**Supplementary material for**

**Pyric herbivory increases soil microbial diversity but has a site-dependent effect on soil mesofauna in the mid-term**

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**Box 1. Methodological details for fungi and bacteria qPCR**

For qPCR Fungi we designed a hydrolysis (TaqMan-like) probe (ITS4\_Fungi\_Probe 5’ 6FAM-GTTCAGCGGGTAKYCYTRCCTGAT-BHQ1 3’) targeting the conserved region amplified by these primers. The dye 6-carboxy-fluorescein (6-FAM) and the Black Hole Quencher (BHQ1) were attached to the probes’ 5’ and 3’ ends, respectively.

Prior PCR tests were conducted to generate the standard curves. qPCRs for fungi and bacteria were carried out in a final volume of 20 μL, containing 10 μL of NZY qPCR Probe Master Mix ROX plus (NZYTech), 0.25 μM of the probe, 0.9 μM of the amplification primers, 2 μL of template DNA, and ultrapure water up to 20 μL. The reaction mixture for fungi was incubated as follows: an initial incubation at 95 °C for 10 min, followed by 40 cycles of denaturation at 95 °C for 15 s, annealing at 48 °C for 1 min, extension at 60 °C for 1 min. The reaction mixture for bacteria was incubated as follows: an initial incubation at 95 °C for 10 min, followed by 40 cycles of denaturation at 95 °C for 15 s, annealing at 51 °C for 1 min, extension at 60 °C for 1 min; and a final extension step at 60 °C for 30 s. Negative qPCR controls that contained no DNA were included to check for cross-contamination. qPCR reactions were performed in triplicate on each sample and controls.

**Box 2. Methodological details for DNA metabarcoding**

The fungal and bacterial PCR cycle for library preparation consisted of an initial denaturation step at 95 °C for 5 min, followed by 25 cycles of 95 °C for 30 s, 47 °C for 45 s, 72 °C for 45 s, and a final extension step at 72 °C for 7 min. The oligonucleotide indices which are required for multiplexing different libraries in the same sequencing pool were attached in a second PCR round with identical conditions but only 5 cycles and 60 °C as the annealing temperature. Libraries were purified using the Mag-Bind RXNPure Plus magnetic beads (Omega Biotek), pooled in equimolar amounts according to the quantification data provided by the Qubit dsDNA HS Assay (Thermo Fisher Scientific), and sequenced in 2 GB of a NovaSeq PE250 run (Illumina).

The 16S and ITS amplicon reads were processed using QIIME2 (release 2021.11) (Bolyen *et al.*, 2019)(Bolyen *et al.*, 2019). We removed the PCR primers, filtered the reads according to their quality, denoised, merged the reads, removed the chimeric reads, and clustered the resulting sequences into amplicon sequence variants (ASVs) using DADA2 (Callahan *et al.*, 2016), implemented in QIIME2. The taxonomy was assigned to ASVs using a pre-trained classifier of the UNITE reference database for fungi (Abarenkov et al., 2020) (updated on May 2021) and a pre-trained classifier of the SILVA reference database for bacteria (Quast *et al.*, 2013) release 138.1 August 2020). For assigning fungi and bacteria taxa, we used the feature-classifier classify-sklearn approach (Bokulich *et al.*, 2018) implemented in QIIME2. Singletons (i.e., ASVs containing only one member sequence in the whole data set), ASVs occurring at a frequency below 0.01 % in each sample, unassigned ASVs, and non-Fungi or non-Bacteria ASVs were removed from their respective ASV tables. ASVs were grouped using the CD-HIT program at 97 % identity threshold (Li and Godzik, 2006; Huang *et al.*, 2010). We considered that the clustered ASVs were the same taxon.

**References:**

Bokulich, N. A. *et al.* (2018) ‘Optimizing taxonomic classification of marker-gene amplicon sequences with QIIME 2’s q2-feature-classifier plugin’, *Microbiome*, 6(1), p. 90. doi: 10.1186/s40168-018-0470-z.

Bolyen, E. *et al.* (2019) ‘Reproducible, interactive, scalable and extensible microbiome data science using QIIME 2’, *Nature Biotechnology*, 37(8), pp. 852–857. doi: 10.1038/s41587-019-0209-9.

