

Microbial keyplayers involved in P turnover differ in artificial soil mixtures depending on clay mineral composition

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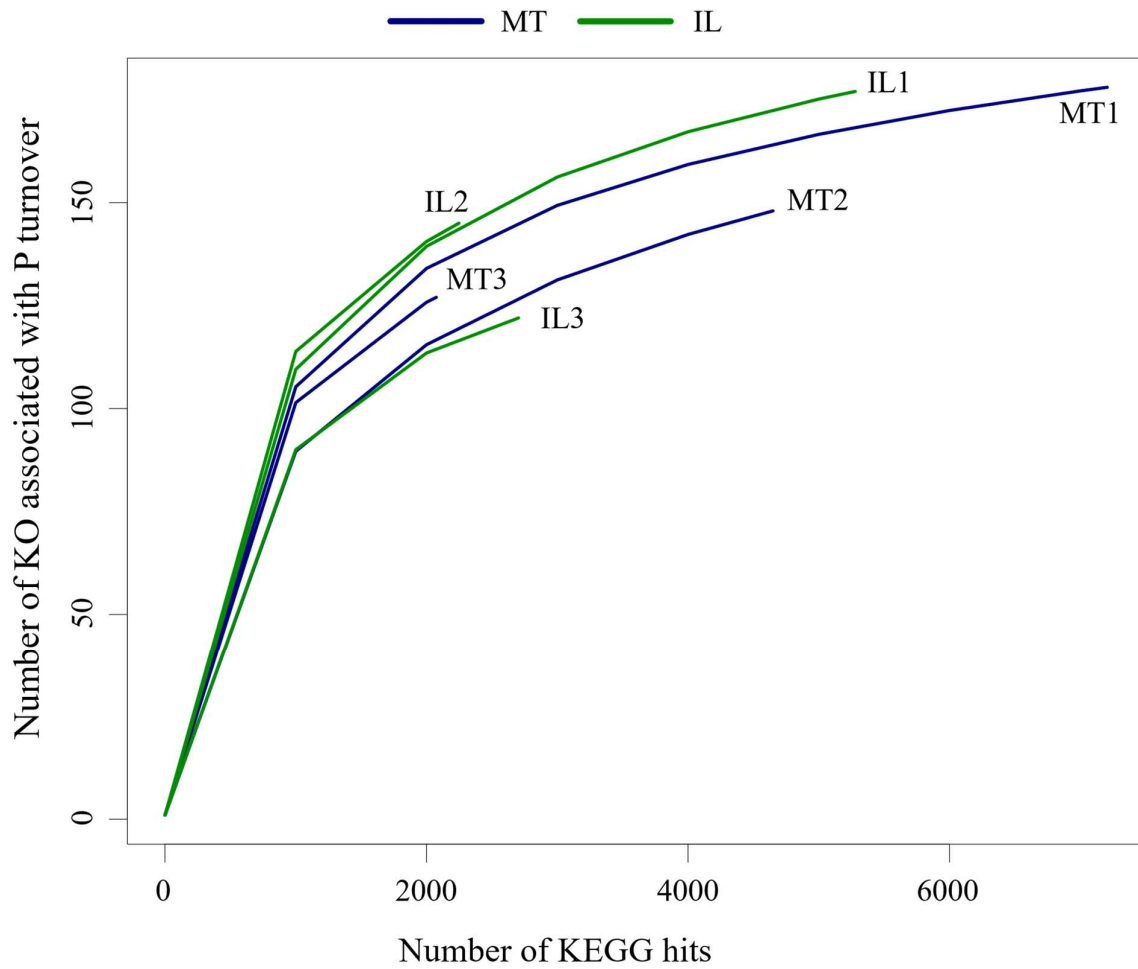
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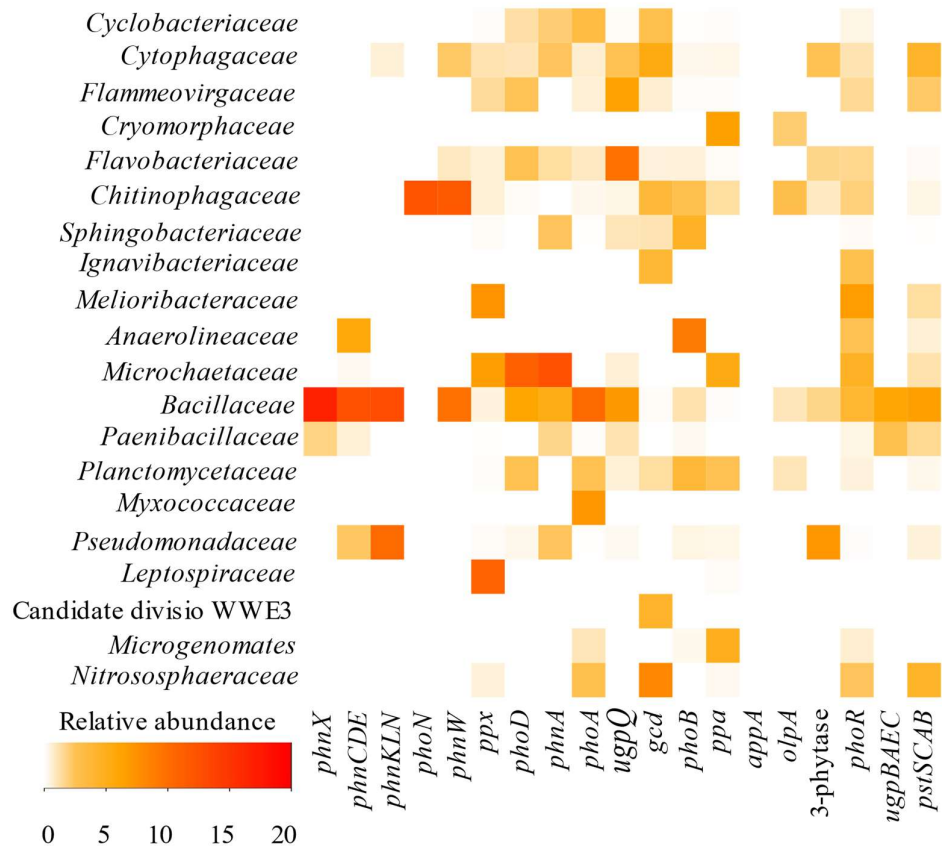
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Supplementary Figure S1: Rarefaction curves based on KO numbers associated with P turnover in montmorillonite and illite. Depicted is the number of KEGG hits associated with P turnover against all KEGG hits.



