Assemblage and functioning of bacterial communities in soil and rhizosphere

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Chapter 3

Soil characteristics determine the assemblage of bacterial communities in terrestrial ecosystems

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Manuscript

Abstract

In a previous study we showed that the soil had a strong effect on the assemblage of bacterial communities after inoculation of a sterilized soil with bacterial suspensions of that same soil in a dilution series. Here, we continued our investigation on the impact of soil on the assemblage of bacterial communities and checked the concept of the overriding effect of the soil on shaping bacterial communities after inoculation of suspensions obtained from different soils. Diluted suspensions from different soils harboring different bacterial diversities were crossed inoculated into three pre-sterilized soils. We used 16S rRNA gene amplicon sequencing to determine the bacterial community structure of the suspensions and the soils. In a multivariate analysis the different regrown soil bacterial communities after inoculation of different diluted suspensions in a particular soil clustered together while the same suspensions inoculated in different soils were separated. Diversity indices of the suspensions were reduced significantly upon dilution. The strength of selection of the soil on the bacterial communities was stronger for the undiluted 10^{-1} soil samples than of the diluted 10^{-9} soil samples. Permanova tests showed that dilution had a slightly larger effect on the community structure than soil had, and both main effects were larger than their interactions. Meijendel soil was characterized by the highest organic matter, ammonium and nitrate concentrations and pH, while Clue soil was characterized by the highest phosphorus concentration, and Utrecht soil was characterized by the highest C:N ratio. These differences in abiotic environmental factors may explain the variation in bacterial communities across these soils.

Keywords

Microbial biodiversity | Soil selection | Soil chemical factors

3.1. Introduction

Soil-borne bacteria represent an essential component of terrestrial ecosystems, which are key to many vital ecosystem functions (Philippot et al 2013). With an estimate of hundreds to thousands of taxa per gram of soil, their diversity provides the majority of biodiversity in terrestrial ecosystems (Torsvik and Ovreas 2002). Abiotic factors such as pH (Fierer and Jackson 2006), moisture (Brockett et al 2012) and salinity (Crump et al 2004), phosphate availability (Faoro et al 2010), and organic matter content (Verbruggen et al 2010) as well as biotic interactions with local communities of macro- and micro-organisms are known to drive the activity of soil-borne bacteria and to shape their community structure (Garbeva et al 2004, Berg and Smalla 2009).

In order to be able to assess and predict the dynamics of microbial communities in soil, proper understanding of the mechanisms of the assemblage of microbial communities in soil and other natural environments is a long-standing goal of microbial ecology. Microorganisms are dispersed globally and able to propagate in any habitat with suitable environmental conditions (Martiny et al 2006). Upon their arrival in a new environment or upon drastic changes of their current environment, microorganisms may either be assembled into distinct, new, community profiles (Panke-Buisse et al 2015) or functionally adapt to the local habitat without dramatic changes in community composition (Comte and del Giorgio 2010). Thus, bacteria colonizing the soil may be included into a specific microbiome of distinct structure and functionality.

Many studies have addressed the factors responsible for the structuring of microbial communities in soils (Pavon-Jordan et al 2013), but often such studies typically focus on the importance of a single factor without considering the full complexity of all edaphic properties for which an increasing amount of evidence is being generated as critical drivers for the shaping of microbial communities (Kuramae et al 2012, Navarrete et al 2013). Moreover, only few studies have examined the development of communities after addition of diverse inocula in soils, and, when they were done, such studies are often restricted in analysis depth providing little detailed information on taxonomy (Garland and Lehman 1999, Franklin et al 2001, Griffiths et al 2001, Matos et al 2005, Franklin and Mills 2006). Therefore, it is difficult to predict with confidence how soil microbial communities are assembled in different soil habitats.