Callahan, B. J. *et al.* (2016) ‘DADA2: High-resolution sample inference from Illumina amplicon data’, *Nature Methods*, 13(7), pp. 581–583. doi: 10.1038/nmeth.3869.

Huang, Y. *et al.* (2010) ‘CD-HIT Suite: a web server for clustering and comparing biological sequences’, *Bioinformatics*, 26(5), pp. 680–682. doi: 10.1093/bioinformatics/btq003.

Li, W. and Godzik, A. (2006) ‘Cd-hit: A fast program for clustering and comparing large sets of protein or nucleotide sequences’, *Bioinformatics*, 22(13), pp. 1658–1659. doi: 10.1093/bioinformatics/btl158.

Quast, C. *et al.* (2013) ‘The SILVA ribosomal RNA gene database project: improved data processing and web-based tools’, *Nucleic Acids Research*, 41(D1), pp. D590–D596. doi: 10.1093/nar/gks1219.

![Diagrama, Esquemático

Descripción generada automáticamente]()

Figure S1. Variance of soil properties on the top 3 cm of burned and burned soils. Error bars indicate confidence intervals at 95%

![Gráfico, Gráfico de dispersión

Descripción generada automáticamente]()

![Gráfico, Diagrama

Descripción generada automáticamente]()

Figure S2. Contour maps and semi variograms of soil compaction

![Diagrama

Descripción generada automáticamente]()

Figure S3. Variance of soil properties and vegetation structure under different managements. B-G-, no burning and no grazing, B+G- no burning and grazing, B+G+ burning and grazing. Error bars represent confidence intervals at 95%.

|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  | **Richness** | |  | **Shannon** | |  | **Simpson** | |  | | **N copy** | | | |
|  | F | p |  | F | p |  | F | p |  | | F | | p | |
| **Fungi** |  |  |  |  |  |  |  |  |  | |  | |  | |
| Site (S) | 3.9 | *0.063* |  | 18.0 | **<0.001** |  | 7.9 | **0.012** |  | | 6.1 | | **0.024** | |
| Management (M) | 22.3 | **<0.001** |  | 14.3 | **<0.001** |  | 4.7 | **0.023** |  | | 0.1 | | 0.892 | |
| S x M | 0.9 | 0.405 |  | 0.2 | 0.816 |  | 0.1 | 0.878 |  | | 2.5 | | 0.107 | |
| **Bacteria** |  |  |  |  |  |  |  |  | |  | |  | |
| Site (S) | 6.7 | **0.018** |  | 18.2 | **<0.001** |  | 39.7 | **<0.001** |  | | 0.1 | | 0.697 | |
| Management (M) | 6.6 | **0.007** |  | 6.5 | **0.007** |  | 5.1 | **0.017** |  | | 1.2 | | 0.335 | |
| S x M | 0.6 | 0.556 |  | 1.0 | 0.382 |  | 1.1 | 0.364 |  | | 0.3 | | 0.722 | |

Table S1. Anova table of management effects on the differences on richness, diversity and biomass of fungi and bacteria after treatment.

![Imagen que contiene Calendario

Descripción generada automáticamente]()

Figure S4. Response of richness, diversity and biomass of fungi and bacteria to management.; B+G- burning and no grazing, B+G+ burning and grazing.

Table S2. Mesofauna abundances.

