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1

2 # Total RNA-sequencing reveals multi-level microbial community
3 changes and functional responses to wood ash application in
4 agricultural and forest soil

5

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17

18 ## Abstract

19 Recycling of wood ash from energy production may counteract soil acidification and return
20 essential nutrients to soils. However, wood ash amendment affects soil physicochemical
21 parameters that control composition and functional expression of the soil microbial
22 community. Here, we applied Total RNA-sequencing to simultaneously assess the impact
23 of wood ash amendment on the active soil microbial communities and the expression of
24 functional genes from all microbial taxa. Wood ash significantly affected the taxonomic
25 (rRNA) as well as functional (mRNA) profiles of both agricultural and forest soil. Increase
26 in pH, electrical conductivity, dissolved organic carbon and phosphate were the most
27 important physicochemical drivers for the observed changes. Wood ash amendment
28 increased the relative abundance of the copiotrophic groups Chitinonophagaceae
29 (Bacteroidetes) and Rhizobiales (Alphaproteobacteria) and resulted in higher expression
30 of genes involved in metabolism and cell growth. Finally, Total RNA-sequencing allowed
31 us to show that some groups of bacterial feeding protozoa increased concomitantly to the

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32 enhanced bacterial growth, which shows their pivotal role in the regulation of bacterial
33 abundance in soil.
34
35 *Keywords: Metatranscriptomics, Total RNA, wood ash, soil micro-biome, biodiversity, soil biota, mRNA,*
36 *rRNA, renewable energy, protozoa.*

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37 **1. Introduction**

38 Wood ash from energy production is often considered a waste product (Vance, 1996;
39 Demeyer *et al.*, 2001) despite that recycling of wood ash may have beneficial effects as it
40 counteracts acidification and returns essential nutrients to soil (Demeyer *et al.*, 2001;
41 Augusto *et al.*, 2008). Wood combustion is becoming more popular in several countries
42 and increased reuse of wood ash as soil amendment holds the potential to improve the
43 sustainability of this practice (Karltun *et al.*, 2008; Huotari *et al.*, 2015). However, wood ash
44 application affects several soil physicochemical parameters important to the structure and
45 function of microbial communities; e.g. pH, electrical conductivity and dissolved organic
46 carbon (DOC), (Ohno & Susan Erich, 1990; Demeyer *et al.*, 2001; Pitman, 2006; Augusto
47 *et al.*, 2008; Maresca *et al.*, 2017; Hansen *et al.*, 2017). As the soil micro-biota carries out
48 an array of key biochemical processes (Blagodatskaya & Kuzyakov, 2013), knowledge of
49 its response to disturbance is important; not least in production soils due to potential
50 impact on soil fertility.

51 The soil micro-biome, which includes prokaryotes as well as micro-eukaryotes, is one of
52 the most diverse and complex biomes on Earth. It has a pivotal role in nutrient cycling and
53 carbon sequestration and is a key component in the maintenance of soil fertility of
54 managed ecosystems (Wall *et al.*, 2012; Fierer, 2017). Wood ash amendment causes
55 changes in soil micro-biome composition, activity and quantity (Perkiömäki & Fritze, 2002;
56 Aronsson & Ekelund, 2004; Huotari *et al.*, 2015). Ash amendment induces changes in
57 community structure followed by increased microbial activity and growth, which is usually
58 explained by the increased soil pH brought about by the alkaline oxides in the ash (Cruz-
59 Paredes *et al.*, 2017; Vestergård *et al.*, 2018). Still, some studies show no or only minor
60 microbial response to wood ash application (Aronsson et al. 2004; Huotari et al. 2015).

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61 Only few studies have concomitantly analysed microorganisms from all domains of life (i.e.
62 Archaea, Bacteria, Eukaryotes) and most of these rely on cultivation, model organisms or
63 molecular fingerprinting, which only provide limited resolution of taxonomical and
64 functional responses. Total RNA-sequencing, or metatranscriptomics, makes it possible to
65 investigate active soil microbial communities from all domains of life, incl. their
66 transcriptional activity, simultaneously. Because total RNA-sequencing allows for the study
67 of immediate regulatory responses to environmental changes (Carvalhais *et al.*, 2012), it
68 has also proven useful in the assessment of active microbial communities' functional roles
69 in soil (Urich *et al.*, 2008; Hultman *et al.*, 2015; Epelde *et al.*, 2015; Geisen *et al.*, 2015;
70 Schostag *et al.*, 2019).

71 We therefore aimed to investigate how the active soil prokaryotic and micro-eukaryotic
72 communities in agricultural and forest soil responded structurally and functionally
73 (transcriptional) to wood ash application. Both soil types are relevant for large scale
74 application of wood ash. We applied wood ash in concentrations corresponding to field
75 application of 0, 3, 12 and 90 t ha^{-1}, where 3 t ha^{-1} is the currently allowed dose in
76 Scandinavian countries. We expected wood ash to increase soil pH, electrical conductivity
77 and dissolved organic carbon and therefore hypothesised that (I) the pH increase would
78 favour bacteria more than fungi, (II) the nutrients in the wood ash would benefit the
79 copiotrophic microbial groups, (III) multitrophic responses would appear gradually over
80 time after wood ash application, and (IV) that microbial stress responses would be
81 observable in the transcriptome.

82 2. Materials & Methods

83 2.1. Soils and wood ash

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84 We used two contrasting soils for the experiment. The first was a loamy sand (Typic
85 Hapludult) from the plough layer (0-10 cm) of an agricultural field (Research Center
86 Foulum, DK, 56°29’42”N 9°33’36”E). The other was from the O-horizon (0-10 cm) of a
87 forest (Gedhus, DK, 56°16’38”N 09°05’12”E). The forest is a second-generation Norway
88 spruce stand (*Picea abies* (L.) Karst.) on Podzol heathland. Qin *et al.* (2017) provide soil
89 characteristics for both soils. On both sites, we removed visible plant parts before taking
90 ten 100 g soil samples within a 30 m^2 area. The 10 samples from each site were sieved (4
91 mm), pooled and stored in the dark at 4 °C until further processing.

92 Wood ash was a mixture of bottom- and fly-ash from a heating plant (Brande, Denmark)
93 produced by combustion of wood chips from predominantly coniferous trees. We
94 homogenized the ash by sieving (2 mm). Maresca et al. (2017) provide a list of mineral
95 nutrients and heavy metals in the ash.

96

97 **2.2. Microcosm set-up and incubation**

98 We prepared microcosms in triplicates of 50 g soil in 250 ml sterilised airtight glass jars.
99 We mixed the ash thoroughly with soil to ash concentrations corresponding to field
100 application of 0, 3, 12 and 90 t ash ha^{-1}. The water content was adjusted to 50 % of the
101 water holding capacity of the two soils. We prepared 12 microcosms for each soil-ash
102 combination to allow four destructive samplings; i.e. a total of 96 microcosms. Samples
103 were also collected at the start of the experiment. Microcosms were incubated at 10 °C in
104 the dark and all microcosms were opened once a week inside a LAF-bench to maintain
105 aerobic conditions.

106

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107 **2.3. Physicochemical soil parameters**

108 At destructive sampling, after 3, 10, 30 and 100 days of incubation, we prepared soil
109 extracts from 15 g soil and 75 ml sterile ddH_2O followed by 1 h shaking and settling for 0.5
110 h. In the supernatant, we measured electrical conductivity using a TetraCon 325 electrode
111 adapted to a conductivity meter Cond 340i (WTW, Weilheim, Germany) and pH using a pH
112 electrode (Sentix Mic) connected to pH meter Multi 9310 (WTW). The remaining
113 supernatant was filtered (5C filters, Advantec, Tokyo, Japan; 1 µm pore size) and analysed
114 for dissolved organic carbon (DOC), nitrate (NO_3^-), ammonium (NH_4^+) and phosphate
115 (PO_4^{3-}). DOC concentrations were determined on a TOC-5000A (Shimadzu,Kyoto, Japan).
116 Nitrate, ammonium and phosphate concentrations were determined by flow injection
117 analysis (FIAstar™ 5000, FOSS, Hillerød, Denmark) following manufacturer's instructions.

118

119 **2.4. Nucleic acid extraction, qPCR and library preparation for sequencing**

120 RNA and DNA were co-extracted from 2 g soil samples using the RNA PowerSoil Total
121 RNA Isolation Kit (MOBIO, Carlsbad, USA) in combination with DNA Elution Accessory Kit
122 (MOBIO) following manufacturer's protocol. Agricultural soil amended with the highest ash
123 concentration had an RNA yield below detection limit and was not sequenced.

124 We quantified 16S rRNA and ITS2 gene copies (DNA level) using qPCR. 16S rRNA genes
125 were amplified in technical duplicates using a CFX Connect (Bio-Rad, Richmond, USA).
126 We used a dilution series of genomic DNA from *Escherichia coli* K-12 (with 7 copies of
127 16S rRNA genes) as a standard (Blattner *et al.*, 1997). The master-mix consisted of 2 µl
128 bovine serum albumin (BSA) (20mg/ml; BIORON, Ludwigshafen, Germany), 10 µl SsoFast
129 EvaGreen Supermix (Bio-Rad), 0.8 µl of primer 341f (5′-CCTAYGGGRBGCASCAG-3′),

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130 0.8 µl of primer 806r (5′-GGACTACNNGGGTATCTAAT-3′) (Hansen *et al.*, 2012); 1 µl of
131 10× diluted template, and 5.4 µl sterile DEPC-treated water. PCR conditions for 16S rRNA
132 gene amplification were 98°C for 15 min, followed by 35 cycles of 98°C for 30 s, 56°C for
133 30 s, and 72°C for 30 s (with fluorescence measurements) and ending with 72°C for 7 min
134 and production of melt curves. The PCR efficiencies for the 16S assays were 96.1±1.0%
135 (SEM, n=3) with R^2 = 0.99±0.001. ITS gene copies were quantified as described for the
136 16S rRNA above with minor modifications: Vector cloned ITS2 DNA regions from
137 *Aureobasidium pullulans* were included as standards, primers used were gITS7 (5′-
138 GTGARTCATCGARTCTTTG-3′ (Ihrmark *et al.*, 2012)) and ITS4 (5′-
139 TCCTCCGCTTATTGATATGC-3′ (White *et al.*, 1990)), annealing temperature was 60 °C
140 and 40 amplification cycles were used. The PCR efficiencies for the ITS assays were
141 106.0±4.6% with R^2 = 0.99±0.003.

142 Prior to Total RNA library building, we removed potential DNA carryovers using the DNase
143 Max Kit (MOBIO) following manufacturer's protocol. Successful DNA removal of RNA
144 extracts were tested with the 16S qPCR protocol described above but with 50 amplification
145 cycles: All DNase treated RNA extracts had higher or equal Cq values than the negative
146 samples (sterile DEPC-treated water as template) and DNA was thereby not present.

147 Quality of the DNase treated RNA was tested using RNA 6000 Nano Kit (Agilent, Santa
148 Clara, USA) on a 2100 Bioanalyzer System (Agilent) following manufacturer's protocol
149 (Average RIN number was 7.85±0.13 (SEM, n=69)

150 Subsequently, DNase treated RNA extracts from time points 0, 3, 30 and 100 days were
151 fragmented into ~150 bp segments and prepared for sequencing using the NEBNext Ultra
152 Directional RNA Library Prep Kit for Illumina in combination with the NEBNext Multiplex

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153 Oligos for Illumina (New England BioLabs, Ipswich, USA) according to the manufacturer's
154 protocol. We sequenced the resulting metatranscriptome libraries using HiSeq 2500
155 (Illumina Inc., San Diego, USA) in high output mode (8 HiSeq lanes, 125bp, paired end
156 reads) at the National High-throughput DNA Sequencing Centre (Copenhagen, Denmark).

