups ranged between 0 and 2% in forest soils (Fig. 1). Diversity indices indicated that SEG was the most diverse and that SEW soils were the least diverse (Fig. 1; see also Table S3). SEW soils exhibited the most pronounced phylogenetic clustering (see also Table S3). Based on LIBSHUFF analysis (55), the composition of each library differed significantly ($P < 0.001$) from that of the others, except for the two forest soil libraries, AEW and HEW. Only two OTUs were shared among all soils, whereas 217 out of 414 OTUs were unique to only one soil (data not shown). OTU3, representing species of subgroup Gp5, represented the most abundant OTU among all soils (3.5%), representing up to 7% of sequences in a single library. OTU114 (subgroup Gp1) comprised even 12% of all sequences of SEW soils (see Table S4 in the supplemental material). Further in-depth phylogenetic analyses were based on the nearly full-length 16S rRNA gene sequence data. The phylogenetic divergence (range of sequence identities) of clones falling into acidobacterial subgroups was most pronounced in subgroup Gp4 (82 to 100% sequence identity), followed by Gp6 (85 to 100%), Gp1 (86 to 100%), and Gp17 (87 to 100%), whereas subgroup Gp5 was least diverse (90 to 100%). Certain clone sequences were closely related to sequences from cultivated *Acidobacteria* (99% sequence identity; *Edaphobacter*, Gp1, and "*Candidatus* Solibacter usitatus," Gp3) while others were only distantly related (84% sequence identity; *Bryobacter aggregatus*, Gp3).

Diversity patterns of acidobacterial communities across 57 soils. Changes in acidobacterial community composition across all 57 individual soils were assessed using two different 16S rRNA gene fingerprinting methods (T-RFLP and DGGE). After normalization and standardization, 98 T-RFs and 101 DGGE bands remained for further analysis. We compared the two fingerprinting methods by ANOSIM and PCA. Acidobacterial T-RFLP patterns differed significantly from DGGE fingerprinting patterns (ANOSIM, $R = 0.72$, $P < 0.001$), as well as in PCA ordination (see Fig. S1 in the supplemental material), which is not surprising, since the fingerprinting techniques differ with respect to the underlying principle. However, both fingerprinting methods showed significant differences among regions (ANOSIM, $R = 0.16$ and 0.17, $P < 0.001$) as well as between grassland and forest soils (ANOSIM, $R = 0.35$ and 0.24, $P < 0.001$). Differences between exploratories were even more pronounced, when grassland (ANOSIM, $R = 0.36$ and 0.35, $P < 0.001$) and forest (ANOSIM, $R = 0.34$ and 0.38, $P < 0.001$) soils were compared separately. Furthermore, soil type affected the acidobacterial community

TABLE 1 Results of PLSR analysis[a]

Parameter	Result by sample type and analysis					
	All ($n = 57$)		Grassland ($n = 27$)		Forest ($n = 30$)	
	T-RFLP	DGGE	T-RFLP	DGGE	T-RFLP	DGGE
Explained variance (%)						
In fingerprinting pattern	**72.1**	**8.8**	**58.0**	**10.4**	**70.4**	**11.1**
Of component by predictors	**19.5**	**22.9**	**14.4**	**12.3**	**20.2**	**31.1**
Square wt of predictors						
pH	**0.270**	**0.221**	***0.073***	0.029	**0.226**	**0.136**
C_{org}	0.019	0.028	**0.186**	**0.125**	**0.122**	**0.116**
Nitrogen (N)	0.037	0.049	**0.188**	**0.112**	**0.148**	**0.143**
Phosphorus	**0.176**	**0.194**	***0.094***	**0.350**	**0.142**	**0.106**
C_{org}/N ratio	**0.203**	**0.206**	0.014	0.027	**0.131**	**0.146**
Soil moisture	0.030	0.039	***0.055***	***0.058***	**0.114**	**0.115**
Soil temp	0.030	0.020	**0.100**	0.026	0.008	0.047
Soil respiration	0.042	***0.054***	***0.067***	0.027	0.025	0.031
Ciliates	0.017	0.029	***0.087***	0.021	0.003	0
Land use intensity	—[b]	—[b]	0.047	***0.090***	0.009	0.002
Ammonium	**0.100**	***0.084***	***0.062***	**0.113**	0.041	0.039
Nitrate	0.035	0.044	0	0.004	0.027	***0.085***

[a] Results include the explanatory capacity of the first component as well as the square weight of each predictor within each component to estimate significant (>0.10 in bold, >0.05 in bold italics) and nonsignificant (<0.05) predictors in each component. The abundance of amoebae and flagellates and the number of vascular plant species were included in the calculations but did not show any significant correlations and, therefore, have been excluded from the table.

