Supplementary Materials

Title: Comparison of two bioinformatics tools used to characterize the microbial diversity and predictive functional attributes of microbial mats from Lake Obersee, Antarctica.

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Running Title: Comparison of PICRUSt and Tax4Fun on Lake Obersee microbial mats metagenome

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Supplementary material S1: Screenshots figures (S1.1 and S1.2) represent KEGG annotated sequence files generated from two different bioinformatics tools; 1) PICRUSt and 2) Tax4Fun.

S1.1: Screenshot of the first page of a KEGG annotated sequence file generated from PICRUSt. A total 6909 KEGGID has found and contained in the KEGG annotated sequence file for comparison analysis. The file was saved as comma delimited (.csv) file format with desired file name ("KEGG_Table_PICRUSt.csv") to upload into RStudio. In this study, we saved "KEGG_Table_PICRUSt.csv" file into "C:\#your user name\Documents\LakeObersee" directory.

#KEGGID	OB12-Pie	OB13-Pie
K00001	0.000613	0.000416
K00002	6.52E-06	6.90E-06
K00003	0.000609	0.000492
K00004	6.75E-06	1.25E-06
K00005	0.000166	8.33E-05
K00007	5.14E-06	1.11E-05
K00008	0.000238	0.000197
K00009	2.89E-05	9.63E-06
K00010	6.57E-05	5.36E-05
K00011	7.51E-06	1.84E-05
K00012	0.000737	0.000745
K00013	0.00056	0.000577
K00014	0.000782	0.000628
K00015	7.80E-05	6.39E-05
K00016	0.000435	6.88E-05
K00018	0.000282	6.52E-05
K00019	8.28E-05	0.000253
K00020	0.000306	0.000539
K00021	3.43E-06	8.75E-06
K00022	0	0
K00023	9.24E-05	0.0004
K00024	0.000498	0.000525
K00025	1.04E-05	1.17E-07
K00026	0	0
K00027	0.000289	0.000122
K00028	2.07E-07	4.68E-08
K00029	0.000304	0.000473
K00030	0.000212	0.00028

S1.2: Screenshot of the first page of a KEGG annotated sequence file generated from Tax4Fun. A total 6559 KEGGID has identified and included in the KEGG annotated sequence file for comparison analysis. The file was saved as comma delimited (.csv) file format with desired file name ("KEGG_Table_Tax4Fun.csv") to upload into RStudio. In this study, we saved "KEGG_Table_Tax4Fun.csv" file into "C:\#your user name\Documents\LakeObersee" directory.

#KEGGID	OB13-T4F	OB12-T4F
K00001; alcohol dehydrogenase [EC:1.1.1.1]	0.000463	0.000356
K00002; alcohol dehydrogenase (NADP+) [EC:1.1.1.2]	1.87E-05	1.20E-05
K00003; homoserine dehydrogenase [EC:1.1.1.3]	0.000523	0.000634
K00004; (R,R)-butanediol dehydrogenase / diacetyl reductase [EC:1.1.1.4 1.1.1.303]	3.37E-06	7.60E-06
K00005; glycerol dehydrogenase [EC:1.1.1.6]	0.000118	0.000127
K00007; D-arabinitol 4-dehydrogenase [EC:1.1.1.11]	1.70E-05	6.78E-06
K00008; L-iditol 2-dehydrogenase [EC:1.1.1.14]	6.76E-05	8.87E-05
K00009; mannitol-1-phosphate 5-dehydrogenase [EC:1.1.1.17]	2.41E-06	3.89E-05
K00010; myo-inositol 2-dehydrogenase [EC:1.1.1.18]	0.000198	0.000142
K00011; aldehyde reductase [EC:1.1.1.21]	9.60E-07	4.77E-07
K00012; UDPglucose 6-dehydrogenase [EC:1.1.1.22]	0.000862	0.000993
K00013; histidinol dehydrogenase [EC:1.1.1.23]	0.000773	0.000732
K00014; shikimate dehydrogenase [EC:1.1.1.25]	0.000569	0.000604
K00015; glyoxylate reductase [EC:1.1.1.26]	9.22E-05	0.000141
K00016; L-lactate dehydrogenase [EC:1.1.1.27]	5.25E-05	0.000411
K00018; glycerate dehydrogenase [EC:1.1.1.29]	5.38E-05	0.000247
K00019; 3-hydroxybutyrate dehydrogenase [EC:1.1.1.30]	0.000169	7.05E-05
K00020; 3-hydroxyisobutyrate dehydrogenase [EC:1.1.1.31]	0.000555	0.000354
K00021; hydroxymethylglutaryl-CoA reductase (NADPH) [EC:1.1.1.34]	6.55E-06	2.49E-06
K00023; acetoacetyl-CoA reductase [EC:1.1.1.36]	0.000198	4.83E-05
K00024; malate dehydrogenase [EC:1.1.1.37]	0.000486	0.000543
K00027; malate dehydrogenase (oxaloacetate-decarboxylating) [EC:1.1.1.38]	0.000189	0.000415
K00029; malate dehydrogenase (oxaloacetate-decarboxylating)(NADP+) [EC:1.1.1.40]	0.000951	0.000828
K00030; isocitrate dehydrogenase (NAD+) [EC:1.1.1.41]	0.0003	0.000259

