Fluctuations of soil bacterial communities under potato cropping

Ö. İnceoğlu, L. van Overbeek, J. Falcão Salles and J. D. van Elsas.
Submitted to *Applied Environmental Microbiology*

**Abstract**

It is important to understand the underlying factors that lead to shifts in soil microbial communities, not simply for the characterization of these complex biotic systems, but also to understand the impact that changes in microbial community composition may have on terrestrial ecosystems. In this study, the impact of genetically different potato (*Solanum tuberosum*) plants growing in arable soil on the soil bacterial community composition was investigated. It was hypothesized that the modifications in the tuber starch content of these plants might lead to changes in root exudation, resulting in changes in bacterial composition near the roots. In a crop rotation schedule, including potato-barley-potato, plants of different potato cultivars were grown, including one genetically modified (GM) line that was modified in its tuber starch content. Thus, to establish the normal operating range (NOR) of soil bacterial community make-up in the production system, the variability of the bacterial communities in soil under the subsequent cropping regimes was determined. The results showed that the effect of the GM potato line on soil bacteria fitted within the NOR. The bacterial community structure of the potato rhizosphere samples was mainly affected by plant growth stage. Besides, clone library and PCR-DGGE analyses indicated that the bulk soil community fluctuated over the three growth seasons, concomitant with the crop rotation and fertilization regime. Some bacterial species found in the bulk soil were specific for the plant species under investigation (barley and potato) or plant growth stage. *Arthrobacter, Streptomyces, Rhodanobacter* and *Dokdonella* were only found at flowering potato plants in both seasons, whereas *Rhodoplanes* and *Sporosarcina* were only observed in the bulk soil with barley.
Introduction

Genetically modified (GM) plants may offer great perspectives in food production and industrial use, but potential risks related to their effects once introduced into the ecosystem need to be better defined. For the risk assessments that are required prior to the production of GM plants, effects on aboveground organisms as well as on the soil biota need to be considered. In particular, soil-borne micro-organisms play important roles in plant growth and health. This pertains mainly to their functioning, which is dependent on their success in energy and carbon sequestration (4). Root exudation, resulting in rhizodeposition, has been identified as the most important factor controlling the microbial communities in the rhizosphere (22). The root-released products can vary according to plant species, soil type and the plant developmental stage. It is possible that GM plants change the bacterial community structure in the rhizosphere due to the release of altered root exudates, and hence a focus should be placed on this potential effect (2, 25, 35). However, no significant effect of the GM plants on microbial communities has been demonstrated in several studies (15, 26, 34). Given the inherent uncertainty, it is also important to assess whether the characteristics of the soil microbiota remain unaltered after removal of a GM plant (4, 20). If changes in microbial composition occur, one needs to assess whether it will affect growth of the next crop (3). However, and very importantly, the potential effects of GM plants on soil microbial communities need to be evaluated in the light of the natural variation (establishing the normal operating range or NOR) among cultivars of the same plant species (19). In addition, to establish the limits of the NOR, it is also necessary to understand to which extent the abiotic and biotic factors of soil affect the NOR of microbial communities under potato. Moreover, to understand the stability of the respective soil microbiota, it is important to assess microbial community compositions over time, with respect to the abundances of the individual populations (14, 33). The measured variation then will form a reference matrix for the variation that can occur under agricultural practices.

It is key to evaluations of the impact of GM plants, that their effects on soil microorganisms are evaluated against the NOR. Many features of agricultural soil, i.e. management of soil, soil properties, the presence and growth stage of specific plant species, natural fluctuations (temperature, pH and moisture) and crop rotation, affect microbial community structures (10). Thus, these factors need to be included in definition of the NOR as well as in appropriate risk assessment studies.

