Supplemental material

Soil microbial communities vary in composition and functional strategy across soil aggregate size class regardless of tillage

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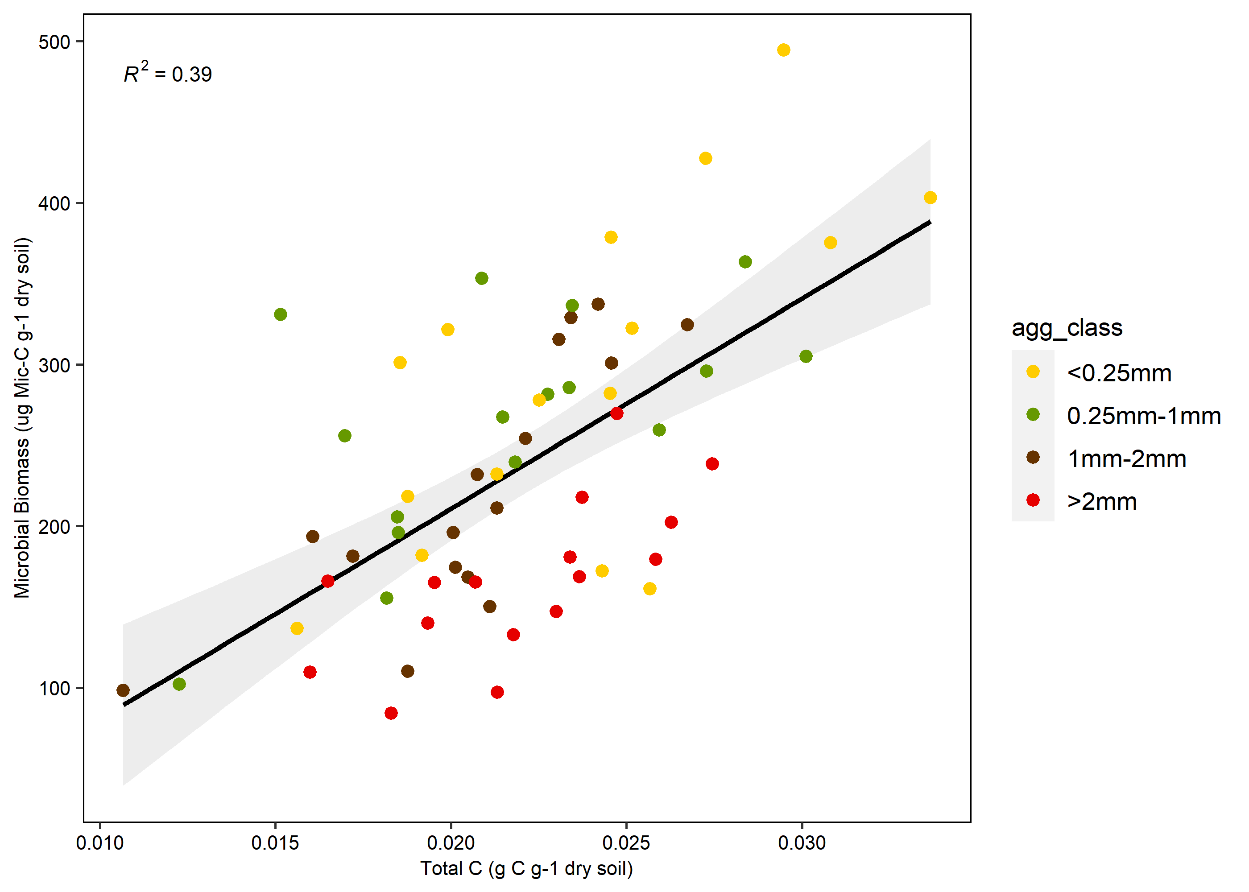
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Chart, line chart

Description automatically generated

**Figure S1: Rarefaction curve showing amplicon sequence variants (ASVs) observed at various sequencing depths**

The colors represent aggregate size classes. Light blue- 1 mm- 2 mm; Dark orange 0.25 mm- 1 mm; Light orange <0.25 mm; Dark blue extraction blanks. The boxes along each curve are the median and 1st and 3rd quartiles of the data for observed ASVs at various sequencing depths.



**Figure S2: Linear regression of microbial biomass** **vs total carbon**

Biomass was calculated via glucose respiration

with the equation from Anderson and Domsch, 1978 and plotted against total carbon. Linear modeling determined that the variables were significantly correlated p < 0.001.

