

Experimental procedures

Sampling and chemical characterization

Bulk (rain +dry) deposition was sampled at the shore of Gossenköllesee, an alpine lake located above the treeline at 2417 m a.s.l. in the Tyrolean Alps, Austria (47°13'N, 11°00'E). Deposition was collected using acid-washed plastic funnels (35 cm diameter) connected to sterile 1 liter glass bottles that were muffled for 4 h at 450 °C. In total, three distinct rain events without dust influence and three events with Sahara dust influence were collected between June 2007 and October 2008 (Table S1). Collectors were exposed for one to nine days during extended periods of rain. Rainfall was recorded by an on-site weather station and the duration of rain during the exposure time in combination with the volume of rainwater collected was used to calculate deposition rates. Rain was falling for 35 to 85% of the exposure times. Saharan dust concentrations in rainwater was assessed by gravimetry after filtration onto glass fiber filters GF/F (Whatman), drying at 60 °C for three days, and combustion at 450 °C for 5 h. Weight differences were used to calculate dust deposition as $\text{g dust m}^{-2} \text{ h}^{-1}$ and $\text{g C m}^{-2} \text{ h}^{-1}$. Conductivity and pH in the collected rain water were measured with an LF196 (WTW) and an Orion 960 meter (Thermo Fischer Scientific), respectively. For the analysis of total phosphorus (TP) and total dissolved phosphorous (TDP), rain water samples were placed in polyethylene bottles (HCl-cleaned and rinsed several times with Milli-Q and sample water) and transported to the laboratory at *in situ* temperature, where they were kept unfiltered (TP) or filtered through a glass-fiber filter (Whatman, GF/F). The concentration of TP and TDP was determined spectrophotometrically using the molybdate method after digestion with sulfuric acid and hydrogen peroxide. To analyze water soluble organic carbon (WSOC, Yang et al. 2003) and total soluble nitrogen (TSN), the samples were transferred into precombusted (4 h at 450 °C) glass bottles with glass stoppers (100 ml, Schott) and filtered within 4 h through two glass-fiber filters (Whatman, GF/F; precombusted for 2 h at 450 °C) using a stainless-steel syringe holder. The filters and the syringe holder

were rinsed with 20 ml of Milli-Q and 10 ml of sample water. Filtered samples were collected in precombusted (4 h at 450 °C) glass vials (40 ml, Shimadzu), acidified with HCl to pH 2, and stored in the dark at 4 °C until further analysis (within 48 h). Both, WSOC measured as dissolved organic carbon (DOC, non purgeable organic carbon) and TSN concentrations were measured with a total organic carbon analyzer (Shimadzu TOC-Vc series) equipped with a total nitrogen module. The instrument for DOC analysis was calibrated with potassium hydrogen phthalate in the range of 0.4 to 4 mg l⁻¹, while calibration for the TSN analysis was done with potassium nitrate in the range of 0.1 to 2 mg l⁻¹ (for both calibrations a four-point calibration curve was used; $r^2 > 0.999$). Concentrations of WSOC and TSN were detected simultaneously after combustion and catalytic oxidation of the injected sample. Three to five injections were analyzed for each sample. Water-soluble chromophoric dissolved organic matter (CDOM) composition was estimated using absorbance slope ratios (Helms *et al.*, 2008). The ratio of the slopes of log-transformed absorbance over two wavelength regions (275-295 and 350-400 nm) is inversely related to the molecular weight of CDOM in the water sample. Concentration of anions and cations were analyzed by ion chromatography in a Dionex ICS 1000 and 1010 system.

Growth experiments

To assess the immigration potential of bacteria associated with rain events into Gossenköllesee, we inoculated 100 ml of rain water into 1 liter of sterilized lake water using two Saharan dust intrusions (11 September 2008 and 14 October 2008, Table 1). Prior to the experiments, an integrated lake water sample was taken using a modified Schindler-Patalas sampler from 1 m depth intervals above the deepest point of the lake (9 m). The integrated lake water sample was filtered through precombusted glass fiber filters (GF/F, Whatman) to remove particles and subsequently autoclaved. The communities present in rain water with Saharan dust were inoculated, allowed to grow for 4 to 5 days at *in situ* temperature in

darkness, and then sampled for bacterial abundance and DNA extraction, cloning, and sequencing.