Supplementary material S2: Description of the R code for comparison analysis between "KEGG_Table_PICRUSt.csv" and "KEGG_Table_Tax4Fun.csv" file used in this study.

RStudio_workflow
Description of elements. To excute below commands, both sequence files ("KEGG_Table_PICRUSt.csv" and "KEGG_Table_Tax4Fun.csv") need to be saved on "C:\#your user name\Documents\LakeObersee" directory.

Type: setwd("C:\#your user name\Documents\LakeObersee")
#This command will set up the directory folder path in the RStudio. One can change the folder path according to the file location.

Type:
keggtable_Tax4Fun.data <- read.csv("KEGG_Table_Tax4Fun.csv ", sep=",", header=TRUE, na.strings = "?", stringsAsFactors = FALSE)
This command will load "KEGG_Table_Tax4Fun.csv" file into RStudio. File name can change accordingly.

keggtable_PICRUSt.data <- read.csv("KEGG_Table_PICRUSt.csv ", sep=",", header=TRUE, na.strings = "?", stringsAsFactors = FALSE)
This command will load "KEGG_Table_PICRUSt.csv" file into RStudio. File name can change accordingly.

Type: keggtable_Tax4Fun.data $X.KEGGID <- gsub("(K[0-9]+);.*", "\\1", keggtable_Tax4Fun.data $X.KEGGID)
This command will be applied only for the "KEGG_Table_Tax4Fun.csv" file. Since "KEGG_Table_Tax4Fun.csv" file has extra information on KEGGID column compared to the "KEGG_Table_PICRUSt.csv" file, this command will help to remove the extra information and leave only KEGGID numbers on the KEGGID column.

Type (optional): write.table(keggtable_Tax4Fun.data, " keggtable_Tax4Fun.tsv", sep="\t", row.names=FALSE)
This command will be applied only for the "KEGG_Table_Tax4Fun.csv" file. After removed all extra information from the KEGGID column, this command will help to export a filtered "keggtable_Tax4Fun.tsv", which can be used to compare with "keggtable_PICRUSt.data".

Type: merge_table <- merge(keggtable_Tax4Fun.data, keggtable_PICRUSt.data, all =TRUE)
This command will help to merge "keggtable_Tax4Fun.data" and "keggtable_PICRUSt.data" into one table.

Type: merge_table[is.na(merge_table)] <- "NA"
This command will find an empty space in merge_table data, and then replace it to "NA (character)".

Type (optional): write.table(merge_table, "merged_Tax4Fun_PICRUSt.tsv", sep="\t")
This command will export merge_table file into an actual table file format ("merged_Tax4Fun_PICRUSt.tsv").

