

Supplementary files

Table S1. Treatment means and standard deviation in brackets of the soil physiochemical variables at 0-10 cm depth.

| Date | Treatment ¹ | pH | EC ² | TOC | Nt | C/N | CEC | Bs |
|----------|------------------------|-----------|-----------------|------------|-----------|------------|------------|------------|
| May 2018 | A | 6.3 (0.1) | 0.8 (0.1) | 27.4 (2.8) | 1.8 (0.3) | 15.2 (0.8) | 26.4 (2.3) | 23.2 (2.2) |
| | B | 6.1 (0.3) | 1.0 (0.1) | 29.0 (2.6) | 2.0 (0.2) | 14.4 (0.9) | 25.8 (3.7) | 21.4 (4.8) |
| | C | 6.3 (0.2) | 0.8 (0.1) | 28.5 (4.7) | 2.1 (0.4) | 13.8 (0.5) | 26.4 (2.9) | 23.3 (2.9) |
| | D | 6.1 (0.1) | 1.0 (0.1) | 27.6 (5.8) | 2.2 (0.5) | 12.8 (0.8) | 24.4 (3.2) | 19.8 (3.4) |
| Oct 2018 | A | 6.3 (0.1) | 0.9 (0.2) | 26.9 (3.3) | 1.8 (0.2) | 14.6 (1.0) | 25.8 (3.8) | 22.2 (4.1) |
| | B | 6.2 (0.2) | 1.0 (0.1) | 31.1 (3.4) | 2.3 (0.2) | 13.8 (0.6) | 24.0 (3.1) | 20.9 (3.3) |
| | C | 6.1 (0.2) | 1.6 (0.1) | 28.1 (4.8) | 2.0 (0.4) | 14.3 (0.2) | 25.2 (1.9) | 20.7 (2.7) |
| | D | 6.0 (0.3) | 1.5 (0.3) | 26.8 (4.9) | 1.9 (0.4) | 13.9 (0.6) | 26.0 (3.9) | 19.5 (3.5) |
| Oct 2019 | A | 6.3 (0.1) | 0.6 (0.0) | 26.8 (3.7) | 2.2 (0.3) | 12.4 (1.1) | 21.6 (2.0) | 19.9 (2.4) |
| | B | 6.2 (0.1) | 0.7 (0.1) | 29.1 (3.0) | 2.5 (0.2) | 11.8 (0.4) | 18.5 (2.7) | 17.3 (3.5) |
| | C | 6.1 (0.1) | 0.9 (0.1) | 28.2 (3.4) | 2.3 (0.3) | 12.4 (0.9) | 20.8 (2.3) | 18.5 (2.2) |
| | D | 6.2 (0.1) | 0.8 (0.1) | 30.2 (8.0) | 2.6 (0.7) | 11.8 (0.6) | 22.0 (1.2) | 20.1 (1.7) |
| Oct 2020 | A | 6.3 (0.1) | 0.7 (0.0) | 25.9 (2.5) | 1.6 (0.3) | 16.8 (2.5) | 22.0 (2.0) | 19.1 (1.7) |
| | B | 6.2 (0.3) | 0.8 (0.1) | 28.1 (3.8) | 2.0 (0.3) | 14.4 (1.0) | 21.2 (3.3) | 17.7 (3.5) |
| | C | 6.2 (0.2) | 0.8 (0.0) | 29.4 (4.9) | 2.0 (0.4) | 14.7 (0.8) | 22.8 (1.9) | 19.1 (1.1) |
| | D | 6.2 (0.1) | 0.8 (0.1) | 24.9 (3.4) | 1.8 (0.4) | 14.3 (1.4) | 22.8 (3.5) | 19.2 (3.0) |

EC, Electrical Conductivity [dS/m]; TOC, Total Organic C [g/kg]; Nt, total N [g/kg]; C/N, Ratio C and N; CEC, Cation Exchange Capacity [cmol/kg]; Bs, sum of base cations [cmol/kg]

¹Treatments (A and C) were conventionally tilled (CT; moldboard ploughing to a depth of 20 cm) and treatments (B and D) were no-till (NT) treatments for 10 years until May 2018. Thereafter all treatments started with barley and the division between CT and NT continued but A was diversified with a cover crop in Oct 2018, B continued as a monoculture and treatments C and D received a rotation in Oct 2019.