Advanced sequencing approaches now allow for a more accurate and detailed assessment of the assemblage and the structuring of microbial communities in soils. The major aim of this study was to assess the shaping of bacterial communities after inoculating different suspensions varying in bacterial diversity into different soils, so to detect the impact of soil on the assemblage of microbial communities. We sequenced the 16S rRNA gene marker to provide information about initial inocula and soil bacterial communities after regrowth in soils. A previous study had already shown that the dilution approach is suitable for manipulating the diversity of bacterial communities, and that soil had a strong selective power in shaping the microbial community after inoculation of the different suspensions leading to a rather uniform structure of the regrown microbial community (Chapter 2). In this study, we took this approach one step further by inoculating into three soils two dilutions of the suspensions of these three different soils in a cross-inoculation design. We addressed two basic questions: 1) Does soil determine the composition of the bacterial community after incubation following the inoculation of suspensions with different bacterial diversity? 2) If so, which are the main soil characteristics explaining the structure of the final bacterial community?

3.2. Material and Methods

3.2.1. Soil sampling and treatment

Three field soils were selected across The Netherlands: soils from the surroundings of Utrecht (52°03′N, 5°13′E), from the so-called Clue fields (52°03′N, 5°45′E) and from the Meijendel dunes (52°9′N, 4°22′E). Ten liter of each soil were collected at a depth of around 15 cm in each field. The soil was sieved (5mm) and homogenized, and aliquots of 50 g were stored in plastic bags (Whirl-Pak sampling bag, 100 ml; Sigma-Aldrich). One bag of each soil was kept separately to serve as inoculum. The bags with soil were sterilized by gamma irradiation (> 35 kGy; Isotron, Ede, The Netherlands). As compared to autoclaving and freezing this way of sterilizing soils minimizes the effects on abiotic soil properties. Sterility was checked by spreading 0.5 g of sterilized soil onto Reasoner's 2A (R2A) and potato-dextrose agar (PDA) media and incubated for one week. No bacterial or fungal growth on agar plates for six

replicates was observed in the sterile soil samples after incubation. To control the sterility during the experiment, plates were incubated for the duration of the experiment ay 28 °C and no colonies were observed during the entire incubation period. Three sterilized bags of each soil were inoculated with autoclaved demineralized water to be used as controls. A subsample of the fresh soil was taken to determine soil moisture (24 h, 105°C).

Soil suspensions 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. The obtained suspension 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 subsequently shaken by hand for 1 min. This procedure was repeated for several times until 10^{-9} dilutions were obtained. Subsequently, 2.5 ml of the respective dilutions were added to 25 g of soil in the bags and additional demineralized water was given to bring the moisture level of the inoculated soil at around 20%, which is roughly similar to the average level of the prevailing moisture conditions at the sides from where the soil was taken.

The experiment was designed as a cross inoculation experiment in which we inoculated suspensions of the three soils in each of the sterilized soils. The experiment consisted of twelve treatments in a factorial design, with 108 samples, i.e. three soils × three inocula × two dilutions × six replicates, including 9 sterile controls (three soils × three replicates). The remaining suspensions were centrifuged at 3000 g for 10 min at 4°C and the pellets were stored at -20°C for further analysis. After inoculation, soil bags were incubated at 20°C using sterilized cotton plug caps to ensure gas exchange with 70% humidity in the climate chamber. The soils were turned over regularly once a week to enable homogeneous microbial growth. After 9 weeks of incubation soil samples were taken, under laminar flow conditions, to determine the microbial abundance in all treatments by quantitative real time PCR (qPCR).

Total DNA was extracted from the incubated soil using the MoBio Power Soil Extraction Kit according to the supplier's manual. Total DNA concentration was quantified and qualified on ND-1000 spectrophotometer (Nanodrop Technology, Wilmington, DE). Amplification of the 16S rRNA

