Driving forces of soil bacterial community structure, diversity, and function in temperate grasslands and forests

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Soil bacteria provide a large range of ecosystem services such as nutrient cycling. Despite their important role in soil systems, compositional and functional responses of bacterial communities to different land use and management regimes are not fully understood. Here, we assessed soil bacterial communities in 150 forest and 150 grassland soils derived from three German regions by pyrotag sequencing of 16S rRNA genes. Land use type (forest and grassland) and soil edaphic properties strongly affected bacterial community structure and function, whereas management regime had a minor effect. In addition, a separation of soil bacterial communities by sampling region was encountered. Soil pH was the best predictor for bacterial community structure, diversity and function. The application of multinomial log-linear models revealed distinct responses of abundant bacterial groups towards pH. Predicted functional profiles revealed that differences in land use not only select for distinct bacterial populations but also for specific functional traits. The combination of 16S rRNA data and corresponding functional profiles provided comprehensive insights into compositional and functional adaptations to changing environmental conditions associated with differences in land use and management.

Soil bacteria play an important role in biogeochemical cycles1,2. They control soil processes such as decomposition2 and mineralization, including the associated release of greenhouse gases such as carbon dioxide (CO₂), nitrous oxide (N₂O), and methane (CH₄)4,5 into the atmosphere. Moreover, several soil bacteria promote plant growth and productivity2,6. As soil represents a highly dynamic and complex environment, bacterial communities living in this ecosystem are influenced by a multitude of different biotic and abiotic factors. Previous studies showed that soil pH is a major driver of these communities7–9. Lauber and colleagues8 observed that the overall bacterial community composition in different soils from across South and North America was significantly correlated with soil pH. This was confirmed by a study of bacterial communities in German grassland and forest soils9. Other studies investigating the effect of edaphic parameters on soil bacteria found that these communities were influenced by the availability of nutrients such as carbon, nitrogen10,11, and soil moisture in grasslands12 and forests13.

In recent years, the impact of land use intensification on bacterial community diversity and composition, e.g. by fertilization in grasslands, has been frequently investigated14–17. In a study by Herzog et al.15, composition and diversity of entire and active bacterial communities were altered by fertilizer application. Lauber et al.16 analyzed soil bacterial communities across different land use types such as grasslands and forests. For soil bacteria in forest systems, soil disturbance and organic matter removal18,19 as well as the dominant tree species20 have been shown to influence community composition. This provides evidence that land use intensification can alter soil bacterial community composition. However, most studies have focused on a limited number of soil samples in one region. Therefore, the response of bacterial communities in grasslands and forests to land use intensification and

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Betaproteobacteria exploratories showed that bacterial diversity was influenced by land use intensity and land use type. Bacterial similar way within the same land use system.

(ANOVA, P < 0.5 in all cases). Significant differences in soil parameters between the different management regimes were not recorded followed by the Hainich-Dün soils. The highest clay amounts were determined for the Schwäbische Alb grassland soils. Forest soil samples derived from acidic, had a higher C:N ratio and smaller clay amount than grassland soils. Forest soils were more forest and grassland by capital letters according to Dunn’s test (P < 0.05).

Table 1. Edaphic properties among different land uses and exploratories (median ± SD). Significant differences between study regions are indicated by lowercase letters and between forest and grassland by capital letters according to Dunn’s test (P < 0.05).

Results and Discussion

General characteristics of the soil samples. Soil samples showed significant differences with respect to soil texture and edaphic properties (Table 1, Supplementary Material Tables S1 and S2). Forest soils were more acidic, had a higher C:N ratio and smaller clay amount than grassland soils. Forest soil samples derived from the different exploratories exhibited significant differences in all measured edaphic properties. The Schorfheide-Chorin forest soils were more acidic and had higher C:N ratios compared to the Hainich-Dün and Schwäbische Alb soils, which did not differ significantly. In addition, Schorfheide-Chorin forest soils also exhibited the lowest gravimetric water content, clay and silt amount of all exploratories.

Grassland soil samples derived from the different exploratories also exhibited significant differences between all measured edaphic properties. The Hainich-Dün grassland soils had the highest pH values, lowest gravimetric water content and highest silt amount compared to the Schorfheide-Chorin and Schwäbische Alb soil, which did not differ significantly. The Schorfheide-Chorin grassland soils were compared to the other two exploratories. Clay amount was lowest in the Schorfheide-Chorin grassland soils, followed by the Hainich-Dün soils. The highest clay amounts were determined for the Schwäbische Alb grassland soils. Significant differences in soil parameters between the different management regimes were not recorded (ANOVA, P > 0.5 in all cases).

Soil bacterial communities. Composition and diversity of soil bacterial communities were assessed by pyrotag sequencing of 16S rRNA genes. After quality filtering, denoising, and removal of potential chimeras and non-bacterial sequences, approximately 2,700,000 high quality sequences with an average read length of 525 bp were obtained for further analyses. All sequences were classified below phylum level. Based on richness estimator data (Michaels-Menten fit; Supplementary Material Table S3) 78–88% of the operational taxonomic units (OTUs) at 80% identity (phylum level) and 27–55% of the OTUs at 97% identity (species level) were covered by the surveying effort (for rarefaction curves, see Supplementary Material Figs S1 and S2).