²The estimated means of the CT treatment (A, C) differed significantly ($p = 0.034$) from the NT treatment (B, D) in May 2018.

Table S2. Differentially abundant fungal OTUs according to paired comparisons by ANCOM-BC analysis. Log2FoldChange (lfc) value below 2 and above -2 are used as threshold levels for differential abundance with significance p<0.05. If there was more than one OTU representing the same taxa, only the highest or lowest lfc are shown.

| Comparison | OTU | lfc | Phylum | Class | Order | Family | Genus | Function |
|-------------------------|----------|------|--------------------|----------------------|------------------|--------------------------|-----------------------|----------|
| May 18 AC (CT) | OTU_12 | -5.1 | unknown | unknown | unknown | unknown | unknown | |
| | OTU_1067 | -4.2 | Basidiomycota | Geminibasidiomycetes | Geminibasidiales | <i>Geminibasidiaceae</i> | <i>Geminibasidium</i> | SAP |
| | OTU_21 | -3.9 | Ascomycota | Leotiomycetes | unknown | unknown | unknown | |
| | OTU_1046 | -3.6 | Mortierellomycota | Mortierellomycetes | Mortierellales | <i>Mortierellaceae</i> | <i>Mortierella</i> | SAP |
| | OTU_93 | -3.0 | Ascomycota | unknown | unknown | unknown | unknown | |
| | OTU_1072 | -2.6 | Ascomycota | Leotiomycetes | Helotiales | unknown | unknown | |
| | OTU_139 | -2.5 | unknown | unknown | unknown | unknown | unknown | |
| May 18 BD (NT) | OTU_45 | 2.5 | Ascomycota | Leotiomycetes | Helotiales | <i>Helotiaceae</i> | unknown | |
| | OTU_86 | 3.0 | Glomeromycota | Glomeromycetes | Glomerales | <i>Glomeraceae</i> | unknown | SYM |
| | OTU_1057 | 3.3 | Ascomycota | Pezizomycetes | Pezizales | <i>Pezizaceae</i> | <i>Peziza</i> | SAP-SYM |
| | OTU_64 | 3.4 | Ascomycota | Pezizomycetes | Pezizales | <i>Pyronemataceae</i> | unknown | |
| Oct 19 AB (barley) | OTU_51 | -3.5 | Basidiomycota | Agaricomycetes | Agaricales | unknown | unknown | |
| | OTU_1069 | -3.4 | Ascomycota | Leotiomycetes | Helotiales | <i>Helotiaceae</i> | unknown | |
| | OTU_88 | -2.7 | Ascomycota | Leotiomycetes | Helotiales | unknown | unknown | |
| | OTU_58 | -2.5 | unknown | unknown | unknown | unknown | unknown | |
| Oct 19 CD (rapeseed) | OTU_155 | 2.7 | Ascomycota | Leotiomycetes | Helotiales | unknown | unknown | |
| | OTU_114 | 3.1 | Ascomycota | Leotiomycetes | Helotiales | <i>Helotiaceae</i> | <i>Pseudohelotium</i> | |
| | OTU_40 | 3.4 | Ascomycota | Eurotiomycetes | Chaetothyriales | unknown | unknown | |
| | OTU_1133 | 3.5 | Basidiomycota | Agaricomycetes | Sebacinales | <i>Serendipitaceae</i> | unknown | SYM |
| | OTU_1059 | 3.9 | Glomeromycota | Paraglomeromycetes | Paraglomerales | unknown | unknown | SYM |
| | OTU_1062 | 4.1 | Olpidiomycota | Olpidiomycetes | Olpidiales | <i>Olpidiaceae</i> | <i>Olpidium</i> | PAT |
| | OTU_59 | 4.5 | Blastocladiomycota | unknown | unknown | unknown | unknown | |
| | OTU_74 | 4.6 | Basidiomycota | Tremellomycetes | Filobasidiales | <i>Filobasidiaceae</i> | <i>Naganishia</i> | SAP |
| | OTU_17 | 5.6 | Ascomycota | unknown | unknown | unknown | unknown | |
| | OTU_1054 | 5.6 | Basidiomycota | Agaricomycetes | Thelephorales | <i>Thelephoraceae</i> | <i>Amaurodon</i> | SYM |

