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# Long-term stability of soil bacterial and fungal community structures revealed in their abundant and rare fractions

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## Abstract

Despite the importance of soil microorganisms for ecosystem services, long-term surveys of their communities are largely missing. Using metabarcoding, we assessed temporal dynamics of soil bacterial and fungal communities in three land-use types, i.e., arable land, permanent grassland, and forest, over five years. Soil microbial communities remained relatively stable and differences over time were smaller than those among sites. Temporal variability was highest in arable soils. Indications for consistent shifts in community structure over five years were only detected at one site for bacteria and at two sites for fungi, which provided further support for long-term stability of soil microbial communities. A sliding window analysis was applied to assess the effect of OTU abundance on community structures. Partial communities with decreasing OTU abundances revealed a gradually decreasing structural similarity with entire communities. This contrasted with the steep decline of OTU abundances, as subsets of rare OTUs (<0.01%) revealed correlations of up to 0.97 and 0.81 with the entire bacterial and fungal communities. Finally, 23.4% of bacterial and 19.8% of fungal OTUs were identified as scarce, i.e., neither belonging to site-cores nor correlating to environmental factors, while 67.3% of bacterial and 64.9% of fungal OTUs were identified as rare but not scarce. Our results demonstrate high stability of soil microbial communities in their abundant and rare fractions over five years. This provides a step towards defining site-specific normal operating ranges of soil microbial communities, which is a prerequisite for detecting community shifts that may occur due to changing environmental conditions or anthropogenic activities.

**KEYWORDS**
next-generation biomonitoring, normal operating range, rare biosphere, soil quality monitoring, temporal dynamics

## 1 | INTRODUCTION

Soil bacterial and fungal communities impact ecosystem services such as crop production (Hu et al., 2018) or nutrient cycling (Regan et al., 2017), and therefore have a major influence on soil quality. Maintaining soil quality requires the maintenance of stable soil microbial communities, because changes in their structures may induce disturbances in ecosystem processes. However, soil quality assessment and especially long-term soil monitoring rarely include high-throughput DNA sequencing of soil microbial communities in addition to soil physicochemical analyses (van Leeuwen et al., 2017). A major reason for this gap in soil quality assessments is the largely missing information on temporal dynamics of soil bacterial and fungal communities over multiple years.

The temporal stability of microbial communities in soil has been shown to be higher as compared to the stability of microbial communities living in water, air or host-associated environments (Shade et al., 2013). The first evidence showing temporal stability of soil bacterial communities over 1 year was obtained using fingerprinting techniques (Gelsomino et al., 1999). Since then, high-throughput DNA sequencing has been developed enabling the assessment of entire soil bacterial and fungal communities to a depth that reflects their diversity. A 3-year survey using high-throughput sequencing revealed a stable fungal community until a heavy rainfall occurred in the last year and resulted in soil water saturation (Barnes et al., 2018). Larger differences were also observed for soil bacterial communities among sites as compared to temporal variability over 6 months (Carini et al., 2020; Lauber et al., 2013). In general, soil bacterial and fungal communities appear to be relatively stable over several months to a few years, but empirical data are scarce.

Temporal stability of communities depends on the one hand on their resistance and resilience to environmental fluctuations, where resistance is defined as the insensitivity of a community to a disturbance, and resilience as the ability of a community to return to its initial, predisturbed state (Shade et al., 2012). Furthermore, temporal stability depends on ecological stochasticity including ecological drift and random dispersal (see Zhou & Ning, 2017 for a review), which may cause communities to shift over time. Monitoring programmes may allow to identify community shifts over longer time periods in natural habitats. Long-term community shifts continuously drive a community away from its initial state (Figure S1), which can be assessed by linking community dissimilarities to the elapsed time between two sampling time points. This may be achieved by analysing time–decay relationships (e.g. Berg & Bengtsson, 2007; Chow et al., 2013; Shade et al., 2013), which are analogous to distance–decay relationships (Nekola & White, 1999). Time–decay analyses allow identification of steady shifts in microbial communities. However, they do not allow identifying specific time points when changes occur, because time lags rather than single time points are assessed in these analyses. The identification of time points when community shifts occur is of particular importance for long-term monitoring systems. Therefore, long-term biomonitoring of macro-organisms often relies on comparison to a reference point for identifying community shifts (Magurran et al., 2010). Due to the lack of long-term surveys of soil microbial organisms, it is currently unknown whether continuous shifts of soil microbial communities occur over extended time periods.

Assessment of temporal variability of soil microbial communities in ecosystems that are not subject to exceptional disturbances allows definition of their normal operating ranges (NORs). An NOR describes the multivariate space in which the states of an undisturbed ecosystem occur (Kersting, 1984; van Straalen, 2002). For soils, Semenov et al. (2014) established NORs for sandy and clay soils based on 21 parameters including chemical (e.g., pH and nitrogen content) as well as biological (e.g., gene abundances and bacterial diversity) parameters that were determined at eight agricultural sites over 3 years. In a second step, these authors applied weak and strong temperature and flooding stresses to these soils in microcosms and compared the measured parameters against the NOR established based on undisturbed soils. While control microcosms remained within the NOR, the stressors caused increasing distances of soil parameters to the NOR. However, due to the lack of long-term surveys using high-throughput sequencing of soil microbial communities, NORs of soil microbial community structures are currently unknown. Along with Semenov et al. (2014), who showed different NORs for different soil types (i.e., clay and sandy soils), Lauber et al. (2013) have shown that arable soils harbour temporally less stable bacterial communities as compared to grassland, which suggests that the NOR of soil microbiomes depends on environmental settings and soil management. Furthermore, the NOR may be influenced to different degrees by abundant and rare operational taxonomic units (OTUs), as rare OTUs are hypothesized to over-proportionally affect temporal variability (Shade et al., 2014).

In changing environments rare microbial OTUs may become more abundant (Aanderud et al., 2015; Barnes et al., 2018), and thus temporal variability may be more strongly influenced by conditionally rare as compared to abundant OTUs (Shade et al., 2014). Among the rare taxa, some are present in a dormant state and build a seed bank from which components of a community can be recruited in case conditions would become favourable (Shade et al., 2014). Other rare microbial taxa can be continuously active despite their rarity (Campbell et al., 2011; Hausmann et al., 2019). These would therefore be stably detected at low abundances. Finally, parts of a community appearing as rare OTUs may be due to analytical artefacts, such as polymerase chain reaction (PCR) amplification errors (Potapov & Ong, 2017). With the exception of sequence-dependent errors leading for instance to chimera formation during PCR (Haas et al., 2011), these will occur randomly and represent analytical background noise. Therefore, two groups of rare OTUs may conceptually be distinguished: (i) consistent, rare OTUs that include microorganisms living in rare microniches, and (ii) rare OTUs that occur randomly in highly fluctuating soil conditions (e.g., flooding) or very infrequently not yielding information to differentiate microbial systems. The second group, termed scarce OTUs here, includes biological as well as erroneous sequences, which cannot be reliably distinguished due to their infrequent occurrence. To distinguish consistently rare OTUs

from scarce OTUs within a community, spatial and temporal replication is needed.

Here, we assessed the temporal dynamics of soil bacterial and fungal communities over 5 years at 30 different long-term monitoring sites of the Swiss Soil Monitoring Network (NABO). The sites represented the three different land-use types arable land, permanent grassland and forest, with 10 sites each. The overarching goal of this study was to identify NORs of soil bacterial and fungal communities in these three land-use types and thus to provide the basis for the development of reference baselines for variations of soil microbial communities. We defined four main research objectives, which focused exclusively on the microbial community level, rather than on individual microbial taxa, their identities and their functions. Moreover, the analytical focus was on temporal shifts of microbial communities and differences among land-use types as well as sites rather than detailed effects of soil properties or soil textural classes. The objectives were: (i) to identify factors that influence the long-term dynamics of soil microbial communities, (ii) to screen microbial communities for consistent community shifts over 5 years, (iii) to compare community structures and notably temporal stability of their abundant and rare fractions, and (iv) to differentiate rare from scarce OTUs.

## 2 | MATERIAL AND METHODS

### 2.1 | Sampling design and physicochemical soil analyses

Soils were sampled yearly in spring over 5 years at 30 long-term monitoring sites of the NABO and were located across Switzerland (Figure S2A). Ten sites were sampled for each of three land-use types (i.e., arable land, permanent grassland and forest), and which covered eight USDA soil textural classes (Table S1; Soil Science Division Staff, 2017). At each sampling, three composite samples consisting of 25 soil cores of 20 cm depth and 2.5 cm diameter were taken in a square area of 10 × 10 m (Figure S2B) resulting in a total of 450 samples (5 years × 30 sites × 3 replicates). The samples were taken following the standardized sampling protocol of the NABO (Gubler et al., 2019). The standardized sampling within the framework of the NABO, which was established in 1985 (Gubler et al., 2015), ensures methodological stability and robustness, and furthermore facilitated interpretation of the soil microbial data due to numerous associated data, which are available for these sites. The physicochemical properties determined for each sample were: soil pH, total and organic carbon, total nitrogen, C/N ratio and bulk density. For this purpose, soils were dried at 40°C for 48 h and sieved (2 mm). Soil pH was determined in 0.01 M $CaCl_2$ using a pH-meter including an Expert Pro-ISM electrode (SevenMulti; Mettler-Toledo). Total carbon and nitrogen contents were measured with a TruSpec CN analyser (Leco) using 0.5 g soil. Organic carbon was determined by subtracting 12% of the total calcium carbonate from total carbon, where total calcium carbonate was measured in soils with a pH higher than 6.3 as the $CO_2$ production after the addition of hydrochloric acid (Harris et al., 2001). Bulk density was calculated by dividing the weight of the dried fine fraction (<2 mm) by the volume of the soil sample. Furthermore, mean annual precipitation and temperature, elevation, percentage of coarse soil fraction (>2 mm) and soil texture were obtained to characterize all sites. Mean annual precipitation and temperature were obtained based on the years from 1981 to 2015 from MeteoSwiss (http://www.meteoswiss.admin.ch). Soil texture was determined by sedimentation after humus removal using $H_2O_2$ (International Organization for Standardization [ISO], 2009). A summary of the environmental factors and how these differ between land-use types is given in Table S2.

