**Supplementary Information**

**Only Minor Changes in the Soil Microbiome of a Sub-Alpine Forest After 20 Years of Moderately Increased Nitrogen Loads**

Beat Frey1, Monique Carnol2, Alexander Dharmarajah1, Ivano Brunner1, Patrick Schleppi1

1Forest Soils and Biogeochemistry, Swiss Federal Research Institute WSL, 8903 Birmensdorf, Switzerland

2 Laboratory of Plant and Microbial Ecology, InBioS, University of Liège, 4000 Liège, Belgium

\*Correspondence: Beat Frey, beat.frey@wsl.ch

**Supplemental Figure 1**. Differently abundant bacterial taxa (statistical difference *p* < 0.1), which respond to N treatments across all soil horizons with a log2fold change > 0.5. Negative values (blue) refer to Control-upregulated genera, positive values (red) to N-Addition-upregulated genera. Bar diagrams on the right shows the relative abundance.

**Supplemental Figure 2**. Differently abundant fungal genera (statistically different *p* < 0.05), which respond to N treatments in the horizons of the mineral soil in the control and N-addition plots (A = A horizon; B = oxic B horizon; C = reduced B horizon) with a log2-fold change > 1.0. Bar diagrams on the right shows the relative abundance. Negative values (blue) refer to Control-upregulated genera, positive values (red) to N-Addition-upregulated genera.

***Supplementary Results***

***Overall microbial community composition***

Among the 30 samples of N-treated and control soils, a total of 793,235 bacterial sequences with an average number of 26,441 (± 4,386) sequences per sample was identified. These sequences were distributed among 6,098 OTUs (2183 ± 357 per sample) of which 99.6% could be classified at phylum and 25.8% at genus level. We further identified 5544 archaeal sequences, which were not analysed further. The predominant bacterial phyla were *Proteobacteria* (27.7% of total sequences; 1125 OTUs), *Acidobacteria* (15.6%; 425 OTUs), *Verrucomicrobia* (12.8%; 377 OTUs), *Chloroflexi* (12.4%; 575 OTUs) and *Planctomycetes* (10.6%; 831 OTUs). For fungi, sequencing analysis yielded a total of 828,050 ITS2 sequences with an average of 27,602 (± 7,227) sequences per sample. The total number of sequences represented 1,749 (292 ± 112 per sample) fungal OTUs. 94.9% of OTUs could be classified at phylum and 82.4% at genus level. *Basidiomycota* (54.0%; 540 OTUs), *Ascomycota* (38.0%; 901 OTUs), *Mortierellomycota* (1.3%; 48 OTUs) and *Rozellomycota* (1.2%; 77 OTUs) were found to be the most abundant fungal phyla.

**Supplementary Table 1**: Primer pairs and annealing temperatures used in real-time quantitative PCR analysis for functional genes.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Genes | Primer name | Primer sequence (5’-3’) | Annealing temperature | Reference |
| aamoA | Arch-amoAF | STAATGGTCTGGCTTAGACG | 53 °C | Francis et al. (2005) |
| Arch-amoAR | GCGGCCATCCATCTGTATGT |
| bamoA | amoA-1F | GGGGTTTCTACTGGTGGT | 55 °C | Rotthauwe et al. (1997) |
| amoA-2R | CCCCTCKGSAAAGCCTTCTTC |
| nrxB | nrxB-169F | TACATGTGGTGGAACA | 57 °C | Pester et al. (2014) |
| nrxB-638R | CGGTTCTGGTCRATCA |
| nirS | nirS-cd3AF | GTSAACGTSAAGGARACSGG | 58 °C | Throbäck et al. (2004) |
| nirS\_R3cd | GASTTCGGRTGSGTCTTGA |
| nosZ | nosZ-1F | WCSYTGTTCMTCGACAGCCAG | 62 °C | Henry et al. (2006) |
| nosZ-1R | ATGTCGATCARCTGVKCRTTYTC |
| nifH | Pol-F | TGCGAYCCSAARGCBGACTC | 55 °C | Poly et al. (2001) |
| Pol-R | ATSGCCATCATYTCRCCGGA |
| mcrA | ML-F | GGTGGTGTMGGATTCACACARTAYGCWACAGC | 50 °C | Luton et al. (2002) |
| ML-R | TTCATTGCRTAGTTWGGRTAGTT |

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**Supplemental Table 2**. Treatment (N addition) and spatial (horizon) effects on phylogenetic and functional marker genes in the Alptal N addition experiment.

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
|  | Treatment\* | | Horizon | | Treatment x Horizon | |
| Gen | *F*(1/24) † | *p* | *F*(2/24) | *p* | *F*(2/24) | *p* |
| 16S | 0.79 | 0.38 | 0.92 | 0.41 | 0.07 | 0.93 |
| ITS | 0.03 | 0.86 | 1.20 | 0.31 | 0.32 | 0.72 |
| bamoA | 0.25 | 0.62 | 2.36 | 0.12 | 0.06 | 0.95 |
| aamoA | 0.45 | 0.51 | 1.52 | 0.24 | 0.44 | 0.65 |
| nifH | 3.26 | *0.08* | 5.55 | **0.01** | 0.79 | 0.46 |
| nrxB | 3.94 | *0.06* | 9.28 | **0.001** | 0.31 | 0.73 |
| nirS | 2.07 | 0.16 | 4.43 | **0.02** | 0.68 | 0.52 |
| nosZ | 0.24 | 0.62 | 0.67 | 0.52 | 0.24 | 0.79 |
| mcrA | 2.43 | 0.13 | 2.03 | 0.15 | 0.81 | 0.45 |

\*Effects of main factors and their interactions assessed by analysis of variance (ANOVA).

+Values represent the *F*-ratio (degrees of freedom for each factor are given in brackets); significant values (*p*<0.05) are in bold letters and values in italics represent *p*<0.1.