The objective of the current study was to ascertain the structure and dynamics of bacterial communities in bulk soil under a potato–barley–potato rotation regime. Furthermore, the bacterial communities in the rhizosphere of potato plants of different cultivars, including a GM line, were assessed and cultivars/GM line differed in tuber starch contents. The studies with potato included two years and three growth stages per season. It was hypothesized that modifications in the tuber starch content might lead to changes in root exudate composition, resulting in a shifted bacterial community structure at the roots. The impact of this effect was evaluated in the light of other, possibly more important, factors, governing the bacterial community composition in bulk soils in succeeding years.
Materials and Methods

Soils and soil sampling. Three experiments were conducted over three successive years (2008 through 2010) in the field located near the village of Buinen, The Netherlands. Soil in this field was characterized as a loamy sand, consisting of 5% OM [pH 5.0] (19). The fields were treated under an agricultural rotation scheme common for potato production in The Netherlands. This scheme consisted of potato growth with barley as preceding crop. Potato was grown with rotation of barley between 2008 and 2010. In 2008, artificial fertilizer was applied, whereas pig manure was used in 2010 (25 ton/ha). The surface of the soil was shoveled to remove the plants and to improve aeration in all three years preceding each growth season.

There were nine sampling moments between 2008 and 2010. They were April 2008 (before planting of potato), May 2008 (young potato plant), June 2008 (flowering), September-2008 (senescence), December 2008 (after removal of potato plants), June 2009 (soil under barley-during the flowering of potato plants in the adjacent field), May 2010 (young potato plant), July 2010 (flowering) and September 2010 (senescence).

The fields were treated under a routine agricultural regime, common for potato cultivation practice in The Netherlands. A randomized plot design was applied for the studies in the field, including four replicate plots for each cultivar. At the start of the growth season, twenty tubers were planted in each plot. Samples were taken at the young plant [EC30], flowering [EC60] and senescence stages [EC99] (11). Time of sampling differed per cultivar, depending on time of flowering. At sampling, plants were taken from each plot, making composite samples consisting of four plants (rhizosphere). Further, six composite bulk soil samples, each consisting of four cores of each cultivar area outside of the reach of plant roots, were collected. All samples were taken to the laboratory and processed within 5h. Soil loosely adhering to roots was first shaken off, and the soil more tightly adhering to the root surface was brushed off. This soil was considered as rhizosphere soil.

Six different potato cultivars: Aveka [A], Aventra [Av], Karnico [K], Modena ([M]; genetically modified derivative from Karnico with lower amylose content) and Désirée [D] were used. Cultivars were genetically different from each other and differed in pedigree. For instance, cultivar A was related to D in the fifth generation and to K in the third generation (7). Also the physiologies of the cultivars differed, cultivars A, Av, K and M produced tubers with high starch contents and had low and/or medium growth rates, whereas cultivars P and D yielded tubers with relatively low starch contents and had higher plant growth rates.

Soil DNA extraction. Pooled samples of fresh bulk soil samples were used directly for DNA extraction, whereas three of four replicates of rhizosphere soil were used. For soil DNA extraction, the PowerSoil DNA extraction kit (Mo Bio Laboratories Inc., NY, USA) was applied using the modified protocol previously described in Inceoglu et al. (19). Amount, quality and purity of the DNA were checked in agarose gels, run for an hour at 90V and stained with ethidium bromide, upon exposure to UV.
Quantitative PCR. Quantification of 16S bacterial rRNA genes was performed with primers 341F and 518R according to (19, 29), using an annealing temperature of 55°C. qPCR standards (between $10^6$-$10^{10}$ molecules per reaction) were prepared using PCR products made from the *Variovorax paradoxus* type strain DSM30034. Two tailed students t-tests were performed to compare differences in cell equivalent numbers in the rhizosphere and bulk soils of the different cultivars at three growth stages over the two years of potato growth and bulk soil from barley field in 2009, before and after planting of potato in 2008.

