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ORIGINAL ARTICLE

Species sorting and neutral processes are both important during the initial assembly of bacterial communities

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Many studies have shown that species sorting, that is, the selection by local environmental conditions is important for the composition and assembly of bacterial communities. On the other hand, there are other studies that could show that bacterial communities are neutrally assembled. In this study, we implemented a microcosm experiment with the aim to determine, at the same time, the importance of species sorting and neutral processes for bacterial community assembly during the colonisation of new, that is, sterile, habitats, by atmospheric bacteria. For this we used outdoor microcosms, which contained sterile medium from three different rock pools representing different environmental conditions, which were seeded by rainwater bacteria. We found some evidence for neutral assembly processes, as almost every 4th taxon growing in the microcosms was also detectable in the rainwater sample irrespective of the medium. Most of these taxa belonged to widespread families with opportunistic growth strategies, such as the *Pseudomonadaceae* and *Comamonadaceae*, indicating that neutrally assembled taxa may primarily be generalists. On the other hand, we also found evidence for species sorting, as one out of three media selected a differently composed bacterial community. Species sorting effects were relatively weak and established themselves via differences in relative abundance of generalists among the different media, as well as media-specific occurrences of a few specific taxa. In summary, our results suggest that neutral and species sorting processes interact during the assembly of bacterial communities and that their importance may differ depending on how many generalists and specialists are present in a community.
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Introduction

Understanding the mechanisms underlying the assembly of bacterial communities is a major goal in microbial community ecology. Community assembly can be either regulated by the local environment (so-called species-sorting), including selection of taxa by abiotic conditions and inter-specific competition, or by regional factors related to dispersal that determine rates of immigration to the local community. Species sorting has shown to be important in many bacterial communities, ranging from those in lakes (Van der Gucht *et al.*, 2007; Logue and Lindström, 2010) and soils (Fierer and Jackson, 2006) to rock pools (Langenheder and Ragnarsson, 2007), sunken wood particles (Palacios *et al.*, 2009), human skin (Costello *et al.*, 2009) and human gut (Trosvik *et al.*, 2010). On the other hand, it is frequently observed that local bacterial communities are dominated by taxa that are also dominant at the regional scale (Sloan *et al.*, 2006; Woodcock *et al.*, 2007; Östman *et al.*, 2010). In other words, widespread and abundant taxa seem to be the ones that are also most abundant at the local scale. This so-called regional invariance fits well with the prediction made by the neutral theory (Hubbell, 2001), according to which species share similar traits and fitness. Thus, local community composition is mostly regulated by immigration and emigration, as well as random genetic drift leading to speciation and extinctions. Regional invariance should also be predominant if bacterial communities consisted of generalists with wide niche widths, that is, taxa that occur over a wider range of local environmental conditions (Östman *et al.*, 2010, and references therein).

Hence, we are currently facing a situation where we have studies that provide support for both species-sorting and neutral dynamics in bacterial communities, and only very few studies have made the explicit attempt to study both mechanisms at the same time (van der Gast *et al.*, 2008; Ofiteru *et al.*, 2010). A special situation that will help us to

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understand the principle mechanisms of bacterial community assembly arises during the colonization of new, that is, sterile, or heavily disturbed habitats by atmospheric bacteria. Assembly patterns of bacterial communities have been studied in sterile environments, such as pitcher plants (Koopman *et al.*, 2010) and infant guts (Trosvik *et al.*, 2010). Others have looked at the re-assembly of bacterial communities in the overlaying water column from dried sediments (Fazi *et al.*, 2008), as well as in re-wettened soils after droughts (Fierer *et al.*, 2003; Clark *et al.*, 2009), all of them, however, without specific focus on testing underlying community assembly mechanisms. The scenario where a pristine or heavily disturbed habitat is (re)-formed from scratch from atmospheric deposition offers the opportunity to specifically investigate the importance of species sorting and neutral processes during early assembly from a common species pool, that is, in a stage during community development where local competition and source-sink dynamics are presumably of minor importance.

