Supplementary material

Evidence of Ash Tree (*Fraxinus* spp*.*) Specific Associations with Soil Bacterial Community Structure and Functional Capacity

**Title: Analysis of “healthy ash” sites**

**Supplementary Table 1.** Summary statistics of re-analyzed data using only sites that have ash canopy condition (AC) values less than or equal to 3, resulting in 2 sites (BHN and KRS), consisting of 6 ash plots (meanAC=2.42±0.30) and 4 non-ash plots. Adonis tests were used to analyze differences in overall bacterial community structure and overall soil chemical characteristics between categorical variables (a). Continuous variables were analysed individually (b) for differences between ash and non-ash plots, and differences between sites using Mann-Whitney *U* tests, and for correlations between overall bacterial community structure and individual variables using Mantel tests. Text in bold and italics represents a significant result (p<0.05).

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| (a) |  | Adonis test | | | | | | | | | | | |
|  |  | Bacterial community | | | | |  | | Soil environment | | | | |
| Categorical variables |  | *R2* | | *p*-value | | |  | | *R2* | | | *p*-value | |
| Ash *vs*. Non-ash |  | ***0.457*** | | ***0.029*** | | |  | | 0.139 | | | 0.222 | |
| Forest site |  | 0.045 | | 0.660 | | |  | | 0.232 | | | 0.055 | |
| (b) |  | Mann-Whitney *U* test | | | | | | | |  | Mantel test  (Bacterial community) | | |
|  | (Ash *vs.* Non-ash) | | |  | (Forest site; n=2) | | | |
| Continuous variables |  | *W* | *p*-value | |  | *W* | | *p*-value | |  | *r*-statistic | | *p*-value |
| Mean AC (ash only) |  | - | - | |  | 0 | | 0.064 | |  | 0.066 | | 0.333 |
| Mean Stems (#/ha) |  | 19 | 0.158 | |  | 5.5 | | 0.167 | |  | 0.112 | | 0.197 |
| Mean BA (m2/ha) |  | 1 | 0.694 | |  | 18 | | 0.310 | |  | -0.137 | | 0.797 |
| Ash (%) |  | - | - | |  | 10 | | 0.666 | |  | ***0.553*** | | ***0.007*** |
| Maple (%) |  | 14 | 0.762 | |  | ***23*** | | ***0.032*** | |  | 0.271 | | 0.059 |
| Oak (%) |  | 4 | 0.088 | |  | 20 | | 0.119 | |  | 0.140 | | 0.165 |
| Beech (%) |  | 12 | 1.000 | |  | ***2*** | | ***0.036*** | |  | 0.156 | | 0.182 |
| Hickory (%) |  | 18 | 0.149 | |  | 20 | | 0.072 | |  | -0.213 | | 0.957 |
| α-diversity (tree) |  | 20.5 | 0.087 | |  | 19.5 | | 0.173 | |  | -0.077 | | 0.644 |
| α-diversity (bacteria) |  | 21 | 0.067 | |  | 6 | | 0.222 | |  | -0.120 | | 0.744 |
| Soil pH |  | 20 | 0.114 | |  | 7 | | 0.310 | |  | ***0.513*** | | ***0.010*** |
| %C |  | 17.5 | 0.285 | |  | 13.5 | | 0.917 | |  | -0.204 | | 0.937 |
| %N |  | 19 | 0.163 | |  | ***22.5*** | | ***0.046*** | |  | -0.221 | | 0.958 |
| C:N |  | 11.5 | 1.000 | |  | 6.5 | | 0.249 | |  | -0.234 | | 0.985 |
| Ca |  | 17 | 0.352 | |  | 4 | | 0.095 | |  | ***0.502*** | | ***0.011*** |
| K |  | 15 | 0.610 | |  | 6 | | 0.222 | |  | ***0.312*** | | ***0.044*** |
| Mg |  | 19 | 0.171 | |  | 6 | | 0.222 | |  | ***0.461*** | | ***0.008*** |
| P |  | 14 | 0.762 | |  | ***2*** | | ***0.032*** | |  | -0.009 | | 0.482 |
| Al |  | 9 | 0.610 | |  | ***25*** | | ***0.008*** | |  | ***0.387*** | | ***0.023*** |
| B |  | 11.5 | 1.000 | |  | 8.5 | | 0.463 | |  | -0.001 | | 0.473 |
| Cu |  | 4.5 | 0.134 | |  | 12.5 | | 1.000 | |  | ***0.306*** | | ***0.045*** |
| Fe |  | ***2*** | ***0.038*** | |  | 15 | | 0.691 | |  | ***0.606*** | | ***0.005*** |
| Mn |  | 14 | 0.762 | |  | 13 | | 1.000 | |  | -0.125 | | 0.783 |
| Na |  | 12 | 1.000 | |  | 8 | | 0.421 | |  | -0.069 | | 0.644 |
| S |  | 8 | 0.476 | |  | 15 | | 0.691 | |  | -0.048 | | 0.592 |
| Zn |  | 7 | 0.352 | |  | ***2*** | | ***0.032*** | |  | 0.165 | | 0.149 |