Bacterial community analyses on PCR-denaturing gradient gel electrophoresis (DGGE) fingerprints. PCR amplifications were done on all DNA extracts from bulk over three years and rhizosphere soils taken at three growth stages over two growth seasons, using the DGGE primers GC-341 and 518R (35 cycles) as it is described previously (19). All DGGE profiles were generated in the Ingeny Phor-U system (Ingeny International, Goes, The Netherlands). The PCR products obtained from the soil DNAs, at estimated concentrations of ~200 ng, were loaded onto polyacrylamide gels [6% (wt/vol) acrylamide in 0.5x Tris-acetate-EDTA (TAE) buffer (2.42 g Tris-base, 0.82 g sodium acetate, 0.185 g EDTA, H$_2$O 1L)]. The bacterial amplicons were run on 35-65% denaturant gradient gels at 100V for 16h at 60°C. All gels were silver-stained (16) and air-dried, after which they were digitized for further analyses.

Pictures were taken from resulting DGGE profiles and stored as TIFF files for further analysis with GelCompar II software (Applied Maths, Sint-Martens-Latem, Belgium). Similarity matrices consisting of defined numbers within each gel were generated using Pearson’s correlation coefficient ($r$). Subsequently, the patterns were clustered using the unweighted pair group method with arithmetic averages (UPGMA) with GelCompar II software (Applied Maths, Sint-Martens-Latem, Belgium). Besides, data derived on the basis of band analysis, were used for redundancy analysis (RDA) using CANOCO (Microcomputer Power, Ithaca, NY) (19). Community similarities based on relative band intensities and position were analyzed by performing canonical correspondence analyses with Monte Carlo permutation tests (CANOCO 4.0, Microcomputer Power). The Monte Carlo tests were based on 499 random permutations of the data to establish statistical significance.

Moving window analyses (MWA) were used to calculate the rate-of-change parameter (Δt) for bulk soil during the season. First, the similarities of the densitometric curves of DGGE patterns were calculated based on the Pearson correlation coefficient. The percentage change (%change = 100 - %similarity) was recalculated as explained by Marzorati et al (23). The %change value matrix is used to perform moving window analysis by plotting the values between consecutive sampling points but also change between the first and last sampling moment was calculated.

Rhizosphere samples from 2008 and 2010 were also compared with GelCompar. Principal component analysis (PCA) was performed, and analysis of similarity (ANOSIM) using Euclidean distance. ANOSIM was carried out using PRIMER 6 (Primer v6, Plymouth), based on transformed data (fourth square). Samples were also grouped per growth stage and sampling year. Two-way crossed analyses with replicates were done with 5000 permutations. The global R value, varying between -1 and 1, was used. R=0 indicated
completely random grouping while $R=1$ indicated samples within a soil were more similar to each other than to any samples from the other soil. A significant global $R$ indicated that there are differences between soil types somewhere in the analysis.

Cloning and sequencing of 16S bacterial rRNA gene amplicons generated from selected samples. Six clone libraries of 16S bacterial rRNA gene fragments were generated with replicates (using primers 27F and 518R) from the bulk soils taken in April 2008 (before planting), in June 2008 (flowering of potato) in December 2008 (after removal of plants), in June 2009 (barley bulk soil during the flowering of potato plants), in July 2010 (flowering of potato) and September 2010 (during the senescence of potato plants) to be able to monitor the changes in the bacterial community make-up over three years. The amplicons were ligated into pGEM®-T easy vectors (pGEM®242-T Vector System II, Promega, Madison, WI, USA), followed by introduction into competent *Escherichia coli* JM109 cells by transformation according to the manufacturer’s instructions. White colonies (containing intact inserts) were picked and diluted in 10µl sterile water and used as template for PCR using the universal M13f/M13r vector primers. The samples were sequenced by LGC (Berlin, Germany). Differences in the community structures of clone libraries were analyzed with UNIFRAC (12), using maximum-likelihood-based and jackknife trees.

