Assemblage and functioning of bacterial communities in soil and rhizosphere

Yan, Y.

Chapter 2

Revisiting the dilution procedure used to manipulate microbial biodiversity in terrestrial systems

Yan Yan, Eiko E. Kuramae, Peter G.L. Klinkhamer, Johannes A. van Veen

Applied and Environmental Microbiology (2015)

Abstract

It is hard to assess experimentally the importance of microbial diversity in soil for the functioning of terrestrial ecosystems. An approach that is often used to make such assessment is the so-called dilution method. This method is based on the assumption that the biodiversity of the microbial community is reduced after dilution of a soil suspension and that the reduced diversity persists after incubation of more or less diluted inocula in soil. However, little is known how the communities develop in soil after inoculation. In this study, serial dilutions of a soil suspension were made and reinoculated into the original soil previously sterilized by γ-irradiation. We determined the structure of the microbial communities in the suspensions and the inoculated soils using 454-pyrosequencing of 16S rRNA genes. Upon dilution, several diversity indices showed that, indeed, the diversity of the bacterial communities in the suspensions reduced dramatically, with *Proteobacteria* as the dominant phylum of bacteria detected in all dilutions. The structure of the microbial community was changed considerably in soil with *Proteobacteria*, *Bacteroidetes* and *Verrucomicrobia* as the dominant groups in most diluted samples, indicating the importance of soil- related mechanisms operating in the assembly of the communities. We found unique operational taxonomic units (OTUs) even in the highest dilution both in the suspensions and in the incubated soil samples. We conclude that the dilution approach reduces the diversity of microbial communities in soil samples but that it does not allow for accurate predictions on the community assemblage during incubation of (diluted) suspensions in soil.

2.1. Introduction

The significance of biodiversity for terrestrial ecosystem processes continues to be a matter of much debate (Sala et al 2000, Magurran and Henderson 2003, Butchart et al 2010). Compared to the importance of plants and animals, the role of microbial biodiversity is still poorly understood. This lack of knowledge is of great concern as soil microbes, particularly bacteria, represent the major source of biodiversity in terrestrial ecosystems and are known to carry out numerous essential ecosystem functions, including nutrient cycling and facilitating plant nutrition (Philippot et al 2013).

The biggest obstacle to a better understanding of the importance of microbial biodiversity for the functioning of terrestrial ecosystems is the lack of sound experimental approaches to make directed and predictable changes in the diversity of microbial communities in soil. One of the most interesting approaches so far is the so-called dilution method. This method involves the inoculation of sterilized soils with more or less diluted inocula derived from suspensions of the same soil (Salonius 1981, Garland and Lehman 1999, Franklin et al 2001, Griffiths et al 2001, Matos et al 2005, Franklin and Mills 2006, Wertz et al 2006, Hol et al 2010, Philippot et al 2013, Vivant et al 2013). However, previous studies were often limited by the depth and extent of the analytical methodology applied and focused only on the structure of the microbial community after regrowth in the soil. As a consequence, they do not provide information about the community from which the different communities after incubation originated and the process of community assemblage. Therefore, these studies do not allow testing of the assumption that dilution mainly influences the diversity through the reduction of the number of the less abundant, rare, species. In reality, rare species in the original community may have become common after incubation or vice versa.

High-throughput next-generation sequencing technologies have allowed researchers to use deeper sampling depths by providing large numbers of reads by cost-effective means to detect microbial phylogenetic diversity (Margulies et al 2005). This has provided new insights into the details of microbial communities in natural ecosystems (Sogin et al 2006, Huber et al 2007, Neufeld et al 2008) and human body (Turnbaugh et al 2008). One of the exciting possibilities provided by this technology is the ability to estimate accurately the

assembly processes and structure of microbial communities, including the long tail of less abundant microbes, that is evident in graphs of relative abundances of microbial species, which may lead to a better understanding of the relevance of microbial biodiversity in soil.

The major aim of this study was to determine the changes and the associated variation in the composition of a soil microbial community brought about by inoculation of serial dilutions of suspensions of that soil and to detect how the microbial community structure develops during regrowth in soil. This analysis will allow evaluation of the suitability of the dilution approach as a tool for the manipulation of microbial biodiversity and for the separation of rare from abundant species. It will also lead to a better understanding of the selective pressure of the soil environment on the assembly of microbial communities. We addressed three basic questions: 1) does the dilution procedure reduce the diversity of the microbial community after inoculation and subsequent incubation of soil suspensions in soil? 2) does the composition of the microbial community change during incubation in soil? 3) is the dilution procedure effective in separating more and less abundant species so to allow an assessment of their specific roles? In order to answer these questions, we established a range of microbial communities through the inoculation of serial dilutions of microbial suspensions from nonsterilized soil samples into the same soil after sterilization.

2.2. Materials and methods

2.2.1. Soil sampling and treatment

Thirty liters of soil was collected at a depth of around 15 cm from dune sandy soil in Meijendel, The Netherlands (52°9'N, 4°22'E). Soil organic matter content (%) was 9.11 ± 0.36 (n=6), soil pH was 7.4 ± 0.005 (n=6), NO_3^- content (mg/kg) was 30.43 ± 0.85 (n=6), NH_4^+ content (mg/kg) was 2.23 ± 0.25 (n=6), P content (mg/kg) was 15.16 ± 0.41 (n=6). The soil had a sandy texture, with more than 99% of the grains greater than 75 µm. The soil was sieved, homogenized and aliquots of 500 g were stored in plastic bags (Whirl-pak sampling bag, 720 ml, Sigma-aldrich). The bags containing soil were gamma irradiation sterilized (> 25 kGray, Isotron, Ede, the Netherlands). One bag of

soil was kept separately to serve as inoculum. Sterility was checked by spreading 0.5 g sterilized soil from the inoculum bag onto TSA and PDA media. No bacterial and fungal growth on agar plates for 6 replicates was observed in the sterilized soil samples after 6 days. Three gamma irradiation sterilized soil bags were inoculated with autoclaved demineralized water to be used as control. A subsample of the fresh soil was taken to determine soil moisture (24 h, 105 ^{o}C).

Soil suspensions for inoculation were made by mixing 20 g fresh soil and 190 ml autoclaved demineralized water with a blender for 2 minutes. This procedure was repeated 3 times and in between the blender was cooled down on ice for 2 minutes. This was called the 10^{-1} dilution. 100 ml of 10^{-1} dilution was transferred to a bottle containing 900 ml of autoclaved demineralized water and followed the bottle shaking by hands for 1 min. This procedure was repeated for several times until 10^{-6} and 10^{-9} dilutions were made. Subsequently, 25 ml of each dilution were added to 500 g of soil in the bags, and additional autoclaved demineralized water was added to bring the moisture level of the inoculated soil at around 20%, which is roughly similar to the average level at the prevailing climatic conditions at the side from where the soil was taken. In total, 39 bags of soil (i.e. six replicated samples of three dilutions in duplicates plus three controls) were used. We kept the six replicate samples (and the duplicates) per dilution separated throughout the experiment in order to be able to assess the variance caused by the dilution procedure. The remaining suspensions were centrifuged at 3,000 g for 10 min at 4 ^{o}C, and the pellets were stored at -20 ^{o}C for further analysis. After inoculation, the soil bags were incubated at 20 ^{o}C using sterilized cotton plug caps to ensure gas exchange. The soils were turned over regularly once a week to homogenize microbial growth. The aim was to reach similar microbial abundances in the different dilution treatments. After 8 weeks of incubation under laminar flow conditions, soil samples were taken to determine the microbial abundance in all treatments by quantitative real time PCR (qPCR) using Eub 338 (Lane 1991) and Eub 518 (Muyzer et al 1993) primer set for 16S rRNA gene. Total DNA was extracted from the incubated soil using the MoBio Power Soil Extraction Kit according to the supplier's instructions. Each 25 µl reaction consisted of 12.5 µl Sybr green mix (Bioline, GC-Biotech) with 4 mg/ml bovine serum albumin (BSA) in a total volume of 25 µl, 5 µM of each primer, 5 µl template DNA (5 ng/µl). For bacteria, the standard curves were generated using 10-fold dilution series from 10^{8} to 10^{3} of

plasmid DNA. PCRs were run on a Rotor-Gene 3000 (Qiagen) and started with 15 min at 95 ^{o}C, followed by 40 amplification cycles each of 95 ^{o}C for 60 sec, 53 ^{o}C 50 sec and 72 ^{o}C 60 sec. A subsample of soil from each bag was stored at -20 ^{o}C for further analysis. Triplicate reaction mixtures per DNA sample and the appropriate set of standards were used. For qPCR assays, there was a linear relationship between the log of the plasmid DNA copy number and the calculated threshold cycle (C_T value). PCR efficiencies were 99%, and correlation coefficients (R^2) for standard curves were 0.99. Bacterial abundance was similar for all dilution treatments after 8 weeks of incubation as determined by quantitative real time PCR (Fig. 2.1). I also measured fungal abundance by quantitative real-time PCR using the primer of 5.8S and internal transcribed spacer 1 (ITS1) genes. For fungi, the standard curves were generated using 10-fold dilution series from 10^8 to 10^3 of plasmid DNA obtained from fungi. Because of the difficulties in assessing fungal abundance by quantitative real-time PCR due to heterogeneity in ribosomal operon number per fungal species/phylum, we decided to ignore the fungal community in the rest of our analyses. The primers we used for pyrosequencing target both bacteria and archaea. There were no significant numbers of archaea sequences; therefore we did not include archaea in our analyses.

2.2.2. DNA extraction, PCR reaction and 16S rRNA gene fragment pyrosequencing

Total DNA was extracted from the soil suspensions and from incubated soil to determine the composition of the respective microbial communities by 454-pyrosequencing. DNA was extracted using the MoBio Power Soil Extraction Kit according to the supplier's manual (MO BIO Laboratories, Carlsbad, CA, USA). Total DNA concentration was qualified on ND-1000 spectrophotometer (Nanodrop Technology, Wilmington, DE). For DNA concentrations below 5 ng/µl, *i.e.* five samples of 10^{-6} and four samples of 10^{-9} suspension, nested PCR was performed. The general bacterial primer 27F and 1492R (Lane 1991) were used for the first

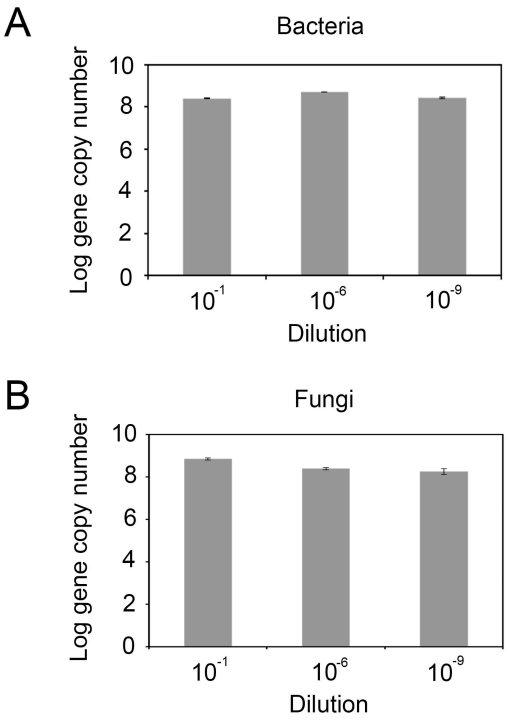

Figure 2.1. Bacterial abundance after incubation among dilution treatments as estimated by real time PCR (Mean ± SE, n=5).

amplification, and then 2 µl of the amplified products from the first round was used as template for the second round PCR using barcoded primers 515F and 806R (Bergmann et al 2011). Five ng/µl of DNA/sample of the diluted samples was used as template for the first round of nested PCR with the PCR program of 95 ^{o}C for 5 min followed by 25 cycles each of 95 ^{o}C for 30 s, 55 ^{o}C 1 min and 72 ^{o}C 10 min. For PCR reactions using barcoded primers were performed using 5 µM of each forward (515F) and reverse (806R) primers, 5 mM dNTPs (Invitrogen, Carlsbad, CA), 1 unit of *Taq* polymerase (Roche, Indianapolis, IN), and 5 ng/µl of sample DNA as the template in a total volume of 25 µl. The PCR conditions for the barcoded primer were similar to the first PCR round except for 25 cycles with 52 ^{o}C annealing temperature. To control for contamination during PCR preparation, one negative control (water in place of DNA) was included for all PCR reactions. PCR products of each subsample from the barcoded primers were generated in six replicates and purified using the Wizard® SV Gel and PCR Clean-Up System (Promega). Equimolar purified

PCR products that were quantified by picogreen assays were mixed and sequenced using Roche Genome Sequencer FLX Titanium 454 sequencing platform (Macrogen, Seoul, South Korea).