Obtained sequences clustered into 203,530 OTUs (97% identity) and were assigned to 51 bacterial phyla, 574 orders and 1,215 families. The dominant phyla and proteobacterial classes (>1% of all sequences across all samples) were Actinobacteria (23.75% ± 8.55%), Alphaproteobacteria (20.43% ± 5.21%), Acidobacteria (18.39% ± 9.19%), Deltaproteobacteria (7.22% ± 2.84%), Bacteroidetes (5.15% ± 2.60%), Chloroflexi (5.09% ± 2.10%), Betaproteobacteria (4.64% ± 2.38%), Gammaproteobacteria (4.32% ± 1.23%), Gemmatimonadetes (1.88% ± 0.92%), Firmicutes (1.18% ± 3.20%), and Nitrospirae (1.14% ± 1.10%). These phylogenetic groups were present in all samples and accounted for more than 95% of all sequences analyzed in this study (Fig. 1). These results are consistent with previous studies on grasslands and temperate beech forests. The most abundant phylotype (3.99% ± 2.44) is an uncultured member of the Subgroup 6 of the Acidobacteria. The five most abundant phylotypes that could be assigned to a genus are Bradyrhizobium (2.66% ± 1.45%), Candidatus Solibacter (2.00% ± 1.86%), Halothiobacillus (1.39% ± 0.74%), Variovorax (1.36% ± 0.58%) and Gaiella (1.34% ± 1.31%) of all sequences, respectively.
Biogeographic variations of soil bacterial diversity and community composition. Diversity (represented by the Shannon index $H'$) and community structure of soil bacteria (PERMANOVA, $P < 0.001$) differed between the three Biodiversity Exploratories. The Hainich-Dün exploratory harbored the most diverse bacterial community ($H' = 10.22$) compared to Schorfheide-Chorin ($H' = 9.72$) and the Schwäbische Alb ($H' = 9.92$). Furthermore, grassland soils are significantly more diverse than forest soils ($H' = 10.12$ and $H' = 9.48$, respectively, with $P < 0.001$), which supports previous findings of Nacke and colleagues$^9$, who reported that bacterial communities were more diverse in grasslands at phylum level. As samples derived from forest soils were more acidic ($P < 0.001$), the difference in pH might explain the difference in diversity (Table 1).

The most dominant bacterial orders of the complete dataset differed in their distribution across the three exploratories. These differences most likely arose from differences in edaphic properties in the exploratories. Therefore, we tested for correlation of environmental factors by NMDS analysis based on Bray-Curtis dissimilarities. Fitting the edaphic properties to the ordination revealed the pH as the strongest driver of the community. Additional canonical correspondence analysis (CCA) using pH as constrain showed that pH explains 26% of the variation in community structure ($P < 0.001$, Supplementary Figure S3). We additionally found a separation of soil bacterial communities by sampling region (PERMANOVA, $P < 0.001$) and the two land use types grassland and forest (PERMANOVA, $P < 0.001$) (Supplementary Figure S4). Therefore, we were interested in a detailed analysis of the factors driving the changes in the structure of bacterial communities in each exploratory. We further split the data between grasslands and forests due to the strong separation between the community structure of both land use types.

Figure 1. Abundances of bacterial orders in Schorfheide-Chorin, Hainich-Dün and Schwäbische Alb grassland and forest soils. Mean abundances of the most abundant bacterial orders (>1% of the total bacterial community) for each exploratory and land use are given. Rare: sum of bacterial orders contributing <1% to the total bacterial community per exploratory.
Key drivers of bacterial communities. To identify the key drivers of soil bacterial community structure for each land use type in each exploratory, we performed NMDS analysis for the six subsets. The soil pH was the only property, which affected the community structure in each subset (Fig. 2). Another property influencing the community structure in grasslands and forests was soil texture (amount of clay, sand and/or silt), which represents pore size, water and gas fluxes, and nutrient availability. Moreover, soil texture is important for niche separation and protection from predation.

In grassland soils, the C:N ratio influenced bacterial community structure in the Schwäbische Alb and Schorfheide-Chorin, but not in the Hainich-Dün. This is supported by a PFLA-based study on soil bacterial communities, in which edaphic properties such as soil texture, pH, and C and N concentration were involved in structuring soil bacterial communities. The land use intensity index (LUI) was only correlated with the Schwäbische Alb grassland community. However, the LUI only accounts for the amount and not for the source of fertilization. In the Schwäbische Alb grasslands, most plots received organic fertilizer (manure, dung), whereas fertilization in the Hainich-Dün and Schorfheide-Chorin was predominated by mineral fertilizer application. These findings support a recent study, in which soil microbial communities of farming systems receiving organic fertilizer were different compared to those of conventional, mineraly fertilized systems and control soils. In agreement with Geisseler and Scow, clear trends suggesting bacterial community structural shifts due to long-term mineral fertilizer application, were not found in our survey.

