*Supplementary Material*

# Tree species shape soil bacterial community structure and function in temperate deciduous forests

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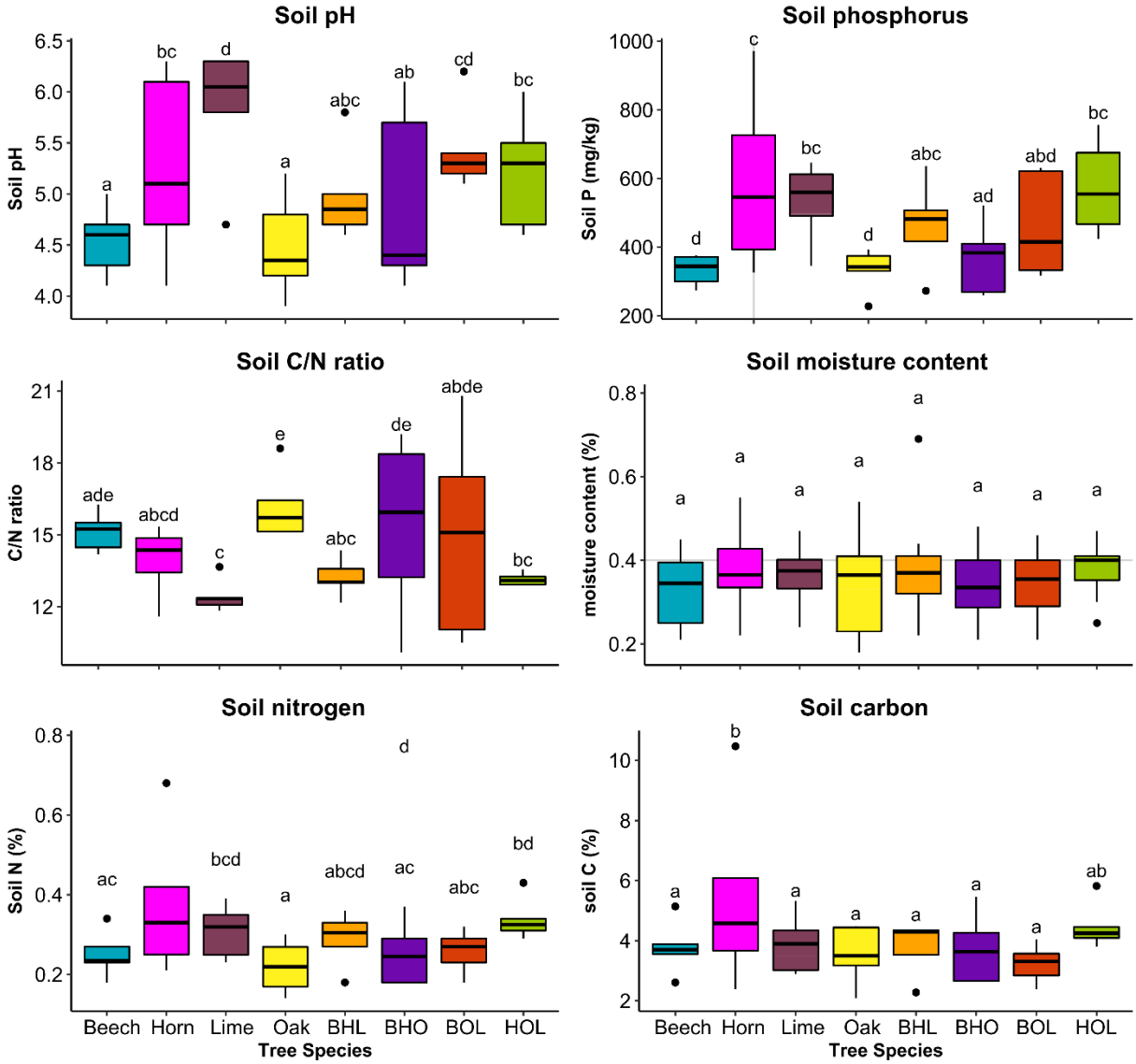
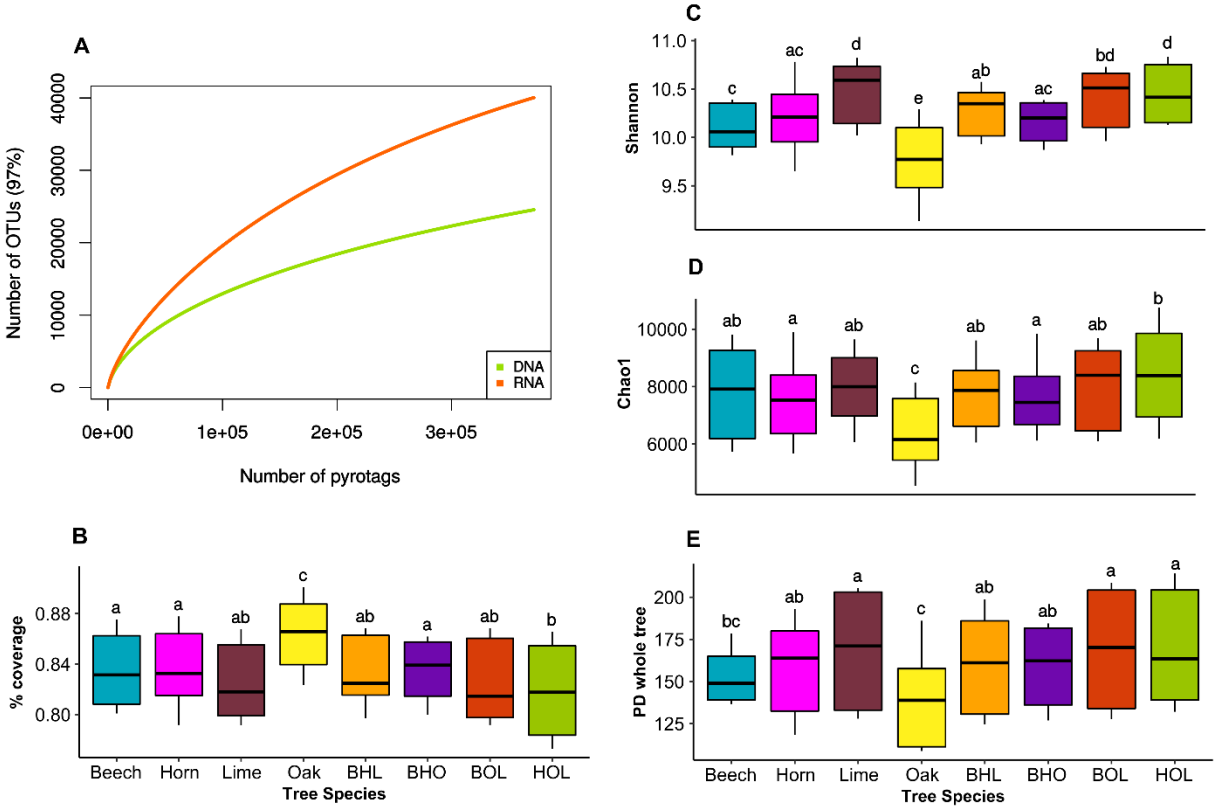


