The following supplement accompanies the article

Distribution of *Bacteria* and *Archaea* in meromictic tropical Lake Kivu (Africa)

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Materials and methods

Nucleic acid extraction. Water samples (0.5–1.0 L) for nucleic acid extraction were processed as previously described (Llirós *et al.*, 2010). Briefly, water samples were first passed through 5-µm-pore-size polycarbonate filters (ISOPORE, Millipore, MA) to remove the particulate debris as well as large protozoa. Filtrates were then subsequently passed through 0.22-µm-pore-size polycarbonate filters (ISOPORE, Millipore, Billerica, MA, USA) to retain free-living prokaryotes. Total nucleic acids were extracted using a combination of enzymatic cell lysis and cetyltrimethyl ammonium bromide (CTAB) extraction protocol as previously described by Llirós *et al*. (2008). Dry DNA pellets were finally rehydrated in 50 µL of 10 mM Tris-HCl buffer (pH 7.4).

Quantitative real-time PCR (qPCR). The bacterial and archaeal 16S rRNA gene copy numbers were determined by using the 341f-534r (Lopez-Gutierrez *et al*., 2004) and 519f-915r (Coolen *et al.,* 2007) primer combinations specific for *Bacteria* and *Archaea*, respectively. All reactions were carried out in MicroAmp optical 96-well reaction plates covered with optical adhesive covers (Applied Biosystems). Mixtures (20 µL) for qPCR reactions contained 10 µL of *Power* SYBR® Green PCR Master Mix (Applied Biosystems), 10 µM of each corresponding primer, molecular biology-grade water (Eppendorf), and 9 µL of template DNA (40 ng of DNA). qPCR assays were performed using a StepOnePlus Thermal cycler system (Applied Biosystems) and the data were analysed with the StepOne Plus software v2.1 (Applied Biosystems). Bacterial qPCR consisted of an initial denaturing step for 15 min at 95°C, followed by 60 cycles of amplification at 60°C for 30 s, and data collection was read after an elongation step of each cycle at 80°C for 30 s to ensure stringent product quantification. In turn, archaeal qPCR consisted of an initial denaturing step for 4 min at 95°C, followed by 60 cycles of amplification at 63°C for 40 s and data collection was read after an elongation step of each cycle at 78°C for 32 s to ensure stringent product quantification. All reactions were performed in triplicate, with standard curves spanning from 10^1 to 10^7 gene copy numbers. Standard curves were generated from serial dilutions of previously titrated suspensions of conventionally PCR-amplified environmental clones (FN691587 for archaea), purified (QIAquick; Qiagen) and quantified. Overall, average efficiencies for all quantification reactions ranged from 74.2% to 98.6% and from 77.6% to 92.2% for archaeal and bacterial qPCRs, respectively, with R^2 values equal to *ca.* 0.995. The specificity of reactions was confirmed by melting curve analyses and by separating the amplicons obtained on 1.5% agarose gel electrophoresis to identify unspecific PCR products such as primer dimers or gene fragments of unexpected length (data not shown).

Results

Bacterial generalists and specialists. Each operational taxonomic unit (OTU. defined at a 0.03 cut-off threshold) represented by more than one sequence was drawn to split the set of taxa into two categories: generalists, or broadly distributed microbial taxa (defined as present in ≥ 15 samples over the three sampling campaigns), and specialists (representing more than 0.5% of the sequences in each sample, but only found in fewer than six samples) (Fig. S5). Based on our criteria, 16.7% of the $OTUs_{0.03}$ in Gisenyi and 12.7% of the OTUs in Ishungu were considered as generalists, while *ca.* 1% fell into the specialist category for both stations. There was a significant positive relationship between the relative abundance and occupancy of OTUs.