Table S3. Differentially abundant bacterial OTUs according to paired comparisons by ANCOM-BC analysis. Log2FoldChange (lfc) value below 2 and above -2 are used as threshold levels for differential abundance with significance p<0.0001. If there was more than one OTU representing the same taxa, only the highest or lowest lfc are shown.

| Comparison | OTU | lfc | Phylum | Class | Order | Family | Genus |
|-------------------------|----------|------|----------------|---------------------|-----------------------|-------------------------------|-----------------------|
| May 18 AC (CT) | OTU_1807 | -3.0 | Actinobacteria | Actinobacteria | Propionibacterales | <i>Nocardioidaceae</i> | <i>Nocardioides</i> |
| | OTU_1038 | -2.8 | Acidobacteria | Thermoanaerobaculia | Thermoanaerobaculales | <i>Thermoanaerobaculaceae</i> | unknown |
| | OTU_2849 | -2.5 | Bacteroidetes | Bacteroidia | Chitinophagales | <i>Chitinophagaceae</i> | <i>Flavolibacter</i> |
| May 18 BD (NT) | OTU_8307 | 2.6 | Rokubacteria | NC10 | Rokubacterales | unknown | unknown |
| | OTU_3890 | 3.1 | Chloroflexi | Ktedonobacteria | Ktedonobacterales | <i>Ktedonobacteraceae</i> | unknown |
| | OTU_36 | 3.3 | Acidobacteria | Acidobacterii | Acidobacterales | <i>Koribacteraceae</i> | <i>Can Koribacter</i> |
| | OTU_1157 | 3.4 | Actinobacteria | Acidimicrobiia | unknown | unknown | unknown |
| | OTU_5778 | 3.5 | Proteobacteria | Alphaproteobacteria | Elsterales | unknown | unknown |
| | OTU_7913 | 3.5 | Proteobacteria | Gammaproteobacteria | Diplorickettsiales | <i>Diplorickettsiaceae</i> | <i>Aquicella</i> |
| | OTU_5814 | 3.6 | Proteobacteria | Alphaproteobacteria | Micropepsales | <i>Micropepsaceae</i> | unknown |
| | OTU_3270 | 3.7 | Chloroflexi | AD3 | unknown | unknown | unknown |
| | OTU_6480 | 4.5 | Proteobacteria | Deltaproteobacteria | Desulfuromonadales | <i>Geobacteraceae</i> | <i>Geobacter</i> |
| Oct 19 AB (barley) | - | - | - | - | - | - | - |
| Oct 19 CD (rapeseed) | - | - | - | - | - | - | - |

Table S4. The estimated means of fixed effects for the fungal (ITS) or bacterial (16S) alpha-diversity measures HillQ0 (species richness), HillQ1 and HillQ2. Comparisons denoted with the *different* letter differed statistically significantly from each other ($p<0.10$). The letters from a to c are used for row-wise comparisons and the letters from u to v for column-wise comparisons, respectively. The values in the parentheses are the lower and upper limits of 95% confidence intervals of the estimates.