**Title: Forest site characteristics**

**Supplementary Table 2.** Means and standard errors of all measured variables separated by forest site. Alpha diversity was calculated using the Shannon diversity index (*H*). Kruskal Wallis test significance (p<0.05) is indicated by “\*”. Nemenyi post hoc test significant differences (p<0.05) are indicated by superscripts “a” and “b”.

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| --- | --- | --- | --- | --- | --- | --- | --- |
|  | Forest Site | | | | | | |
| Variables | BHN (n=5) |  | KRS (n=5) |  | SYM (n=4) |  | STR (n=6) |
| AC (ash only) | 1.83±0.33 a |  | 3.00±0.00 ab |  | 4.40±0.20 ab |  | 4.83±0.17 b |
| Stems (#/ha) \* | 323.0±24.1 ab |  | 385.0±48.5 ab |  | 600.0±103.6 b |  | 295.8±36.2 a |
| BA (m2/ha) | 37.72±2.57 |  | 34.75±2.99 |  | 25.98±2.97 |  | 33.93±5.05 |
| α-diversity (tree) | 1.164±0.155 |  | 1.065±0.070 |  | 1.415±0.124 |  | 1.281±0.162 |
| α-diversity (bacteria) | 8.221±0.095 |  | 8.432±0.090 |  | 8.619±0.072 |  | 8.523±0.284 |
| pH | 4.78±0.05 |  | 4.99±0.12 |  | 4.98±0.09 |  | 4.97±0.09 |
| %C \* | 3.26±0.48 ab |  | 2.91±0.15 ab |  | 2.18±0.14 b |  | 3.66±0.18 a |
| %N \* | 0.37±0.03 a |  | 0.28±0.02 ab |  | 0.21±0.01 b |  | 0.31±0.01 ab |
| C:N \* | 8.80±0.80 a |  | 10.29±0.33 ab |  | 10.41±0.47 ab |  | 11.78±0.31 b |
| Ca | 554.0±117.6 |  | 1046.8±203.1 |  | 601.3±63.4 |  | 811.9±119.6 |
| K | 73.22±10.75 |  | 90.04±10.08 |  | 87.33±13.23 |  | 69.67±9.63 |
| Mg | 116.43±18.77 |  | 168.46±27.49 |  | 102.38±9.84 |  | 146.57±21.67 |
| P \* | 1.14±0.09 ab |  | 1.52±0.08 a |  | 0.58±0.07 b |  | 0.98±0.15 ab |
| Al \* | 188.64±10.27 a |  | 104.65±22.37 b |  | 124.89±15.31 ab |  | 144.28±21.83 ab |
| B | 0.11±0.01 |  | 0.14±0.02 |  | 0.09±0.01 |  | 0.13±0.02 |
| Cu | 0.21±0.03 |  | 0.20±0.02 |  | 0.19±0.03 |  | 0.26±0.06 |
| Fe | 23.90±3.60 |  | 20.24±6.78 |  | 16.87±4.36 |  | 21.56±5.21 |
| Mn | 61.25±16.67 |  | 54.36±11.36 |  | 34.72±5.23 |  | 41.16±6.68 |
| Na | 7.18±0.54 |  | 7.89±0.57 |  | 5.95±0.39 |  | 7.51±0.67 |
| S | 10.87±0.67 |  | 9.98±0.70 |  | 9.48±1.70 |  | 9.89±0.47 |
| Zn \* | 1.84±0.27 ab |  | 2.97±0.32 a |  | 1.20±0.21 b |  | 2.63±0.70 ab |

**Title: Tree health, dominance, and diversity**

**Supplementary Table 3.** Plot level characteristics of soil pH, tree community health (AC), total basal area (BA), percent dominance of tree genera by BA, and alpha-diversity as calculated by the Shannon diversity index.