Type: collist <- c("OB12_pie", "OB13_pie", "OB12_t4f", "OB13_t4f")
This command will need to include all column list. In this study, we put "OB12_pie", "OB13_pie", "OB12_t4f", "OB13_t4f" as a column list.

Type:
sel <- apply(merge_table[,collist], 1, function(row) "NA" %in% row)
and then type,
selected_NA <- merge_table[sel,]
These two commands will help to select NA (character) from your merge_table data.

Type: write.table(selected_NA, "selected_NA.tsv", sep="\t", row.names=FALSE)
This command will export comparison results (selected_NA) into an actual table file format (selected_NA.tsv).

Type: sel2 <- apply (selected_NA[,collist], 1, function(row) all(row !=0))
and then type,
filtering_all_zero <- selected_NA[sel2,]
These two commands will help to discard all "0" from your selected_NA table.

Type: write.table(filtering_all_zero, "filtering_all_zero.tsv", sep="\t", row.names=FALSE)
This command will export filtered results (filtering_all_zero) into an actual table file format (filtering_all_zero.tsv).

Type (optional): T4F_filtered_KEGGIDS <- subset(filtering_all_zero, OB12_t4f == "NA" & OB13_t4f =="NA", select = c(X.KEGGID, OB12_t4f, OB13_t4f), header = "true")
This command will show all filtered KEGGIDS results, which includes "NA" only in the OB12_t4f and OB13_t4f. One can change the column name and filter KEGGIDS.

Type (optional): Filter_KEGGIDS_ONLY <- subset(filtering_all_zero, OB12_t4f == "NA" & OB13_t4f =="NA", select = c(X.KEGGID), header = "true")
This command will filter only KEGGIDS when the selected column includes "NA."

Supplementary Material S3: A list of differences in the taxonomic information identified by Silva and Greengene Database. A blank space indicates the taxa that were not identified by the respective database.

	Silva	**Greengenes**
Undetected Phyla		
		Armatimonadetes
		TM6
		Tenericutes
		Hydrogenedentes
		Parcubacteria
Undetected Genus		
	A1-B1	Acidibacter
	Actinomycetales	Acidimicrobiaceae
	Anaerospora	Acidocella
	Bacteriovoracaceae	Albidiferax
	C111	Algoriphagus
	Candidatus Xiphinematobacter	Alistipes
	Chloronema	Amphritea
	Clostridiales	Anaerostipes
	Clostridium	Armatimonadetes others
	Cryomorphaceae	Bacteroidales
	DH61	Bdellovibrio
	Enterobacteriaceae	Blastocatella
	Kaistobacter	Blastopirellula
	LD19	Brevundimonas
	Lysobacter	Candidatus Chloroploca
	Methylibium	Candidatus Methylacidiphilum
	Mycoplana	Comamonas
	Oxalobacteraceae	Cyanobacteria;SubsectionIII
	Paucibacter	EB1017
	Pirellulaceae	Ellin6067
	Piscirickettsiaceae	Ellin6075
	Pseudanabaena	Cytophaga
	Rikenellaceae	Duganella
	Rubrivivax	Ferruginibacter
	S24-7	Flavobacteriales
	Salinibacterium	Frigoribacterium
	Sinobacteraceae	Fusicatenibacter
	SM1D11	Gammaproteobacteria
	Solibacteraceae	Gardnerella

