## Supplementary information

# Dry habitats sustain high CO2 emissions from temporary ponds across seasons

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## S1. Hydrological year and hydroperiod of the ponds

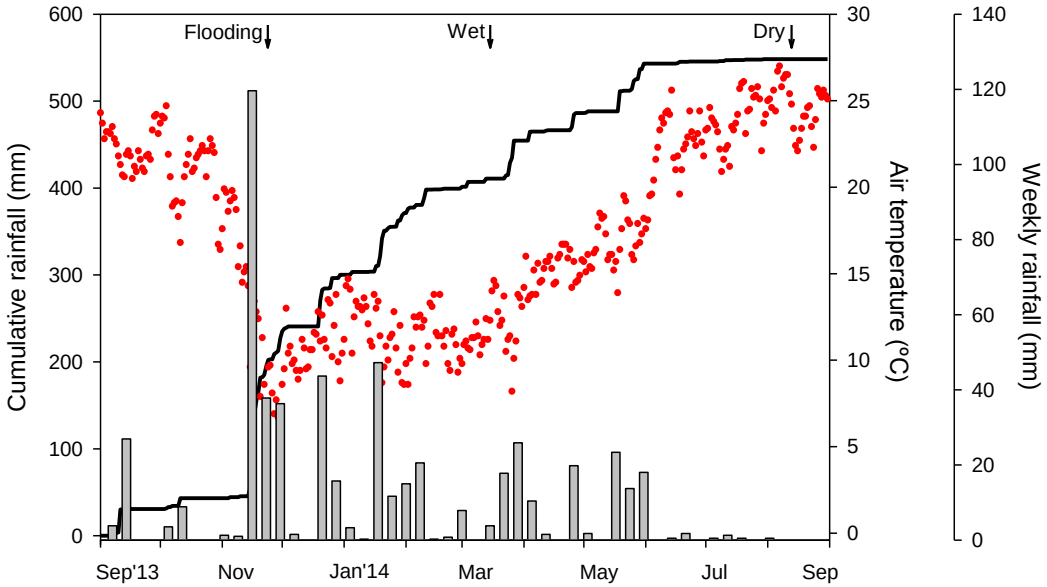


**Fig. S1.** Seasonal evolution of cumulative precipitation (bars), weekly rainfall (black line), and air temperature (red dots) during the hydrological year of 2014 (from September 2013 to September 2014) in the island of Menorca. The arrows indicate the samplings corresponding to the flooding, wet and dry phases of the ponds.

**Table S1.** Percent surface area of each of the three habitats for each pond across the sampled periods. *Denotes systems classified as ephemeral based on their hydroperiod (Ref. 46)

| | | Flooding phase | | Wet phase | | | Dry phase | | |
|---|---|---|---|---|---|---|---|---|---|
| | | Dry habitats | | | Dry habitats | | | Dry habitats | |
| Pond | Inundated habitat (%) | Emerged bare sediments (%) | Emerged vegetated sediments (%) | Inundated habitat (%) | Emerged bare sediments (%) | Emerged vegetated sediments (%) | Inundated habitat (%) | Emerged bare sediments (%) | Emerged vegetated sediments (%) |
| Son Morell* | 15 | 20 | 65 | 9 | 32 | 59 | 0 | 10 | 90 |
| Curniola | 0 | 71 | 29 | 100 | 0 | 0 | 0 | 71 | 29 |
| Verda d'Algaiarens | 24 | 0 | 76 | 58 | 21 | 21 | 0 | 5 | 95 |
| Torrellafuda | 13 | 24 | 63 | 21 | 0 | 79 | 0 | 0 | 100 |
| Mal Lloc | 39 | 0 | 61 | 100 | 0 | 0 | 0 | 5 | 95 |
| Verda des compte* | 0 | 100 | 0 | 0 | 60 | 40 | 0 | 100 | 0 |
| Armaris | 0 | 0 | 100 | 90 | 0 | 10 | 0 | 0 | 100 |
| Sa Mesquida | 78 | 11 | 11 | 96 | 0 | 4 | 0 | 0 | 100 |
| Cap Negre* | 28 | 0 | 72 | 1 | 84 | 15 | 0 | 0 | 100 |
| Marina Curniola* | 0 | 55 | 45 | 20 | 24 | 56 | 0 | 55 | 45 |