### 2.2 | Analyses of soil microbial communities

DNA extraction and the assessment of soil bacterial and fungal communities using metabarcoding followed the description of Gschwend et al. (2020). Briefly, DNA of every sample of 0.5 g fresh and homogenized soil was extracted three times. Variable regions 3 and 4 of the bacterial 16S rRNA gene were amplified using primers 341F (5′-CCTAYGGGDBGCWSCAG-3′) and 806R (5′-GGACTACNVGGGTHTCTAAT-3′) developed by Frey et al. (2016), and the fungal internal transcribed spacer 2 (ITS2) was amplified using primers ITS3 (5′-CAHCGATGAAGAACGYRG-3′) and ITS4 (5′-TCCTSCGCTTATTGATATGC-3′) developed by Tedersoo et al. (2014). Sequence library preparation, which included individual labelling samples using the Fluidigm Access Array technology prior to pooling, as well as sequencing on the Illumina MiSeq platform was performed by the Génome Québec Innovation Center at McGill University (Montréal, Canada). Amplicon sequences were filtered based on a custom pipeline (Frey et al., 2016) mainly relying on USEARCH version 9 (Edgar, 2010). In brief, sequence analyses included removal of phiX contaminants, merging of paired-end sequences, trimming of primer sequences, where all sequences without detectable primers were discarded, quality filtering of sequences with a maximum expected error above 1, removal of singletons, de novo chimera detection, verification of their ribosomal origin using METAXA for bacterial (Bengtsson-Palme et al., 2015) and ITSX for fungal sequences (Bengtsson-Palme et al., 2013), as well as comparison against taxonomic reference databases to ensure bacterial and fungal origins. Furthermore, only sequences occurring with 100% identity in at least two of the 450 samples were allowed to build OTU centroid sequences to avoid PCR or sequencing errors from inflating soil microbial diversity. High-quality sequences were clustered into OTUs at 97% sequence identity using USEARCH version 9. Raw sequences have been archived in NCBI SRA with the project number PRJNA660320. In total, 9,020,192 quality filtered bacterial sequences were clustered into 18,140 OTUs, and 11,958,695 quality filtered fungal sequences into 8477 OTUs. This corresponds to a mean Good's coverage of 0.91 (±0.020), 0.91 (±0.016) and 0.93 (±0.023) for bacteria in arable land, permanent grassland, and forest, respectively, and 0.99 (±0.003), 0.98 (±0.004) and 0.98 (±0.004) for

fungi. Given the high sequencing coverage obtained for all samples, sequencing depth of each sample was standardized using relative abundances. This correlated highly to community structures based on subsampling to the minimum sequencing depth (Bacteria = 0.9996, Fungi = 1). Community structures were compared using Bray–Curtis dissimilarities. Since Bray–Curtis dissimilarity is based on differences in relative abundance and emphasizes dominant OTUs, two additional dissimilarity metrics were used to assess the structural similarities of community subsets. These were Jaccard dissimilarity, which is based on presence–absence data and therefore equally weighs all OTUs, and Canberra dissimilarity, which is based on relative abundances, but which gives less weight to abundant OTUs as compared to Bray–Curtis dissimilarity (Locey et al., 2020).

## 2.3 | Statistical analyses of soil microbial communities

All analyses, unless stated otherwise, were performed in R (R Core Team, 2016; RStudio, 2015). Centroids of communities from all 15 samples from each site (site centroids), and centroids from the three samples from each site and year (replicate centroids) were obtained using the function "betadisper" of the R package "vegan" (Oksanen et al., 2018). This function implements the approach described by Anderson et al. (2006), in which non-Euclidean dissimilarities are first transferred into the Euclidean space by principal coordinate analysis (PCoA), then distances between samples and centroids are retrieved. Based on distances between replicate centroids (i.e., centroids of yearly triplicates), land-use- and site-specificity of soil microbial communities were assessed by canonical analysis of principal coordinates (CAP) as implemented in the function "CAPdiscrim" of the R package "BiodiversityR" (Kindt & Coe, 2005). Nonmetric multidimensional scaling (NMDS) was used for unconstrained ordination of soil microbial communities. To assess the variance of soil microbial communities explained by land-use type, site and time, we used permutational multivariate analysis of variance (PERMANOVA) implemented in the "PERMANOVA+ add-on" (Anderson et al., 2008) of the PRIMER software version 7 (Clarke et al., 2014). Bray–Curtis dissimilarities used for PERMANOVA were based on mean relative abundances of the three samples collected at the same time point. To account for the repeated measurements, sites were nested within land-use types in the PERMANOVA design (Anderson et al., 2008). Square roots of components of variation (√CV), which are expressed as Bray–Curtis dissimilarities, were used to quantify effects of land-use types, sites and year.

The temporal dynamics of soil microbial communities was analysed in two steps (Concepts visualized in Figure S1). First, temporal variability of soil microbial communities was assessed for each site (Figure S1A), and, second, community shifts over time were determined (Figure S1B). Temporal variability was assessed as distance of yearly replicate centroids to site centroids in PCoA-ordination space taking into account all PCoA-axes (Figure S1A). A further indication of overall temporal variability was obtained by nested PERMANOVA. For comparison of the temporal variability of soil microbial communities in different land-use types or Spearman correlations of temporal variability to environmental factors, median values were taken per site. Differences in temporal variability between land-use types were tested using Dunn's test, which is the pairwise post hoc test of the Kruskal–Wallis test, implemented in the R package "FSA" (Ogle et al., 2018). Community shifts over time were assessed for each site based on the distances of each microbial community to the replicate centroid of the first year (Figure S1B). Indications for a consistent community shift at a site were obtained, if distances to the first year steadily increased over time, and if these distances of at least two consecutive years were significantly increased in comparison to the change between the first and second year. The significance was tested using Dunn's test.

Structural similarity between entire and partial communities was assessed using Mantel statistics implemented in the R package "vegan" (Oksanen et al., 2018) and based on the Spearman correlation coefficient. Randomly selected microbial communities with predefined numbers of OTUs (i.e., 10, 25, 50, 100, 150, 200, 500, 1000, 2000, 5000) were taken to identify the number of OTUs needed to obtain representative community subsets. For each subset size, 10,000 random subsets were generated without replacement from the OTU table containing relative abundances. Only those samples were used to determine structural similarities that contained OTUs present in the subsets. To identify the impact of OTU abundance on structural similarities of partial communities, a sliding window approach was chosen. For this, OTUs were ordered and numbered according to their abundance, and a subset of 500 consecutive OTUs (defined as a window) was taken starting with the most abundant OTUs. The window was then shifted throughout the community by steps of 100 OTUs from abundant to rare OTUs. Therefore, the first window corresponds to OTUs 1–500, the second window to OTUs 101–600, and window 6 to OTUs 501–1000. Window 6 is thus the first independent community subset in comparison to the 500 most abundant OTUs. In total, 177 windows were obtained for bacteria and 80 for fungi.

## 2.4 | Indicative, core, abundant, rare and scarce OTUs

To assess community structures in greater detail, we partitioned OTUs into five groups: indicative, core, abundant, rare and scarce OTUs. An indicative OTU was defined as significantly correlating (|Spearman rho| > 0.4) with at least one of the environmental factors considered in this study. Bacterial and fungal indicative OTUs occurred in at least 27 and 28 samples, respectively. A core OTU was defined as occurring in at least 12 of the 15 samples from one site and therefore represents an OTU with a high occupancy at a site. Indicative and core OTUs have also been used and further characterized by Gschwend et al. (2021). A rare OTU was defined

as occurring in a window where all OTUs had a relative abundance below 0.01%. Consequently, all OTUs occurring in a window where at least one OTU had a relative abundance of 0.01% or more were defined as abundant. The threshold for scarce OTUs was defined at the abundance level, where windows contained neither indicative nor core OTUs. Indicative OTUs represent ecological signals that depend on the factors considered and core OTUs represent consistently detected OTUs that depend on sequencing depth. Therefore, the partitioning of OTUs into the five groups is operationally and system-dependent. For the comparison of community structures we included in addition to the abundant (a), rare (r) and scarce (s) OTUs, the two combinations rare minus scarce OTUs (r-s), which corresponds to the rare OTUs excluding the scarce OTUs, as well as abundant plus rare minus scarce OTUs (a+r-s), which corresponds to all except the scarce OTUs.

## 3 | RESULTS

### 3.1 | Temporal stability of soil microbial communities

The greatest variation of soil bacterial and fungal communities was detected between sites (Table 1, $\sqrt{CV}_{Bacteria} = 0.39$, $\sqrt{CV}_{Fungi} = 0.51$), and was followed by land-use type ($\sqrt{CV}_{Bacteria} = 0.28$, $\sqrt{CV}_{Fungi} = 0.31$). Overall, time had only a small effect ($\sqrt{CV}_{Bacteria} = 0.05$, $\sqrt{CV}_{Fungi} = 0.06$), indicating relatively stable soil microbial communities and no systematic artefacts among years. Nevertheless, the residuals of the PERMANOVA model show that temporal effects can be detected at the site level ($\sqrt{CV}_{Bacteria} = 0.15$, $\sqrt{CV}_{Fungi} = 0.29$). Reclassification success in a leave-one-out cross-validation to land-use type and site was 100% for bacterial and fungal communities. Consequently, differences of soil bacterial and fungal communities over time were smaller than those among land-use types or among sites.