2.2.3. Data analysis

The raw sequence data were processed using the QIIME v.1.6.0 pipeline (Caporaso et al 2010). Low quality sequences less than 150 bp in length or with average quality score of less than 25 were removed. After denoising the sequences using Denoiser, version 0.91 (Reeder and Knight 2010), and checking for chimeras using USEARCH, Operational Taxonomic Units (OTUs) were identified using the UCLUST 1.2.21 algorithm (Edgar 2010) with a phylotype defined at the 97% sequence similarity level. The resulting OTUs were aligned against the Ribosomal Database Project (RDP) database (Cole et al 2009).

Alpha diversity calculation was performed based on the rarefied OTU table to compare the diversity among samples at the given level of a sampling effort (Hughes and Hellmann 2005). The OTU table was rarefied to 1,535 reads by "single rarefaction" QIIME script since this number was the lowest number of reads for all samples. The average reads from the three sterilized controls were used as baseline that was subtracted from the reads of the other 36 samples. The OTU table after subtraction of the control was used for further statistical analysis. Chao1 richness and Simpson and Shannon diversity and evenness indices were determined with the "vegan" package (Dixon 2003) in R (The R Foundation for Statistical Computing). The percentage of coverage was calculated by Good's method using the following formula: $\% = [1-(n/N)] \times 100$, where n means the number of phylotypes represented by singletons and N is the total number of sequences (Good 1953).

To compare the communities from the different dilution treatments, Nonmetric MultiDimensional Scaling (NMDS) plots were used to visualize the structure among samples at genus level. The plots were generated from Bray-Curtis similarity index matrices of all samples. NMDS was calculated by using the PAST software (Hammer et al 2001).

2.3. Results

2.3.1. Effect of dilution and incubation on bacterial community diversity

Several indices were used to assess the diversity in the soil suspension dilutions and in the associated soil communities after incubation on the basis of OTU detection (Table 2.1). Remarkably, all indices for the diluted inocula of 10^{-6} and 10^{-9} were significantly higher after incubation than the indices of the associated suspensions, while the indices were lower for the 10^{-1} dilution after incubation in soil. Good's estimator of coverage increased with increasing dilution, indicating that microbial species were lost through dilution.

Table 2.1. Estimators of sequence library diversity, evenness and coverage in soil suspensions at three dilutions and the related samples after incubation in soil.

Treatment	Dilution	S.obs	S.chao-1	Shannon	Simpson	Evenness	Good's estimator of coverage
Suspension	10^{-1}	131.00±3.27	169.15±7.80	3.986±0.036	0.966±0.002	0.41±0.01	97.61±0.12
Soil	10^{-1}	107.20±1.27	134.37±2.96	3.719±0.019	0.954±0.002	0.38±0.01	97.56±0.11
P		*	*	*	*	*	
Suspension	10^{-6}	44.80±7.98	53.09±10.33	2.383±0.416	0.774±0.124	0.24±0.07	99.32±0.18
Soil	10^{-6}	70.09±2.13	89.64±4.46	3.208±0.040	0.934±0.004	0.35±0.01	97.95±0.21
P		*	*	*	NS	*	
Suspension	10^{-9}	17.00±2.17	19.54±2.46	1.462±0.293	0.623±0.128	0.25±0.05	99.77±0.03
Soil	10^{-9}	55.83±1.14	81.82±3.37	2.633±0.042	0.867±0.006	0.25±0.01	97.27±0.24
P		*	*	*	*	NS	

Estimators were calculated for each dilution treatment of soil suspensions (n = 5-6) and incubated soil samples (n = 11-12) as well as significant comparisons ($P < 0.05$) among phylogenetic profile (species level). S.obs is the observed number of OTUs. NS means not significant.

2.3.2. Effect of dilution and incubation on bacterial community composition

After the OTUs were classified according to the RDP database, the soil microbial community consisted of 18 phyla (Fig. 2.2). Phylum-level taxonomic assignments indicated that *Proteobacteria*, followed by *Actinobacteria*, *Bacteroidetes*, *Acidobacteria*, *Verrucomicrobia*, *Planctomycetes* and *Firmicutes* dominated the microbial communities in the original non-diluted (10^{-1}) soil suspension (> 90% of all sequences). The variance in the abundance of the seven dominating phyla among the replicated suspension samples increased from the low-dilution treatments to the high dilution treatments (Table 2.2 and

Fig. 2.3). The same was true for the incubated samples while in general the variance among the replicates of the incubated samples was lower than the variance among the replicated samples of the soil suspensions (Table 2.2 and Fig. 2.4).

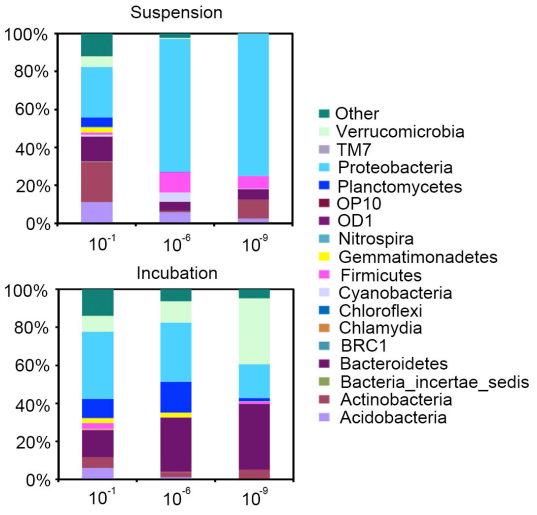

Figure 2.2. Bacterial community composition at phylum level of soil suspensions and incubated soil samples in relative abundances.

To test the selective power of soil further, we analyzed the major phyla at the family level. Visually, we noticed that the diversity of the communities in the incubated soil samples, and in particular, those which were incubated with the highest, i.e. 10^{-9} dilution, suspensions differed strongly from the diversity of the inoculated suspension (Fig. 2.5). Thus, we compared the diversity of the communities in both the suspensions and the inoculated soil samples. Remarkably, for most phyla we found that the Shannon diversity index was significantly higher in the incubated soil samples than in the corresponding suspensions of the 10^{-6} and 10^{-9} dilution (Table 2.3), while they were lower in the soil samples than in the associated suspensions at the 10^{-1} dilutions.

Table 2.2. Coefficients of variation (%) for each phylum measured in soil suspensions at the three dilution levels and in related soil samples after incubation

	Suspension			Soil		
Dilution	10^{-1}	10^{-6}	10^{-9}	10^{-1}	10^{-6}	10^{-9}
Proteobacteria	13.94	46.66	26.66	6.05	20.73	50.69
Actinobacteria	24.95	146.26	205.76	10.97	60.29	77.59
Bacteroidetes	10.03	107.73	192.35	20.30	20.24	31.30
Acidobacteria	29.84	67.97	222.38	13.31	43.77	65.31
Verrucomicrobia	39.95	188.99	223.61	26.50	28.35	43.43
Planctomycetes	39.94	121.61	-	24.56	32.27	107.52
Firmicutes	14.97	64.43	135.96	31.49	109.87	96.17

The table depicts the coefficient of variation (CV) of each phylum based on absolute reads in soil suspensions and incubated soil samples. CV (%) = Standard deviation/mean*100. "-" data are not present.

Table 2.3. Shannon diversity of major phyla

Phylum	Suspension	Soil	*P*	Suspension	Soil	*P*	Suspension	Soil	*P*
	10^{-1}			10^{-6}			10^{-9}		
Acidobacteria	1.60±0.04	1.16±0.04	*	0.73±0.17	0.85±0.07	NS	0.00±0.00	0.54±0.15	*
Actinobacteria	2.49±0.03	2.34±0.03	*	0.96±0.25	1.78±0.07	*	0.01±0.01	1.46±0.16	*
Bacteroidetes	1.49±0.05	1.29±0.04	*	0.61±0.18	1.27±0.06	*	0.00±0.00	1.16±0.07	*
Firmicutes	1.17±0.05	1.04±0.04	NS	0.94±0.27	0.23±0.12	*	0.22±0.19	0.52±0.11	NS
Verrucomicrobia	1.15±0.07	1.23±0.03	NS	0.07±0.07	0.96±0.06	*	0.00±0.00	0.81±0.07	*
Alphaproteobacteria	1.95±0.01	1.88±0.02	NS	1.05±0.19	1.69±0.04	*	0.66±0.07	1.37±0.12	*
Betaproteobacteria	1.39±0.02	1.50±0.03	NS	0.65±0.21	0.75±0.14	NS	0.41±0.12	0.91±0.08	*
Deltaproteobacteria	1.16±0.03	1.31±0.08	NS	0.02±0.02	0.78±0.13	*	0.00±0.00	0.74±0.09	*
Gammaproteobacteria	1.18±0.02	0.94±0.04	*	0.79±0.09	0.95±0.07	NS	0.33±0.16	0.47±0.11	NS

Diversity was calculated for each dilution of soil suspensions (n = 5-6) and incubated soil samples (n = 11-12) as well as the level of significance ($P < 0.05$) for each major phylum based on the phylogenetic profile at the family level. NS means not significant.

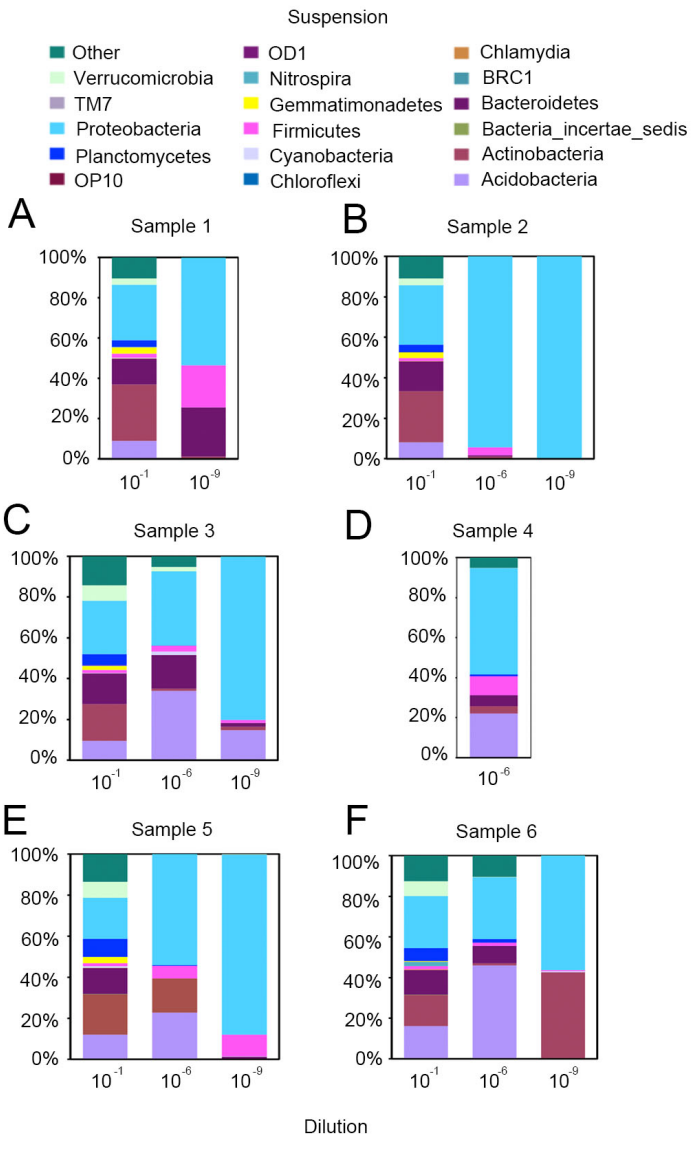

Figure 2.3. Bacterial community composition at phylum level from six samples of soil suspensions. Dilution level is shown below each bar. 10^{-1} and 10^{-9} dilutions of sample 4 are not available due to technical issues.

