**Supplementary materials**

**Supplementary Figure 1**. Summary of sequence read counts for each sample.



**Supplementary Figure 2**. Rarefaction curve.



**Supplementary Figure 3.** Alpha diversity indices based on sampling lakes. Independent *t*- test was employed to examine the differences between lakes. The significance is shown by small letters a and b. Boxes with different letters show significant difference (*p <0.05*).

**Supplementary Table 1**: Relative abundance of microbial communities at the family level. Results are expressed as the mean ± standard error of mean (SEM).

|  |  |
| --- | --- |
| **Family** | **Mean±SEM** |
| Lake Awassa | Lake Chamo | Lake Hashengie | Lake Tana |
| Acetobacteraceae | 0.0001±0.0001 | 0.0044±0.0018 | 0.0007±0.0005 | 0 |
| Aeromonadaceae | 0.0000± | 0.0014±0.0009 | 0.0070±0.0043 | 0 |
| Bacillaceae | 0.0003±0.0002 | 0.0072±0.0024 | 0.0067±0.0051 | 0.0053±0.0035 |
| Barnesiellaceae | 0.0081±0.0073 | 0.0178±0.0107 | 0.0001±0.0001 | 0.0134±0.0042 |
| Beijerinckiaceae | 0.0049±0.0016 | 0.0022±0.0012 | 0.0092±0.0069 | 0.0013±0.0010 |
| Caldilineaceae | 0.0024±0.0010 | 0.0041±0.0019 | 0.0003±0.0002 | 0 |
| Clostridiaceae | 0.3930±0.0774 | 0.2413±0.0381 | 0.1657±0.0474 | 0.0777±0.0262 |
| Cyanobiaceae | 0.0072±0.0023 | 0.0001±0.0001 | 0 | 0.0006±0.0003 |
| Enterobacteriaceae | 0.0058±0.0056 | 0.0085±0.0034 | 0.0092±0.0048 | 0.0015±0.0005 |
| Erysipelotrichaceae | 0.1473±0.0293 | 0.0167±0.0047 | 0.0007±0.0004 | 0.0119±0.0062 |
| Fusobacteriaceae | 0.0155±0.0078 | 0.1456±0.0429 | 0.4752±0.1075 | 0.6692±0.0563 |
| Hyphomicrobiaceae | 0.0027±0.0013 | 0.0022±0.0008 | 0.0004±0.0004 | 0.0006±0.0005 |
| Kineosporiaceae | 0 | 0.0012±0.0004 | 0.0003±0.0003 | 0 |
| Lachnospiraceae | 0 | 0.0009±0.0004 | 0.0151±0.0096 | 0 |
| Legionellaceae | 0.0044±0.0020 | 0 | 0.0273±0.0156 | 0 |
| Methylococcaceae | 0.0031±0.0012 | 0.0363±0.0171 | 0.0016±0.0007 | 0 |
| Microbacteriaceae | 0 | 0 | 0.0021±0.0014 | 0 |
| Micrococcaceae | 0 | 0.0004±0.0003 | 0.0012±0.0007 | 0 |
| Microcystaceae | 0.0139±0.0032 | 0.0044±0.0022 | 0 | 0.0006±0.0003 |
| Microtrichaceae | 0.0018±0.0005 | 0 | 0 | 0 |
| Mycobacteriaceae | 0.0056±0.0023 | 0.0005±0.0003 | 0.0003±0.0003 | 0.0001±0.0001 |
| Nocardioidaceae | 0 | 0.0034±0.0015 | 0.0001±0.0001 | 0 |
| Oligoflexaceae | 0.0049±0.0024 | 0 | 0 | 0.0002±0.0002 |
| Peptostreptococcaceae | 0.3509±0.0661 | 0.3901±0.0688 | 0.2540±0.0809 | 0.1447±0.0197 |
| Rhizobiales\_Incertae\_Sedis | 0.0046±0.0036 | 0.0095±0.0042 | 0.0017±0.0011 | 0.0020±0.0011 |
| Rhodobacteraceae | 0 | 0.0065±0.0024 | 0.0001±0.0001 | 0 |
| Rickettsiaceae | 0.0030±0.0008 | 0.0021±0.0014 | 0 | 0.0009±0.0004 |
| Ruminococcaceae | 0.0025±0.0010 | 0 | 0 | 0 |
| SC\_I\_84 | 0 | 0.0041±0.0021 | 0 | 0.0004±0.0002 |
| Shewanellaceae | 0 | 0.0013±0.0007 | 0 | 0.0004±0.0003 |
| Silvanigrellaceae | 0 | 0 | 0 | 0.0351±0.0120 |
| Solirubrobacteraceae | 0 | 0 | 0.0037±0.0034 | 0 |
| Steroidobacteraceae | 0 | 0.0384±0.0198 | 0.0011±0.0007 | 0.0006±0.0003 |
| Sutterellaceae | 0.0001±0.0001 | 0.0102±0.0054 | 0.0001±0.0001 | 0.0003±0.0002 |
| Tannerellaceae | 0.0012±0.0012 | 0.0255±0.0134 | 0.0003±0.0003 | 0.0027±0.0011 |
| UBA12409 | 0.0025±0.0009 | 0 | 0 | 0 |
| uncultured | 0 | 0.0033±0.0014 | 0.0022±0.0021 | 0 |
| V19 | 0.0090±0.0030 | 0.0080±0.0029 | 0.0037±0.0027 | 0.0041±0.0026 |
| Vibrionaceae | 0.0052±0.0028 | 0.0022±0.0019 | 0.0101±0.0055 | 0.0265±0.0123 |