To obtain a more profound understanding of the temporal dynamics of soil microbial communities, we first assessed the temporal variability as the Euclidean distances in the multidimensional ordination space from replicate centroids to the site centroids (conceptually visualized in Figure S1A). In a two-dimensional ordination, this corresponds to the lengths of the lines shown in Figure 1. We subsequently screened the communities for community shifts over 5 years (Figure S1B). Temporal variability of bacterial and fungal communities was highest in soils from arable land and no difference in this temporal variability was detected between forest and grassland soils (Figure 2, Dunn's test, $p < .0001$). The difference between temporal variability in arable land as compared to grassland and forest was lower for bacteria (+12%) than for fungi (+36%, Table S3). Arable soils are characterized by a more intense management, which includes site-adjusted crop rotations, tillage, fertilization and plant protection regimes, as well as by environmental factors that significantly differed from forest and grassland soils. For instance, soil pH and bulk density were higher, while carbon content and elevation were lower in arable soils (Table S2). These environmental factors also correlated with the temporal variability of microbial communities (Table S4). The environmental factors with strongest correlation to temporal variability of bacterial communities were bulk density (rho = 0.68, $p = .0005$), pH (rho = 0.58, $p = .0025$), organic carbon (rho = −0.66, $p = .0005$), total carbon (rho = −0.63, $p = .0008$) and elevation (rho = −0.52, $p = .0081$). Temporal variability of fungal communities was correlated to soil pH (rho = 0.64, $p = .0017$), bulk density (rho = 0.60, $p = .0023$), organic carbon (rho = −0.59, $p = .0023$), elevation (rho = −0.58, $p = .0026$) and total carbon (rho = −0.56, $p = .0031$). Separate analyses for each land-use type did not reveal any significant correlations of temporal variability of community structures and environmental factors.

Community shifts over time were determined for each site by comparing microbial communities of each year to the community detected in the first year (2012). This was achieved by determining the distances of microbial communities to the replicate centroids from the first year (Figure S1B). These distances were then compared to the distance between the first (2012) and the second (2013) year. Significantly different distances were observed at 12 sites for bacterial communities (Figure S3) and at four sites for fungal communities (Figure S4). However, indications for consistent community shifts (i.e., significantly increased distances to the first year of at least two consecutive years) were only observed in three cases: at site 77 for bacteria and at sites 25 and 68 for fungi. The absence of indications for consistent community shifts at 27 sites underlines the stability of soil microbial community structures over 5 years.

**TABLE 1** Variation of soil bacterial and fungal communities by land-use type, site and time assessed using nested PERMANOVA

| | Bacteria | | | Fungi | | |
|---|---|---|---|---|---|---|
| Factor | Pseudo-*F* | *p*-value | √CV[a] | Pseudo-F | *p*-value | √CV[a] |
| LUT | 6.1 | .0001 | 0.28 | 4.4 | .0001 | 0.31 |
| Site | 33.6 | .0001 | 0.39 | 16.9 | .0001 | 0.51 |
| Time | 4.2 | .0001 | 0.05 | 2.2 | .0001 | 0.06 |
| LUT × time | 1.6 | .0001 | 0.04 | 1.3 | .0001 | 0.05 |
| Residuals | | | 0.15 | | | 0.29 |

Abbreviation: LUT, land-use type.

[a]√CV: square root of components of variation expressed as Bray–Curtis dissimilarity.

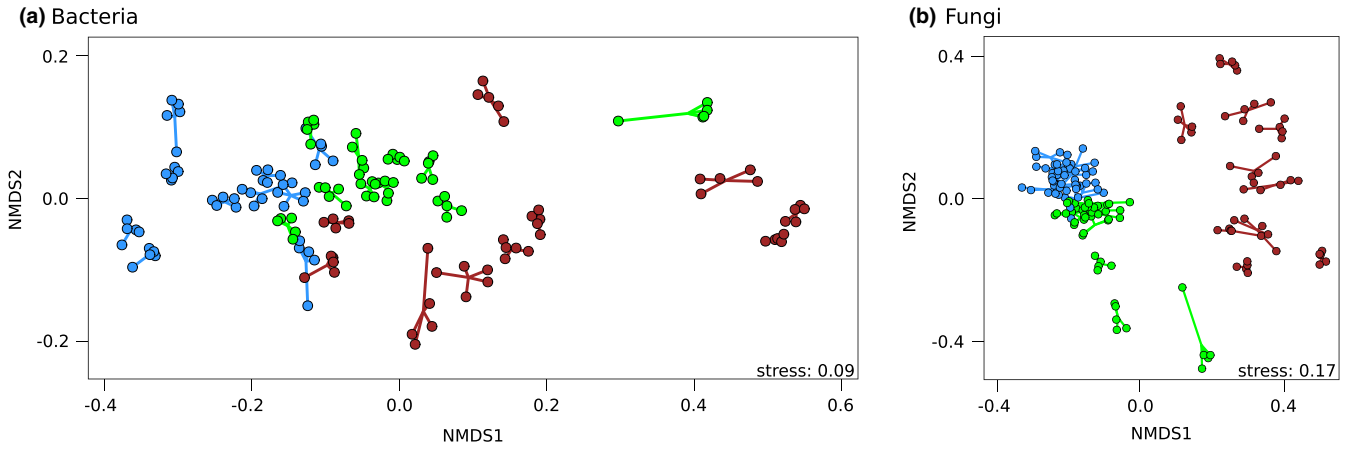


FIGURE 1 Bacterial (a) and fungal (b) communities of 30 sites from three land-use types: arable land (blue), permanent grassland (green) and forest (brown). Each site was sampled with yearly triplicates during 5 years. Centroids of yearly triplicates (replicate centroids) are shown resulting in five dots per site ($N = 150$), which are connected by lines to the centroid of the site. Unconstrained ordinations are based on nonmetric multidimensional scaling (NMDS)

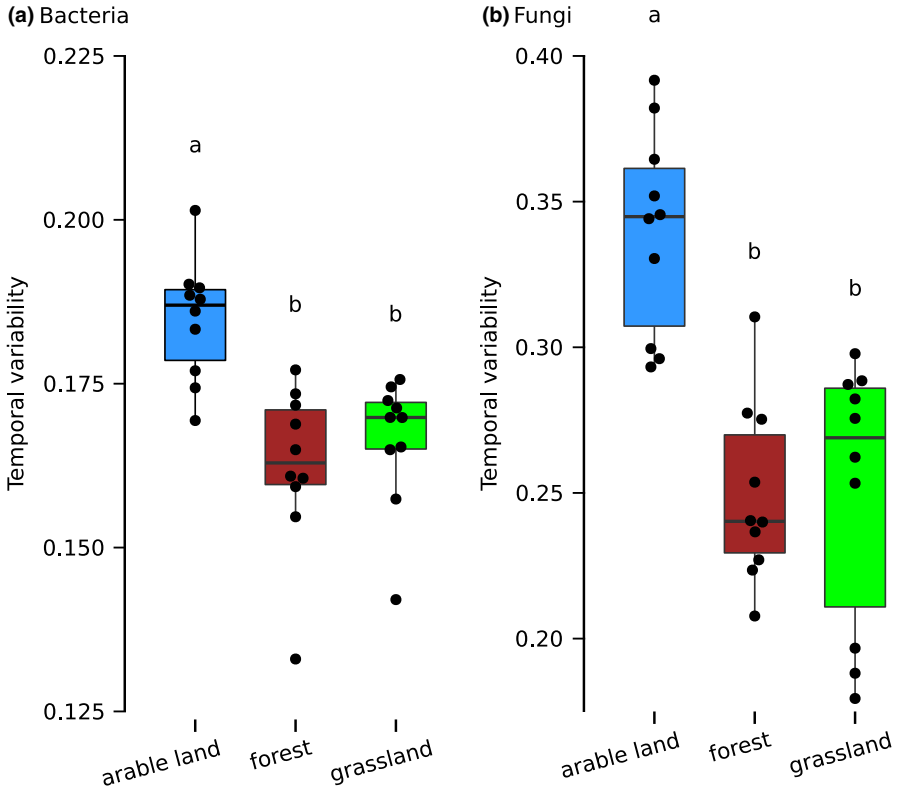


FIGURE 2 Temporal variability of soil bacterial (a) and fungal (b) communities in the three land-use types. Letters indicate significant differences among groups determined using Dunn's test ($p < .01$). Temporal variability was recorded as distances between the centroid of triplicates of each year and the centroid of the corresponding site (Figure S1A). Note the different scales of the *y*-axes

### 3.2 | Structural similarities of entire and partial communities

The high temporal stability of soil microbial communities and their distinctness among sites together raises the question of which fractions of soil microbial communities are responsible for this. Therefore, identification of partial communities that are representative for the site-specificity of a community was approached. To determine the number of OTUs that is required to build such representative community subsets we prepared random subsets of different sizes (i.e., 10 to 5000 OTUs) with 10,000 iterations and compared these to the entire original communities using the Mantel test. Correlations between entire and partial communities increased with increasing numbers of OTUs in the partial communities (Figure 3). For example, mean values of randomly assembled partial communities composed of 500 OTUs showed a correlation of .92 (±.026) and .84 (±.040) to entire bacterial and fungal communities, respectively, while random sets of 10 OTUs yielded correlations of .41 (±.14) for bacteria and .42 (±.13), for fungi. In a few cases, even partial communities composed of 10 OTUs yielded correlations >.8 to entire communities. When, for example, the 10 most abundant OTUs were selected, their community structures were almost identical to the one of the

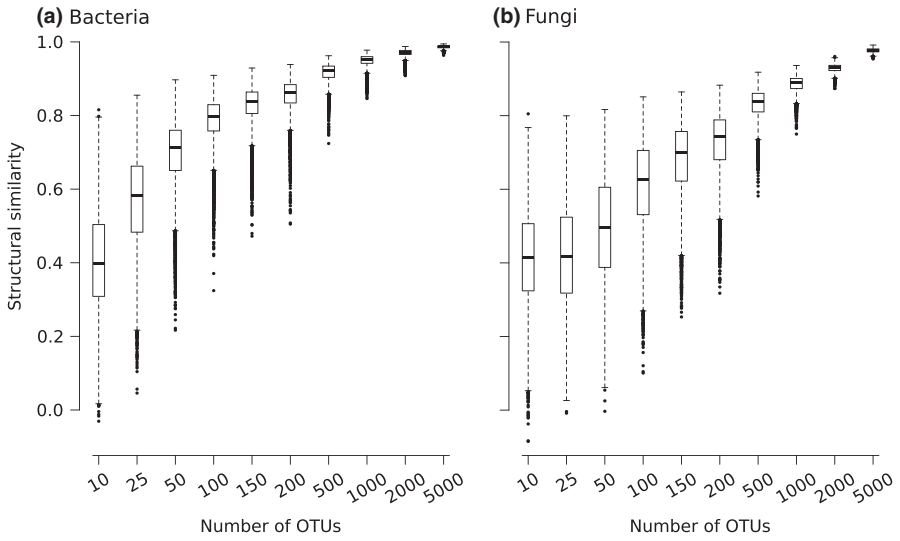


**FIGURE 3** Structural similarity of entire and partial communities composed of 10 to 5000 randomly selected bacterial (a) and fungal (b) OTUs assessed using the Mantel test with Spearman correlations. For each subset size, 10,000 random partial communities were generated. The entire data set included 450 samples with 18,140 bacterial and 8477 fungal OTUs

entire communities with correlations of .99 ($p < .0001$) for bacteria, and 1.00 ($p < .0001$) for fungi.