gene was performed using the primer set Eub 338 and Eub 518 (Muyzer et al 1993). Each 25 µl reaction solution consisted of 12.5 µl Sybergreen mix (Bioline, GC-Biotech) with 4 mg/ml 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 obtained from *Firmicutes*. Polymerase Chain Reactions were run on a Rotor-Gene 3000 (Qiagen) and started with 15 min at 95°C, followed by 40 amplification cycles each of 95°C for 60 sec, 53°C 50 sec and 72°C 60 sec. A subsample of the soil from each bag was stored at -20°C for further analysis. Triplicate reactions per DNA sample and the appropriate set of standards were used. For qPCR assays, a linear relationship was presumed between the log of the plasmid DNA copy number and the calculated threshold cycle (Ct value). PCR efficiencies were 99% and correlation coefficients for standard curves were $R^2 = 0.99$. Because there were differences of bacterial abundance between the undiluted 10^{-1} samples and diluted 10^{-9} samples after 9 weeks of incubation (Fig. 3.1), the relative abundance of species was used for further analysis and comparison among samples.

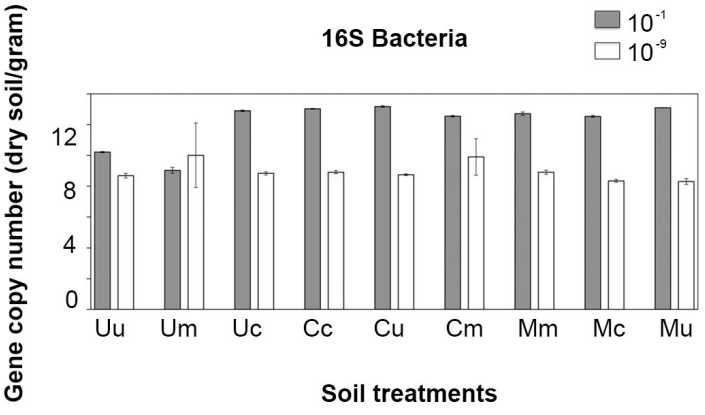

Figure 3.1. Real time PCR of bacterial abundance after 9 weeks incubation. Capital letter means suspension; lower case means incubated soil. Error bars mean standard errors (n=3).

3.2.2. 16S rRNA amplicon sequencing

Total DNA was extracted from the soil suspensions and incubated soil, as described above, to determine the composition of the respective microbial communities by 16S rRNA illumina Miseq. For DNA concentrations below 5 ng/µl, *i.e.* some of the 10^{-9} soil suspension samples, nested PCR was performed and for DNA concentrations above 5 ng/µl, direct PCR was performed. The general bacterial primer set 27F and 1492R (Lane 1991) was used for the first amplification, and subsequently 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 (Caporaso et al 2012). The PCR program used included incubation at 95°C for 5 min followed by 25 cycles each of 95°C for 30 s, 55°C 1 min and 72°C 10 min. For PCR reactions 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 were used. The PCR conditions for the second round were similar to the first PCR round except for 25 cycles with 52°C annealing temperature. To control for contamination during PCR amplification, one negative control (water instead of DNA) was included for all PCR reactions. PCR products of each subsample from the barcoded primers were generated in six replicates per sample and purified using the Agencourt AMPure XP beads kit. Equimolar purified PCR products that were quantified by fragment analyzer (Advanced Analytical Technologies, GmbH, Germany) were mixed and sequenced using Miseq sequencing from Illumina (Argonne Institute, USA).

3.2.3 Sequence analysis

The RDP extention to PANDASeq (Masella et al., 2012) named Assembler (Cole et al., 2014) was used to merge paired-end reads with a minimum overlap of 10bp and at least a PHRED score of 25. Primer sequences were removed from the per sample FASTQ files using Flexbar version 2.5 (Dodt et al., 2012). Sequences were converted to FASTA format and concatenated into a single file. All reads were clustered into OTUs using the UPARSE strategy by dereplication, sorted by abundance with at least two sequences and clustered using the UCLUST smallmem algorithm (Edgar 2010). These steps were performed with VSEARCH version 1.0.10 (Rognes et al., 2015), which is an

open-source and 64-bit multithreaded compatible alternative to USEARCH. Subsequently, chimeric sequences were detected using the UCHIME algorithm (Edgar et al., 2011) implemented in VSEARCH. All reads before the dereplication step were mapped to OTUs using the usearch_global method implemented in VSEARCH to create an OTU table and converted to BIOM format 1.3.1 (McDonald et al., 2012). Finally, taxonomic information for each OTU was added to the BIOM file by using the RDP Classifier version 2.10 (Cole et al., 2014). All steps were implemented in a Snakemake workflow (Köster and Rahmann, 2012).