Bacterial abundance

Bacteria in the collected rain water and during the experiments were enumerated by DNA staining with 4',6-diamidino-2-phenylindole (DAPI) and epifluorescence microscopy. For this, 50 ml of sample water were filtered onto 0.2 μm black polycarbonate filters and stained with DAPI for 5 min. Subsequently, cells were counted under an epifluorescence microscope (Axiophot, Zeiss, Germany) at 1250 x magnification until at least 200 cells were counted.

DNA extraction, cloning and sequencing

Cells were concentrated on 0.2 μm membrane filters by gentle vacuum filtration. Between 600 and 1100 mL of water were filtered for each sample. Filters were directly used for DNA extraction using the Powersoil DNA extraction kit (Mobio) following the manufacturer's protocol. DNA was stored frozen at $-80\text{ }^{\circ}\text{C}$ until PCR and cloning was done. Bacterial 16S rRNA genes were amplified using PCR and the bacterial primer 27F (5'-AGRGTGGATCMTGGCTCAG-3') and 1492 R (5'-GGYTACCTTGTTACGACTT-3'). Triplicated PCR reactions contained 1-5 ng template DNA, 1 x buffer (Invitrogen, CA, USA), 200 $\mu\text{mol l}^{-1}$ of each dNTP, 100 nmol l^{-1} of the forward and reverse primers and 0.02 U μl^{-1} of Taq DNA polymerase (Invitrogen). PCR reactions of 25 μL volume were amplified using an initial denaturation at 94°C for 5 min, followed by 25 cycles of 1 min at 94°C , 1 min at 55°C and 3 min at 72°C . A final extension was performed at 72°C for 7 min. Replicated PCRs were pooled and purified using the QIAquick gel extraction kit (Qiagen). For each sample, 5-10 ng of PCR product was cloned into the pCR 4-TOPO TA vector and transformed into competent *E. coli* using the TOPO TA cloning kit for Sequencing (Invitrogen). Positive clones were selected and transferred into 96 well plates containing LB medium and 50 $\mu\text{g l}^{-1}$ kanamycin. Cells were grown over night at $37\text{ }^{\circ}\text{C}$ and harvested by centrifugation. After resuspending and washing the cells in MQ water, they were lysed by heating to $98\text{ }^{\circ}\text{C}$ for 10 min. The lysates

were diluted tenfold with ultrapure water and used as template for PCR using the M13f-20 (5'-GTAAAACGACGGCCAG-3') and M13r (5'-CAGG AAACAGCTATGAC-3'); Invitrogen) vector primers. PCR conditions were same as described above. PCR products were sent to Macrogen (Korea) for purification and sequencing. In total, 664 clones were sequenced of which 573 good quality sequences were obtained and deposited at GenBank under accession numbers KJ404278 - KJ404850.

Data and statistical analyses

NOAA's online HYSPLIT model was used to display backward trajectories for each sampled event and to determine air mass origin (Draxler and Rolph, 2013). The Absorbing Aerosol Index, which indicates the presence of elevated absorbing aerosols (namely of desert dust and biomass burning aerosols) in the atmosphere, for the respective sampling dates were obtained from NASA's Ozone Monitoring Instrument.

We used mothur v. 1.31.2 (Schloss *et al.*, 2009) to trim, clean, and assign taxonomic affiliation to the sequences and to calculate bacterial community descriptors. First, the sequences were aligned against the Silva seed database for bacteria. Short sequences and sequences with more than 5 ambiguous bases were removed from the dataset. Chimera were identified and removed using the uchime implementation in mothur with default settings. Taxonomic affiliation was assigned using the consensus based algorithm of mothur. Intra-sample rarefaction curves were generated using a resampling approach without replacement, which provides an indication of sampling intensity. Alpha diversity indices (Chao1, Abundance-based Coverage Estimator (ACE), Shannon, and Inverse Simpson diversity) and differences in community composition using libshuff were also calculated using mothur. Chao1 corrects the total number of observed OTUs by considering the frequency of singletons and doubletons, while ACE incorporates all OTUs with less than 10 individuals (Hughes *et al.*, 2001). Shannon diversity takes into account the number of individuals, as well as the number of taxa. Its value varies between 0 for communities with only a single taxon to high

values for communities with many taxa, each with few individuals. Inverse Simpson diversity indices account for differences in dominance, where communities with all taxa equally present obtain a value of 0 and communities dominated by a single species obtain a value of 1. Libshuff scores were used to compare libraries of 16S rRNA gene sequences using the integral form of the Cramer-von Mises statistic and 10000 randomizations (Schloss *et al.*, 2004). Figures were created using R 3.0.1. (R Development Core Team, 2008) and Cytoscape vers. 3.0.1.