	Solibacterales	Geitlerinema
	Spirobacillales	Gemmobacter
	Streptophyta	Glaciecola
		Granulicatella
		Halomicronema
		Hydrogenedentes
		Illumatobacter
		Intestinibacter
		Ketogulonicigenium
		Lachnoclostridium
		Leptothrix
		Marvinbryantia
		Massilia
		Methylobacteriaceae
		Nannocystis
		Nitrosomonadaceae
		Noviherbaspirillum
		Oligoflexaceae
		Oligoflexales
		OPB35
		Parabacteroides
		Parasutterella
		Parcubacteria
		PeM15
		Peredibacter
		Pirellula
		Planctomycetaceae
		Porphyrobacter
		Pseudoalteromonas
		Pseudobutyrivibrio
		Pseudoxanthomonas
		Rhodobacter
		Rhodovarius
		Roseomonas
		Sandaracinaceae
		Sandaracinus
		Sarcina
		Silanimonas
		SJA-149
		SM1A02
		Sphingomonas

		Sphingopyxis
		Sporichthya
		Stenotrophomonas
		Subdoligranulum
		TA18
		Terrisporobacter
		TM6
		vadinHA49
		Vampirovibrionales
		Xanthomonadales

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Supplementary Material S4: A list of KEGG categories was not able to be identified using (a) Tax4Fun and (b) PICRUSt bioinformatics tools. Sample list (a) through (h) are presented solely for the purpose of organizing the KEGG numbers. OB12_t4f: OB12 microbial mat sample analyzed by using Tax4Fun; OB13_t4f: OB13 microbial mat sample analyzed by using Tax4Fun; OB12_pie: OB12 microbial mat sample analyzed by using PICRUSt; OB13_pie: OB13 microbial mat sample analyzed by using PICRUSt.

(a)		
X.KEGGID	**OB12_t4f**	**OB13_t4f**
K00025	NA	NA
K00028	NA	NA
K00037	NA	NA
K00071	NA	NA
K00079	NA	NA
K00106	NA	NA
K00178	NA	NA
K00222	NA	NA
K00227	NA	NA
K00300	NA	NA
K00306	NA	NA
K00353	NA	NA
K00369	NA	NA
K00421	NA	NA
K00429	NA	NA
K00504	NA	NA
K00607	NA	NA
K00660	NA	NA
K00702	NA	NA
K00743	NA	NA
K00811	NA	NA
K00827	NA	NA
K00904	NA	NA
K00935	NA	NA
K00964	NA	NA
K01013	NA	NA
K01044	NA	NA
K01121	NA	NA
K01132	NA	NA
K01152	NA	NA
K01188	NA	NA
K01280	NA	NA
K01514	NA	NA

(b)		
X.KEGGID	**OB12_pie**	**OB13_pie**
K00410	NA	NA
K03318	NA	NA
K03365	NA	NA
K03468	NA	NA
K03929	NA	NA
K04765	NA	NA
K05286	NA	NA
K05602	NA	NA
K05828	NA	NA
K05829	NA	NA
K06580	NA	NA
K08086	NA	NA
K10824	NA	NA
K10831	NA	NA
K11130	NA	NA
K11131	NA	NA
K11691	NA	NA
K11692	NA	NA
K12055	NA	NA
K12516	NA	NA
K13037	NA	NA
K13877	NA	NA
K14387	NA	NA
K14561	NA	NA
K14564	NA	NA
K14568	NA	NA
K14574	NA	NA
K14683	NA	NA
K14978	NA	NA
K14979	NA	NA
K14980	NA	NA
K14981	NA	NA
K14982	NA	NA

K01516	NA	NA
K01557	NA	NA
K01634	NA	NA
K01648	NA	NA
K01718	NA	NA
K01762	NA	NA
K01789	NA	NA
K01850	NA	NA
K01930	NA	NA
K01970	NA	NA
K02266	NA	NA
K02312	NA	NA
K02330	NA	NA
K02750	NA	NA
K02753	NA	NA
K02764	NA	NA
K02765	NA	NA
K02779	NA	NA
K02787	NA	NA
K02788	NA	NA
K02790	NA	NA
K02791	NA	NA
K02808	NA	NA
K02809	NA	NA
K02810	NA	NA
K02817	NA	NA
K02818	NA	NA
K02819	NA	NA
K03295	NA	NA
K03743	NA	NA
K03927	NA	NA
K03943	NA	NA
K04340	NA	NA
K04708	NA	NA
K04779	NA	NA
K05344	NA	NA
K05692	NA	NA
K05899	NA	NA
K05929	NA	NA
K05993	NA	NA
K06014	NA	NA