Supplementary Figure S2: The distribution of microbial genes associated with P turnover across twenty most abundant families. Obtained sequences were assigned on the functional level by aligning against KEGG database and on taxonomic level against NCBI-nr database. Twenty most abundant families are depicted. The values represent mean relative abundance of both soils of assigned sequences per gene.

Supplementary Table S1: Genes and corresponding KEGG orthology numbers (KO) investigated and detected in this study.

| Investigated enzymes | Corresponding gene | KEGG KO number | Detected in dataset | Reference |
|---|---------------------------|-----------------------------|----------------------------|------------------|
| PQQGDH | <i>gcd</i> | KO0117 | + | [1] |
| Acid phosphatase class A | <i>phoN</i> | KO9474 | + | [2] |
| Acid Phosphatase class B | <i>aphA</i> | KO3788 | - | [2] |
| Acid Phosphatase class C | <i>olpA</i> | KO1078 | + | [2] |
| Alkaline phosphatase | <i>phoA</i> | KO1077 | + | [3] |
| Alkaline phosphatase | <i>phoD</i> | KO1113 | + | [4] |
| Alkaline Phosphatase | <i>phoX</i> | no KEGG KO number available | - | [5] |
| Glycerophosphoryl diester phosphodiesterase | <i>ugpQ</i> | KO1126 | + | [6] |
| Phosphotriesterase | <i>opd</i> | KO7048 | - | [7] |
| 4-phytase | <i>appA</i> | KO1093 | + | [8] |
| 3-phytase | <i>phyK</i> | KO1083 | + | [9] |
| 2-aminoethylphosphonate-pyruvate transaminase | <i>phnW</i> | KO3430 | + | [10] |
| Phosphonoacetaldehyde hydrolase | <i>phnX</i> | KO5306 | + | [10] |
| Phosphonoacetate hydrolase | <i>phnA</i> | KO6193 | + | [10] |
| Inorganic pyrophosphatase | <i>ppa</i> | KO1507 | + | [11] |
| Exopolyphosphatase | <i>ppx</i> | KO1524 | + | [12] |
| C-P lyase subunit | <i>phnF</i> | KO2043 | - | [10] |
| C-P lyase subunit | <i>phnG</i> | KO6166 | - | [10] |
| C-P lyase subunit | <i>phnH</i> | KO6165 | - | [10] |
| C-P lyase subunit | <i>phnI</i> | KO6164 | - | [10] |
| C-P lyase subunit | <i>phnJ</i> | KO6163 | - | [10] |
| C-P lyase subunit | <i>phnK</i> | KO5781 | + | [10] |
| C-P lyase subunit | <i>phnL</i> | KO5780 | + | [10] |
| C-P lyase subunit | <i>phnM</i> | KO6162 | - | [10] |
| C-P lyase subunit | <i>phnN</i> | KO5774 | + | [10] |
| C-P lyase subunit | <i>phnO</i> | KO9994 | - | [10] |
| C-P lyase subunit | <i>phnP</i> | KO6167 | - | [10] |