Sequences were checked for chimera using BELLEROPHON v.3 (http://greengenes.lbl.gov) (17). The gene sequences were compared with sequences in GenBank using nucleotide–nucleotide comparisons using BLAST (BLAST-N)-assisted searches in publically available databases to obtain the nearest phylogenetic neighbors (http://www.ncbi.nlm.nih.gov/BLAST). Sequences were then processed in MEGA 4 (31). Finally, an RDP library comparison was done at a confidence threshold level of 80% (6). The program DOTUR (28) was used to create rarefaction curves, where a conservative operational taxonomic unit’s cutoff of 97% similarity was used to determine the bias-corrected Chao1 estimator of richness.

Deposition of sequences. The sequences generated in this study were deposited in Genbank under numbers JF910290-JF911347.

Results

Plant development over the growth season. In 2008, the young plant stage [EC30] occurred around 30 days post-planting (dpp), i.e. at the end of June. The flowering stages [EC60] occurred between 50 and 60 dpp for cultivars D and P and between 80 and 85 dpp for cultivars A, Av, K and M (June). Finally, the senescence stages [EC99] were between 110 and 115 dpp for P and D, between 135 and 140 dpp for A and between 145 and 150 dpp for Av, K and M. In the second season (2010), flowering occurred between 50 and 60 dpp for all cultivars, and the senescence stage was around 120 dpp for all cultivars. Since the Premiere cultivar already started to decline after July 2010, these plants were removed from the field. The field was planted with barley in 2009 and sampled in June 2009 when barley plants were prior to flowering. This time in the season coincided with the flowering stage of potato grown in an adjacent field.
Dynamics of bacterial abundances in bulk and rhizosphere soils as assessed by 16S rRNA gene-based qPCR. The abundance of the bacterial populations, assessed in bulk soil samples over three years on the basis of the bacterial 16S rRNA gene, was in the range of between $3 \times 10^7$ to $8 \times 10^8$ gene copies / g soil. There was no clear trend in the dynamics of the bacterial cell numbers over the seasons. In April 2008, gene copy numbers initially dropped from $7.2 \times 10^8$ to $5.9 \times 10^7$, and then remained stable at levels of between $1.6 \times 10^8$ and $1.2 \times 10^8$ until September. Then, numbers dropped again to a level of $3.6 \times 10^7$ in December (Fig. 1) (19). In the next year, barley was grown on the field. The measurement taken at a time coinciding with the flowering stage of potato plants growing in an adjacent field revealed raised bacterial numbers, i.e. $1.17 \times 10^9$ gene copies / g soil in bulk soil. In 2010, the field was again cropped with potato. The bacterial numbers in bulk soil increased from $1.39 \times 10^7$ gene copies / g soil in May to $8.4 \times 10^8$ gene copies / g soil in September. It was interesting to note that, although there were fluctuations over the samples taken along the three years, the abundance found in September 2010 was very similar to that determined at the start of the experiment, in April 2008. With respect to the bacterial abundances in the rhizosphere, different trends were observed per cultivar over time in the two potato growth seasons on the same field (19). In the 2008 growth season, significant positive rhizosphere effects on total bacterial abundances were observed for cultivars A, M, P and D in the young plant stage and for all cultivars in the flowering stage (Fig. 2).

Considering the bacterial dynamics over time, in the 2008 growth season the total bacterial abundances increased from the young plant to the flowering stages, after which they decreased to the senescence stage for cultivars A, Av, K and M ($p<0.05$, except for cultivar A). In contrast, the bacterial abundance at cultivar D showed a significant increase from $1.4 \times 10^9$ gene copies / g soil in the flowering to $6.2 \times 10^9$ gene copies in the senescence stage.
stage. In addition, we also found significant effects of the rhizosphere for cultivars Av, K, M and D in the senescence stage. In the 2010 growth season, rhizosphere effects were observed in all growth stages (except Av, K at senescence stage). In the 2010 growth season, the total bacterial abundances showed an increasing trend, from young plant to senescence stage, for cultivars A (from 1.4x10^8 to 9.5x10^9 gene copies per g dry soil) and D (from 1.1x10^9 to 1.7x10^10 gene copies per g dry soil), whereas a decreasing trend was noted for cultivar Av, i.e. from 1.7x10^9 to 1.5x10^8 gene copies per g dry soil. The bacterial abundances at cultivars K and M increased from the young plant to flowering stage and decreased in the senescence stage.