Alpha diversity was calculated based on the rarefied OTU table (Hughes and Hellmann 2005). Eight samples were deleted after rarefaction since those samples had substantially lower reads in comparison to the other ones. The average reads from 3 sterilized controls of each soil were used as baseline, which was subtracted from the reads of each soil samples, respectively. The OTU table after subtraction of the control was used for further statistical analysis. Shannon diversity 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 formula % = [1-(n/N)] × 100, where n means the number of phylotypes represented by singletons and N is the total number of sequences (Good 1953). Good's method equation gives an estimate of the coverage of an entire sampled community.

To assess the differences between communities of different treatments, Nonmetric Multi-Dimensional Scaling (NMDS) plots were used to visualize the structure among samples at OTU level. Canonical Correlation Analysis (CCA) was used to evaluate the linkages between soil microbial structure and soil chemical characteristics. The plots were generated from Bray-Curtis similarity index matrices of all samples. The effects of soil and dilution on bacterial community composition were tested by a two-way PERMANOVA test for each inoculum, respectively. All the multivariate analyses were performed using the PAST software (Hammer et al 2001).

3.3. Results

3.3.1. Chemical characteristics of three field soils

Soil chemical analysis showed that Meijendel soil had the highest pH and had higher concentrations of NO^{3-}, NH^{4+} and organic matter than the other soils, while Utrecht soil had highest C:N ratio and the lowest pH. Clue soil had the highest phosphorus concentration (Table 3.1).

Table 3.1. Chemical properties of each field soil

Chemical properties	Field soil		
	Utrecht	Clue	Meijendel
OM (%)	4.67±0.18 a	3.97±0.29 a	9.11±0.36 b
NO_3^- (mg/kg)	0.02±0.02 a	6.50±0.51 b	30.43±0.85 c
NH_4^+ (mg/kg)	0.92±0.20 a	1.21±0.18 ab	2.23±0.25 b
Phosphorus (mg/kg)	2.28±0.35 a	80.84±3.56 b	15.16±0.41 c
C:N ratio	20.30±1.22 a	14.81±0.69 ab	12.16±0.26 b
pH (H_2O)	4.61±0.023 a	5.77±0.015 b	7.47±0.005 c

Values are mean ±SE, n = 6. Within columns, means followed by the same letter are not significantly different ($p < 0.05$) based on Tukey HSD test. OM means organic matter. Data was transformed to fit normal distribution when needed.

3.3.2. Effect of dilution and soil on bacterial community diversity

Alpha diversity indices reduced significantly upon dilution for each of the three soil suspensions (Table 3.2). Diversity indices changed substantially after incubation in the different soils. This is especially true for Clue and Meijendel inocula. In general, the diversity of the soil with 10^{-9} diluted inocula increased compared to that of 10^{-9} suspension. Good's estimator of coverage of all samples was above 99% indicating that sequencing depth was enough to detect most species in this study.

Table 3.2. Estimators of sequence library diversity and coverage in soil suspensions and incubated soil samples.