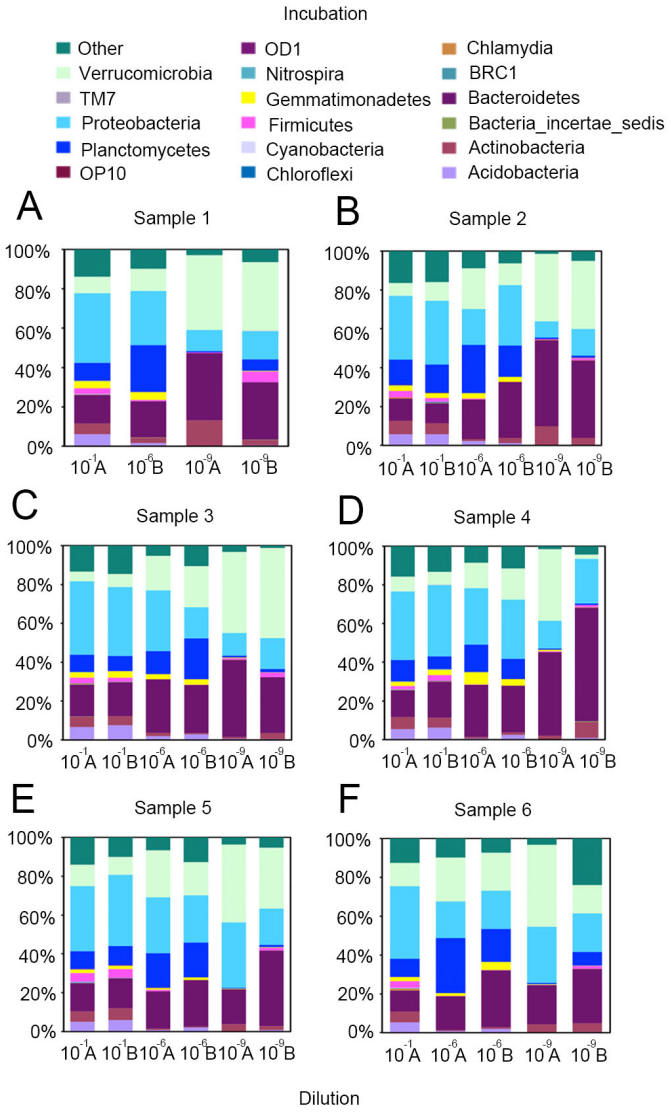

Figure 2.4. Bacterial community composition at phylum level of six replicate samples of the incubated soil. Dilution level is shown below each bar. A, B indicates the duplicates from the same dilution level. 10^{-1}B, 10^{-6}A of sample 1 and 10^{-1}B of sample 6 are not available due to technical issues.

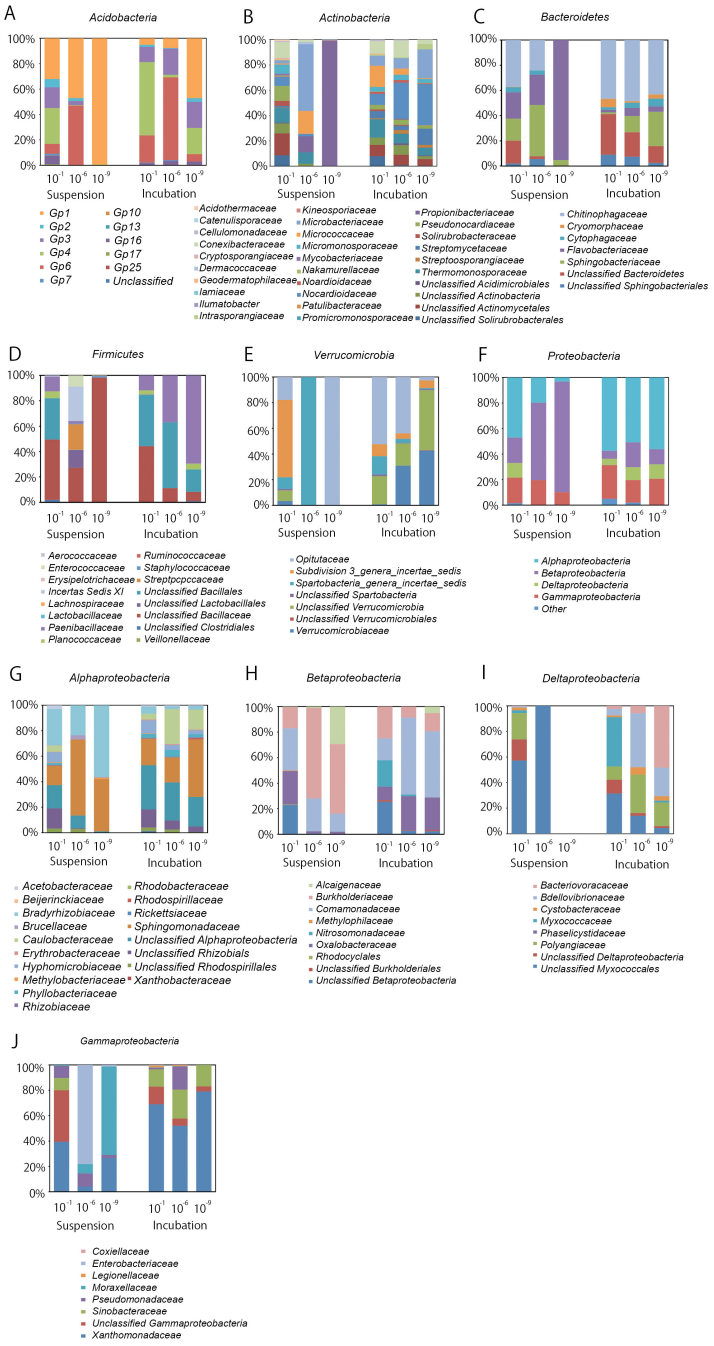

Figure 2.5. Bacterial community composition at the family level of soil suspensions and incubated soil samples.

To compare the overall community structure of the different dilution treatments and differences before/after incubation, the taxonomic abundance profiles were used to compute Bray-Curtis similarity matrix, coordinated into two dimensions by using NMDS (Fig. 2.6). Samples were grouped according to before/after incubation. This analysis revealed clear differences in the microbial community structure between before and after incubation. The community structures of the soil samples after incubation were more similar to each other than to the associated suspension samples. This may hint to selective processes in the soil leading to more equal communities. One-way analysis of similarities (ANOSIM) showed that the dilution treatment had a significant ($R = 0.28$, $P < 0.001$) overall effect on the structure of the bacterial community in the suspension and the soil samples after incubation.

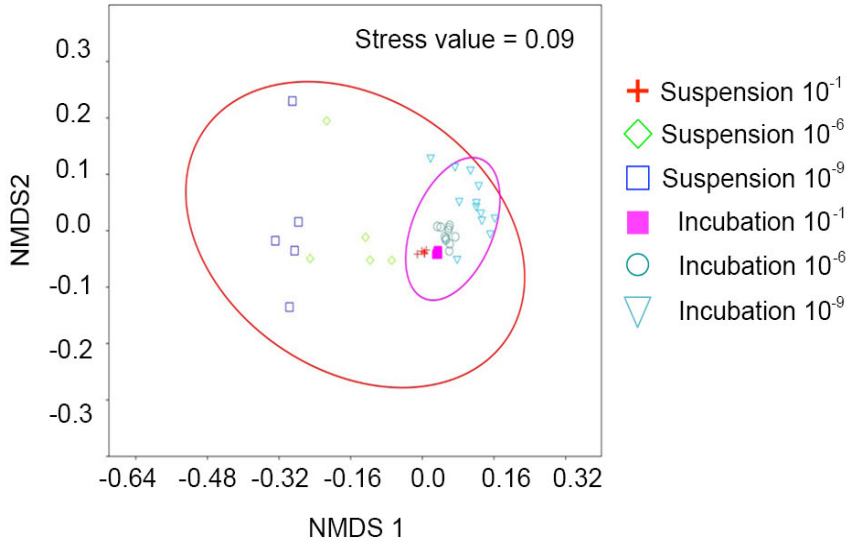

Figure 2.6. NMDS of Bray-Curtis similarity matrix among soil suspensions and incubated soil samples.

2.3.3. Effect of dilution on rare/abundant OTUs

A possibility to determine if the dilution approach is appropriate to separate rare species from abundant ones is to make Venn diagrams to assess the shared and

unique OTUs between dilution treatments in the soil suspensions (Fig. 2.7) and incubated soil (Fig. 2.7). We found 954, 77 and 10 unique OTUs in the 10^{-1}, 10^{-6} and 10^{-9} dilution samples of the soil suspensions, respectively, and 386, 96 and 88 unique OTUs in the respective dilution treatments of the incubated soil samples. To identify the unique OTUs in the different treatments, the phylogenetic affiliation was done at the genus level. From the unique OTUs that were assigned to the genus, a total of 158, 38, 10 unique genera were detected in 10^{-1}, 10^{-6} and 10^{-9} dilutions of soil suspensions, respectively (Table 2.4) and 84, 33 and 34 unique genera were detected in 10^{-1}, 10^{-6} and 10^{-9} of the incubated soil samples, respectively (Table 2.5).

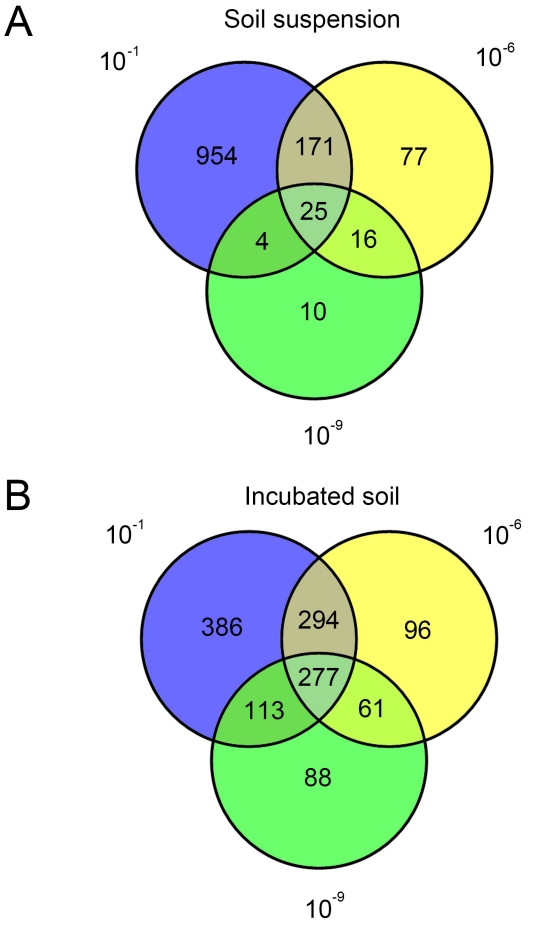

Figure 2.7. Venn diagram of shared and unique OTUs in each dilution of (A) soil suspensions and (B) incubated soil samples.

Table 2.4. The phylogenetic affiliation of the unique OTUs in the six replicate samples of the three dilutions of a soil suspension.

Dilution	Phylum	Class	Order	Family	Genus	Abundance	SE
10^-1	Other	Other	Other	Other	Other	0.0984	0.0083
10^-1	Acidobacteria	Other	Other	Other	Other	0.0003	0.0003
10^-1	Acidobacteria	Acidobacteria_Gp1	Gp1	Other	Other	0.0063	0.002
10^-1	Acidobacteria	Acidobacteria_Gp10	Gp10	Other	Other	0.0001	0.0001
10^-1	Acidobacteria	Acidobacteria_Gp13	Gp13	Other	Other	0.0001	0.0001
10^-1	Acidobacteria	Acidobacteria_Gp16	Gp16	Other	Other	0.0005	0.0005
10^-1	Acidobacteria	Acidobacteria_Gp17	Gp17	Other	Other	0.0007	0.0002
10^-1	Acidobacteria	Acidobacteria_Gp2	Gp2	Other	Other	0.0012	0.0005
10^-1	Acidobacteria	Acidobacteria_Gp3	Gp3	Other	Other	0.007	0.0011
10^-1	Acidobacteria	Acidobacteria_Gp4	Gp4	Other	Other	0.0081	0.0044
10^-1	Acidobacteria	Acidobacteria_Gp6	Gp6	Other	Other	0.0054	0.0017
10^-1	Acidobacteria	Acidobacteria_Gp7	Gp7	Other	Other	0.0007	0.0003
10^-1	Actinobacteria	Actinobacteria	Other	Other	Other	0.0093	0.0006
10^-1	Actinobacteria	Actinobacteria	Acidimicrobiales	Other	Other	0.0013	0.0004
10^-1	Actinobacteria	Actinobacteria	Actinomycetales	Other	Other	0.02	0.0032
10^-1	Actinobacteria	Actinobacteria	Actinomycetales	Acid Othermaceae	Acid Othermus	0.0022	0.0007
10^-1	Actinobacteria	Actinobacteria	Actinomycetales	Catenulisporaceae	Catenulispora	0.0004	0.0003
10^-1	Actinobacteria	Actinobacteria	Actinomycetales	Cellulomonadaceae	Cellulomonas	0.0014	0.0005
10^-1	Actinobacteria	Actinobacteria	Actinomycetales	Cryptosporangiaceae	Cryptosporangium	0.0013	0.0002
10^-1	Actinobacteria	Actinobacteria	Actinomycetales	Geodermatophilaceae	Other	0.0013	0.0005
10^-1	Actinobacteria	Actinobacteria	Actinomycetales	Geodermatophilaceae	Modestobacter	0.0001	0.0001
10^-1	Actinobacteria	Actinobacteria	Actinomycetales	Intrasporangiaceae	Phycicoccus	0.0009	0.0003
10^-1	Actinobacteria	Actinobacteria	Actinomycetales	Kineosporiaceae	Other	0.0004	0.0003
10^-1	Actinobacteria	Actinobacteria	Actinomycetales	Microbacteriaceae	Other	0.005	0.0011
10^-1	Actinobacteria	Actinobacteria	Actinomycetales	Microbacteriaceae	Leucobacter	0.0001	0.0001
10^-1	Actinobacteria	Actinobacteria	Actinomycetales	Micromonosporaceae	Other	0.0134	0.0016
10^-1	Actinobacteria	Actinobacteria	Actinomycetales	Micromonosporaceae	Actinoplanes	0.0005	0.0002
10^-1	Actinobacteria	Actinobacteria	Actinomycetales	Micromonosporaceae	Rugosimonospora	0.0014	0.0007
10^-1	Actinobacteria	Actinobacteria	Actinomycetales	Nakamurellaceae	Humicoccus	0.0016	0.0005
10^-1	Actinobacteria	Actinobacteria	Actinomycetales	Nocardiaceae	Nocardia	0.0001	0.0001
10^-1	Actinobacteria	Actinobacteria	Actinomycetales	Nocardiaceae	Rhodococcus	0.0003	0.0003
10^-1	Actinobacteria	Actinobacteria	Actinomycetales	Nocardioidaceae	Other	0.0017	0.0004
10^-1	Actinobacteria	Actinobacteria	Actinomycetales	Nocardioidaceae	Actinopolymorpha	0.0003	0.0002
10^-1	Actinobacteria	Actinobacteria	Actinomycetales	Nocardioidaceae	Aeromicrobium	0.0036	0.0006