Chart, scatter chart

Description automatically generated

**Figure S3: Linear regression of microbial biomass against soil DNA measured**

Recovered DNA calculated as ng DNA per g dry soil-1

. Biomass calculated via glucose respiration with the equation from Anderson and Domsch, 1978. Linear modeling determined that the variables were significantly correlated P<0.001.

Chart, waterfall chart

Description automatically generated

**Figure S4:** **Aggregate size class distribution in no-till (left) and full-till (right) treatments**

Lower case letters denote significance within tillage treatment. p-values denote significant differences between aggregates within the same size class when comparing no-till and full-till. There was no difference between the 0.25- 1mm aggregate size classes between no-till and full-till. Within full-till sites the <0.25 mm size class differed from both the 0.25-1 mm and >2 mm size class (p < 0.001) and the 1-2 mm size class differed from the 0.25- 1 and >2 mm size class (p <0.001). Within no-till sites, every aggregate size class differed significantly from one another (p < 0.001 for all comparrisons). p-values and significance within tillage treatment determined using two-way ANOVA and Tukey HSD post-hoc test.

**Table S1: Community weighted NSTI values for samples included in Picrust2 analysis**

Lower NSTI values indicate samples with good reference representation. NSTI values between 0.06 < NSTI < 0.15 are considered reliable values. Any OTU’s with NSTI > 2 standard deviations from mean were discarded from analysis. After quality control, 8149 OTU’s were retained.

|  |  |
| --- | --- |
| **sample** | **weighted\_NSTI** |
| LB\_16S\_1 | 0.081055 |
| LB\_16S\_2 | 0.093029 |
| LB\_16S\_3 | 0.085708 |
| LB\_16S\_4 | 0.096764 |
| LB\_16S\_5 | 0.110303 |
| LB\_16S\_6 | 0.102728 |
| LB\_16S\_7 | 0.10931 |
| LB\_16S\_8 | 0.095926 |
| LB\_16S\_10 | 0.112773 |
| LB\_16S\_11 | 0.106172 |
| LB\_16S\_12 | 0.097453 |
| LB\_16S\_14 | 0.105701 |
| LB\_16S\_15 | 0.109831 |
| LB\_16S\_16 | 0.099343 |
| LB\_16S\_18 | 0.094098 |
| LB\_16S\_19 | 0.094662 |
| LB\_16S\_20 | 0.094207 |
| LB\_16S\_21 | 0.118401 |
| LB\_16S\_22 | 0.101297 |
| LB\_16S\_23 | 0.107071 |
| LB\_16S\_24 | 0.094269 |
| LB\_16S\_25 | 0.10373 |
| LB\_16S\_26 | 0.098978 |
| LB\_16S\_27 | 0.112357 |
| LB\_16S\_28 | 0.101437 |
| LB\_16S\_29 | 0.110311 |
| LB\_16S\_30 | 0.103154 |
| LB\_16S\_31 | 0.104774 |
| LB\_16S\_32 | 0.067374 |
| LB\_16S\_34 | 0.098465 |
| LB\_16S\_35 | 0.101493 |
| LB\_16S\_36 | 0.087087 |
| LB\_16S\_37 | 0.090882 |
| LB\_16S\_38 | 0.089149 |
| LB\_16S\_39 | 0.101833 |
| LB\_16S\_40 | 0.084779 |
| LB\_16S\_41 | 0.112045 |
| LB\_16S\_42 | 0.096271 |
| LB\_16S\_43 | 0.113692 |
| LB\_16S\_44 | 0.122051 |
| LB\_16S\_45 | 0.100425 |
| LB\_16S\_46 | 0.100742 |
| LB\_16S\_47 | 0.110877 |
| LB\_16S\_48 | 0.094318 |
| LB\_16S\_49 | 0.115414 |
| LB\_16S\_50 | 0.093069 |
| LB\_16S\_51 | 0.097761 |
| LB\_16S\_53 | 0.09217 |
| LB\_16S\_54 | 0.1118 |
| LB\_16S\_55 | 0.107273 |
| LB\_16S\_57 | 0.104035 |
| LB\_16S\_58 | 0.099947 |
| LB\_16S\_59 | 0.106719 |
| LB\_16S\_60 | 0.105085 |
| LB\_16S\_61 | 0.097653 |
| LB\_16S\_62 | 0.101817 |
| LB\_16S\_63 | 0.103257 |
| LB\_16S\_64 | 0.106076 |