157

158 **2.5. Bioinformatic processing**

159 We obtained a total of 3.3 billion paired sequences (SRA accession number:
160 PRJNA512608) and processed them through the following bioinformatic pipeline.
161 Adapters, poly-A tails, sequences shorter than 60 nt and nucleotides with phred score
162 below 20 at the 5' and 3' end of sequences were removed using Cutadapt v.1.9.1 (Martin,
163 2011). Five samples were removed prior to subsequent processing due to low quality of
164 reads (one replicate of 3 t ha^{-1}, day 100 from the agricultural soil and two replicates of 0 t
165 ha^{-1}, day 0 and two replicates 0 t ha^{-1}, day 100 from the forest soil). Sequences were then
166 sorted into small subunit (SSU) rRNA, large subunit (LSU) rRNA and non-rRNA
167 sequences using SortMeRNA v.2.1 (Kopylova *et al.*, 2012).

168

169 **2.5.1. rRNA**

170 A subset of 1.5 million randomly chosen SSU rRNA sequences per sample were
171 assembled into longer SSU rRNA contigs using EMIRGE (Miller *et al.*, 2011). Contigs were
172 taxonomically classified using CREST (Lanzén *et al.*, 2012) and rRNA reads were mapped
173 to resulting EMIRGE contigs using BWA (Li & Durbin, 2009), as in Epelde *et al.* (2015),
174 resulting in a table of taxonomically annotated read abundance across samples
175 (Supplementary Datasheet 1).

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176

177 **2.5.2. mRNA**

178 A combined pool of non-ribosomal sequences from all samples was assembled using
179 trinity v.2.0.6 (Grabherr *et al.*, 2011). From the resulting assembled contigs, non-coding
180 RNA contigs were filtered away by aligning contigs to the Rfam database v.12.0 (Nawrocki
181 *et al.*, 2015) using cmsearch v.1.1.1 with a significant e-value threshold of $<10^{-3}$. Input
182 sequences used for non-ribosomal RNA assembly were then mapped to coding mRNA
183 contigs. We normalized the contigs by removing those with relative expression lower than
184 1 out of the number of sequences in the dataset with least number of sequences.
185 EMBOSS (Rice *et al.*, 2000) was used to search six possible open reading frames (ORFs)
186 of the contigs. SWORD (Vaser *et al.*, 2016) was used to align ORFs against the Md5nr
187 protein database (Wilke *et al.*, 2012). The output was then parsed with custom Python
188 scripts and filtered hits with minimum e-value of 10^{-5} as threshold. Best hit for each contig
189 was then selected based on alignment statistics and annotated against the eggnog
190 hierarchical database v.4.5 (Jensen *et al.*, 2008). The output was an abundance table of
191 numbers of sequences assigned to groups of different functional genes (COGs)
192 (Supplementary Datasheet 2).

193

194 **2.6. Statistical analysis and data processing**

195 Statistical validation for both taxonomy and functional abundance was done in R v.3.4.0 (R
196 Core Team, 2015) using *vegan* (Oksanen *et al.*, 2008). The rRNA abundance was
197 converted into relative abundance and collapsed taxonomically into Archaea, Bacteria and
198 Eukaryota. We further grouped Eukaryota into Fungi, Metazoa, and protists (with main

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199 focus on bacterivorous protozoa). We calculated Richness and Shannon diversity on the
200 total number of rRNA contigs and abundance of sequence reads mapped to them. Non-
201 metric multidimensional scaling (NMDS) was carried out using Bray-Curtis dissimilarities of
202 community composition between samples. Soil physicochemical parameters were fitted to
203 the resulting NMDS using the function *envfit*. Variables explaining overall differences in
204 community composition were evaluated using the function *Adonis*, which performs
205 permutational analysis of variance (PERMANOVA; 10,000 permutations) using Bray-Curtis
206 dissimilarities as response variable. A forward selection strategy was carried out to only
207 include explanatory variables with significant p-values in *Adonis* models.

208 Significant effects of wood ash amendment and incubation time on taxonomic groups were
209 determined using non-parametric Kruskal-Wallis tests (due to the non-normal distribution
210 of taxon abundances). To separate the pronounced changes in community responses
211 observed at the 90 t ha^{-1} amendment in the forest soil from the less pronounced changes
212 observed at 0-12 t ha^{-1}, we performed Kruskal-Wallis tests with wood ash concentration as
213 independent variable for both the range of 0-12 t ha^{-1} and 0-90 t ha^{-1}. We also used
214 Kruskal-Wallis to test the effect of time on differential abundances within the wood ash
215 concentrations separately. P-values were adjusted for false discovery rate (FDR) using the
216 Benjamini–Hochberg method in all tests. NMDS on Bray Curtis dissimilarities of gene
217 compositions and *Adonis* testing were carried out as described above. Gene counts
218 between samples were normalized using the DESeq2 algorithm (Love *et al.*, 2014).
219 Significantly differentially expressed genes were analysed using the DESeq2 module of
220 SARTools (Varet *et al.*, 2016). These analyses were conducted by pairwise comparisons
221 of gene transcription levels between samples of increasing wood ash concentration to
222 control samples (0t ha^{-1}) at different incubation times. For the forest samples at time 100

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223 days, only one replicate remained for the 0 t ha^{-1} treatment. Therefore we compared
224 instead the 12 and 90 t ha^{-1} to the 3 t ha^{-1}.

225 We used linear Pearson regression to test for significant correlations between wood ash
226 concentration and time against measured physicochemical parameters. Additionally, we
227 performed two-way ANOVAs with Tukey's post-hoc tests using wood ash concentration
228 and time as explanatory variables, with all physicochemical parameters as dependent
229 variables. Variance homogeneity was tested using Levenes's test and normal distribution
230 of data was tested using the Shapiro-Wilk test in combination with QQ-plots prior to
231 ANOVA tests.

232 We used a significance level of 0.05, unless otherwise explicitly mentioned, and the results
233 section provide descriptions at this significance level.

234

235 **3. Results**

236 **3.1. Physicochemical parameters**

237 Soil pH, electrical conductivity and DOC correlated positively with wood ash concentration
238 for both soils (Table 1). For the 90 t ha^{-1} ash amendment, soil pH increased from 6.4 to
239 11.5 in the agricultural and from 4.1 to 8.5 in the forest soil (Supplementary Figure 1).
240 Similarly, the 90 t ha^{-1} resulted in 15 and 19-fold increases in electrical conductivity for the
241 agricultural and forest soil, respectively. In the agricultural soil, ammonium increased with
242 time in samples both with and without ash amendment, while nitrate showed no significant
243 changes. In the forest soil, ammonium and nitrate increased after 3 days in the 90 t ha^{-1}
244 amendment, followed by a decrease after 30 days. In the other treatments, increased
245 concentrations were observed during the entire incubation period. In both soils,

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246 concentrations of dissolved phosphate increased up to 12 t ash ha^{-1} followed by a
247 decrease at 90 t ha^{-1}.

248

249 **3.2. Quantitative PCR**

250 Prokaryotic abundance (number of 16S rRNA gene copies) increased in the agricultural
251 soil after the wood ash application of 12 t ha^{-1}, but decreased after application of 90 t ha^{-1}
252 (Figure 1). Fungal abundance (number of ITS copies) remained fairly unchanged over
253 time regardless of ash application with the exception of an increase after 100 days at 90 t
254 ha^{-1}. In the forest soil prokaryotic abundance increased over time for all treatments (Figure
255 1); however, addition of 12 and 90 t ha^{-1} resulted in a stronger increase. The fungal
256 abundance in the forest soil showed higher abundance for most of the period with wood
257 ash concentrations of 90 t ha^{-1}.

258

259 **3.3. rRNA - Community composition**

260 The number of unique rRNA contigs ranged from 1,216 – 5,931 per sample and originated
261 from all domains of life. Community composition differed significantly ($p < 0.001$; $R^2 = 0.86$;
262 *Adonis*) between the two soil types. For forest soil, amendment with 90 t ha^{-1} resulted in
263 highly altered community composition (Figure 2) compared to 0-12 t ha^{-1}. Though less
264 pronounced, changes from 0-3-12 t ha^{-1} were also clearly visible for both soil types (Figure
265 2). Moreover, microcosms for particular soil type/ash dose combinations were clearly
266 separated by sampling times (Figure 2).

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267 In both soils, wood ash dose, incubation time, pH and electrical conductivity correlated to
268 the transformed NMDS community space (Figure 2). Optimized *Adonis* models (Table 2)
269 supported that wood ash concentration, time, pH, and electrical conductivity together
270 significantly explained the variation in microbial communities after ash application in both
271 soils. Additionally, dissolved phosphate significantly explained the variation in microbial
272 communities in both soils up to 12 t ha^{-1} ash amendments and DOC, ammonium and
273 nitrate in the forest soil.

274

275 **3.4. rRNA – Taxonomic distribution and diversity**

276 A majority (85%) of sequence reads, mapped to contigs, could be annotated to order rank
277 (99% to phylum and 97% to class rank) (Figure 3A). Fewer sequences could be assigned
278 lower taxonomic ranks (60% and 27% to family and genus level, respectively). Therefore,
279 we evaluated possible significant differences in abundance of taxa at order level (see
280 Supplementary Datasheet 3 and 4 for p-values and averages of relative abundances,
281 respectively). Richness and Shannon diversity decreased in the unamended agricultural
282 soil over time, while ash amendments of 3 and 12 t ha^{-1} counteracted this decrease
283 (Figure 3B). In the forest soil these measures generally remained unchanged up to 12 t ha^{-1}
284 amendments (with a single exception of increased richness at 3 t ha^{-1} after 100 days of
285 incubation), while the 90 t ha^{-1} amendment caused reduction of Shannon diversity.

286

287 **3.4.1. Prokaryotic community**

288 In both soil types, the relative abundance of Chitinophagaceae (Bacteroidetes) increased
289 with wood ash application (Figure 3A). In the agricultural soil, ash-amendment also caused

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290 increases in Alphaproteobacteria and Betaproteobacteria. In the forest soil, the 3 and 12 t
291 ha^{-1} ash-amendments increased Myxococcales (Deltaproteobacteria), while Acidimicrobiia
292 (Actinobacteria) decreased.

293 In the forest soil, the 90 t ha^{-1} ash-amendment resulted in major prokaryotic community
294 changes. Actinobacteria, Acidobacteria, Armatimonadetes, and Verrucomicrobia
295 decreased, while Firmicutes, Bacteroidetes and Proteobacteria increased. Firmicutes
296 dominated after 3 days, with *Paenibacillus* as most abundant with relative abundance of
297 21.3%, followed by a gradual decrease towards 1.1% after 100 days. Similarly,
298 Gammaproteobacteria decreased during incubation after an initial increase.
299 Chitinophagaceae and Rhizobiales (Alphaproteobacteria) showed the opposite temporal
300 trend and were most abundant after100 days.