Dilution	Phylum	Class	Order	Family	Genus	Abundance	SE
10^-1	Actinobacteria	Actinobacteria	Actinomycetales	Nocardioidaceae	Kribbella	0.0046	0.0012
10^-1	Actinobacteria	Actinobacteria	Actinomycetales	Nocardioidaceae	Nocardioides	0.0009	0.0004
10^-1	Actinobacteria	Actinobacteria	Actinomycetales	Promicromonosporaceae	Promicromonospora	0.0004	0.0003
10^-1	Actinobacteria	Actinobacteria	Actinomycetales	Pseudonocardiaceae	Other	0.0042	0.0008
10^-1	Actinobacteria	Actinobacteria	Actinomycetales	Pseudonocardiaceae	Amycolatopsis	0.0096	0.0019
10^-1	Actinobacteria	Actinobacteria	Actinomycetales	Pseudonocardiaceae	Kutzneria	0.0008	0.0003
10^-1	Actinobacteria	Actinobacteria	Actinomycetales	Pseudonocardiaceae	Pseudonocardia	0.0104	0.003
10^-1	Actinobacteria	Actinobacteria	Actinomycetales	Streptosporangiaceae	Other	0.0007	0.0004
10^-1	Actinobacteria	Actinobacteria	Actinomycetales	Streptosporangiaceae	Nonomuraea	0.0004	0.0002
10^-1	Actinobacteria	Actinobacteria	Actinomycetales	Streptosporangiaceae	Streptosporangium	0.0001	0.0001
10^-1	Actinobacteria	Actinobacteria	Actinomycetales	Thermomonosporaceae	Other	0.0154	0.0012
10^-1	Actinobacteria	Actinobacteria	Actinomycetales	Thermomonosporaceae	Actinoallomurus	0.0091	0.0013
10^-1	Actinobacteria	Actinobacteria	Solirubrobacterales	Other	Other	0.0043	0.0005
10^-1	Actinobacteria	Actinobacteria	Solirubrobacterales	Conexibacteraceae	Conexibacter	0.0067	0.0014
10^-1	Actinobacteria	Actinobacteria	Solirubrobacterales	Solirubrobacteraceae	Solirubrobacter	0.0062	0.0023
10^-1	Bacteria_incertae_sedis	Ktedonobacteria	Ktedonobacterales	Ktedonobacteraceae	Ktedonobacter	0.0004	0.0002
10^-1	Bacteroidetes	Other	Other	Other	Other	0.0149	0.0025
10^-1	Bacteroidetes	Flavobacteria	Flavobacteriales	Cryomorphaceae	Fluviicola	0.0003	0.0002
10^-1	Bacteroidetes	Flavobacteria	Flavobacteriales	Flavobacteriaceae	Other	0.0004	0.0003
10^-1	Bacteroidetes	Flavobacteria	Flavobacteriales	Flavobacteriaceae	Chryseobacterium	0.0003	0.0002
10^-1	Bacteroidetes	Flavobacteria	Flavobacteriales	Flavobacteriaceae	Flavobacterium	0.002	0.0007
10^-1	Bacteroidetes	Sphingobacteria	Sphingobacteriales	Other	Other	0.0022	0.0004
10^-1	Bacteroidetes	Sphingobacteria	Sphingobacteriales	Chitinophagaceae	Other	0.0148	0.0019
10^-1	Bacteroidetes	Sphingobacteria	Sphingobacteriales	Chitinophagaceae	Ferruginibacter	0.0012	0.001
10^-1	Bacteroidetes	Sphingobacteria	Sphingobacteriales	Chitinophagaceae	Flavisolibacter	0.0003	0.0002
10^-1	Bacteroidetes	Sphingobacteria	Sphingobacteriales	Chitinophagaceae	Sediminibacterium	0.0032	0.0006
10^-1	Bacteroidetes	Sphingobacteria	Sphingobacteriales	Chitinophagaceae	Terrimonas	0.0012	0.0003
10^-1	Bacteroidetes	Sphingobacteria	Sphingobacteriales	Cytophagaceae	Other	0.0003	0.0002
10^-1	Bacteroidetes	Sphingobacteria	Sphingobacteriales	Cytophagaceae	Cytophaga	0.0041	0.0012
10^-1	Bacteroidetes	Sphingobacteria	Sphingobacteriales	Cytophagaceae	Dyadobacter	0.0005	0.0002
10^-1	Bacteroidetes	Sphingobacteria	Sphingobacteriales	Cytophagaceae	Spirosoma	0.0005	0.0004
10^-1	Bacteroidetes	Sphingobacteria	Sphingobacteriales	Sphingobacteriaceae	Other	0.0001	0.0001
10^-1	Bacteroidetes	Sphingobacteria	Sphingobacteriales	Sphingobacteriaceae	Mucilaginibacter	0.0014	0.0004
10^-1	Bacteroidetes	Sphingobacteria	Sphingobacteriales	Sphingobacteriaceae	Pedobacter	0.0004	0.0003

Dilution	Phylum	Class	Order	Family	Genus	Abundance	SE
10^-1	BRC1	BRC1_genera_incertae_sedis	Other	Other	Other	0.0003	0.0002
10^-1	Chlamydiae	Chlamydiae	Chlamydiales	Other	Other	0.0008	0.0003
10^-1	Chlamydiae	Chlamydiae	Chlamydiales	Parachlamydiaceae	Other	0.0004	0.0002
10^-1	Chlamydiae	Chlamydiae	Chlamydiales	Parachlamydiaceae	Neochlamydia	0.0001	0.0001
10^-1	Chlamydiae	Chlamydiae	Chlamydiales	Simkaniaceae	Simkania	0.0003	0.0002
10^-1	Chloroflexi	Other	Other	Other	Other	0.0001	0.0001
10^-1	Chloroflexi	Anaerolineae	Anaerolineales	Anaerolineaceae	Other	0.0004	0.0002
10^-1	Cyanobacteria	Cyanobacteria	Chloroplast	Bacillariophyta	Other	0.0001	0.0001
10^-1	Cyanobacteria	Cyanobacteria	Chloroplast	Chlorophyta	Other	0.0004	0.0003
10^-1	Cyanobacteria	Cyanobacteria	Family I	GpI	Other	0.0001	0.0001
10^-1	Cyanobacteria	Cyanobacteria	Family V	GpV	Other	0.0001	0.0001
10^-1	Firmicutes	Bacilli	Bacillales	Other	Other	0.0009	0.0002
10^-1	Firmicutes	Bacilli	Bacillales	Bacillaceae	Bacillus	0.0003	0.0002
10^-1	Firmicutes	Bacilli	Bacillales	Bacillaceae	Oceanobacillus	0.0001	0.0001
10^-1	Firmicutes	Bacilli	Bacillales	Paenibacillaceae	Other	0.0001	0.0001
10^-1	Firmicutes	Bacilli	Bacillales	Paenibacillaceae	Ammoniphilus	0.0001	0.0001
10^-1	Firmicutes	Bacilli	Bacillales	Paenibacillaceae	Cohnella	0.0003	0.0002
10^-1	Firmicutes	Bacilli	Bacillales	Paenibacillaceae	Paenibacillus	0.0011	0.0004
10^-1	Firmicutes	Clostridia	Clostridiales	Other	Other	0.0003	0.0002
10^-1	Gemmatimonadetes	Gemmatimonadetes	Gemmatimonadales	Gemmatimonadaceae	Gemmatimonas	0.0261	0.0015
10^-1	Nitrospira	Nitrospira	Nitrospirales	Nitrospiraceae	Nitrospira	0.0029	0.0005
10^-1	OD1	OD1_genera_incertae_sedis	Other	Other	Other	0.0001	0.0001
10^-1	OP10	OP10_genera_incertae_sedis	Other	Other	Other	0.0005	0.0002
10^-1	Planctomycetes	Planctomycetacia	Planctomycetales	Planctomycetaceae	Other	0.0345	0.0077
10^-1	Planctomycetes	Planctomycetacia	Planctomycetales	Planctomycetaceae	Blastopirellula	0.0001	0.0001
10^-1	Planctomycetes	Planctomycetacia	Planctomycetales	Planctomycetaceae	Gemmata	0.0033	0.0014
10^-1	Planctomycetes	Planctomycetacia	Planctomycetales	Planctomycetaceae	Pirellula	0.0044	0.0011
10^-1	Planctomycetes	Planctomycetacia	Planctomycetales	Planctomycetaceae	Planctomyces	0.0005	0.0002
10^-1	Planctomycetes	Planctomycetacia	Planctomycetales	Planctomycetaceae	Schlesneria	0.0005	0.0002
10^-1	Planctomycetes	Planctomycetacia	Planctomycetales	Planctomycetaceae	Singulisphaera	0.0032	0.0013
10^-1	Planctomycetes	Planctomycetacia	Planctomycetales	Planctomycetaceae	Zavarzinella	0.002	0.0006
10^-1	Proteobacteria	Other	Other	Other	Other	0.0037	0.0006
10^-1	Proteobacteria	Alphaproteobacteria	Other	Other	Other	0.0173	0.0009
10^-1	Proteobacteria	Alphaproteobacteria	Caulobacterales	Caulobacteraceae	Other	0.0001	0.0001