For a more detailed understanding of the influence of relative OTU abundances on the structural similarity between entire and partial communities, a sliding window approach was used. For this approach, we first chose three dissimilarity indices (i.e., Bray–Curtis, Canberra and Jaccard), which differently weight rare and abundant OTUs, as well as different window sizes ranging from 100 to 1000 OTUs (Figure S5). All revealed highly similar results correlating by at least .96. We used Bray–Curtis dissimilarities and a window size of 500 OTUs for more detailed analyses. Partial communities composed of abundant OTUs revealed higher structural similarities with the entire communities. For example, community structures of the 500 most abundant OTUs (i.e., window 1) almost perfectly matched the structures of entire communities with correlations of .99 for bacteria and of 1.00 for fungi ($p < .0001$, Table 2). These windows contained only 2.8% (total: 18,140) of bacterial and 5.9% (total: 8477) of fungal OTUs but accounted for 58.5% of total bacterial and 75.3% of total fungal relative abundances (Table 2). Land-use- and site-specificity for window 1 also revealed high CAP reclassifications of at least 99.6% (Table 2). The structural similarity between partial and the entire community decreased with decreasing OTU abundances for bacteria and fungi (Figure 4a–d). Remarkably, the summed relative abundance of OTUs within the sliding windows dropped much faster than the correlations between entire and partial communities. This is, for instance, illustrated by the windows at the border of abundant and rare OTUs (Figure 4, red line), that is windows 18 for bacteria and 14 for fungi. Bacterial window 18 had an overall relative abundance of 3.9% and revealed a correlation of .97 to the entire community (Table 2). For fungi, window 14 had an overall relative abundance of 3.5% while revealing a correlation of .81 to the entire community. Consequently, even windows exclusively composed of rare OTUs may be highly representative of entire communities. For example, land-use- and site-specificity remained very high, with 100% reclassification success rate for bacterial window 18 and at least 99.8% for fungal window 14 (Table 2). Further scanning through the communities by decreasing relative abundance decreased the correlation to the entire community, which eventually reached .13 for the bacterial and .17 for the fungal windows with the lowest abundances (Figure 4c,d).

## 3.3 | Identification of scarce OTUs

Scarce OTUs were defined as OTUs that are not consistently detected at a site and that do not respond to an environmental factor. Therefore, we identified windows that did not contain core (Figure 4e,f; orange line) or indicative OTUs (Figure 4e,f; green line). For bacteria and fungi, core OTUs were detected at lower abundances as compared to indicative OTUs (Figure 4e,f). The thresholds defining scarce bacterial and fungal OTUs were therefore based on the absence of core OTUs. Modifying this threshold by defining scarce OTUs based on indicative OTUs (Figure 4e,f, green line) did not affect the community structures revealed by rare and scarce OTUs (Table S5). Therefore, our analysis was robust to modifications of the criteria selected to define rare and scarce OTUs. The determined thresholds of relative abundances of scarce OTUs in the present study (Figure 4e,f; orange line) were at 0.00016% for bacteria and 0.00015% for fungi (Table 2) and thus about 60 times lower than the 0.01% thresholds to separate abundant from rare OTUs. This revealed that rare OTUs can be robustly detected using a metabarcoding approach. Scarce OTUs, which accounted for 4240 (23.4%) of bacterial and 1677 (19.8%) of fungal OTUs, were individually little informative, but in combination still explained differences among land-use types and sites (Table 3).

## 3.4 | Temporal stability of abundant and rare community fractions

Removing the scarce OTUs from the communities resulted in community structures that were perfectly correlated to entire

**TABLE 2** Abundance, structural similarity, and grouping by land-use type and site of selected windows composed of 500 bacterial or fungal OTUs, which corresponds to 2.8% of the 18,140 bacterial and 5.9% of the 8477 fungal OTUs

| | Bacteria | | | | | Fungi | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| **Definition**[a] | ***a*** | ***b*** | ***c*** | ***d*** | ***e*** | ***a*** | ***b*** | ***c*** | ***d*** | ***e*** |
| Window number | 1 | 6 | 18 | 94 | 140 | 1 | 6 | 14 | 41 | 69 |
| Included OTUs[b] | 1–500 | 501–1000 | 1701–2200 | 9301–9800 | 13,901–14,400 | 1–500 | 501–1000 | 1301–1800 | 4001–4500 | 6801–7300 |
| Rel. abund.[c] | 0.585 | 0.130 | 0.039 | 0.002 | 0.001 | 0.753 | 0.115 | 0.035 | 0.004 | 0.001 |
| Most abund. OTU[d] | $3.7 \times 10^{-2}$ | $3.9 \times 10^{-4}$ | $9.4 \times 10^{-5}$ | $4.9 \times 10^{-6}$ | $1.6 \times 10^{-6}$ | $2.9 \times 10^{-2}$ | $3.6 \times 10^{-4}$ | $9.4 \times 10^{-5}$ | $8.5 \times 10^{-6}$ | $1.5 \times 10^{-6}$ |
| Least abund. OTU[e] | $3.9 \times 10^{-4}$ | $1.8 \times 10^{-4}$ | $6.5 \times 10^{-5}$ | $4.3 \times 10^{-6}$ | $1.4 \times 10^{-6}$ | $3.6 \times 10^{-4}$ | $1.4 \times 10^{-4}$ | $5.3 \times 10^{-5}$ | $6.1 \times 10^{-6}$ | $1.1 \times 10^{-6}$ |
| Structural similarity[f] | 0.99 | 0.98 | 0.97 | 0.62 | 0.31 | 1.00 | 0.89 | 0.81 | 0.38 | 0.20 |
| CAP[g] (LUT) [%] | 100 | 100 | 100 | 99.8 | 96.4 | 100 | 99.8 | 99.8 | 99.6 | 94.7 |
| CAP[g] (Site) [%] | 100 | 100 | 100 | 97.6 | 84.0 | 99.6 | 99.6 | 99.8 | 96.7 | 79.8 |

Abbreviation: LUT, land-use type.

[a]*a*: 500 most abundant OTUs, *b*: first window which is independent of *a*, *c*: first window containing only rare OTUs (red line, Figure 4), *d*: first window containing no indicative OTUs (green line, Figure 4), *e*: first window containing only scarce OTUs (orange line, Figure 4), *e* covered 449 and 430 of the 450 samples for bacteria and fungi.

[b]OTUs ranked by relative abundance.

[c]Rel. abund.: relative abundance, sum of all OTU abundances within a window.

[d]Abundance of the most abundant OTU in a window.

[e]Abundance of the least abundant OTU in a window.

[f]Mantel test, Spearman correlation to entire community.

[g]CAP: canonical analysis of principal coordinates, reclassification successes are shown.