| Response variable | Treatment | May 2018 | Oct 2018 | Oct 2019 | Oct 2020 |
|-----------------------|-----------|--------------------------------|--------------------------------|--------------------------------|--------------------------------|
| ITS ^{HillQ0} | A | 250 ^{ab} [214, 285] | 223 ^{a,u} [191, 256] | 244 ^{ab} [198, 290] | 282 ^b [229, 335] |
| | B | 209 ^a [175, 243] | 221 ^{a,u} [188, 254] | 283 ^b [237, 329] | 281 ^b [228, 334] |
| | C | 249 [216, 283] | 241 ^{uv} [209, 274] | 289 [243, 334] | 279 [225, 332] |
| | D | 216 ^a [182, 250] | 270 ^{b,v} [237, 302] | 304 ^b [259, 350] | 295 ^b [242, 348] |
| ITS ^{HillQ1} | A | 12.7 ^{ab} [8.1, 19.8] | 11.9 ^a [7.6, 18.5] | 13.6 ^{ab} [8.7, 21.1] | 14.8 ^b [9.5, 23.0] |
| | B | 12.3 ^a [7.9, 19.2] | 9.0 ^b [5.8, 14.1] | 12.2 ^a [7.9, 19.0] | 11.6 ^a [7.5, 18.1] |
| | C | 12.1 ^a [7.8, 18.9] | 12.1 ^a [7.8, 18.7] | 14.7 ^{ab} [9.5, 22.9] | 15.2 ^b [9.8, 23.7] |
| | D | 10.0 ^a [6.4, 15.5] | 14.1 ^b [9.1, 21.9] | 16.0 ^b [10.3, 24.9] | 14.9 ^b [9.6, 23.1] |
| ITS ^{HillQ2} | A | 5.9 [3.8, 8.1] | 5.3 [3.3, 7.4] | 5.8 [3.8, 7.9] | 6.5 [4.5, 8.6] |
| | B | 6.2 ^a [4.2, 8.3] | 4.1 ^b [2.0, 6.2] | 5.6 ^a [3.5, 7.6] | 5.2 ^{ab} [3.1, 7.2] |
| | C | 5.2 ^{ab} [3.2, 7.3] | 4.8 ^a [2.7, 6.8] | 6.0 ^{ab} [4.0, 8.1] | 6.6 ^b [4.6, 8.7] |
| | D | 4.2 ^a [2.1, 6.2] | 6.0 ^b [3.9, 8.0] | 6.4 ^b [4.3, 8.4] | 5.3 ^{ab} [3.3, 7.4] |
| 16S ^{HillQ0} | A | 750 [474, 1026] | 642 ^u [503, 780] | 842 [614, 1070] | 927 [571, 1282] |
| | B | 769 ^a [493, 1045] | 806 ^{a,v} [668, 944] | 612 ^a [384, 840] | 1296 ^b [940, 1651] |
| | C | 866 ^{ab} [590, 1143] | 668 ^{a,uv} [530, 806] | 694 ^a [465, 922] | 1266 ^b [910, 1622] |
| | D | 716 ^a [440, 992] | 717 ^{a,uv} [579, 885] | 768 ^{ab} [540, 997] | 1247 ^b [892, 1603] |
| 16S ^{HillQ1} | A | 474 [310, 638] | 416 [332, 499] | 544 [397, 691] | 548 [350, 746] |
| | B | 470 ^a [306, 634] | 511 ^a [428, 594] | 393 ^a [247, 540] | 835 ^b [637, 1033] |
| | C | 552 ^{ab} [388, 716] | 438 ^a [355, 521] | 448 ^a [301, 595] | 812 ^b [614, 1010] |
| | D | 440 ^a [276, 604] | 461 ^a [377, 544] | 493 ^a [346, 639] | 798 ^b [600, 996] |
| 16S ^{HillQ2} | A | 272 [165, 380] | 244 [0, 493] | 330 [229, 431] | 312 ^u [196, 428] |
| | B | 262 ^a [154, 369] | 302 ^a [54, 551] | 224 ^a [123, 325] | 505 ^{b,v} [389, 621] |
| | C | 313 ^a [206, 421] | 255 ^a [6, 504] | 261 ^a [159, 362] | 491 ^{b,uv} [375, 607] |
| | D | 252 ^a [145, 360] | 269 ^a [20, 518] | 283 ^a [182, 384] | 484 ^{b,uv} [368, 600] |

Table S5. The estimated means of fixed effects for relative proportion of fungal guilds SYM, SAP, PAT, qPCR of genes involved in the N cycle (*amoA*, *narG* and *nirK*), and potential nitrification (PN). Comparisons denoted with the *different* letter differed statistically significantly from each other ($p<0.10$). The letters from a to c are used for row-wise comparisons and the letters from u to x for column-wise comparisons, respectively. The values in the parentheses are the lower and upper limits of 95% confidence intervals of the estimates.