[b] Land use intensity was calculated from different variables for grassland and forest and therefore cannot be compared between all sites.

composition for both fingerprinting methods (ANOSIM, $R = 0.24$ and 0.3, $P < 0.001$), whereas land use type did not show any influence. PCA revealed that acidobacterial communities in grassland soils were more similar to each other than were those in forest soils. Predominantly, the DGGE and, though less pronounced, the T-RFLP profiles showed little variation between all grassland and all forest plots within the same region. This was especially prominent for Schorfheide forest plots (see Fig. S1).

Effects of soil parameters, plant diversity, and land use on the composition of *Acidobacteria*. Because of colinearity among environmental variables (see Table S5 in the supplemental material), potential effects of environmental variables (soil chemical parameters, plant diversity, soil fauna, and land use intensity) on acidobacterial community composition were assessed by PLSR of the 16S rRNA gene patterns (Table 1). Soil pH predominantly affected the acidobacterial community composition in all 57 soils, but C/N ratio, ammonium, and P concentration exerted an additional effect. Specific effects on acidobacterial community composition were detected for C and N contents in grasslands and for soil moisture in forests. Only minor and selective influences were detected for soil temperature and respiration, abundance of ciliates, nitrate concentration, and land use intensity (Table 1). These correlations of acidobacterial diversity with environmental parameters were also supported by RDA of T-RFLP (Fig. 2) and DGGE (see Fig. S2 in the supplemental material) patterns, but in addition, RDA allowed detection of those T-RFs and DGGE bands that were explaining most of the variance observed. In grassland soils, the first RDA axis could significantly explain 54% of the variance in acidobacterial community composition and was positively correlated with C and N content (only with T-RFLP analysis). In forest soils, the first RDA axis could significantly explain 57% of the variance in acidobacterial community composition and was positively correlated with pH, soil moisture, and C and N content