| **Prot** | **Diplu** | **Colle** | **Zyg** | **Psoc** | **Hemi** | **Thys** | **Coleo** | **Hyme** | **Dipt** | **Holo** | **Aca** | **Ara** | **Pseu** | **Iso** | **Chilo** | **Diplo** | **Paur** | **Symp** | **T** | **D** | **S** |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| 0 | 0 | 11 | 0 | 0 | 0 | 1 | 4 | 0 | 0 | 0 | 280 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | B-G- | 0-2 | 1 |
| 7 | 0 | 18 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 222 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | B-G- | 2-4 | 1 |
| 0 | 0 | 530 | 0 | 0 | 5 | 8 | 12 | 3 | 0 | 0 | 1730 | 0 | 0 | 0 | 5 | 0 | 0 | 0 | B-G- | 0-2 | 1 |
| 0 | 0 | 46 | 0 | 0 | 1 | 0 | 3 | 1 | 2 | 0 | 34 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | B-G- | 2-4 | 1 |
| 1 | 2 | 116 | 0 | 0 | 0 | 2 | 5 | 3 | 2 | 0 | 310 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | B-G- | 0-2 | 1 |
| 0 | 0 | 23 | 0 | 1 | 0 | 0 | 3 | 0 | 3 | 0 | 116 | 0 | 1 | 0 | 2 | 0 | 0 | 0 | B-G- | 2-4 | 1 |
| 0 | 1 | 190 | 0 | 0 | 2 | 0 | 4 | 0 | 0 | 0 | 350 | 0 | 0 | 1 | 3 | 0 | 0 | 0 | B+G- | 0-2 | 1 |
| 0 | 1 | 30 | 0 | 0 | 1 | 0 | 1 | 0 | 2 | 0 | 17 | 1 | 0 | 0 | 1 | 0 | 0 | 1 | B+G- | 2-4 | 1 |
| 0 | 2 | 80 | 0 | 0 | 3 | 0 | 3 | 0 | 11 | 0 | 400 | 3 | 0 | 0 | 5 | 0 | 0 | 2 | B+G- | 0-2 | 1 |
| 0 | 12 | 10 | 0 | 0 | 0 | 0 | 1 | 0 | 1 | 0 | 29 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | B+G- | 2-4 | 1 |
| 12 | 0 | 280 | 0 | 0 | 1 | 9 | 8 | 1 | 2 | 0 | 1270 | 6 | 0 | 0 | 8 | 1 | 0 | 0 | B+G- | 0-2 | 1 |
| 14 | 8 | 30 | 0 | 0 | 0 | 1 | 6 | 0 | 1 | 0 | 51 | 2 | 1 | 0 | 3 | 0 | 0 | 1 | B+G- | 2-4 | 1 |
| 0 | 3 | 6 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 57 | 0 | 0 | 0 | 5 | 0 | 0 | 0 | B+G+ | 0-2 | 1 |
| 0 | 2 | 5 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 30 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | B+G+ | 2-4 | 1 |
| 6 | 0 | 4 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 70 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | B+G+ | 0-2 | 1 |
| 0 | 0 | 2 | 0 | 0 | 2 | 0 | 0 | 0 | 0 | 0 | 10 | 0 | 0 | 0 | 2 | 0 | 0 | 0 | B+G+ | 2-4 | 1 |
| 2 | 2 | 21 | 0 | 0 | 1 | 0 | 0 | 0 | 3 | 0 | 50 | 0 | 0 | 0 | 2 | 1 | 0 | 1 | B+G+ | 0-2 | 1 |
| 7 | 8 | 8 | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 0 | 46 | 0 | 0 | 0 | 1 | 1 | 0 | 1 | B+G+ | 2-4 | 1 |
| 0 | 0 | 10 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 35 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | B+G+ | 0-2 | 1 |
| 0 | 2 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 33 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | B+G+ | 2-4 | 1 |
| 0 | 0 | 17 | 0 | 0 | 0 | 1 | 0 | 2 | 1 | 2 | 46 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | B+G+ | 0-2 | 1 |
| 0 | 0 | 62 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 157 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | B+G+ | 2-4 | 1 |
| 0 | 0 | 24 | 0 | 0 | 2 | 0 | 1 | 4 | 4 | 0 | 93 | 0 | 0 | 0 | 3 | 1 | 0 | 0 | B+G+ | 0-2 | 1 |
| 1 | 0 | 18 | 0 | 0 | 0 | 0 | 0 | 1 | 4 | 0 | 218 | 0 | 0 | 0 | 2 | 0 | 0 | 1 | B+G+ | 2-4 | 1 |
| 0 | 0 | 4 | 0 | 0 | 0 | 3 | 0 | 0 | 0 | 0 | 130 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | B+G+ | 0-2 | 1 |
| 0 | 0 | 5 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 65 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | B+G+ | 2-4 | 1 |
| 0 | 0 | 39 | 0 | 0 | 18 | 9 | 1 | 3 | 1 | 0 | 60 | 0 | 0 | 0 | 2 | 0 | 0 | 0 | B+G+ | 0-2 | 1 |
| 2 | 0 | 27 | 0 | 0 | 16 | 0 | 0 | 0 | 1 | 0 | 105 | 0 | 0 | 0 | 2 | 0 | 0 | 0 | B+G+ | 2-4 | 1 |