| Response variable | Treatment ^a | May 2018 | Oct 2018 | Oct 2019 | Oct 2020 |
|------------------------------|------------------------|---------------------------------------|--|---|---|
| SYM | A | 0.011 [0.006, 0.021] | 0.019 [0.011, 0.032] | 0.016 ^{uv} [0.009, 0.027] | 0.015 ^{uv} [0.009, 0.026] |
| | B | 0.008 [0.005, 0.014] | 0.011 [0.006, 0.020] | 0.013 ^u [0.008, 0.022] | 0.015 ^u [0.009, 0.025] |
| | C | 0.010 ^a [0.006, 0.017] | 0.012 ^a [0.007, 0.019] | 0.032 ^{b,v} [0.019, 0.054] | 0.034 ^{b,v} [0.020, 0.057] |
| | D | 0.011 ^a [0.006, 0.018] | 0.014 ^{ab} [0.008, 0.024] | 0.024 ^{b,uv} [0.014, 0.040] | 0.018 ^{ab,uv} [0.011, 0.031] |
| SAP | A | 0.026 [0.000, 0.093] | 0.018 ^u [0.007, 0.029] | 0.021 [0.010, 0.031] | 0.021 [0.008, 0.033] |
| | B | 0.095 ^a [0.038, 0.152] | 0.015 ^{b,u} [0.004, 0.026] | 0.024 ^u [0.014, 0.035] | 0.018 ^b [0.005, 0.030] |
| | C | 0.022 [0.000, 0.079] | 0.021 ^{a,uv} [0.010, 0.032] | 0.018 ^b [0.008, 0.029] | 0.021 [0.008, 0.033] |
| | D | 0.019 ^{ab} [0.000, 0.076] | 0.038 ^{a,v} [0.027, 0.049] | 0.032 ^{ab} [0.021, 0.042] | 0.025 ^b [0.012, 0.037] |
| PAT | A | 0.00003 ^a [0.0002, 0.0007] | 0.00004 ^{a,uv} [0.0002, 0.0007] | 0.00014 ^{b,u} [0.0008, 0.0025] | 0.00004 ^{ab,uv} [0.0003, 0.0008] |
| | B | 0.00004 [0.0002, 0.0007] | 0.00003 ^v [0.0002, 0.0006] | 0.00005 ^x [0.0003, 0.0009] | 0.00004 ^v [0.0002, 0.0007] |
| | C | 0.00006 ^a [0.0004, 0.0011] | 0.00004 ^{a,uv} [0.0002, 0.0007] | 0.00064 ^{b,v} [0.0037, 0.0110] | 0.00008 ^{a,uv} [0.0005, 0.0014] |
| | D | 0.00004 ^a [0.0003, 0.0008] | 0.00009 ^{ab,u} [0.0005, 0.0016] | 0.0071 ^{c,v} [0.0041, 0.0122] | 0.0014 ^{b,u} [0.0008, 0.0025] |
| amoA (values $\times 10^8$) | A | 0.84 [0.61, 1.15] | 0.83 ^u [0.61, 1.14] | 1.01 ^{uv} [0.73, 1.38] | 0.77 [0.56, 1.06] |
| | B | 1.06 ^{ab} [0.77, 1.46] | 0.80 ^{a,u} [0.58, 1.10] | 1.21 ^{b,u} [0.88, 1.67] | 0.99 ^{ab} [0.72, 1.36] |
| | C | 0.75 [0.55, 1.03] | 0.79 ^u [0.58, 1.09] | 0.68 ^v [0.50, 0.94] | 0.83 [0.61, 1.14] |
| | D | 0.79 ^a [0.57, 1.08] | 1.51 ^{b,v} [1.10, 2.08] | 1.67 ^{b,u} [1.22, 2.29] | 0.80 ^a [0.58, 1.09] |
| narG (values $\times 10^8$) | A | 1.95 [1.35, 2.56] | 1.74 ^{uv} [1.09, 2.39] | 1.48 [0.69, 2.28] | 1.48 [-, -] |
| | B | 1.80 ^a [1.24, 2.36] | 1.41 ^{ab,v} [0.77, 2.05] | 1.80 ^{ab} [1.08, 2.51] | 1.24 ^b [-, -] |
| | C | 1.29 ^a [0.51, 2.07] | 1.98 ^{b,uv} [1.27, 2.69] | 1.53 ^{ab} [0.88, 2.17] | 1.46 ^{ab} [0.07, 2.84] |
| | D | 1.76 ^{ab} [1.14, 2.38] | 2.37 ^{a,u} [1.62, 3.11] | 1.89 ^{ab} [1.29, 2.49] | 1.47 ^b [0.93, 2.01] |
| nirK (values $\times 10^8$) | A | 0.85 ^{a,u} [0.65, 1.13] | 0.85 ^a [0.65, 1.13] | 1.86 ^{b,u} [1.40, 2.45] | 1.19 ^{a,u} [0.90, 1.58] |
| | B | 0.55 ^{a,v} [0.42, 0.73] | 0.66 ^a [0.50, 0.87] | 2.28 ^{b,x} [1.73, 3.02] | 1.11 ^{c,u} [0.84, 1.46] |
| | C | 0.60 ^{a,uv} [0.45, 0.79] | 0.80 ^a [0.60, 1.05] | 0.55 ^{a,v} [0.42, 0.73] | 1.61 ^{b,uv} [1.22, 2.13] |
| | D | 0.52 ^{a,v} [0.39, 0.68] | 0.78 ^b [0.59, 1.03] | 0.61 ^{a,uv} [0.46, 0.81] | 1.99 ^{c,v} [1.51, 2.63] |
| PN | A | 168 ^{ab} [112, 224] | 131 ^{a,u} [74, 187] | 184 ^{ab,u} [128, 240] | 224 ^{b,u} [168, 281] |
| | B | 163 ^a [107, 219] | 199 ^{ab,u} [143, 255] | 255 ^{b,u} [199, 311] | 202 ^{ab,u} [146, 258] |
| | C | 175 ^{ab} [119, 231] | 129 ^{a,uv} [73, 185] | 222 ^{bc,u} [165, 278] | 276 ^{c,u} [220, 332] |
| | D | 180 ^a [124, 236] | 240 ^{a,v} [183, 296] | 342 ^{b,v} [285, 398] | 379 ^{b,v} [323, 436] |