K14983	NA	NA
K14986	NA	NA
K14987	NA	NA
K14988	NA	NA
K14989	NA	NA
K15011	NA	NA
K15012	NA	NA
K15045	NA	NA
K15051	NA	NA
K15052	NA	NA
K15054	NA	NA
K15058	NA	NA
K15059	NA	NA
K15064	NA	NA
K15066	NA	NA
K15067	NA	NA
K15125	NA	NA
K15126	NA	NA
K15226	NA	NA
K15228	NA	NA
K15229	NA	NA
K15230	NA	NA
K15231	NA	NA
K15232	NA	NA
K15234	NA	NA
K15242	NA	NA
K15253	NA	NA
K15256	NA	NA
K15257	NA	NA
K15268	NA	NA
K15269	NA	NA
K15270	NA	NA
K15311	NA	NA
K15312	NA	NA
K15313	NA	NA
K15314	NA	NA
K15315	NA	NA
K15320	NA	NA
K15327	NA	NA
K15328	NA	NA
K15329	NA	NA

K06021	NA	NA
K06026	NA	NA
K06867	NA	NA
K06873	NA	NA
K06883	NA	NA
K06884	NA	NA
K06885	NA	NA
K06887	NA	NA
K06888	NA	NA
K06889	NA	NA
K06890	NA	NA
K06892	NA	NA
K06893	NA	NA
K06894	NA	NA
K06896	NA	NA
K06897	NA	NA
K06898	NA	NA
K06900	NA	NA
K06903	NA	NA
K06904	NA	NA
K06905	NA	NA
K06906	NA	NA
K06907	NA	NA
K06908	NA	NA
K06910	NA	NA
K06911	NA	NA
K06914	NA	NA
K06915	NA	NA
K06916	NA	NA
K06918	NA	NA
K06921	NA	NA
K06922	NA	NA
K06923	NA	NA
K06926	NA	NA
K06927	NA	NA
K06929	NA	NA
K06934	NA	NA
K06936	NA	NA
K06937	NA	NA
K06938	NA	NA
K06940	NA	NA

K15330	NA	NA
K15337	NA	NA
K15342	NA	NA
K15344	NA	NA
K15345	NA	NA
K15346	NA	NA
K15347	NA	NA
K15349	NA	NA
K15350	NA	NA
K15351	NA	NA
K15352	NA	NA
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K15354	NA	NA
K15355	NA	NA
K15357	NA	NA
K15358	NA	NA
K15366	NA	NA
K15367	NA	NA
K15368	NA	NA
K15371	NA	NA
K15372	NA	NA
K15373	NA	NA
K15383	NA	NA
K15395	NA	NA
K15396	NA	NA
K15408	NA	NA
K15429	NA	NA
K15431	NA	NA
K15460	NA	NA
K15461	NA	NA
K15466	NA	NA
K15467	NA	NA
K15468	NA	NA
K15469	NA	NA
K15470	NA	NA
K15471	NA	NA
K15473	NA	NA
K15474	NA	NA
K15475	NA	NA
K15476	NA	NA
K15477	NA	NA

K06942	NA	NA
K06944	NA	NA
K06945	NA	NA
K06946	NA	NA
K06948	NA	NA
K06951	NA	NA
K06952	NA	NA
K06953	NA	NA
K06954	NA	NA
K06955	NA	NA
K06956	NA	NA
K06960	NA	NA
K06962	NA	NA
K06966	NA	NA
K06971	NA	NA
K06972	NA	NA
K06973	NA	NA
K06974	NA	NA
K06975	NA	NA
K06976	NA	NA
K06977	NA	NA
K06978	NA	NA
K06979	NA	NA
K06980	NA	NA
K06981	NA	NA
K06983	NA	NA
K06986	NA	NA
K06987	NA	NA
K06988	NA	NA
K06990	NA	NA
K06991	NA	NA
K06992	NA	NA
K06995	NA	NA
K06996	NA	NA
K06997	NA	NA
K06998	NA	NA
K07000	NA	NA
K07002	NA	NA
K07003	NA	NA
K07004	NA	NA
K07005	NA	NA