In addition, the indicator value (INDVAL; Dufrene & Legendre, 1997) was used to identify the strict specialists at the genus level. We detected 90 genera in Gisenyi and 62 genera in Ishungu that are strict specialists (INDVAL >0.3, p<0.05), accounting for 24.4% and 17% of the total genera data set in Gisenyi and Ishungu, respectively. The distribution of such strict specialist genera among the zone-based oxygen concentrations was: oxic zone (27 and 19), transition zone (18 and 7) and anoxic zone (49 and 36) in Gisenyi and Ishungu, respectively. The highest number of specialists was found in the anoxic waters of both sampling sites. In Ishungu, *Legionella*, *Prochlorococcus*, *Planctomyces*, *Hyphomicrobium*, *Chryseobacterium*, *Agrococcus* were the strict specialists of the oxic zone, whereas methane-oxidising bacteria *Methylotenera*, *Methylomonas* and *Methyloversatilis* were strict transition zone specialists and *Desulfomonile*, *Desulfocapsa and Geobacter* were strict anoxic zone specialists. In Gisenyi, *Leptolyngbya*, *Prochlorococcus*, *Cylinderospermosis*, *Fluviiocola*, *Gemmatimonas*, *Roseomonas*, *Arcobacter* were the strict specialists of the oxic zone, whereas *Nitrospira*, *Dietzia and Candidatus Solibacter* were the strict specialists of the transition zone, as *Sulfuricurvum, Sulphurimonas, Methylomonas, Methyloversatilis, Methylocaldum, Acholoeplasma, Desulfocapsa, Desulfomonile, Caldilinea, Treponema, Spirochoeta* were the specialists of the anoxic zone in Gisenyi based on the data collected in three sampling campaigns.

References

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Table S1. Diversity indices for bacterial OTUs without subsampling in the water compartments analysed in Lake Kivu defined according to dissolved oxygen concentrations. Sobs: number of observed species; Chao1: Chao1 richness estimate; Shannon: diversity index; Shannoneven: species evenness index; Coverage: non-parametric coverage estimator.

Gisenyi	Sobs	Chao1	ACE	Shannon	Coverage
oxic-Oct10	320	732	1160	4.58	0.86
oxic-Jun11	750	1726	2830	5.43	0.84
oxic-Feb12	781	1938	2673	5.64	0.80
transition-Oct10	367	790	1276	4.78	0.84
transition-Jun11	865	2179	3841	5.93	0.75
transition-Feb12	1831	4186	6467	6.37	0.84
anoxic-Oct10	1197	3384	5917	6.13	0.77
anoxic-Jun11	1398	4065	7178	6.28	0.73
anoxic-Feb12	2097	5393	9034	7.08	0.71

Ishungu	Sobs	Chao1	ACE	Shannon	Coverage
oxic-Oct10	323	721	1256	4.26	0.86
oxic -Jun11	522	1250	2162	5.02	0.81
oxic -Fb12	587	1732	2927	5.27	0.80
transition-Oct10	380	890	1466	5.33	0.70
transition-June11	671	2060	3620	5.72	0.74
transition-Fb12	921	2234	4014	5.91	0.78
anoxic-Oct10	894	2551	4812	6.15	0.67
anoxic-Jun11	1133	3189	5648	6.51	0.65
anoxic-Fb12	3021	8759	14201	7.21	0.72