^aSee Table 1 for explanations

Table S6. Differences between tillage and non-tillage in May 2018. Bonferroni-level is now calculated 0.05 divided by the number of comparisons (=15). Thus, a significant p-value should be under that.

| Variable | Effect | Label | Difference | t Value | P value | Bonferroni level | Decision |
|------------|----------------|------------------|------------|---------|---------|------------------|-----------------|
| ITS_HillQ0 | Treatment*Time | CT vs NT, time 1 | 28.97 | 2.34 | 0.045 | 0.003 | Significant |
| ITS_HillQ1 | Treatment*Time | CT vs NT, time 1 | -0.07 | -0.33 | 0.749 | 0.003 | No significance |
| ITS_HillQ2 | Treatment*Time | CT vs NT, time 1 | -1.11 | -0.98 | 0.340 | 0.003 | No significance |
| 16S_HillQ0 | Treatment*Time | CT vs NT, time 1 | 7.51 | 0.05 | 0.960 | 0.003 | No significance |
| 16S_HillQ1 | Treatment*Time | CT vs NT, time 1 | 18.53 | 0.21 | 0.837 | 0.003 | No significance |
| 16S_HillQ2 | Treatment*Time | CT vs NT, time 1 | 17.07 | 0.3 | 0.763 | 0.003 | No significance |
| SYM | Treatment*Time | CT vs NT, time 1 | 0.25 | 0.9 | 0.376 | 0.003 | No significance |
| SAP | Treatment*Time | CT vs NT, time 1 | -0.07 | -2.35 | 0.037 | 0.003 | No significance |
| PAT | Treatment*Time | CT vs NT, time 1 | 0.22 | 0.73 | 0.469 | 0.003 | No significance |
| PN | Treatment*Time | CT vs NT, time 1 | 10.98 | 0.38 | 0.708 | 0.003 | No significance |
| amoA | Treatment*Time | CT vs NT, time 1 | -0.31 | -1.72 | 0.095 | 0.003 | No significance |
| narG | Treatment*Time | CT vs NT, time 1 | -1.4E+07 | -0.46 | 0.648 | 0.003 | No significance |
| nirK | Treatment*Time | CT vs NT, time 1 | 0.13 | 0.88 | 0.383 | 0.003 | No significance |