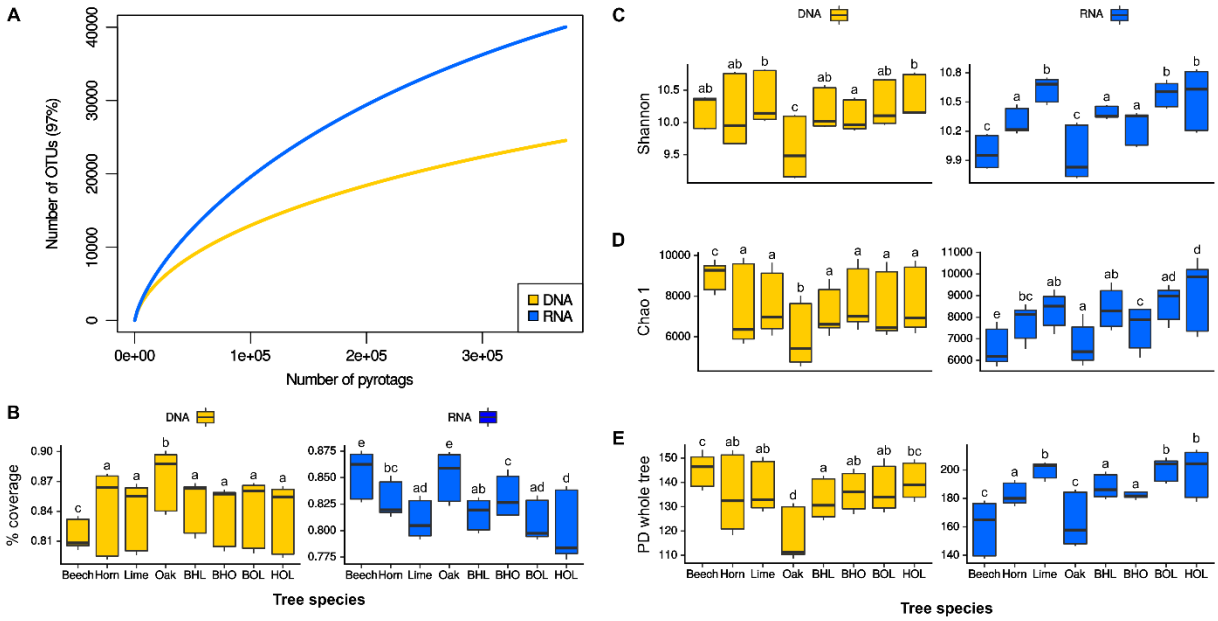

**Supplementary Figure S1.|** Boxplots showing soil environmental parameters in mono and mixed stands. Mean values (n = 6 replicated stand) are given. Horizontal bars show median value. Mean values with similar letters determined by ANOVA with Tukey HSD post-hoc test shared significant similarities (*p* 0.05). Soil data was previously collected and described by Schmidt et al. (2015). Abbreviations: BHL, beech, hornbeam, lime; BHO, beech, hornbeam, oak; BOL, beech, oak, lime; HOL, hornbeam, oak, lime.

Reference:

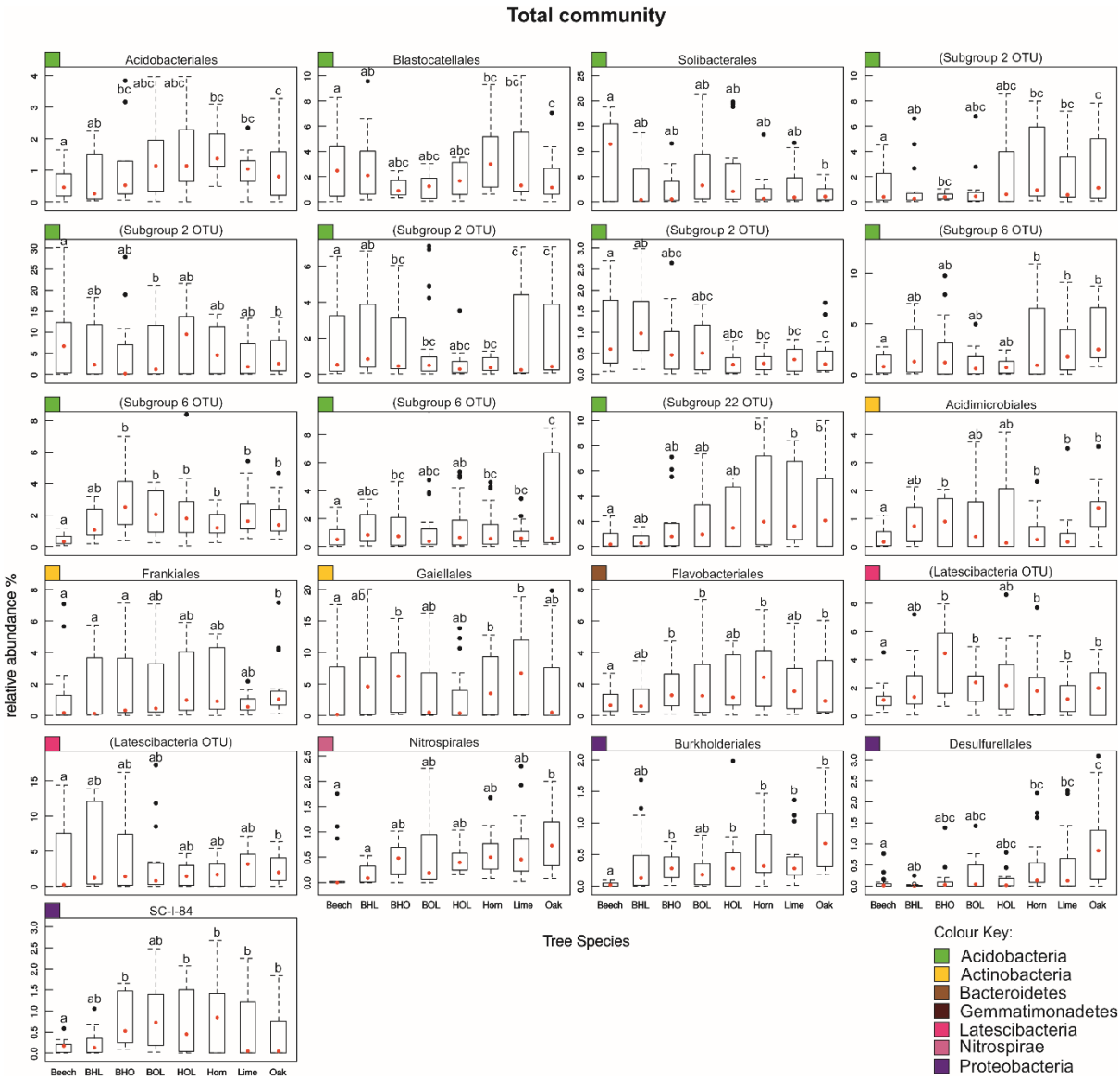
Schmidt, M., Veldkamp, E., and Corre, M. D. (2015). Tree species diversity effects on productivity, soil nutrient availability and nutrient response efficiency in a temperate deciduous forest. *For. Ecol. Manage.* 338, 114–123. doi:10.1016/j.foreco.2014.11.021