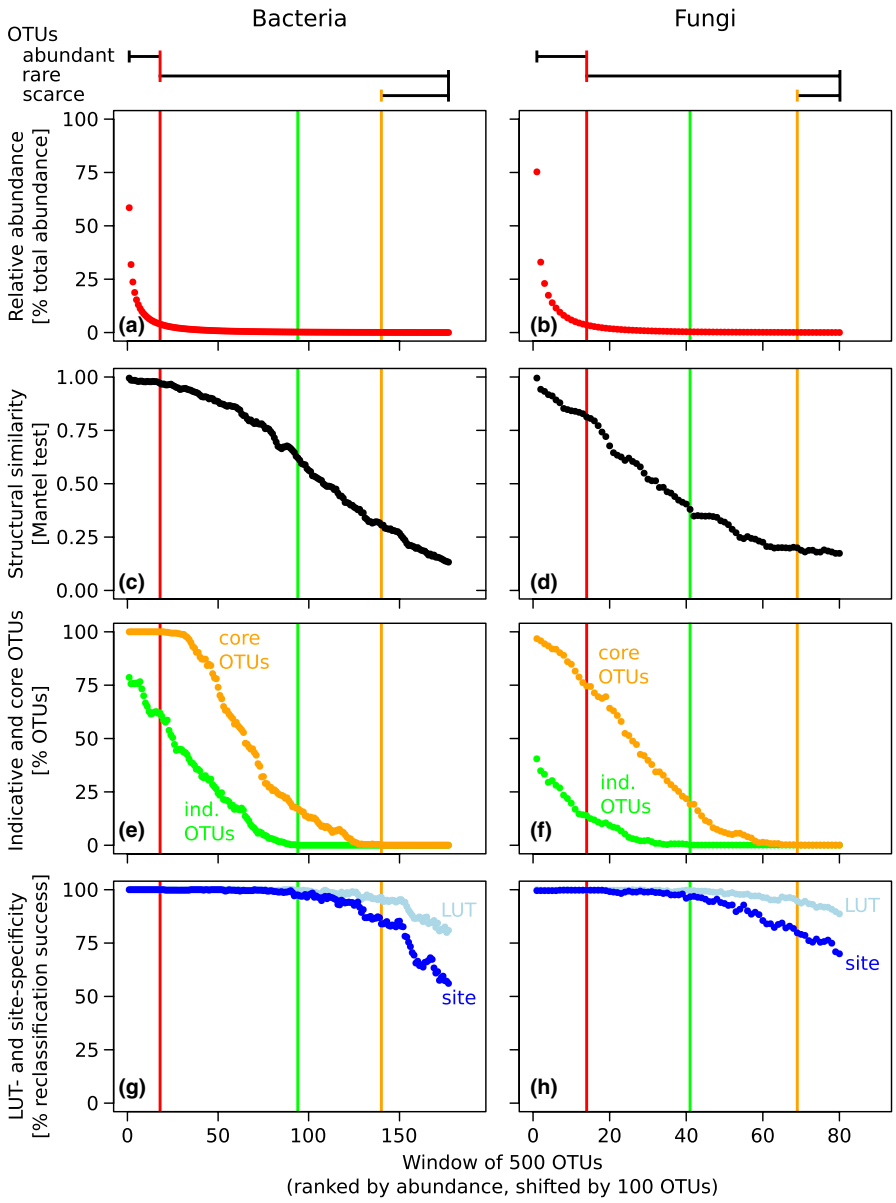


**FIGURE 4** Sliding window analysis to scan soil bacterial (left panels) and fungal (right panels) communities for relative abundance (a,b), structural similarity (c,d), indicative and core OTUs (e,f), and land-use- and site specificity (g,h). Sliding windows were composed of 500 OTUs of decreasing relative abundance and shifted by 100 OTUs. Panels (a) and (b) show the summed relative abundances of windows. Structural similarity, shown in panels (c) and (d) is defined as the Spearman correlation between windows and entire communities. Panels (e) and (f) show the percentage of indicative (green) and core (orange) OTUs within the windows; indicative OTUs were defined as OTUs correlated with an environmental factor (|rho| > 0.4), and core OTUs (orange) as OTUs that occur in at least 12 of the 15 samples from a site. The specificity to land-use type (LUT, light blue) and site (blue), shown in panels (g) and (h) were determined as the reclassification success in leave-one-out cross-validations based on canonical analysis of principal coordinates (CAP). Coloured lines indicate thresholds to differentiate abundant from rare OTUs at 0.01% relative abundance (red), and thresholds to delimit scarce OTUs as determined based on the absence of indicative (green) and core OTUs (orange). A summary of the characteristics of the first window below these thresholds, as well as for the most abundant window, is given in Table 2. The entire data set included 450 samples with 18,140 bacterial and 8477 fungal OTUs

communities (rho = 1, Table 3). Therefore, the removal of 23.4% bacterial and 19.8% fungal OTUs did not affect discrimination power of microbial community structures, supporting the robustness of structure-based analyses of soil microbial communities. Community structures of the rare OTUs excluding scarce OTUs were highly correlated to the structures of the entire communities with correlations of .97 for bacteria and .83 for fungi. Furthermore, land-use as well as site specificities of communities composed of abundant or rare OTUs without scarce OTUs were also high with reclassification successes of at least 99.8% (Table 3). Dissimilarities among sites were higher than dissimilarities among years, which again indicated the high temporal stability of rare OTUs (excluding scarce OTUs). However, PERMANOVA revealed a higher temporal variability within sites for communities composed of rare excluding scarce

**TABLE 3** Characteristics of assembled communities composed of abundant, rare and scarce OTUs

| | Bacteria | | | | | Fungi | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Abundant (a)[a] | Rare (r)[b] | Scarce (s)[c] | (r-s)[d] | (a+r-s)[e] | Abundant (a)[a] | Rare (r)[b] | Scarce (s)[c] | (r-s)[d] | (a+r-s)[e] |
| Overview | | | | | | | | | | |
| OTUs [%] | 9.4 | 90.6 | 23.4 | 67.3 | 76.6 | 15.3 | 84.7 | 19.8 | 64.9 | 80.2 |
| Abundance [%] | 80.7 | 19.3 | 0.4 | 18.9 | 99.6 | 90.2 | 9.8 | 0.1 | 9.6 | 99.9 |
| Mantel test[f] | 1.00 | 0.97 | 0.47 | 0.97 | 1.00 | 1.00 | 0.84 | 0.20 | 0.83 | 1.00 |
| CAP[h] | | | | | | | | | | |
| LUT [%] | 100 | 100 | 99.8 | 100 | 100 | 100 | 99.8 | 97.5 | 99.8 | 100 |
| Site [%] | 100 | 100 | 99.1 | 100 | 100 | 99.8 | 99.8 | 87.2 | 99.8 | 99.8 |
| PERMANOVA[g] | | | | | | | | | | |
| LUT | 0.28 | 0.27 | 0.04 | 0.27 | 0.28 | 0.31 | 0.19 | 0.03 | 0.20 | 0.31 |
| Site | 0.37 | 0.47 | 0.28 | 0.47 | 0.39 | 0.51 | 0.50 | 0.25 | 0.50 | 0.51 |
| Time | 0.05 | 0.06 | $0.02^{n.s}$ | 0.06 | 0.05 | 0.06 | 0.05 | $0.02^{n.s}$ | 0.05 | 0.06 |
| LUT × Time | 0.03 | 0.04 | $-0.01^{n.s}$ | 0.04 | 0.04 | 0.05 | 0.07 | $0.02^{n.s}$ | 0.07 | 0.05 |
| Residuals | 0.12 | 0.31 | 0.65 | 0.31 | 0.15 | 0.27 | 0.44 | 0.66 | 0.44 | 0.29 |

[a]Abundant OTUs: OTUs occurring in windows where at least one OTU has a relative abundance of 0.01% (Figure 4, red line).

[b]Rare OTUs: OTUs occurring in windows where all OTUs have a relative abundance below 0.01%.

[c]Scarce OTUs: OTUs occurring in windows without core or indicative OTUs (Figure 4, orange line), scarce fungal OTUs occurred in 142 of the 150 samples.

[d]Rare OTUs excluding scarce OTUs.

[e]All OTUs except scarce OTUs.

[f]Structural similarity between entire and partial communities, i.e., Mantel test using Spearman correlations.

[g]Square root of components of variation are shown (i.e., Bray–Curtis dissimilarity); nonsignificant values ($p > .05$) are indicated by n.s.

[h]CAP: canonical analysis of principal coordinates; reclassification successes to land-use type (LUT) and site are shown.

OTUs ($\sqrt{CV}_{Bacteria} = 0.31$, $\sqrt{CV}_{Fungi} = 0.44$) as compared to abundant OTUs ($\sqrt{CV}_{Bacteria} = 0.12$, $\sqrt{CV}_{Fungi} = 0.27$) (Table 3). Consequently, while major community characteristics were maintained throughout the majority of taxa as well as from abundant to rare OTUs, temporal variability increased slightly from abundant to rare community fractions.

## 4 | DISCUSSION

### 4.1 | Temporal stability of soil bacterial and fungal communities

Our survey of 30 long-term monitoring sites over 5 years revealed a high land-use- and site-specificity of soil bacterial and fungal community structures, along with comparatively small temporal variability (Figure 1; Table 1). A higher spatial variability relative to the temporal variability was also reported by Carini et al. (2020), who analysed the development of soil bacterial and fungal communities at two mountain slopes over 6 months. Interestingly, consistent differences not only between the two sites but also within sites at the metre scale were detected over time. To prevent spatial variability at the metre scale from masking temporal effects in our study, we took advantage of the sampling design of the Swiss long-term soil monitoring network (NABO) and collected bulk samples of 25 cores from exactly the same locations of the 100-$m^2$ plots every year (Figure S2). Despite the stability and high site-specificity of soil microbial community structures, temporal variability was detect at the site level, with arable sites showing significantly more temporal variability when compared to permanent grassland and forest sites (Figure 2). In agreement with our findings, Lauber et al. (2013) detected more variable bacterial communities in arable land as compared to a grassland plot at a single site over 6 months during one growing season. The authors attributed the increased temporal variability of bacterial communities in arable land to the land management and to the plant community, which developed over the growing season. All arable sites we assessed were managed with crop rotations, which included three to six different crops, and with one exception they were conventionally tilled. Land management of arable sites, such as crop rotations (Peralta et al., 2018), tillage (Degrune et al., 2017), fertilization (Hartmann et al., 2015) and plant protection (Rivera-Becerril et al., 2017), has been shown to affect the structures of soil microbial communities, and may therefore reduce their temporal stability. Beside the influence of land management, temporal variability of soil microbial communities may also be caused by environmental factors. Lauber et al. (2013) hypothesized, for example, that environmental selection of microorganisms with different life-strategies may lead to varying temporal stability of soil microbial communities. However, environmental factors that correlated with temporal stability of soil bacterial and fungal communities in our survey were also significantly different between arable sites and the other land-use types (Tables S2 and S4). Within land-use types no significant correlations were detected, and temporal variability of soil bacterial and fungal communities was always highest in arable soils regardless of the soil texture class (Figure S6). Together, this indicates that the increased temporal variability of bacterial and fungal communities at arable sites is likely explained by land management, and that environmental factors appear to have a minor effect on temporal stability of soil microbial communities. Interestingly, the increase in temporal variability at arable sites as compared to the other two land-use types was larger for fungal than for bacterial communities (Figure 2; Table S3). This may be due to a stronger dependence of certain fungal taxa on specific plant species (Ai et al., 2018; Fox et al., 2020) or a stronger disturbance of fungal communities in tilled systems (Schmidt et al., 2019).