301

302 **3.4.2. Fungal community**

303 The 3 and 12 t ha^{-1} ash amendments did not affect fungal community composition in the
304 agricultural soil (Figure 3A). In the forest soil no major changes were found at low
305 amendments, while application of 90 t ha^{-1} resulted in increase in *Mortierella*, Hypocreales
306 (Sordariomycetes) and *Peziza* (Pezizomycetes).

307

308 **3.4.3. Micro-eukaryotic community**

309 In the agricultural soil, the relative abundances of Tubulinea (Amoebozoa),
310 Thaumatomonadida (Cercozoa) and Silicofilosea (Cercozoa) increased over time in all
311 treatments (Figure 3A). In the forest soil, *Colpoda* (Ciliophora) increased with time in all

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312 treatments, though more pronounced at higher wood ash amendments. Further, Tubulinea
313 (Amoebozoa), Heteromitidae (Cercozoa) and Silicofilosea (Cercozoa) increased in the 12
314 and 90 t ha^{-1} amendments.

315

316 **3.5. mRNA - Functional genes**

317 A total of 0.9 million sequences were mapped to 463 mRNA contigs (Supplementary
318 Figure 2 and Supplementary Datasheet 2). The two soils possessed distinct pools of
319 expressed genes ($p < 0.001$; $R^2 = 0.82$; A*donis*). Overall, Bray-Curtis dissimilarities, based
320 on mRNA profiles, and fitting of physicochemical parameters to these, revealed similar
321 trends as for rRNA taxonomic communities (Figure 2 and Table 2).

322 In the agricultural soil, we observed only minor functional gene responses to time and ash-
323 amendment, while more genes were differentially expressed in the forest soil, especially at
324 the 90 t ha^{-1} amendment (Figure 4; full list of differential expressed genes in
325 Supplementary Datasheet 5). Of the well characterized genes, four functional categories
326 contained most of the differentially expressed genes; i.e. “Post-translation modification,
327 protein turnover, and chaperones”, “Transcription”, “Replication, recombination and repair”
328 and “Carbohydrate transport and metabolism”. Furthermore, genes related to stress
329 responses increased mainly in the forest soil at 90 t ha^{-1} ash amendments (Supplementary
330 Figure 3).

331

332

333

334 **4. Discussion**

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335 Here, we present the first detailed analysis of changes in soil microbial prokaryotic and
336 eukaryotic communities after amendment with ash using the Total RNA-sequencing
337 procedure.

338

339 **4.1. Bacterial responses to wood ash application**

340 The general copiotrophic groups of bacteria, i.e. Bacteroidetes, Alphaproteobacteria and
341 Betaproteobacteria were stimulated by wood ash application. Members of Bacteroidetes
342 benefit from wood ash application (Noyce *et al.*, 2016; Bang-Andreasen *et al.*, 2017); they
343 are initial metabolizers of labile carbon and respond positively to increased soil pH and
344 electrical conductivity (Fierer *et al.*, 2007; Lauber *et al.*, 2009; Kim *et al.*, 2016). Alpha- and
345 Betaproteobacteria are also generally copiotrophic (Fierer *et al.*, 2007; Cleveland *et al.*,
346 2007); Betaproteobacteria thrive in soils with higher pH (Kim *et al.*, 2016), whereas
347 Alphaproteobacteria are favoured at high N availability (Nemergut *et al.*, 2010; Fierer *et al.*,
348 2012).

349 Acidobacteria and Verrucomicrobia declined after the 90 t ha^{-1} amendment to the forest
350 soil. These phyla are considered oligotrophic (Fierer *et al.*, 2007; Bergmann *et al.*, 2011;
351 Ramirez *et al.*, 2012; Cederlund *et al.*, 2014; Kielak *et al.*, 2016) and Acidobacteria are
352 generally most abundant under acidic conditions (Rousk *et al.*, 2010; Kielak *et al.*, 2016).
353 Thus, increases in pH, bioavailable DOC and nutrients induced by wood-ash allow
354 copiotrophic groups to thrive on the expense of oligotrophic groups. The shift towards a
355 more copiotrophic dominated community after ash amendment was further supported by
356 the mRNA profile of the soil. Here, an increasing number of functional genes involved in

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357 metabolism and cell growth ("Translation", "Transcription" and "Replication") showed
358 significant higher transcription levels.

359 Of the Bacteroidetes, Chitinonophagaceae showed the strongest positive response to
360 wood ash application. Members of this family can degrade a broad spectrum of carbon
361 compounds (Kämpfer *et al.*, 2006; Hanada *et al.*, 2014). Thus, they are well suited for the
362 ash-induced increased DOC availability. Rhizobiales dominated the increasing
363 Alphaproteobacterial fraction of the forest soil after ash amendment. They are copiotrophs
364 (Starke *et al.*, 2016; Lladó & Baldrian, 2017) and can degrade organic pollutants and cope
365 with heavy metals (Teng *et al.*, 2015). Probably advantageous properties, as the wood ash
366 induces increase of heavy metals and nutrients in the soils. Deltaproteobacterial
367 Myxococcales responded positively to wood ash amendment in the forest soil. Noteworthy,
368 the increase in Myxococcales occurred late in the incubation where especially
369 Chitinophagaceae and Alphaproteobacteria decreased. Myxococcales are 'micropredators'
370 and attack and lyse other bacteria which might explain the increased dominance of this
371 group on the expense of other bacterial groups (Reichenbach, 1999).

372 The increase in 16S rRNA gene copy numbers after ash amendment (up to 12 t ha^{-1} and
373 90 t ha^{-1} for the agricultural and forest soil, respectively) is consistent with other reports of
374 increasing bacterial numbers after wood ash application (Bååth & Arnebrant, 1994; Fritze
375 *et al.*, 2000; Perkiömäki & Fritze, 2002; Bang-Andreasen *et al.*, 2017; Vestergård *et al.*,
376 2018). The large increase in the forest soil is further consistent with the increased pH as
377 most bacteria thrive better at pH around 7 (Rousk *et al.*, 2009). Prokaryotic growth as well
378 as a change towards a more copiotrophic community with higher average 16S rRNA gene
379 number per genome is likely causing the 16S rRNA gene copy increase (Klappenbach *et*
380 *al.*, 2000; Roller *et al.*, 2016).

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381 The 90 t ha^{-1} ash amendment to the forest soil caused immediate dominance of Firmicutes
382 and Gammaproteobacteria. Both groups are copiotrophs that thrives upon addition of
383 easily degradable carbon and nitrogen to soil which probably partly explain their success
384 upon ash application (Fierer *et al.*, 2007; Cleveland *et al.*, 2007; Nemergut *et al.*, 2010;
385 Ramirez *et al.*, 2012; Fierer *et al.*, 2012). However, bacteria from these phyla are also
386 known to be tolerant to heavy metals (Jacquiod *et al.*, 2017). Moreover, within Firmicutes
387 the endospore-forming genus *Paenibacillus* dominated (de Hoon *et al.*, 2010), and we
388 found increased transcription of genes involved in sporulation in these samples.
389 Combined, these capabilities probably enable members of these groups to withstand the
390 initial wood ash induced changes to the soil, including increased heavy metal
391 concentrations, thereby allowing them to be initial utilizers of newly available labile
392 resources. Reduced diversity at this ash dose further indicates that less organisms can
393 cope with the ash induced changes to the soil system

394

395 **4.2. Fungal responses to wood ash application**

396 In both soil types, fungal response to ash amendment was slight compared to the
397 prokaryotic response. Likewise, Cruz-Paredes et al. (2017), Högberg et al. (2007) and
398 Rousk *et al.*, (2009, 2011) found bacteria to be more stimulated by nutrient addition and
399 increases in pH than fungi. Similarly effects of ash amendment have been reported by
400 Noyce *et al.* (2016) and Mahmood *et al.* (2003). The 90 t ha^{-1} amendment in the forest soil
401 caused increased ITS gene copy numbers and a fungal community shift with increased
402 dominance of *Mortierella*, *Peziza* and Hypocreales. These fungi are opportunistic
403 saprotrophs with high growth rates and can exploit readily availible nutrients before other

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404 fungi arrive (Carlile *et al.*, 2001; Tedersoo *et al.*, 2006; Druzhinina *et al.*, 2012). Further,
405 some *Peziza* spp. are early post-fire colonizers adapted to ash conditions (Egger, 1986;
406 Rincón *et al.*, 2014). The increase in these groups further supports that copiotrophic-like
407 lifestyles are favoured by wood ash application.

408

409 **4.3. Micro-eukaryote responses to wood ash application**

410 The micro-eukaryotes also responded to wood ash application in the forest soil, probably
411 because the stimulation of copiotrophic bacteria and fungi provided more food for
412 nematodes and protozoa (Rønn *et al.*, 2012). Ciliates (*Colpoda*), amoebae (Tubulinea)
413 and small heterotrophic flagellates (Heteromitidae and Silicofilosea) increased with more
414 pronounced responses at the later incubation times. Protozoa generally have longer
415 generation times than prokaryotes, and thus need longer time to increase in population
416 size. Further, they cannot start growth before a reasonable bacterial population has been
417 formed (Fenchel, 1987; Ekelund *et al.*, 2002). The protozoan increase may explain the
418 small decrease in prokaryotic 16S rRNA gene copies at day 100, where we observed the
419 largest fraction of protozoa. The positively responding protozoa were likely primarily
420 bacterivorous (Ekelund & Rønn, 1994; Ekelund, 1998), consistent with the decreasing
421 relative fraction of bacterial rRNA sequences and the increasing relative fraction of fungal
422 and protozoan rRNA sequences in the later incubation times after the application of 12 and
423 90 t ha^{-1} ash. Thus, preferential protozoan grazing on bacteria can explain the relative
424 larger rRNA-fraction of fungi and protozoa at day 100. We found no significant effect of
425 ash-amendment on micro-eukaryotes in the agricultural soil, which is consistent with the
426 relative minor effects on prokaryotes and fungi in this soil.

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427

428 **4.5. Stress responses at high wood ash amendments**

429 We recorded increased transcription of stress-response genes at the 90 t ha^{-1}
430 amendments, which supports that this high dose exerts harmful effects on many members
431 of the micro-biome. For example, chaperones ensure correct folding of proteins and are
432 involved in cellular coping with stress-induced denaturation of proteins (Feder & Hofmann,
433 1999) and the increase in transcription level of these probably is a stress response. Also,
434 transmembrane transporter proteins balance osmotic pressure of cells, regulate cytosolic
435 pH and can export toxins such as metals from the cell (Alberts *et al.*, 2002; Ma *et al.*, 2009;
436 Wilkens, 2015). Increased activity of transmembrane transporters is probably a response
437 to wood ash induced osmotic changes to the soil system, increased pH, metal
438 concentration and other toxic compounds.

439

440 **4.6. The changes in the microbial communities are linked to physicochemical soil**
441 **parameters**

442 We found that ash-amendment strongly increased soil pH, which is a strong driver of
443 microbial community composition and functioning (Fierer & Jackson, 2006; Rousk *et al.*,
444 2010) also after wood ash application (Frostegård *et al.*, 1993; Zimmermann & Frey, 2002;
445 Högberg *et al.*, 2007; Peltoniemi *et al.*, 2016; Bang-Andreasen *et al.*, 2017). DOC and
446 phosphate concomitantly increased. Several factors may contribute to this: (I) pH
447 dependent changes in solubility (Evans *et al.*, 2012; Maresca *et al.*, 2017), (II) release from
448 dead organisms incapable of coping with the wood ash or wood ash induced changes to
449 the soil system, (III) increased mineralization rates after wood ash application (Bååth &

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450 Arnebrant, 1994; Vestergård *et al.*, 2018) and (IV) the phosphorous in the bio-ash (Pitman,
451 2006; Maresca *et al.*, 2017).