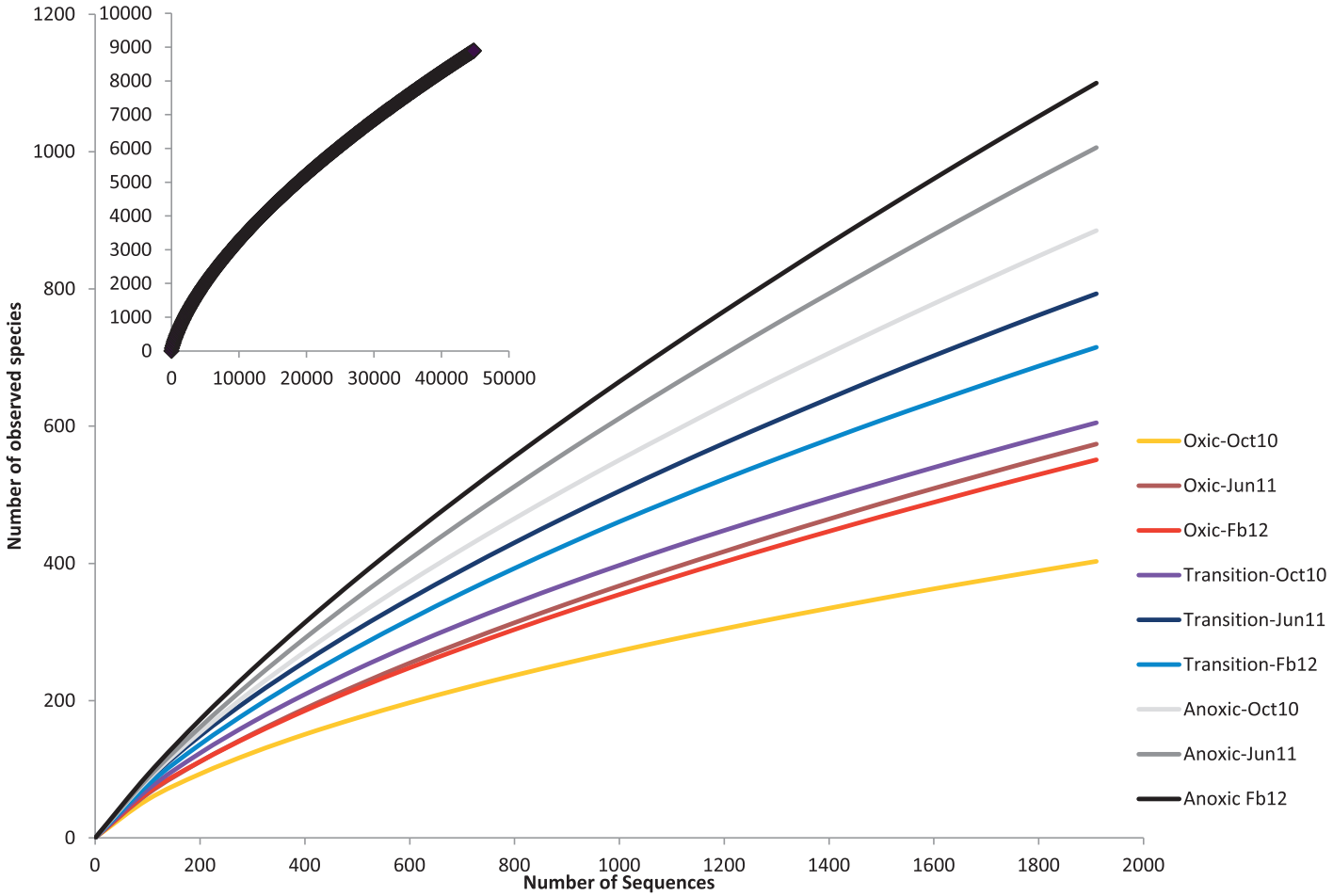

Fig S1A. Bacterial rarefaction curves for pooled samples (inset, all samples from two sampling stations, three sampling campaigns and depths) at 97% cut-off and rarefaction curves for each analysed water compartment based on dissolved oxygen concentrations and randomly subsampled using Mothur to the same size based on the sample with the smallest sampling size at each sampling time