In forest soils, the tree species was correlated with bacterial community structure in all exploratories, while the silvicultural management index (SMI) only significantly influenced the community structure in the Schorfheide-Chorin (Fig. 2). Soil bacterial communities under broadleaved (Fagus and Quercus) and coniferous (Pinus and Picea) trees formed distinct patterns. This is in accordance with results of previous studies. Nacke et al. analyzed a subset of soil samples derived from the Schwäbische Alb and found that the bacterial community structure was different under beech (Fagus) and spruce (Picea). This is consistent with a study comparing bacterial communities under coniferous and broadleaved trees. We did not observe a difference between the two broadleaved tree species, although differences in soil community structure between broadleaved trees have been described for Fagus versus Tilia and Acer. These effects might be partly due to the reduced soil acidification and higher turnover rates of the leaf litter of Tilia and Acer. Coniferous tree species such as spruce (Picea abies) and pine (Pinus sylvestris) are known to significantly decrease the soil pH (reviewed in ref. 33) due to the special chemical structure of evergreen litter or capture of atmospheric acidic compounds. This would result in an indirect pH effect on soil bacteria. Additionally, this might be one of the reasons why tree species play an important role in the structuring of bacterial communities in all forest samples analyzed.

According to our hypothesis that bacterial community structure and diversity would be affected in similar ways under the same land use, we compared the bacterial diversity, represented by the Shannon index (H’), between the different management regimes (Supplementary Material Table S4). Differences in diversity were detected for the tree species in the Schwäbische Alb and Schorfheide-Chorin.

Interestingly, the management regimes in grasslands (meadow, pasture, mown pasture) and forests (unmanaged forest, age-lass forest, selection forest) exhibited no significant effect on bacterial diversity (PERMANOVA, P < 0.05). This is in contrast to a previous study by Will et al., who found a higher bacterial diversity in grassland soils of low land use intensity in the Hainich-Dün. In contrast, Tardy et al. investigated bacterial diversity along gradients of land use intensity and observed the highest bacterial diversity in moderately managed soils.

The authors suggest that this effect is related to the stress response of the bacterial community. In highly stressed environments, as under high land use intensity, diversity decreases due to the dominance of competitive species and competitive exclusion, while in unstressed environments diversity decreases due to the dominance of adapted species through selection. In accordance with our hypothesis, we could find soil conditions such as pH that consistently drive bacterial community structure as well as diversity, while management regimes and therefore land use intensity have no significant influence. In addition, we could show that pH is the best predictor of bacterial communities.

Bacterial functioning in grassland and forest soils. We further hypothesized that bacterial functioning was driven in a similar manner as bacterial community structure and diversity. To clarify this hypothesis, we focused on pathways involved in the cycling of carbon, nitrogen, phosphorus, and sulfur (Fig. 3) and compared the relative abundances of key enzyme-encoding genes between the two land uses grassland and forest.

Abundances of the enzyme-encoding genes were derived from a novel bioinformatic tool Taxa4Fun. Taxa4Fun transforms the SILVA-based OTUs into a taxonomic profile of KEGG organisms, which is normalized by the 16S rRNA copy number (obtained from NCBI genome annotations). As soils harbor unknown or uncultured organisms, not all 16S sequences can be mapped to KEGG organisms. Spearman correlation analysis of functional profiles derived from whole metagenome sequencing and profiles deduced from 16S rRNA gene sequences revealed a median of the correlation coefficient of 0.8706 for soils. This indicated that Taxa4Fun provides a good approximation to functional profiles obtained from metagenomic shotgun sequencing approaches. This is especially valuable to deduce functional profiles for a large number of samples derived from complex environments, as achieving representative coverage for each sample of a large sample set by metagenome shotgun sequencing would be a daunting task.

Most key enzyme-encoding genes involved in the cycling of C, N, S, and P are either more abundant in grassland or forest soils (Mann-Whitney test, P < 0.05, Supplementary Material Table S5). For example, genes that encode acid phosphatases were observed at 1.4-fold higher abundances in the functional profile of the forest soils than in the grassland soils, while alkaline phosphatases showed the opposite trend. We assume that this effect could be attributed to the difference in pH between the land use types, as we showed that pH is the best predictor for bacterial communities. The genes encoding urease were 1.2-fold more abundant in the grassland. The availability of urea was higher in the grassland samples, as these are partly fertilized with manure or dung or were...
grazed by animals. Chitinase genes also showed a 1.2-fold higher abundance in grasslands compared to forest soils. This might result from the higher abundance of Actinobacteria in grassland soils, as this group is known...
to harbor a high number of chitinase genes. Genes involved in polyaromatic hydrocarbon (PAH, here lignin) degradation are more abundant in grasslands. In forest systems, this process is primarily performed by ligninolytic fungi (mainly saprotrophic basidiomycetes), which are able to degrade wooden biomass. One key enzyme for aerobic methane oxidation, methanol dehydrogenase, was notably more abundant in forest soils. Methane oxidation in forest soils is the largest biological sink for atmospheric methane and therefore plays a critical role in the flux of this greenhouse gas. Additionally, nitrous-oxide (N\(_2\)O) reductase, which catalyzes the last step in denitrification and reduces N\(_2\)O to N\(_2\), is also more abundant in forest soils (data not shown). These results indicate that temperate forest ecosystems not only play a crucial role in the regulation and removal of methane, but also of the greenhouse gas nitrous oxide.