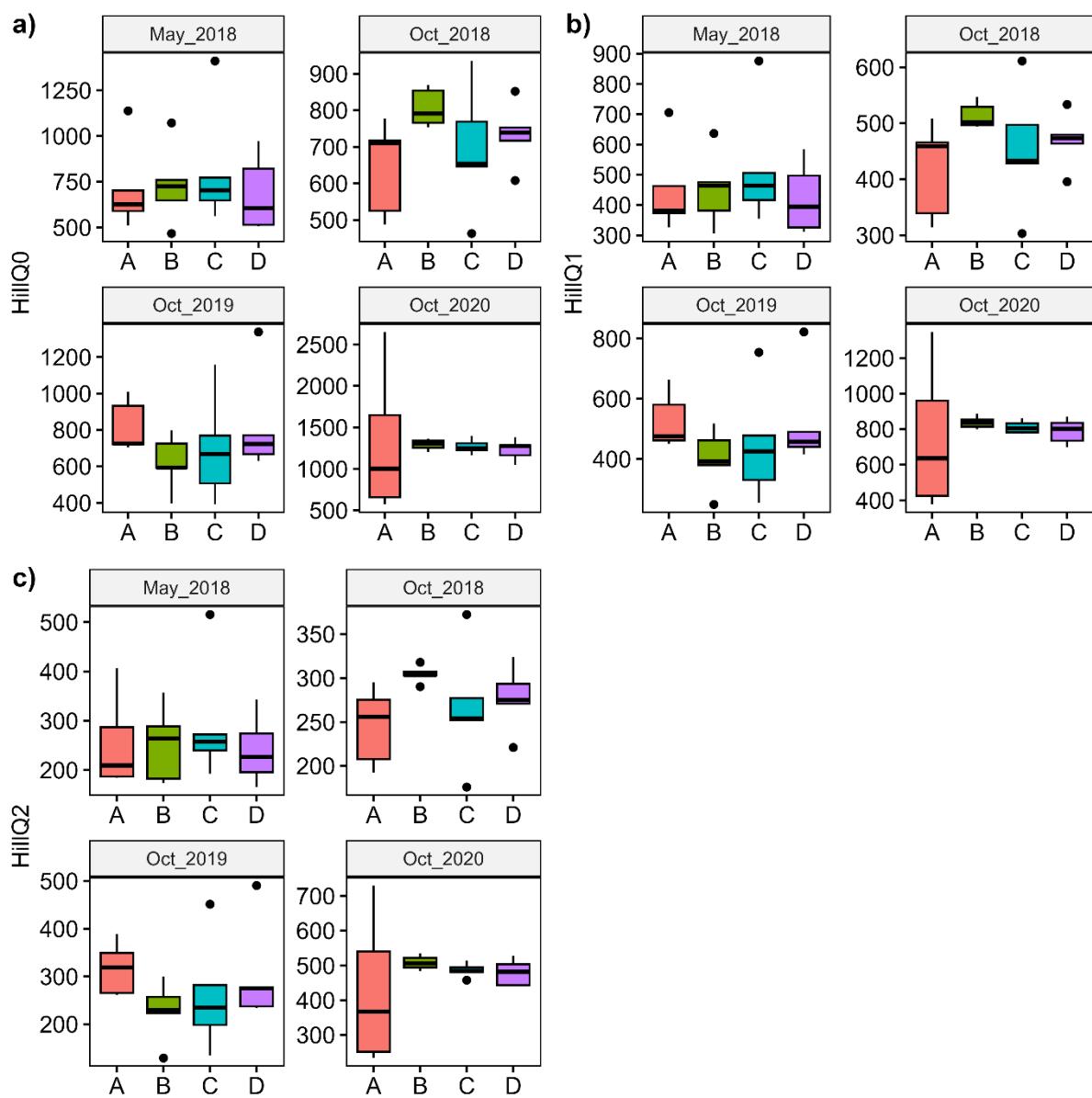


Figure S1. Boxplots representing the alpha diversity measures as Hills numbers a) Q0, b) Q1 and c) Q2 for fungal ITS data in different treatments (A, B, C, D) and sampling times (May 2018, October 2018, October 2019, October 2020).

