**Supplementary Information:**

**Evaluating the lingering effect of livestock grazing on functional potentials of microbial communities in Tibetan grassland soils**

Mengmeng Wanga, Shiping Wangb,c, Linwei Wua, Depeng Xua,d, Qiaoyan Line, Yigang Hue,f, Xiangzhen Lig, Jizhong Zhoua,d,h,i and Yunfeng Yanga,i,\*

aState Key Joint Laboratory of Environment Simulation and Pollution Control, School of Environment, Tsinghua University, Beijing 100084, China

bKey Laboratory of Alpine Ecology and Biodiversity, Institute of Tibetan Plateau Research, Chinese Academy of Sciences, Beijing 100101, China

cCAS Center for Excellence in Tibetan Plateau Earth Science, Beijing 100101, China

dInstitute for Environmental Genomics and Department of Botany and Microbiology, University of Oklahoma, Norman, OK 73019, USA

eKey Laboratory of Adaption and Evolution of Plateau Biota, Northwest Institute of Plateau Biology, Chinese Academy of Sciences, Xining 810008, China

fShapotou Desert Experiment and Research Station, Cold and Arid Regions and Environmental & Engineering Research Institute, Chinese Academy of Sciences, Lanzhou 730000, China

gChengdu Institute of Biology, Chinese Academy of Sciences, Chengdu 610041, China

hEarth Sciences Division, Lawrence Berkeley National Laboratory, Berkeley, CA 94720, USA

iCollaborative Innovation Center for Regional Environmental Quality, School of Environment, Tsinghua University, Beijing 100084, China