Interestingly, the key enzyme of nitrogen fixation, the nitrogenase, is less abundant in grassland than in forest soils. In this study, only bulk soil was sampled and therefore presumably only free-living nitrogen-fixing bacteria could be detected. It is possible, that nitrogen fixation by free-living bacteria plays a greater role in forest systems, whereas symbiotic and rhizospheric bacteria, which were not covered by the study, carry out the major part of nitrogen fixation in grassland systems.

The obtained results suggest that the different land uses grassland and forest not only select for distinct bacterial populations, but also for specific functional traits within their bacterial communities. As the grasslands and forests analyzed in the present study are long-term established systems, it would be interesting to evaluate if a similar adaptation is also present in younger systems.

Soil pH is the best predictor of bacterial communities. In the present study, pH was the only factor, which influenced the bacterial community regardless of exploratory and land use. Furthermore, it not only affected bacterial community structure, but also the functional profile of the soil bacteria. As already mentioned, CCA analysis revealed that pH explains 26% of total variance in the community profile (Supplementary Figure S5). Thus, the pH was the strongest predictor for bacterial community structure.

We hypothesized that bacterial community structure and functioning would be shaped in a similar manner. Environmental correlations with the Tax4Fun-derived functional profile were tested by NMDS based on Bray-Curtis dissimilarities (Fig. 4). The results are similar to those obtained for the community structure. The pH played an important role in shaping the functional profile and explained 32% of the variance (tested by CCA, P < 0.001, Supplementary Figure S5). This supports our hypothesis that structure and functions of bacterial communities are shaped by similar mechanisms. The functional profile also showed a separation between grassland and forest systems.

Additionally, we found that pH is the strongest predictor of soil bacterial diversity (P < 0.001, \(R^2 = 0.4\)) (Fig. 5). It has already been shown that diversity of soil bacterial communities in the exploratories is positively correlated with pH. However, our results indicate a more complex relationship between pH and diversity. Diversity was lowest at low pH, then increased and appeared to be stable between pH 5 and 7 and increases again under slightly alkaline conditions. This is in contrast to Fierer and Jackson and Lauber et al., who described a peak of soil bacterial diversity in near neutral soils.

Multinomial regression models revealed multiple responses of bacterial orders to soil pH. To better understand the complex relationship of single bacterial groups and soil pH, we applied multinomial regression models on the 30 most abundant orders of the dataset (Supplementary Material Figure S6). Four general
responses were observed: (1) decrease in abundance with increasing pH (Acidobacteriales, acidobacterial subgroup 3, Frankiales, Corynebacteriales), (2) increase in abundance with increasing pH (acidobacterial subgroup 6, Gaiellales, Acidimicrobiales, Propionibacteriales), (3) narrow pH range with high abundance (Rhizobiales, Rhodospirillales), and (4) relatively constant abundance across pH range (Bacillales, Gemmatimonadales, Sphingobacteriales) (Fig. 6). In their publication on niche theory, Austin and Smith 37 described pH as a direct physiological gradient acting on organisms, resulting in unimodal, or skewed unimodal response curves restricted by growth limiting conditions at one end, and competition at the other end. This is supported by our observation of few highly abundant orders at low pH and many less abundant orders in near neutral soils. The ability to grow at low pH values is known as ATR (acid tolerance response) and confers a competitive advantage compared to other bacteria in soils.

To test which mechanisms are involved in acid tolerance of soil bacteria, we chose those genes reported to be involved in acid tolerance in Rhizobia 38 and Gram positive bacteria 39 that were present in the functional profile. Additionally, we analyzed the genes present of the KEGG pathway for biosynthesis of unsaturated fatty acids (ko001040) as well as 3-trans-2-decenoyl isomerase. This enzyme is involved in the generation of unsaturated fatty acids in Streptococcus mutans by changing cell membrane composition 40. We found that the genes for biosynthesis of unsaturated fatty acids were highly abundant in low pH samples (pH 3–4), while decenoyl isomerase did not follow this trend (Fig. 7). Therefore, this gene might not be generally involved in acid tolerance in soil. Additionally, most genes involved in alkali production, two
component systems and repair of macromolecules were more abundant in the low pH samples compared to more neutral samples. Several genes involved in DNA repair were probably also involved in the ATR of soil-inhabiting bacteria, as well as levansucrase, a gene involved in biofilm formation. Our results suggest that bacteria can apply an active mechanism to cope with stressful pH conditions. Alkali production increases the pH in the immediate environment, improving bacterial survival chances. Additionally, macromolecule repair-enzymes protect and repair DNA and proteins, and bacteria seem to enhance pH tolerance by altering their cell wall components or protect themselves within biofilms.