The major aim of this study was therefore to investigate whether species sorting or neutral processes predominate during the early assembly of microbial communities. For this we implemented an experiment with outdoor microcosms consisting of three treatments, which contained sterile medium from three different rock pools representing different environmental conditions. Rock pools were chosen because, as other natural microcosms, they offer very suitable model systems to study community assembly patterns (Srivastava *et al.*, 2004), in particular as they go through repeated desiccation and community re-assembly cycles each year. Thus, the microcosm study performed here fulfills two purposes. First, it enables us to make some general statements about the assembly of bacterial communities in 'empty' local environments; second, it will help us to better understand assembly patterns of bacterial communities in natural rock pools and other temporary water bodies. Once prepared in the lab, the microcosms were placed back into their natural environment and opened until they were 'seeded' by rainwater and then incubated under constant temperature conditions for 5 days to allow sufficient build-up of biomass that was not yet top-down controlled by predators. Bacterial community composition in the microcosms was measured by 454-pyrosequencing of the *16S rRNA* gene and similarities between microcosms, as well as with the original pool and rainwater communities were determined.

We hypothesise the following: If species sorting effects are strong this should lead to growth of different communities in microcosms with different media. Moreover, if species sorting effects are very strong, at least some of the taxa growing in the microcosms should resemble those in the respective source pools. This assumes that the global diversity is high (Fenchel and Finlay, 2004) so that a considerable fraction of 'habitat specialists' from the original pool communities are present in the rainwater inoculum and able to readily colonise and grow in the microcosms. If, on the contrary, neutral effects are strong, community composition in microcosms based on different media should not differ from each other. Moreover, taxa that occur at high relative abundances in the source community (rain) should also be found at high relative abundance in the microcosms.

Material and methods

Experimental set-up

Water was collected from three freshwater rock pools from Marskäret in Sikhälma (N60°34′45.5″; O17°49′2.25″) located on the Hållnäs peninsula at the Swedish Baltic Sea Coast on August 20, 2008. The pools were chosen to differ clearly with regard to basic water chemical parameters (Table 1). Briefly, Pool 1 had high chlorophyll a concentration, Pool 2 was humic and relatively nutrient rich, whereas Pool 3 was clear and relatively nutrient poor. The water was sequentially filtered through a GF/C (Whatman) and a 0.2 μm Gelman Membrane Supor filter (Pall, Lund, Sweden) and autoclaved. As autoclaving increased the pH in the water, it was set back to initial levels with hydrochloric acid and autoclaved again, after which the pH remained stable. A measure of 200 ml of water from the three pools, from now on called medium 1, medium 2 and medium 3, were filled into sterile microcosms (Kilner jars), replicated six times, and stored at room temperature until the set-up of the experiment. The experiment was implemented on August 28 at the same site from where the water was collected. The microcosms were placed out randomly in a 5×5 metre grid consisting of 400 squares and opened. They were placed at an elevated position ~30 metres from the coastline to protect them from sea-spray. We also included controls (three for each medium), which consisted of unopened microcosms, and rain collectors, which were simply empty sterile microcosm (six in total). The aim was to wait until the microcosms had been inoculated with rainwater at ~10% of the total volume in the microcosms. After some minor drizzle throughout the afternoon, there was a heavy rain shower

Table 1 Some characteristics of the three rock pools used for the preparation of the media

Pool	*pH*	*Temp (°C)*	*Conductivity (μS m⁻¹)*	*Abs₄₃₆*	*Chla (μg l⁻¹)*	*Tot-P (μg l⁻¹)*	*BA (10⁶ ml⁻¹)*
1	7.28	22.5	199.2	0.057	627	20.8	10.9
2	6.55	22.5	90.5	0.249	71	62.5	14.0
3	7.49	22.1	175.5	0.048	0	14.6	4.58

Abbreviations: Abs_{436}, absorbance at 436 nm; BA, Bacterial abundance; Chla, chlorophyll a; Temp, temperature; Tot-P, total phosphorus.
All measurements were made before the water was sterilized.

~8 h after the start of the experiment, and the experiment was ended briefly after its onset. In total the microcosms had been exposed for 8.5 h to atmospheric deposition. During this time period 38 ± 11 ml (range: 31–45 ml) had accumulated in the rain collectors, that is, the microcosms were inoculated with ~20% (v/v) of rainwater. The microcosms were then closed, transported to the laboratory and incubated for 5 days in the dark at 20 °C, which was close to the *in situ* temperature in the pools at the time of sampling. The microcosms were incubated in the dark to specifically focus on the early assembly of heterotrophic bacteria without interference from photosynthetic organisms.