**Supplementary Table 2**: Microbial communities identified at genus level from all samples. Results are expressed as the mean ± standard error of mean (SEM).

|  |  |
| --- | --- |
| **Genus** | **Mean±SEM** |
| Lake Awassa | Lake Chamo | Lake Hashengie | Lake Tana |
| Aeromonas | 0 | 0.0014±0.0009 | 0.0070±0.0043 | 0 |
| Aurantimicrobium | 0 | 0 | 0.0021±0.0014 | 0 |
| Bacillus | 0.0003±0.0002 | 0.0072±0.0024 | 0.0067±0.0051 | 0.0053±0.0035 |
| Candidatus\_Megaira | 0 | 0.0021±0.0014 | 0 | 0.0007±0.0004 |
| Candidatus\_Soleaferrea | 0.0025±0.0010 | 0 | 0 | 0 |
| Cetobacterium | 0.0124±0.0074 | 0.1355±0.0410 | 0.4750±0.1074 | 0.6628±0.0580 |
| Clostridium\_sensu\_stricto\_1 | 0.1983±0.0622 | 0.0691±0.0143 | 0.1388±0.0362 | 0.0678±0.0231 |
| Clostridium\_sensu\_stricto\_13 | 0 | 0.0112±0.0065 | 0.0008±0.0007 | 0.0025±0.0022 |
| Cyanobium\_PCC\_6307 | 0.0072±0.0023 | 0.0001±0.0001 | 0 | 0.0006±0.0003 |
| Epulopiscium | 0 | 0.0009±0.0004 | 0.0151±0.0096 | 0 |
| Hyphomicrobium | 0.0027±0.0013 | 0.0022±0.0008 | 0.0004±0.0004 | 0.0006±0.0005 |
| Legionella | 0.0022±0.0013 | 0 | 0.0243±0.0133 | 0 |
| Macellibacteroides | 0.0012±0.0012 | 0.0255±0.0134 | 0.0003±0.0003 | 0.0027±0.0011 |
| Methylocaldum | 0.0003±0.0002 | 0.0009±0.0005 | 0.0012±0.0007 | 0 |
| Methylocystis | 0.0003±0.0003 | 0.0004±0.0002 | 0.0092±0.0069 | 0 |
| Methyloparacoccus | 0.0015±0.0010 | 0.0039±0.0019 | 0.0004±0.0003 | 0 |
| Microcystis\_PCC\_7914 | 0.0139±0.0032 | 0.0044±0.0022 | 0 | 0.0006±0.0003 |
| Mycobacterium | 0.0056±0.0023 | 0.0005±0.0003 | 0.0003±0.0003 | 0.0001±0.0001 |
| Nocardioides | 0 | 0.0014±0.0006 | 0 | 0 |
| Plesiomonas | 0.0058±0.0056 | 0.0085±0.0034 | 0.0092±0.0048 | 0.0015±0.0005 |
| Romboutsia | 0.0006±0.0003 | 0.0852±0.0264 | 0.2132±0.0814 | 0.0230±0.0056 |
| Roseomonas | 0.0001±0.0001 | 0.0044±0.0018 | 0.0007±0.0005 | 0 |
| Shewanella | 0 | 0.0013±0.0007 | 0 | 0.0004±0.0003 |
| Silvanigrella | 0 | 0 | 0 | 0.0351±0.0120 |
| Turicibacter | 0.1473±0.0293 | 0.0167±0.0047 | 0.0007±0.0004 | 0.0119±0.0062 |
| Uncultured | 0.0253±0.0087 | 0.0800±0.0283 | 0.0063±0.0032 | 0.0166±0.0038 |
| V2 | 0.5725±0.0536 | 0.5370±0.0553 | 0.0885±0.0201 | 0.1679±0.0256 |