452 Since pH, conductivity, DOC and phosphate all correlated positively to wood ash
453 concentrations it is difficult to disentangle the direct effect of these components as they
454 might all be covariates of the wood ash amendments. pH-changes induce a cascade of
455 effects in soil parameters and therefore affect mineral nutrient availability, salinity, metal
456 solubility and organic C (Lauber *et al.*, 2009). Many of the wood ash induced changes
457 were likely caused directly or indirectly by the pH increase, which is probably the major
458 reason that pH is an essential driver of taxonomic and functional soil characteristics
459 (Lauber *et al.*, 2009; Rousk *et al.*, 2010; Fierer, 2017; Vestergård *et al.*, 2018).

460 Wood ash contains virtually no nitrogen, hence measurable effects on soil nitrate and
461 ammonium are probably caused by pH effects on microbial N mineralization (Vestergård *et*
462 *al.*, 2018) and ion solubility (Pitman, 2006). Changes in nitrate and ammonium were
463 significant as explanatory variables on the observed rRNA and mRNA dissimilarity profiles
464 of the forest soil but not in the agricultural soil. Forest soil is generally more N limited than
465 agricultural soil, where N is kept at a high level through fertilization.

466

467 **4.7. Conclusions**

468 We used detailed total RNA-Sequencing to demonstrate drastic taxonomic and functional
469 changes in the active prokaryotic and eukaryotic micro-biomes of agricultural and forest
470 soil after wood ash amendment. Our analyses suggested that increase in pH, electrical
471 conductivity, dissolved organic carbon and phosphate were the main drivers of the
472 observed changes. Wood ash amendment of 3 and 12 t ha^{-1} resulted in increased

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473 prokaryotic abundance and dominance of copiotrophic groups and elevated expression of
474 genes involved in metabolism and cell growth. Amendment of 90 t ha^{-1} caused collapse of
475 the micro-biome in the agricultural soil, while in the forest the copiotrophic micro-biome,
476 also including fast-growing saprotrophic fungi, was further stimulated. However, diversity
477 was reduced, and expression of stress response genes increased. Bacterivorous
478 protozoan groups increased as a response to enhanced bacterial growth, which supports
479 that the protozoa have a pivotal role in controlling bacterial abundance in soil following
480 wood ash application. Overall, prokaryotic community and quantity responded more
481 pronounced to wood ash amendment than fungi in both forest and agricultural soil.

482

483 **Conflict of Interest**

484 The authors declare no conflict of interest.

485

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494

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497 **References**

498 Alberts B, Johnson A, Lewis J, Raff M, Roberts K, Walter P. (2002). Molecular Biology of the Cell.
499 4th ed. Garland Science: New York.

500 Aronsson KA, Ekelund NGA. (2004). Biological effects of wood ash application to forest and
501 aquatic ecosystems. *J Environ Qual* **33**:1595–1605.

502 Augusto L, Bakker MR, Meredieu C. (2008). Wood ash applications to temperate forest
503 ecosystems—potential benefits and drawbacks. *Plant Soil* **306**:181–198.

504 Bang-Andreasen T, Nielsen JT, Voriskova J, Heise J, Rønn R, Kjøller R, *et al.* (2017). Wood ash
505 induced pH changes strongly affect soil bacterial numbers and community composition. *Front*
506 *Microbiol* **8**:1400.

507 Bergmann GT, Bates ST, Eilers KG, Lauber CL, Caporaso JG, Walters WA, *et al.* (2011). The
508 under-recognized dominance of Verrucomicrobia in soil bacterial communities. *Soil Biol Biochem*
509 **43**:1450–1455.

510 Blagodatskaya E, Kuzyakov Y. (2013). Active microorganisms in soil: Critical review of estimation
511 criteria and approaches. *Soil Biology and Biochemistry* **67**:192–211.

512 Blattner FR, Plunkett G, Bloch CA, Perna NT, Burland V, Riley M, *et al.* (1997). The complete
513 genome sequence of Escherichia coli K-12. *Science* **277**:1453–1462.

514 Bååth E, Arnebrant K. (1994). Growth rate and response of bacterial communities to pH in limed
515 and ash treated forest soils. *Soil Biology and Biochemistry* **26**:995–1001.

516 Carlile MJ, Watkinson SC, Gooday GW. (2001). The Fungi. 2nd ed. Academic Press: San Diego,
517 Calif.

518 Carvalhais LC, Dennis PG, Tyson GW, Schenk PM. (2012). Application of metatranscriptomics to
519 soil environments. *J Microbiol Methods* **91**:246–251.

520 Cederlund H, Wessén E, Enwall K, Jones CM, Juhanson J, Pell M, *et al.* (2014). Soil carbon
521 quality and nitrogen fertilization structure bacterial communities with predictable responses of
522 major bacterial phyla. *Applied Soil Ecology* **84**:62–68.

523 Cleveland CC, Nemergut DR, Schmidt SK, Townsend AR. (2007). Increases in soil respiration
524 following labile carbon additions linked to rapid shifts in soil microbial community composition.
525 *Biogeochemistry* **82**:229–240.

526 Cookson WR, Osman M, Marschner P, Abaye DA, Clark I, Murphy DV, *et al.* (2007). Controls on
527 soil nitrogen cycling and microbial community composition across land use and incubation
528 temperature. *Soil Biology and Biochemistry* **39**:744–756.

529 Cruz-Paredes C, Wallander H, Kjøller R, Rousk J. (2017). Using community trait-distributions to
530 assign microbial responses to pH changes and Cd in forest soils treated with wood ash. *Soil*
531 *Biology and Biochemistry* **112**:153–164.

532 Demeyer A, Voundi Nkana J., Verloo M. (2001). Characteristics of wood ash and influence on soil
533 properties and nutrient uptake: an overview. *Bioresour Technol* **77**:287–295.

534 Druzhinina IS, Shelest E, Kubicek CP. (2012). Novel traits of Trichoderma predicted through the
535 analysis of its secretome. *FEMS Microbiol Lett* **337**:1–9.

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536 Egger KN. (1986). Substrate Hydrolysis Patterns of Post-Fire Ascomycetes (Pezizales). *Mycologia*
537 **78**:771.

538 Ekelund F. (1998). Enumeration and abundance of mycophagous protozoa in soil, with special
539 emphasis on heterotrophic flagellates. *Soil Biology and Biochemistry* **30**:1343–1347.

540 Ekelund F, Frederiksen HB, Rønn R. (2002). Population dynamics of active and total ciliate
541 populations in arable soil amended with wheat. *Appl Environ Microbiol* **68**:1096–1101.

542 Ekelund F, Rønn R. (1994). Notes on protozoa in agricultural soil with emphasis on heterotrophic
543 flagellates and naked amoebae and their ecology. *FEMS Microbiol Rev* **15**:321–353.

544 Epelde L, Lanzén A, Blanco F, Urich T, Garbisu C. (2015). Adaptation of soil microbial community
545 structure and function to chronic metal contamination at an abandoned Pb-Zn mine. *FEMS*
546 *Microbiol Ecol* **91**:1–11.

547 Evans CD, Jones TG, Burden A, Ostle N, Zieliński P, Cooper MDA, *et al.* (2012). Acidity controls
548 on dissolved organic carbon mobility in organic soils. *Glob Change Biol* **18**:3317–3331.

549 Feder ME, Hofmann GE. (1999). Heat-shock proteins, molecular chaperones, and the stress
550 response: evolutionary and ecological physiology. *Annu Rev Physiol* **61**:243–282.

551 Fenchel T. (1987). Ecology of Protozoa. 1st ed. Springer: Berlin, Heidelberg doi:10.1007/978-3-
552 662-25981-8.

553 Fierer N. (2017). Embracing the unknown: disentangling the complexities of the soil microbiome.
554 *Nat Rev Microbiol* **15**:579–590.

555 Fierer N, Bradford MA, Jackson RB. (2007). Toward an ecological classification of soil bacteria.
556 *Ecology* **88**:1354–1364.

557 Fierer N, Jackson RB. (2006). The diversity and biogeography of soil bacterial communities. *Proc*
558 *Natl Acad Sci U S A* **103**:626–631.

559 Fierer N, Lauber CL, Ramirez KS, Zaneveld J, Bradford MA, Knight R. (2012). Comparative
560 metagenomic, phylogenetic and physiological analyses of soil microbial communities across
561 nitrogen gradients. *ISME J* **6**:1007–1017.

562 Fritze H, Perkiömäki J, Saarela U, Katainen R, Tikka P, Yrjälä K, *et al.* (2000). Effect of Cd-
563 containing wood ash on the microflora of coniferous forest humus. *FEMS Microbiol Ecol* **32**:43–51.

564 Frostegård A, Tunlid A, Bååth E. (1993). Phospholipid Fatty Acid composition, biomass, and
565 activity of microbial communities from two soil types experimentally exposed to different heavy
566 metals. *Appl Environ Microbiol* **59**:3605–3617.

567 Frostegård Å., Bååth E, Tunlio A. (1993). Shifts in the structure of soil microbial communities in
568 limed forests as revealed by phospholipid fatty acid analysis. *Soil Biology and Biochemistry*
569 **25**:723–730.

570 Geisen S, Tveit AT, Clark IM, Richter A, Svenning MM, Bonkowski M, *et al.* (2015).
571 Metatranscriptomic census of active protists in soils. *ISME J* **9**:2178–2190.

572 Grabherr MG, Haas BJ, Yassour M, Levin JZ, Thompson DA, Amit I, *et al.* (2011). Full-length
573 transcriptome assembly from RNA-Seq data without a reference genome. *Nat Biotechnol* **29**:644–
574 652.

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575 Hanada S, Tamaki H, Nakamura K, Kamagata Y. (2014). Crenotalea thermophila gen. nov., sp.
576 nov., a member of the family Chitinophagaceae isolated from a hot spring. *Int J Syst Evol Microbiol*
577 **64**:1359–1364.

578 Hansen CHF, Krych L, Nielsen DS, Vogensen FK, Hansen LH, Sørensen SJ, *et al.* (2012). Early
579 life treatment with vancomycin propagates Akkermansia muciniphila and reduces diabetes
580 incidence in the NOD mouse. *Diabetologia* **55**:2285–2294.

581 Hansen M, Bang-Andreasen T, Sørensen H, Ingerslev M. (2017). Micro vertical changes in soil pH
582 and base cations over time after application of wood ash on forest soil. *Forest Ecology and*
583 *Management* **406**:274–280.

584 De Hoon MJL, Eichenberger P, Vitkup D. (2010). Hierarchical evolution of the bacterial sporulation
585 network. *Curr Biol* **20**:R735–45.

586 Hultman J, Waldrop MP, Mackelprang R, David MM, McFarland J, Blazewicz SJ, *et al.* (2015).
587 Multi-omics of permafrost, active layer and thermokarst bog soil microbiomes. *Nature* **521**:208–
588 212.

589 Huotari N, Tillman-Sutela E, Moilanen M, Laiho R. (2015). Recycling of ash – For the good of the
590 environment? *Forest Ecology and Management* **348**:226–240.