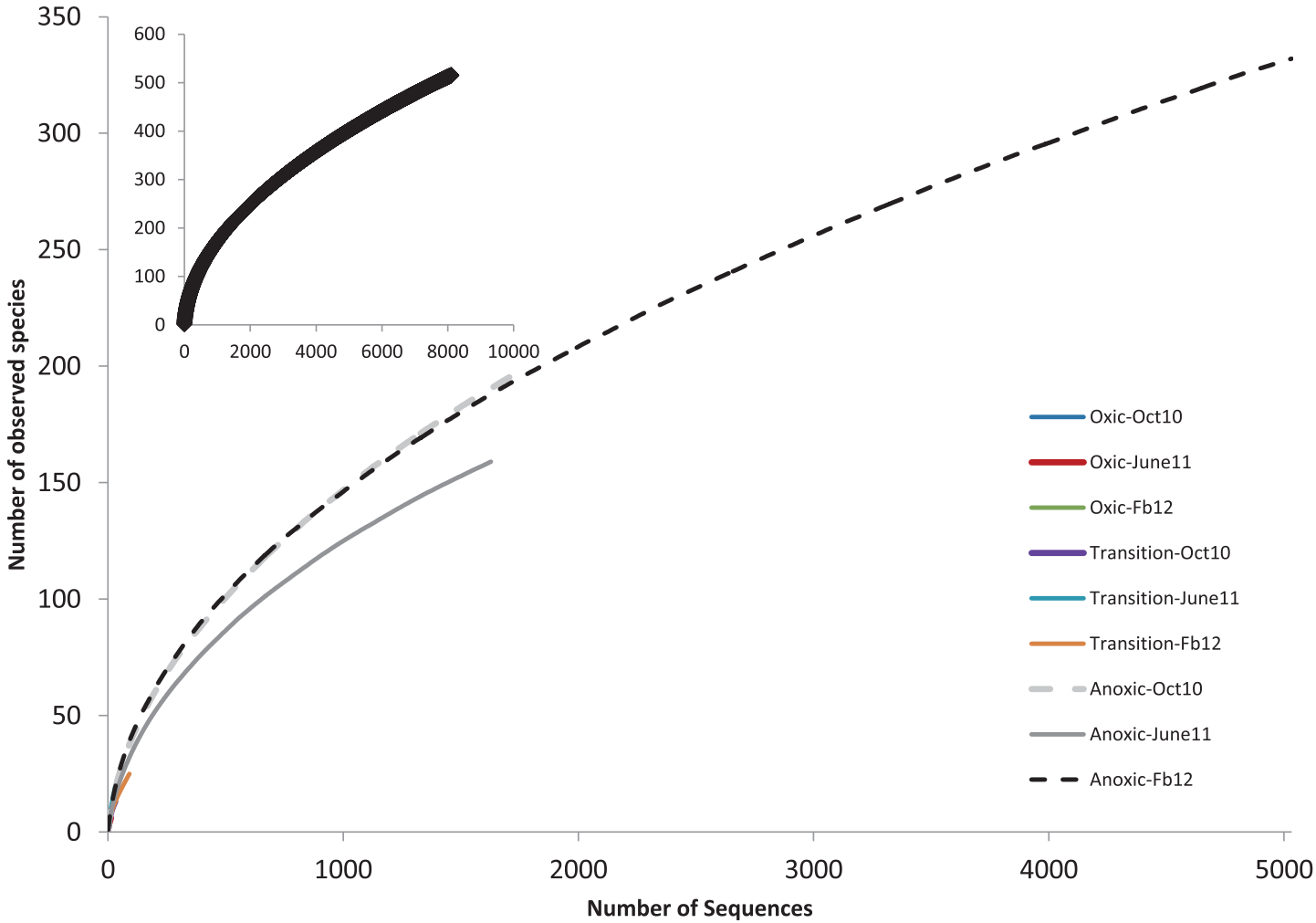

Fig S1B. Archaeal rarefaction curves for pooled samples (inset, all samples from two sampling stations, three campaigns and depths) at 97% cut-off and rarefaction curves for each analysed water compartment based on dissolved oxygen concentrations.