| | | | | |
|---|-------------|--------|---|------|
| Phosphate inorganic transporter | <i>pit</i> | KO3306 | - | [13] |
| Phosphonate transport system subunit | <i>phnC</i> | KO2041 | + | [14] |
| Phosphonate transport system subunit | <i>phnD</i> | KO2044 | + | [14] |
| Phosphonate transport system subunit | <i>phnE</i> | KO2042 | + | [14] |
| Phosphate-specific transport system subunit | <i>pstA</i> | KO2038 | + | [14] |
| Phosphate-specific transport system subunit | <i>pstB</i> | KO2036 | + | [14] |
| Phosphate-specific transport system subunit | <i>pstC</i> | KO2037 | + | [14] |
| Phosphate-specific transport system subunit | <i>pstS</i> | KO2040 | + | [14] |
| Glycerol-3-phosphate transporter subunit | <i>ugpA</i> | KO5814 | + | [14] |
| Glycerol-3-phosphate transporter subunit | <i>ugpB</i> | KO5813 | + | [14] |
| Glycerol-3-phosphate transporter subunit | <i>ugpC</i> | KO5816 | + | [14] |
| Glycerol-3-phosphate transporter subunit | <i>ugpE</i> | KO5815 | + | [14] |
| Phosphate regulon response regulator | <i>phoB</i> | KO7657 | + | [14] |
| Phosphate regulon sensor histidine kinase | <i>phoR</i> | KO7636 | + | [14] |
| PhoR/PhoB Inhibitor Protein (PhoU) | <i>phoU</i> | KO2039 | - | [14] |

Supplementary Table S2: Relative abundance of significantly different sequences annotated to the prokaryotic families in artificial soils are shown. Relative abundances were calculated based on the number of all assigned reads. Values are shown as mean with standard deviation of three independent replicates (n = 3). 4 archaeal families were detected and are marked in grey. Asterisks indicate families whose abundance exceeds 0.005%. Sequences were aligned against NCBI Non-redundant protein (NCBI-nr) database and annotated with MEGAN5.