**Supplementary Table 3**: Alpha diversity indexes and goods coverage of microbial 16S rRNA sequences from the gut of Nile tilapia collected from the four sampling lakes.

|  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| No. | Lake | Samples | Observed | Chao 1 | ACE | Shannon | Simpson | Fisher | Goods |
| 1 | Tana | Au1CH | 27 | 33 | 39.038 | 1.439 | 0.562 | 5.323 | 99 |
| 2 | Tana | Au2CH | 13 | 23 | 19.078 | 0.796 | 0.351 | 2.181 | 99 |
| 3 | Tana | Au3CH | 25 | 25 | 25.326 | 2.092 | 0.796 | 4.838 | 100 |
| 4 | Tana | Au4CH | 16 | 17.5 | 18.741 | 1.613 | 0.676 | 2.801 | 100 |
| 5 | Tana | Au5CH | 24 | 26.143 | 27.854 | 1.392 | 0.568 | 4.600 | 99 |
| 6 | Tana | Au6CH | 35 | 37.625 | 41.672 | 2.395 | 0.833 | 7.369 | 99 |
| 7 | Tana | Au7CH | 27 | 42 | 41.938 | 2.064 | 0.818 | 5.323 | 99 |
| 8 | Tana | Au8CH | 36 | 41.143 | 43.229 | 2.217 | 0.805 | 7.636 | 99 |
| 9 | Tana | Au9CH | 34 | 35.5 | 35.939 | 2.879 | 0.925 | 7.104 | 100 |
| 10 | Tana | Au10CH | 22 | 27 | 30.148 | 1.928 | 0.754 | 4.132 | 99 |
| 11 | Tana | Au11CH | 18 | 20 | 21.670 | 1.168 | 0.477 | 3.232 | 100 |
| 12 | Tana | Au12CH | 29 | 32 | 36.161 | 1.959 | 0.744 | 5.819 | 99 |
| 13 | Chamo | C1CH | 61 | 66 | 66.704 | 3.282 | 0.939 | 15.093 | 99 |
| 14 | Chamo | C2CH | 51 | 54.273 | 56.308 | 2.543 | 0.822 | 11.937 | 99 |
| 15 | Chamo | C3CH | 56 | 59 | 61.621 | 3.117 | 0.927 | 13.486 | 99 |
| 16 | Chamo | C5CH | 49 | 53.5 | 55.591 | 2.919 | 0.908 | 11.333 | 99 |
| 17 | Chamo | C7CH | 35 | 54.5 | 50.799 | 1.696 | 0.658 | 7.369 | 98 |
| 18 | Chamo | C8CH | 27 | 27.857 | 30.300 | 2.156 | 0.812 | 5.323 | 100 |
| 19 | Chamo | C9CH | 55 | 61.429 | 60.833 | 2.686 | 0.839 | 13.172 | 99 |
| 20 | Chamo | C10CH | 32 | 41 | 40.570 | 2.108 | 0.796 | 6.582 | 99 |
| 21 | Chamo | C11CH | 31 | 40.333 | 37.899 | 2.182 | 0.823 | 6.325 | 99 |
| 22 | Chamo | C12CH | 52 | 63.143 | 61.899 | 2.849 | 0.906 | 12.242 | 98 |
| 23 | Hashengie | H1CH | 44 | 55 | 53.045 | 2.427 | 0.800 | 9.863 | 99 |
| 24 | Hashengie | H2CH | 30 | 33.75 | 34.353 | 2.272 | 0.844 | 6.071 | 99 |
| 25 | Hashengie | H3CH | 16 | 21 | 21.836 | 0.709 | 0.308 | 2.801 | 99 |
| 26 | Hashengie | H4CH | 26 | 28.143 | 31.243 | 2.204 | 0.859 | 5.079 | 99 |
| 27 | Hashengie | H5CH | 26 | 26.75 | 27.332 | 2.058 | 0.739 | 5.079 | 100 |
| 28 | Hashengie | H6CH | 24 | 24.6 | 25.972 | 1.764 | 0.648 | 4.600 | 100 |
| 29 | Hashengie | H7CH | 21 | 24 | 23.185 | 1.704 | 0.734 | 3.903 | 100 |
| 30 | Hashengie | H9CH | 22 | 25.333 | 25.707 | 1.074 | 0.401 | 4.132 | 99 |
| 31 | Hashengie | H10CH | 31 | 36.6 | 39.330 | 2.360 | 0.844 | 6.325 | 99 |
| 32 | Awassa | Aw4CH | 47 | 49.333 | 52.220 | 2.620 | 0.878 | 10.738 | 99 |
| 33 | Awassa | Aw5CH | 24 | 25.667 | 27.752 | 1.570 | 0.726 | 4.600 | 99 |
| 34 | Awassa | Aw6CH | 29 | 42.2 | 38.133 | 1.393 | 0.625 | 5.819 | 99 |
| 35 | Awassa | Aw8CH | 32 | 41.167 | 43.018 | 1.760 | 0.714 | 6.582 | 99 |
| 36 | Awassa | Aw9CH | 28 | 43 | 41.428 | 2.019 | 0.803 | 5.569 | 99 |
| 37 | Awassa | Aw10CH | 33 | 33.125 | 33.690 | 1.608 | 0.571 | 6.842 | 100 |
| 38 | Awassa | Aw11CH | 30 | 41 | 48.034 | 1.975 | 0.811 | 6.071 | 99 |
| 39 | Awassa | Aw12CH | 27 | 31 | 37.102 | 1.933 | 0.804 | 5.323 | 99 |