## S2. Characterization of organic matter

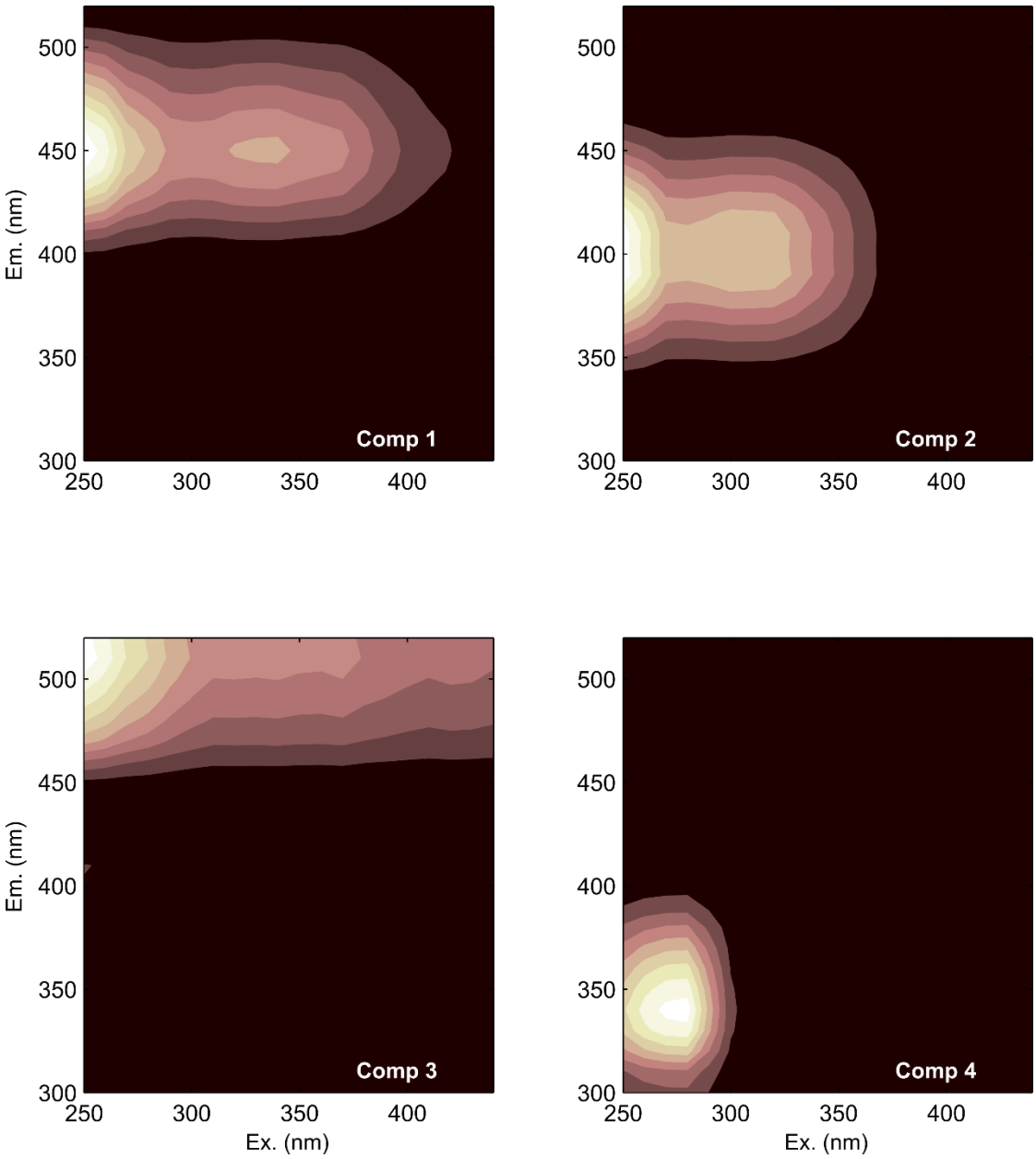


**Fig. S2.** Fingerprint of the four components validated in the PARAFAC model, referred as C1-C4 in the main text. A description is provided in Table S2.