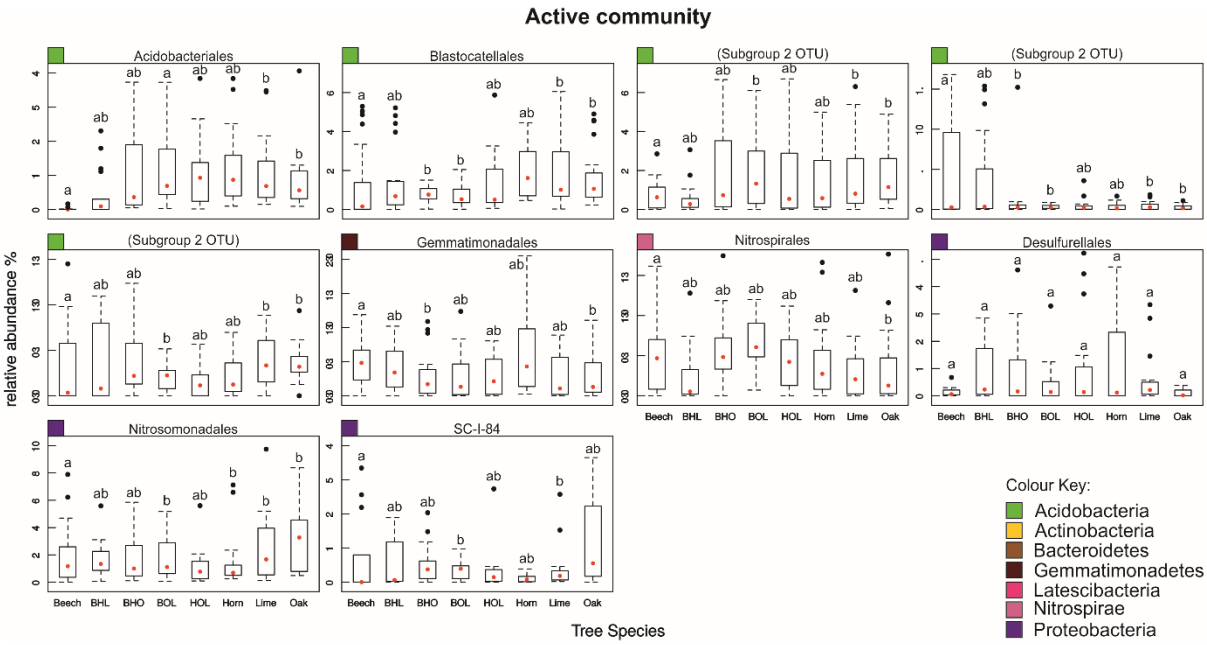

**Supplementary Figure S2.**| Alpha diversity metrics of soil bacterial OTUs across different tree stands of the entire dataset. **(A)** Rarefaction curves of subsampled OTUs for environmental DNA and RNA, at 97% similarity. **(B)** Good's coverage, **(C)** Shannon richness index, **(D)** Chao1 estimator and **(E)** phylogenetic diversity (PD). Mean values are given (n = 6). Horizontal bars represent the median value. Mean values with similar letters determined by ANOVA with Tukey HSD post-hoc test shared significant similarities ($p \leq 0.05$). Abbreviations: BHL, beech, hornbeam, lime; BHO, beech, hornbeam, oak; BOL, beech, oak, lime; HOL, hornbeam, oak, lime.