![Figure 2](image)

**Figure 2** – Abundance of bacterial 16S rRNA genes in rhizosphere and bulk soil per growth stage in Buinen soil. Error bars indicate standard error. Black bars – young plant, grey bars – flowering, white bars – senescence

**Dynamics of bacterial diversity and community composition in bulk and rhizosphere soils as assessed by PCR-DGGE.** Bacterial PCR-DGGE patterns from bulk and rhizosphere soils generated from all four replicate plots revealed high within-treatment similarities per treatment (cultivar) and sampling moment (data not shown). This suggested generally low variability resulting from plot, sampling and sample processing.

We then applied moving window analysis to the fingerprinting data. First, the similarities of the densitometric curves of DGGE patterns were calculated based on the Pearson correlation coefficient. The percentage change (%change= 100 - %similarity) was recalculated as explained by Marzorati et al (23). The bacterial PCR-DGGE patterns generated from bulk soils revealed around 40% change over the 2008 season, based on
this analysis. Specifically, the patterns were different from those before planting in the B soil in June 2008, and differences increased in the patterns of July and September 2008 (Fig. 3). Besides, the patterns generated from bulk soil collected in December 2008 (after removal of plants) and June 2009 (barley) were again different from the foregoing ones (Fig. 3). Then, in May 2010 (young plant stage) the community had changed by 60% in comparison to June 2010, whereas the difference was only 20% (as compared to foregoing sampling moment) in July and in September 2010. An analysis of the total percentual change of the community structure from April 2008 to September 2010 indicated that this amounted to 60±15%. Conversely, this also indicated that at least 40% of the dominant community detected by DGGE, was stable over a period of three years. We also compared samples from May 2008 to May 2010, June 2008 to July 2010 and September 2008 and September 2010, since they represented the same growth stages of the potato plant. The change between May 2008 and May 2010 was 25±15%, whereas it was higher for June 2008 and July 2010 and also September 2008 and 2010 with 50±10% changes. This indicated similarities of about 50 to 75% in the structures of the communities that were compared. Moreover, the differences across two years might have been the result of the different fertilization regimes used (pig manure vs. mineral fertilizers), next to dependency on the growth stage of the plants.

Figure 3 – Moving window analyses to evaluate the percentage of community change for bacterial communities in B soil through different sampling times. (∆t) the rate of change was calculated as the average of the respective moving window curve data points

In all potato plant growth stages in the 2008 and 2010 growth seasons, the rhizosphere bacterial PCR-DGGE patterns grouped apart from the corresponding bulk soil patterns, thus indicating clear rhizosphere effects on the soil bacterial community compositions (19). In the 2008 growth season, the bacterial PCR-DGGE patterns from five of the six
cultivars (exception: cultivar A) grouped together in the young plant stage. However, in the flowering stage cultivars M, P and D grouped together (p>0.05), while A, Av and K clustered apart, each as a separate unit (p<0.05). In the senescence stage, two main clusters were obtained as in the flowering stage (19). In the young plant stage of the 2010 season, cultivars K, M, P and D grouped together, while A and Av clustered apart. On the other hand, in the flowering stage cultivars P and D (low starch) clustered together, whereas A, Av, K and M (high starch) grouped together. In the senescence stage, cultivar P could not be sampled, so it was excluded. Cultivars A, Av and K grouped together, while M and D formed another group (data not shown). Two main groupings of rhizosphere samples were observed in the two sampling years, that is, cultivars A, Av and K forming one group and cultivars P and D a second group. Cultivar M appeared to be grouped with one of the groups based on the growth stage.