**Supplementary Tables**

**Table S1.** Plant biomass of each species within different plant functional group (g·m-2).

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Species | Functional groupa | 3200-m |  | 3400-m |
| Control | Post-grazing | *P*e |  | Control | Post-grazing | *P* |
| *Poa annua* | GG | 16.3 | 1.4 | 0.42 |  | 4.2 | 5.0 | 0.84 |
| *Elymus dahuricus* | GG | - | - | - |  | 0.1 | - | 0.37 |
| *Elymus nutans* | GG | 21.7 | 23.2 | 0.77 |  | 8.7 | 7.4 | 0.70 |
| *Festuca*b | GG | 42.0 | 34.8 | 0.44 |  | 9.0 | 8.1 | 0.92 |
| *Festuca ovina* | GG | 11.0 | 4.0 | 0.58 |  | 2.0 | - | 0.37 |
| *Graminoids*c | GG | 42.4 | 54.9 | 0.68 |  | 13.1 | 10.8 | 0.83 |
| *Koeleria glauca* | GG | - | - | - |  | 0.1 | 2.3 | 0.36 |
| *Stipa aliena* | GG | 1.9 | 16.1 | 0.17 |  | - | - | - |
| *Stipa capillata* | GG | 17.2 | 16.1 | 0.95 |  | 8.9 | 2.0 | 0.31 |
| *Carex tristachya* | SG | 0.3 | 3.9 | 0.08 |  | **2.1** | **0.5** | **0.01** |
| *Kobresia capillifolia* | SG | - | - | - |  | - | 0.3 | 0.37 |
| *Kobresia humilis* | SG | 6.7 | 12.9 | 0.10 |  | 3.2 | 8.5 | 0.34 |
| *Kobresia tibetica* | SG | 1.4 | 12.1 | 0.26 |  | 1.4 | 0.5 | 0.33 |
| *Scripus distigmaticus* | SG | 8.1 | 23.2 | 0.19 |  | **0.8** | **3.0** | **0.04** |
| *Astragalus membranaceus* | LG | 0.3 | 0.5 | 0.65 |  | 2.8 | 0.3 | 0.19 |
| *Gueldenstaedtia multiflora* | LG | 6.0 | 21.8 | 0.14 |  | 0.1 | 0.1 | 0.80 |
| *Leguminosae* | LG | **1.6** | **7.4** | **0.03**f |  | 1.4 | 6.3 | 0.24 |
| *Medicago ruthenica* | LG | 4.8 | 9.9 | 0.15 |  | - | - | 0.37 |
| *Oxytropis* | LG | - | - | 0.37 |  | 2.1 | 7.0 | 0.41 |
| *Oxytropis deflexa* | LG | 4.9 | 5.1 | 0.97 |  | - | - | - |
| *Ajania tenuifolia* | FG | - | 0.8 | 0.37 |  | 0.2 | 1.9 | 0.42 |
| *Allium ramosum* | FG | - | - | - |  | - | - | 0.37 |
| *Anaphalis lactea* | FG | - | - | - |  | 1.0 | 0.4 | 0.53 |
| *Anaphalis sinica Hance* | FG | - | - | - |  | - | 0.3 | 0.37 |
| *Aster tataricus* | FG | - | 0.2 | 0.21 |  | 9.9 | 5.7 | 0.28 |
| *Chrysosplenium sinicum* | FG | - | - | - |  | 0.3 | 0.2 | 0.83 |
| *Compositae sp.* | FG | 0.4 | - | 0.37 |  | - | - | - |
| *Cruciferae* | FG | - | - | 0.37 |  | - | - | - |
| *Euphrasia pectinata Ten* | FG | **0.1** | **0.5** | **0.05** |  | **0.7** | **2.7** | **0.02** |
| *Geranium pylzowianum* | FG | - | - | 0.37 |  | 6.4 | 7.6 | 0.64 |
| *Herba Taraxaci* | FG | 1.4 | 4.6 | 0.27 |  | 1.3 | 2.9 | 0.27 |
