**Additional information**

**EVALUATION OF MICROBIAL SHIFTS CAUSED BY A SILVER NANOMATERIAL: COMPARISON OF FOUR TEST SYSTEMS**

Kerstin Hund-Rinke \*a, Anna Hümmler a, Ruben Schlinkert a, Franziska Wege a, Gabriele Broll b

a Fraunhofer Institute for Molecular Biology and Applied Ecology IME, Schmallenberg, Germany

b Osnabrück University, Institute of Geography, Osnabrück, Germany

Table S1: Effect of Ag NM (NM-300K) on potential ammonium oxidation activity.

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| Ag NM Conc. [mg/kg] | NO2--N  [ng/g /h] | SD a | NO2--N  [ng/g /h] | SD | NO2--N  [ng/g /h] | SD |
| Day | 7 |  | 14 |  | 28 |  |
| Control 1 | 62.0 | 2.2 | 51.3 | 5.1 | 57.0 | 2.7 |
| Control 2 | 63.7 | 4.3 | 49.9 | 2.2 | 54.4 | 1.5 |
| 0.19 | 66.0 | 2.8 | 49.0 | 1.9 | 52.4 | 2.4 |
| 0.56 | 59.1 | 5.3 | 54.9 | 4.8 | 53.4 | 2.9 |
| 1.67 | 50.1 | 0.7 | 38.9 | 1.7 | 35.6 | 1.4 |
| 5.0 | 13.3 | 2.0 | 3.3 | 2.3 | 3.9 | 2.8 |
| 15.0 | 2.1 | 1.2 | 0.5 | 1.0 | 1.3 | 1.6 |

a SD = standard deviation

Table S2: Effect of Ag NM (NM-300K) on substrate induced microbial respiration activity (MicroResp™) with the carbon sources glucose, cellobiose, L-alanine, cysteine.

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| Ag NM Conc. [mg/kg] | CO2-C  [µg/g TM/h] | SD a | CO2-C  [µg/g TM/h] | SD | CO2-C [µg/g TM/h] | SD |
| Day | 7 |  | 14 |  | 28 |  |
| Glucose |  |  |  |  |  |  |
| Control 1 | 0.90 | 0.28 | 0.59 | 0.09 | 0.27 | 0.02 |
| Control 2 | 0.89 | 0.32 | 0.98 | 0.12 | 0.29 | 0.02 |
| 0.19 | 1.10 | 0.39 | 0.85 | 0.14 | 0.27 | 0.03 |
| 0.56 | 0.69 | 0.11 | 0.81 | 0.10 | 0.25 | 0.02 |
| 1.67 | 0.64 | 0.05 | 0.92 | 0.07 | 0.22 | 0.01 |
| 5.0 | 0.41 | 0.03 | 0.39 | 0.03 | 0.16 | 0.00 |
| 15.0 | 0.36 | 0.02 | 0.38 | 0.03 | 0.13 | 0.01 |
| Cellobiose |  |  |  |  |  |  |
| Control 1 | 1.06 | 0.63 | 0.66 | 0.13 | 0.26 | 0.01 |
| Control 2 | 0.82 | 0.41 | 0.88 | 0.09 | 0.27 | 0.01 |
| 0.19 | 1.05 | 0.38 | 0.88 | 0.13 | 0.27 | 0.04 |
| 0.56 | 0.55 | 0.07 | 0.91 | 0.20 | 0.23 | 0.01 |
| 1.67 | 0.55 | 0.06 | 0.86 | 0.03 | 0.20 | 0.01 |
| 5.0 | 0.42 | 0.04 | 0.42 | 0.09 | 0.16 | 0.01 |
| 15.0 | 0.35 | 0.03 | 0.36 | 0.02 | 0.12 | 0.00 |
| L-alanine |  |  |  |  |  |  |
| Control 1 | 0.51 | 0.13 | 0.52 | 0.07 | 0.23 | 0.01 |
| Control 2 | 0.49 | 0.17 | 0.56 | 0.04 | 0.25 | 0.01 |
| 0.19 | 0.48 | 0.06 | 0.45 | 0.07 | 0.19 | 0.04 |
| 0.56 | 0.44 | 0.06 | 0.58 | 0.08 | 0.17 | 0.01 |
| 1.67 | 0.39 | 0.02 | 0.61 | 0.03 | 0.15 | 0.01 |
| 5.0 | 0.35 | 0.05 | 0.34 | 0.05 | 0.14 | 0.00 |
| 15.0 | 0.32 | 0.02 | 0.35 | 0.03 | 0.13 | 0.00 |
| Cysteine |  |  |  |  |  |  |
| Control 1 | 0.43 | 0.06 | 0.49 | 0.07 | 0.21 | 0.02 |
| Control 2 | 0.38 | 0.06 | 0.51 | 0.04 | 0.23 | 0.02 |
| 0.19 | 0.43 | 0.06 | 0.45 | 0.10 | 0.15 | 0.01 |
| 0.56 | 0.40 | 0.08 | 0.54 | 0.07 | 0.15 | 0.01 |
| 1.67 | 0.37 | 0.06 | 0.58 | 0.05 | 0.15 | 0.00 |
| 5.0 | 0.42 | 0.06 | 0.42 | 0.04 | 0.13 | 0.00 |
| 15.0 | 0.32 | 0.01 | 0.42 | 0.05 | 0.13 | 0.01 |