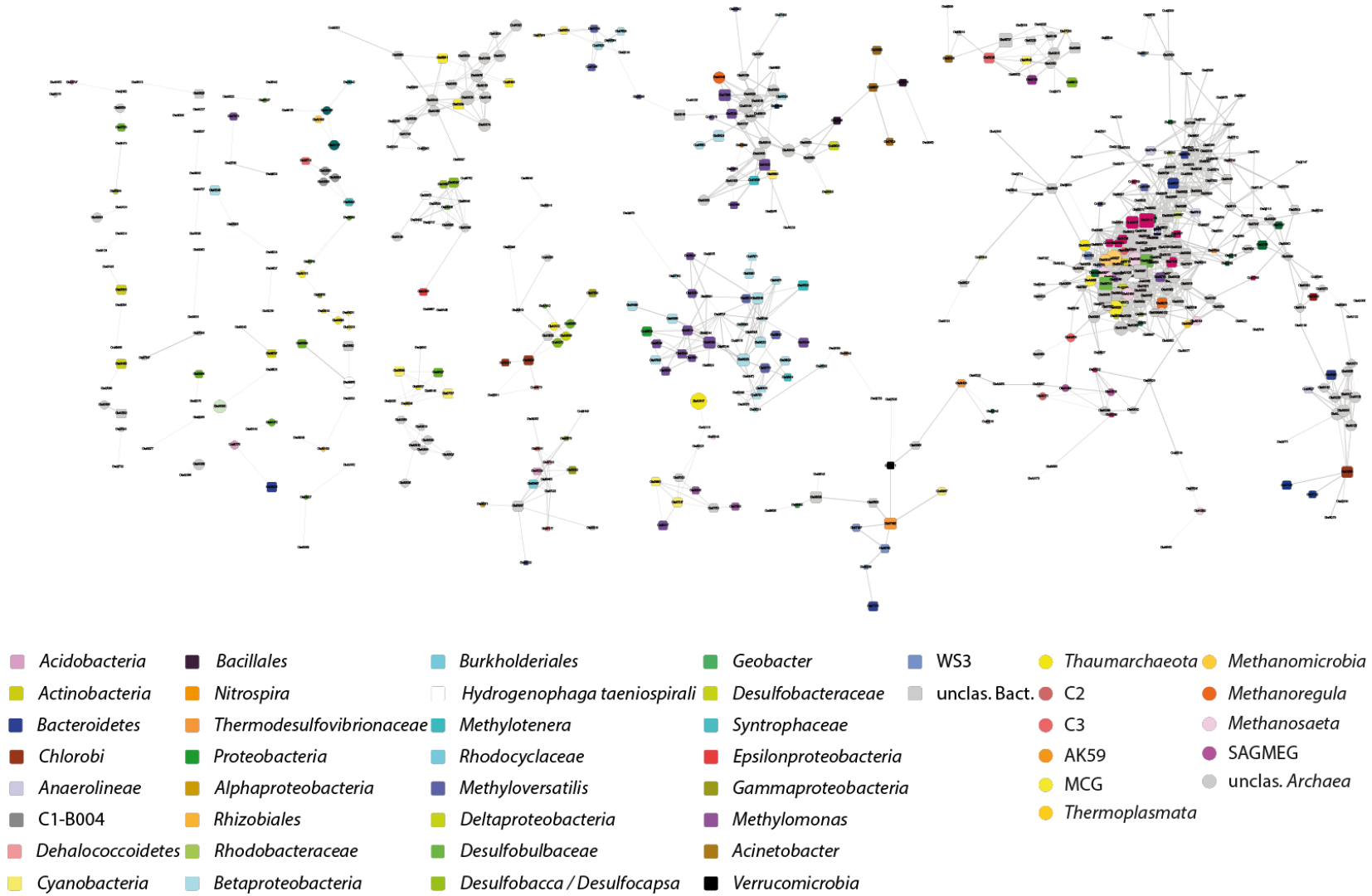

Fig S2. Network of co-occurring OTUs in Lake Kivu based on correlation analysis. A connection stands for a strong (Pearson r >0.8) and significant (P-value 0.01) correlation. The size of each node is proportional to the number of connections. Legend: squares refers to bacterial groups, whereas circles refers to archaeal groups.