Time	Suspension	Soil	Dilution	Observed	Shannon	Good's estimator of coverage
Suspension	Utrecht		10^{-1}	134±2	3.40±0.04	0.995
Suspension	Utrecht		10^{-9}	100±2	2.28±0.03	0.998
Suspension	Clue		10^{-1}	190±4	3.79±0.08	0.993
Suspension	Clue		10^{-9}	50±2	0.91±0.02	0.997
Suspension	Meijendel		10^{-1}	190±3	3.59±0.11	0.994
Suspension	Meijendel		10^{-9}	80±8	2.17±0.11	0.999
Soil	Utrecht	Utrecht soil	10^{-1}	111±6	3.27±0.07	0.994
Soil	Clue	Utrecht soil	10^{-1}	91±4	3.13±0.06	0.995
Soil	Meijendel	Utrecht soil	10^{-1}	57±4	2.46±0.22	0.998
Soil	Utrecht	Clue soil	10^{-1}	138±4	3.54±0.06	0.995
Soil	Clue	Clue soil	10^{-1}	163±3	3.79±0.06	0.995
Soil	Meijendel	Clue soil	10^{-1}	131±8	3.32±0.06	0.995
Soil	Utrecht	Meijendel soil	10^{-1}	132±3	3.58±0.11	0.997
Soil	Clue	Meijendel soil	10^{-1}	189±1	4.25±0.04	0.995
Soil	Meijendel	Meijendel soil	10^{-1}	164±2	3.86±0.06	0.995
Soil	Utrecht	Utrecht soil	10^{-9}	113±12	2.21±0.42	0.995
Soil	Clue	Utrecht soil	10^{-9}	113±13	2.59±0.31	0.995
Soil	Meijendel	Utrecht soil	10^{-9}	121±19	2.16±0.45	0.996
Soil	Utrecht	Clue soil	10^{-9}	142±19	2.53±0.41	0.994
Soil	Clue	Clue soil	10^{-9}	173±7	3.48±0.22	0.993
Soil	Meijendel	Clue soil	10^{-9}	128±17	2.51±0.22	0.994
Soil	Utrecht	Meijendel soil	10^{-9}	140±9	2.77±0.38	0.990
Soil	Clue	Meijendel soil	10^{-9}	122±6	1.85±0.19	0.988
Soil	Meijendel	Meijendel soil	10^{-9}	100±7	1.56±0.23	0.988

Estimators and statistical significance ($P < 0.05$) were calculated for each dilution treatment of incubated soil samples (n = 5-6) on the basis of the phylogenetic profile at the species level. S.obs is the observed number of OTUs. NS means not significant.

3.3.3. Effects of dilution and soil on bacterial community composition

To explain the variability of the community composition in the different treatments, relative abundances were used to compute the Bray-Curtis similarity matrix (Fig. 3.2). Samples were grouped according to soils and dilutions

(ANOSIM: R = 0.80, $P < 0.001$). We also assessed, by CCA (Fig. 3.2B), which of the soil characteristics could be responsible for the separation of the communities, including organic matter (OM), ammonium and nitrate concentrations and pH, phosphorus concentration, and C:N ratio.