Dilution	Phylum	Class	Order	Family	Genus	Abundance	SE
10^-1	Proteobacteria	Alphaproteobacteria	Caulobacterales	Caulobacteraceae	Asticcacaulis	0.0005	0.0002
10^-1	Proteobacteria	Alphaproteobacteria	Caulobacterales	Caulobacteraceae	Caulobacter	0.0008	0.0004
10^-1	Proteobacteria	Alphaproteobacteria	Caulobacterales	Caulobacteraceae	Phenylobacterium	0.0041	0.0011
10^-1	Proteobacteria	Alphaproteobacteria	Rhizobiales	Other	Other	0.008	0.0012
10^-1	Proteobacteria	Alphaproteobacteria	Rhizobiales	Hyphomicrobiaceae	Other	0.0025	0.0006
10^-1	Proteobacteria	Alphaproteobacteria	Rhizobiales	Hyphomicrobiaceae	Devosia	0.0001	0.0001
10^-1	Proteobacteria	Alphaproteobacteria	Rhizobiales	Hyphomicrobiaceae	Hyphomicrobium	0.0016	0.0006
10^-1	Proteobacteria	Alphaproteobacteria	Rhizobiales	Hyphomicrobiaceae	Prosthecomicrobium	0.0001	0.0001
10^-1	Proteobacteria	Alphaproteobacteria	Rhizobiales	Methylobacteriaceae	Methylobacterium	0.0003	0.0003
10^-1	Proteobacteria	Alphaproteobacteria	Rhizobiales	Methylobacteriaceae	Microvirga	0.0004	0.0003
10^-1	Proteobacteria	Alphaproteobacteria	Rhizobiales	Phyllobacteriaceae	Mesorhizobium	0.0008	0.0004
10^-1	Proteobacteria	Alphaproteobacteria	Rhizobiales	Rhizobiaceae	Rhizobium	0.0003	0.0002
10^-1	Proteobacteria	Alphaproteobacteria	Rhizobiales	Xanthobacteraceae	Other	0.0001	0.0001
10^-1	Proteobacteria	Alphaproteobacteria	Rhizobiales	Xanthobacteraceae	Labrys	0.0004	0.0003
10^-1	Proteobacteria	Alphaproteobacteria	Rhodospirillales	Other	Other	0.0037	0.0009
10^-1	Proteobacteria	Alphaproteobacteria	Rhodospirillales	Acetobacteraceae	Other	0.0022	0.0004
10^-1	Proteobacteria	Alphaproteobacteria	Rhodospirillales	Acetobacteraceae	Acidisoma	0.0005	0.0001
10^-1	Proteobacteria	Alphaproteobacteria	Rhodospirillales	Acetobacteraceae	Roseomonas	0.0001	0.0001
10^-1	Proteobacteria	Alphaproteobacteria	Rhodospirillales	Acetobacteraceae	Stella	0.0001	0.0001
10^-1	Proteobacteria	Alphaproteobacteria	Rhodospirillales	Rhodospirillaceae	Other	0.0004	0.0003
10^-1	Proteobacteria	Alphaproteobacteria	Rhodospirillales	Rhodospirillaceae	Inquilinus	0.0001	0.0001
10^-1	Proteobacteria	Alphaproteobacteria	Sphingomonadales	Erythrobacteraceae	Other	0.0003	0.0002
10^-1	Proteobacteria	Alphaproteobacteria	Sphingomonadales	Erythrobacteraceae	Porphyrobacter	0.0001	0.0001
10^-1	Proteobacteria	Alphaproteobacteria	Sphingomonadales	Sphingomonadaceae	Other	0.0013	0.0006
10^-1	Proteobacteria	Alphaproteobacteria	Sphingomonadales	Sphingomonadaceae	Sphingomonas	0.0005	0.0003
10^-1	Proteobacteria	Betaproteobacteria	Other	Other	Other	0.004	0.0008
10^-1	Proteobacteria	Betaproteobacteria	Burkholderiales	Burkholderiaceae	Burkholderia	0.0007	0.0003
10^-1	Proteobacteria	Betaproteobacteria	Burkholderiales	Comamonadaceae	Other	0.0034	0.0008
10^-1	Proteobacteria	Betaproteobacteria	Burkholderiales	Oxalobacteraceae	Other	0.0003	0.0002
10^-1	Proteobacteria	Betaproteobacteria	Methylophilales	Methylophilaceae	Methylophilus	0.0003	0.0002
10^-1	Proteobacteria	Betaproteobacteria	Rhodocyclales	Rhodocyclaceae	Other	0.0001	0.0001
10^-1	Proteobacteria	Deltaproteobacteria	Other	Other	Other	0.005	0.0012
10^-1	Proteobacteria	Deltaproteobacteria	Bdellovibrionales	Bacteriovoracaceae	Peredibacter	0.0001	0.0001
10^-1	Proteobacteria	Deltaproteobacteria	Bdellovibrionales	Bdellovibrionaceae	Bdellovibrio	0.0003	0.0002

Dilution	Phylum	Class	Order	Family	Genus	Abundance	SE
10^-1	Proteobacteria	Deltaproteobacteria	Myxococcales	Other	Other	0.0062	0.0013
10^-1	Proteobacteria	Deltaproteobacteria	Myxococcales	Cystobacteraceae	Other	0.0007	0.0002
10^-1	Proteobacteria	Deltaproteobacteria	Myxococcales	Myxococcaceae	Corallococcus	0.0007	0
10^-1	Proteobacteria	Deltaproteobacteria	Myxococcales	Polyangiaceae	Other	0.0034	0.0005
10^-1	Proteobacteria	Deltaproteobacteria	Myxococcales	Polyangiaceae	Byssovorax	0.0017	0.0002
10^-1	Proteobacteria	Deltaproteobacteria	Myxococcales	Polyangiaceae	Chondromyces	0.0011	0.0002
10^-1	Proteobacteria	Gammaproteobacteria	Other	Other	Other	0.0034	0.0009
10^-1	Proteobacteria	Gammaproteobacteria	Pseudomonadales	Moraxellaceae	Alkanindiges	0.0004	0.0003
10^-1	Proteobacteria	Gammaproteobacteria	Pseudomonadales	Pseudomonadaceae	Cellvibrio	0.0008	0.0006
10^-1	Proteobacteria	Gammaproteobacteria	Xanthomonadales	Sinobacteraceae	Alkanibacter	0.0011	0.0003
10^-1	Proteobacteria	Gammaproteobacteria	Xanthomonadales	Sinobacteraceae	Nevskia	0.0003	0.0003
10^-1	Proteobacteria	Gammaproteobacteria	Xanthomonadales	Sinobacteraceae	Steroidobacter	0.0011	0.0002
10^-1	Proteobacteria	Gammaproteobacteria	Xanthomonadales	Xanthomonadaceae	Other	0.0092	0.0022
10^-1	Verrucomicrobia	Other	Other	Other	Other	0.0047	0.0007
10^-1	Verrucomicrobia	Opitutae	Opitutales	Opitutaceae	Other	0.0018	0.0007
10^-1	Verrucomicrobia	Opitutae	Opitutales	Opitutaceae	Opitutus	0.0084	0.0021
10^-1	Verrucomicrobia	Spartobacteria	Other	Other	Other	0.0007	0.0003
10^-1	Verrucomicrobia	Spartobacteria	Spartobacteria_genera_incertae_sedis	Other	Other	0.0061	0.0011
10^-1	Verrucomicrobia	Subdivision3	Subdivision3_genera_incertae_sedis	Other	Other	0.005	0.0006
10^-1	Verrucomicrobia	Verrucomicrobiae	Verrucomicrobiales	Verrucomicrobiaceae	Other	0.0013	0.0003
10^-1	Verrucomicrobia	Verrucomicrobiae	Verrucomicrobiales	Verrucomicrobiaceae	Prosthecobacter	0.0005	0.0001
10^-1	Verrucomicrobia	Verrucomicrobiae	Verrucomicrobiales	Verrucomicrobiaceae	Verrucomicrobium	0.0001	0.0001
10^-6	Other	Other	Other	Other	Other	0.0258	0.0151
10^-6	Acidobacteria	Other	Other	Other	Other	0.0001	0.0001
10^-6	Acidobacteria	Acidobacteria_Gp1	Gp1	Other	Other	0.0022	0.0022
10^-6	Acidobacteria	Acidobacteria_Gp3	Gp3	Other	Other	0.0013	0.0008
10^-6	Acidobacteria	Acidobacteria_Gp4	Gp4	Other	Other	0.0014	0.0009
10^-6	Acidobacteria	Acidobacteria_Gp6	Gp6	Other	Other	0.0007	0.0005
10^-6	Bacteroidetes	Other	Other	Other	Other	0.0026	0.0026
10^-6	Bacteroidetes	Flavobacteria	Flavobacteriales	Flavobacteriaceae	Flavobacterium	0.0001	0.0001
10^-6	Bacteroidetes	Sphingobacteria	Sphingobacteriales	Chitinophagaceae	Other	0.006	0.006
10^-6	Bacteroidetes	Sphingobacteria	Sphingobacteriales	Sphingobacteriaceae	Pedobacter	0.0001	0.0001
10^-6	Cyanobacteria	Cyanobacteria	Chloroplast	Bacillariophyta	Other	0.0025	0.0025
10^-6	Firmicutes	Bacilli	Bacillales	Paenibacillaceae	Paenibacillus	0.0022	0.0022
10^-6	Firmicutes	Bacilli	Lactobacillales	Other	Other	0.0004	0.0004

Table 2.5. The phylogenetic affiliation of the unique OTUs in the soil samples after incubation of three dilutions of the soil suspension dilutions of a soil suspension.

	Phylum	Class	Order	Family	Genus	Abundance	SE
10^-6	Firmicutes	Bacilli	Lactobacillales	Aerococcaceae	Aerococcus	0.0003	0.0003
10^-6	Firmicutes	Bacilli	Lactobacillales	Lactobacillaceae	Lactobacillus	0.0013	0.0011
10^-6	Firmicutes	Bacilli	Lactobacillales	Streptococcaceae	Lactococcus	0.0014	0.0014
10^-6	Firmicutes	Bacilli	Lactobacillales	Streptococcaceae	Streptococcus	0.0007	0.0007
10^-6	Firmicutes	Clostridia	Clostridiales	Veillonellaceae	Veillonella	0.0003	0.0003
10^-6	Planctomycetes	Planctomycetacia	Planctomycetales	Planctomycetaceae	Other	0.0007	0.0007
10^-6	Planctomycetes	Planctomycetacia	Planctomycetales	Planctomycetaceae	Pirellula	0.0017	0.0017
10^-6	Planctomycetes	Planctomycetacia	Planctomycetales	Planctomycetaceae	Singulisphaera	0.0004	0.0004
10^-6	Proteobacteria	Other	Other	Other	Other	0.0033	0.0031
10^-6	Proteobacteria	Alphaproteobacteria	Other	Other	Other	0.0001	0.0001
10^-6	Proteobacteria	Alphaproteobacteria	Rhizobiales	Other	Other	0.0005	0.0005
10^-6	Proteobacteria	Alphaproteobacteria	Rhizobiales	Bradyrhizobiaceae	Other	0.0004	0.0004
10^-6	Proteobacteria	Alphaproteobacteria	Rhizobiales	Brucellaceae	Pseudochrobactrum	0.0003	0.0003
10^-6	Proteobacteria	Alphaproteobacteria	Rhizobiales	Rhizobiaceae	Rhizobium	0.0001	0.0001
10^-6	Proteobacteria	Alphaproteobacteria	Rhodospirillales	Acetobacteraceae	Other	0.001	0.001
10^-6	Proteobacteria	Betaproteobacteria	Burkholderiales	Alcaligenaceae	Other	0.0004	0.0003
10^-6	Proteobacteria	Betaproteobacteria	Burkholderiales	Oxalobacteraceae	Janthinobacterium	0.0001	0.0001
10^-6	Proteobacteria	Deltaproteobacteria	Other	Other	Other	0.0001	0.0001
10^-6	Proteobacteria	Gammaproteobacteria	Other	Other	Other	0.0012	0.0005
10^-6	Proteobacteria	Gammaproteobacteria	Enterobacteriales	Enterobacteriaceae	Other	0.0005	0.0005
10^-6	Proteobacteria	Gammaproteobacteria	Pseudomonadales	Pseudomonadaceae	Other	0.0001	0.0001
10^-6	Proteobacteria	Gammaproteobacteria	Xanthomonadales	Xanthomonadaceae	Dyella	0.0001	0.0001
10^-6	Proteobacteria	Gammaproteobacteria	Xanthomonadales	Xanthomonadaceae	Stenotrophomonas	0.0004	0.0004
10^-6	TM7	TM7_genera_incertae_sedis	Other	Other	Other	0.0001	0.0001
10^-6	Verrucomicrobia	Spartobacteria	Spartobacteria_genera_incertae_sedis	Other	Other	0.0003	0.0003
10^-9	Actinobacteria	Actinobacteria	Actinomycetales	Dermacoccaceae	Dermacoccus	0.0004	0.0004
10^-9	Firmicutes	Bacilli	Lactobacillales	Enterococcaceae	Vagococcus	0.0003	0.0003
10^-9	Firmicutes	Erysipelotrichi	Erysipelotrichales	Erysipelotrichaceae	Turicibacter	0.0001	0.0001
10^-9	Proteobacteria	Alphaproteobacteria	Rhizobiales	Methylobacteriaceae	Methylobacterium	0.0003	0.0003
10^-9	Proteobacteria	Alphaproteobacteria	Sphingomonadales	Sphingomonadaceae	Sphingopyxis	0.0001	0.0001
10^-9	Proteobacteria	Alphaproteobacteria	Sphingomonadales	Sphingomonadaceae	Sphingopyxis	0.0004	0.0004
10^-9	Proteobacteria	Betaproteobacteria	Burkholderiales	Alcaligenaceae	Alcaligenes	0.005	0.005
10^-9	Proteobacteria	Betaproteobacteria	Burkholderiales	Oxalobacteraceae	Herbaspirillum	0.0012	0.001
10^-9	Proteobacteria	Gammaproteobacteria	Pseudomonadales	Pseudomonadaceae	Pseudomonas	0.0001	0.0001
10^-9	Verrucomicrobia	Opitutae	Opitutales	Opitutaceae	Opitutus	0.0001	0.0001