591 Högberg MN, Högberg P, Myrold DD. (2007). Is microbial community composition in boreal forest
592 soils determined by pH, C-to-N ratio, the trees, or all three? *Oecologia* **150**:590–601.

593 Ihrmark K, Bödeker ITM, Cruz-Martinez K, Friberg H, Kubartova A, Schenck J, *et al.* (2012). New
594 primers to amplify the fungal ITS2 region--evaluation by 454-sequencing of artificial and natural
595 communities. *FEMS Microbiol Ecol* **82**:666–677.

596 Jacquiod S, Cyriaque V, Riber L, Al-soud WA, Gillan DC, Wattiez R, *et al.* (2017). Long-term
597 industrial metal contamination unexpectedly shaped diversity and activity response of sediment
598 microbiome. *J Hazard Mater* **344**:299–307.

599 Jensen LJ, Julien P, Kuhn M, von Mering C, Muller J, Doerks T, *et al.* (2008). eggNOG: automated
600 construction and annotation of orthologous groups of genes. *Nucleic Acids Res* **36**:D250–4.

601 Karltun E, Saarsalmi A, Ingerslev M, Mandre M, Andersson S, Gaitnieks T, *et al.* (2008). Wood ash
602 recycling – possibilities and risks. In:*Sustainable use of forest biomass for energy*, Röser, D,
603 Asikainen, A, Raulund-Rasmussen, K, & Stupak, I (eds), Springer Netherlands: Dordrecht, pp. 79–
604 108.

605 Kielak AM, Barreto CC, Kowalchuk GA, van Veen JA, Kuramae EE. (2016). The Ecology of
606 Acidobacteria: Moving beyond Genes and Genomes. *Front Microbiol* **7**:744.

607 Kim JM, Roh A-S, Choi S-C, Kim E-J, Choi M-T, Ahn B-K, *et al.* (2016). Soil pH and electrical
608 conductivity are key edaphic factors shaping bacterial communities of greenhouse soils in Korea. *J*
609 *Microbiol* **54**:838–845.

610 Klappenbach JA, Dunbar JM, Schmidt TM. (2000). rRNA operon copy number reflects ecological
611 strategies of bacteria. *Appl Environ Microbiol* **66**:1328–1333.

612 Kopylova E, Noé L, Touzet H. (2012). SortMeRNA: fast and accurate filtering of ribosomal RNAs in
613 metatranscriptomic data. *Bioinformatics* **28**:3211–3217.

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614 Kämpfer P, Young C-C, Sridhar KR, Arun AB, Lai WA, Shen FT, *et al.* (2006). Transfer of
615 [Flexibacter] sancti, [Flexibacter] filiformis, [Flexibacter] japonensis and [Cytophaga] arvensicola to
616 the genus Chitinophaga and description of Chitinophaga skermanii sp. nov. *Int J Syst Evol*
617 *Microbiol* **56**:2223–2228.

618 Lanzén A, Jørgensen SL, Huson DH, Gorfer M, Grindhaug SH, Jonassen I, *et al.* (2012). CREST--
619 classification resources for environmental sequence tags. *PLoS ONE* **7**:e49334.

620 Lauber CL, Hamady M, Knight R, Fierer N. (2009). Pyrosequencing-based assessment of soil pH
621 as a predictor of soil bacterial community structure at the continental scale. *Appl Environ Microbiol*
622 **75**:5111–5120.

623 Li H, Durbin R. (2009). Fast and accurate short read alignment with Burrows-Wheeler transform.
624 *Bioinformatics* **25**:1754–1760.

625 Lladó S, Baldrian P. (2017). Community-level physiological profiling analyses show potential to
626 identify the copiotrophic bacteria present in soil environments. *PLoS ONE* **12**:e0171638.

627 Love MI, Huber W, Anders S. (2014). Moderated estimation of fold change and dispersion for
628 RNA-seq data with DESeq2. *Genome Biol* **15**:550–550.

629 Ma Z, Jacobsen FE, Giedroc DP. (2009). Metal Transporters and Metal Sensors: How
630 Coordination Chemistry Controls Bacterial Metal Homeostasis. *Chem Rev* **109**:4644–4681.

631 Mahmood S, Finlay RD, Fransson A-M, Wallander H. (2003). Effects of hardened wood ash on
632 microbial activity, plant growth and nutrient uptake by ectomycorrhizal spruce seedlings. *FEMS*
633 *Microbiol Ecol* **43**:121–131.

634 Maresca A, Hyks J, Astrup TF. (2017). Recirculation of biomass ashes onto forest soils: ash
635 composition, mineralogy and leaching properties. *Waste Manag* **70**:127–138.

636 Martin M. (2011). Cutadapt removes adapter sequences from high-throughput sequencing reads.
637 *EMBnet.journal* **17**:10.

638 Miller CS, Baker BJ, Thomas BC, Singer SW, Banfield JF. (2011). EMIRGE: reconstruction of full-
639 length ribosomal genes from microbial community short read sequencing data. *Genome Biol*
640 **12**:R44.

641 Nawrocki EP, Burge SW, Bateman A, Daub J, Eberhardt RY, Eddy SR, *et al.* (2015). Rfam 12.0:
642 updates to the RNA families database. *Nucleic Acids Res* **43**:D130–7.

643 Nemergut DR, Cleveland CC, Wieder WR, Washenberger CL, Townsend AR. (2010). Plot-scale
644 manipulations of organic matter inputs to soils correlate with shifts in microbial community
645 composition in a lowland tropical rain forest. *Soil Biology and Biochemistry* **42**:2153–2160.

646 Noyce GL, Fulthorpe R, Gorgolewski A, Hazlett P, Tran H, Basiliko N. (2016). Soil microbial
647 responses to wood ash addition and forest fire in managed Ontario forests. *Applied Soil Ecology*
648 **107**:368–380.

649 Ohno T, Susan Erich M. (1990). Effect of wood ash application on soil pH and soil test nutrient
650 levels. *Agriculture, Ecosystems & Environment* **32**:223–239.

651 Oksanen J, Kindt R, Legendre P, O'Hara B, Simpson GL, Solymos P, *et al.* (2008). vegan:
652 Community Ecology Package. https://cran.r-project.org/web/packages/vegan/.

bioRxiv preprint doi: https://doi.org/10.1101/621557; this version posted April 30, 2019. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. All rights reserved. No reuse allowed without permission.

653 Peltoniemi K, Pyrhönen M, Laiho R, Moilanen M, Fritze H. (2016). Microbial communities after
654 wood ash fertilization in a boreal drained peatland forest. *European journal of soil biology* **76**:95–
655 102.

656 Perkiömäki J, Fritze H. (2002). Short and long-term effects of wood ash on the boreal forest humus
657 microbial community. *Soil Biology and Biochemistry* **34**:1343–1353.

658 Pitman RM. (2006). Wood ash use in forestry - a review of the environmental impacts. *Forestry*
659 **79**:563–588.

660 Qin J, Hovmand MF, Ekelund F, Rønn R, Christensen S, Groot GA de, *et al.* (2017). Wood ash
661 application increases pH but does not harm the soil mesofauna. *Environ Pollut* **224**:581–589.

662 R Core Team. (2015). R: A language and environment for statistical computing. http://www.R-
663 project.org/.

664 Ramirez KS, Craine JM, Fierer N. (2012). Consistent effects of nitrogen amendments on soil
665 microbial communities and processes across biomes. *Glob Change Biol* **18**:1918–1927.

666 Reichenbach H. (1999). The ecology of the myxobacteria. *Environ Microbiol* **1**:15–21.

667 Rice P, Longden I, Bleasby A. (2000). EMBOSS: the european molecular biology open software
668 suite. *Trends Genet* **16**:276–277.

669 Rincón A, Santamaría BP, Ocaña L, Verdú M. (2014). Structure and phylogenetic diversity of post-
670 fire ectomycorrhizal communities of maritime pine. *Mycorrhiza* **24**:131–141.

671 Roller BRK, Stoddard SF, Schmidt TM. (2016). Exploiting rRNA operon copy number to investigate
672 bacterial reproductive strategies. *Nature microbiology* **1**:16160.

673 Rousk J, Brookes PC, Bååth E. (2009). Contrasting soil pH effects on fungal and bacterial growth
674 suggest functional redundancy in carbon mineralization. *Appl Environ Microbiol* **75**:1589–1596.

675 Rousk J, Brookes PC, Bååth E. (2011). Fungal and bacterial growth responses to N fertilization
676 and pH in the 150-year “Park Grass” UK grassland experiment. *FEMS Microbiol Ecol* **76**:89–99.

677 Rousk J, Bååth E, Brookes PC, Lauber CL, Lozupone C, Caporaso JG, *et al.* (2010). Soil bacterial
678 and fungal communities across a pH gradient in an arable soil. *ISME J* **4**:1340–1351.

679 Rønn R, Vestergård M, Ekelund F. (2012). Interactions Between Bacteria, Protozoa and
680 Nematodes in Soil. *Acta Protozoologica* **51**:223–235.

681 Schostag M, Priemé A, Jacquiod R, Russel J, Ekelund F, Jacobsen CS. (2019). Bacterial and
682 protozoan dynamics upon thawing and freezing of an active layer permafrost soil. *ISME J*
683 doi:10.1038/s41396-019-0351-x.

684 Starke R, Kermer R, Ullmann-Zeunert L, Baldwin IT, Seifert J, Bastida F, *et al.* (2016). Bacteria
685 dominate the short-term assimilation of plant-derived N in soil. *Soil Biology and Biochemistry*
686 **96**:30–38.

687 Tedersoo L, Hansen K, Perry BA, Kjøller R. (2006). Molecular and morphological diversity of
688 pezizalean ectomycorrhiza. *New Phytol* **170**:581–596.

689 Teng Y, Wang X, Li L, Li Z, Luo Y. (2015). Rhizobia and their bio-partners as novel drivers for
690 functional remediation in contaminated soils. *Front Plant Sci* **6**:32.

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691 Urich T, Lanzén A, Qi J, Huson DH, Schleper C, Schuster SC. (2008). Simultaneous assessment
692 of soil microbial community structure and function through analysis of the meta-transcriptome.
693 *PLoS ONE* **3**:e2527.

694 Vance ED. (1996). Land Application of Wood-Fired and Combination Boiler Ashes: An Overview.
695 *Journal of Environment Quality* **25**:937.

696 Varet H, Brillet-Guéguen L, Coppée J-Y, Dillies M-A. (2016). SARTools: A DESeq2- and EdgeR-
697 Based R Pipeline for Comprehensive Differential Analysis of RNA-Seq Data. *PLoS ONE*
698 **11**:e0157022.

699 Vaser R, Pavlović D, Šikić M. (2016). SWORD-a highly efficient protein database search.
700 *Bioinformatics* **32**:i680–i684.

701 Vestergård M, Bang-Andreasen T, Buss SM, Cruz-Paredes C, Bentzon-Tilia S, Ekelund F, *et al.*
702 (2018). The relative importance of the bacterial pathway and soil inorganic nitrogen increase
703 across an extreme wood-ash application gradient. *Glob Change Biol Bioenergy*.
704 doi:10.1111/gcbb.12494.

705 Wall DH, Bardgett RD, Behan-Pelletier V, Herrick JE, Jones TH, Ritz K, *et al.* (2012). Soil ecology
706 and ecosystem services. 1st ed. Oxford University Press: USA.