**Conclusion**

During the last years, several studies targeting soil microbial communities and their driving forces came to the same conclusion that soil pH is the major driver of bacterial communities. This statement, however, falls short as it provides no direct answer about the complex interaction of soil bacteria with pH. We showed that soil bacteria respond differently to changing pH conditions, being adapted to certain pH ranges or even stable over a broad pH range. Obtained data suggest that this adaptation is attributed to different mechanisms including...
alkali production and alteration of cell wall components. In addition to soil pH, it is generally assumed that land use intensity drives bacterial community composition and diversity. However, the present study demonstrated that land use intensity plays a minor role, or that its effect is concealed by the tree species effect in forest. Biogeographic variations and the corresponding changing edaphic properties resulted in distinct patterns of soil bacteria, which explains regional differences and also the distinct patterns of bacterial communities in grasslands and forests. This is in line with our first and second hypothesis.

Large comparative studies are required to unravel the diverse interactions between bacteria and their environments, and how changes in community structure might reflect changes in bacterial functioning. With a total of 300 samples representing different land uses and gradients of land use intensity, this study provides comprehensive insights into soil bacterial communities present in temperate systems. Taking the enormous size and diversity of soil microbial communities into account, functional information on soil bacterial communities has been limited as it was so far mainly derived from small-scale comparative metagenomic approaches with a rather low coverage. However, the ability to focus on functional genes and enzymes offers novel insights in the nutrient cycling potential of soil bacterial communities. Consequently, the application of novel bioinformatic and statistical approaches, such as Tax4Fun and multinomial log-linear models, in microbial ecology resulted in a more holistic understanding of the links between bacteria and their environment.

Materials and Methods
Study regions. The present study was conducted as part of the German Biodiversity Exploratories initiative, which is a project investigating large-scale and long-term relationships of biodiversity and land use in Central European grasslands and forests21. Its unique design allows detailed analysis of bacterial communities along a regional north-south gradient in Germany. The study is based on 30 study regions (exploratories). They are located in the Schorfheide-Chorin, the Hainich-Dün and the Schwäbische Alb. Each study region covers the land use types forest and grassland. Grassland plots are 50 m × 50 m and forest plots are 100 m × 100 m in size.

The grassland land use-intensity gradient was represented by three different management regimes (meadows, pastures and mown pastures) that are non-fertilized or fertilized. Fertilization always represents higher land use intensity. The land use intensity index (LUI41) combines and equally weights the three components which is a project investigating large-scale and long-term relationships of biodiversity and land use in Central European grasslands and forests21. Its unique design allows detailed analysis of bacterial communities along a regional north-south gradient in Germany. The study is based on 30 study regions (exploratories). They are located in the Schorfheide-Chorin, the Hainich-Dün and the Schwäbische Alb. Each study region covers the land use types forest and grassland. Grassland plots are 50 m × 50 m and forest plots are 100 m × 100 m in size.

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In forests, the land use intensity-gradient was represented by different forest management systems (age class forest, selection forest and unmanaged forest). Additionally, forest plots were dominated by one of the following tree species: (1) European beech (Fagus sylvatica), (2) sessile/pedunculate oak (Quercus petraea/Quercus robur), (3) Scots pine (Pinus sylvestris) or (4) Norway spruce (Picea abies). The silvicultural management index (SMI) was used to assess the impact of management intensity in forest systems (Supplementary Table S1). This index integrates three characteristics of forest stands: (1) tree species, (2) stand age and (3) aboveground, living and dead wooden biomass52. Detailed information on land use, the applied management, dominant tree species, soil type and fertilization for every experiment is provided in Supplementary Material Table S2.

Sampling and soil properties. Soil samples were collected from all 300 experimental plots in May 2011. In brief, plots were sampled along two 36 m transects in forests and along two 18 m transects in grasslands. The top 10 cm of the soil layer were taken from 14 locations along the two transects in each plot with a split tube auger of 5 cm diameter. At forest sites, the litter layer was removed with a metal frame (15 × 15 cm) prior to sampling. The soil cores were pooled and sieved to remove stones >0.5 cm and roots.

Ten grams of the pooled soil samples were used to determine the gravimetric water content, which represents the water content of the respective sample at the sampling time. The subsamples were weighted and dried at 105 °C to a constant weight. Air-dried soil samples sieved to <2 mm were used for the determination of soil texture, soil pH, and carbon (C) and nitrogen (N) concentrations as described previously43. Detailed information on soil characteristics is given in Supplementary Material Table S1.

DNA extraction, amplification of 16S rRNA genes and pyrosequencing. Total microbial community DNA was isolated from approximately 0.25 g soil per sample using the MoBio Power Soil DNA isolation kit (MoBio laboratories, Carlsbad, CA, USA) following the manufacturer’s recommendations. This method was recently shown to perform equally well over a range of different soils46. It produces similar amounts of DNA and 16S rRNA gene copies for each soil tested and does not overestimate any of the abundant phyla detected throughout the soils. Therefore, extraction biases were limited and comparable given for all DNA extractions. DNA concentrations were quantified using a NanoDrop ND-1000 UV-Vis Spectrophotometer (NanoDrop Technologies, USA) as recommended by the manufacturer.