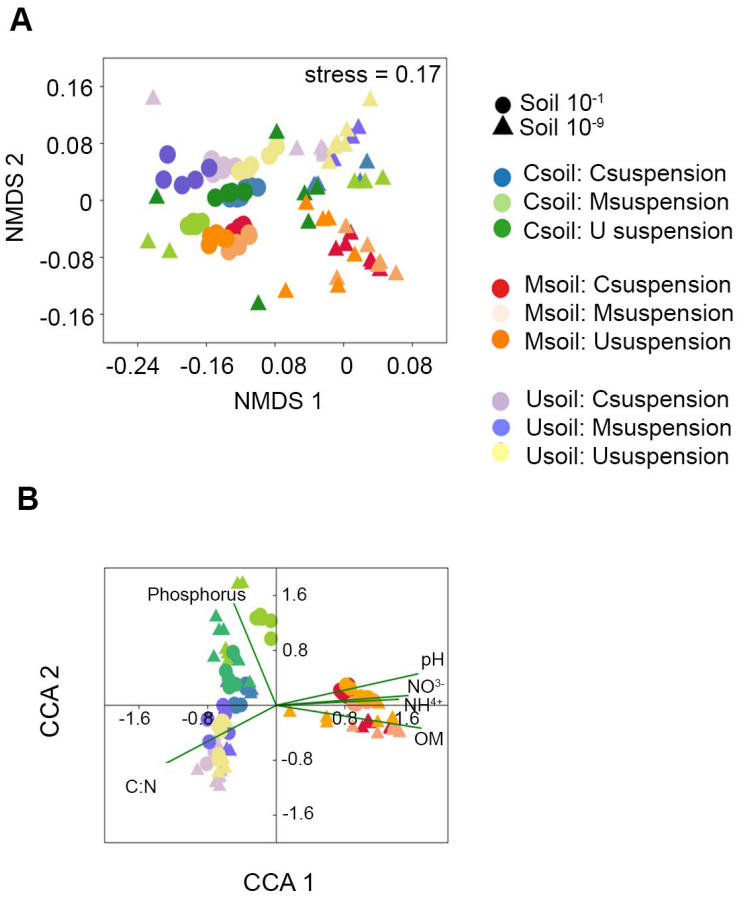

Figure 3.2. Redundancy analysis of bacterial community patterns and soil characteristics from samples. (A) NMDS plot of Bray-Curtis similarity matrix among six replicate samples of the two dilutions of the incubated soil samples. (B) CCA plot of bacterial community patterns and soil chemical characteristics for the incubated soil samples.

Furthermore, we quantified the individual effects of soil and dilution on the regrown bacterial communities with different inoculations. Based on the associated *F*-values, for all three soils, the dilution effect was slightly stronger than the soil effects and both individual effects were larger than their interactions. Permanova test for these two factors yielded statistically

significant results regarding dilutions and soils, as well as their interactions (Table 3.3).

Table 3.3. Results from two-way PERMANOVA analysis using Bray-Curtis similarity showing the effects of soil, dilution and their interaction on the composition of bacterial communities.

Inocula	Factors	Sum of sqrs	df	Mean Square	*F*	p
U suspension	Soil	2.88	2	1.44	10.94	0.0001
	Dilution	1.76	1	1.76	13.38	0.0001
	Interaction	1.41	2	0.70	5.34	0.0001
C suspension	Soil	3.03	2	1.52	22.45	0.0001
	Dilution	2.47	1	2.47	36.64	0.0001
	Interaction	2.15	2	1.08	15.94	0.0001
M suspension	Soil	3.22	2	1.61	17.73	0.0001
	Dilution	2.18	1	2.18	24	0.0001
	Interaction	2.09	2	1.04	11.47	0.0001

The most dominant phyla in the undiluted 10^{-1} suspension samples were *Proteobacteria*, *Acidobacteria*, *Actinobacteria*, *Planctomycetes*, *Bacteroidetes* and *Verrucomicrobia* (Fig. 3.3). Some interesting trends in the relative abundance of phyla were observed. The abundance of *Proteobacteria* dominated in the three soils of the undiluted 10^{-1} soil suspension samples, and *Actinobacteria*, *Deinococcus* and *Proteobacteria* comprised around 80% of the total population of the, diluted, 10^{-9} Utrecht and Meijendel soil suspension samples, respectively (Fig. 3.3A). The communities that developed after inoculation and incubation of a suspension derived from that same soil showed to be highly similar with regard to phyla abundance, whereas communities developed in other soils were less similar. The differences in phylum abundance after incubation were larger for the diluted 10^{-9} samples than for the 10^{-1} undiluted samples. This holds for all three soils.

