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 fauna 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 fauna 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...
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 n fauna 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 Thuringen, 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), latitude (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 intensively 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 CaCl₂ solution (ratio of soil to liquid, 1:2.5). Ground soil samples were taken for total carbon and total nitrogen (N [g kg⁻¹]) by dry combustion (Vario Max; Elementar Analyse- sensysteme 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 CaCl₂, 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 CO₂ m⁻² s⁻¹) was measured (after aboveground vegetation removal) with a Licor 6400 soil respiration chamber calibrated against reference CO₂ concentrations. The soil n fauna 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, 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 MgCl₂, 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 CO₂ m⁻² s⁻¹) was measured (after aboveground vegetation removal) with a Licor 6400 soil respiration chamber calibrated against reference CO₂ concentrations. The soil n fauna 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.

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October 2012 Volume 78 Number 20 aem.asm.org 7399

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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-isooamyl 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 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 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 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 an initial denaturation step at 94°C for 3 min. The final extension step at 72°C was carried out for 7 min.

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 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) show 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).

**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 nanoa fauna 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.

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,
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

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Result by sample type and analysis</th>
<th>T-RFLP</th>
<th>DGGE</th>
<th>T-RFLP</th>
<th>DGGE</th>
<th>T-RFLP</th>
<th>DGGE</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>All (n = 57)</td>
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<td></td>
<td></td>
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<td></td>
<td>Grassland (n = 27)</td>
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<tr>
<td></td>
<td>Forest (n = 30)</td>
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<td>Explained variance (%)</td>
<td>In fingerprinting pattern</td>
<td>72.1</td>
<td>8.8</td>
<td>58.0</td>
<td>10.4</td>
<td>70.4</td>
<td>11.1</td>
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<td>Of component by predictors</td>
<td>19.5</td>
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<td>20.2</td>
<td>31.1</td>
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<td>Square wt of predictors</td>
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<td>0.029</td>
<td>0.226</td>
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<td>Soil temp</td>
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<td>Ciliates</td>
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</table>

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.

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 acido- bacterial community composition were assessed by PLSR of the 16S rRNA gene patterns (Table 1). Soil pH predominantly affected the acido bacterial 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 cor-
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 ammonium concentration, P content, soil

---

**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

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>T-RF 82,</th>
<th>T-RF 86,</th>
<th>T-RF 90,</th>
<th>T-RF 256,</th>
<th>T-RF 142,</th>
<th>T-RF 139,</th>
<th>T-RF 134,</th>
<th>T-RF 163,</th>
<th>T-RF 189,</th>
<th>T-RF 192,</th>
<th>T-RF 283,</th>
<th>T-RF 460,</th>
<th>T-RF 272,</th>
<th>T-RF 100,</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH</td>
<td>-0.56*</td>
<td>0.16</td>
<td>0.65***</td>
<td>-0.14</td>
<td>-0.37</td>
<td>-0.10</td>
<td>-0.05</td>
<td>0.06</td>
<td>0.02</td>
<td>-0.23</td>
<td>0.06</td>
<td>-0.11</td>
<td>-0.11</td>
<td>-0.02</td>
</tr>
<tr>
<td>Corg/N ratio</td>
<td>0.12</td>
<td>0.14</td>
<td>0.52**</td>
<td>0.56*</td>
<td>0.49</td>
<td>-0.74***</td>
<td>-0.47</td>
<td>-0.53*</td>
<td>-0.19</td>
<td>-0.56*</td>
<td>-0.59*</td>
<td>-0.55*</td>
<td>0.56*</td>
<td>0.59**</td>
</tr>
<tr>
<td>Nitrogen (N)</td>
<td>0.12</td>
<td>0.17</td>
<td>0.52*</td>
<td>0.59**</td>
<td>0.4</td>
<td>-0.79***</td>
<td>-0.48</td>
<td>-0.53*</td>
<td>-0.19</td>
<td>-0.53*</td>
<td>-0.56*</td>
<td>-0.59**</td>
<td>0.59**</td>
<td>0.59**</td>
</tr>
<tr>
<td>Soil moisture</td>
<td>0.59**</td>
<td>0.02</td>
<td>-0.63***</td>
<td>0.35</td>
<td>0.54*</td>
<td>0.27</td>
<td>-0.20</td>
<td>-0.21</td>
<td>-0.22</td>
<td>-0.02</td>
<td>-0.30</td>
<td>-0.69**</td>
<td>-0.44</td>
<td>0.45</td>
</tr>
<tr>
<td>Soil temp</td>
<td>-0.29</td>
<td>0.51*</td>
<td>0.43</td>
<td>0.45</td>
<td>0.23</td>
<td>0.56*</td>
<td>-0.57*</td>
<td>-0.37</td>
<td>-0.44</td>
<td>-0.52*</td>
<td>-0.44</td>
<td>-0.67**</td>
<td>0.49</td>
<td>0.52</td>
</tr>
<tr>
<td>Soil respiration</td>
<td>-0.33</td>
<td>0.32</td>
<td>0.45</td>
<td>0.52*</td>
<td>0.33</td>
<td>0.50*</td>
<td>-0.67**</td>
<td>-0.60**</td>
<td>-0.40</td>
<td>-0.36</td>
<td>-0.45</td>
<td>-0.49</td>
<td>-0.66**</td>
<td>0.62**</td>
</tr>
<tr>
<td>Ciliates</td>
<td>-0.12</td>
<td>0.24</td>
<td>0.15</td>
<td>0.49</td>
<td>0.63***</td>
<td>0.52*</td>
<td>-0.74***</td>
<td>-0.46</td>
<td>-0.54*</td>
<td>-0.25</td>
<td>0.53*</td>
<td>-0.56*</td>
<td>-0.59*</td>
<td>0.59*</td>
</tr>
</tbody>
</table>