Abundances of bacteria and heterotrophic nanoflagellates (HNFs)

For estimation of abundances of bacteria and heterotrophic flagellates (HNFs), samples from the microcosms, rain collectors and controls were preserved with 4% formaldehyde (final concentration v/v) and counted under the epifluoresence microscope after staining with DAPI (4,6-diamidino-2-phenyl indole) as described (Langenheder and Jürgens, 2001). Pearson's product moment correlations between bacterial and HNF abundance were calculated at the end of the experiment to test for potential predation control of bacterial abundance.

Nucleic acid extraction and 454 sequencing

For DNA extraction, 150 ml of water from the microcosms and the original pools were filtered onto 0.2 μm Gelman Supor filtered and stored at −80 °C. For the rainwater sample water from six replicate rain collectors was pooled and filtered to obtain one composite sample. Nucleic acids were then extracted with the Easy DNA kit (Invitrogen, Paisley, UK) following the protocol #3 for small amounts of cells, but included an extra bead-beating step using 0.2 ml of 0.1-mm silica beads. For 454-pyrosequencing, the V3 and V4 regions of bacterial *16S rRNA* genes were amplified using the B-341 forward primer (5′-CCTACGGGNGGCWGCAG-3′) and the A-barcode-805 reverse primer (5′-GACTACHVGGG TATCTAATCC-3′) where A and B are the 454 Life Science's sequencing adapters fused to the 5′ end of the respective primer and the barcode is a sample specific pentameric tag. PCR reactions were performed in three separate 20-μl reactions using ~0.05 ng μl^{-1} final concentration of template DNA and Phusion high-fidelity DNA polymerase (Finnzymes, Espoo, Finland) according to the protocol of the manufacturer. Cycling conditions were as follows: an initial denaturation step at 95 °C for 5 min; followed by 25 cycles of 95 °C 40 s, 48 °C for 40 s, and 72 °C for 1 min; and a final 7-min extension at 72 °C. After purification with the QIAquick PCR Purification Kit (Qiagen, Hilden, Germany) and equal mixing of the barcoded PCR products, pyrosequencing was performed on a 454 GS20 FLX platform at KTH Biotechnology Sequencing Center (Stockholm, Sweden). Sequences that passed through the quality filter of 454 software were processed using in-house developed Perl scripts (Andersson *et al.*, 2010, http://www.perl.org). Briefly, sequences were grouped by barcodes and trimmed to equal length (230 bp without primers). Sequences were aligned and clustered into 2% dissimilarity operational taxonomic units (OTUs) using the ribosomal database project (RDP) pyrosequencing pipeline (http://pyro.cme.msu.edu/). Closest relatives were identified with BLASTN (Altschul *et al.*, 1997) against 986907 bacterial 16S rRNA sequences with good Pintail scores downloaded from RDP release 10, update 17 (RDP; http://rdp.cme.msu.edu/) (Cole *et al.*, 2003). Only sequences with at least 85% similarity to their closest RDP sequence match were included in further analyses. All selected sequences have been deposited to GenBank under accession numbers HQ144256—HQ147562. Before the statistical analyses described below the samples were normalised in the following way: the relative abundance of each OTU was calculated and OTUs with a lower relative abundance compared than that corresponding to one sequence in the sample with the lowest total sequence number were excluded from further analyses. In addition, to remove biases from sequencing errors, OTUs with only one sequence in one out of the six replicates (singletons) were also deleted except from the unreplicated original pool and rainwater samples.

Rank abundance lists for the 10 most abundant OTUs were prepared for each microcosm, and the corresponding rank of each of the most abundant taxa in each other treatment, the rainwater and original pool community was recorded as well (Table 2).

Statistical analysis

Detrended correspondence analysis, based on relative abundance data, was used to explore differences in community composition between all samples. Differences in bacterial community composition among treatments were tested by one-way analysis of similarities (ANOSIM). To remove the weight of rare species, we used a cut-off of 1 % relative abundance for at least one replicate, that is, OTUs that did not achieve higher abundances than 1% in at least one out of six replicates were removed. Bray–Curtis similarities were used and the significance test was done using 10 000 permutations. *P*-values in pairwise comparisons were Bonferroni corrected. Analysis of variance (ANOVA) was used to test differences between treatments with regard to bacterial abundance and origin of OTUs. Before this, the data was checked for normality

Table 2 List of the 10 most abundant OTUs and their taxonomic affiliation in each treatment and their abundance ranks