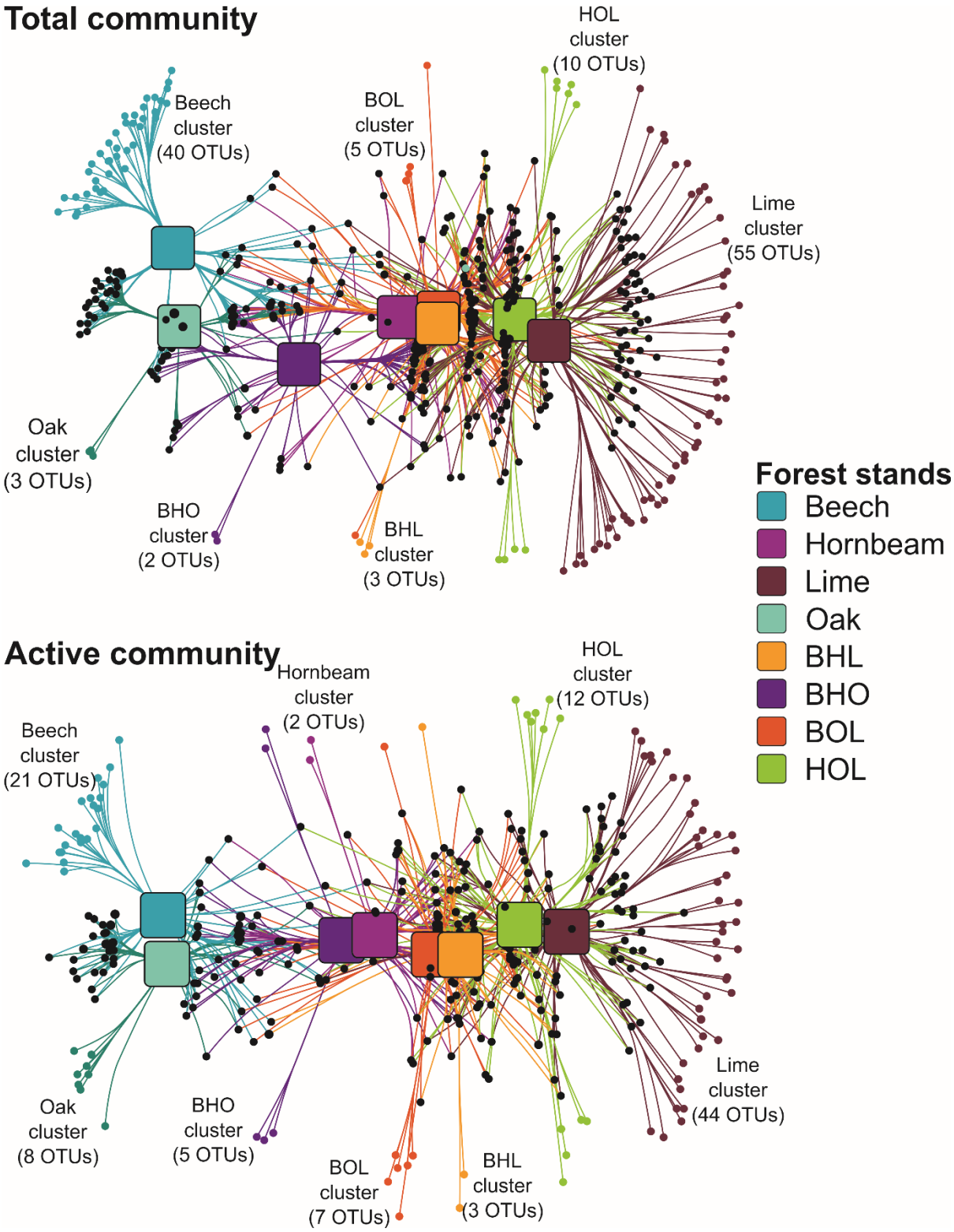

**Supplementary Figure S3**. Alpha diversity metrics for DNA-derived and RNA-derived OTUs, across different tree stands. **(A)** Rarefaction curves of subsampled OTUs for environmental DNA and RNA, at 97% similarity. **(B)** Good's coverage, **(C)** Shannon richness index, **(D)** Chao1 estimator and **(E)** phylogenetic diversity (PD). Mean values are given (n = 6). Horizontal bars represent the median value. Mean values with similar letters determined by ANOVA with Tukey HSD post-hoc test shared significant similarities ($p \leq 0.05$). Abbreviations: BHL, beech, hornbeam, lime; BHO, beech, hornbeam, oak; BOL, beech, oak, lime; HOL, hornbeam, oak, lime.



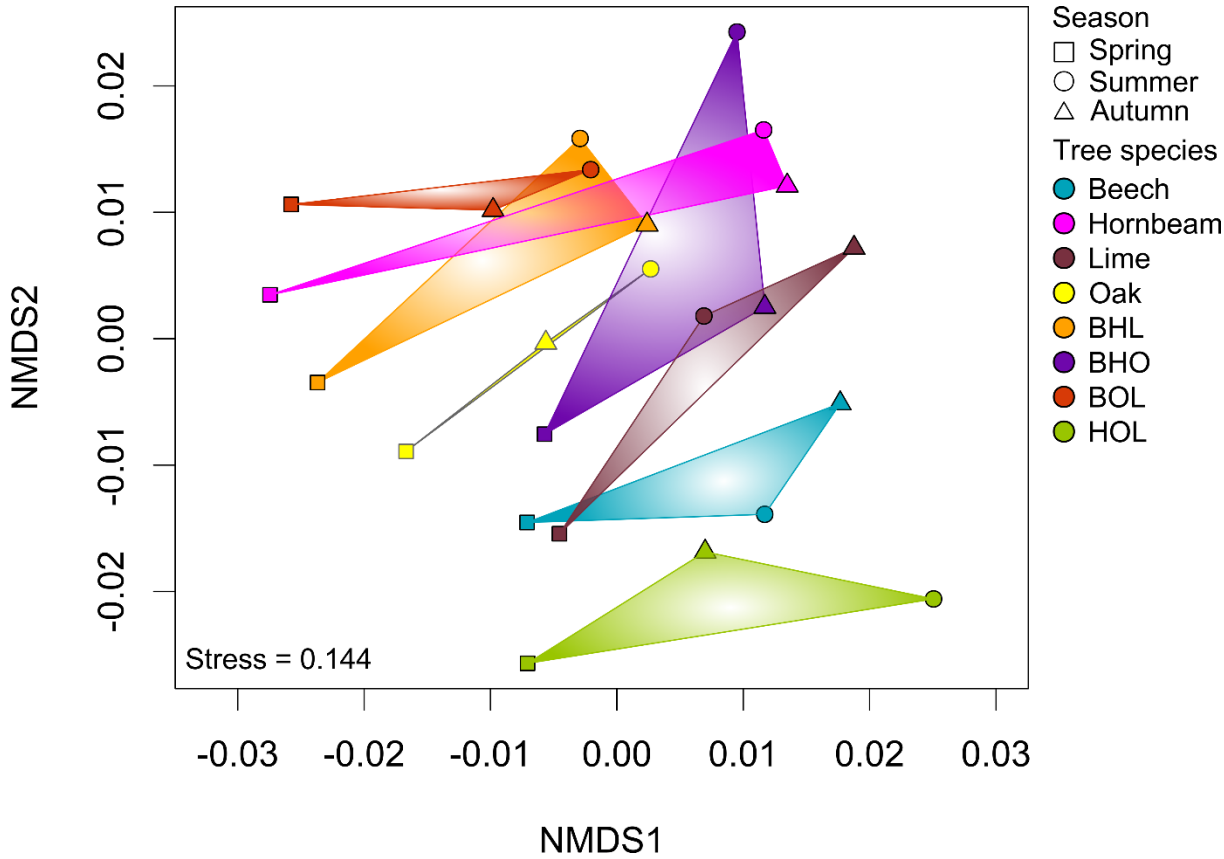

**Supplementary Figure S4.**| Bacterial orders at total community level showing significant change in relative abundance across different tree stands. Only bacteria orders belonging to dominant phyla (relative abundance higher than 1 %) are shown. In the case that the order could not be assigned, the taxonomic name at the highest determined taxonomic resolution is given in parenthesis. Mean values of replicate stands ($n$ = 6) for three seasons are given. Statistical calculations employed Kruskal-Wallis test with Dunn's post hoc test. A $p \leq 0.05$ was considered statistically significant. Mean values with similar letters determined by ANOVA with Tukey HSD post-hoc test shared significant similarities ($p \leq 0.05$). Red dots represent the median value. Black dots represent outlying data. Abbreviations: BHL, beech, hornbeam, lime; BHO, beech, hornbeam, oak; BOL, beech, oak, lime; HOL, hornbeam, oak, lime.




**Supplementary Figure S5.** Bacterial orders at potentially active community level showing significant change in relative abundance across different tree stands. Only bacteria orders belonging to dominant phyla (greater than 1 %) are shown. In the case that the order could not be assigned, the taxonomic name at the highest determined taxonomic resolution is given in parenthesis. Mean values of replicate stands ($n$ = 6) for three seasons are given. Statistical calculations employed Kruskal-Wallis test with Dunn's post hoc test. Mean values with similar letters determined by ANOVA with Tukey HSD post-hoc test share significant similarities ($p \leq 0.05$). Red dots represent the median value. Black dots represent outlying data. Abbreviations: BHL, beech, hornbeam, lime; BHO, beech, hornbeam, oak; BOL, beech, oak, lime; HOL, hornbeam, oak, lime.