**Table S2.** Components identified in the PARAFAC model and results of the query on the OpenFluor database (http://www.openfluor.org, January 2016). The matched components and corresponding model (TCC = 96%) are shown. The description of the models in our model is based on those previous studies.

| Components | N* | Components and PARAFAC models* matched | Description/ Classification |
|---|---|---|---|
| **C1** | 2 | C1 (FCE)C1 (Model A) | Ubiquitous humic-like, terrestrially derived organic matter, similar to peaks A+C |
| **C2** | 13 | C2 (DONKEY); C2 (JapanSea); C2 (Model A); C2 (Model C); C2 (Model D); C2 (Omdrev); C2 (Recycle_PC); C2 (Recycle_WRAMS); C3 (Recycle_RH); C3 (SAB6_2001_2005); C4 (Horsens5); C6 (Antarctic) | Humic-like, terrestrially delivered reprocessed organic matter, microbial humic-like fluorescence, similar to peak M. |
| **C3** | 9 | C1 (NeusePOMDOM); C2 (ONR); C2 (SharkBay); C2 (TropicalRivers); C2 (Umeå); C2 (drEEM); C2 (graeber2012); C3 (PARTNERS); C5 (FCE) | Terrestrial humic-like, ubiquitous fulvic acid, reduced terrestrial semi-quinone fluorophore (SQ1)c |
| **C4** | 5 | C1 (NeuseBEPOM); C2 (CorrOrganicCompounds2); C5 (GoMaine); C5 (NeusePOMDOM); C6 (Quebec Boreal) | Protein-like, microbial derived or freshly produced organic material |

*N denotes the number of models listed below matched with our PARAFAC model.

| | |
|---|---|
| Antarctic | Stedmon C.A. et al. 2011. J. Geophys. Res. 116(G03027)1-9 |
| CorrOrganicCompounds2 | Wünsch UJ et al. 2016. Front. Mar. Sci. 3:9 |
| DONKEY | Stedmon, C. A et al. 2007. Mar. Chem. 104: 227-240. |
| drEEM | Murphy, K.R. et al. 2013. Anal. Methods, 5: 6557-6566 |
| FCE: | Yamashita, Y. et al. 2010. Ecosystems 13: 1006-1019. |
| GoMaine | Cawley et al., 2012. Mar. Pollution Bulletin, v64 : 1678-1687 |
| graeber2012 | Graeber, D. et al. 2012. Sci. Tot. Environ.,438 : 435-446 |
| Horsens5 | Murphy, K.R. et al. 2014, in: Coble, P. et al. (Eds.). Aquatic organic matter fluorescence. Cambridge University Press, New York. |
| JapanSea | Tanaka, K. et al. 2014. Sci. Rep. 4, 5292. |
| Quebec Boreal | Lapierre J. F. and del Giorgio P. A., 2014. Biogeosciences, 11: 5969–5985 |
| Model A , C and D | Shutova et al., 2014. Water Res. 54: 159-169 |
| NeuseBEPOM | Brym, A. et al. 2014. Mar. Chem., 162 : 96-113. |
| NeusePOMDOM | Osburn, C.L. et al. 2012. Environ. Sci. Technol. 46: 8628−8636 |
| Omdrev | Kothawala, D. N. et al. 2013. Glob Chang Biol.20: 1101-1114 |
| ONR | Osburn C.L. et al. 2011. Mar. Chem., 126: 281-294 |
| PARTNERS | Walker S. A. et al. 2014. J. Geophys. Res. Biogeosci., 118: 1689-1702. |
| Recycle_PC, RH and WRAMS | Murphy, K.R. et al. 2011. Environ Sci Technol 45: 2909-2916 |
| SAB6_2001_2005 | Kowalczuk, P et al. 2009. Mar. Chem. 113: 182-196 |
| SharkBay | Cawley et al., 2012. Mar. Freshwater Res. 63: 1098-1107. |
| TropicalRivers | Yamashita, Y. et al. 2010. J. Geophs. Res. 115, G00F10. |
| Umeå | Stedmon, C.A. et al. 2007. Environ. Sci. Technol. 41: 7273-7279. |

## S3. Variables in the PLS analysis and VIP values

**Table S3.1.** Drivers of the total $CO_2$ flux (T-$FCO_2$) determined by the (PLS) analysis. Variables are ordered by decreasing VIP (variable importance to the projection, shown with their ± SE). The analysis explained 92% of the variance in the T-$FCO_2$. Only the highly and moderately influential variables (i.e. VIP > 0.8) are shown.