*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, ammonium, nitrate, and phosphorus content; and Corg/N ratio were included in the calculations but did not show any significant correlations and, therefore, have been excluded from the table.

---

**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

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>T-RF 90,</th>
<th>T-RF 256,</th>
<th>T-RF 142,</th>
<th>T-RF 134,</th>
<th>T-RF 163,</th>
<th>T-RF 189,</th>
<th>T-RF 192,</th>
<th>T-RF 281,</th>
<th>T-RF 283,</th>
<th>T-RF 460,</th>
<th>T-RF 130,</th>
<th>T-RF 429,</th>
<th>T-RF 554</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH</td>
<td>0.60***</td>
<td>-0.67***</td>
<td>0.42</td>
<td>0.87***</td>
<td>0.73***</td>
<td>0.88***</td>
<td>0.83***</td>
<td>0.85***</td>
<td>0.75***</td>
<td>0.49*</td>
<td>0.83***</td>
<td>-0.89***</td>
<td>-0.77***</td>
</tr>
<tr>
<td>Corg/N ratio</td>
<td>0.52</td>
<td>-0.50*</td>
<td>0.31</td>
<td>0.71***</td>
<td>0.56*</td>
<td>0.75**</td>
<td>0.74**</td>
<td>0.79**</td>
<td>0.58**</td>
<td>0.39</td>
<td>0.72***</td>
<td>-0.70***</td>
<td>-0.61***</td>
</tr>
<tr>
<td>Nitrogen (N)</td>
<td>0.54**</td>
<td>-0.56**</td>
<td>0.38</td>
<td>0.74***</td>
<td>0.69**</td>
<td>0.79**</td>
<td>0.78**</td>
<td>0.84***</td>
<td>0.67**</td>
<td>0.44</td>
<td>0.73***</td>
<td>-0.76***</td>
<td>-0.63***</td>
</tr>
<tr>
<td>Phosphorus</td>
<td>0.36</td>
<td>-0.53**</td>
<td>0.40</td>
<td>0.71***</td>
<td>0.48*</td>
<td>0.68**</td>
<td>0.72**</td>
<td>0.79**</td>
<td>0.60**</td>
<td>0.47</td>
<td>0.72***</td>
<td>-0.72***</td>
<td>-0.57**</td>
</tr>
<tr>
<td>Soil moisture</td>
<td>0.40</td>
<td>-0.51*</td>
<td>0.59**</td>
<td>0.58**</td>
<td>0.41</td>
<td>0.67**</td>
<td>0.66**</td>
<td>0.71**</td>
<td>0.71**</td>
<td>0.30</td>
<td>0.67***</td>
<td>-0.72***</td>
<td>-0.57**</td>
</tr>
<tr>
<td>Amoebae</td>
<td>0.16</td>
<td>-0.31</td>
<td>0.32</td>
<td>0.34</td>
<td>0.27</td>
<td>0.44</td>
<td>0.42</td>
<td>0.444</td>
<td>0.43</td>
<td>0.17</td>
<td>0.47</td>
<td>-0.35</td>
<td>-0.51*</td>
</tr>
<tr>
<td>Nitrate</td>
<td>0.19</td>
<td>-0.27</td>
<td>0.46</td>
<td>0.18</td>
<td>0.38</td>
<td>0.36</td>
<td>0.39</td>
<td>0.54*</td>
<td>0.21</td>
<td>0.26</td>
<td>0.35</td>
<td>-0.35</td>
<td>-0.12</td>
</tr>
</tbody>
</table>