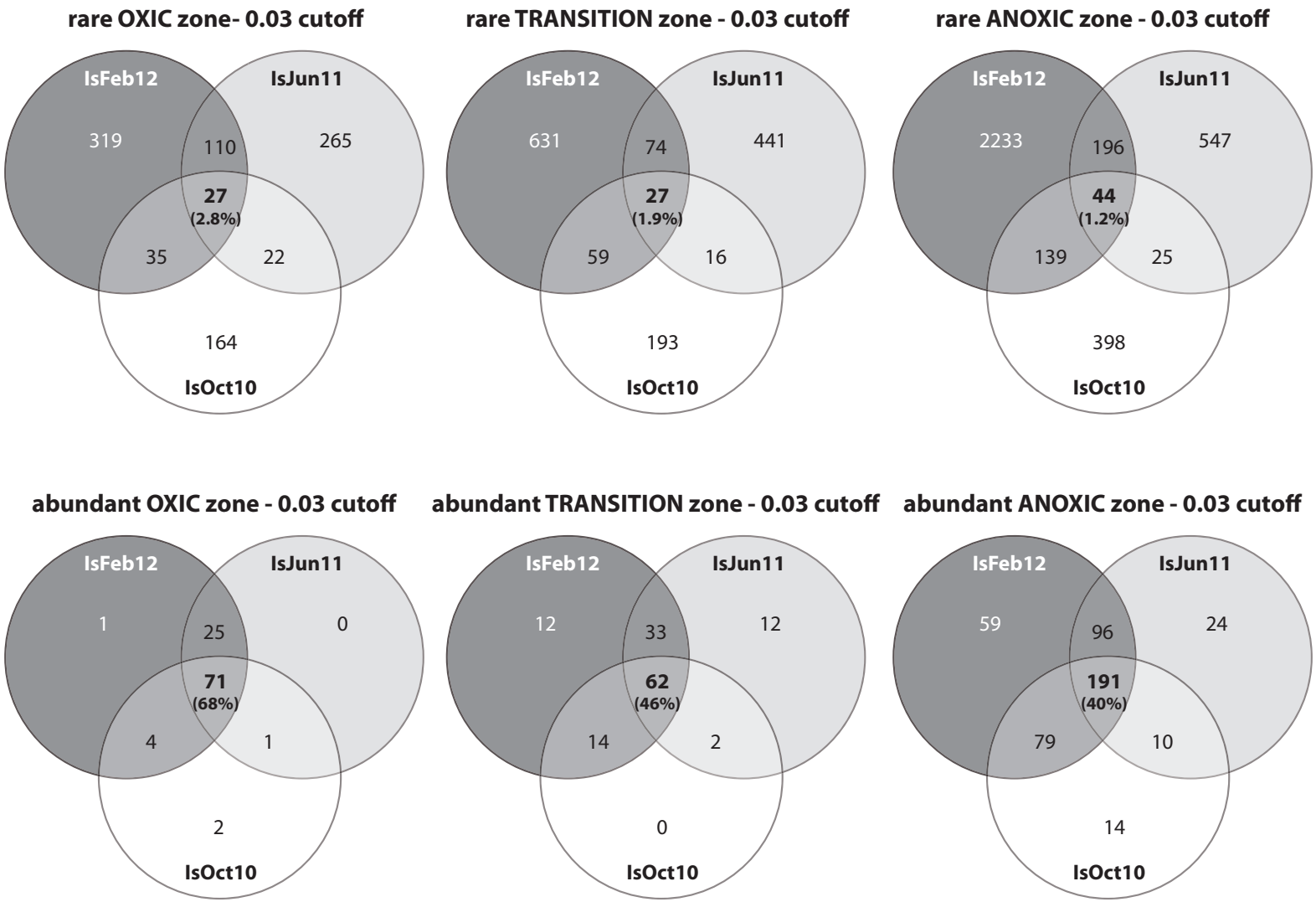

Fig S3. Venn diagrams showing the distribution of OTUs among the 3 sampling campaigns over the oxic, transition and anoxic zones in Ishungu. Abundant OTUs are defined as taxa represented by more than 6 sequences at each depth, whereas rare OTUs are defined as taxa represented by fewer than 6 sequences.