707 White T, Bruns R, Lee S, Taylor J. (1990). Amplification and direct sequencing of fungal
708 ribosomalrna genes for phylogenetics. In:*PCR protocols - a guide to methods and applications*,
709 Innis, M, Gelfand, D, Sninsky, J, & White, T (eds), Academic Press: New York, US, pp. 315–322.

710 Wilke A, Harrison T, Wilkening J, Field D, Glass EM, Kyrpides N, *et al.* (2012). The M5nr: a novel
711 non-redundant database containing protein sequences and annotations from multiple sources and
712 associated tools. *BMC Bioinformatics* **13**:141.

713 Wilkens S. (2015). Structure and mechanism of ABC transporters. *F1000Prime Rep* **7**:14.

714 Zimmermann S, Frey B. (2002). Soil respiration and microbial properties in an acid forest soil:
715 effects of wood ash. *Soil Biology and Biochemistry* **34**:1727–1737.

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719 **Figure Legends**

720 **Figure 1:** Numbers of 16S rRNA gene copies (top row) and ITS gene copies (bottom row) per g-1 DW of the
721 agricultural soil (left panel) and the forest soil (right panel) across wood ash concentrations and incubation
722 times. Symbols represents averages with SEM (n=3). The presented data are results from qPCR on DNA.
723 Note logarithmic y-axes and different ranges of values on y-axes.

724 **Figure 2:** NMDS plots based on Bray-Curtis dissimilarities of the taxonomic (rRNA; top row) and functional
725 (mRNA; bottom row) profiles of an agricultural soil and a forest soil amended with wood-ash. Dashed lines
726 represent 95% confidence ellipses around samples with same wood ash concentration. Arrows indicate the
727 direction of fitted physiochemical parameters (using envfit function; only significant parameters shown) onto
728 the NMDS ordination space (longer arrows indicate better fit). To improve the resolution of the forest soil at
729 wood ash concentrations 0-12 t ha-1, we removed the 90 t ha-1 samples and repeated the analysis
730 (rightmost two panels).

731 **Figure 3:** Community composition and diversity across the two soils at increasing wood ash amendment and
732 incubation times based on PCR-free, total RNA-Seq. (A). The most abundant taxonomic groups (cutoff levels
733 of average relative abundances are shown in legend header) are presented in upper panel (Overall
734 taxonomy); i.e. Archaea, Bacteria, Fungi, Protists, and Metazoa. Bars represente averages of triplicates
735 (excluding agricultural soil 3 t ha^{-1} at 100 days (n=2), forest soil 0t ha^{-1} at 0 days (n=1) and forest soil 0t ha^{-1}
736 at 100 days (n=1)). (B) Richness and Shannon diversity. Statistically significant different richness and
737 diversity measures ($p < 0.05$) between samples within each measure and soil is indicated by different letters.
738 Symbols represent averages, as described for the bar plots.

739 **Figure 4:** Numbers of differentially expressed genes within functional categories across agricultural and
740 forest soil by pairwise comparisons of gene transcription levels between samples of increasing wood ash
741 concentration to reference samples without ash-amendment at different incubation times. “0vs3, “0vs12” and
742 “0vs90” denote the wood ash doses compared, i.e. wood ash dose 0 t ha-1 compared to 3 t ha-1 is written
743 “0vs3”. Increasing and decreasing gene transcription levels are presented above and below the black
744 horizontal zero-line, respectively. The pairwise comparisons for forest soil, 100 days, were carried out using
745 3 t ha-1, 100 days, as reference samples because only one replicate was acquired from the 0 t ha-1, 100
746 days, samples (hence the empty plot in 0vs3, 100 days, forest plot). Digits above/below bars represent the
747 number of differentially expressed genes within a gene category.

748 **Supplementary Figure 1:** Metadata results across soil types, wood ash concentrations and incubation time.
749 Different letters denote significant ($p < 0.05$) difference between samples within the same plot (Tukey post-
750 hoc pairwise comparisons). Bars represents averages of triplicates with SEM (n = 3). Bars without errorbars
751 represents values of 1 replicate. Note different range of y-axis values between the two soils for the same
752 metadata category.

753 **Supplementary Figure 2:** Functional gene compositions in (A) relative abundance and (B) absolute
754 abundance (note log10 y-axis). “Poorly characterized” genes are excluded from the relative abundance plots
755 to increase resolution of genes with known function. Bars are averages of triplicates with SEM as errorbars
756 (excluding agricultural soil 3 t $ha^{-1,}$ 100 days (n=2) and forest soil 0t ha after 0 (n=1) and 100 days (n=1)).

757 **Supplementary Figure 3:** Functional genes involved in (A) chaperones, (B) sporulation, (C)
758 Transmembrane transporters and (D) general stress response. The presented functional genes (with unique
759 COG IDs) are all differentially expressed after wood ash amendment and are presented as relative
760 abundance of total mRNA profile. Symbols are averages of triplicates with SEM as errorbars (excluding
761 agricultural soil 3 t $ha^{-1,}$ 100 days (n=2) and forest soil 0t ha after 0 (n=1) and 100 days (n=1)).

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763 ## Tables

764 **Table 1:** Pearson correlation values (r) and associated significance levels between ash dose (field

765 equivalents 0, 3, 12 and 90 t ha-1) and incubation time, and soil physicochemical parameters.

	Agricultural soil		Forest soil	
Explanatory variable	Ash dose ($t\ ha^{-1}$)	Time (days)	Ash dose ($t\ ha^{-1}$)	Time (days)
pH	0.76***	0.15	0.98***	0.07
Conductivity ($\mu s\ cm^{-1}$)	0.82***	0.14	0.99***	0.07
DOC ($mg\ g^{-1}$ DW soil)	0.74***	0.33*	0.91***	0.05
Ammonium ($\mu g\ g^{-1}$ DW soil)	0.05	0.57***	0.40**	0.36
Nitrate ($\mu g\ g^{-1}$ DW soil)	-0.45***	0.28*	0.63***	-0.15
Phosphate ($\mu g\ g^{-1}$ DW soil)	-0.61*	-0.07	0.26	-0.04

766 *) p<0.05, **) p<0.01, ***) p< 0.001.

767

768 **Table 2:** Explanatory strength of physiochemical variables on rRNA and mRNA dissimilarity profiles of the

769 two soils after ash amendment tested using Permutational Multivariate Analysis Of Variance (*Adonis*)

	rRNA			mRNA		
Explanatory variable	Agriculture (0–12 $t\ ha^{-1}$)	Forest (0–90 $t\ ha^{-1}$)	Forest (0–12 $t\ ha^{-1}$)	Agriculture (0–12 $t\ ha^{-1}$)	Forest (0–90 $t\ ha^{-1}$)	Forest (0–12 $t\ ha^{-1}$)
pH	0.184***	0.536***	0.216***	0.079*	0.386***	0.224***
Conductivity ($\mu s\ cm^{-1}$)	0.081***	0.056***	0.108***	0.140*	0.061***	0.100***
Wood ash concentration ($t\ ha^{-1}$)	0.113***	0.044***	0.041*	0.063*	0.049***	0.051**
Time (days)	0.089***	0.068***	0.173***	0.092*	0.086***	0.258***
Phosphate ($\mu g\ g^{-1}$ DW soil)	0.039*	-	0.076***	0.065*	-	0.118***
DOC ($mg\ g^{-1}$ DW soil)	-	0.094***	0.038*	-	0.162***	0.033**
Ammonium ($\mu g\ g^{-1}$ DW soil)	-	0.034**	0.029*	-	0.066***	0.040**

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Nitrate (µg g^{-1} DW soil)	-	0.027**	0.036*	-	0.038***	0.028*
Wood ash concentration:Time	0.064***	0.015*	0.043*	-	0.025**	0.039**
Residuals (unexplained variance)	0.430	0.127	0.239	0.560	0.126	0.109