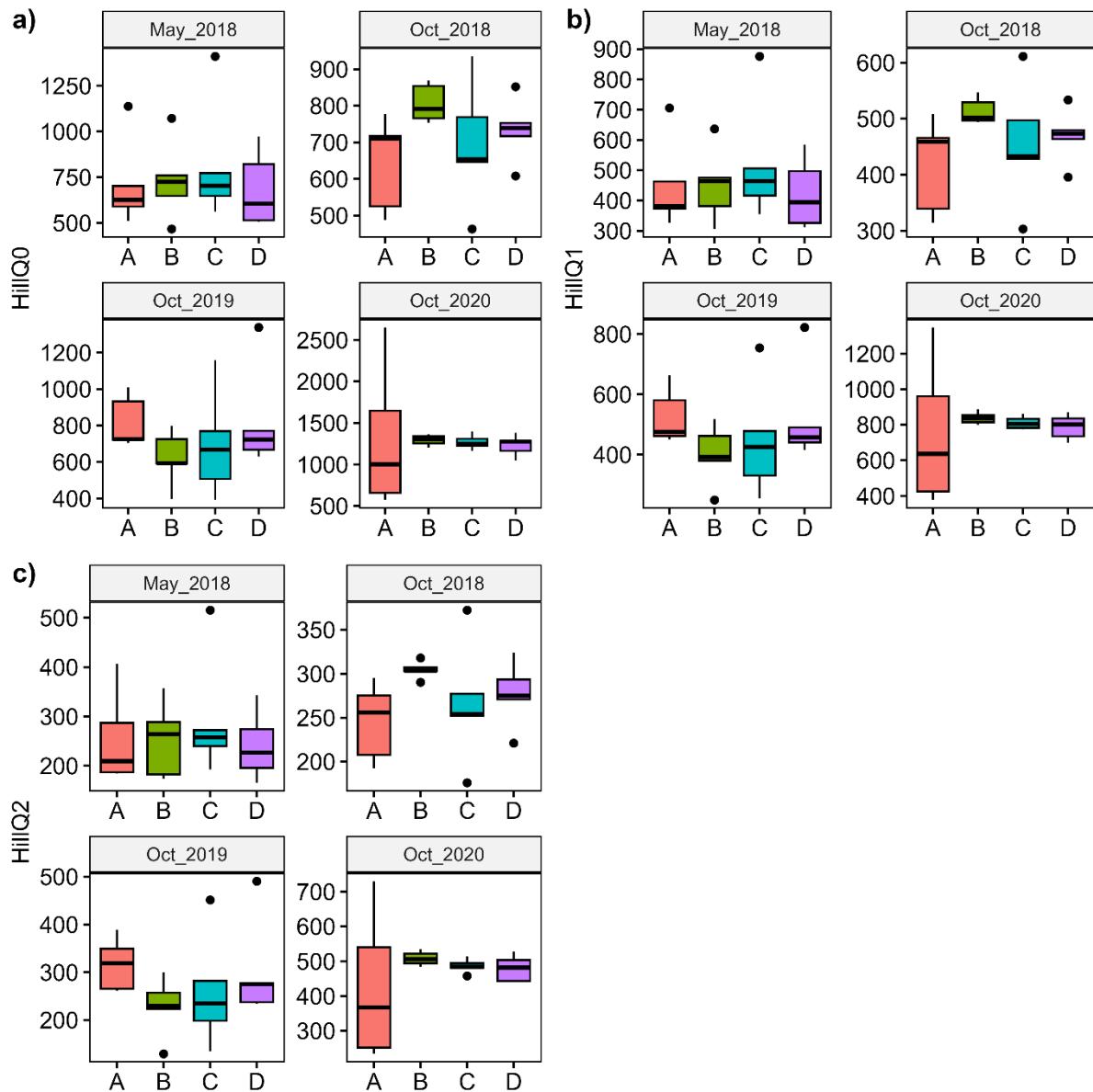


Figure S2. Boxplots representing the alpha diversity measures as Hills numbers a) Q0, b) Q1 and c) Q2 for bacterial 16S rRNA data in different treatments (A, B, C, D) and sampling times (May 2018, October 2018, October 2019, October 2020)