| Abbreviation | Variable | VIP |
|---|---|---|
| SI_C2 | Inundated habitat, component 2 (%) | 1.74 ± 0.71 |
| SI_%MO | Inundated habitat, organic matter content (LOI; %) | 1.58 ± 0.19 |
| W_TN | Water, total nitrogen (mgN $L^{-1}$) | 1.28 ± 0.69 |
| SE_%MO | Emerged sediment, organic matter content (LOI; %) | 1.24 ± 0.42 |
| SE_ $T^a$ | Emerged sediment, temperature (ºC) | 1.23 ± 0.37 |
| W_Alk | Water Alkalinity (meq $L^{-1}$) | 1.22 ± 0.98 |
| SE_DOC_Extract | Emerged sediment, DOC extract (WEOM; mg $L^{-1}$) | 1.21 ± 0.46 |
| SI_C4 | Inundated habitat, component 4 (%) | 1.19 ± 0.54 |
| SI_C1 | Inundated habitat, component 1 (%) | 1.19 ± 0.52 |
| SE_ RH | Emerged sediment, relative humidity (%) | 1.17 ± 0.92 |
| W_DOC | Water, DOC (mg $L^{-1}$) | 1.11 ± 0.64 |
| Env_Depth | Depth of the pond (cm) | 1.03 ± 0.61 |
| SE_HIX | Emerged sediment, humification index | 1.01 ± 0.24 |
| SE_C1 | Emerged sediment, component 1 (%) | 0.89 ± 0.24 |
| W_BIX | Water, biological index | 0.85 ± 0.57 |
| SI_C3 | Inundated habitat, component 3 (%) | 0.84 ± 0.54 |
| W_Chla | Water, Chl-a (µg $L^{-1}$) | 0.82 ± 0.57 |
| SE_C4 | Emerged sediment, component 4 (%) | 0.82 ± 0.29 |

**Table S3.2.** Drivers of the $CO_2$ flux from inundated habitats (W-$FCO_2$) determined by the (PLS) analysis. Variables are ordered by decreasing VIP (variable importance to the projection, shown with their ± SE). The analysis explained 86% of the variance in the W-$FCO_2$. Only the highly and moderately influential variables (i.e. VIP > 0.8) are shown.

| **Abbreviation** | **Variable** | **VIP ± SE** |
|---|---|---|
| HIX | Humification index | 1.45 ± 0.14 |
| C4 | Fluorescence component 4 (%) | 1.35 ±0.14 |
| C1 | Fluorescence component 1 (%) | 1.35 ± 0.14 |
| $S_R$ | Absorbance slope ratio | 1.24 ± 0.22 |
| Cond. | Conductivity | 1.21 ± 0.40 |
| DOC | Dissolved organic carbon (mg $L^{-1}$) | 1.06 ± 0.31 |
| C2 | Fluorescence component 2 (%) | 1.06 ± 0.34 |
| C3 | Fluorescence component 3 (%) | 1.05 ± 0.15 |
| BIX | Biological Index | 1.05 ± 0.12 |
| $O_2$ | Oxygen concentration (mg $L^{-1}$) | 1.00 ± 0.32 |
| TFDOM | Total fluorescence (Raman units) | 0.90 ± 0.13 |