| 0 | 0 | 81 | 0 | 0 | 4 | 10 | 6 | 0 | 0 | 0 | 251 | 0 | 0 | 0 | 3 | 0 | 0 | 0 | B+G+ | 0-2 | 1 |
| 1 | 3 | 20 | 0 | 0 | 2 | 3 | 2 | 0 | 0 | 0 | 140 | 0 | 0 | 0 | 5 | 1 | 0 | 3 | B+G+ | 2-4 | 1 |
| 2 | 4 | 1650 | 0 | 0 | 4 | 0 | 5 | 0 | 2 | 0 | 2500 | 1 | 0 | 0 | 1 | 0 | 0 | 0 | B-G- | 0-2 | 2 |
| 17 | 9 | 265 | 0 | 0 | 0 | 0 | 4 | 0 | 1 | 0 | 261 | 0 | 0 | 0 | 4 | 1 | 0 | 2 | B-G- | 2-4 | 2 |
| 3 | 0 | 630 | 0 | 0 | 2 | 2 | 4 | 0 | 3 | 0 | 1020 | 2 | 0 | 0 | 5 | 0 | 0 | 0 | B-G- | 0-2 | 2 |
| 9 | 2 | 218 | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 544 | 0 | 0 | 0 | 4 | 0 | 1 | 0 | B-G- | 2-4 | 2 |
| 0 | 1 | 645 | 0 | 0 | 3 | 2 | 3 | 1 | 3 | 0 | 1150 | 1 | 0 | 1 | 1 | 0 | 0 | 0 | B-G- | 0-2 | 2 |
| 0 | 0 | 9 | 0 | 0 | 1 | 0 | 0 | 1 | 0 | 0 | 61 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | B-G- | 2-4 | 2 |
| 15 | 18 | 584 | 0 | 0 | 0 | 0 | 0 | 0 | 3 | 0 | 500 | 1 | 0 | 0 | 3 | 1 | 0 | 0 | B+G- | 0-2 | 2 |
| 1 | 7 | 13 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 26 | 1 | 0 | 0 | 0 | 0 | 0 | 1 | B+G- | 2-4 | 2 |
| 0 | 0 | 330 | 0 | 0 | 0 | 0 | 4 | 0 | 0 | 0 | 200 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | B+G- | 0-2 | 2 |
| 0 | 1 | 49 | 0 | 0 | 1 | 0 | 1 | 0 | 4 | 0 | 32 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | B+G- | 2-4 | 2 |
| 0 | 2 | 298 | 0 | 0 | 3 | 0 | 2 | 0 | 8 | 0 | 265 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | B+G- | 0-2 | 2 |
| 22 | 9 | 101 | 0 | 0 | 1 | 2 | 2 | 0 | 1 | 0 | 310 | 0 | 2 | 0 | 7 | 0 | 3 | 2 | B+G- | 2-4 | 2 |
| 0 | 3 | 38 | 0 | 0 | 7 | 1 | 1 | 1 | 0 | 0 | 1050 | 0 | 0 | 0 | 4 | 0 | 0 | 0 | B+G+ | 0-2 | 2 |
| 16 | 13 | 14 | 0 | 0 | 2 | 1 | 2 | 0 | 2 | 0 | 330 | 0 | 0 | 0 | 2 | 0 | 1 | 0 | B+G+ | 2-4 | 2 |
| 1 | 9 | 140 | 0 | 0 | 0 | 3 | 2 | 1 | 1 | 0 | 810 | 2 | 0 | 0 | 1 | 0 | 0 | 0 | B+G+ | 0-2 | 2 |
| 7 | 40 | 44 | 0 | 0 | 1 | 0 | 1 | 0 | 1 | 0 | 520 | 0 | 1 | 0 | 6 | 0 | 0 | 0 | B+G+ | 2-4 | 2 |
| 2 | 0 | 150 | 0 | 0 | 12 | 1 | 1 | 0 | 3 | 0 | 530 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | B+G+ | 0-2 | 2 |
| 1 | 0 | 22 | 0 | 0 | 2 | 0 | 1 | 0 | 1 | 0 | 113 | 0 | 0 | 0 | 2 | 0 | 2 | 0 | B+G+ | 2-4 | 2 |
| 0 | 0 | 20 | 0 | 0 | 0 | 2 | 1 | 0 | 0 | 1 | 160 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | B+G+ | 0-2 | 2 |
| 1 | 1 | 2 | 0 | 0 | 1 | 1 | 1 | 0 | 0 | 0 | 183 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | B+G+ | 2-4 | 2 |
| 0 | 0 | 45 | 0 | 0 | 0 | 5 | 1 | 0 | 2 | 0 | 300 | 1 | 0 | 0 | 0 | 0 | 0 | 1 | B+G+ | 0-2 | 2 |
| 0 | 1 | 29 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 340 | 1 | 0 | 0 | 1 | 0 | 1 | 0 | B+G+ | 2-4 | 2 |
| 0 | 2 | 120 | 0 | 0 | 2 | 5 | 0 | 0 | 0 | 0 | 460 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | B+G+ | 0-2 | 2 |
| 0 | 1 | 21 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 7 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | B+G+ | 2-4 | 2 |
| 0 | 2 | 40 | 0 | 0 | 0 | 1 | 1 | 0 | 2 | 1 | 368 | 0 | 0 | 0 | 0 | 0 | 0 | 2 | B+G+ | 0-2 | 2 |
| 2 | 1 | 18 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 330 | 1 | 0 | 0 | 1 | 0 | 3 | 13 | B+G+ | 2-4 | 2 |
| 0 | 0 | 17 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 96 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | B+G+ | 0-2 | 2 |
| 1 | 0 | 27 | 0 | 0 | 0 | 1 | 1 | 0 | 0 | 0 | 121 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | B+G+ | 2-4 | 2 |
| 0 | 0 | 130 | 0 | 0 | 3 | 18 | 3 | 0 | 3 | 0 | 230 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | B+G+ | 0-2 | 2 |
| 0 | 0 | 31 | 0 | 0 | 3 | 0 | 0 | 1 | 1 | 0 | 90 | 1 | 0 | 0 | 0 | 0 | 3 | 0 | B+G+ | 2-4 | 2 |