**Supplementary Figure S6.|** Bipartite association networks between soil bacterial communities (genus level) and tree stands. Source nodes (rounded squares) represent tree stands and edges represent associations between stands and bacterial OTUs (circles, target nodes). Edges are coloured according to the source tree stand and the length of edges is weighted according to association strength. Unique clusters, which associate with one tree species, consist of nodes coloured as the corresponding stand. Numbers of OTUs making up respective unique clusters are given in parenthesis. Black circles represent OTUs with significant cross association between two or more stands. Target node sizes represent mean relative abundance of OTUs across all mono plots. Data only represents OTUs that showed significant positive association with tree stands ($p \leq 0.05$). For visualisation, edges were bundled with a stress value of 3.




**Supplementary Figure S7.|** Non-metric multidimensional scaling (NMDS) of potential gene functions in methane metabolism. Significant grouping of metabolic genes according to tree species (*p* 0.033) is given. NMDS was constructed with a Bray Curtis distance matrix using KEGG orthologs predicted with Tax4fun (Aßhauer et al., 2015). Genes used to construct the plot are listed in Supplementary Table S5.

Reference:

Aßhauer, K. P., Wemheuer, B., Daniel, R., and Meinicke, P. (2015). Tax4Fun: Predicting functional profiles from metagenomic 16S rRNA data. *Bioinformatics* 31, 2882–2884. doi:10.1093/bioinformatics/btv287

**Supplementary Table S1**. Summary of sequence data generated with high throughput pyrotag sequencing.

| **Reads after key steps of sequencing pipeline** | **Template** | |
|---|---|---|
| | DNA | RNA[a] |
| 1. Raw reads | 3,335,821 | 1,308,705 |
| 2. Quality filtering | 3,052,200 | 1,291,122 |
| 3. Chimera and singleton removal | 2,402,787 | 857,868 |
| 4. Taxonomy filtering | 2,401,078 | 857,464 |
| % Recovery | 72.4 | 65.7 |
| Total OTUs[b] | **40,385** | **52,277** |

[a] Reads for RNA were generated via cDNA
[b] OTUs generated after clustering of abovementioned reads. Numbers represent total count across all plots in spring, summer and autumn from subsampled datasets of environmental DNA and RNA.
[c] Recovery refers to the percentage of sequence reads after processing over the total reads.

**Supplementary Table S2**. Statistical tests of tree stand effects on bacterial community structure. Results of ANOSIM and PERMANOVA were calculated using weighted UniFrac matrices representing the total (DNA-based) and potentially active (RNA-based) bacterial community. A *p* value ≤ 0.05 was considered significant.

| Test | ANOSIM | | | | PERMANOVA | | | |
|---|---|---|---|---|---|---|---|---|
| | DNA | | RNA | | DNA | | RNA | |
| | *p*-value | R | *p*-value | R | *p*-value | Pseudo F | *p*-value | Pseudo F |
| Tree species | 0.001 | 0.72 | 0.001 | 0.63 | 0.001 | 9.4 | 0.001 | 4.9 |
| Stand type | 0.008 | 0.22 | 0.002 | 0.23 | 0.1 | 2.4 | 0.044 | 2.9 |
| Season | 0.464 | -0.009 | 0.038 | 0.132 | 0.529 | 0.73 | 0.132 | 1.7 |

| NMDS goodness of fit | | | |
|---|---|---|---|
| | *p* value | $R^2$ | Significance |
| Template | 0.001 | 0.439 | *** |
| Tree species | 0.001 | 0.506 | *** |
| Stand type | 0.043 | 0.068 | * |
| Season | 0.858 | 0.014 | NS |

| Environmental parameters | | | | Mantel test | | |
|---|---|---|---|---|---|---|
| | *p* value | $R^2$ | Significance | *p* value | $R^2$ | Significance |
| pH | 0.001 | 0.809 | *** | 0.001 | 0.518 | *** |
| N | 0.001 | 0.353 | *** | 0.001 | 0.215 | *** |
| C/N ratio | 0.001 | 0.683 | *** | 0.001 | 0.384 | *** |
| P | 0.001 | 0.589 | *** | 0.001 | 0.418 | *** |
| MC | 0.001 | 0.655 | *** | 0.001 | 0.430 | *** |
| C | 0.490 | 0.341 | NS | 0.936 | -0.079 | NS |

Abbreviations: N, nitrogen; C/N, carbon/nitrogen; P, phosphorus; MC, moisture content; C, carbon

Significance 0 = ***, 0.001 = **, 0.01 = *, 0.05 = *, greater than 0.05 = not significant (NS)

**Supplementary Table S3**. Summary of abundant soil bacterial genera across mono and mixed stands at entire community level. Values represent mean relative abundance for 6 replicate stands in spring, summer and autumn. Values are given for total (DNA-based) community. Only genera from abundant orders (greater than 1 %) are given. In the case that the order or genus could not be assigned, the taxonomic name at the highest determined taxonomic resolution is given in parenthesis.