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| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Site / Plot |  | Soil pH |  | Mean AC |  | Total BA (m2/ha) |  | Ash (%) |  | Maple (%) |  | Oak (%) |  | Beech (%) |  | Hickory (%) |  | α-diversity (H) |
| BHN1-Ash |  | 4.86 |  | 2 |  | 44.67 |  | 44.3 |  | 16.3 |  | 11.0 |  | 24.4 |  | 3.9 |  | 1.287 |
| BHN2-Ash |  | 4.80 |  | 2.3 |  | 40.07 |  | 43.0 |  | 27.9 |  | - |  | 11.2 |  | 5.6 |  | 1.264 |
| BHN3-Ash |  | 4.73 |  | 1.2 |  | 36.36 |  | 60.7 |  | 21.5 |  | 6.6 |  | 0.9 |  | 1.0 |  | 1.475 |
| BHN1-NonAsh |  | 4.87 |  | 1 |  | 29.00 |  | - |  | 16.6 |  | 54.3 |  | - |  | - |  | 1.228 |
| BHN2-NonAsh |  | 4.63 |  | 1 |  | 38.53 |  | - |  | 49.0 |  | 51.0 |  | - |  | - |  | 0.566 |
| KRS1-Ash |  | 5.31 |  | 3 |  | 33.96 |  | 58.5 |  | 16.7 |  | - |  | 22.1 |  | - |  | 1.040 |
| KRS2-Ash |  | 5.14 |  | 3 |  | 42.96 |  | 59.1 |  | 5.2 |  | - |  | 35.7 |  | - |  | 1.160 |
| KRS3-Ash |  | 5.10 |  | 3 |  | 36.11 |  | 72.1 |  | 11.8 |  | - |  | 12.4 |  | - |  | 1.228 |
| KRS1-NonAsh |  | 4.65 |  | 1 |  | 24.42 |  | - |  | 3.8 |  | - |  | 91.6 |  | - |  | 1.082 |
| KRS2-NonAsh |  | 4.75 |  | 1 |  | 36.30 |  | - |  | 5.6 |  | 45.9 |  | 47.6 |  | - |  | 0.817 |
| SYM1-Ash |  | 5.23 |  | 5 |  | 31.29 |  | 40.5 |  | 11.2 |  | 25.2 |  | - |  | - |  | 1.477 |
| SYM2-Ash |  | 5.00 |  | 4.5 |  | 19.81 |  | 52.4 |  | 38.6 |  | - |  | - |  | - |  | 1.075 |
| SYM1-NonAsh |  | 4.78 |  | 5 |  | 30.82 |  | 3.9 |  | 22.6 |  | 73.6 |  | - |  | - |  | 1.437 |
| SYM2-NonAsh |  | 4.97 |  | 1 |  | 21.99 |  | 1.5 |  | 11.7 |  | 11.5 |  | - |  | 70.9 |  | 1.672 |
| STR1-Ash |  | 4.96 |  | 1 |  | 45.10 |  | 29.0 |  | 34.7 |  | 27.5 |  | 8.9 |  | - |  | 1.221 |
| STR2-Ash |  | 4.97 |  | 1 |  | 12.46 |  | 17.0 |  | 36.0 |  | - |  | - |  | 13.9 |  | 1.408 |
| STR3-Ash |  | 5.09 |  | 4.6 |  | 46.25 |  | 60.6 |  | 3.5 |  | 31.5 |  | - |  | - |  | 1.622 |
| STR1-NonAsh |  | 5.03 |  | 4.2 |  | 31.09 |  | - |  | 60.4 |  | 33.2 |  | - |  | 6.4 |  | 0.849 |
| STR2-NonAsh |  | 4.55 |  | 1 |  | 37.33 |  | - |  | 32.5 |  | 50.1 |  | 16.4 |  | - |  | 1.386 |
| STR3-NonAsh |  | 5.23 |  | 1 |  | 31.36 |  | - |  | 5.4 |  | 92.0 |  | - |  | - |  | 1.265 |

**Title: Twenty most abundant bacterial classes**



**Figure S1.** Boxplot comparing the average Hellinger transformed abundances of the 20 most abundant bacterial/archael classes between ash (blue) and non-ash (orange) plots. Mann-Whitney U-test significance is denoted by asterisks, where \* = p<0.05.

**Title: Thirty most abundant bacterial orders**



**Figure S2.** Boxplot comparing the average Hellinger transformed abundances of the 30 most abundant bacterial/archael orders between ash (blue) and non-ash (orange) plots. Mann-Whitney U-test significance is denoted by asterisks, where \* = p<0.05.