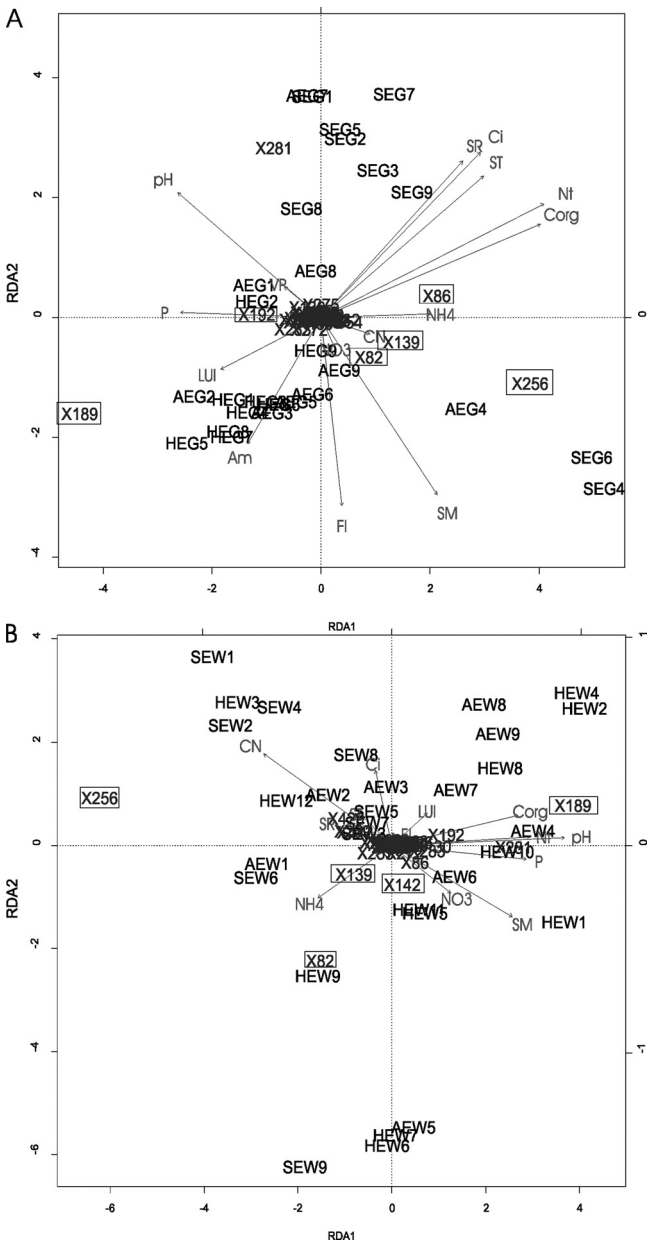

FIG 2 RDA of 16S rRNA gene T-RFLP patterns and 15 environmental variables. (A) T-RFLP grassland, $n = 27$, RDA1 = 54%, RDA2 = 15%; 81% of total variation in the data could be explained by the environmental variables. (B) T-RFLP forest, $n = 30$, RDA1 = 57%, RDA2 = 7%; 71% of total variation in the data could be explained by environmental variables. Distinct T-RFs (e.g., X256) are indicated by a box, and environmental variables are indicated by arrows. The longer the arrow and the smaller the angle to a significant axis, the more likely that this variable influences the overall community composition. Abbreviations: Corg, organic carbon; Nt, nitrogen; CN, Corg/N ratio; SM, soil moisture; ST, soil temperature; SR, soil respiration; Am, abundance of amoebae; Fl, abundance of flagellates; Ci, abundance of ciliates; VP, number of vascular plant species; LUI, land use intensity; G, grassland; W, woodland (forest); NH4, ammonium; NO3, nitrate; P, phosphorus.

TABLE 2 Spearman's rank correlation coefficient rho of relative 16S rRNA gene T-RF abundances across all grassland samples with soil and site characteristics[a]

Characteristic ($n = 27$ samples)	T-RF 82, Gp1	T-RF 86, Gp1	T-RF 90, Gp1	T-RF 256, Gp1	T-RF 142, Gp3	T-RF 139, Gp4	T-RF 134, Gp5	T-RF 163, Gp6	T-RF 189, Gp6	T-RF 192, Gp6	T-RF 283, Gp6	T-RF 460, Gp11	T-RF 272, Gp18	T-RF 554
pH	**−0.56***	0.16	**0.65*****	−0.14	−0.37	−0.10	−0.05	0.06	0.02	−0.23	0.06	−0.11	−0.11	−0.02
C_{org}	−0.12	0.42	0.11	**0.52***	**0.56***	0.49	**−0.74*****	−0.47	**−0.53***	−0.19	**−0.56***	**−0.59***	**−0.55***	**0.56***
Nitrogen (N)	−0.12	0.38	0.14	**0.52***	**0.59****	0.4	**−0.79*****	−0.48	**−0.53***	−0.19	**−0.53***	**−0.56***	**−0.59****	**0.59****
Soil moisture	**0.59****	0.02	**−0.63*****	0.35	**0.54***	0.27	−0.20	−0.21	−0.22	−0.02	−0.30	**−0.69****	−0.44	0.45
Soil temp	−0.29	**0.51***	0.43	0.45	0.23	**0.56***	**−0.57***	−0.37	−0.44	**−0.52***	−0.44	**−0.67****	−0.49	**0.52***
Soil respiration	−0.33	0.32	0.45	**0.52***	0.33	**0.50***	**−0.67****	**−0.60****	−0.40	−0.36	−0.45	−0.49	**−0.66*****	**0.62****
Ciliates	−0.12	0.24	0.15	0.49	**0.63*****	**0.52***	**−0.74*****	−0.46	**−0.54***	−0.25	**−0.53***	**−0.56***	**−0.59****	**0.59****

[a] Only T-RFs with significant (*, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$) correlations are shown in bold. *P* values have been adjusted for multiple comparisons by the FDR approach. The predominant acidobacterial subgroup represented by the analyzed T-RFs is mentioned. Abundance of amoebae and flagellates; the number of vascular plant species; land use intensity; ammonium, nitrate, and phosphorus content; and C_{org}/N ratio were included in the calculations but did not show any significant correlations and, therefore, have been excluded from the table.

and negatively correlated with C/N ratio (T-RFLP and DGGE, Fig. 2; see also Fig. S2).