**Table S2:** **Soil and microbial community properties for no-till and full-till aggregate size classes.**

Mean and standard error values are reported. Bold letters denote significant differences between aggregate size classes within tillage treatment analyzed by ANOVA and Tukey HSD post hoc test.

|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Soil properties** | **<0.25 mm** | | | | **0.25-1 mm** | | | | **1- 2 mm** | | | | **>2 mm** | | | |
| **NT** | | **FT** | | **NT** | | **FT** | | **NT** | | **FT** | | **NT** | | **FT** | |
|  | Mean | + SE | Mean | + SE | Mean | + SE | Mean | + SE | Mean | + SE | Mean | + SE | Mean | + SE | Mean | + SE |
| pH | 6.52 | 0.11 | 6.28 | 0.13 | 6.63 | 0.16 | 6.37 | 0.13 | 6.93 | 0.28 | 6.44 | 0.11 | 6.46 | 0.095 | 6.3 | 0.11 |
| EC | 32.72 | 2.89 | 21.25 | 2.17 | 31.07 | 3.41 | 18.29 | 2.15 | 26.04 | 2.6 | 18.97 | 1.86 | 29.97 | 2.94 | 20.18 | 2.67 |
| Total Ca | 0.027 | 1.60E-03 | 0.021 | 1.10E-03 | 0.023 | 1.90E-03 | 0.02 | 1.40E-03 | 0.022 | 1.10E-03 | 0.019 | 1.40E-03 | 0.024 | 1.20E-03 | 0.02 | 8.00E-03 |
| Total Nb | 0.003 | 1.4e-04**a** | 0.0019 | 8.77E-05 | 0.0021 | 1.4e-04**b** | 0.0018 | 1.02E-04 | 0.002 | 6.7e-05**b** | 0.0017 | 1.13E-04 | 0.0022 | 8.8e-05**ab** | 0.0018 | 6.68E-05 |
| C:N | 10.53 | 0.13 | 10.48 | 0.12 | 10.9 | 0.23 | 10.69 | 0.21 | 10.85 | 0.22 | 10.95 | 0.19 | 10.87 | 0.2 | 10.68 | 0.12 |
| Mic. Biomassc | 332.2 | 41.9**a** | 253.67 | 27.44**a** | 315.8 | 12.9**a** | 213.41 | 21.76 **a** | 274.64 | 20.65**ab** | 172.46 | 21.97**ab** | 200.17 | 14.25**b** | 132.81 | 11.65**b** |
| WHC | 0.73 | 0.015**a** | 0.67 | 0.018**a** | 0.65 | 0.018**b** | 0.57 | 0.018**ab** | 0.58 | 0.02**bc** | 0.51 | 0.019**bc** | 0.52 | 0.02**c** | 0.46 | 0.02**c** |
| ***Enzyme Activity (*µ*mol h-1 g dry soil -1)*** | | | | | | | | | | | | | | | | |
| PHOS | 1003 | 318.71 | 734.04 | 79.50**a** | 813.86 | 168.85 | 511.45 | 113.51**ab** | 753.41 | 140.2 | 676.64 | 98.16**ab** | 613.56 | 81.87 | 374.45 | 31.91**b** |
| BG | 401.2 | 76.83 | 228.09 | 33.09 | 370.17 | 47.28 | 199.76 | 32.6 | 322.29 | 43.02 | 249.5 | 41.24 | 365.67 | 48.41 | 151.99 | 13.67 |
| NAG | 98.88 | 15.28 | 57.73 | 8.88a | 94.94 | 8.302 | 54.63 | 7.66 | 84.01 | 11.11 | 65.46 | 9.64 | 101.12 | 5.28 | 48.57 | 7.61 |
| LAP | 88.67 | 14.94 | 76.64 | 9.83 | 88.86 | 13 | 56.09 | 13.16 | 72.52 | 7.99 | 70.11 | 15.21 | 72.04 | 7.89 | 42.43 | 5.12 |
| CBH | 92.76 | 16.77 | 40.66 | 5.93 | 85.94 | 11.01 | 36.61 | 6.68 | 73.97 | 10.19 | 41.64 | 7.7 | 79.63 | 5.98 | 31.64 | 6.45 |
| ***Multiple substrate induced respiration (µg CO2-C g-1 dry soil h-1)*** | | | | | | | | | | | | | | | | |
| Arginine | 1.25 | 0.42 | 0.97 | 0.22 | 1.07 | 0.15 | 0.62 | 0.17 | 1.28 | 0.21 | 0.82 | 0.16 | 0.89 | 0.15 | 0.67 | 0.15 |
| Lysine | 0.40 | 0.15 | 0.23 | 0.08 | 0.20 | 0.05 | 0.17 | 0.07 | 0.47 | 0.07 | 0.25 | 0.06 | 0.34 | 0.07 | 0.24 | 0.08 |
| Citric Acid | 1.81 | 0.33 | 1.13 | 0.27 | 2.10 | 0.15 | 1.12 | 0.24 | 2.72 | 0.11 | 1.81 | 0.22 | 2.30 | 0.3 | 1.54 | 0.23 |
| Malic Acid | 1.97 | 0.56 | 1.25 | 0.26 | 1.85 | 0.21 | 0.92 | 0.17 | 1.33 | 0.14 | 0.74 | 0.15 | 1.34 | 0.25 | 1.01 | 0.19 |
| Trehalose | 1.69 | 0.39 | 1.09 | 0.23 | 1.63 | 0.15 | 0.88 | 0.14 | 1.44 | 0.12 | 0.78 | 0.18 | 1.31 | 0.2 | 1.09 | 0.16 |
| Arabinose | 0.91 | 0.21 | 0.65 | 0.15 | 1.19 | 0.18 | 0.54 | 0.13 | 1.47 | 0.11 | 0.91 | 0.15 | 1.30 | 0.16 | 0.86 | 0.12 |
| Glucose | 2.30 | 0.5 | 1.78 | 0.29**a** | 2.44 | 0.17 | 1.48 | 0.19**ab** | 2.30 | 0.18 | 1.19 | 0.26**ab** | 1.44 | 0.23 | 0.23 | 0.12**b** |
| Total MSIR | 10.34 | 2.44 | 7.1 | 1.32 | 10.49 | 0.81 | 5.73 | 0.86 | 11.03 | 0.77 | 6.5 | 0.11 | 8.94 | 1.24 | 6.22 | 0.94 |
| Basal Resp.d | 1.76 | 0.19 | 1.32 | 0.19 | 1.42 | 0.13 | 1.12 | 0.14 | 1.05 | 0.11 | 0.92 | 0.11 | 1.00 | 0.09 | 0.79 | 0.08 |