**Analysis of bacterial 16S rRNA gene clone libraries generated from of bulk soil samples.**
Since the bacterial PCR-DGGE analyses had revealed clear differences between the communities in bulk soils collected at different time points, these samples were selected for the construction of six 16S rRNA bacterial gene clone libraries with two replicates each. After quality and chimera checks, totals of 185, 176, 181, 183, 164 and 170 sequences were obtained from bulk soils from April 2008 (before planting), June 2008 (flowering), December 2008 (after removal of plants), June 2009 (barley), July 2010 (flowering potato) and September 2010 (senescence potato), respectively. Libshuff analysis (30) showed that replicate libraries were similar to each other for each treatment (p<0.05), whereas different samples were significantly different from any of the other ones. Beside, fast unifrac analysis also showed significant differences between the bulk soil samples of different treatments (12). Rarefaction curves of the six analyzed samples were then generated to assess the depth of sampling and the richness of the libraries; 97% cut-off criteria were used for the grouping of the OTUs. Complete coverage of a data set would be expected to result in a plateau-shaped curve. Since none of the curves reached the plateau level (data not shown), the analyses suggested that the number of clones was insufficient to reach saturation and to describe completely the diversity of bacterial phylotypes in the bulk soils with the clone libraries obtained. Although we obviously did not sample to saturation, the rarefaction analysis did not show any significant effect of sampling time on the bacterial community. Based on the CHAO1 richness estimator, the estimated richness in the bulk soil December 2008 sample was highest (average value 86 / 180 analyzed clones), whereas bulk soil collected in June 2008 had the lowest richness estimation (49 / 177 analyzed clones). The rest had an estimation in the same range (70) – Fig. 4).

Using the RDP library and a comparison at a confidence threshold of 80%, the majority of the sequences was affiliated to recognized classes of bacteria, whereas the remainder was affiliated with as-yet-unclassified bacteria (17-24%; Fig. 5). There were significant differences in bacterial community make-up, with respect to the prevalence of particular groups, between the bulk soil samples. For instance, sequences assigned to the *Actinobacteria* accounted for 15 to 22% of the clones from the bulk soils of April 2008 (before planting), June 2008 (flowering), December 2008 (after removal of plants), June 2009 (Barley) and September 2010 (senescence potato), whereas this group made up 37%
of the amplicons generated from the bulk soil of July 2010. In contrast, sequences affiliated with the *Alphaproteobacteria* were highly dominant in the aforementioned bulk soil samples (30-40%, Fig. 5), while they made up only 19% of the clone library from June 2010 bulk soil (Fig. 6A). We observed that *Actinobacteria* and *Alphaproteobacteria* were apparently selected at the different growth stages, and reach up to the same level in total for each sampling moment (Fig. 6A). The prevalence of sequences affiliated with *Betaproteobacteria* and *Deltaproteobacteria* was quite stable over the season, whereas that of *Gammaproteobacteria* peaked in June 2008 (Fig. 6B). Besides, neither *Acidobacteria* nor *Gemmatimonadetes* appeared to be highly selected in June and September 2010, and *Firmicutes* showed a rapid increase in the June 2010 sample (Fig. 6B). Moreover, the relative abundances of *Gamma* - and *Delta-proteobacteria* were higher and that of *Bacteriodetes* lower in June 2008 than the general trend of the season. Also, the relative abundances of *Actinobacteria* and *Firmicutes* were higher and that of *Alphaproteobacteria* lower than the overall trends in July 2010 (Fig. 6).

![Figure 4](image_url) – CHAO 1 estimation over the sampling moments for 97% cut off. Bulk soils from April 2008 (before planting), June 2008 (flowering), December 2008 (after removal of plants), June 2009 (barley), July 2010 (flowering potato) and September 2010 (senescence potato).

The relative abundance data from June 2008 and July 2010 were found to be outliers of the general trend in the overall results: some phyla/classes were detected which were obviously lower or higher than the remainder, e.g. increased relative abundances of *Actinobacteria* in June 2008 and *Gammaproteobacteria* in July 2010 (Fig. 5).