Abundant T-RFs explaining most of the variance observed (positioned far from RDA origin, Fig. 2) were analyzed in detail. T-RFs (see Table S6 in the supplemental material) were assigned to acidobacterial subgroups based on *in silico* analysis of clone sequences. In grassland and forest soils, acidobacterial 16S rRNA gene patterns, i.e., individual T-RFs, showed significant correlations with soil and site characteristics such as pH, organic C content, N content, and soil moisture (Tables 2 and 3). In addition, populations in grassland soils were correlated significantly with soil temperature, soil respiration, and abundance of ciliates, whereas in forest soils *Acidobacteria* were correlated with C/N ratio, the abundance of amoebae, and nitrate content. However, most important were patterns of correlations observed among populations representing acidobacterial subgroups. For example, in forest soil, Gp1 populations consisted of those positively (T-RF 90) or negatively (T-RF 256) correlated with N content. Likewise, in grassland soils, subgroup Gp1 populations were positively (T-RF 82) or negatively (T-RF 90) correlated with soil moisture (Table 2).

The availability of nearly full-length 16S rRNA gene sequences from six combined soil samples allowed assessment of correlations of environmental parameters with acidobacterial communities, e.g., down to the level of individual populations (OTUs). Certain correlations with environmental parameters were in agreement with results from fingerprinting methods. For example, the relative abundances of Gp1 OTUs (Table 4) and Gp1 T-RF 90 (Table 3) were strongly negatively affected by P concentration. However, distinct correlation with some environmental parameters such as abundance of amoebae, number of vascular plant species, and ammonium and nitrate concentration became apparent only by sequence analysis (Table 4). For example, the relative abundance of subgroup Gp5 clone clusters (OTU3 and OTU15) was significantly positively correlated with vascular plant diversity, and subgroup Gp1 sequences were strongly negatively correlated with abundance of amoebae (Table 4).

DISCUSSION

Acidobacteria in soils are still a conundrum: their diversity is among the highest encountered in soil, but yet their role in biogeochemical cycling, as well as their influence on the diversity of higher organisms, is mostly unknown. The present work exploited large data sets for extensively characterized soils from interdisciplinary biodiversity study sites to evaluate potential determinants of soil acidobacterial diversity. We found (i) novel interrelations of environmental parameters with acidobacterial populations (ii) not only at the phylum and subgroup levels (iii) but even down to the level of individual populations.

pH is one of the strongest predictors of acidobacterial community composition (5, 17, 29, 43). Besides a number of additional factors such as total C and N content, C/N ratios have been assessed at the acidobacterial subgroup level (29, 47, 61); most other soil properties, such as ammonia concentration, P content, soil

TABLE 3 Spearman's rank correlation coefficient rho of relative 16S rRNA gene T-RF abundances across all forest samples with soil and site characteristics[a]

Characteristic ($n = 30$ samples)	T-RF 90, Gp1	T-RF 256, Gp1	T-RF 142, Gp3	T-RF 134, Gp5	T-RF 163, Gp6	T-RF 189, Gp6	T-RF 192, Gp6	T-RF 281, Gp6	T-RF 283, Gp6	T-RF 460, Gp11	T-RF 130, Gp17	T-RF 429	T-RF 554
pH	**0.60*****	**−0.67*****	0.42	**0.87*****	**0.73*****	**0.88*****	**0.83*****	**0.85*****	**0.75*****	**0.49***	**0.83*****	**−0.89*****	**−0.77*****
C_{org}	**0.52***	**−0.50***	0.31	**0.71*****	**0.56****	**0.75*****	**0.74*****	**0.79*****	**0.58****	0.39	**0.72*****	**−0.70*****	**−0.61*****
Nitrogen (N)	**0.54***	**−0.56****	0.38	**0.74*****	**0.60****	**0.79*****	**0.78*****	**0.84*****	**0.67*****	0.44	**0.73*****	**−0.76*****	**−0.63*****
C_{org}/N ratio	−0.32	**0.57****	**−0.69*****	**−0.66*****	**−0.49***	**−0.70*****	**−0.70*****	**−0.81*****	**−0.77*****	−0.41	**−0.70*****	**0.85*****	**0.58****
Phosphorus	0.36	**−0.53***	0.40	**0.71*****	**0.48***	**0.68*****	**0.72*****	**0.79*****	**0.60****	0.47	**0.72*****	**−0.72*****	**−0.57****
Soil moisture	0.40	**−0.51***	**0.59****	**0.58****	0.41	**0.67*****	**0.61*****	**0.62*****	**0.71*****	0.30	**0.67*****	**−0.72*****	**−0.57****
Amoebae	0.16	−0.31	0.32	0.34	0.27	0.44	0.42	0.444	0.43	0.17	0.47	−0.35	**−0.51***
Nitrate	0.19	−0.27	0.46	0.18	0.16	0.38	0.36	0.39	**0.54***	0.21	0.26	−0.35	−0.12

[a] Only T-RFs with significant (*, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$) correlations are shown in bold. *P* values have been adjusted for multiple comparisons by the FDR approach. The predominant acidobacterial subgroup represented by the analyzed T-RFs is mentioned. Soil temperature, soil respiration, abundance of flagellates and ciliates, the number of vascular plant species, land use intensity, C/N ratio, and ammonium content were included in the calculations but did not show any significant correlations and, therefore, have been excluded from the table.