Dilution	Phylum	Class	Order	Family	Genus	Abundance	SE
10^-1	other	other	other	other	other	0.1403	0.0059
10^-1	Acidobacteria	other	other	other	other	0.0002	0.0001
10^-1	Acidobacteria	Acidobacteria_Gp1	Gp1	other	other	0.0031	0.0004
10^-1	Acidobacteria	Acidobacteria_Gp3	Gp3	other	other	0.0072	0.0011
10^-1	Acidobacteria	Acidobacteria_Gp4	Gp4	other	other	0.0346	0.0027
10^-1	Acidobacteria	Acidobacteria_Gp6	Gp6	other	other	0.0128	0.0017
10^-1	Acidobacteria	Acidobacteria_Gp7	Gp7	other	other	0.0002	0.0001
10^-1	Actinobacteria	Actinobacteria	other	other	other	0.0032	0.0005
10^-1	Actinobacteria	Actinobacteria	Actinomycetales	other	other	0.0050	0.0009
10^-1	Actinobacteria	Actinobacteria	Actinomycetales	Cryptosporangiaceae	Cryptosporangium	0.0001	0.0001
10^-1	Actinobacteria	Actinobacteria	Actinomycetales	Geodermatophilaceae	other	0.0003	0.0003
10^-1	Actinobacteria	Actinobacteria	Actinomycetales	Micrococcaceae	Arthrobacter	0.0094	0.0011
10^-1	Actinobacteria	Actinobacteria	Actinomycetales	Micromonosporaceae	other	0.0021	0.0005
10^-1	Actinobacteria	Actinobacteria	Actinomycetales	Nocardiaceae	Nocardia	0.0003	0.0002
10^-1	Actinobacteria	Actinobacteria	Actinomycetales	Nocardioidaceae	other	0.0004	0.0001
10^-1	Actinobacteria	Actinobacteria	Actinomycetales	Pseudonocardiaceae	other	0.0013	0.0004
10^-1	Actinobacteria	Actinobacteria	Actinomycetales	Pseudonocardiaceae	Amycolatopsis	0.0002	0.0002
10^-1	Actinobacteria	Actinobacteria	Solirubrobacterales	other	other	0.0044	0.0007
10^-1	Actinobacteria	Actinobacteria	Solirubrobacterales	Conexibacteraceae	Conexibacter	0.0055	0.0007
10^-1	Bacteroidetes	other	other	other	other	0.0459	0.0041
10^-1	Bacteroidetes	Sphingobacteria	Sphingobacteriales	other	other	0.0128	0.0034
10^-1	Bacteroidetes	Sphingobacteria	Sphingobacteriales	Chitinophagaceae	other	0.0553	0.0037
10^-1	Bacteroidetes	Sphingobacteria	Sphingobacteriales	Chitinophagaceae	Ferruginibacter	0.0014	0.0004
10^-1	Bacteroidetes	Sphingobacteria	Sphingobacteriales	Chitinophagaceae	Flavisolibacter	0.0001	0.0001
10^-1	Bacteroidetes	Sphingobacteria	Sphingobacteriales	Chitinophagaceae	Terrimonas	0.0085	0.0007
10^-1	Bacteroidetes	Sphingobacteria	Sphingobacteriales	Cytophagaceae	other	0.0002	0.0001
10^-1	Bacteroidetes	Sphingobacteria	Sphingobacteriales	Cytophagaceae	Larkinella	0.0001	0.0001
10^-1	BRC1	BRC1_genera_incertae_sedis	other	other	other	0.0009	0.0002
10^-1	Chlamydiae	Chlamydiae	Chlamydiales	other	other	0.0008	0.0004
10^-1	Chlamydiae	Chlamydiae	Chlamydiales	Parachlamydiaceae	other	0.0023	0.0005
10^-1	Chloroflexi	other	other	other	other	0.0003	0.0002
10^-1	Chloroflexi	Anaerolineae	Anaerolineales	Anaerolineaceae	other	0.0007	0.0002
10^-1	Chloroflexi	Caldilineae	Caldilineales	Caldilineaceae	Caldilinea	0.0004	0.0002
10^-1	Cyanobacteria	Cyanobacteria	Chloroplast	other	other	0.0007	0.0002

Dilution	Phylum	Class	Order	Family	Genus	Abundance	SE
10^-1	Firmicutes	Bacilli	Bacillales	other	other	0.0122	0.0017
10^-1	Firmicutes	Bacilli	Bacillales	Bacillaceae	other	0.0124	0.0012
10^-1	Firmicutes	Bacilli	Bacillales	Bacillaceae	Bacillus	0.0007	0.0002
10^-1	Firmicutes	Bacilli	Bacillales	Bacillaceae	Oceanobacillus	0.0002	0.0001
10^-1	Firmicutes	Bacilli	Bacillales	Bacillaceae	Tumebacillus	0.0001	0.0001
10^-1	Firmicutes	Bacilli	Bacillales	Staphylococcaceae	Staphylococcus	0.0001	0.0001
10^-1	Firmicutes	Clostridia	Clostridiales	Ruminococcaceae	other	0.0001	0.0001
10^-1	Gemmatimonadetes	Gemmatimonadetes	Gemmatimonadales	Gemmatimonadaceae	Gemmatimonas	0.0251	0.0020
10^-1	Nitrospira	Nitrospira	Nitrospirales	Nitrospiraceae	Nitrospira	0.0016	0.0004
10^-1	OD1	OD1_genera_incertae_sedis	other	other	other	0.0016	0.0004
10^-1	Planctomycetes	Planctomycetacia	Planctomycetales	Planctomycetaceae	other	0.0564	0.0046
10^-1	Planctomycetes	Planctomycetacia	Planctomycetales	Planctomycetaceae	Gemmata	0.0089	0.0011
10^-1	Planctomycetes	Planctomycetacia	Planctomycetales	Planctomycetaceae	Pirellula	0.0101	0.0012
10^-1	Planctomycetes	Planctomycetacia	Planctomycetales	Planctomycetaceae	Planctomyces	0.0080	0.0008
10^-1	Planctomycetes	Planctomycetacia	Planctomycetales	Planctomycetaceae	Singulisphaera	0.0068	0.0010
10^-1	Planctomycetes	Planctomycetacia	Planctomycetales	Planctomycetaceae	Zavarzinella	0.0012	0.0004
10^-1	Proteobacteria	other	other	other	other	0.0172	0.0019
10^-1	Proteobacteria	Alphaproteobacteria	other	other	other	0.0707	0.0038
10^-1	Proteobacteria	Alphaproteobacteria	Caulobacterales	Caulobacteraceae	other	0.0039	0.0008
10^-1	Proteobacteria	Alphaproteobacteria	Caulobacterales	Caulobacteraceae	Phenylobacterium	0.0048	0.0007
10^-1	Proteobacteria	Alphaproteobacteria	Rhizobiales	other	other	0.0288	0.0016
10^-1	Proteobacteria	Alphaproteobacteria	Rhizobiales	Bradyrhizobiaceae	other	0.0002	0.0001
10^-1	Proteobacteria	Alphaproteobacteria	Rhizobiales	Hyphomicrobiaceae	Devosia	0.0148	0.0012
10^-1	Proteobacteria	Alphaproteobacteria	Rhizobiales	Hyphomicrobiaceae	Hyphomicrobium	0.0024	0.0004
10^-1	Proteobacteria	Alphaproteobacteria	Rhizobiales	Methylobacteriaceae	Microvirga	0.0008	0.0003
10^-1	Proteobacteria	Alphaproteobacteria	Rhodospirillales	other	other	0.0058	0.0007
10^-1	Proteobacteria	Alphaproteobacteria	Rhodospirillales	Acetobacteraceae	Stella	0.0002	0.0002
10^-1	Proteobacteria	Alphaproteobacteria	Rhodospirillales	Rhodospirillaceae	other	0.0004	0.0002
10^-1	Proteobacteria	Alphaproteobacteria	Rhodospirillales	Rhodospirillaceae	Inquilinus	0.0001	0.0001
10^-1	Proteobacteria	Alphaproteobacteria	Rickettsiales	Rickettsiaceae	Rickettsia	0.0006	0.0002
10^-1	Proteobacteria	Alphaproteobacteria	Sphingomonadales	Erythrobacteraceae	Porphyrobacter	0.0003	0.0001
10^-1	Proteobacteria	Betaproteobacteria	other	other	other	0.0057	0.0007
10^-1	Proteobacteria	Betaproteobacteria	Burkholderiales	Burkholderiaceae	Burkholderia	0.0044	0.0007
10^-1	Proteobacteria	Deltaproteobacteria	other	other	other	0.0019	0.0006

Dilution	Phylum	Class	Order	Family	Genus	Abundance	SE
10^-1	Proteobacteria	Deltaproteobacteria	Bdellovibrionales	Bacteriovoracaceae	Peredibacter	0.0009	0.0002
10^-1	Proteobacteria	Deltaproteobacteria	Bdellovibrionales	Bdellovibrionaceae	Bdellovibrio	0.0000	0.0000
10^-1	Proteobacteria	Deltaproteobacteria	Myxococcales	other	other	0.0056	0.0009
10^-1	Proteobacteria	Deltaproteobacteria	Myxococcales	Cystobacteraceae	other	0.0002	0.0002
10^-1	Proteobacteria	Deltaproteobacteria	Myxococcales	Polyangiaceae	other	0.0018	0.0003
10^-1	Proteobacteria	Gammaproteobacteria	other	other	other	0.0131	0.0012
10^-1	Proteobacteria	Gammaproteobacteria	Enterobacteriales	Enterobacteriaceae	other	0.0003	0.0002
10^-1	Proteobacteria	Gammaproteobacteria	Legionellales	Legionellaceae	other	0.0007	0.0003
10^-1	Proteobacteria	Gammaproteobacteria	Legionellales	Legionellaceae	Legionella	0.0007	0.0003
10^-1	Proteobacteria	Gammaproteobacteria	Pseudomonadales	Moraxellaceae	Alkanindiges	0.0002	0.0002
10^-1	Proteobacteria	Gammaproteobacteria	Xanthomonadales	Sinobacteraceae	other	0.0003	0.0001
10^-1	Proteobacteria	Gammaproteobacteria	Xanthomonadales	Xanthomonadaceae	other	0.0522	0.0037
10^-1	Proteobacteria	Gammaproteobacteria	Xanthomonadales	Xanthomonadaceae	Pseudoxanthomonas	0.0034	0.0007
10^-1	TM7	TM7_genera_incertae_sedis	other	other	other	0.0001	0.0001
10^-1	Verrucomicrobia	Opitutae	Opitutales	Opitutaceae	Opitutus	0.0293	0.0032
10^-1	Verrucomicrobia	Spartobacteria	other	other	other	0.0011	0.0004
10^-1	Verrucomicrobia	Spartobacteria	Spartobacteria_genera_incertae_sedis	other	other	0.0078	0.0017
10^-1	Verrucomicrobia	Subdivision3	Subdivision3_genera_incertae_sedis	other	other	0.0118	0.0009
10^-6	other	other	other	other	other	0.0912	0.0074
10^-6	Acidobacteria	Acidobacteria_Gp1	Gp1	other	other	0.0014	0.0006
10^-6	Acidobacteria	Acidobacteria_Gp3	Gp3	other	other	0.0036	0.0011
10^-6	Actinobacteria	Actinobacteria	Actinomycetales	other	other	0.0010	0.0003
10^-6	Bacteroidetes	other	other	other	other	0.0454	0.0118
10^-6	Bacteroidetes	Flavobacteria	Flavobacteriales	Cryomorphaceae	Fluviicola	0.0031	0.0015
10^-6	Bacteroidetes	Flavobacteria	Flavobacteriales	Flavobacteriaceae	Flavobacterium	0.0151	0.0040
10^-6	Bacteroidetes	Sphingobacteria	Sphingobacteriales	other	other	0.0172	0.0052
10^-6	Bacteroidetes	Sphingobacteria	Sphingobacteriales	Chitinophagaceae	other	0.1008	0.0083
10^-6	Bacteroidetes	Sphingobacteria	Sphingobacteriales	Sphingobacteriaceae	other	0.0008	0.0003
10^-6	Bacteroidetes	Sphingobacteria	Sphingobacteriales	Sphingobacteriaceae	Pedobacter	0.0204	0.0077
10^-6	Cyanobacteria	Cyanobacteria	Chloroplast	Streptophyta	other	0.0001	0.0001
10^-6	Gemmatimonadetes	Gemmatimonadetes	Gemmatimonadales	Gemmatimonadaceae	Gemmatimonas	0.0300	0.0046
10^-6	Planctomycetes	Planctomycetacia	Planctomycetales	Planctomycetaceae	other	0.1121	0.0138
10^-6	Planctomycetes	Planctomycetacia	Planctomycetales	Planctomycetaceae	Zavarzinella	0.0035	0.0017
10^-6	Proteobacteria	Alphaproteobacteria	Rhizobiales	other	other	0.0091	0.0015