Prot, Protura; Diplu, Diplura; Colle, Collembola; Zyg, Zygentoma; Psoc, Psocoptera; Hemi, Hemiptera; Thys, Thysanoptera; Coleo, Coleoptera; Hyme, Hymenoptera; Dipt, Diptera; Holo, Holometabolous; Aca, Acari; Ara, Araneae, Pseu, Pseudoscorpiones; Iso, Isopoda; Chilo, Chilopoda; Diplo, Diplopoda; Paur, Pauropoda; Symp, Symphyla; T, Treatment; B-G-, no burning no grazing; B+G-, burning no grazing; B+G+, burning and grazing; D, depth (cm); S, Site.

Table S3. ANOVA table of management effects on soil mesofauna

|  |  |  |  |
| --- | --- | --- | --- |
| **Variable** | **Factor** | **F** | **p** |
| Shannon | Site | 0.2 | 0.619 |
|  | Management | 0.4 | 0.650 |
|  | Depth | 0.4 | 0.505 |
|  | Site:Management | 1.4 | 0.244 |
|  | Site:Depth | 0.0 | 0.986 |
|  | Management:Depth | 4.3 | 0.019\* |
| Simpson | Site | 2.0 | 0.165 |
|  | Management | 2.7 | 0.078+ |
|  | Depth | 0.0 | 0.915 |
|  | Site:Management | 2.7 | 0.077+ |
|  | Site:Depth | 0.2 | 0.616 |
|  | Management:Depth | 2.0 | 0.139 |