TABLE 4 Spearman's rank correlation coefficient rho of OTUs with more than 20 sequences across all six clone libraries with soil and site characteristics[a]

Characteristic	OTU7, Gp1	OTU94, Gp1	OTU32, Gp1	OTU119, Gp1	OTU2, Gp1	OTU3, Gp5	OTU15, Gp5	OTU9, Gp6	OTU115, Gp6	OTU6, Gp6	OTU92, Gp6	OTU45, Gp6	OTU99, Gp6
pH	−0.84	−0.88	−0.88	−0.88	−0.90	0.03	−0.37	−0.64	**0.97****	0.26	0.88	0.90	0.75
Phosphorus	**−0.99*****	**−0.94***	**−0.94***	**−0.94***	**−0.93***	0.46	0.03	−0.29	0.88	0.71	**0.94***	0.81	0.70
C_{org}/N ratio	0.90	**0.94***	**0.94***	**0.94***	0.84	−0.12	0.28	0.58	**−0.97****	−0.37	**−0.94***	**−0.99*****	−0.67
Soil moisture	−0.74	−0.62	−0.62	−0.62	−0.59	0.88	0.61	0.34	0.51	**0.99*****	0.62	0.43	0.31
Soil temp	−0.32	−0.46	−0.46	−0.46	−0.38	−0.73	**−0.93***	**−0.99*****	0.50	−0.49	0.46	0.61	0.46
Soil respiration	−0.58	−0.70	−0.70	−0.70	−0.64	−0.46	−0.77	**−0.93***	0.71	−0.20	0.70	0.75	0.70
Amoebae	**−0.99*****	**−0.94***	**−0.94***	**−0.94***	**−0.93***	0.32	−0.12	−0.44	**0.97****	0.60	**0.94***	**0.93***	0.61
No. of vascular plant species	−0.22	−0.12	−0.12	−0.12	−0.13	**0.96***	**0.94***	0.79	0.06	0.81	0.12	−0.07	0.02
Ammonium	**0.93***	0.88	0.88	0.88	**0.99*****	−0.38	0.06	0.35	−0.88	−0.60	−0.88	−0.73	−0.78
Nitrate	−0.75	−0.88	−0.88	−0.88	−0.81	0.12	−0.22	−0.49	0.79	0.26	0.88	0.73	**0.99*****

[a] Only OTUs with significant (*, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$) correlations are shown in bold. *P* values have been adjusted for multiple comparisons by the FDR approach. Organic carbon and nitrogen content and abundances of flagellates and ciliates were included in the calculations but did not show any significant correlations and, therefore, have been excluded from the table.

moisture, soil temperature, and soil respiration, were studied at the level of bacterial phyla (18, 47, 51, 61). However, in addition to these known factors, we detected parameters not described hitherto to significantly correlate with the community composition of *Acidobacteria* in soils, such as nitrate concentration, number of vascular plant species, and most notably, abundance of protozoa (amoebae and ciliates). Interrelations with soil P, nitrate, and ammonia contents in soil potentially reflect acidobacterial adaptations toward substrate affinities. The strong negative correlations of Gp1 *Acidobacteria* (T-RF 256) with P, C, and N corroborate a nutrient-limited, possibly oligotrophic lifestyle in low-nutrient soils of the exploratories studied. In fact, *Acidobacteria* are mostly regarded as *K*-strategists and oligotrophic bacteria with low growth rates and seem to be favored under resource-limited conditions because of high substrate affinities (18). Members of subgroups Gp5, Gp6, and Gp17, however, showed highest relative abundances in soils of high nutrient levels, and thus, certain *Acidobacteria* might be more copiotrophic than previously assumed (18).