### 4.2 | Temporal community shifts

Communities may follow various trajectories over time. On the one hand, communities may be disturbed but return to their initial state due to a high resilience (Lamothe et al., 2019). If this trajectory were to occur repeatedly, the community could be considered stable, although with a larger NOR. On the other hand, communities may shift to another state, which may occur rapidly or over longer time periods. Various mechanisms could cause community shifts, including deterministic processes such as short-term (pulse) or long-term (press) disturbances (Shade et al., 2012), and possibly stochastic processes such as ecological drift or random dispersal (Zhou & Ning, 2017) . Notably, continuously changing environmental factors, for instance caused by land management-induced soil compaction (Hartmann et al., 2014), by climate change (Isobe et al., 2020) or by increasing atmospheric nitrogen depositions (Leff et al., 2015; Peñuelas et al., 2012), represent press disturbances (Shade et al., 2012), which could alter the structures of soil microbial communities. Subsequently, ecosystem functions and services provided by soil microbial communities could also be altered (Allison & Martiny, 2008) and might affect soil quality. To assess, whether indications for community shifts over 5 years were detectable in our system, we determined for each site the distance of the communities to the one of the first year (Figures S3 and S4). Only soil microbial communities from three arable sites showed indications for consistent community shifts over at least 2 years. As shifts in a single year were more common (Figures S3 and S4), this may show the high resilience of soil bacterial and fungal community structures to pulse disturbances that may occur throughout the years (e.g., tillage at arable sites or meteorological changes). In agreement with the high temporal stability detected in our study, it has been demonstrated that experimental warming of forest soils lead to shifts in bacterial communities only after 20 years, while no treatment effect was found for warming periods of 5 or 8 years (DeAngelis et al., 2015). Furthermore, by correlating past and present climate data to current soil microbial diversity, Ladau et al. (2018) identified a lag of ~50 years between changes of climate variables and soil microbial diversity. This

indicates that shifts of soil microbial communities may occur over time periods in the order of decades rather than a few years, but particular taxa (populations) responsive to daily varying environmental conditions such as soil moisture, temperature or nutrient levels may be subject to more frequent changes. However, it becomes increasingly evident that soil microbial communities show a high temporal stability, despite their sensitivity to changing environmental factors, and despite their short generation times, which may increase random diversification (Zhou & Ning, 2017). Consequently, long-term experiments and monitoring are clearly needed to assess long-term effects of changing environmental and anthropogenic factors on soil microbial communities.

### 4.3 | Normal operating ranges of soil microbial community structures

With the absence of lasting community shifts over 5 years, the temporal variability assessed for most sites can be considered as within the NOR of these soil microbial communities. The temporal variability differed between land-use types and sites (Figure 2), which indicated that the extent of NORs depend on land-use as well as on specific environmental factors. Therefore, a better understanding of these factors is necessary to reliably define NORs of microbial communities. In the present study, we used the first year as the reference to detect community shifts over time. The results could, therefore, be affected by unusually large differences between the first and the second year. Our analyses also showed that significant differences of a single year were relatively common within 5 years (i.e., this was the case at eight of the 30 sites for bacteria and at two sites for fungi; Figures S3 and S4). Consequently, reference baselines or NORs based on longer time periods will provide more balanced and stable values and thereby enhance the ability to detect relevant community shifts over time. The number of years needed for the definition of a NOR or a significant community change will, however, depend on empirical data of site characteristics. In arable land, it may be necessary to consider entire crop rotations, while reference data sets with lower temporal resolution may be sufficient for permanent grassland or forest sites.

### 4.4 | Similarities of entire communities with abundant and rare fractions

The high temporal stability of soil bacterial and fungal communities raises the question of whether all fractions throughout the community from abundant to rare remain temporally stable over 5 years. Therefore, we first compared groups of 500 randomly selected OTUs with entire communities, which all revealed a high structural similarity (Mantel test, Figure 3). This suggested that subsets of a few hundred OTUs may be representative of entire soil microbial communities composed of several thousand OTUs. In agreement with our findings, 511 dominant bacterial OTUs from a global dataset have been shown to be highly correlated (Mantel test, $r = .92$) to the rest of the bacterial community (Delgado-Baquerizo et al., 2018) and, in an experimental warming experiment of forest soils, the 155 most abundant OTUs were highly correlated (Mantel test, $r = .98$) with the entire community structures and also represented the detected warming effects (DeAngelis et al., 2015). Furthermore, the removal of rare OTUs with increasing abundances had little effects on community structures of bacterial and fungal communities (Botnen et al., 2018, Zinger et al., 2014). These results suggest that community structures and their changes may already become evident based on the most dominant members of soil microbial communities. In cases where coarse differences among land-use types and sites are analysed, soil bacterial and fungal community structures may be correctly assessed using earlier culture-independent techniques such as fingerprinting approaches that assess their dominant members. To assess whether rare OTUs were also representative of entire communities we used a sliding window analysis. This revealed that structures of entire communities were best represented by abundant OTUs, but interestingly, relative abundance dropped much faster as compared to the structural similarity of partial and entire communities (Figure 4). Thus, correlated community structures were consistently detected throughout the majority of the community ranging from abundant to rare OTUs. This suggests that similar processes were driving the assembly of abundant and rare community fractions at the surveyed sites. The high stability and the strong differences among sites and land-use types could indicate that environmental filtering (Yan et al., 2019) was a major determinant of the recovered community structures. The heterogeneity within a soil habitat with interconnected major and minor niches, where abundant and rare organisms thrive, may lead to concerted community structures of abundant and rare fractions of soil bacterial and fungal communities. This may indicate that rare OTUs inhabit small niches, which are specific to a given land-use type or site. As observed for the entire communities, temporal variability remained lower than variability among sites or land-use types for communities composed of rare OTUs (Table 3). Therefore, rare fractions of soil bacterial and fungal communities also remained generally stable over 5 years. This may reflect the stability of rare microniches within soils, which offer a habitat for specialized rare taxa. However, temporal variability was slightly higher in communities composed of rare as compared to abundant OTUs (Table 3), which may be due to OTUs that are occasionally more abundant but that are generally present in low numbers (Shade et al., 2014). Experimentally, this was demonstrated in microcosms, where an initially rare OTU thrived in response to hydrocarbon pollution (Fuentes et al., 2016). Rare OTUs may therefore be more sensitive to environmental changes, on the one hand because they may thrive due to environmental changes, or on the other hand because the microniches they inhabit may be more easily disturbed compared to larger niches, where abundant taxa live.

### 4.5 | Identification of scarce OTUs

Rare OTUs are distinguished from abundant OTUs by relative abundance thresholds (e.g., 0.01%), but no thresholds exist for the definition of scarce OTUs. Scarce OTUs are extremely rare and infrequently detected OTUs, which provide little information on a system. These OTUs may include real biological sequences originating for instance from organisms that could not establish in a particular habitat, but also erroneous sequences that represent analytical failure. The continuous decrease of the structural similarity between entire and partial communities from the most to the least abundant taxa (Figure 4) impedes the definition of a threshold for scarce OTUs. Here, we defined indicative and core OTUs to empirically separate rare from scarce OTUs. This resulted in 23.4% and 19.8% of bacterial and fungal OTUs that were classified as scarce. Despite this relatively large number of scarce OTUs, analyses of community structures were only marginally affected by the inclusion or exclusion of scarce OTUs (Table 3). This is in agreement with analyses of marine and freshwater bacterial communities, where the exclusion of 45% of the OTUs with the lowest abundance showed correlations of at least .95 to entire community structures (Gobet et al., 2010; Liu et al., 2015). Furthermore, our analysis revealed that bacterial communities were composed of 9.4% abundant and 67.3% rare OTUs (excluding scarce OTUs), while these numbers were 15.3% and 64.9% for fungi. Soil microbial communities are therefore mainly composed of rare and temporally stable OTUs (Table 3). The temporal stability of rare OTUs (excluding scarce OTUs) suggests that these can be robustly assessed using metabarcoding. Finally, our analysis revealed that a relative abundance of <0.0002% (Table 2) may represent a first conceptual basis for an operational threshold to define scarce OTUs.

### 4.6 | Consequences for soil quality monitoring

Metabarcoding of soil bacterial and fungal communities has been shown to sensitively detect responses to a multitude of factors such as heavy metal pollution (Frossard et al., 2018) or salinization (Rath et al., 2019). Metabarcoding also had a higher power to discriminate between different soils (i.e., subject to periodic waterlogging or different management), as compared to physicochemical soil analyses (Gschwend et al., 2020). The reproducible detection of soil microbial communities in soils from defined environmental conditions and land-use is a major requirement for the implementation of metabarcoding in long-term monitoring programmes. Therefore, the stability of soil bacterial and fungal community structures over 5 years builds further support for metabarcoding-based soil quality monitoring. Experimental studies are needed to assess the extent of the NOR and its relationship to pulse and press disturbances. The high structural similarities between entire and partial communities also showed that defined partial communities can be used for surveys of effects on soil microbiota at the community level. This may facilitate long-term monitoring as it allows for a robust comparison among recurring sampling campaigns.

## 5 | CONCLUSIONS

Soil bacterial and fungal community structures of 30 sites from three different land-use types (i.e., arable land, permanent grassland and forest) were land-use- and site-specific and stable over 5 years. Consistent community shifts over time were largely absent, which further supports the high temporal stability of soil bacterial and fungal communities. Normal operating ranges of bacterial and fungal community structures depend on site, land management and possibly soil properties. Partial communities composed of abundant or rare OTUs were highly correlated to entire communities, revealing that subsets of a few hundred OTUs can be highly representative of entire communities composed of several thousand OTUs. Focusing on a few hundred OTUs may facilitate the establishment of references for soil identification and long-term soil quality monitoring. Finally, we showed that scarce OTUs, which account for about a third of all OTUs, have little impact on the assessment of community structures, but may be valuable scarce biota performing important soil functions in scarce habitats.

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### CONFLICTS OF INTEREST

The authors declare no conflicts of interest.

### AUTHOR CONTRIBUTIONS

All authors designed the study; F.G., A.H., A.G., R.G.M., B.F. and F.W. collected the data; F.G., M.H. and F.W. analysed the data; F.G. and F.W. drafted the manuscript, to which all authors critically contributed.

### DATA AVAILABILITY STATEMENT

Raw sequences were submitted to NCBI's SRA and can be accessed under project no. PRJNA660320. Site characteristics as well as physicochemical soil data are provided in two supplementary data files.

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## REFERENCES


Aanderud, Z. T., Jones, S. E., Fierer, N., & Lennon, J. T. (2015). Resuscitation of the rare biosphere contributes to pulses of ecosystem activity. *Frontiers in Microbiology*, *6*(24), 24. https://doi.org/10.3389/fmicb.2015.00024

Ai, C., Zhang, S., Zhang, X., Guo, D., Zhou, W., & Huang, S. (2018). Distinct responses of soil bacterial and fungal communities to changes in fertilization regime and crop rotation. *Geoderma*, *319*, 156–166. https://doi.org/10.1016/j.geoderma.2018.01.010

Allison, S. D., & Martiny, J. B. (2008). Resistance, resilience, and redundancy in microbial communities. *Proceedings of the National Academy of Sciences*, *105*, 11512–11519. https://doi.org/10.1073/pnas.0801925105

Anderson, M. J., Ellingsen, K. E., & McArdle, B. H. (2006). Multivariate dispersion as a measure of beta diversity. *Ecology Letters*, *9*(6), 683–693. https://doi.org/10.1111/j.1461-0248.2006.00926.x

Anderson, M., Gorley, R. N., & Clarke, K. (2008). *PERMANOVA+ for primer: Guide to software and statistical methods*. Primer E.