Best RDP	*Taxonomy*	*Type*	*M 1*	*M 2*	*M 3*	*Source*
S001681641, 97.8%	*Betaproteobacteria; Burkholderiales; Oxalobacteraceae*	Uncultured-bacterium clone from Yunnan snub-nosed monkey feces	1	1	1	RW (1), P2(<20)
S001869626, 100%	*Betaproteobacteria; Burkholderiales; Comamonadaceae; Curvibacter*	Uncultured bacterium clone from subsurface saline soil	2	2	3	P3 (<20)
S001028821, 100%	*Betaproteobacteria; Burkholderiales; Comamonadaceae; Delftia*	Uncultured bacterium clone from human skin	3	4	5	n.a.
S000414590, 100%	*Betaproteobacteria; Burkholderiales*	*Leptothrix discophora* (strain SS-1)	4	7	2	P2 (1), P3 (1), RW (8), P1 (<20)
S001022016, 100%	*Gammaproteobacteria; Pseudomonadales; Pseudomonadaceae*	Uncultured *Pseudomonas sp.* clone from cultivated banana plant	5	17	<20	n.a.
S001341834, 100%	*Bacteroidetes; Sphingobacteria; Sphingobacteriales; Cytophagaceae; Emticicia*	Uncultured *Bacteroidetes* bacterium clone from eutrophic lake	6	5	4	n.a.
S000643270, 100%	*Bacteroidetes; Sphingobacteria; Sphingobacteriales; Sphingobacteriaceae; Pedobacter*	Uncultured bacterium clone from *Typha* rhizosphere in constructed wetlands	7	3	6	P1 (<20)
S000426486, 100%	*Gammaproteobacteria; Pseudomonadales; Pseudomonadaceae*	*Pseudomonas sp.* 'LV3 Naranja'	8	9	<20	RW (3)
S001354598, 100%	*Bacteroidetes; Flavobacteriales; Flavobacteriaceae; Flavobacterium*	Uncultured *Flavobacterium sp.* clone from freshwater lake at altitude of 4082 m	9	12	15	n.a.
S001602778, 100%	*Betaproteobacteria; Burkholderiales; Comamonadaceae*	Uncultured bacterium DGGE gel band from *Dendroctonus valens*	10	13	14	P1 (10)
S001341613, 98.7%	*Bacteroidetes; Sphingobacteria; Sphingobacteriales; Cytophagaceae*	Uncultured *Bacteroidetes* bacterium clone from eutrophic lake	n.a.	6	n.a.	n.a.
S001384209, 100%	*Betaproteobacteria; Burkholderiales; Oxalobacteraceae; Janthinobacterium*	Uncultured bacterium clone from ear punch biopsy from mouse	12	8	10	RW (<20)
S000768483, 100%	*Gammaproteobacteria; Pseudomonadales; Pseudomonadaceae; Pseudomonas*	*Pseudomonas savastanoi pv. savastanoi* (plant pathogen)	19	10	<20	RW (13)
S001785874, 100%	*Gammaproteobacteria; Pseudomonadales; Moraxellaceae; Acinetobacter*	Uncultured *Acinetobacter sp.* clone from raw source water	<20	<20	7	n.a.
S001682567, 100%	*Bacteroidetes; Flavobacteriales; Flavobacteriaceae; Flavobacterium*	Uncultured *Bacteriodetes* bacterium clone from soil	<20	18	8	P2 (6), P1 (<20), P3 (<20), RW (<20)
S001794657, 99.1%	*Betaproteobacteria; Burkholderiales; Comamonadaceae*	Bacterium strain SU4 from iron ore	<20	n.a.	9	n.a.

Abbreviations: OTUs, operational taxonomic units; RDP, ribosomal database project.
Rank '1' means that the OTU was the most abundant one in the sample, '2' that it was the second most abundant one and so on. Indicated is also whether and at which rank the OTU could be found in the source community (rainwater, RW) or any of the original pools (P1, P2, P3). The ranks were calculated based on the average of relative abundance values from six replicate microcosms. n.a. means that the OTU was not detected, <20 indicates that it was detected, but rare. 'Best RDP' refers to the RDP sequence number of the closest relative in the RDP database. 'Type' is a summary of the information that could be retrieved about the closest relative from the database.

(Shapiro–Wilk test) and homogeneity of variances (Levene's test). All analyses were carried out using the PAST software package (Hammer *et al.*, 2001).