The V3-V5 region of the 16S rRNA gene was amplified by PCR. The PCR reaction mixture (50 μl) contained 10 μl 5-fold reaction buffer, 200 μM of each of the four deoxyribonucleoside triphosphates, 2% DMSO, 2% BSA, 0.25 μM of each of the primers, 0.5 U of Phusion High fidelity DNA polymerase (Thermo Scientific, Waltham, MA, USA) and approximately 50 ng of isolated DNA as template. The V3-V5 region was amplified with the following set of primers containing the Roche 454 pyrosequencing adaptors and a unique MID per sample (underlined): V3for 5′-CCATCTCTACCTCCTGCGGTGTCTCAG-MID-TACGGRAGGCAGCAG-3′45 and V5rev 5′-CTATCCCTGCTGCTCTTGGGAGTCACG-MID-CCGTAATCMTTTGTAGT-3′45. The following thermal cycling scheme was used: initial denaturation at 98 °C for 3 min, 25 cycles of denaturation at 98 °C for 10 s, annealing at 58 °C for 30 s, and extension at 72 °C for 30 s followed by a final extension at 72 °C for 10 min. All samples were amplified in triplicate, pooled in equal amounts and purified by gel electrophoresis using peqGOLD
Gel Extraction kit as recommended by the manufacturer (Peqlab Biotechnologie GmbH, Erlangen, Germany). PCR products were quantified using the Quant-iT dsDNA HS assay kit and a Qubit fluorometer (Invitrogen GmbH, Karlsruhe, Germany) as recommended by the manufacturer. The Göttingen Genomics Laboratory determined the 16S RNA gene sequences employing the Roche GS-FLX+ pyrosequencer with Titanium chemistry (Roche, Mannheim, Germany).

**Analysis of pyrosequencing data.** Pyrosequencing-derived 16S rRNA gene sequences were processed using the QIIME software package version 1.8.47. Following the extraction of raw data, reads shorter than 300 bp, with long homopolymer stretches (>8 bp), or primer mismatches (>3) were removed. Subsequently, sequences were denoised employing Acacia version 1.53b48. Cutadapt49 was employed to truncate remaining primer sequences. Chimeric sequences were removed using UCHIME implemented in USEARCH version (8.0.1623) first in de novo and subsequently in reference mode using the SILVA SSURef 123 NR database as reference database50,51. Afterwards, processed sequences were clustered with UCLUST version 1.2.2q in operational taxonomic units (OTUs) at 97% and 80% genetic identity representing species and phylum level, respectively52. OTUs were classified by BLAST alignment against the most recent SILVA database (see above). Rarefaction curves, alpha diversity indices (Chao1, Shannon, Simpson) and Michaelis-Menten-Fit were determined using QIIME according to Wemheuer et al.53. The analysis was performed by using 5,311 sequences per sample (Supplementary Material Table S3). Non-metric multidimensional scaling plots were generated based on Bray Curtis dissimilarities or weighed UniFrac distances in R using the metaMDS function to visualize differences in bacterial community composition.

**Statistical analyses.** All statistical analyses were conducted employing R version 3.1.14. The results of all statistical tests were regarded significant with \( P \leq 0.05 \), and only significant results are shown and described throughout the manuscript. The median is used throughout the manuscript instead of the mean value, except stated otherwise. For all statistical analysis, the dataset calculated for 97% identity (species level) was used.

The Mann-Whitney test and non-parametric Kruskal-Wallis one-way analysis of variance (ANOVA) were used due to the non-normal distribution of the data. They were performed to test for differences in soil parameters and bacterial diversity between land use systems, exploratories and management regimes. The effects of environmental parameters onto the variance of bacterial communities were analyzed using the envfit function as described previously55. Canonical correspondence analysis (CCA) on single soil properties was carried out using the cca function and subsequently tested for significance applying the permu.test function with 1000 permutations. All these functions are contained in the vegan package56. Response curves of bacterial orders toward pH were calculated employing a multilog linear model (function multinom contained in the nnet package).

Functional profiles were predicted from obtained 16S rRNA gene data using Tax4Fun57. Genes involved in acid tolerance (ATR) and encoding key enzymes in nutrient cycling were identified in the resulting profiles using their KEGG orthologs. The heatmap, based on the ATR-involved genes was calculated using the heatmap.2 function as described previously55. Canonical correspondence analysis (CCA) on single soil properties was carried out using the cca function and subsequently tested for significance applying the permu.test function with 1000 permutations. All these functions are contained in the vegan package56. Response curves of bacterial orders toward pH were calculated employing a multilog linear model (function multinom contained in the nnet package).