| **Total community** | | **Relative abundance (%) in forest stands** | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Order | Genus | Mono stands | | | | Mixed stands | | | | |
| | | **Beech** | **Horn** | **Lime** | **Oak** | **BHL** | **BHO** | **BOL** | **HOL** | **Average** |
| ***Acidobacteria*** | | | | | | | | | | |
| *Acidobacteriales* | *(Acidobacteriaceae)* | 13.74 | 6.87 | 1.17 | 12.84 | 5.24 | 9.31 | 5.11 | 3.32 | 7.20 |
| *Solibacterales* | *Bryobacter* | 6.20 | 4.34 | 1.52 | 6.90 | 3.57 | 5.28 | 4.11 | 2.78 | 4.34 |
| | *Candidatus Solibacter* | 5.05 | 3.85 | 2.61 | 4.30 | 4.09 | 4.20 | 3.61 | 3.18 | 3.86 |
| *Subgroup 2* | *(Subgroup 2)* | 13.20 | 6.57 | 1.29 | 11.99 | 4.28 | 7.67 | 4.48 | 3.39 | 6.61 |
| *Subgroup 6* | *(Subgroup 6)* | 2.11 | 4.35 | 8.01 | 2.10 | 5.55 | 3.49 | 4.92 | 5.85 | 4.55 |
| | *(Subgroup 6)* | 0.86 | 2.06 | 3.41 | 0.85 | 2.68 | 1.50 | 2.22 | 2.62 | 2.02 |
| ***Actinobacteria*** | | | | | | | | | | |
| *Acidimicrobiales* | *(Acidimicrobiales)* | 0.77 | 0.99 | 0.95 | 0.83 | 0.93 | 0.93 | 1.02 | 1.07 | 0.94 |
| *Frankiales* | *Acidothermus* | 2.53 | 4.03 | 0.91 | 3.02 | 2.21 | 3.19 | 2.25 | 1.95 | 2.51 |
| *Gaiellales* | *(Gaiellales)* | 1.15 | 2.24 | 2.14 | 1.08 | 2.30 | 1.78 | 2.47 | 2.62 | 1.97 |
| *Solirubrobacterales* | *(Solirubrobacterales)* | 0.17 | 0.48 | 0.71 | 0.17 | 0.48 | 0.39 | 0.59 | 0.70 | 0.46 |
| ***Bacteroidetes*** | | | | | | | | | | |
| *Cytophagales* | *(Cytophagaceae)* | 0.66 | 1.32 | 1.58 | 0.94 | 1.46 | 1.33 | 1.28 | 1.80 | 1.29 |
| *Flavobacteriales* | *Flavobacterium* | 0.33 | 1.02 | 1.79 | 0.45 | 0.81 | 0.73 | 1.12 | 1.69 | 0.99 |
| *Sphingobactriales* | *(Chitinophagaceae)* | 0.97 | 1.01 | 0.89 | 1.04 | 0.97 | 1.06 | 1.09 | 1.04 | 1.01 |
| ***Gemmatimonadetes*** | | | | | | | | | | |
| *Gemmatimonadales* | *Gemmatimonas* | 1.46 | 1.19 | 1.58 | 1.09 | 1.61 | 1.10 | 1.89 | 1.43 | 1.42 |
| | *(Gemmatimonadaceae)* | 1.25 | 1.10 | 1.34 | 0.71 | 1.15 | 1.02 | 1.49 | 1.17 | 1.15 |
| ***Latescibacteria*** | | | | | | | | | | |
| *(Latescibacteria)* | *(Latescibacteria)* | 0.20 | 0.62 | 1.48 | 0.16 | 0.90 | 0.58 | 0.81 | 0.89 | 0.70, |
| | *(Latescibacteria)* | 0.07 | 0.22 | 0.42 | 0.09 | 0.45 | 0.13 | 0.34 | 0.46 | 0.27 |
| | *(Latescibacteria)* | 0.02 | 0.09 | 0.27 | 0.03 | 0.15 | 0.06 | 0.12 | 0.11 | 0.11 |
| ***Nitrospirae*** | | | | | | | | | | |
| *Nitrorpirales* | *(Nitrospirales)* | 0.04 | 0.44 | 1.38 | 0.06 | 0.62 | 0.35 | 0.56 | 0.62 | 0.51 |
| | *Nitrospira* | 0.35 | 0.26 | 0.51 | 0.20 | 0.30 | 0.19 | 0.37 | 0.30 | 0.31 |
| ***Proteobacteria*** | | | | | | | | | | |
| *Burkholderiales* | *(Comamonadaceae)* | 0.31 | 0.57 | 0.92 | 0.37 | 0.74 | 0.47 | 0.68 | 0.93 | 0.62 |
| | *Variovorax* | 0.10 | 0.20 | 0.27 | 0.13 | 0.23 | 0.14 | 0.26 | 0.26 | 0.20 |

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| *1* | *Paraburkholderia* | 0.15 | 0.08 | 0.11 | 0.13 | 0.05 | 0.14 | 0.05 | 0.04 | 0.09 |
| | *Rhizobacter* | 0.03 | 0.07 | 0.14 | 0.03 | 0.11 | 0.06 | 0.09 | 0.14 | 0.08 |
| *Desulfurellales* | *(Desulfurellaceae)* | 0.29 | 0.54 | 1.33 | 0.25 | 0.74 | 0.47 | 0.77 | 0.86 | 0.66 |
| | *(Desulfurellaceae)* | 0.00 | 0.02 | 0.06 | 0.00 | 0.02 | 0.01 | 0.02 | 0.04 | 0.02 |
| *Myxococcales* | *Haliangium* | 0.85 | 1.38 | 1.38 | 1.17 | 1.41 | 1.30 | 1.72 | 1.51 | 1.34 |
| | *Sorangium* | 0.60 | 0.50 | 0.35 | 0.58 | 0.45 | 0.49 | 0.55 | 0.38 | 0.49 |
| *Nitrosomonadales* | *(Nitrosomonadaceae)* | 1.30 | 1.78 | 2.64 | 1.21 | 2.19 | 1.95 | 2.40 | 2.05 | 1.95 |
| | *Nitrosospira* | 0.00 | 0.01 | 0.01 | 0.00 | 0.01 | 0.01 | 0.01 | 0.01 | 0.01 |
| *Rhizobiales* | *(Xanthobacteraceae)* | 1.20 | 2.83 | 3.89 | 1.37 | 3.40 | 2.05 | 3.12 | 3.61 | 2.68 |
| | *Bradyrhizobium* | 1.79 | 2.57 | 2.45 | 2.19 | 2.74 | 2.61 | 2.52 | 2.83 | 2.46 |
| | *Rhizomicrobium* | 2.77 | 2.25 | 0.75 | 3.15 | 2.05 | 2.82 | 1.71 | 1.47 | 2.12 |
| | *Variibacter* | 1.67 | 1.80 | 1.51 | 1.48 | 1.82 | 1.52 | 1.75 | 1.76 | 1.66 |
| *Rhodospirillales* | *(DA111)* | 2.01 | 1.78 | 2.25 | 1.40 | 2.08 | 1.50 | 2.00 | 2.13 | 1.89 |
| | *(Acetobacteraceae)* | 1.97 | 1.67 | 0.86 | 2.55 | 1.67 | 2.33 | 1.20 | 1.02 | 1.66 |
| | *Reyranella* | 0.62 | 1.46 | 2.47 | 0.84 | 1.88 | 1.12 | 1.37 | 1.70 | 1.43 |
| *Xanthomonadales* | *Rhodanobacter* | 1.87 | 1.87 | 1.09 | 2.17 | 1.83 | 1.54 | 2.68 | 1.85 | 1.86 |
| | *Acidibacter* | 1.58 | 1.37 | 1.13 | 1.72 | 1.07 | 1.46 | 1.03 | 0.94 | 1.29 |
| | *(Xanthomonadales)* | 1.58 | 1.32 | 0.19 | 1.20 | 0.78 | 2.00 | 0.69 | 0.54 | 1.13 |

Abbreviations: BHL, beech, hornbeam, lime; BHO, beech, hornbeam, oak; BOL, beech, oak, lime; HOL, hornbeam, oak, lime

**Supplementary Table S4.** Summary of abundant soil bacterial genera across mono and mixed stands at potentially active community level. Values represent mean relative abundance for 6 replicate stands in spring, summer and autumn. Values are given for the potentially active (RNA-based) community. Only genera from abundant orders (greater than 1 %) are given. In the case the order or genus could not be assigned, the taxonomic name at the highest determined taxonomic resolution is given in parenthesis.