Results

Abundance of bacteria and HNF

The bacterial abundance in rainwater was $3.60 \pm 0.64 \times 10^4$ ($n = 6$). Hence, each microcosm was inoculated by roughly 1.4×10^6 cells, yielding an estimated initial concentration of roughly 7000 cells ml^{-1}. After 5 days of incubation, bacterial abundances differed significantly between the different media (One-way ANOVA, $F_{2,15} = 98.44$, $P < 0.0001$, with significant Tukey's pairwise comparisons in all cases) and had increased to $3.08 \pm 0.29 \times 10^6$ ($n = 6$) in microcosms with medium 1, $3.79 \pm 0.52 \times 10^6$ ($n = 6$) in microcosms with medium 2 and $1.02 \pm 0.24 \times 10^6$ ($n = 6$) in microcosms with medium 3 (Figure 1). Importantly, there were no significant correlations between bacterial and HNF abundances (Figure 1). This shows that bacterial communities in the microcosms were not influenced by predation by HNF when the experiment was stopped indicating that top-down control did not interfere with our interpretation of species sorting and neutral processes during community assembly.

Bacterial community composition

A total number of 20 043 good quality sequences corresponding to 3308 unique 230 bp long sequences were obtained from the 21 samples analysed by 454-pyrosequencing (average number of sequences per sample: 933; minimum: 295; maximum: 2088). Those sequences could be assigned to between 51–337 OTUs before and 23–55 OTUs

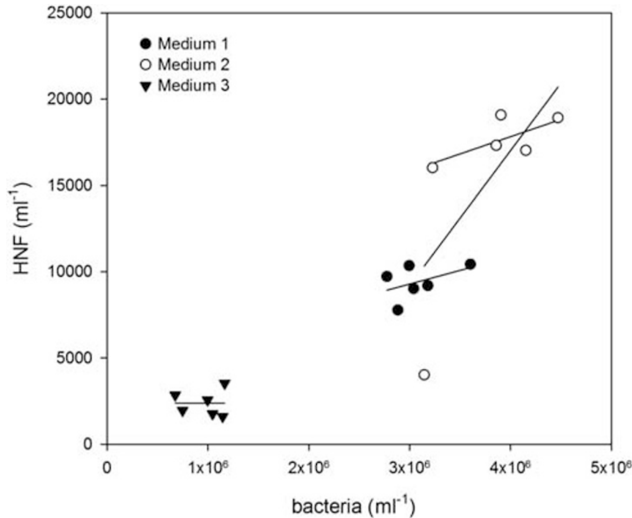

Figure 1 Linear regressions for bacterial versus HNF-abundances in the microcosms. For medium 2, two regressions with and without the outlier value, are included. Medium 1: $y = 0.016x + 4536$, $r^2 = 0.217$. Medium 2: first equation, $y = 0.078x - 1430$, $r^2 = 0.508$; second equation, $y = 0.019x + 9834$, $r^2 = 0.49$. Medium 3: $y = 7.7 \times 10^6 x + 2373$, $r^2 \sim 0$. Pearson's product moment correlations were not significant in any case.

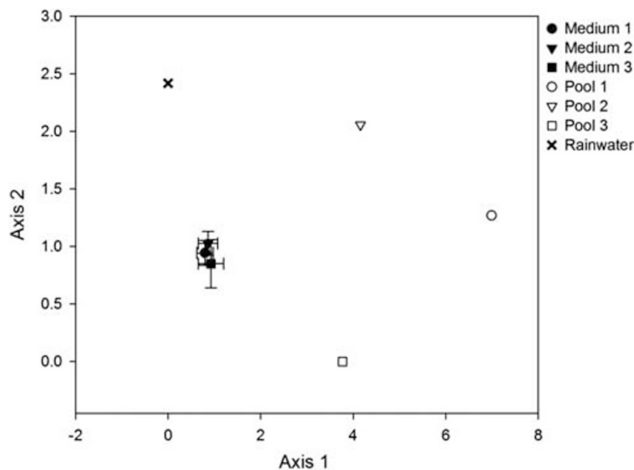

Figure 2 Detrended correspondence analysis-plot showing differences in bacterial community composition between the microcosms, the rainwater samples and the original pools. For medium 1, 2 and 3 error bars indicate s.d. from the mean ($n = 6$). The eigenvalue is 0.852 for the first axis and 0.201 for the second.

after normalisation and removal of singletons, and, despite the short sequence length, almost half of the OTUs (41%) could be classified to genus level. There were clear differences in community composition between the original pools, the rainwater sample and the microcosms, and the communities growing in the microcosms were more similar to the rainwater sample than to any of the original pool communities (see separation along axis 1 in Figure 2). Communities in microcosms with medium 3 were significantly different in composition compared with the microcosms with medium 1 and 2 (One-way ANOSIM, $R = 0.5449$; $P < 0.001$).