*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, ammonium, nitrate, and phosphorus content; and Corg/N ratio were included in the calculations but did not show any significant correlations and, therefore, have been excluded from the table.
### TABLE 4

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>OTU7, Gp1</th>
<th>OTU94, Gp1</th>
<th>OTU32, Gp1</th>
<th>OTU119, Gp1</th>
<th>OTU2, Gp1</th>
<th>OTU3, Gp5</th>
<th>OTU15, Gp6</th>
<th>OTU9, Gp6</th>
<th>OTU115, Gp6</th>
<th>OTU92, Gp6</th>
<th>OTU45, Gp6</th>
<th>OTU99, Gp6</th>
<th>**/H11002</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phosphorus</td>
<td>0.99</td>
<td>***</td>
<td>0.88</td>
<td>0.93</td>
<td>0.93</td>
<td>0.90</td>
<td>0.93</td>
<td>0.94</td>
<td>0.94</td>
<td>0.93</td>
<td>0.94</td>
<td>0.94</td>
<td>0.93</td>
</tr>
<tr>
<td>Soil moisture</td>
<td>0.74</td>
<td>0.73</td>
<td>0.81</td>
<td>0.85</td>
<td>0.85</td>
<td>0.73</td>
<td>0.85</td>
<td>0.85</td>
<td>0.85</td>
<td>0.73</td>
<td>0.85</td>
<td>0.85</td>
<td>0.85</td>
</tr>
<tr>
<td>Soil temp</td>
<td>0.32</td>
<td>0.67</td>
<td>0.67</td>
<td>0.97</td>
<td>0.97</td>
<td>0.67</td>
<td>0.97</td>
<td>0.97</td>
<td>0.97</td>
<td>0.67</td>
<td>0.97</td>
<td>0.97</td>
<td>0.97</td>
</tr>
<tr>
<td>Nitrate</td>
<td>0.75</td>
<td>0.75</td>
<td>0.75</td>
<td>0.75</td>
<td>0.75</td>
<td>0.75</td>
<td>0.75</td>
<td>0.75</td>
<td>0.75</td>
<td>0.75</td>
<td>0.75</td>
<td>0.75</td>
<td>0.75</td>
</tr>
<tr>
<td>Ammonium</td>
<td>0.88</td>
<td>0.88</td>
<td>0.88</td>
<td>0.88</td>
<td>0.88</td>
<td>0.88</td>
<td>0.88</td>
<td>0.88</td>
<td>0.88</td>
<td>0.88</td>
<td>0.88</td>
<td>0.88</td>
<td>0.88</td>
</tr>
</tbody>
</table>

**Notes:**
- P-values have been adjusted for multiple comparisons by the FDR approach.
- Significant correlations are shown in bold. 
- Only OTUs with more than 20 sequences across all six clone libraries with soil and site characteristics and abundances of organisms and controls were included in the calculations. Differences shown above significant correlations and therefore, have been excluded from the table.

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 Gp4 (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.

ACKNOWLEDGMENTS

This work has been funded by the DFG Priority Program 1374 "Infrastructure-Biodiversity-Exploratories" (grants OV 20/18-1 and -18-2 and FR 1151/5-1 and -5-2), the Max Planck Society (Munich), and the University of Bremen.

Field work permits were issued by the responsible state environmental offices of Baden-Württemberg, Thüringen, and Brandenburg (according to §72 BbgNatSchG). We thank the management teams of the three exploratories for their work in maintaining the plot infrastructure and Jens Nieschulze and the BEXS database team (Max-Planck-Institut für Biogeochemie, Jena) for their excellent support. Special thanks go to Marion Schrumpf and Nadine Herold (Max-Planck-Institut für Biogeochemie, Jena) for providing soil data; to Ernst-Detlef Schulze for supporting the joint soil sampling campaigns (Max-Planck-Institut für Biogeochemie, Jena); to Daniel Prati, Stephanie Socher, Jörg Müller, and Steffen Boch (University of Bern) for providing data on plant diversity; and to Nico Blüthgen (Technical University of Darmstadt) for providing data for the grassland land use index. We thank Bianca Pommerenke (MPI Marburg) for excellent technical assistance, Werner Wosniok (Faculty of Mathematics, University of Bremen) for advice in statistical analyses, and Alban Ramette (Max-Planck-Institut für Marine Mikrobiologie) for performing FDR calculations.

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