**Supplementary Table 4**: Spearman correlation between the relative abundances of gut microbial communities at genus level and altitude. \*\*. Correlation is significant at the 0.01 level (2-tailed). \*. Correlation is significant at the 0.05 level (2-tailed).

|  |  |  |
| --- | --- | --- |
| Taxa | Correlation Coefficient | Sig. (2-tailed) |
| Aeromonas | 0.196 | 0.233 |
| Aurantimicrobium | 0.398\* | 0.012 |
| Bacillus | -0.166 | 0.313 |
| Candidatus\_Megaira | -0.214 | 0.190 |
| Candidatus\_Soleaferrea | -0.171 | 0.299 |
| Cetobacterium | 0.576\*\* | 0.000 |
| Clostridium\_sensu\_stricto\_1 | 0.042 | 0.802 |
| Clostridium\_sensu\_stricto\_13 | -0.380\* | 0.017 |
| Cyanobium\_PCC\_6307 | -0.199 | 0.224 |
| Epulopiscium | 0.267 | 0.100 |
| Hyphomicrobium | -0.409\*\* | 0.010 |
| Legionella | 0.570\*\* | 0.000 |
| Macellibacteroides | -0.380\* | 0.017 |
| Methylocaldum | -0.042 | 0.801 |
| Methylocystis | 0.259 | 0.111 |
| Methyloparacoccus | -0.479\*\* | 0.002 |
| Microcystis\_PCC\_7914 | -0.499\*\* | 0.001 |
| Mycobacterium | -0.285 | 0.079 |
| Nocardioides | -0.510\*\* | 0.001 |
| Plesiomonas | -0.039 | 0.813 |
| Romboutsia | 0.192 | 0.242 |
| Roseomonas | -0.370\* | 0.020 |
| Shewanella | -0.319\* | 0.048 |
| Silvanigrella | 0.208 | 0.203 |
| Turicibacter | -0.604\*\* | 0.000 |
| Uncultured | -0.494\*\* | 0.001 |
| V2 | -0.788\*\* | 0.000 |
|  |
|  |

**Supplementary Table 5**: Microbial communities showing a significant difference at family level between sampling lakes. Comparison was done by independent t-test.