**Sequence data deposition.** Sequence data were deposited in the Sequence Read Archive (SRA) of the National Center for Biotechnology Information (NCBI) under the accession number SRP065604.

**References**

1. Mooshammer, M. et al. Adjustment of microbial nitrogen use efficiency to carbon:nitrogen imbalances regulates soil nitrogen cycling. *Nat Commun.*, **5**, 3694 (2014).
2. van der Heijden, M. G., Bardgett, R. D. & van Straalen, N. M. The unseen majority: soil microbes as drivers of plant diversity and productivity in terrestrial ecosystems. *Ecol Lett.*, **11**, 296–310 (2008).
3. Stursová, M., Žižlaková, L., Leigh, M. B., Burgess, R. & Balďánek, P. Cellulose utilization in forest litter and soil: identification of bacterial and fungal decomposers. *FEMS Microbiol Ecol.*, **80**, 735–746 (2012).
4. Riihola, S. The quest for atmospheric methane oxidizers in forest soils. *Environ Microbiol Rep.*, **1**, 336–346 (2009).
5. Thomas, A. J., Giannopoulos, G., Pretty, L., Bagg, E. M. & Richardson, D. J. Biological sources and sinks of nitrous oxide and strategies to mitigate emissions. *Philos Trans R Soc Lond B Biol Sci.*, **367**, 1157–1168 (2012).
6. Hayat, R., Ali, S., Amara, U., Khalid, R. & Ahmed, I. Soil beneficial bacteria and their role in plant growth promotion: a review. *Ann Microbiol.*, **60**, 579–598 (2010).
7. Fierer, N. & Jackson, R. B. The diversity and biogeography of soil bacterial communities. *Proc Natl Acad Sci USA.*, **103**, 626–631 (2006).
8. Lauber, C. L., Hamady, M., Knight, R. & Fierer, N. Pyrosequencing-based assessment of soil pH as a predictor of soil bacterial community structure at the continental scale. *Appl Environ Microbiol.*, **75**, 5111–5120 (2009).
9. Sacke, H. et al. Pyrosequencing-based assessment of bacterial community structure along different management types in German forest and grassland soils. *PLoS One.*, **6**, e17000 (2011).
10. de Vries, F. T. et al. Abiotic drivers and plant traits explain landscape-scale patterns in soil microbial communities. *Ecol Lett.*, **15**, 1230–1239 (2012).
11. Rasche, F. et al. Seasonality and resource availability control bacterial and archaeal communities in soils of a temperate beech forest. *ISME J.*, **5**, 389–402 (2011).
12. Cruz-Martinez, K., Stuttle, K. B., Brodie, E. L., Power, M. E., Andersen, G. L. & Banfield, J. F. Despite strong seasonal responses, soil microbial consortia are more resilient to long-term changes in rainfall than overlying grassland. *ISME J.*, **3**, 738–744 (2009).
13. Brockett, B. F. T., Prescott, C. E. & Grayston, S. J. Soil moisture is the major factor influencing microbial community structure and enzymes across seven biogeoclimatic zones in western Canada. *Soil Biol Biochem.*, **44**, 9–20 (2010).
14. Fierer, N., Lauber, C. L., Ramireza, K. S., Zaneveld, J., Bradford, M. A. & Knight, R. Comparative metagenomic, phylogenetic and physiological analyses of soil microbial communities across nitrogen gradients. *ISME J.*, **6**, 1007–1017 (2012).
15. Herroz, S., Wemheuer, E., Wemheuer, B. & Daniel, R. Effects of fertilization and sampling time on composition and diversity of entire and active bacterial communities in german grassland soils. *PLoS One.*, **10**, e0145575 (2015).
16. Lauber, C. L., Hamady, M., Knight, R. & Fierer, N. Pyrosequencing-based assessment of soil pH as a predictor of soil bacterial community structure at the continental scale. Appl Environ Microbiol. 79, 3283–3291 (2013).

17. Tardy, V. et al. Analysis of soil bacterial communities by pyrosequencing: effects of forest practices on diversity and composition. Sci Total Environ. 411, 2199–2211 (2011).

18. Söderström, E. et al. Variability of soil microbial communities in temperate forests: A comparison of pyrosequencing and traditional methods. ISME J. 5, 1275–1286 (2011).

19. Prober, S. M. et al. Long-term effects of grasslands and forests on soil bacterial communities. ISME J. 5, 1275–1286 (2011).

20. Urbanová, S., Snajdr, J. & Baldařík, P. Composition of fungal and bacterial communities in forest litter and soil is largely determined by dominant trees. Soil Biol Biochem. 42, 226–244 (2010).

21. Fischer, M. et al. Implementing large-scale and long-term functional biodiversity research: The Biodiversity Exploratories. Basic App Ecol. 11, 473–485 (2010).

22. Will, C. et al. Horizon-specific bacterial community composition of German grassland soils, as revealed by pyrosequencing-based analysis of 16S rRNA genes. Appl Environ Microbiol. 76, 6751–6759 (2010).