| **Active Community** | | **Forest stands** | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | Mono stands | | | | Mixed stands | | | | |
| Order | Genus | **Beech** | **Horn** | **Lime** | **Oak** | **BHL** | **BHO** | **BOL** | **HOL** | **Average** |
| ***Acidobacteria*** | | | | | | | | | | |
| *Acidobacteriales* | *(Acidobacteriaceae)* | 8.29 | 4.07 | 0.70 | 9.53 | 3.44 | 5.45 | 3.21 | 1.88 | 4.57 |
| *Solibacterales* | *Candidatus Solibacter* | 6.81 | 3.45 | 2.09 | 6.47 | 2.69 | 3.56 | 3.73 | 2.48 | 3.91 |
| | *Bryobacter* | 5.89 | 4.21 | 1.00 | 7.00 | 2.72 | 3.44 | 3.13 | 1.53 | 3.62 |
| *Subgroup 2* | *(Subgroup 2)* | 7.57 | 3.77 | 0.84 | 6.74 | 3.16 | 3.82 | 2.37 | 1.72 | 3.749 |
| *Subgroup 6* | *(Subgroup 6)* | 0.88 | 1.56 | 3.46 | 0.73 | 1.92 | 1.62 | 2.01 | 2.26 | 1.80 |
| | *(Subgroup 6)* | 0.34 | 0.63 | 1.43 | 0.27 | 0.86 | 0.75 | 0.90 | 1.07 | 0.78 |
| ***Actinobacteria*** | | | | | | | | | | |
| *Acidimicrobiales* | *(Acidimicrobiales)* | 0.79 | 0.75 | 0.858 | 0.82 | 0.74 | 0.84 | 0.75 | 0.70 | 0.78 |
| *Frankiales* | *Acidothermus* | 4.51 | 5.56 | 1.189 | 5.04 | 3.74 | 5.69 | 3.11 | 2.90 | 3.97 |
| *Gaiellales* | *(Gaiellales)* | 0.65 | 0.91 | 0.897 | 0.59 | 1.01 | 0.83 | 0.87 | 1.05 | 0.85 |
| *Solirubrobacterales* | *(Solirubrobacterales)* | 0.05 | 0.15 | 0.280 | 0.03 | 0.17 | 0.10 | 0.12 | 0.20 | 0.14 |
| ***Bacteroidetes*** | | | | | | | | | | |
| *Cytophagales* | *(Cytophagaceae)* | 0.18 | 0.23 | 0.55 | 0.16 | 0.40 | 0.49 | 0.36 | 0.53 | 0.36 |
| *Flavobacteriales* | *Flavobacterium* | 0.24 | 1.11 | 1.79 | 0.32 | 0.88 | 0.83 | 2.72 | 2.23 | 1.26 |
| *Sphingobactriales* | *(Chitinophagaceae)* | 1.89 | 2.48 | 2.75 | 2.17 | 2.35 | 2.15 | 3.21 | 2.88 | 2.49 |
| ***Gemmatimonadetes*** | | | | | | | | | | |
| *Gemmatimonadales* | *Gemmatimonas* | 0.48 | 0.31 | 0.46 | 0.34 | 0.55 | 0.28 | 0.46 | 0.43 | 0.41 |
| | *(Gemmatimonadaceae)* | 0.31 | 0.22 | 0.40 | 0.13 | 0.25 | 0.22 | 0.25 | 0.28 | 0.26 |
| ***Latescibacteria*** | | | | | | | | | | |
| *(Latescibacteria)* | *(Latescibacteria)* | 0.06 | 0.10 | 0.36 | 0.02 | 0.16 | 0.15 | 0.19 | 0.22 | 0.16 |
| | *(Latescibacteria)* | 0.04 | 0.08 | 0.18 | 0.03 | 0.17 | 0.08 | 0.18 | 0.24 | 0.12 |
| | *(Latescibacteria)* | 0.01 | 0.00 | 0.03 | 0.01 | 0.00 | 0.02 | 0.01 | 0.02 | 0.01 |
| ***Nitrospirae*** | | | | | | | | | | |
| *Nitrorpirales* | *(Nitrospirales)* | 0.19 | 0.09 | 0.17 | 0.08 | 0.12 | 0.12 | 0.13 | 0.10 | 0.12 |
| | *Nitrospira* | 0.01 | 0.06 | 0.26 | 0.00 | 0.06 | 0.09 | 0.07 | 0.13 | 0.09 |
| ***Proteobacteria*** | | | | | | | | | | |
| *Burkholderiales* | *(Comamonadaceae)* | 0.81 | 1.68 | 2.30 | 0.87 | 2.44 | 1.40 | 2.06 | 2.38 | 1.74 |
| | *Variovorax* | 0.38 | 0.57 | 0.82 | 0.40 | 0.70 | 0.41 | 0.75 | 0.95 | 0.62 |

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | *Rhizobacter* | 0.10 | 0.25 | 0.57 | 0.13 | 0.45 | 0.26 | 0.43 | 0.57 | 0.34 |
| *Desulfurellales* | *(Desulfurellaceae)* | 0.37 | 0.67 | 1.06 | 0.26 | 0.66 | 0.53 | 0.60 | 0.73 | 0.61 |
| | *(Desulfurellaceae)* | 0.00 | 0.04 | 0.16 | 0.00 | 0.06 | 0.04 | 0.05 | 0.08 | 0.06 |
| *Myxococcales* | *Haliangium* | 3.70 | 5.39 | 8.29 | 3.92 | 7.48 | 5.18 | 6.65 | 7.35 | 6.00 |
| | *Sorangium* | 3.66 | 2.59 | 2.43 | 3.06 | 2.70 | 2.27 | 2.44 | 2.10 | 2.66 |
| *Nitrosomonadales* | *(Nitrosomonadaceae)* | 0.95 | 1.23 | 1.99 | 0.81 | 1.70 | 1.66 | 1.88 | 1.49 | 1.46 |
| | *Nitrosospira* | 0.02 | 0.03 | 0.04 | 0.01 | 0.06 | 0.04 | 0.06 | 0.07 | 0.04 |
| *Rhizobiales* | *(Xanthobacteraceae)* | 2.96 | 5.33 | 5.73 | 2.54 | 5.98 | 4.40 | 5.46 | 5.89 | 4.79 |
| | *Variibacter* | 3.28 | 4.02 | 2.81 | 2.54 | 3.42 | 3.78 | 3.20 | 3.35 | 3.30 |
| | *Bradyrhizobium* | 2.46 | 3.51 | 3.07 | 2.27 | 3.30 | 3.29 | 2.75 | 3.98 | 3.17 |
| | *Rhizomicrobium* | 1.21 | 0.90 | 0.26 | 1.37 | 0.67 | 0.82 | 0.98 | 0.42 | 0.83 |
| *Rhodospirillales* | *(DA111)* | 4.05 | 3.11 | 2.94 | 2.78 | 3.74 | 2.82 | 2.86 | 2.88 | 3.15 |
| | *(Acetobacteraceae)* | 2.59 | 2.16 | 0.74 | 3.07 | 1.67 | 2.57 | 1.39 | 0.71 | 1.86 |
| | *Reyranella* | 0.44 | 0.67 | 0.72 | 0.50 | 0.63 | 0.47 | 0.74 | 0.57 | 0.59 |
| *Xanthomonadales* | *Rhodanobacter* | 1.90 | 1.32 | 0.06 | 2.81 | 0.94 | 2.32 | 0.75 | 0.24 | 1.29 |
| | *Acidibacter* | 1.42 | 1.43 | 0.93 | 1.93 | 1.04 | 1.51 | 0.98 | 0.91 | 1.27 |
| | *(Xanthomonadales)* | 1.01 | 1.15 | 0.47 | 1.23 | 0.68 | 1.10 | 0.71 | 0.56 | 0.87 |