770 Values refer to R^2 values of the *Adonis* test on Bray-Curtis dissimilarities between samples.

771 Asterisks refers to significance level (* is 0.01<p<0.05, ** is 0.001<p<0.01, *** is p<0.001).

772 Non-significant (p>0.05) parameters are written as "– ".

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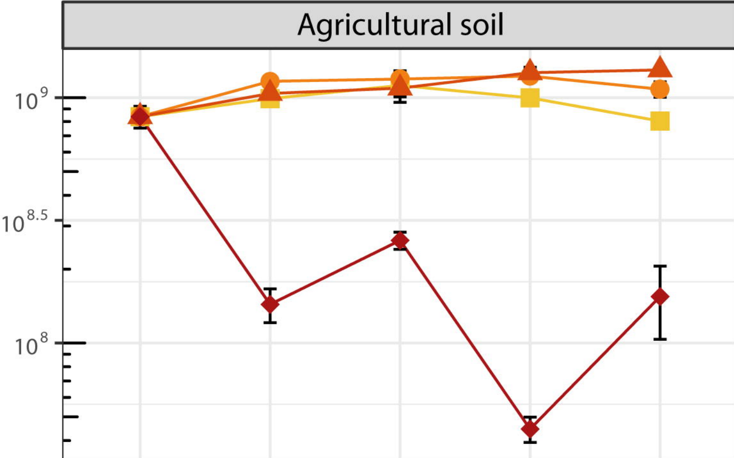
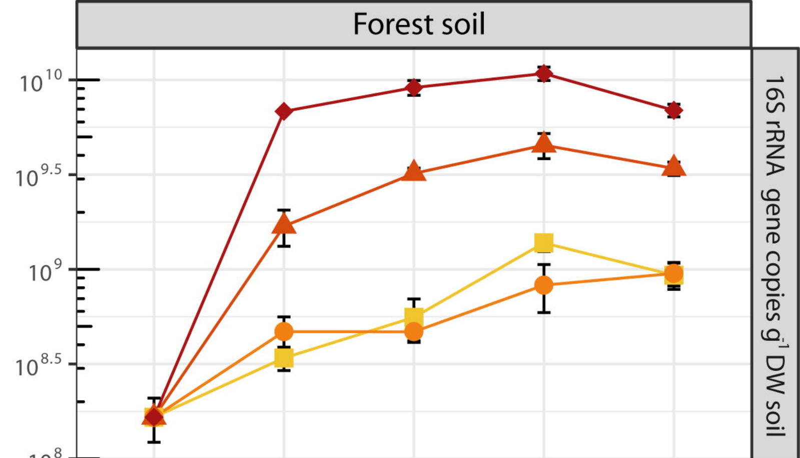
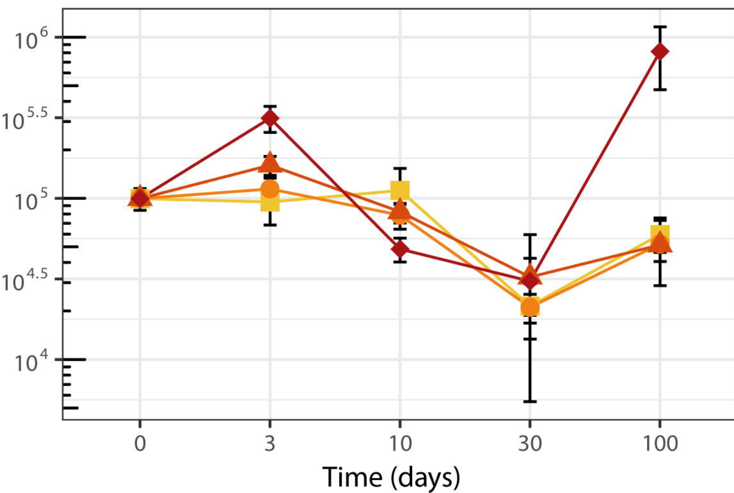
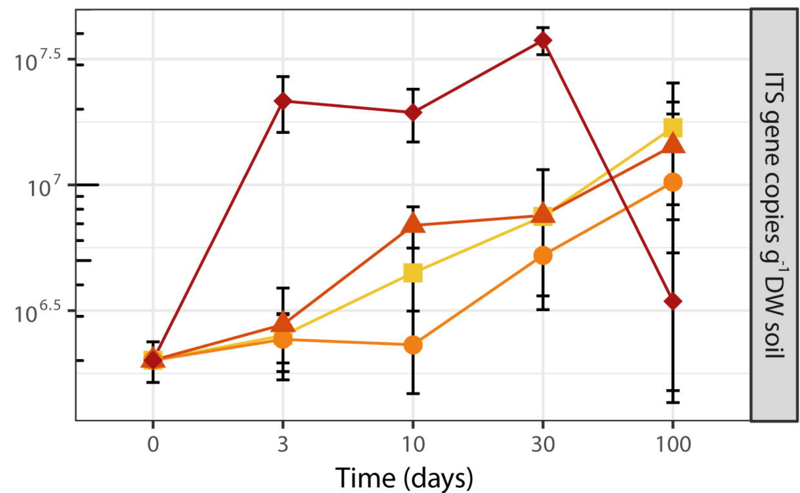
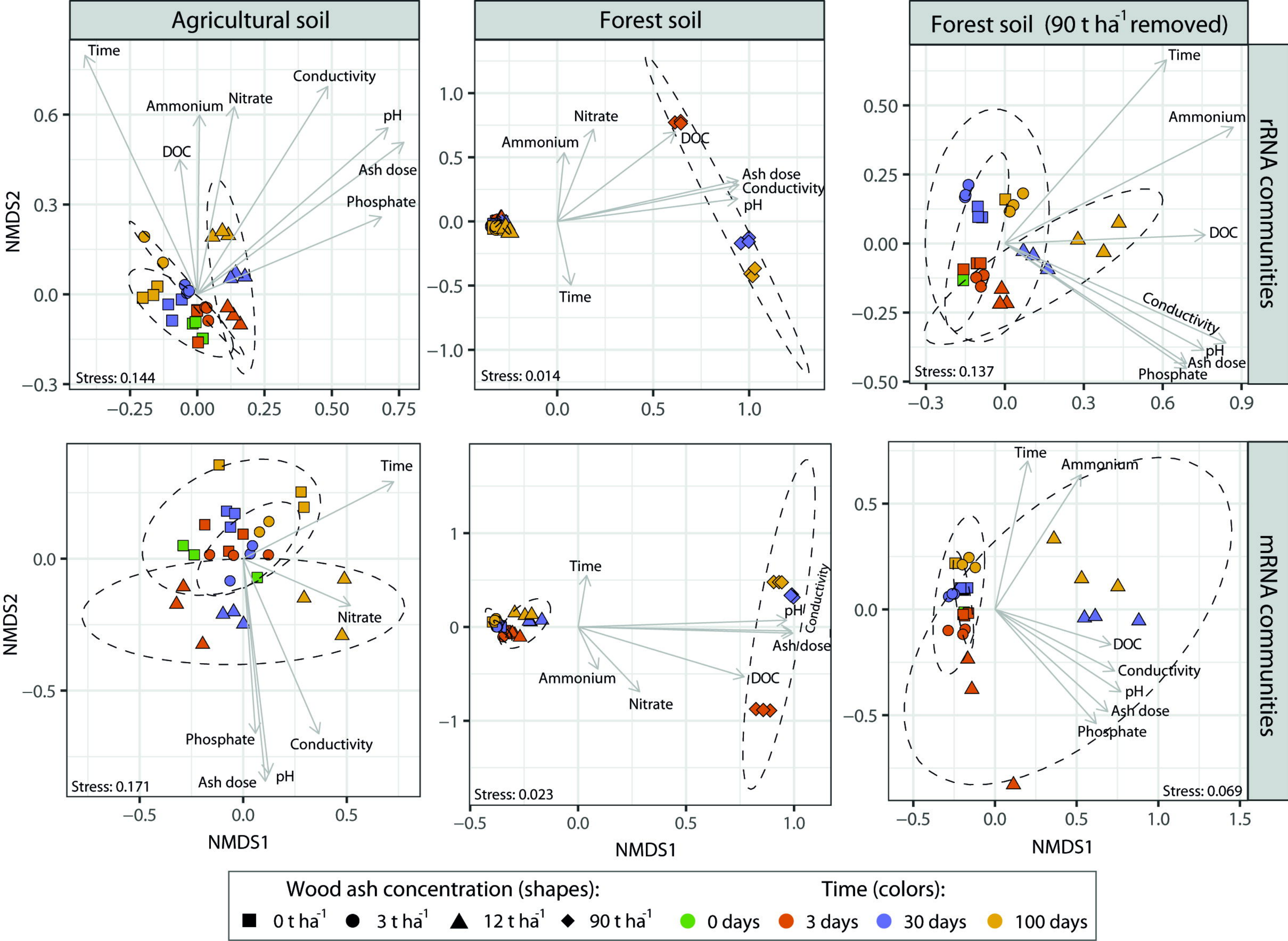