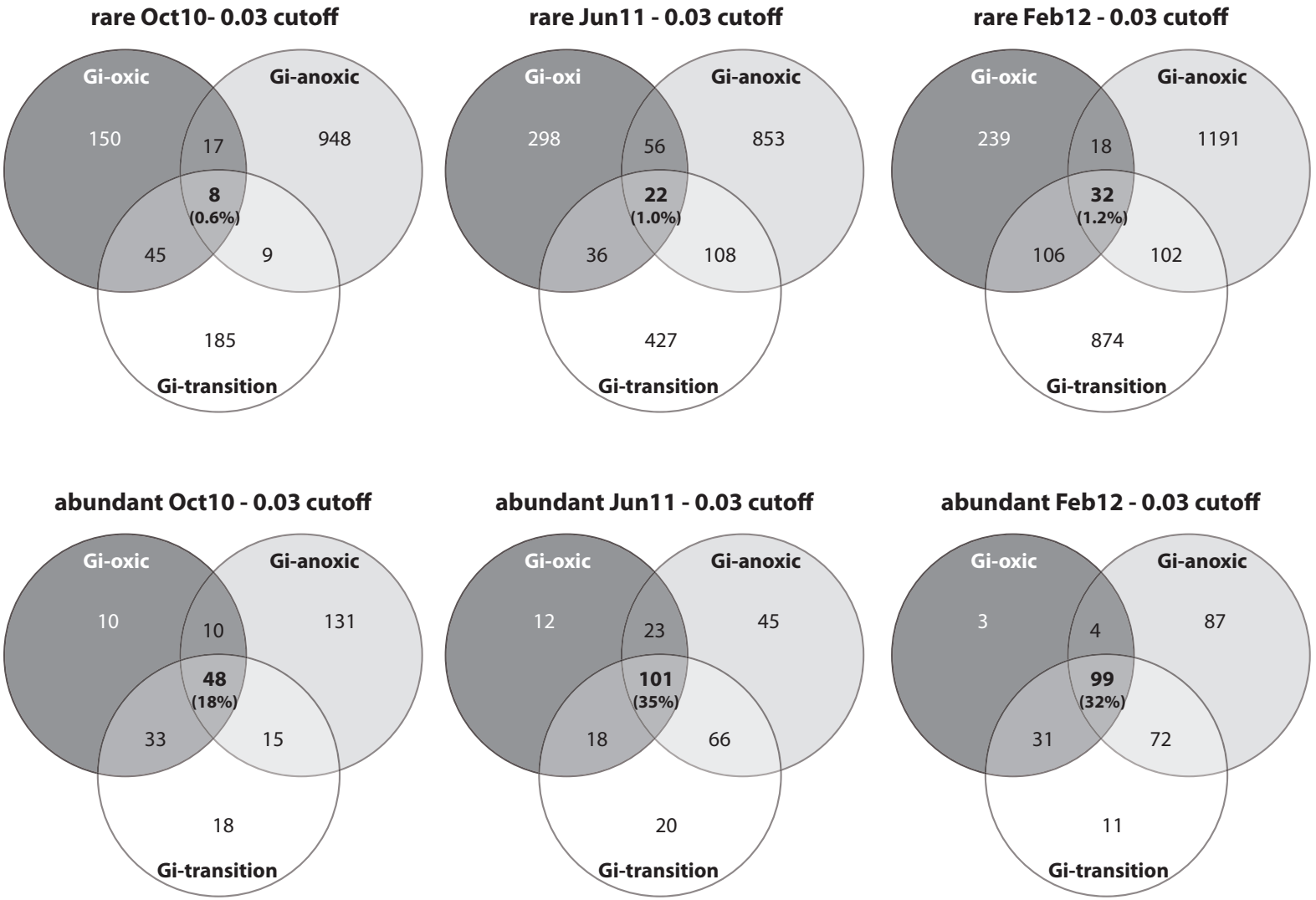

Fig S4. Venn diagrams showing the distribution of rare and abundant OTUs with respect to each sampling campaign and water compartment analysed according to dissolved oxygen concentrations. Abundant and rare OTUs are defined as taxa represented by more than 6 sequences at each depth, whereas rare OTUs are defined as taxa represented by fewer than 6 sequences.