| *Hippophae rhamnoides* | FG | - | - | - |  | 3.6 | - | 0.37 |
| *Iris tectorum* | FG | 1.2 | - | 0.35 |  | - | - | - |
| *Lancea tibetica* | FG | 1.0 | 4.0 | 0.12 |  | 7.8 | 10.4 | 0.63 |
| *Leontopodium leontopodioides* | FG | 0.2 | 0.5 | 0.36 |  | 3.2 | 5.4 | 0.47 |
| *Microula sikkimensis Hemsl* | FG | - | - | - |  | 0.4 | - | 0.37 |
| *Morina coulteriana* | FG | - | 3.6 | 0.38 |  | - | - | - |
| *Notopterygium incisum* | FG | 0.2 | 0.1 | 0.51 |  | 0.1 | 0.1 | 0.54 |
| *Pedicularis kansuensis* | FG | 0.7 | 0.6 | 0.82 |  | 0.8 | 4.5 | 0.42 |
| *Plantago asiatica* | FG | - | 0.1 | 0.37 |  | - | - | - |
| *Polygonum sibioicum* | FG | - | - | 0.37 |  | 0.4 | 0.1 | 0.23 |
| *Polygonum viviparum* | FG | - | - | - |  | 11.9 | 9.1 | 0.12 |
| *Potentilla anserina* | FG | 23.9 | 8.1 | 0.14 |  | 3.0 | 6.4 | 0.07 |
| *Potentilla bifurca* | FG | 0.8 | 0.4 | 0.31 |  | 0.2 | - | 0.18 |
| *Potentilla fruticosa* | FG | - | - | - |  | 3.3 | 3.0 | 0.89 |
| *Potentilla nivea* | FG | 10.0 | 7.5 | 0.72 |  | 3.0 | 5.3 | 0.18 |
| *Saussurea* | FG | - | - | - |  | - | - | 0.37 |
| *Saussurea japonica* | FG | - | - | 0.37 |  | - | - | - |
| *Saussurea nigrescens* | FG | 0.5 | 0.2 | 0.40 |  | 3.6 | 4.4 | 0.69 |
| *Saussurea superba Anthony* | FG | **3.4** | **5.8** | **0.02** |  | - | 0.8 | 0.37 |
| *Saxifraga stolonifera* | FG | - | - | - |  | - | - | 0.37 |
| *Unclassified*d | FG | 1.4 | 4.4 | 0.15 |  | **1.6** | **22.9** | **0.02** |
| *Anemone obtusiloba* | NG | - | - | 0.37 |  | 2.5 | 1.7 | 0.56 |
| *Delphinium caeruleum* | NG | 0.2 | 0.3 | 0.79 |  | - | - | - |
| *Gentiana farreri* | NG | 1.4 | 0.4 | 0.24 |  | 0.1 | - | 0.37 |
| *Gentiana scabra Bunge* | NG | - | 0.1 | 0.37 |  | 0.7 | 0.5 | 0.62 |
| *Gentiana spathulifolia* | NG | 0.4 | 1.1 | 0.12 |  | - | - | - |
| *Gentiana straminea* | NG | **35.3** | **16.6** | **0.02** |  | - | - | - |
| *Gentianopsis paludosa* | NG | 0.2 | 1.2 | 0.33 |  | - | - | - |
| *Ligularia virgaurea* | NG | - | - | - |  | 6.8 | 4.8 | 0.39 |
| *Ranunculus* | NG | - | 0.1 | 0.55 |  | - | - | - |
| *Ranunculus tanguticus* | NG | **-** | **0.9** | **0.01** |  | 1.4 | 0.9 | 0.50 |
| *Stellariamedia* | NG | 1.6 | 1.5 | 0.91 |  | 1.5 | 0.3 | 0.06 |
| *Swertia* | NG | - | 0.2 | 0.18 |  | 0.2 | 0.2 | 0.94 |
| *Swertia tetraptera Maxim.* | NG | - | - | - |  | 0.1 | 0.1 | 0.97 |
| *Thalictrum alpinum* | NG | 4.6 | 4.7 | 0.97 |  | 1.7 | 5.9 | 0.17 |