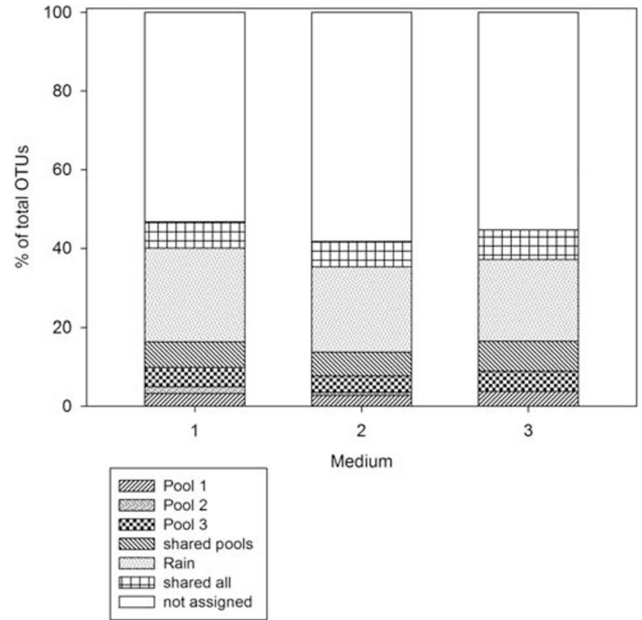

Figure 3 Origin of OTUs (% of total) in microcosms based on media 1, 2 and 3. The following fractions are shown: the three original pools (Pool 1, Pool 2 and Pool 3), the rainwater sample (Rain), OTUs that were detected in more than one pool (shared pools), OTUs that were found in the rainwater, as well as in at least one of the pools (shared all), and finally, those that were not detected in any of the pools nor the rainwater (not assigned). The fractions were calculated based on the presence or absence of an OTU in the respective source community and mean values calculated from six replicate microcosms are shown.

More than 40% of all OTUs detected in the microcosms were found in one of the original pools, the rainwater, or both, respectively (Figure 3). On average 22 ± 5% ($n = 18$, minimum: 12%; maximum: 35%) of the OTUs in the microcosms were detected in the rainwater only (Figure 3). According to a one-way ANOVA there were no significant differences between the three media with regard to the fractions of OTUs that could be tracked back to OTUs in the rainwater or original pool communities. There was a consistent shift in relative abundance of some of the major phylogenetic groups growing in the microcosms. Although *Betaproteobacteria* and *Bacteriodetes* were the most abundant groups in the original pools at approximately similar relative abundances, this ratio shifted towards a stronger dominance of the *Betaproteobacteria* in the microcosms, even though the *Bacteriodetes* still remained the second most abundant phyla in the microcosms (Figure 4). Moreover, the relative abundance of *Gammaproteobacteria* increased in the microcosms compared with the original pools. Notably the relative abundance of *Gammaproteobacteria* was quite high in the rainwater samples, which generally showed a higher phylogenetic richness and evenness compared with any of the pools or microcosms (Figure 4). Irrespective of the origin of the medium, *Alphaproteobacteria*, *Actinobacteria* and *Cyanobacteria* decreased in relative abundance