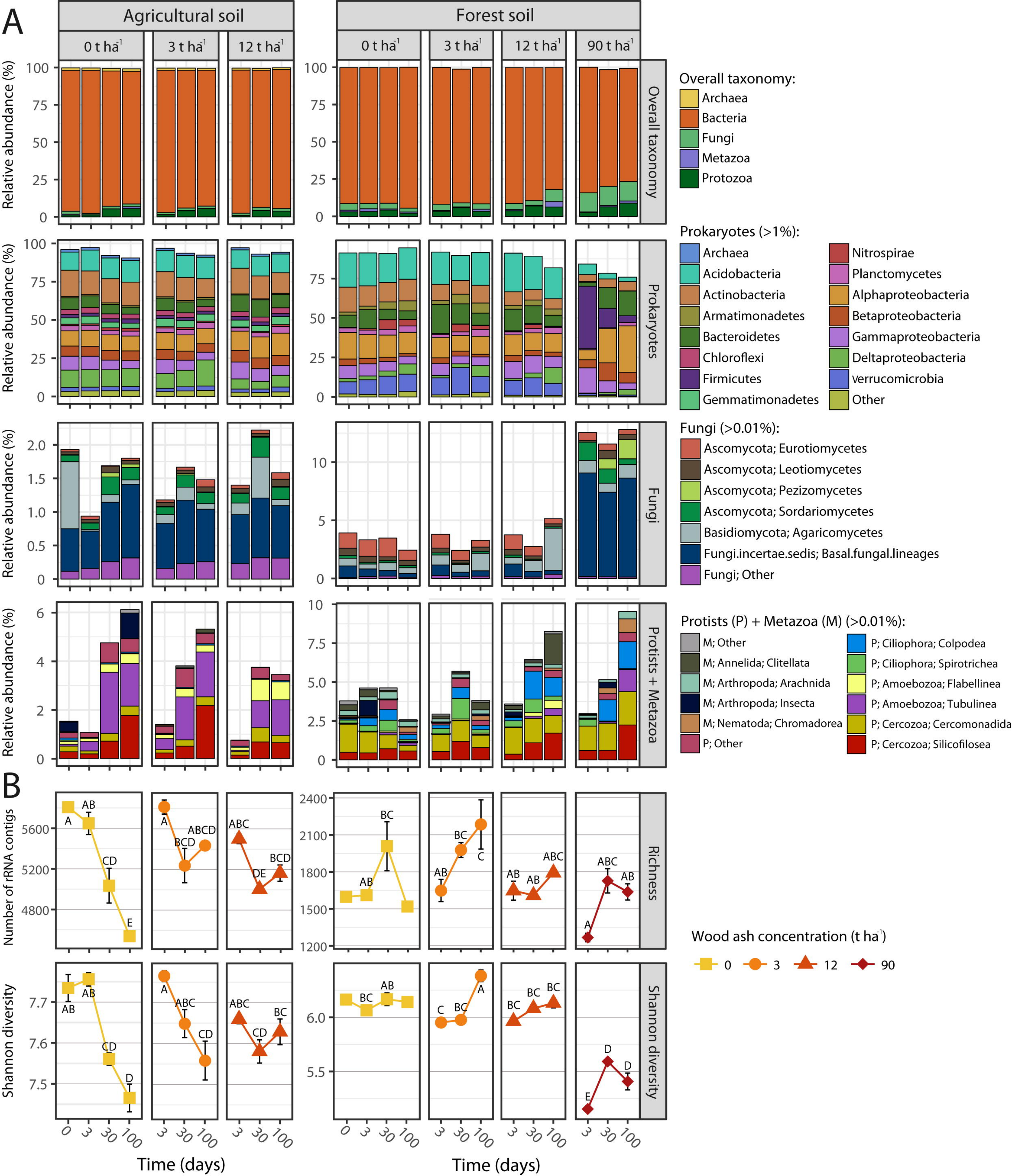


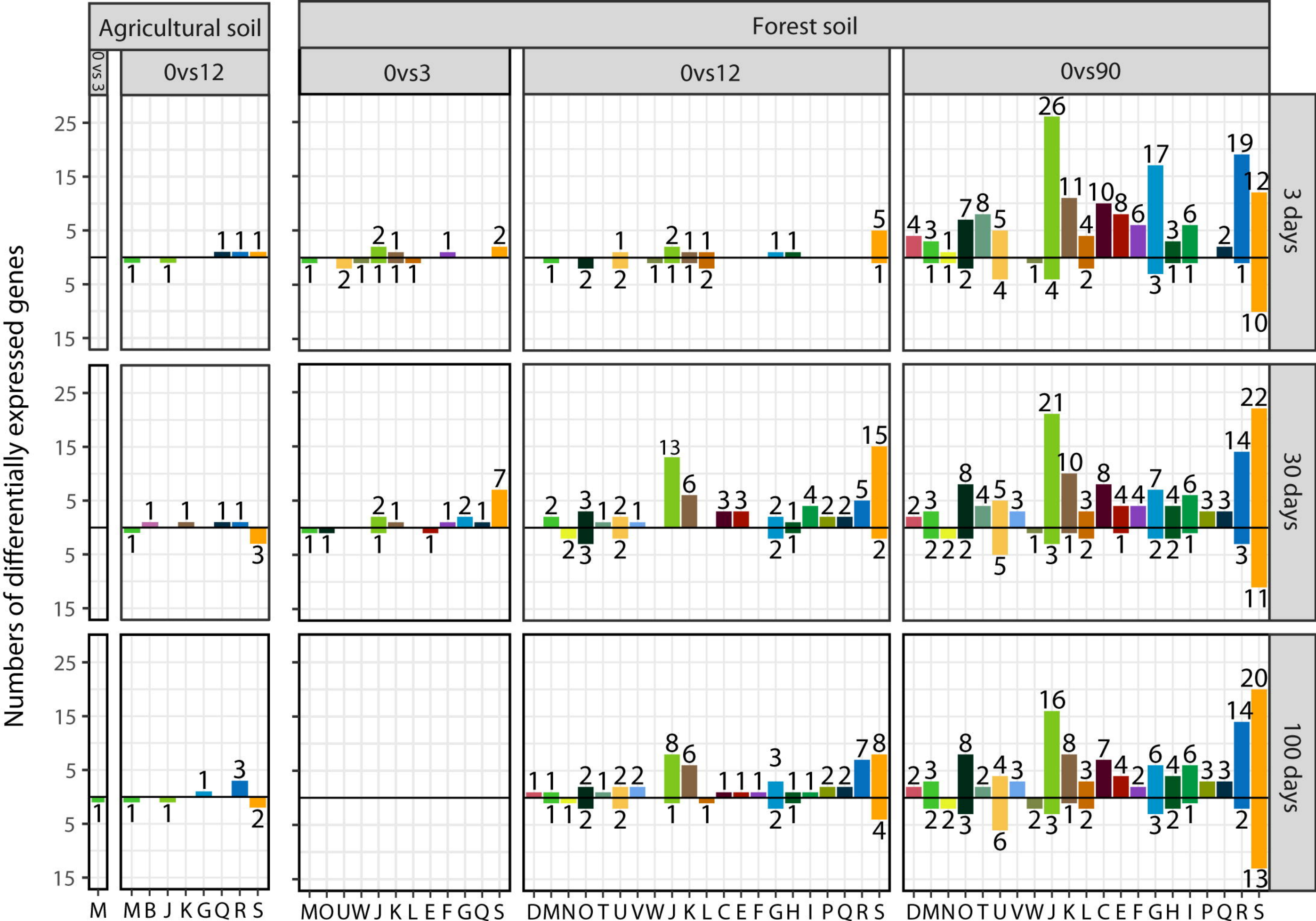




Wood ash concentration (t ha^{-1})


Agricultural soil
Forest soil
Forest soil (90 t ha-1 removed)
rRNA communities
mRNA communities
NMDS2
NMDS1
Time
Ammonium
Nitrate
Conductivity
pH
DOC
Ash dose
Phosphate
Stress: 0.144
Stress: 0.014
Stress: 0.137
Stress: 0.171
Stress: 0.023
Stress: 0.069
Wood ash concentration (shapes):
0 t ha-1
3 t ha-1
12 t ha-1
90 t ha-1
Time (colors):
0 days
3 days
30 days
100 days


A
Agricultural soil
Forest soil
0 t ha-1
3 t ha-1
12 t ha-1
90 t ha-1
Relative abundance (%)
Overall taxonomy
Prokaryotes
Fungi
Protists + Metazoa
Overall taxonomy:
Archaea
Bacteria
Fungi
Metazoa
Protozoa
Prokaryotes (>1%):
Archaea
Acidobacteria
Actinobacteria
Armatimonadetes
Bacteroidetes
Chloroflexi
Firmicutes
Gemmatimonadetes
Nitrospirae
Planctomycetes
Alphaproteobacteria
Betaproteobacteria
Gammaproteobacteria
Deltaproteobacteria
Verrucomicrobia
Other
Fungi (>0.01%):
Ascomycota; Eurotiomycetes
Ascomycota; Leotiomycetes
Ascomycota; Pezizomycetes
Ascomycota; Sordariomycetes
Basidiomycota; Agaricomycetes
Fungi.incertae.sedis; Basal.fungal.lineages
Fungi; Other
Protists (P) + Metazoa (M) (>0.01%):
M; Other
M; Annelida; Clitellata
M; Arthropoda; Arachnida
M; Arthropoda; Insecta
M; Nematoda; Chromadorea
P; Other
P; Ciliophora; Colpodea
P; Ciliophora; Spirotrichea
P; Amoebozoa; Flabellinea
P; Amoebozoa; Tubulinea
P; Cercozoa; Cercomonadida
P; Cercozoa; Silicofilosea
B
Number of rRNA contigs
Richness
Shannon diversity
Wood ash concentration (t ha-1)
0
3
12
90
Time (days)



Gene catagories

Cellular processes and signalling:

- [D] Cell cycle control, cell division, chromosome partitioning
- [M] Cell wall/membrane/envelope biogenesis
- [N] Cell motility
- [O] Post–translational modification, protein turnover, and chaperones
- [T] Signal transduction mechanisms
- [U] Intracellular trafficking, secretion, and vesicular transport
- [V] Defense mechanisms
- [W] Extracellular structures

Information storage and processing:

- [B] Chromatin structure and dynamics
- [J] Translation, ribosomal structure and biogenesis
- [K] Transcription
- [L] Replication, recombination and repair

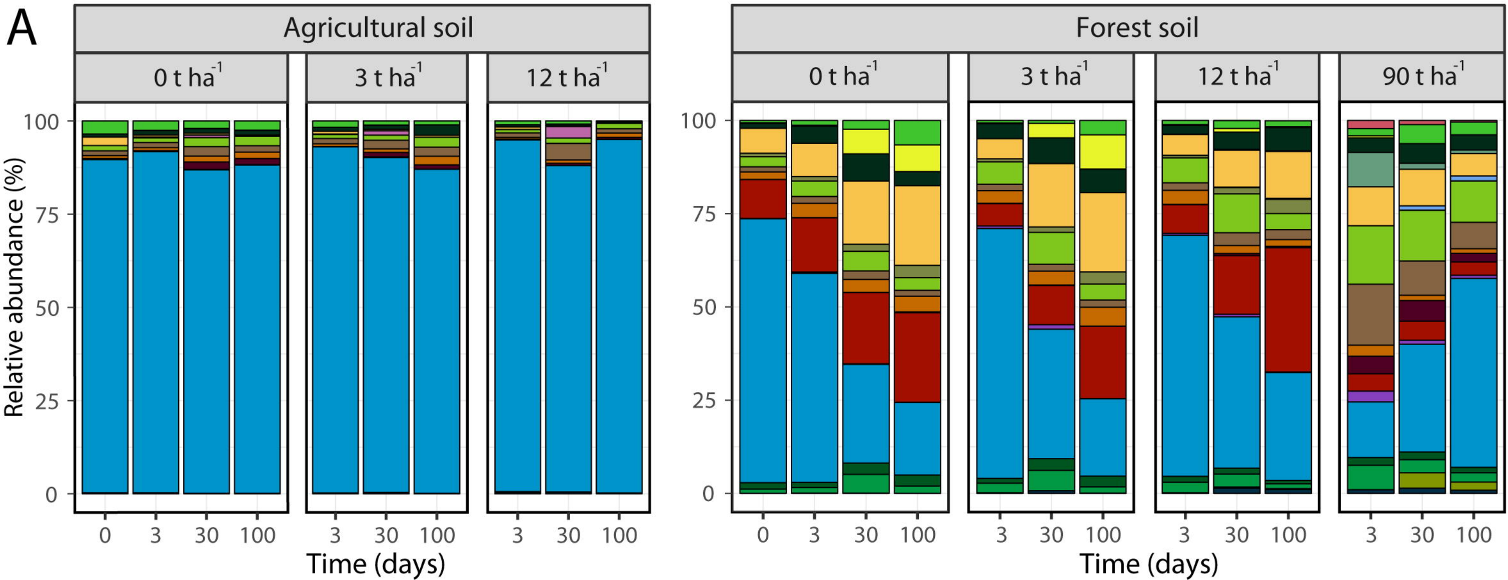
Metabolism:

- [C] Energy production and conversion
- [E] Amino acid transport and metabolism
- [F] Nucleotide transport and metabolism
- [G] Carbohydrate transport and metabolism
- [H] Coenzyme transport and metabolism
- [I] Lipid transport and metabolism
- [P] Inorganic ion transport and metabolism
- [Q] Secondary metabolites biosynthesis, transport, and catabolism

Poorly characterized:

- [R] General function prediction only
- [S] Function unknown

Agricultural soil
Forest soil
pH
Conductivity (µs cm-1)
DOC (mg g-1)
Ammonium (µg g-1)
Nitrate (µg g-1)
Phosphate (µg g-1)
0
3
10
30
100
Time (days)
Wood ash concentration (t ha-1)
0
3
12
90



B
Agricultural soil
Forest soil
0 t ha^{-1} 3 t ha^{-1} 12 t ha^{-1} 90 t ha^{-1}
Gene counts
0 days
3 days
30 days
100 days
10^4 10^3 10^2 10^1 0
M O U W J L E G I Q S
D N T V B K C F H P R

Gene catagories

Cellular processes and signalling:
- [D] Cell cycle control, cell division, chromosome partitioning
- [M] Cell wall/membrane/envelope biogenesis
- [N] Cell motility
- [O] Post-translational modification, protein turnover, and chaperones
- [T] Signal transduction mechanisms
- [U] Intracellular trafficking, secretion, and vesicular transport
- [V] Defense mechanisms
- [W] Extracellular structures
- [B] Chromatin structure and dynamics

Information storage and processing:
- [J] Translation, ribosomal structure and biogenesis
- [K] Transcription
- [L] Replication, recombination and repair

Metabolism:
- [C] Energy production and conversion
- [E] Amino acid transport and metabolism
- [F] Nucleotide transport and metabolism
- [G] Carbohydrate transport and metabolism
- [H] Coenzyme transport and metabolism

Metabolism (continued):
- [I] Lipid transport and metabolism
- [P] Inorganic ion transport and metabolism
- [Q] Secondary metabolites biosynthesis, transport, and catabolism

Poorly characterized:
- [R] General function prediction only
- [S] Function unknown

A Chaperones

Agriculture | Forest

Molecular chaperone (COG0443)

ATPases with chaperone activity (COG0542)

FKBP-type peptidyl-prolyl cis-trans isomerase (trigger factor) (COG0544)

FKBP-type peptidyl-prolyl cis-trans isomerase (COG0545)

B Sporulation

Agriculture | Forest

DNA segregation ATPase FtsK/SpoIIIE and related proteins (COG1674)

Spore germination protein (COG5401)

Stage IV sporulation protein A (NOG05030)

Protein involved in asexual sporulation (NOG07100)

Stage III sporulation protein (NOG08145)

Stage III sporulation protein D (NOG08151)

Prespore-Specific transcriptional protein (NOG12545)

Small, acid-soluble spore protein (NOG140080)

C Transmembrane transporters

ABC-type phosphate transport system, periplasmic component (COG0226)

ABC-type antimicrobial peptide transport system, permease component (COG0577)

ABC-type transport system involved in Fe-S cluster assembly (COG0719)

Cation/multidrug efflux pump (COG0841)

ABC-type antimicrobial peptide transport system, ATPase component (COG1136)

ABC-type sugar transport systems, permease components (COG1175)

ABC-type transport system (COG1744)

ABC-type sugar transport system, periplasmic component (COG1653)

ABC-type sugar transport systems, ATPase components (COG3839)

Putative Mg2+ and Co2+ transporter CorB (COG4536)

Extracellular solute binding protein family 1 (NOG285286)

D General stress response

Exopolyphosphatase related proteins (COG0618)

Predicted membrane GTPase involved in stress response (COG1217)

Ribosomal protein L25 (general stress protein Ctc) (COG1825)

Phage shock protein A (IM30) (COG1842)

ATPase related to phosphatestarvation inducible protein PhoH (COG1875)

Relative abundance (%)

Time (days): 0, 3, 30, 100

Wood ash concentration (t ha^{-1}): 0, 3, 12, 90