aPlants can be divided into five functional groups based on selective consumption by livestock: graminoids species group (GG), sedge species group (SG) and leguminous species group (LG) are palatable; forbs species group (FG) are less palatable; and noxious species group (NG) are noxious for animals.

b*Festuca* is the sum of plant species belonging to genus of *Festuca*. It is difficult to examine at the species level.

cGraminoids is the sum of plant species in the category of grass. It is difficult to examine those plant species at a finer level.

dUnclassified is the sum of unclassified species.

eDifferences between control and post-grazing grassland samples were examined by two tailed *t*-test.

fSignificantly (*P*<0.05) changed values are shown in bold.

**Table S2.** Functional genes detected by GeoChip 4.0**.**

|  |  |  |
| --- | --- | --- |
| **Gene category** | **Number of detected genes**  | **Number of detected probes** |
| **Carbon cycling** | **41** | **11 034** |
| Acetogenesis | 1 | 52 |
| Methane cycling | 3 | 251 |
| Carbon fixation | 4 | 1077 |
| Carbon degradation | 33 | 4759 |
| Cellulose | 4 | 412 |
| Chitin | 3 | 776 |
| Hemicellulose | 5 | 749 |
| Lignin | 4 | 486 |
| Pectin | 1 | 40 |
| Starch | 8 | 1207 |
| Others | 8 | 1089 |
| **Nitrogen cycling** | **17** | **4305** |
| Ammonification | 2 | 518 |
| Anammox | 1 | 18 |
| Assimilatory N reduction | 4 | 261 |
| Dissimilatory N reduction | 2 | 337 |
| Nitrification | 2 | 625 |
| Denitrification | 5 | 1720 |
| Nitrogen fixation | 1 | 826 |
| **Phosphorus utilization** | **3** | **792** |
| **Sulphur** | **6** | **1794** |
| Adenylsulphate reductase | 3 | 260 |
| Sulphite reductase | 2 | 1232 |
| Sulphur oxidation | 1 | 302 |
| **Energy process** | **4** | **530** |
| Cytochrome | 1 | 378 |
| Hydrogenase | 2 | 127 |
| P450 | 1 | 25 |
| **Metal resistance** | **44** | **5515** |
| Aluminium | 1 | 67 |
| Arsenic | 5 | 551 |
| Cadmium | 2 | 396 |
| Cadmium, cobalt, zinc | 3 | 882 |
| Chromium | 1 | 637 |
| Cobalt | 1 | 30 |
| Cobalt, nickel | 3 | 12 |
| Copper | 5 | 1041 |
| Lead | 3 | 38 |
| Mercury | 7 | 480 |
| Nickel | 1 | 24 |
| Selenium | 1 | 2 |
| Silver | 4 | 285 |
| Tellurium | 4 | 703 |
| Zinc | 2 | 350 |
| Miscellaneous | 1 | 17 |
| **Complex C metabolism** | **182** | **10 949** |
| Aromatics | 131 | 8220 |
| Aromatic carboxylic acid | 37 | 3813 |
| BTEX and related aromatics | 21 | 679 |
| Chorinated aromatics | 11 | 485 |
| Heterocyclic aromatics | 9 | 94 |
| Nitoaromatics | 11 | 698 |
| Polycyclic aromatics | 19 | 591 |
| Other aromatics | 23 | 1652 |
| Chlorinated solvents | 6 | 376 |
| Herbicides related compounds | 13 | 948 |
| Pesticides related compounds | 4 | 317 |
| Other hydrocarbons | 14 | 515 |
| Others | 14 | 573 |
| **Antibiotic resistance** | **11** | **1,730** |
| Transporters | 5 | 1186 |
| β-lactamases | 4 | 377 |
| Others | 2 | 167 |
| **Stress** | **45** | **11 367** |
| Cold shock | 4 | 36 |
| Heat shock | 5 | 858 |
| Glucose limitation | 2 | 39 |
| Nitrogen limitation | 3 | 893 |
| Osmotic stress | 4 | 240 |
| Oxygen limitation | 7 | 557 |
| Oxygen stress | 7 | 2666 |
| Phosphate limitation | 6 | 2646 |
| Protein stress | 2 | 310 |
| Radiation stress | 1 | 665 |
| Sigma factors | 4 | 2457 |
| **Bacteria phage** | **39** | **391** |
| Replication | 24 | 261 |
| Lysis | 7 | 71 |
| Structural | 6 | 35 |
| Host recognition/structural | 2 | 24 |
| **Bioleaching** | **121** | **642** |
| **Virulence** | **13** | **1879** |
| **Other (gyrB, bchY)** | **2** | **1243** |
| **Total** | **528** | **47 327** |

**Supplementary Figures**

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**Figure S1.** Dissimilarity of functional gene compositions between control and post-grazing grassland samples based on Bray-Curtis coefficients. Significance was examined by ANOVA: ‘\*’ *P*<0.05.

(A)



(B)

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**Figure S2.** Relative abundances of functional genes associated with C fixation at (A) the 3200-m site and (B) the 3400-m site. The normalized signal intensity for each gene was the sum of signal intensities of detected genes divided by detected gene number across all samples. Significance was examined by two-tailed *t*-test: ‘\*’ *P*<0.05 and ‘\*\*’ *P*<0.01.

(A)



(B)



**Figure S3.** Relative abundances of functional genes associated with CH4 production and oxidation at (A) the 3200-m site and (B) the 3400-m site. The normalized signal intensity for each gene was the sum of signal intensities of detected genes divided by detected gene number across all samples. Significance was examined by two-tailed *t*-test: ‘\*’ *P*<0.05.

 (A)





(B)

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****

**Figure S4.** Response ratio analyses of genes associated with (A) different gene categories and (B) complex carbon metabolism genes at the confidence level of 0.95.