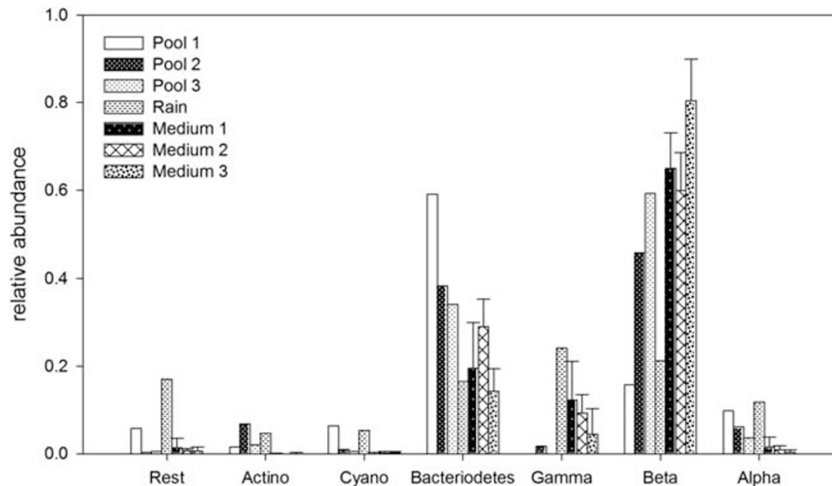

Figure 4 Relative abundance of different phyla in microcosms, rainwater and pools. Error bars indicate s.d. from the mean ($n=6$). Alpha, *Alphaproteobacteria*; Beta, *Betaproteobacteria*; Gamma, *Gammaproteobacteria*; Cyano, *Cyanobacteria*; Actino, *Actinobacteria*.

in comparison with the original pools and the rainwater (Figure 4).

In general, there was considerable overlap in the most abundant OTUs growing in the microcosms, that is, the majority of OTUs growing in one medium were also found at high abundance ranks in the other media (Table 2). About half of those dominant OTUs were detectable also in the rainwater and/or any of the original pools, whereas the other half was not (Table 2). Notably, the most abundant OTU in all microcosms (relative abundances in medium 1: 19.5 ± 5.7%, in medium 2: 16.0 ± 4.4%, in medium 3:42.2 ± 13.3%) was also the most abundant taxon in the rainwater (12%) (Table 2). The second most abundant OTU (medium 1: 12.9 ± 10.4 %, medium 2: 14.3 ± 6.8 %, medium 3: 6.4 ± 5.5%), however, was not found in the rainwater sample, but instead detectable in one of the original pools at low abundance (<0.5%) (Table 2). It was also remarkable that OTUs growing in the microcosms had closest relatives that originated from a vast variety of different habitat types (Table 2). Moreover, a large proportion of the most abundant OTUs belonged to various families within the orders *Burkholderiales, Pseudomonadales* and *Sphingobacteriales,* and particularly the families *Pseudomonadaceae*, *Comamonadaceae* and *Flavobacteriaceae.*

Discussion

The major aim of this experiment was to investigate whether species sorting or neutral effects predominate during the early assembly of bacterial communities in pristine local environments and we found indications that both mechanisms are important. There was evidence that neutral effects had a strong role during early community assembly in our experiment. This was suggested by the fact that the proportion of OTUs growing in the microcosms that was also present at detectable numbers in the rain was considerable: almost every 4th OTUs growing in the microcosms was also found in the rainwater, and the majority of them was unique, that is, did not overlap with OTUs found in any of the original pools. This indicates that many of the taxa that seeded the microcosms were readily growing independent of the local environmental conditions, that is, the medium in the microcosms. A strict application of the predictions made by the neutral theory would mean that rank abundance curves should be the same in the rainwater and in the microcosms (Sloan *et al.*, 2006), that is, a taxon that occurs with a certain relative abundance in the source community should be found at a very similar relative abundance in the microcosms and ranks among taxa should be conserved, that is, the most abundant OTU in the rain should also be the most abundant one in the microcosms and so on. Even though the most abundant taxon in the microcosms was also the most abundant one in the rainwater sample, the subsequent ranks did not follow the predicted pattern in a consistent way. Hence, the neutral model did not apply to 100%, which indicates that other community assembly mechanisms have operated at the same time.

It was interesting to see that the abundance of OTUs that belong to phyla with opportunistic growth strategies and many culturable representatives, such as the *Betaproteobacteria, Gammaproteobacteria* and *Bacteriodetes* (Pernthaler and Amann, 2005; Fierer *et al.*, 2007) was relatively high in the rainwater and that those phyla were readily growing and achieving high relative abundances in the microcosms. The finding of preferential growth of *Beta*- and *Gammaproteobacteria* in the microcosms agrees well with findings from another study where those two groups were also readily growing in microcosms based on water from four mountain lakes that were inoculated with

atmospheric dust from two sources (Hervas *et al.*, 2009). It was also interesting to note that the vast majority of the most abundant OTUs in the microcosms belonged to families, which are globally widespread and present in many different environments, namely the *Pseudomonadaceae*, *Comamonadaceae* and *Flavobacteriaceae* (Tamames *et al.*, 2010). The origin of the closest relatives of OTUs growing in the microcosms was very diverse, ranging from soil, freshwater over plant pathogens, to human skin and animal fecal samples and reflects the high phylogenetic diversity and various origins that are typical for bacterial communities in the air (Fierer *et al.*, 2008; Hervas and Casamayor, 2009; Fahlgren *et al.*, 2010).