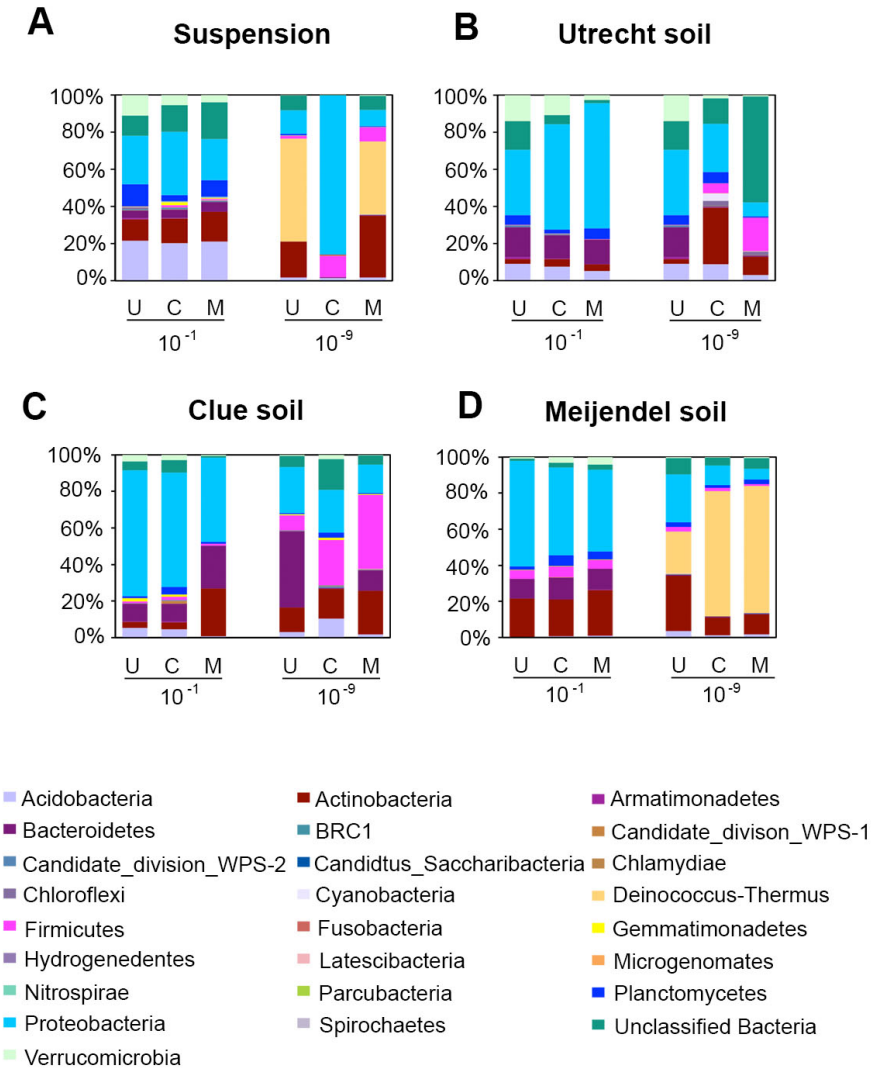

Figure 3.3. Bacterial community composition based on relative abundances at the phylum level of soil suspensions and incubated soil samples. (A) suspension samples, the origins are indicated at the bottom of each plot; (B-D) incubated soil samples from Utrecht soil, Clue soil and Meijendel soil.

3.4. Discussion

In a previous study (Chapter 2) we already found strong indications that soil is a major driving force shaping the structure of bacterial communities that develop after inoculation of suspensions with different bacterial diversities and compositions. The current study was designed to test the concept of the overriding impact of soil on the assemblage of bacterial communities leading to homogeneous community structures even after inoculation of suspensions from different soils and thus different bacterial communities. The composition of the regrown soil bacterial communities was strongly determined by the soil in which they were inoculated such that these communities showed great similarity within each of the three inoculated soils while across the three inoculated soils there were large differences. This result confirmed the earlier formulated concept of the strong impact of soil on the assemblage of bacterial communities. The observations of the soil effect were clear both at the phylum (Fig. 3.3) and the OTU (Fig. 3.2) levels.

Also we observed that dilution had a strong effect on bacterial diversities in the different soils (Table 3.2). Similarly, as was already demonstrated in Chapter 2, diluting soil suspensions to 10^{-9} dilutions led to a significant reduction of the diversity of the bacterial communities. Considering that we inoculated sterile soil, it is fair to assume that the soil itself did not add a substantial inoculum to the community. The observed increased diversity of some of the communities that developed after incubation of diluted 10^{-9} suspensions as compared to the original suspension, may reflect the failure of the technological approach to detect all organisms in a sample, although Good's coverage was large enough to assume with confidence that the largest proportion of the present community was included in the sequence process. Remarkably, the diluted 10^{-9} suspension of Clue soil had a much lower diversity than the other 10^{-9} dilution suspensions (Table 3.2), which was associated with a dominant proportion of *Proteobacteria* in these 10^{-9} dilution Clue suspension (Fig. 3.3). We do not have a straight explanation for this observation; the diversity index for the undiluted Clue suspension was not strongly aberrant from the other soil suspensions. Neither can we explain the observation that the diluted 10^{-9} soil samples that were regrown in Meijendel soil showed remarkably lower diversity indices for both Clue and Meijendel inocula, respectively (Table 3.2). Meijendel soil was characterized by the highest pH