## S4. Microbial community composition

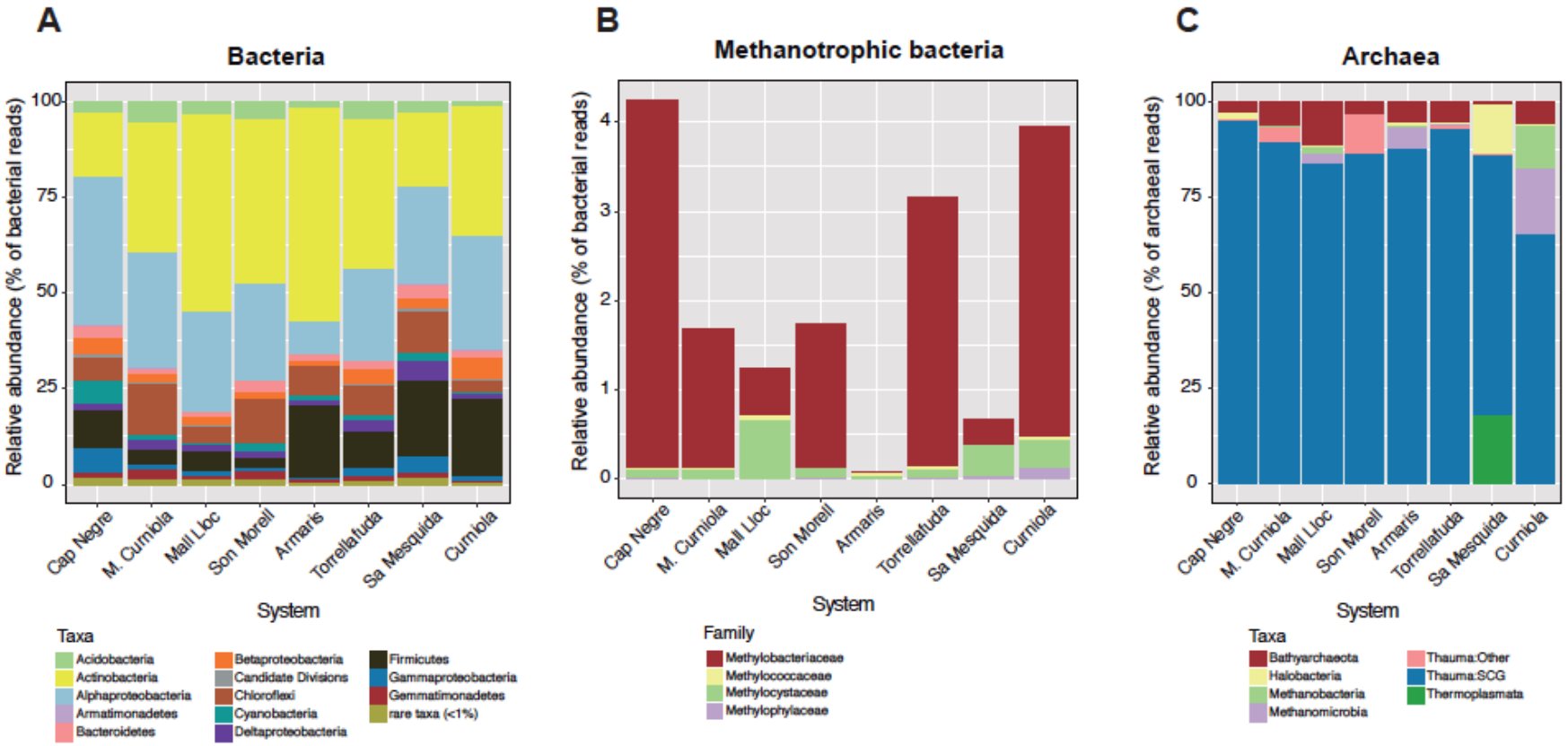


**Fig. S4.** Composition of a) bacterial, b) methanotrophic bacterial and c) archaeal sediment communities in the studied ponds.

**Table S4.** Distribution of (A) bacterial and (B) archaeal sequence reads across studied systems. The number of observed OTUs and estimators of richness (Chao1) and diversity (Shannon) are also shown. The number of sequences per sample was normalized to 28,000 and 12,000 for Bacteria and Archaea, respectively, to avoid biases due to different sequencing effort across samples.

A) Bacteria

| | |
|---|---|
| Total reads: | 512,136 |
| Total OTUs: | 8,621 |

| System | Number of reads | Observed OTUs | Chao1[a] | Shannon[b] | Phylogenetic Diversity[c] |
|---|---|---|---|---|---|
| Armaris | 29,142 | 1,296 | 1623 | 7.9 | 61.1 |
| Cap Negre | 121,157 | 2,442 | 3562 | 9.6 | 107 |
| Curniola | 28,910 | 1,443 | 1843 | 8.7 | 63.4 |
| Mal Lloc | 32,619 | 1,565 | 1860 | 9.1 | 70.4 |
| Marina Curniola | 80,380 | 2,139 | 2985 | 9.0 | 93.2 |
| Sa Mesquida | 151,517 | 1,527 | 1763 | 9.3 | 87.5 |
| Son Morell | 31,954 | 1,771 | 2352 | 8.3 | 79.3 |
| Torrellafuda | 36,457 | 1,635 | 1945 | 9.2 | 77.2 |