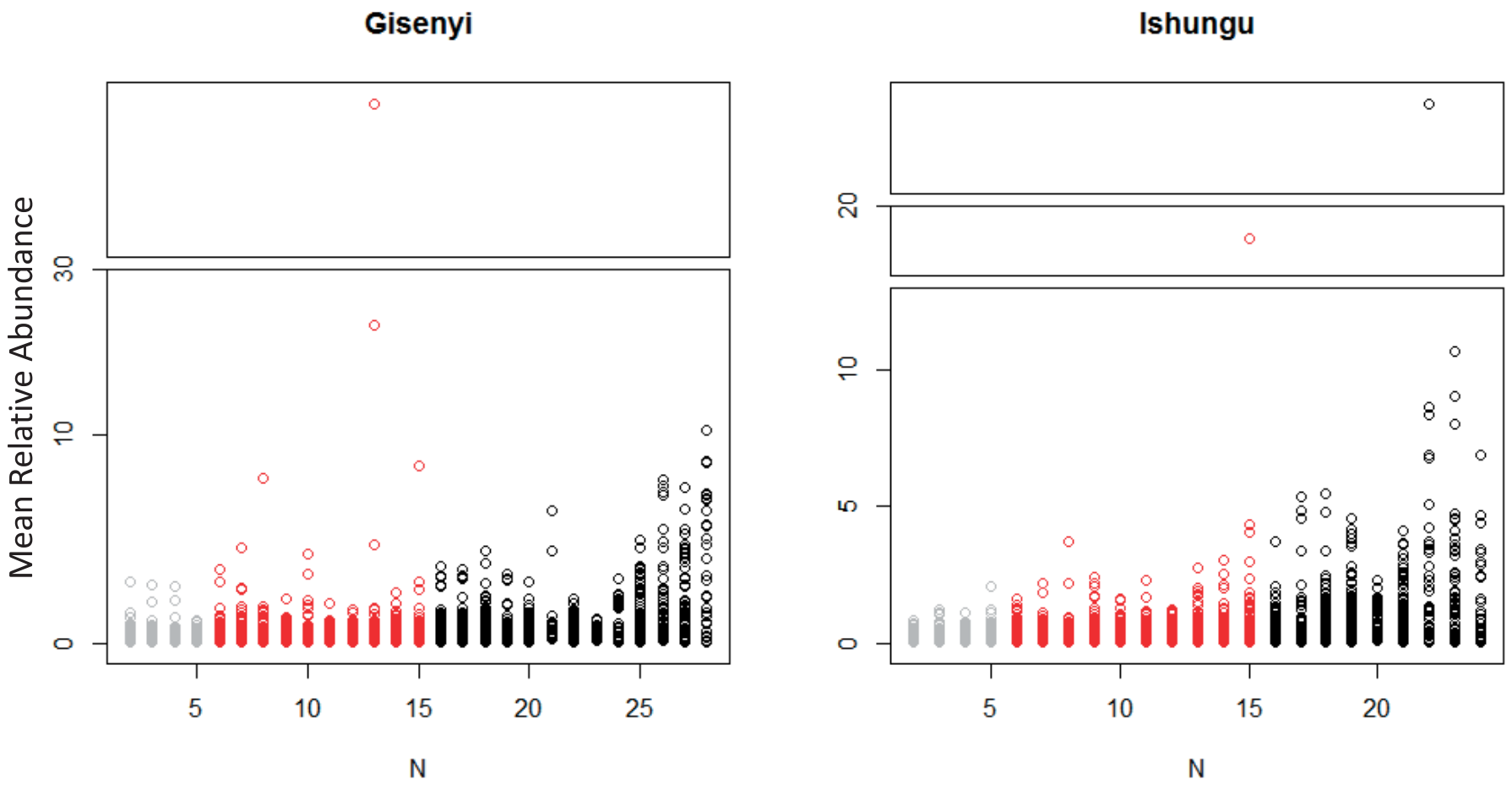

Fig S5. Scatter plot of average relative abundance of OTUs (Y axis) versus occupancy (depths sampled in the three seasons, X axis) for Gisenyi (left) and Ishungu (right) sampling sites. OTUs were split as habitat generalists (in black; defined as OTUs appearing in more than 15 samples (i.e., depths) across the three sampling campaigns) and habitat specialists (in grey; defined as OTUs locally abundant (more than 0.5%) and appearing in 6 samples). OTUs not matching this definition were marked in red. Maximum scale of Y axis for panel A is 40 and for B is 30.