Jones and McMahon (2009) found that immigration by atmospheric bacteria did not strongly influence bacterial community composition in seepage lakes. However, the situation in our experiment was obviously different from that found in natural systems, as immigrants did not need to compete with a resident community. Accordingly, Reche *et al*. (2009) found that dust addition did not change the composition of lake water communities, but let to growth of distinct communities on sterile-filtered water from the same lake. Our results nevertheless indicate that atmospheric deposition might have an important effect on bacterial community structure in systems that are small (pools, ponds and so on) and/or when deposition rates are high (such as during heavy rainfall and storms) and fuel those systems with fast growing, generalists that readily exploit the available resources.

There were also indications for species sorting to be operating during the early assembly of bacterial communities. Accordingly, medium 3 lead to the growth of communities that were significantly different in composition compared with medium 1 and 2, which, on the other hand, did not differ in composition. We also detected OTUs from the original pools in the microcosms. These were, however, generalist taxa that occurred in all microcosms irrespective of the source of the medium. Thus, even though we found species sorting processes, they were relatively weak and established themselves via differences in relative abundances of generalist taxa among the different media, as well as media-specific occurrences of a few specific taxa. It has been shown earlier that species sorting has strong effects on specialists, whereas regional forces (patch or neutral dynamics) are stronger for generalists (Pandit *et al.*, 2009). Hence, the lack of strong species sorting effects could be because of the fact that specialists are disfavoured during the growth in initially empty patches, which selected fast growing, opportunistic species. Another reason for the relatively weak species sorting effect could be that the local habitat, that is, the local conditions, did not differ enough from each other. At the first glance, this seems unlikely, as there were clear differences between the three media with regard to organic carbon concentration and composition, which are known to have strong effects on bacterial community composition in aquatic systems (Judd *et al.*, 2006; Kritzberg *et al.*, 2006). However, it is important to point out that the perception of habitat differences differs between specialists and generalists. There were several dominant OTUs, which were found in all three media that were undetectable in any of the source communities that is, they were rare in the rainwater and original pools. It is possible that those taxa had such large niche widths that they were not ‘sorted’ by the different media. The fact that two of the three media did not differ in their bacterial community composition supports this view. Hence, future studies should make an effort to more specifically study assembly mechanisms in dependence on local habitat heterogeneity and differentiate generalists and specialists.

In general, there are other mechanisms apart from species sorting and neutral processes that can influence the assembly of communities, in particular patch dynamics, which focus on colonisation-extinction dynamics between identical patches (patch-dynamics) and mass effect, which can be found in systems where dispersal rates among patches are very high so that immigrants are found in local communities as ‘time is too short’ to be outcompeted by the better adapted resident community (Leibold *et al.*, 2004). Our experiment was designed to particularly focus on species sorting and neutral processes. It did not address patch-dynamics, because patches, that is, microcosms, were heterogeneous and it did not address mass effects because maladapated taxa that seeded the microcosms should not have been able to grow to detectable numbers in the experiment. However, designing clear-cut experiment that tests all four metacommunity perspectives is a difficult, if not impossible, task. It will be one of the challenges during the coming years to disentangle the importance of these different assembly mechanisms and to study where and when any of them might be of particular importance.

In this study we used 454-pyrosequencing at a low sampling depths, that is, a comparatively low amount of sequences was analysed for each sample compared with studies that use the same method for an in-depth analysis of the microbial diversity in natural aquatic environments (Galand *et al.*, 2009; Andersson *et al.*, 2010; Kirchman *et al.*, 2010). With this approach we probably missed most of the rare species (Pedrós-Alió, 2006), however, the goal in this experiment was not to obtain a full coverage of the diversity in the samples, but rather to use 454-sequencing as a tool that replaces previous fingerprinting methods such as denaturing gradient gel electrophoresis (DGGE) and terminal restriction fragment length polymorphism (TRFLP), that is, targeting the most abundant taxa. Compared with the traditional fingerprinting methods, 454-pyrosequencing has the advantage that also some information about

the phylogenetic composition of the bacterial community can be obtained.

In summary, our study indicates that generalists, that are at least partly neutrally assembled, have an important role during the early colonisation of empty patches by airborne bacteria. Moreover, we also found indications of species sorting indicating that both, species sorting and neutral processes, function in concert during the early assembly of bacterial communities.

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