K15478	NA	NA
K15479	NA	NA
K15480	NA	NA
K15481	NA	NA
K15482	NA	NA
K15483	NA	NA
K15484	NA	NA
K15486	NA	NA
K15487	NA	NA
K15488	NA	NA
K15490	NA	NA
K15491	NA	NA
K15492	NA	NA
K15495	NA	NA
K15496	NA	NA
K15497	NA	NA
K15509	NA	NA
K15510	NA	NA
K15511	NA	NA
K15512	NA	NA
K15513	NA	NA
K15514	NA	NA
K15515	NA	NA
K15518	NA	NA
K15519	NA	NA
K15520	NA	NA
K15521	NA	NA
K15524	NA	NA
K15525	NA	NA
K15526	NA	NA
K15527	NA	NA
K15531	NA	NA
K15532	NA	NA
K15533	NA	NA
K15534	NA	NA
K15536	NA	NA
K15539	NA	NA
K15540	NA	NA
K15545	NA	NA
K15546	NA	NA
K15547	NA	NA

K07006	NA	NA
K07007	NA	NA
K07009	NA	NA
K07011	NA	NA
K07012	NA	NA
K07013	NA	NA
K07014	NA	NA
K07015	NA	NA
K07016	NA	NA
K07017	NA	NA
K07018	NA	NA
K07019	NA	NA
K07020	NA	NA
K07021	NA	NA
K07024	NA	NA
K07027	NA	NA
K07028	NA	NA
K07029	NA	NA
K07030	NA	NA
K07032	NA	NA
K07033	NA	NA
K07034	NA	NA
K07035	NA	NA
K07037	NA	NA
K07043	NA	NA
K07044	NA	NA
K07045	NA	NA
K07046	NA	NA
K07047	NA	NA
K07050	NA	NA
K07051	NA	NA
K07052	NA	NA
K07053	NA	NA
K07054	NA	NA
K07057	NA	NA
K07059	NA	NA
K07062	NA	NA
K07063	NA	NA
K07064	NA	NA
K07065	NA	NA
K07066	NA	NA

K15548	NA	NA
K15549	NA	NA
K15550	NA	NA
K15551	NA	NA
K15552	NA	NA
K15553	NA	NA
K15554	NA	NA
K15555	NA	NA
K15576	NA	NA
K15577	NA	NA
K15578	NA	NA
K15579	NA	NA
K15580	NA	NA
K15581	NA	NA
K15582	NA	NA
K15583	NA	NA
K15584	NA	NA
K15585	NA	NA
K15586	NA	NA
K15587	NA	NA
K15598	NA	NA
K15599	NA	NA
K15600	NA	NA
K15629	NA	NA
K15632	NA	NA
K15633	NA	NA
K15634	NA	NA
K15635	NA	NA
K15640	NA	NA
K15641	NA	NA
K15642	NA	NA
K15643	NA	NA
K15644	NA	NA
K15645	NA	NA
K15646	NA	NA
K15647	NA	NA
K15649	NA	NA
K15650	NA	NA
K15651	NA	NA
K15652	NA	NA
K15653	NA	NA

K07068	NA	NA
K07069	NA	NA
K07070	NA	NA
K07071	NA	NA
K07072	NA	NA
K07074	NA	NA
K07075	NA	NA
K07076	NA	NA
K07077	NA	NA
K07078	NA	NA
K07079	NA	NA
K07080	NA	NA
K07081	NA	NA
K07083	NA	NA
K07084	NA	NA
K07086	NA	NA
K07088	NA	NA
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