| Family | MT | IL | Top 20 taxa | p-value |
|--|----------------|---------------|-------------|---------|
| <i>Actinomycetaceae</i> | 0.013 ± 0.003 | 0.023 ± 0.005 | | 0.039 |
| <i>Dermacoccaceae</i> | 0.002 ± 0.001 | 0.003 ± 0.000 | | 0.038 |
| <i>Porphyromonadaceae</i> | 0.431 ± 0.038 | 0.344 ± 0.034 | | 0.042 |
| <i>Prevotellaceae</i> | 0.221 ± 0.018 | 0.163 ± 0.024 | | 0.027 |
| <i>Amoebophilaceae</i> | 0.226 ± 0.038 | 0.047 ± 0.026 | | 0.003 |
| environmental Bacteroidetes | 0.079 ± 0.008 | 0.033 ± 0.010 | | 0.003 |
| <i>Cryomorphaceae</i> | 0.242 ± 0.037 | 0.410 ± 0.039 | | 0.006 |
| <i>Ignavibacteriaceae</i> | 6.601 ± 0.526 | 2.837 ± 1.332 | * | 0.010 |
| <i>Caldiseriaceae</i> | 0.012 ± 0.003 | 0.004 ± 0.001 | | 0.017 |
| <i>Criblamydiaceae</i> | 0.024 ± 0.002 | 0.012 ± 0.005 | | 0.018 |
| environmental Acidobacteriales | 0.003 ± 0.000 | 0.012 ± 0.004 | | 0.023 |
| unclassified Acidobacteria | 0.210 ± 0.008 | 0.117 ± 0.042 | | 0.019 |
| <i>Chitinivibrionaceae</i> | 0.025 ± 0.006 | 0.012 ± 0.005 | | 0.048 |
| <i>Bacillaceae</i> | 10.576 ± 2.505 | 6.093 ± 1.017 | * | 0.045 |
| <i>Paenibacillaceae</i> | 1.465 ± 0.017 | 0.874 ± 0.161 | * | 0.003 |
| <i>Peptoniphilaceae</i> | 0.094 ± 0.012 | 0.045 ± 0.022 | | 0.027 |
| <i>Symbiobacteriaceae</i> | 0.015 ± 0.001 | 0.010 ± 0.002 | | 0.024 |
| <i>Halanaerobiaceae</i> | 0.024 ± 0.000 | 0.017 ± 0.002 | | 0.002 |
| Thermoanaerobacterales Family IV. Incertae Sedis | 0.015 ± 0.002 | 0.008 ± 0.002 | | 0.023 |
| <i>Fusobacteriaceae</i> | 0.112 ± 0.014 | 0.073 ± 0.020 | | 0.049 |

| | | | |
|-----------------------------------|---------------|---------------|-------|
| <i>Gemmatimonadaceae</i> | 0.201 ± 0.046 | 0.076 ± 0.031 | 0.018 |
| unclassified Rhizobiales | 0.016 ± 0.002 | 0.010 ± 0.002 | 0.028 |
| <i>Anaplasmataceae</i> | 0.022 ± 0.003 | 0.012 ± 0.004 | 0.023 |
| <i>Candidatus Midichloriaceae</i> | 0.001 ± 0.001 | 0.003 ± 0.001 | 0.039 |
| <i>Gallionellaceae</i> | 0.007 ± 0.000 | 0.013 ± 0.003 | 0.021 |
| unclassified Methylophilales | 0.003 ± 0.000 | 0.001 ± 0.001 | 0.010 |
| environmental Desulfobacterales | 0.013 ± 0.002 | 0.007 ± 0.002 | 0.033 |
| <i>Desulfomicrobiaceae</i> | 0.004 ± 0.001 | 0.007 ± 0.001 | 0.036 |
| <i>Desulfurellaceae</i> | 0.054 ± 0.004 | 0.026 ± 0.014 | 0.033 |
| environmental delta subdivision | 0.015 ± 0.001 | 0.008 ± 0.003 | 0.014 |
| <i>Myxococcaceae</i> | 0.198 ± 0.005 | 0.122 ± 0.035 | 0.020 |
| environmental Myxobacteria | 0.001 ± 0.000 | 0.000 ± 0.000 | 0.025 |
| <i>Labilitrachaceae</i> | 0.125 ± 0.020 | 0.075 ± 0.019 | 0.035 |
| unclassified Myxococcales | 0.052 ± 0.002 | 0.033 ± 0.006 | 0.006 |
| <i>Syntrophobacteraceae</i> | 0.189 ± 0.003 | 0.083 ± 0.046 | 0.016 |
| <i>Syntrophorhabdaceae</i> | 0.046 ± 0.005 | 0.028 ± 0.003 | 0.006 |
| <i>Acidithiobacillaceae</i> | 0.007 ± 0.002 | 0.013 ± 0.003 | 0.035 |
| <i>Pseudoalteromonadaceae</i> | 0.125 ± 0.003 | 0.106 ± 0.006 | 0.008 |
| <i>Psychromonadaceae</i> | 0.009 ± 0.001 | 0.016 ± 0.002 | 0.007 |
| <i>Chromatiaceae</i> | 0.110 ± 0.007 | 0.162 ± 0.024 | 0.023 |
| <i>Ectothiorhodospiraceae</i> | 0.110 ± 0.016 | 0.182 ± 0.011 | 0.003 |
| <i>Hahellaceae</i> | 0.061 ± 0.009 | 0.094 ± 0.011 | 0.017 |
| <i>Pasteurellaceae</i> | 0.043 ± 0.003 | 0.033 ± 0.002 | 0.015 |
| <i>Gallaecimonas</i> | 0.004 ± 0.000 | 0.009 ± 0.003 | 0.033 |
| <i>Spongiibacter</i> | 0.023 ± 0.003 | 0.012 ± 0.002 | 0.006 |
| <i>Thiobacillus prosperus</i> | 0.019 ± 0.001 | 0.009 ± 0.003 | 0.003 |
| unclassified Gammaproteobacteria | 0.056 ± 0.013 | 0.096 ± 0.011 | 0.015 |
| unclassified Vibrionales | 0.005 ± 0.001 | 0.002 ± 0.001 | 0.044 |
| unclassified Zetaproteobacteria | 0.013 ± 0.002 | 0.006 ± 0.001 | 0.004 |
| <i>Entomoplasmataceae</i> | 0.003 ± 0.001 | 0.001 ± 0.000 | 0.037 |