Sixty-five genera were found altogether in the soils from the six sampling moments. *Acidobacterium* group 2 and *Bradyrhizobium* were the only two genera found at all
sampling moments. Besides, *Burkholderia* and *Gemmatimonas* were found in five out of the six samples, whereas *Blastococcus, Hyphomicrobium, Nitrospira* and *Acidobacterium* groups 1 and 16 were found in four out of the six samples analyzed. The April 2008 and June 2009 samples contained totals of 26 genera, whereas samples of December 2008, June 2008 and September 2010 encompassed only around 20. Besides, samples collected in July 2010 had 29 genera in total. Interestingly, *Arthrrobacter, Streptomycetes, Rhodanobacter* and *Dokdonella* were only found in the presence of potato plants (June 2008, July 2010 - flowering), whereas *Ktedonobacter* was specific to potato bulk soil, independent of time of sampling. *Rhodoplanes* and *Sporosarcina* were only observed in the bulk soil from barley. Sequences of *Aqicella, Acidobacteria Group 7, Methyllobacterium, Patulibacter* and *Phenylbacterium* were found to be specific to samples from December 2008.

**Discussion**

The results of this study showed that the bacterial communities in one field under a short potato rotation scheme (potato-barley-potato) are dynamic and undergo significant changes at temporal scales related to the seasonal measures. Specifically, our results provided evidence for the contention that (1) time is consistent with taxon-specific compositions of the microbial communities, (2) the taxa *Alphaproteobacteria* and *Actinobacteria* are commonly enriched in the soil under study, being inversely related to each other, (3) the soil fertilization regime may contribute to differential diversities and abundances of rhizosphere bacterial communities, (4) the composition of bacterial communities in soil is linked to the tuber starch content in different cultivars/GM line. There is still conflicting evidence as to whether plant communities can influence the bacterial distribution across fields or whether soil (type) exerts the strongest influence in this respect (5, 9). However, there is growing evidence that the microbial community structure in soil is influenced by plants (8, 18). In our study, a clear rhizosphere effect on the potato-associated bacterial communities was observed. We here posit that this effect cannot be directly associated with the bacterial community fluctuations observed in the bulk soil, which may be more strongly influenced by effects of temperature, moisture, tillage and plant type.