Barnes, C. J., van der Gast, C. J., McNamara, N. P., Rowe, R., & Bending, G. D. (2018). Extreme rainfall affects assembly of the root-associated fungal community. *New Phytologist*, *220*(4), 1172–1184. https://doi.org/10.1111/nph.14990

Bengtsson-Palme, J., Hartmann, M., Eriksson, K. M., Pal, C., Thorell, K., Larsson, D. G. J., & Nilsson, R. H. (2015). METAXA2: Improved identification and taxonomic classification of small and large subunit rRNA in metagenomic data. *Molecular Ecology Resources*, *15*(6), 1403–1414.

Bengtsson-Palme, J., Ryberg, M., Hartmann, M., Branco, S., Wang, Z., Godhe, A., De Wit, P., Sánchez-García, M., Ebersberger, I., de Sousa, F., Amend, A. S., Jumpponen, A., Unterseher, M., Kristiansson, E., Abarenkov, K., Bertrand, Y. J. K., Sanli, K., Eriksson, K. M., Vik, U., … Nilsson, R. H. (2013). Improved software detection and extraction of ITS1 and ITS2 from ribosomal ITS sequences of fungi and other eukaryotes for analysis of environmental sequencing data. *Methods in Ecology and Evolution*, *4*(10), 914–919. https://doi.org/10.1111/2041-210X.12073

Berg, M. P., & Bengtsson, J. (2007). Temporal and spatial variability in soil food web structure. *Oikos*, *116*(11), 1789–1804. https://doi.org/10.1111/j.0030-1299.2007.15748.x

Botnen, S. S., Davey, M. L., Halvorsen, R., & Kauserud, H. (2018). Sequence clustering threshold has little effect on the recovery of microbial community structure. *Molecular Ecology Resources*, *18*(5), 1064–1076. https://doi.org/10.1111/1755-0998.12894

Campbell, B. J., Yu, L., Heidelberg, J. F., & Kirchman, D. L. (2011). Activity of abundant and rare bacteria in a coastal ocean. *Proceedings of the National Academy of Sciences*, *108*(31), 12776–12781. https://doi.org/10.1073/pnas.1101405108

Carini, P., Delgado-Baquerizo, M., Hinckley, E.-L. S., Holland-Moritz, H., Brewer, T. E., Rue, G., Vanderburgh, C., McKnight, D., & Fierer, N. (2020). Effects of spatial variability and relic DNA removal on the detection of temporal dynamics in soil microbial communities. *mBio*, *11*(1), e02776–e12719. https://doi.org/10.1128/mBio.02776-19

Chow, C.-E., Sachdeva, R., Cram, J. A., Steele, J. A., Needham, D. M., Patel, A., Parada, A. E., & Fuhrman, J. A. (2013). Temporal variability and coherence of euphotic zone bacterial communities over a decade in the Southern California Bight. *The ISME Journal*, *7*(12), 2259–2273. https://doi.org/10.1038/ismej.2013.122

Clarke, K. R., Gorley, R. N., Somerfield, P. J., & Warwick, R. M. (2014). *Change in marine communities: An approach to statistical analysis and interpretation* (3rd ed.). Primer E.

DeAngelis, K. M., Pold, G., Topçuoğlu, B. D., van Diepen, L. T. A., Varney, R. M., Blanchard, J. L., Melillo, J., & Frey, S. D. (2015). Long-term forest soil warming alters microbial communities in temperate forest soils. *Frontiers in Microbiology*, *6*(104), 104. https://doi.org/10.3389/fmicb.2015.00104

Degrune, F., Theodorakopoulos, N., Colinet, G., Hiel, M.-P., Bodson, B., Taminiau, B., Daube, G., Vandenbol, M., & Hartmann, M. (2017). Temporal dynamics of soil microbial communities below the seedbed under two contrasting tillage regimes. *Frontiers in Microbiology*, *8*(1127), 1127. https://doi.org/10.3389/fmicb.2017.01127

Delgado-Baquerizo, M., Oliverio, A. M., Brewer, T. E., Benavent-González, A., Eldridge, D. J., Bardgett, R. D., Maestre, F. T., Singh, B. K., & Fierer, N. (2018). A global atlas of the dominant bacteria found in soil. *Science*, *359*(6373), 320–325.

Edgar, R. C. (2010). Search and clustering orders of magnitude faster than BLAST. *Bioinformatics*, *26*(19), 2460–2461. https://doi.org/10.1093/bioinformatics/btq461

Fox, A., Lüscher, A., & Widmer, F. (2020). Plant species identity drives soil microbial community structures that persist under a following crop. *Ecology and Evolution*, *10*, 8652–8668. https://doi.org/10.1002/ece3.6560

Frey, B., Rime, T., Phillips, M., Stierli, B., Hajdas, I., Widmer, F., & Hartmann, M. (2016). Microbial diversity in European alpine permafrost and active layers. *FEMS Microbiology Ecology*, *92*(3), fiw018. https://doi.org/10.1093/femsec/fiw018

Frossard, A., Donhauser, J., Mestrot, A., Gygax, S., Bååth, E., & Frey, B. (2018). Long- and short-term effects of mercury pollution on the soil microbiome. *Soil Biology Biochemistry*, *120*, 191–199. https://doi.org/10.1016/j.soilbio.2018.01.028

Fuentes, S., Barra, B., Caporaso, J. G., & Seeger, M. (2016). From rare to dominant: A fine-tuned soil bacterial bloom during petroleum hydrocarbon bioremediation. *Applied and Environmental Microbiology*, *82*(3), 888–896. https://doi.org/10.1128/AEM.02625-15

Gelsomino, A., Keijzer-Wolters, A. C., Cacco, G., & van Elsas, J. D. (1999). Assessment of bacterial community structure in soil by polymerase chain reaction and denaturing gradient gel electrophoresis. *Journal of Microbiological Methods*, *38*(1), 1–15. https://doi.org/10.1016/S0167-7012(99)00054-8

Gobet, A., Quince, C., & Ramette, A. (2010). Multivariate cutoff level analysis (MultiCoLA) of large community data sets. *Nucleic Acids Research*, *38*(15), e155. https://doi.org/10.1093/nar/gkq545

Gschwend, F., Aregger, K., Gramlich, A., Walter, T., & Widmer, F. (2020). Periodic waterlogging consistently shapes agricultural soil microbiomes by promoting specific taxa. *Applied Soil Ecology*, *155*, 103623. https://doi.org/10.1016/j.apsoil.2020.103623

Gschwend, F., Hartmann, M., Mayerhofer, J., Hug, A., Enkerli, J., Gubler, A., Meuli, R. G., Frey, B., & Widmer, F. (2021). Core and indicative bacterial and fungal taxa define characteristic soil communities of arable land, grassland, and forest. *BioRxiv*. 1–34. https://doi.org/10.1101/2021.06.07.447343

Gubler, A., Wächter, D., Blum, F., & Bucheli, T. D. (2015). Remarkably constant PAH concentrations in Swiss soils over the last 30 years. *Environmental Science: Processes & Impacts*, *17*(10), 1816–1828. https://doi.org/10.1039/C5EM00344J

Gubler, A., Wächter, D., Schwab, P., Müller, M., & Keller, A. (2019). Twenty-five years of observations of soil organic carbon in Swiss croplands showing stability overall but with some divergent trends. *Environmental Monitoring and Assessment*, *191*(5), 277. https://doi.org/10.1007/s10661-019-7435-y

Haas, B. J., Gevers, D., Earl, A. M., Feldgarden, M., Ward, D. V., Giannoukos, G., Ciulla, D., Tabbaa, D., Highlander, S. K., Sodergren, E., Methe, B., DeSantis, T. Z., Petrosino, J. F., Knight, R., & Birren, B. W. (2011). Chimeric 16S rRNA sequence formation and detection in Sanger and 454-pyrosequenced PCR amplicons. *Genome Research*, *21*(3), 494–504. https://doi.org/10.1101/gr.112730.110

Harris, D., Horwáth, W. R., & van Kessel, C. (2001). Acid fumigation of soils to remove carbonates prior to total organic carbon or CARBON-13

isotopic analysis. *Soil Science Society of America Journal*, *65*(6), 1853–1856. https://doi.org/10.2136/sssaj2001.1853

Hartmann, M., Frey, B., Mayer, J., Mäder, P., & Widmer, F. (2015). Distinct soil microbial diversity under long-term organic and conventional farming. *The ISME Journal*, *9*, 1177. https://doi.org/10.1038/ismej.2014.210

Hartmann, M., Niklaus, P. A., Zimmermann, S., Schmutz, S., Kremer, J., Abarenkov, K., Lüscher, P., Widmer, F., & Frey, B. (2014). Resistance and resilience of the forest soil microbiome to logging-associated compaction. *The ISME Journal*, *8*(1), 226–244. https://doi.org/10.1038/ismej.2013.141

Hausmann, B., Pelikan, C., Rattei, T., Loy, A., & Pester, M. (2019). Long-term transcriptional activity at zero growth of a cosmopolitan rare biosphere member. *mBio*, *10*(1), e02189-18. https://doi.org/10.1128/mBio.02189-18

Hu, L., Robert, C. A. M., Cadot, S., Zhang, X. I., Ye, M., Li, B., Manzo, D., Chervet, N., Steinger, T., van der Heijden, M. G. A., Schlaeppi, K., & Erb, M. (2018). Root exudate metabolites drive plant-soil feedbacks on growth and defense by shaping the rhizosphere microbiota. *Nature Communications*, *9*(1), 2738. https://doi.org/10.1038/s41467-018-05122-7

International Organization for Standardization. (2009). *Soil quality—determination of particle size distribution in mineral soil material—method by sieving and sedimentation (ISO Standard No 11277)*. Retrieved from https://www.iso.org/standard/54151.html

Isobe, K., Bouskill, N. J., Brodie, E. L., Sudderth, E. A., & Martiny, J. B. H. (2020). Phylogenetic conservation of soil bacterial responses to simulated global changes. *Philosophical Transactions of the Royal Society B: Biological Sciences*, *375*(1798), 20190242. https://doi.org/10.1098/rstb.2019.0242

Kersting, K. (1984). Normalized ecosystem strain: A system parameter for the analysis of toxic stress in (micro-)ecosystems. *Ecological Bulletins*, *36*, 150–153.