a Measured via elemental analysis (g C g-1 dry soil)

b Measured via elemental analysis (g N g-1 dry soil)

c Microbial biomass calculated via method from Anderson and Domsch, 1978 (µg MIC- C g-1 dry soil h-1)

d Basal respiration calculated from water induced respiration during MSIR (µg CO2-C g-1 dry soil h-1

**Table S3:** **Bacterial indicator species in aggregate size classes**

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Indicator species analysis was conducted using the “indicspecies” package in R. There were 2 amplicon sequence variants (ASV) identified as indicators of the <0.25 mm community and 0.25- 1mm communities. There were 5 ASVs identified as indicators of the >2 mm aggregate bacterial community. ASVs with p- values less than 0.05 were considered significant indicators.

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Aggregate Size class** | **Phylum** | **Class** | **Order** | **Family** | **p value** |  |
| <0.25 mm | Actinobacteria | Actinobacteria | Actinomycetales | Nocardioidaceae | 0.001 | \*\*\* |
|  | Actinobacteria | Actinobacteria | Actinomycetales | Sporichthyacaea | 0.007 | \*\* |
| 0.25- 1 mm | Verrucomicrobia | - | - | - | 0.016 | \* |
|  | Actinobacteria | Actinobacteria | Actinomycetales | Actinosynnemataceae | 0.048 | \* |
| >2 mm | Chloroflexi | Anaerolineae | Anaerolineales | Anaerolinaceae | 0.009 | \*\* |
|  | Verrucomicrobia | Pedosphaerae | Pedosphaerales | R4.31B | 0.043 | \* |
|  | Proteobacteria | Alphaproteobacteria | Rhizobiales | Bradyrhizobiaceae | 0.026 | \* |
|  | Gemmatimonadetes | Gemmatimonadetes | Gemmatimonadales | A1.B1 | 0.046 | \* |
|  | Bacteriodetes | Sphingobacteriia | Sphingobacteriales | Sphingobacteriaceae | 0.043 | \* |