Dilution	Phylum	Class	Order	Family	Genus	Abundance	SE
10^-6	Proteobacteria	Alphaproteobacteria	Rhizobiales	Xanthobacteraceae	Labrys	0.0003	0.0001
10^-6	Proteobacteria	Alphaproteobacteria	Rhodobacterales	Rhodobacteraceae	other	0.0002	0.0002
10^-6	Proteobacteria	Alphaproteobacteria	Rhodospirillales	other	other	0.0030	0.0007
10^-6	Proteobacteria	Alphaproteobacteria	Sphingomonadales	Sphingomonadaceae	Sphingomonas	0.0227	0.0034
10^-6	Proteobacteria	Betaproteobacteria	other	other	other	0.0013	0.0004
10^-6	Proteobacteria	Betaproteobacteria	Burkholderiales	Oxalobacteraceae	Herbaspirillum	0.0001	0.0001
10^-6	Proteobacteria	Betaproteobacteria	Burkholderiales	Oxalobacteraceae	Janthinobacterium	0.0003	0.0003
10^-6	Proteobacteria	Deltaproteobacteria	other	other	other	0.0005	0.0002
10^-6	Proteobacteria	Deltaproteobacteria	Myxococcales	other	other	0.0034	0.0014
10^-6	Proteobacteria	Gammaproteobacteria	other	other	other	0.0025	0.0006
10^-6	Proteobacteria	Gammaproteobacteria	Legionellales	Coxiellaceae	Aquicella	0.0002	0.0001
10^-6	Proteobacteria	Gammaproteobacteria	Pseudomonadales	Moraxellaceae	Acinetobacter	0.0001	0.0001
10^-6	Proteobacteria	Gammaproteobacteria	Pseudomonadales	Pseudomonadaceae	other	0.0004	0.0003
10^-6	Proteobacteria	Gammaproteobacteria	Pseudomonadales	Pseudomonadaceae	Pseudomonas	0.0081	0.0046
10^-6	Verrucomicrobia	other	other	other	other	0.0300	0.0079
10^-6	Verrucomicrobia	Opitutae	Opitutales	Opitutaceae	other	0.0318	0.0097
10^-6	Verrucomicrobia	Opitutae	Opitutales	Opitutaceae	Opitutus	0.0468	0.0109
10^-6	Verrucomicrobia	Spartobacteria	Spartobacteria_genera_incertae_sedis	other	other	0.0073	0.0041
10^-6	Verrucomicrobia	Subdivision3	Subdivision3_genera_incertae_sedis	other	other	0.0060	0.0015
10^-6	Verrucomicrobia	Verrucomicrobiae	Verrucomicrobiales	Verrucomicrobiaceae	Verrucomicrobium	0.0002	0.0002
10^-9	other	other	other	other	other	0.0517	0.0175
10^-9	Acidobacteria	Acidobacteria_Gp1	Gp1	other	other	0.0017	0.0005
10^-9	Actinobacteria	Actinobacteria	other	other	other	0.0011	0.0004
10^-9	Actinobacteria	Actinobacteria	Acidimicrobiales	other	other	0.0004	0.0002
10^-9	Actinobacteria	Actinobacteria	Actinomycetales	other	other	0.0024	0.0004
10^-9	Actinobacteria	Actinobacteria	Actinomycetales	Nocardioidaceae	Nocardioides	0.0006	0.0004
10^-9	Actinobacteria	Actinobacteria	Actinomycetales	Pseudonocardiaceae	Pseudonocardia	0.0006	0.0004
10^-9	Actinobacteria	Actinobacteria	Actinomycetales	Streptosporangiaceae	other	0.0002	0.0002
10^-9	Actinobacteria	Actinobacteria	Solirubrobacterales	Patulibacteraceae	Patulibacter	0.0001	0.0001
10^-9	Bacteroidetes	other	other	other	other	0.0444	0.0092
10^-9	Bacteroidetes	Flavobacteria	Flavobacteriales	Flavobacteriaceae	Chryseobacterium	0.0001	0.0001
10^-9	Bacteroidetes	Flavobacteria	Flavobacteriales	Flavobacteriaceae	Cloacibacterium	0.0001	0.0001
10^-9	Bacteroidetes	Flavobacteria	Flavobacteriales	Flavobacteriaceae	Flavobacterium	0.0167	0.0080
10^-9	Bacteroidetes	Sphingobacteria	Sphingobacteriales	other	other	0.0082	0.0032

Dilution	Phylum	Class	Order	Family	Genus	Abundance	SE
10^-9	Bacteroidetes	Sphingobacteria	Sphingobacteriales	Chitinophagaceae	other	0.0742	0.0169
10^-9	Bacteroidetes	Sphingobacteria	Sphingobacteriales	Cytophagaceae	Dyadobacter	0.0098	0.0065
10^-9	Bacteroidetes	Sphingobacteria	Sphingobacteriales	Sphingobacteriaceae	Pedobacter	0.0408	0.0072
10^-9	Chlamydiae	Chlamydiae	Chlamydiales	Parachlamydiaceae	other	0.0002	0.0001
10^-9	Deinococcus-Thermus	Deinococci	Deinococcales	Deinococcaceae	Deinococcus	0.0220	0.0135
10^-9	Firmicutes	Bacilli	Bacillales	other	other	0.0021	0.0005
10^-9	Firmicutes	Bacilli	Bacillales	Paenibacillaceae	Cohnella	0.0065	0.0034
10^-9	Firmicutes	Bacilli	Bacillales	Paenibacillaceae	Paenibacillus	0.0012	0.0004
10^-9	Firmicutes	Clostridia	Clostridiales	other	other	0.0001	0.0001
10^-9	Planctomycetes	Planctomycetacia	Planctomycetales	Planctomycetaceae	other	0.0130	0.0064
10^-9	Planctomycetes	Planctomycetacia	Planctomycetales	Planctomycetaceae	Gemmata	0.0007	0.0003
10^-9	Planctomycetes	Planctomycetacia	Planctomycetales	Planctomycetaceae	Planctomyces	0.0014	0.0006
10^-9	Proteobacteria	Alphaproteobacteria	Rhizobiales	other	other	0.0046	0.0007
10^-9	Proteobacteria	Alphaproteobacteria	Rhizobiales	Brucellaceae	Pseudochrobactrum	0.0001	0.0001
10^-9	Proteobacteria	Alphaproteobacteria	Rhizobiales	Methylobacteriaceae	Methylobacterium	0.0001	0.0001
10^-9	Proteobacteria	Alphaproteobacteria	Rhodospirillales	Rhodospirillaceae	Inquilinus	0.0010	0.0010
10^-9	Proteobacteria	Alphaproteobacteria	Sphingomonadales	Sphingomonadaceae	other	0.0021	0.0006
10^-9	Proteobacteria	Alphaproteobacteria	Sphingomonadales	Sphingomonadaceae	Novosphingobium	0.0003	0.0001
10^-9	Proteobacteria	Betaproteobacteria	other	other	other	0.0005	0.0002
10^-9	Proteobacteria	Betaproteobacteria	Burkholderiales	Alcaligenaceae	other	0.0012	0.0011
10^-9	Proteobacteria	Betaproteobacteria	Burkholderiales	Comamonadaceae	other	0.0119	0.0090
10^-9	Proteobacteria	Deltaproteobacteria	Bdellovibrionales	Bdellovibrionaceae	Bdellovibrio	0.0042	0.0020
10^-9	Proteobacteria	Deltaproteobacteria	Myxococcales	other	other	0.0010	0.0003
10^-9	TM7	TM7_genera_incertae_sedis	other	other	other	0.0001	0.0001
10^-9	Verrucomicrobia	other	other	other	other	0.1487	0.0355
10^-9	Verrucomicrobia	Verrucomicrobiae	Verrucomicrobiales	other	other	0.0012	0.0011
10^-9	Verrucomicrobia	Verrucomicrobiae	Verrucomicrobiales	Verrucomicrobiaceae	other	0.0212	0.0075

2.4. Discussion

A number of studies have used the dilution method approach to artificially change microbial diversity (Salonius 1981, Garland and Lehman 1999, Franklin et al 2001, Griffiths et al 2001, Matos et al 2005, Franklin and Mills 2006, Wertz et al 2006, Hol et al 2010, Philippot et al 2013, Vivant et al 2013). This approach is one of the few available methods to manipulate microbial biodiversity of complex natural ecosystems such as the soil. And, indeed, our results show that dilution reduces the microbial biodiversity in the soil suspension and the soil after incubation of more or less diluted suspensions. Previous studies mostly based their conclusions on community measurements with limited resolution, detecting only the more abundant species since those species can be detected in the easiest way. However, compared to the rare biosphere, the abundant members are only a small fraction of microbial diversity (Sogin et al 2006), and thus, in this way, the real microbial biodiversity in these ecosystems may not be accounted for. Furthermore, none of those studies focused on changes in the community structure from the original more or less diluted inocula into different communities after incubation in soil or on the degree of variation in the suspensions after dilution and the consequences of this variation for the variances in the incubated soils. We suspected that the variance among the replicate samples would be considerable, and therefore we determined this variation in the suspension samples and how the variant communities developed during incubation and regrowth in soil. We were especially interested in the possibilities created by the dilution approach to separate abundant and rare species and thus allow experimental studies on the importance of rare (and abundant) microbes in soil ecosystems.

Although less abundant microbes should be more prone to be lost from the original microbial community at increasing dilution, our results show that unique OTUs still show up in the highest-dilution treatment in the suspensions (Fig. 2.7). Most likely certain microbial species are suppressed or masked for amplicon measurements in the low-dilution samples and only show up in the higher-dilution treatments. An issue that may have played an important role in the preparation of the diluted soil suspensions is the adsorption of cells on soil particles. Bakken (1985) claimed that a satisfactory separation of microorganisms and soil particles is not possible, and thus this could have influenced the structure of the microbial communities in the suspensions and, in

particular, the large variation therein. Moreover, also methodological errors may also have played a role in the failure of the sequencing approach to detect all species in the suspensions. For instance, the nested PCR could be a possible source of bias; therefore, the patterns from nested PCRs between samples were compared with the ones from direct PCRs. The patterns obtained from the nested PCRs were similar to those from the direct PCRs in soil suspension. Only minor variations were observed. In this experiment, the PCR products were purified before sequencing to exclude the nonincorporated primers. Thus, we concluded that the nested PCR approach may not have influenced significantly the results.

Similarly, our results indicate that, most likely, rare species that were suppressed in the low-dilution samples may have acquired an opportunity to develop in the higher-dilution samples because the cellular densities were low in those samples after dilution.

The data shown in Table 2.3 and the diagrams of Figure 2.5 clearly indicate that the present methodology, i.e. 454 pyrosequencing, does not allow for a complete view on the species present even in a dilute suspension as these data show that the diversity of the communities of the diluted samples increased during incubation in soil. We do not know what the precise detection limit is of the 454 pyrosequencing technique for observing microbial species in a suspension, but it is fair to assume that the bacterial species that are detected in soil after incubation were present but not detected in the soil suspensions, most probably because of their low abundance. As mentioned, also the data of the Venn diagram (Fig. 2.7) also clearly indicate the presence of species in all suspensions, including the 10^{-9} dilutions, which were not detected by our methodological approach.

The fact that these organisms were detected in soil but not in suspensions may be because these organisms were better adapted to the prevailing conditions of the soil environment (Brazelton et al 2010) than other organisms that were detected in the suspensions but not in soils. These other taxa may have been lost during incubation since they might have had special requirements not available in soil. It is not possible to conclude that these hidden species are rare species, and, thus, the conclusion is warranted that the dilution approach does not guarantee the identification of rare or less abundant versus abundant species.

Although all inoculated organisms returned into the same environment where they came from originally, the actual conditions for the individual organisms could have changed dramatically due to the difference in spatial arrangements and the large heterogeneity in soil. The factors that are responsible for the selection of microbes in soil resulting in the different communities as found in soil versus the communities in the suspensions are not clear. Previous studies have indicated that soil microbial communities were largely influenced by soil moisture (Schimel et al 1999, Brockett et al 2012). In our study, moisture availability after incubation could be a potential clue for the structuring of the community by selecting for individual microbial species with a relatively high moisture stress resistance. Other factors are said to be key to the shaping of bacterial communities in soil (Fierer et al 2003, Eichorst et al 2007, Kuramae et al 2010, Navarrete et al 2013), but the relevance of these factors for the assemblage of the communities from various inocula, as in this study, is not known.

In this study, we have considered several taxonomic diversity indicators. All indicated that the dilution procedure has a strong reducing effect on the microbial diversity (Table 2.1). We have used these different diversity indices because they give different insights into the diversity of complex communities such as soil microbial communities. In contrast to the richness index (Chao estimator), the diversity indices (Shannon and Simpson) focus on both the richness and evenness of a community. Shannon diversity is often sensitive to the presence of rare species, while the Simpson index emphasizes the dominant members (Nagendra 2002). Haegeman et al. (2013) suggests that community diversity is best estimated by Shannon and Simpson indices, whereas Chao estimator was not a reliable estimator of richness in the presence of rare species. Despite the differences in the focus of the diversity indices used here, all indices showed a similar trend. This strongly suggests that the alpha diversity decreases in response to dilution of microbial communities and that this decrease is reflected in the diversity of the communities after incubation in soil.