a SD = standard deviation

Table S3: Effect of Ag NM (NM-300K) on microbial exoenzyme activity

|  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Ag NM Conc. [mg/kg] | [nmol/min/g] | | SD a | | [nmol/min/g] | | SD | | [nmol/min/g] | | SD | |
| Phosphatase |  | |  | |  | |  | |  | |  | |
| Day | 7 | |  | | 14 | |  | | 28 | |  | |
| Control 1 | 79.49 | | 4.26 | | 78.91 | | 7.24 | | 82.92 | | 13.79 | |
| Control 2 | 70.30 | | 6.74 | | 75.83 | | 3.40 | | 68.39 | | 5.16 | |
| 0.19 | 53.79 | | 5.58 | | 63.64 | | 7.43 | | 76.95 | | 0.71 | |
| 0.56 | 55.22 | | 5.70 | | 50.83 | | 3.69 | | 70.78 | | 6.96 | |
| 1.67 | 43.43 | | 0.41 | | 48.45 | | 7.08 | | 56.58 | | 2.52 | |
| 5.0 | 51.18 | | 5.67 | | 46.19 | | 4.33 | | 57.06 | | 1.25 | |
| 15.0 | 37.00 | | 1.97 | | 47.58 | | 5.71 | | 56.32 | | 2.25 | |
| β-Gluosidase | |  | |  | |  | |  | |  | |  | |
| Control 1 | 13.55 | | 1.48 | | 10.15 | | 0.49 | | 13.18 | | 1.56 | |
| Control 2 | 11.54 | | 1.92 | | 10.23 | | 0.69 | | 9.62 | | 1.40 | |
| 0.19 | 8.88 | | 1.36 | | 9.14 | | 1.80 | | 12.04 | | 0.20 | |
| 0.56 | 8.80 | | 0.47 | | 8.74 | | 0.50 | | 10.86 | | 0.68 | |
| 1.67 | 6.45 | | 0.37 | | 7.81 | | 1.27 | | 9.69 | | 0.86 | |
| 5.0 | 6.25 | | 0.43 | | 6.20 | | 1.23 | | 8.60 | | 1.48 | |
| 15.0 | 5.03 | | 0.60 | | 7.15 | | 1.09 | | 6.64 | | 0.40 | |
| Arylsulfase |  | |  | |  | |  | |  | |  | |
| Day | 7 | |  | | 14 | |  | | 28 | |  | |
| Control 1 | 1.69 | | 0.09 | | 1.46 | | 0.21 | | 1.57 | | 0.17 | |
| Control 2 | 1.50 | | 0.26 | | 1.40 | | 0.10 | | 1.31 | | 0.18 | |
| 0.19 | 1.14 | | 0.13 | | 1.37 | | 0.12 | | 1.45 | | 0.09 | |
| 0.56 | 1.07 | | 0.08 | | 1.13 | | 0.06 | | 1.23 | | 0.07 | |
| 1.67 | 0.96 | | 0.03 | | 1.02 | | 0.05 | | 0.95 | | 0.04 | |
| 5.0 | 0.94 | | 0.12 | | 0.92 | | 0.08 | | 0.90 | | 0.04 | |
| 15.0 | 0.66 | | 0.03 | | 0.71 | | 0.13 | | 0.61 | | 0.06 | |
| Arylamidase |  | |  | |  | |  | |  | |  | |
| Control 1 | 0.44 | | 0.02 | | 0.41 | | 0.03 | | 0.39 | | 0.04 | |
| Control 2 | 0.38 | | 0.01 | | 0.40 | | 0.02 | | 0.34 | | 0.04 | |
| 0.19 | 0.32 | | 0.02 | | 0.39 | | 0.04 | | 0.29 | | 0.01 | |
| 0.56 | 0.35 | | 0.06 | | 0.30 | | 0.01 | | 0.28 | | 0.01 | |
| 1.67 | 0.22 | | 0.04 | | 0.28 | | 0.04 | | 0.18 | | 0.01 | |
| 5.0 | 0.22 | | 0.02 | | 0.32 | | 0.03 | | 0.34 | | 0.01 | |
| 15.0 | 0.26 | | 0.02 | | 0.31 | | 0.05 | | 0.39 | | 0.01 | |

a SD = standard deviation

Table S4: Effect of Ag NM (NM-300K) on genomic diversity (operational taxominic units – 16 S rRNA).