23. Althauer, K. P., Wemheuer, B., Daniel, R. & Meinicke, P. Tax4Fun: predicting functional profiles from metagenomic 16S rRNA data. Bioinformatics. 31, 2882–2884 (2015).

24. Prober, S. M. et al. Plant diversity predicts beta but not alpha diversity of soil microbes across grasslands worldwide. Ecol Lett. 18, 85–95 (2015).

25. Jean bille, M. et al. Soil Parameters Drive the Structure, Diversity and Metabolic Potentials of the Bacterial Communities Across Temperate Beech Forest Soil Sequences. Microbial Ecol. 71, 1–12 (2015).

26. Oades, J. M. Soil organic matter and structural stability: mechanisms and implications for management. Plant Soil. 1984, 319–337 (1984).

27. Schimel, D. S. Microbial decomposition of organic matter under limiting N and P conditions. Basic Appl Ecol. 11, 473–485 (2010).

28. Heijnen, C. E. & Veen, J. A. A determination of protective microhabitats for bacteria introduced into soil. Appl Environ Microbiol. 42, 1558–1560 (2010).

29. Fried, J. S., Boyle, J. R., Tappeiner II, J. C. & Cromack, K. Jr. Effects of bigleaf maple on soils in Douglas-fir forests. Can J For Res. 10–12 (2011).

30. Geisseler, D. & Scow, K. M. Long-term effects of fertilizers on soil microorganisms – A review. Soil Biol Biochem. 33, 519–537 (2001).

31. Thoms, C., Gattinger, A., Jacob, M., Thomas, F. M. & Gleixner, G. Direct and indirect effects of tree diversity drive soil microbial diversity in temperate deciduous forest. Soil Biol Biochem. 42, 1558–1560 (2010).

32. Fried, J. S., Boyle, J. R., Tappeiner II, J. C. & Cromack, K. Jr. Effects of bigleaf maple on soils in Douglas-fir forests. Can J For Res. 10–12 (2011).

33. Hornung, M. Acidification of soils by trees and forests. Can J For Res. 33, 2194–2200 (2011).

34. Schloss, P. D. & Handelsman, J. Introducing DOTUR, a computer program for defining operational taxonomic units and estimating species richness. Appl Environ Microbiol. 71, 1501–1506 (2005).

35. Wemheuer, B. et al. Impact of a phytoplankton bloom on the diversity of the active bacterial community in the southern North Sea as revealed by metatranscriptomic approaches. FEMS Microbiol Ecol. 87, 378–389 (2014).

36. Caporaso, J. G. et al. QIIME allows analysis of high-throughput community sequencing data. Nat Method. 7, 335–336 (2010).

37. Söderström, E. et al. Analysis of soil bacterial communities by pyrosequencing: effects of forest practices on diversity and composition. ISME J. 5, 1275–1286 (2011).

38. Prober, S. M. et al. Long-term effects of grasslands and forests on soil bacterial communities. ISME J. 5, 1275–1286 (2011).

39. Wang, Y. & Qian P. Y. Conservative fragments in bacterial 16S rRNA genes and primer design for 16S ribosomal DNA amplicons in metagenomic studies. PLoS One. 4, e7401 (2009).

40. Caporaso, J. G. et al. QIIME allows analysis of high-throughput community sequencing data. Nat Method. 7, 335–336 (2010).

41. Bragg, L., Stone, G., Imelfort, M., Hugenholtz, P. & Tyson, G. W. Fast, accurate error-correction of amplicon pyrosequences using de novo assembly. Nat Methods. 9, 425–426 (2012).

42. Martin, M. Cutadapt removes adapter sequences from high-throughput sequencing reads. EMBnet.journal. 17, 10–12 (2011).

43. Quast, C. et al. The SILVA ribosomal RNA gene database project: improved data processing and web-based tools. Nucleic Acids Res. 41, D590–D596 (2013).

44. Edgar, R. C., Haas, B. J., Clemente, J. C., Quince, C. & Knight, R. UCHIME improves sensitivity and speed of amplicon pyrosequences using de novo chimera detection. ISME J. 1, 46–56 (2011).

45. Schloss, P. D. & Handelsman, J. Introducing DOTUR, a computer program for defining operational taxonomic units and estimating species richness. Appl Environ Microbiol. 71, 1501–1506 (2005).

46. Wemheuer, B. et al. Impact of a phytoplankton bloom on the diversity of the active bacterial community in the southern North Sea as revealed by metatranscriptomic approaches. FEMS Microbiol Ecol. 87, 378–389 (2014).

47. R Development Core Team. R: A language and environment for statistical computing (R Foundation for Statistical Computing, Vienna, 2015).

48. Wickham, H. ggplot2: elegant graphics for data analysis. (Springer Science & Business Media, 2009).
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Author Contributions
R.D. and H.N. conceived the study and planned the experiments. K.K. and V.K. performed the experiments. K.K. and B.W. analyzed data. I.S. and M.S. contributed data. K.K., B.W., F.W. and R.D. wrote the manuscript. All authors interpreted the results and reviewed the manuscript.

Additional Information
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