References

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Table S1. Physicochemical parameters of rain water collected during summer 2007 and 2008 at the shore of the high mountain lake Gossenköllesee in Austria. Concentrations of dust, organic carbon, and inorganic nutrients are given as deposition rates, i.e. units are adjusted for sampling area and duration of the rain event. The numbers of 16S rDNA clones used for microbial community analysis are indicated for the collected wet deposition samples and for the growth experiments at the beginning and end of the experiments. n.a.: not available.

Date	Dominant source	Sampling duration [d]	Dust [g m ⁻² d ⁻¹]	Dust org. C [g C m ⁻² d ⁻¹]	Spec. cond. [μS cm ⁻¹]	pH	WSOC [μg m ⁻² d ⁻¹]	TP [μg m ⁻² d ⁻¹]	TPD [μg m ⁻² d ⁻¹]	TSN [μg m ⁻² d ⁻¹]	NO ₃ -N [μg m ⁻² d ⁻¹]	NH ₄ -N [μg m ⁻² d ⁻¹]	Na [mg m ⁻² d ⁻¹]	K [mg m ⁻² d ⁻¹]	Mg [mg m ⁻² d ⁻¹]	Ca [mg m ⁻² d ⁻¹]	SO ₄ [mg m ⁻² d ⁻¹]	Cl [mg m ⁻² d ⁻¹]	# of clones
06-17-07	Atlantic	7	n.a.	n.a.	5.0	5.79	3.71	4.3	3.7	563	140.5	166.9	0.13	0.09	0.02	0.14	0.39	0.17	48
06-26-07	Central Europe	9	n.a.	n.a.	10.4	5.95	2.01	8.2	3.8	817	283.7	399.1	0.21	0.10	0.06	0.65	0.94	0.31	96
09-18-07	Central Europe	1	n.a.	n.a.	1.7	6.05	1.71	8.8	1.6	224	82.9	67.4	0.07	0.02	0.02	0.36	0.53	0.35	96
06-03-08	Sahara	3	0.0406	0.0094	24	6.71	4.24	n.a.	24.4	2175	879.7	1199.4	0.66	0.29	0.36	4.57	7.85	7.85	54
09-11-08	Sahara	1	0.1464	0.0206	12.9	6.83	5.43	141.0	32.3	1731	585.9	955.3	0.42	0.40	0.26	8.31	4.67	4.67	48 T0 67 T5
10-14-08	Sahara	4	0.0139	0.0043	9.5	6.15	1.82	32.6	17.5	908	592.8	505.4	0.25	0.21	0.12	2.24	1.53	1.53	96 T0 63 T4

Abbreviations: org. C – organic carbon, Spec. cond. – specific conductivity, WSCO – water soluble organic carbon, TP – total phosphorus, TPD – total dissolved phosphorus, TSN – total soluble nitrogen

Fig. S1. Backwards modeled trajectories (lines) of the air masses contributing to wet deposition at Gossenköllesee (★) located in the Austrian Alps. Red lines depict the trajectories of air masses residing 500 m above ground level at the sampling site, while blue lines show the trajectories of air masses residing 2000 m above ground level. The panels on the right hand side show air masses influenced by Saharan dust and the respective Absorbing Aerosol Index images as measured by the Ozone Monitoring Instrument (OMI, NASA). The left hand side panels show air masses with Atlantic or central European origins. The altitudes in which air masses resided during transport are shown below each panel.

Fig. S2. Absorption slope ratios as proxy for DOC molecular weight distribution in rain samples collected in the absence and presence of Sahara dust influence.

Fig. S3. Rarefaction curves showing the number of OTUs retrieved from samples of rain events with and without Saharan dust influence. Saturation of the line indicates coverage of bacterial diversity in the samples.