|  |  |
| --- | --- |
| **Families** | **t-test for Equality of Means** |
| **Between Lake Tana and Lake Awassa** | **Between Lake Tana and Lake Chamo** |
| **t** | **Sig. (2-tailed)** | **Families** | **t** | **Sig. (2-tailed)** |
| Acetobacteraceae | -1.24 | 0.23 | Acetobacteraceae | -2.96\* | 0.01 |
| Bacillaceae | 0.96 | 0.35 | Aeromonadaceae | -1.74 | 0.10 |
| Barnesiellaceae | 0.76 | 0.46 | Bacillaceae | -0.56 | 0.58 |
| Beijerinckiaceae | -2.17\* | 0.04 | Barnesiellaceae | -0.45 | 0.65 |
| Caldilineaceae | -2.41\* | 0.03 | Beijerinckiaceae | -1.02 | 0.32 |
| Clostridiaceae | -4.31\* | 0.00 | Caldilineaceae | -2.45\* | 0.02 |
| Comamonadaceae | 0.81 | 0.43 | Clostridiaceae | -3.53\* | 0.00 |
| Cyanobiaceae | -3.55\* | 0.00 | Comamonadaceae | -2.77\* | 0.01 |
| Enterobacteriaceae | -0.79 | 0.44 | Cyanobiaceae | 0.91 | 0.37 |
| Erysipelotrichaceae | -5.45\* | 0.00 | Enterobacteriaceae | -1.95 | 0.07 |
| Fusobacteriaceae | 9.49\* | 0.00 | Erysipelotrichaceae | -0.32 | 0.75 |
| Hyphomicrobiaceae | -1.63 | 0.12 | Fusobacteriaceae | 7.27\* | 0.00 |
| Lachnospiraceae | -1.24 | 0.23 | Hyphomicrobiaceae | -0.76 | 0.46 |
| Legionellaceae | -2.02 | 0.06 | Kineosporiaceae | -3.09\* | 0.01 |
| Methylococcaceae | -4.07\* | 0.00 | Lachnospiraceae | -2.48\* | 0.02 |
| Methyloligellaceae | 1.09 | 0.29 | Legionellaceae | -0.57 | 0.57 |
| Microcystaceae | -5.38\* | 0.00 | Methylococcaceae | -2.48\* | 0.02 |
| Microtrichaceae | -3.80\* | 0.00 | Methyloligellaceae | -0.03 | 0.98 |
| Mycobacteriaceae | -3.88\* | 0.00 | Micrococcaceae | -1.55 | 0.14 |
| Oligoflexaceae | -2.22\* | 0.04 | Microcystaceae | -2.09 | 0.05 |
| Peptostreptococcaceae | -3.57\* | 0.00 | Microtrichaceae | 0.91 | 0.37 |
| Rhizobiales\_Incertae\_Sedis | -0.71 | 0.48 | Mycobacteriaceae | -3.30\* | 0.00 |
| Rhodobacteraceae | 0.81 | 0.43 | Nocardioidaceae | -2.38\* | 0.03 |
| Rickettsiaceae | -1.54 | 0.14 | Peptostreptococcaceae | -3.79\* | 0.00 |
| Ruminococcaceae | -2.15 | 0.05 | Rhizobiales\_Incertae\_Sedis | -1.60 | 0.13 |
| SC\_I\_84 | -1.24 | 0.23 | Rhodobacteraceae | -2.37\* | 0.03 |
| Silvanigrellaceae | 2.31\* | 0.03 | Rickettsiaceae | -0.93 | 0.36 |
| Steroidobacteraceae | 1.64 | 0.12 | SC\_I\_84 | -2.35\* | 0.03 |
| Sutterellaceae | 0.24 | 0.81 | Shewanellaceae | -1.75 | 0.09 |
| Tannerellaceae | 2.06 | 0.05 | Silvanigrellaceae | 2.60\* | 0.02 |
| V19 | -2.88\* | 0.01 | Steroidobacteraceae | -1.92 | 0.07 |
| Vibrionaceae | 1.42 | 0.17 | Sutterellaceae | -2.36\* | 0.03 |
|  |  |  | Tannerellaceae | -1.80 | 0.09 |
|  |  |  | uncultured | -2.87\* | 0.01 |
|  |  |  | V19 | -1.32 | 0.20 |
|  |  |  | Vibrionaceae | 1.90 | 0.07 |