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| Taxon | Control 1 | Control 2 | 0.19 mg/kg | 0.56 mg/kg | 1.67 mg/kg | 5 mg/kg | 15 mg/kg |
| c\_\_Acidobacteriia;o\_\_Acidobacteriales | 5391 | 5304 | 4342 | 4304 | 5559 | 6509 | 3223 |
| c\_\_Solibacteres;o\_\_Solibacterales | 556 | 1017 | 695 | 784 | 1068 | 824 | 288 |
| c\_\_Acidimicrobiia;o\_\_Acidimicrobiales | 393 | 412 | 302 | 288 | 389 | 121 | 153 |
| c\_\_Actinobacteria;o\_\_Corynebacteriales | 170 | 105 | 181 | 121 | 366 | 435 | 485 |
| c\_\_Actinobacteria;o\_\_Streptosporangiales | 607 | 523 | 637 | 715 | 716 | 519 | 350 |
| c\_\_Rubrobacteria;o\_\_Gaiellales | 1123 | 842 | 1177 | 1076 | 864 | 1245 | 1332 |
| c\_\_Actinobacteria;o\_\_Micrococcales | 499 | 413 | 419 | 457 | 536 | 192 | 173 |
| c\_\_Actinobacteria;o\_\_Micromonosporales | 335 | 228 | 219 | 53 | 246 | 320 | 0 |
| c\_\_Actinobacteria;o\_\_Nakamurellales | 257 | 229 | 200 | 243 | 246 | 242 | 187 |
| c\_\_Actinobacteria;o\_\_Propionibacteriales | 152 | 167 | 131 | 76 | 69 | 0 | 0 |
| c\_\_Thermoleophilia;o\_\_Solirubrobacterales | 479 | 405 | 494 | 604 | 460 | 905 | 373 |
| c\_\_Chitinophagia;o\_\_Chitinophagales | 279 | 254 | 280 | 221 | 264 | 996 | 251 |
| c\_\_Cytophagia;o\_\_Cytophagales | 0 | 107 | 67 | 103 | 689 | 1686 | 570 |
| c\_\_Sphingobacteriia;o\_\_Sphingobacteriales | 79 | 107 | 110 | 81 | 197 | 1027 | 1087 |
| c\_\_;o\_\_Chroococcales | 1582 | 1496 | 1114 | 906 | 1195 | 1406 | 1270 |
| c\_\_Gemmatimonadetes;o\_\_Gemmatimonadales | 2877 | 3157 | 2441 | 2195 | 2136 | 1974 | 1644 |
| c\_\_Alphaproteobacteria;o\_\_Caulobacterales | 115 | 469 | 73 | 0 | 88 | 1266 | 726 |
| c\_\_Alphaproteobacteria;o\_\_Rhizobiales | 5345 | 5438 | 3885 | 3586 | 5172 | 5193 | 4991 |
| c\_\_Alphaproteobacteria;o\_\_Rhodospirillales | 766 | 772 | 703 | 564 | 422 | 387 | 11 |
| c\_\_Alphaproteobacteria;o\_\_Sphingomonadales | 1651 | 1658 | 1281 | 1061 | 2379 | 4879 | 3166 |
| c\_\_Betaproteobacteria;o\_\_Burkholderiales | 683 | 879 | 594 | 573 | 604 | 683 | 3903 |
| c\_\_Betaproteobacteria;o\_\_Nitrosomonadales | 852 | 776 | 565 | 528 | 481 | 524 | 384 |
| c\_\_Deltaproteobacteria;o\_\_Desulfovibrionales | 72 | 73 | 0 | 0 | 0 | 0 | 0 |
| c\_\_Gammaproteobacteria;o\_\_Nevskiales | 543 | 610 | 381 | 455 | 406 | 710 | 1099 |
| c\_\_Gammaproteobacteria;o\_\_Xanthomonadales | 779 | 982 | 619 | 580 | 1154 | 2799 | 2846 |
| Others | 1765 | 1845 | 1701 | 1659 | 2210 | 1950 | 839 |
|  |  |  |  |  |  |  |  |
| Total number | 27350 | 28268 | 22611 | 21233 | 27916 | 36792 | 29351 |

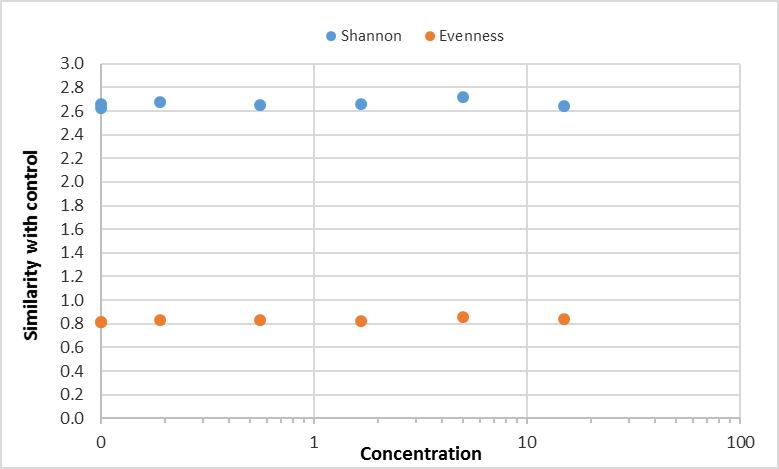


Figure S1: Effect of Ag NM (NM-300K) on genomic diversity expressed as Shannon index and eveness