| | | | |
|---------------------------------|---------------|---------------|-------|
| <i>Caldithrix</i> | 0.500 ± 0.039 | 0.243 ± 0.093 | 0.011 |
| candidate division CPR2 | 0.008 ± 0.007 | 0.022 ± 0.001 | 0.030 |
| candidate division Zixibacteria | 0.732 ± 0.060 | 0.344 ± 0.203 | 0.034 |
| <i>Latescibacteria</i> | 0.007 ± 0.001 | 0.003 ± 0.001 | 0.010 |
| <i>Pyrodictiaceae</i> | 0.001 ± 0.000 | 0.002 ± 0.000 | 0.007 |
| <i>Fervidicoccaceae</i> | 0.009 ± 0.002 | 0.002 ± 0.001 | 0.005 |
| <i>Methanococcaceae</i> | 0.093 ± 0.009 | 0.042 ± 0.025 | 0.031 |
| <i>Methanospirillaceae</i> | 0.003 ± 0.000 | 0.015 ± 0.006 | 0.032 |

Supplementary Table S3 Relative abundance of significantly different sequences annotated to the prokaryotic families involved in P turnover in artificial soils are shown. Relative abundances were calculated based on the number of all assigned reads. Values are shown as mean with standard deviation of three independent replicates (n = 3). 1 archaeal family was detected and marked in grey. Asterisks indicate families whose abundance exceeds 0.005%. Sequences were aligned against NCBI-nr database and annotated with MEGAN5.

| Family | MT | IL | Top 20 taxa | p-value |
|---------------------------------------|----------------|---------------|--------------------|----------------|
| <i>Melioribacteraceae</i> | 11.492 ± 0.635 | 3.604 ± 1.869 | * | 0.002 |
| <i>Paenibacillaceae</i> | 1.530 ± 0.287 | 0.981 ± 0.147 | * | 0.042 |
| <i>Eubacteriaceae</i> | 0.627 ± 0.146 | 0.094 ± 0.106 | | 0.007 |
| <i>Peptococcaceae</i> | 0.132 ± 0.042 | 0.285 ± 0.055 | | 0.019 |
| <i>Thermoanaerobacteraceae</i> | 0.796 ± 0.123 | 0.342 ± 0.138 | | 0.013 |
| <i>Gemmatimonadaceae</i> | 0.111 ± 0.042 | 0.013 ± 0.022 | | 0.022 |
| <i>Rhodobiaceae</i> | 0.005 ± 0.008 | 0.216 ± 0.093 | | 0.017 |
| <i>Myxococcaceae</i> | 1.181 ± 0.193 | 0.194 ± 0.089 | | 0.001 |
| Alteromonadales genera incertae sedis | 0.105 ± 0.008 | 0.159 ± 0.016 | | 0.007 |
| <i>Ectothiorhodospiraceae</i> | 0.030 ± 0.036 | 0.117 ± 0.032 | | 0.034 |
| <i>Oceanospirillaceae</i> | 0.000 ± 0.000 | 0.067 ± 0.026 | | 0.012 |
| <i>Thiotrichaceae</i> | 0.264 ± 0.066 | 0.084 ± 0.045 | | 0.017 |
| unclassified Microgenomates | 0.412 ± 0.572 | 1.794 ± 0.418 | | 0.028 |
| <i>Caldisphaeraceae</i> | 0.077 ± 0.114 | 0.325 ± 0.092 | | 0.043 |

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