In order to understand the effects of seasonal variation on the bacterial community composition and diversity, clone libraries prepared from the field from six different time points — spanning three years — were compared. The six libraries varied in the diversity estimates and the data showed that the soil bacterial consortia are actually quite variable. In the study, some phyla became more dominant at particular sampling moments, but dropped in prevalence at others. For instance, *Alphaproteobacteria* and *Actinobacteria* became dominant at contrasting sampling moments. Where the *Actinobacteria* became highly prevalent in July 2010, the *Alphaproteobacteria* showed a significant decrease. A contrasting case occurred between the two groups in September 2010.
Figure 5 - Normal operating range (NOR) of different phyla and class in the bulk. Boxes show the upper (75%) and the lower (25%) percentiles of the data. (A) Actinobacteria, Alphaproteobacteria and uncultured Bacteria, (B) Beta-, Gamma-, Delta-, uncultured proteobacteria, Firmicutes, Acidobacteria, Gemmatimonadetes, (C) Bacteroidetes, Planctomycetes, Chloroflexi, TM7, W53, bacteria incertae sedis, Nitrospira. Whiskers indicate the highest and the lowest values (SPSS statistics 16). Outliers were also indicated. bf08-june 2008, flowering of potato; bf10 – june 2010, flowering of potato; bsn10-September 2010, senescence of potato; after- December 2008, after removal of potato plants.
Hence, it is plausible that such groups compete for similar or almost similar niches, giving a twist to one group in one sample, but to the other one in another, and never to the two groups simultaneously. Moreover, the relative abundance data from June 2008 and July 2010 were outliers of the general trend in the overall results. Besides, the sample from June 2008 (during flowering of potato) had the lowest number of genera, whereas those of July 2009 (barley) and June 2010 (during flowering of potato) had the highest number of genera. This might be due to the combined effects of (1) the presence of a plant, (2) plant type and (3) environmental conditions such as temperature and moisture. Besides, the presence of some bacterial types was plant-dependent, as we found Arthrobacter, Streptomyces, Rhodanobacter and Dokdonella only during the flowering of potato in both seasons. In contrast, Rhodoplanes and Sporosarcina were only observed in the bulk soil under barley. Arthrobacter has been defined as a plant-growth-promoting bacterium (PGPB), whereas Streptomyces species are key transformers of organic material in the soil, but particular species also cause (scab) disease in potato (27). The drop in the prevalence of Streptomyces types in the other plant growth stages can be a good plea for crop rotation, reducing particular organisms (including plant pathogens) in the soil community. In addition, the PCR-DGGE analysis also revealed differences between the potato-planted bulk soils of the different years. This also provided evidence for the contention that environmental factors (temperature, rain fall) and practices such as digging and application of manure have an additional effect on the soil bacterial community other than the presence of a plant. Moreover, crop rotation might have diversified the microhabitats in the field resulting diverse soil microbial communities. Rhizosphere and cultivar effects were also observed in different ways in the two years when potato was on the field (2008 and 2010). The same cultivars growing in the two different years did not show the same trends. It was also seen that plants at the same growth stages in different years clustered apart. The differences in abundance and community make up might have been caused by the different levels and types of manure application. In the first year, mineral fertilizer was applied, whereas pig manure was applied in the beginning of 2010. This change in the fertilization regime might also have affected the observed growth rate of the plants. Previously, it has been shown that different fertilizers contributed differentiation of rhizosphere microbial communities as well as the abundances of specific microbial groups (1). This might have been mainly due to their different nutrient contents and microbial loads (1). This different level of nutrients might also have caused the differences between the microbial communities of different cultivars that were already in the young plant stage in the 2010 field experiment. This was in contrast to the grouping of all cultivars in the young plant stage in 2008.
Figure 6 – Dynamics of dominating phyla and class (A) Actinobacteria, Alphaproteobacteria and unclassified bacteria, (B) Beta-, Gamma, Deltaproteobacteria, unclassified Proteobacteria, Acidobacteria and Firmicutes.

In this context, the community structure and diversity of the soil microbiota are important determinants of the stability of the key functions of soil, even though the relationship between functioning and microbial diversity in soil is not well defined (13, 21). In this study, although the number of clones was insufficient to reach saturation and to describe completely the different bacterial phylotypes, a major shift in the community composition was observed. Moreover, PCR-DGGE analysis showed that, next to the differences found by moving window analysis, 60% of the community was actually quite stable over the 30 month period. Since PCR-DGGE provides information on organisms down to about 0.1% of the total community (24, 32), a core group of the dominant community members remained relatively stable. Acidobacteria group 2 and Bradyrhizobium also may belong to the core community because they were found in clone libraries over all sampling periods.
This highlighted the existence of a dominant core group in the soil under potato rotation, next to a more variable bacterial group.

Effect of tuber starch content on the community as groupings of high starch and low starch cultivars, especially in the flowering and senescence stage was observed. Although GM line M grouped sometimes apart from its parental cultivar K, it grouped with low starch cultivars. It indicated that the impact of the GM plant M on the plant-associated bacterial communities was only transient and minor. In fact, it was comparable with the dominant variations caused by soil type, plant genotype and vegetation stage. Thus, GM cultivar M fell in the value range of the other cultivars, for all measured variables in the two growth seasons in 2008 and 2010. Thus, it did not leave any relicts of its presence, like a conspicuous and/or lasting effect on the soil bacterial community structure, diversity or abundance.

Acknowledgements

We thank Elco Hoogwout and Emilia Hannula for their help with the soil sampling. The work was executed in fields laid out by Avebe (Foxhol, NL), and we gratefully acknowledge Paul Heeres and Peter Bruinenberg for their help in the field work. This work was supported by the NWO-ERGO program awarded to JDvE.

References