Kindt, R., & Coe, R. (2005). *Tree diversity analysis: A manual and software for common statistical methods for ecological and biodiversity studies*. World Agroforestry Centre (ICRAF).

Ladau, J., Shi, Y. U., Jing, X., He, J.-S., Chen, L., Lin, X., Fierer, N., Gilbert, J. A., Pollard, K. S., & Chu, H. (2018). Existing climate change will lead to pronounced shifts in the diversity of soil prokaryotes. *mSystems*, *3*(5), e00167-18. https://doi.org/10.1128/mSystems.00167-18

Lamothe, K. A., Somers, K. M., & Jackson, D. A. (2019). Linking the ball-and-cup analogy and ordination trajectories to describe ecosystem stability, resistance, and resilience. *Ecosphere*, *10*(3), e02629. https://doi.org/10.1002/ecs2.2629

Lauber, C. L., Ramirez, K. S., Aanderud, Z., Lennon, J., & Fierer, N. (2013). Temporal variability in soil microbial communities across land-use types. *The ISME Journal*, *7*(8), 1641–1650. https://doi.org/10.1038/ismej.2013.50

Leff, J. W., Jones, S. E., Prober, S. M., Barberán, A., Borer, E. T., Firn, J. L., Harpole, W. S., Hobbie, S. E., Hofmockel, K. S., Knops, J. M. H., McCulley, R. L., La Pierre, K., Risch, A. C., Seabloom, E. W., Schütz, M., Steenbock, C., Stevens, C. J., & Fierer, N. (2015). Consistent responses of soil microbial communities to elevated nutrient inputs in grasslands across the globe. *Proceedings of the National Academy of Sciences*, *112*(35), 10967–10972. https://doi.org/10.1073/pnas.1508382112

Liu, L., Yang, J., Yu, Z., & Wilkinson, D. M. (2015). The biogeography of abundant and rare bacterioplankton in the lakes and reservoirs of China. *The ISME Journal*, *9*(9), 2068–2077. https://doi.org/10.1038/ismej.2015.29

Locey, K. J., Muscarella, M. E., Larsen, M. L., Bray, S. R., Jones, S. E., & Lennon, J. T. (2020). Dormancy dampens the microbial distance-decay relationship. *Philosophical Transactions of the Royal Society B: Biological Sciences*, *375*(1798), 20190243. https://doi.org/10.1098/rstb.2019.0243

Magurran, A. E., Baillie, S. R., Buckland, S. T., Dick, J. M. P., Elston, D. A., Scott, E. M., Smith, R. I., Somerfield, P. J., & Watt, A. D. (2010). Long-term datasets in biodiversity research and monitoring: Assessing change in ecological communities through time. *Trends in Ecology & Evolution*, *25*(10), 574–582. https://doi.org/10.1016/j.tree.2010.06.016

Nekola, J. C., & White, P. S. (1999). The distance decay of similarity in biogeography and ecology. *Journal of Biogeography*, *26*(4), 867–878. https://doi.org/10.1046/j.1365-2699.1999.00305.x

Ogle, D. H., Wheeler, P., & Dinno, A. (2018). Package 'FSA'. FSA: Fisheries Stock Analysis. [R package version 0.8.22]. https://github.com/droglenc/FSA

Oksanen, J., Blanchet, F. G., Kindt, R., Legendre, P., Minchin, P. R., O'Hara, R., Simpson, G. L., Solymos, P., Stevenes, M. H. H., & Wagner, H. (2018). Vegan: Community ecology package. [R package version 2.5-1]. https://CRAN.R-project.org/package=vegan

Peñuelas, J., Sardans, J., Rivas-ubach, A., & Janssens, I. A. (2012). The human-induced imbalance between C, N and P in Earth's life system. *Global Change Biology*, *18*(1), 3–6. https://doi.org/10.1111/j.1365-2486.2011.02568.x

Peralta, A. L., Sun, Y., McDaniel, M. D., & Lennon, J. T. (2018). Crop rotational diversity increases disease suppressive capacity of soil microbiomes. *Ecosphere*, *9*(5), e02235. https://doi.org/10.1002/ecs2.2235

Potapov, V., & Ong, J. L. (2017). Examining sources of error in PCR by single-molecule sequencing. *PLoS One*, *12*(1), e0169774. https://doi.org/10.1371/journal.pone.0169774

R Core Team. (2016). *R: A language and environment for statistical computing [Computer software manual]*. https://www.R-project.org/

Rath, K. M., Fierer, N., Murphy, D. V., & Rousk, J. (2019). Linking bacterial community composition to soil salinity along environmental gradients. *The ISME Journal*, *13*(3), 836–846. https://doi.org/10.1038/s41396-018-0313-8

Regan, K., Stempfhuber, B., Schloter, M., Rasche, F., Prati, D., Philippot, L., Boeddinghaus, R. S., Kandeler, E., & Marhan, S. (2017). Spatial and temporal dynamics of nitrogen fixing, nitrifying and denitrifying microbes in an unfertilized grassland soil. *Soil Biology and Biochemistry*, *109*, 214–226. https://doi.org/10.1016/j.soilbio.2016.11.011

Rivera-Becerril, F., van Tuinen, D., Chatagnier, O., Rouard, N., Béguet, J., Kuszala, C., Soulas, G., Gianinazzi-Pearson, V., & Martin-Laurent, F. (2017). Impact of a pesticide cocktail (fenhexamid, folpel, deltamethrin) on the abundance of Glomeromycota in two agricultural soils. *Science of the Total Environment*, *577*, 84–93. https://doi.org/10.1016/j.scitotenv.2016.10.098

RStudio. (2015). *RStudio: Integrated development for R*. RStudio. http://www.rstudio.com

Schmidt, R., Mitchell, J., & Scow, K. (2019). Cover cropping and no-till increase diversity and symbiotroph:saprotroph ratios of soil fungal communities. *Soil Biology and Biochemistry*, *129*, 99–109. https://doi.org/10.1016/j.soilbio.2018.11.010

Semenov, A. V., Pereira e Silva, M. C., Salles, J. F., Schmitt, H., & van Elsas, J. D. (2014). Quantitative assessment of soil functioning across a representative range of Dutch soils. *Ecological Indicators*, *39*, 88–93. https://doi.org/10.1016/j.ecolind.2013.11.009

Shade, A., Gregory Caporaso, J., Handelsman, J., Knight, R., & Fierer, N. (2013). A meta-analysis of changes in bacterial and archaeal communities with time. *The ISME Journal*, *7*, 1493. https://doi.org/10.1038/ismej.2013.54

Shade, A., Jones, S. E., Caporaso, J. G., Handelsman, J., Knight, R., Fierer, N., & Gilbert, J. A. (2014). Conditionally rare taxa disproportionately contribute to temporal changes in microbial diversity. *mBio*, *5*(4), e01371-14. https://doi.org/10.1128/mBio.01371-14

Shade, A., Peter, H., Allison, S. D., Baho, D. L., Berga, M., Bürgmann, H., Huber, D. H., Langenheder, S., Lennon, J. T., Martiny, J. B. H., Matulich, K. L., Schmidt, T. M., & Handelsman, J. O. (2012).

Fundamentals of microbial community resistance and resilience. *Frontiers in Microbiology*, *3*(417), 417. https://doi.org/10.3389/fmicb.2012.00417

Soil Science Division Staff. (2017). Examination and Description of Soil Profiles. In C. Ditzler, K. Scheffe, & H. C. Monger (Eds.), *Soil Survey Manual*. USDA Handbook 18, (pp. 83–233). Washington, D. C.: Government Printing Office.

Tedersoo, L., Bahram, M., Põlme, S., Kõljalg, U., Yorou, N. S., Wijesundera, R., Ruiz, L. V., Vasco-Palacios, A. M., Thu, P. Q., Suija, A., Smith, M. E., Sharp, C., Saluveer, E., Saitta, A., Rosas, M., Riit, T., Ratkowsky, D., Pritsch, K., Põldmaa, K., … Abarenkov, K. (2014). Global diversity and geography of soil fungi. *Science*, *346*(6213), 1256688. https://doi.org/10.1126/science.1256688

van Leeuwen, J. P., Saby, N. P. A., Jones, A., Louwagie, G., Micheli, E., Rutgers, M., Schulte, R. P. O., Spiegel, H., Toth, G., & Creamer, R. E. (2017). Gap assessment in current soil monitoring networks across Europe for measuring soil functions. *Environmental Research Letters*, *12*(12), 124007. https://doi.org/10.1088/1748-9326/aa9c5c

van Straalen, N. M. (2002). Assessment of soil contamination – A functional perspective. *Biodegradation*, *13*(1), 41–52.

Yan, Y., Klinkhamer, P. G. L., van Veen, J. A., & Kuramae, E. E. (2019). Environmental filtering: A case of bacterial community assembly in soil. *Soil Biology and Biochemistry*, *136*, 107531. https://doi.org/10.1016/j.soilbio.2019.107531

Zhou, J., & Ning, D. (2017). Stochastic community assembly: Does it matter in microbial ecology? *Microbiology and Molecular Biology Reviews*, *81*(4). 1–32. https://doi.org/10.1128/MMBR.00002-17

Zinger, L., Boetius, A., & Ramette, A. (2014). Bacterial taxa–area and distance–decay relationships in marine environments. *Molecular Ecology*, *23*(4), 954–964. https://doi.org/10.1111/mec.12640

## SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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