B) Archaea

| | |
|---|---|
| Total reads: | 447,892 |
| Total OTUs: | 240 |

| System | Number of reads | Observed OTUs | Chao1[a] | Shannon[b] | Phylogenetic Diversity[c] |
|---|---|---|---|---|---|
| Armaris | 35,713 | 104 | 112 | 4.8 | 3.9 |
| Cap Negre | 24,967 | 80 | 88 | 3.3 | 2.9 |
| Curniola | 63,184 | 109 | 113 | 5.3 | 3.8 |
| Mal Lloc | 83,789 | 100 | 112 | 3.9 | 4.1 |
| Marina Curniola | 12,494 | 73 | 82 | 4.4 | 2.5 |
| Sa Mesquida | 51,189 | 140 | 154 | 4.1 | 8.5 |
| Son Morell | 83,937 | 69 | 71 | 4.0 | 1.2 |
| Torrellafuda | 92,619 | 83 | 94 | 3.8 | 2.6 |

[a]Chao, A. 1984. *Scandinavian J. Stat.* 11:265-270)
[b]Shannon, C. E. 1948. The Bell Syst.Technic. J., 27: 379–423 and 623–656.
[c]Faith, D.P. 1992. *Biol. Conserv.* 61:1–10

## S5. Supplementary Methods: *Sequencing and phylogenetic analysis*

Genomic DNA from sedimentary communities was used as a template in PCR reactions using primers 28F (Sundquist et al., 2007) and 388R (Turnbaugh et al., 2009) for bacterial 16S rRNA genes and primers 517F (Baker et al., 2003) and 909R (*a.k.a.* 890aR, Burgraff et al., 1997) and then analyzed through MiSeq PE2x250 Illumina chemistry at the RTLGenomics (Lubbock, TX, USA). Both primer pairs were complemented with Illumina-adapters and sample-specific barcodes. Raw sequence dataset was pre-processed at RTLGenomics facilities to reduce noise and sequencing artefacts as well as to merge forward and reverse pairs. Merged sequences were quality-filtered excluding sequences with quality scores <25, with lengths <250 bp or >400 bp, and with erroneous barcodes, ambiguous bases and homopolymers in QIIME (Caporaso et al. 2010a). De-novo and reference-based chimera checking, clustering into Operational Taxonomic Units (OTU, 97% cutoff), identification of representative OTU sequences and construction of OTU table were also carried out using default parameters in QIIME. OTUs with less than 4 members were removed from further analyses. Representative sequences from each OTU were then aligned to the Greengenes imputed core reference alignment (DeSantis et al., 2006) using PyNAST (Caporaso et al., 2010b). Taxonomical assignments for each OTU were carried out in QIIME using the BLAST method and the QIIME-formatted version of the SILVA 111 reference database. For community analysis, the number of sequences in each sample was normalized by randomly selecting a subset of 3,000 sequences from each sample to standardize sequencing effort across samples and to minimize any bias due to different number of total sequences. QIIME was also used to calculate α-diversity indicators of richness (Observed richness and Chao1) and diversity (Shannon index and Phylogenetic Diversity, Faith (1992)) for bacterial and archaeal communities and to calculate community similarity among sites (β-diversity) using unweighted and weighted UniFrac distances (Lozupone and Knight, 2005). All raw bacterial and archaeal DNA sequences retrieved in this study are publicly available through the Sequence Read Archive (SRA) database under accession SRP126916 (https://www.ncbi.nlm.nih.gov/sra).

**References**


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Burggraf S. et al. *Internat. J. Syst. Bacteriol*. 47: 657-660 (1997)

Caporaso J. G. et al. *Nat. Methods*, 7: 335–336 (2010a)

Caporaso J. G. et al. *Bioinformatics*, 26: 266–267. (2010b)

DeSantis T. Z. et al. *Appl. Environ. Microbiol.*, 72: 5069–5072 (2006).

Faith D. P. *Biol. Conserv*., 61: 1–10 (1992)

Lozupone C. and Knight R. *Appl. Environ. Microbiol.*, 71: 8228–8235 (2005).

Sundquist A. et al. *BMC Microbiology* 7: 108 (2007)

Turnbaugh P. J. et al. *Nature* 457: 480–484. (2009)