Up to now, influences of edaphic properties on soil acidobacterial communities were assessed for the whole phylum or at best for single subgroups (29, 35, 47, 61). Important ecological correlations at finer phylogenetic scales, e.g., at the level of individual populations, might be overlooked when focusing on larger phylogenetic groups. Only Jones et al. (29) related the presence of the most numerous *Acidobacteria* phylotypes in North and South American soils to the respective soil pH. In our study, nearly full-length 16S rRNA gene sequences allowed us to evaluate additional correlations of environmental parameters with acidobacterial communities not only at the phylum and subgroup level but even down to the level of individual phylotypes. For example, Gp6 included OTUs (OTU115, -92, and -45) that were positively correlated with the abundance of amoebae (i.e., when abundance of amoebae was high), whereas other Gp6 OTUs and Gp6 T-RFs (as assessed by T-RFLP) were not significantly correlated. Thus, striking differences exist among individual subgroup populations, which suggests that characteristics of acidobacterial subgroups are not homogenous. Protozoan grazing is an important factor known to shape microbial community structure in soil and specifically the rhizosphere of plants (8), but an impact of grazing on soil *Acidobacteria* has not been described so far. Individual Gp6 *Acidobacteria* are obviously capable of surviving grazing or benefit from enhanced grazing pressure by amoebae on other, fast-growing community members (8), the mechanistic details of which remain to be elucidated. However, certain Gp6 populations (as assessed by T-RFLP) were potentially affected by ciliate grazing (i.e., relative abundance was high when ciliate abundance was low; Table 2), whereas other subgroups (Gp3 and Gp4) were not affected.

Changes in plant diversity might influence soil microbial communities and their ecosystem functions (22, 63). Here, an OTU of subgroup Gp5 was more abundant in soils with higher vascular plant diversity, suggesting that changes in biodiversity of higher taxa can indeed affect certain soil bacterial populations. In another study, plant species composition had little direct effect on bacterial community composition in fields subjected to different above-ground biodiversity treatments (33). However, consistent with a soil lysimeter study (65), plant diversity did not affect total *Acidobacteria* community composition in the examined grassland or forest soils in our study.

The basis for detecting differential responses among acidobacterial subgroup populations was clone sequence-based analysis, and accordingly, limitations of this approach apply, e.g., PCR primer selectivity of the *Acidobacteria* specific primer and limited number of clones analyzed. Nevertheless, the relative abundances of *Acidobacteria* subgroups that we determined were comparable to a pyrosequencing-based diversity study of *Bacteria* in the A horizon of Hainich grassland soils (61); *Acidobacteria* were a dominant phylogenetic group (13 to 23% of all *Bacteria*) with subgroups Gp4 (11 to 39%), Gp6 (33 to 57%), Gp7 (3 to 9%), and Gp16 (6 to 21%) being most abundant (61). In soils of the Alb region, subgroups Gp16, Gp6, Gp4, and Gp3 (36, 24, 15, and 10%, respectively) were found to dominate in grasslands and subgroups Gp3, Gp16, Gp6, and Gp1 (34, 14, 14, and 14%, respectively) dominated in forests (47). In our study, for Hainich grassland, Schorfheide grassland, and Schorfheide forest, subgroups Gp6 (62%) and Gp4 (20%), Gp6 (59%) and Gp3 (14%), or Gp1 (85%) and Gp3 (10%) were dominating the community, respectively. In contrast, we found members of subgroup Gp5 at an abundance of 10 to 17% in Hainich grassland and Alb soils but missed two important subgroups found in the pyrosequencing approach (Gp7 and Gp16) due to the limitations of primer 31F (4, 31, 38). In a pyrosequencing analysis of *Acidobacteria* in 87 soils across the United States, subgroups Gp1 to Gp7 and Gp16 dominated, whereas the clone library approach with 22 of these 87 soils with primer 31F showed a predominance of subgroups Gp1, Gp3, Gp4, Gp5, and Gp6 (29). Thus, the relative abundances of acidobacterial subgroups found in grassland and forest soils of the three study regions in Germany are in agreement with the distribution of *Acidobacteria* in soils worldwide (28).

As long as pure cultures of *Acidobacteria* are rare, partitioning the variation in relative community abundance by environmental parameters through statistical analysis can help to discover possible functions of *Acidobacteria* in soils, notwithstanding the limitations of this approach. Most intriguing among the novel potential factors affecting acidobacterial diversity detected were interrelations with abundances of amoebae and ciliates, which may provide new vistas for elucidating adaptations of *Acidobacteria* in soils other than soil properties (37). Potential adaptations of *Acidobacteria* appear to be important at the level of individual populations and, thus, clearly below the level of subgroups. Consequently, large-scale sequencing efforts should consider individual populations for elucidating novel physiological adaptations of *Acidobacteria*.

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