and high proportion of organic matter, nitrate and phosphate. The communities of these soil samples were dominated by *Deinococcus* spp. Neither from our own data nor from literature data on the occurrence of *Deinococcus* spp we can directly relate the high abundance in Meijendel soils directly to these environmental factors.

Generally, a higher similarity in bacterial community composition was found amongst soils after incubation of the more diverse undiluted 10^{-1} samples than that of the less diverse diluted 10^{-9} samples (Fig. 3.2). *Proteobacteria* were dominant in each soil after incubation of undiluted samples, which may explain the relative similarity in the communities after incubation of the undiluted 10^{-1} soil suspensions as compared to the communities that developed after incubation of the diluted 10^{-9} soil suspensions, in which *Proteobacteria* nor any other phylum was consistently dominant (Fig. 3.3). The well known soil bacterial phyla of *Proteobacteria*, *Acidobacteria*, *Actinobacteria*, *Bacteroidetes*, *Deinococcus* and *Verrucomicrobia* (Roesch et al 2007) were observed as dominant members of the communities of each soil.

As the data of Table 3.3 indicate, the effect of dilution is, at least, equal or larger than the effect of soil on the community assemblage process, indicating that the dilution approach not only caused a dramatic reduction in the species diversity but also to rather different communities. The diversity indices of incubated samples were not always highest in their own native soil (Table 3.1). This indicates that only a selected fraction of the original community is able to establish in soil, even in the 'own' soil; more detailed information on the physico-chemical factors that are responsible for this differential species establishment in soil after inoculation of suspensions is needed to predict the outcome of the regrown process on the basis of the original composition of the suspensions.

Logically, on the basis of the afore-described consideration the environmental factors that may determine the assemblage process differ significantly among the soils. Among the soil characteristics that we found to be of significant importance in this study, pH is often regarded as a key factor for shaping bacterial communities (Rousk et al 2010). Remarkably, pH was only indicated as a determining factor in Meijendel soil, which could explain the bacterial communities in Meijendel soil. The abundance of *Acidobacteria* may be another indicator of acidic conditions in soil (Navarrete et al 2013).

Indeed, we observed that the relative abundance of Gp1 and Gp3 of *Acidobacteria* was highest in the most acid Utrecht soils and lowest in the Meijendel soil (Fig. 3.3). Also other soil factors could contribute significantly to the assemblage of the bacterial communities, such as organic matter, nitrite and ammonium, which were found probably to be important for shaping the bacterial communities in Meijendel soil. A previous study reported that the abundance of *Firmicutes* was highly correlated with phosphorus content (Kuramae et al 2012), and, indeed, we found that the abundance of *Firmicutes* was highest in the Clue field soil in which we observed the highest phosphorus content of the three soils. Thus, our study shows that not only pH as suggested by Fierer and Jackson (2006) and Rousk (2010), but also other environmental factors may serve as determinants of the structure of bacterial communities in specific soils.

In conclusion, we have shown that soil characteristics have strong impact on the assemblage of bacterial communities. Soil abiotic factors play a major role in shaping bacterial community structure independent of the diversity of the original suspension inoculated in soil. Indeed, the three soils that were considered in this study modified the bacterial community structure differently by providing specific habitats suitable for the growth of the inocula, which confirmed the concept of the overriding impact of the physicochemical nature of the soil on the assemblage of bacterial communities in terrestrial ecosystems.

3.5. References

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