Interestingly, when we compared the diversity of the different phyla in suspension and in soil after incubation, we observed that the Shannon diversity indices of most phyla decreased from suspension to soil sample for the undiluted (10^{-1}) samples but increased for the most diluted (10^{-9}) samples (Table 2.3). Obviously, there are strong selection mechanisms operating in soil

that lead to a certain homogenization in the communities that are formed after regrowth of the suspensions. That observation is confirmed by the data of Figure 2.6 and Table 2.2, both of which show that the variances in the communities formed in the replicate samples diminished. We are not aware of similar observations presented in literature, but the findings are in line with the wealth of information that indicates that soil is a strong factor shaping the structure of the microbial community inhabiting the soil.

Analysis of the overall microbial community revealed that the community changed through dilution treatment of the soil suspensions and incubated soil at both phylum (Fig. 2.2) and OTU levels (Fig. 2.6). A detailed look at the microbial communities in the original non-diluted (10^{-1}) soil suspension revealed that the core groups comprised the well-known soil microbial phyla of *Proteobacteria*, *Actinobacteria*, *Bacteroidetes*, *Acidobacteria*, *Verrucomicrobia*, *Planctomycetes* and *Firmicutes* (Janssen 2006, Roesch et al 2007). During incubation, the same core groups were observed again, but the relative abundances of each group changed substantially. The largest changes in the occurrences of specific phyla were detected for the phylum of *Proteobacteria*, which was highly dominant especially in the higher dilutions but less dominant in the incubated soil samples, for the phylum of *Bacteroidetes*, which decreased slightly with increasing dilution in the soil suspensions but outgrew and increased significantly in the samples after incubation, and for the phylum of *Verrucomicrobia*, which was not detected in the higher soil suspension dilutions but showed up in high numbers after incubation. At the family level, we detected high proportions of *Beta-proteobacteria* represented by *Alcaligenaceae*, *Burkholderiaceae* and *Comamonadaceae* in the highest suspension dilution. Remarkably, their relative abundance decreased during incubation in soil. That was unexpected as *Proteobacteria* are dominant members in various soils, and as they are mostly fast-growing r-strategists, we expected them to be abundantly present in the incubated soil samples. The result may have been caused by the oligotrophic conditions prevailing in our test soil. However, the same observations after incubation in soil were made for *Acidobacteria*, which are generally considered to be soil-adapted oligotrophic organisms (Eichorst et al 2007), and for other well-known soil inhabitants such as *Actinobacteria*. It is interesting to see that groups such as *Verrucomicrobia*, and *Sphingobacteriaceae* and *Chitinophagaceae* families of *Bacterioidetes*

grew out significantly in all dilution treatments during incubation in soil. This contradicts what is known about *Verrucomicrobia*, which is usually considered a low-abundant phylum in soil (Janssen 2006). *Verrucomicrobia* may highly depend on C availability due to their slow-growing life strategy (Bergmann et al 2011); and that, in combination with the observed results, may indicate that *Verrucomicrobia* is a potential indicator of the response of these taxa to environmental factors (Fierer et al 2007). In summary, our results indicate that the dilution procedure leads to reduction of bacterial diversity, but the assembly of the microbial community during incubation in soil cannot be predicted on the basis of the composition of the inoculum. Obviously, soil has a strong selective power in shaping the microbial community, which leads to more uniform structures of the communities even after inoculation of much more variable suspensions. Also the deep sequencing approach applied here did not allow for a complete view of the microbial species present in even highly diluted suspensions. This also hinders the assessment and identification of rare species in a soil sample as even undetected species in the suspensions could develop into abundant populations after only eight weeks of incubation. In future studies we hope to be able to know more about the functional responses of more or less diverse samples and the consequences of these changes for the functioning of the soil ecosystem.

2.5. References

Bakken LR (1985). Separation and Purification of Bacteria from Soil. *Applied and Environmental Microbiology* **49:** 1482-1487.

Bergmann GT, Bates ST, Eilers KG, Lauber CL, Caporaso JG, Walters WA *et al* (2011). The under-recognized dominance of Verrucomicrobia in soil bacterial communities. *Soil Biology & Biochemistry* **43:** 1450-1455.

Brazelton WJ, Ludwig KA, Sogin ML, Andreishcheva EN, Kelley DS, Shen CC *et al* (2010). Archaea and bacteria with surprising microdiversity show shifts in dominance over 1,000-year time scales in hydrothermal chimneys. *Proceedings of the National Academy of Sciences of the United States of America* **107:** 1612-1617.

Brockett BFT, Prescott CE, Grayston SJ (2012). Soil moisture is the major factor influencing microbial community structure and enzyme activities across seven biogeoclimatic zones in western Canada. *Soil Biology & Biochemistry* **44:** 9-20.

Butchart SHM, Walpole M, Collen B, van Strien A, Scharlemann JPW, Almond REA *et al* (2010). Global Biodiversity: Indicators of Recent Declines. *Science* **328:** 1164-1168.

Caporaso JG, Kuczynski J, Stombaugh J, Bittinger K, Bushman FD, Costello EK *et al* (2010). QIIME allows analysis of high-throughput community sequencing data. *Nature Methods* **7:** 335-336.

Cole JR, Wang Q, Cardenas E, Fish J, Chai B, Farris RJ *et al* (2009). The Ribosomal Database Project: improved alignments and new tools for rRNA analysis. *Nucleic Acids Research* **37:** D141-D145.

Dixon P (2003). VEGAN, a package of R functions for community ecology. *Journal of Vegetation Science* **14:** 927-930.

Edgar RC (2010). Search and clustering orders of magnitude faster than BLAST. *Bioinformatics* **26:** 2460-2461.

Eichorst SA, Breznak JA, Schmidt TM (2007). Isolation and characterization of soil bacteria that define Teniglobus gen. nov., in the phylum Acidobacteria. *Applied and Environmental Microbiology* **73:** 2708-2717.

Fierer N, Allen AS, Schimel JP, Holden PA (2003). Controls on microbial CO2 production: a comparison of surface and subsurface soil horizons. *Global Change Biology* **9:** 1322-1332.

Fierer N, Bradford MA, Jackson RB (2007). Toward an ecological classification of soil bacteria. Ecology **88:** 1354-1364.

Franklin RB, Garland JL, Bolster CH, Mills AL (2001). Impact of dilution on microbial community structure and functional potential: Comparison of numerical simulations and batch culture experiments. *Applied and Environmental Microbiology* **67:** 702-712.

Franklin RB, Mills AL (2006). Structural and functional responses of a sewage microbial community to dilution-induced reductions in diversity. *Microbial Ecology* **52:** 280-288.

Garland JL, Lehman RM (1999). Dilution/extinction of community phenotypic characters to estimate relative structural diversity in mixed communities. *Fems Microbiology Ecology* **30:** 333-343.

Good IJ (1953). The Population Frequencies of Species and the Estimation of Population Parameters. *Biometrika* **40:** 237-264.

Griffiths BS, Ritz K, Wheatley R, Kuan HL, Boag B, Christensen S *et al* (2001). An examination of the biodiversity-ecosystem function relationship in arable soil microbial communities. *Soil Biology & Biochemistry* **33:** 1713-1722.

Haegeman B, Hamelin J, Moriarty J, Neal P, Dushoff J, Weitz JS (2013). Robust estimation of microbial diversity in theory and in practice. *Isme Journal* **7:** 1092-1101.

Hammer et al HD, Ryan PD. (2001). PAST: paleontological statistics software package for education and data analysis. *Palaeontologia Electronica* **4: 9. pp.**

Hol WHG, de Boer W, Termorshuizen AJ, Meyer KM, Schneider JHM, van Dam NM *et al* (2010). Reduction of rare soil microbes modifies plant-herbivore interactions. *Ecology Letters* **13:** 292-301.

Huber JA, Mark Welch D, Morrison HG, Huse SM, Neal PR, Butterfield DA *et al* (2007). Microbial population structures in the deep marine biosphere. *Science* **318:** 97-100.

Hughes JB, Hellmann JJ (2005). The application of rarefaction techniques to molecular inventories of microbial diversity. *Environmental Microbiology* **397:** 292-308.

Janssen PH (2006). Identifying the dominant soil bacterial taxa in libraries of 16S rRNA and 16S rRNA genes. *Applied and Environmental Microbiology* **72:** 1719-1728.

Kuramae EE, Gamper HA, Yergeau E, Piceno YM, Brodie EL, DeSantis TZ *et al* (2010). Microbial secondary succession in a chronosequence of chalk grasslands. *Isme Journal* **4:** 711-715.

Lane DJ (1991). 16S/23S rRNA sequencing, p. 115-175. *In* EStackebrandt and MGoodfellow (ed), Nucleic acid techniques in bacterial systematics John Wiley & Sons, Chichester, United Kingdom.

Magurran AE, Henderson PA (2003). Explaining the excess of rare species in natural species abundance distributions. *Nature* **422:** 714-716.

Margulies M, Egholm M, Altman WE, Attiya S, Bader JS, Bemben LA *et al* (2005). Genome sequencing in microfabricated high-density picolitre reactors. *Nature* **437:** 376-380.

Matos A, Kerkhof L, Garland JL (2005). Effects of microbial community diversity on the survival of Pseudomonas aeruginosa in the wheat rhizosphere. *Microbial Ecology* **49:** 257-264.

Muyzer G, Dewaal EC, Uitterlinden AG (1993). Profiling of Complex Microbial-Populations by Denaturing Gradient Gel-Electrophoresis Analysis of Polymerase Chain Reaction-Amplified Genes-Coding for 16s Ribosomal-Rna. *Applied and Environmental Microbiology* **59:** 695-700.

Nagendra H (2002). Opposite trends in response for the Shannon and Simpson indices of landscape diversity. *Applied Geography* **22:** 175-186.

Navarrete AA, Kuramae EE, de Hollander M, Pijl AS, van Veen JA, Tsai SM (2013). Acidobacterial community responses to agricultural management of soybean in Amazon forest soils. *Fems Microbiology Ecology* **83:** 607-621.

Neufeld JD, Li J, Mohn WW (2008). Scratching the surface of the rare biosphere with ribosomal sequence tag primers. *FEMS Microbiology Letters* **283:** 146-153.

Philippot L, Spor A, Henault C, Bru D, Bizouard F, Jones CM *et al* (2013). Loss in microbial diversity affects nitrogen cycling in soil. *Isme Journal* **7:** 1609-1619.

Reeder J, Knight R (2010). Rapidly denoising pyrosequencing amplicon reads by exploiting rank-abundance distributions. *Nature Methods* **7:** 668-669.

Roesch LF, Fulthorpe RR, Riva A, Casella G, Hadwin AKM, Kent AD *et al* (2007). Pyrosequencing enumerates and contrasts soil microbial diversity. *Isme Journal* **1:** 283-290.

Sala OE, Chapin FS, Armesto JJ, Berlow E, Bloomfield J, Dirzo R *et al* (2000). Biodiversity - Global biodiversity scenarios for the year 2100. *Science* **287:** 1770-1774.

Salonius PO (1981). Metabolic Capabilities of Forest Soil Microbial-Populations with Reduced Species-Diversity. *Soil Biology & Biochemistry* **13:** 1-10.

Schimel JP, Gulledge JM, Clein-Curley JS, Lindstrom JE, Braddock JF (1999). Moisture effects on microbial activity and community structure in decomposing birch litter in the Alaskan taiga. *Soil Biology & Biochemistry* **31:** 831-838.

Sogin ML, Morrison HG, Huber JA, Mark Welch D, Huse SM, Neal PR *et al* (2006). Microbial diversity in the deep sea and the underexplored "rare biosphere". *Proceedings of the National Academy of Sciences of the United States of America* **103:** 12115-12120.

Turnbaugh PJ, Hamady M, Yatsunenko T, Cantarel BL, Duncan A, Ley RE *et al* (2008). A core gut microbiome in obese and lean twins. *Nature* **457:** 480-484.

Vivant AL, Garmyn D, Maron PA, Nowak V, Piveteau P (2013). Microbial Diversity and Structure Are Drivers of the Biological Barrier Effect against Listeria monocytogenes in Soil. *Plos One* **8**.

Wertz S, Degrange V, Prosser JI, Poly F, Commeaux C, Freitag T et al (2006). Maintenance of soil functioning following erosion of microbial diversity. *Environ Microbiol* 8: 2162-2169.