Abbreviations: BHL, beech, hornbeam, lime; BHO, beech, hornbeam, oak; BOL, beech, oak, lime; HOL, hornbeam, oak, lime

**Supplementary Table S5**. Statistical tests of tree stand effects on bacterial community function. NMDS was calculated using Bray Curtis distance matrices representing the genes predicted from the potentially active (RNA-based) bacterial community with Tax4Fun (Aßhauer et al., 2015). Tax4Fun predicted assigned function to 45% of input OTUs from the potentially active community. A $p$ value ≤ 0.05 was considered statistically significant.

| NMDS goodness of fit | *p*-value | $R^2$ | Significance code |
|---|---|---|---|
| **Carbon metabolism** | | | |
| Tree species | 0.079 | 0.45 | NS |
| Stand type | 0.421 | 0.041 | NS |
| Season | 0.001 | 0.42 | *** |
| **Methane metabolism** | | | |
| Tree species | 0.033 | 0.48 | * |
| Stand type | 0.763 | 0.011 | NS |
| Season | 0.001 | 0.39 | *** |
| **Sulphur metabolism** | | | |
| Tree species | 0.078 | 0.44 | NS |
| Stand type | 0.239 | 0.065 | NS |
| Season | 0.001 | 0.42 | *** |
| **Nitrogen metabolism** | | | |
| Tree species | 0.203 | 0.38 | NS |
| Stand type | 0.398 | 0.04 | NS |
| Season | 0.001 | 0.49 | *** |

Significance 0 = ***, 0.001 = **, 0.01 = *, 0.05 = *, greater than 0.1 = not significant (NS)

Reference:
Aßhauer, K. P., Wemheuer, B., Daniel, R., and Meinicke, P. (2015). Tax4Fun: Predicting functional profiles from metagenomic 16S rRNA data. *Bioinformatics* 31, 2882–2884. doi:10.1093/bioinformatics/btv287

**Supplementary Table S6**. List of selected genes associated with energy metabolism. Gene functions were predicted from the potentially active (RNA-based) bacterial community with Tax4Fun (Aßhauer et al., 2015).

| KEGG ortholog | Name | KEGG ortholog | Name |
|---|---|---|---|
| **Calvin Cycle** | | **Hemicellulases** | |
| K00174 - K00177 | 2-oxoglutarate ferredoxin oxidoreductase | K01181 | endo-1,4-beta-xylanase |
| K01601 - K01602 | ribulose-bisphosphate carboxylase | K01198 | xylan 1,4-beta-xylosidase |
| K00239 - K00242 | succinate dehydrogenase | K01218 | mannan endo-1,4-beta-mannosidase |
| **Methane metabolism** | | K01224 | arabinogalactan endo-1,4-beta-galactosidase |
| K14028 - K14029 | methanol dehydrogenase (cytochrome c) | K01684 | galactonate dehydratase |
| K00190 - K00198 | carbon monoxide dehydrogenase / acetyl-CoA synthase | K01811 | alpha-D-xyloside xylohydrolase |
| K03518 - K03520 | carbon-monoxide dehydrogenase small subunit | K15531 | oligosaccharide reducing-end xylanase |
| K16154 - K16162 | methane monooxygenase | K15921 | arabinoxylan arabinofuranohydrolase |
| **Sulphur metabolism** | | K15924 | glucuronoarabinoxylan endo-1,4-beta-xylanase |
| K00380 - K00381 | sulfite reductase (NADPH) flavoprotein | **Cellulases** | |
| K00390 | phosphoadenosine phosphosulfate reductase | K01179 | endoglucanase |
| K00392 | sulfite reductase (ferredoxin) | K01180 | endo-1,3(4)-beta-glucanase |
| K00394 - K00395 | adenylylsulfate reductase | K01182 | oligo-1,6-glucosidase |
| K00860 | adenylylsulfate kinase | K01195 | beta-glucuronidase |
| K00955 | bifunctional enzyme CysN/CysC | K01225 | cellulose 1,4-beta-cellobiosidase |
| K00956 - K00958 | sulfate adenylyltransferase | K05349 | beta-glucosidase |
| K11180 - K11181 | sulfite reductase, dissimilatory-type | K05350 | beta-glucosidase |
| **Nitrogen metabolism** | | K16213 | mannobiose 2-epimerase |
| K00360 - K00363 | nitrate reductase (NADH) | **Chitinases** | |
| K00366 - K00367 | ferredoxin-nitrite reductase | K01183 | chitinase |
| K00368 - K00374 | nitrite reductase (NO-forming) | K01452 | chitin deacetylase |
| K00376 | nitrous-oxide reductase | K03791 | putative chitinase |
| K00531 | nitrogenase | K03933 | chitin-binding protein |
| K01428 - K01430 | urease | K13381 | bifunctional chitinase/lysozyme |
| K02164, K02305, K02448 | nitric oxide reductase NorE, NorD | **Phosphatases** | |
| K02586 - K02588 | nitrogenase molybdenum-iron protein (NifE, NifH) | K01077 | alkaline phosphatase |
| K02591 - K02596, K02597 | nitrogenase molybdenum-iron protein (NifN, NifT, NifV, NifX, NifZ) | K01078 | acid phosphatase |
| K03385 | cytochrome c-552 | K01093 | 4-phytase / acid phosphatase |
| K04561 | nitric oxide reductase subunit B | K01113 | alkaline phosphatase D |
| K04747 - K04748 | nitric oxide reductase protein (NorF, NorQ) | K03788 | acid phosphatase (class B) |
| K10535 | hydroxylamine oxidase | K09474 | acid phosphatase (class A) |
| K10944 - K10946 | ammonia monooxygenase (AmoABC) | | |
| K15864 | nitrite reductase (NO-forming) / hydroxylamine reductase | | |
| K15876 | cytochrome c-type protein | | |

Reference:

Aßhauer, K. P., Wemheuer, B., Daniel, R., and Meinicke, P. (2015). Tax4Fun: Predicting functional profiles from metagenomic 16S rRNA data. *Bioinformatics* 31, 2882–2884. doi:10.1093/bioinformatics/btv287