**Supplementary Information:**

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

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**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.

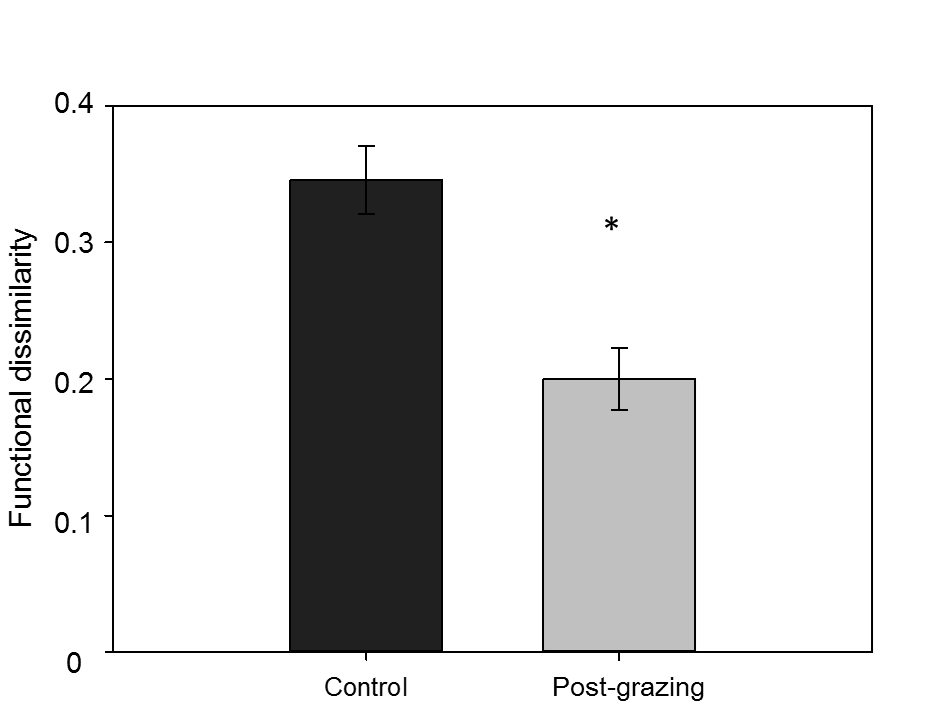
eDifferences between control and post-grazing grassland samples were examined by two tailed *t*-test.

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

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

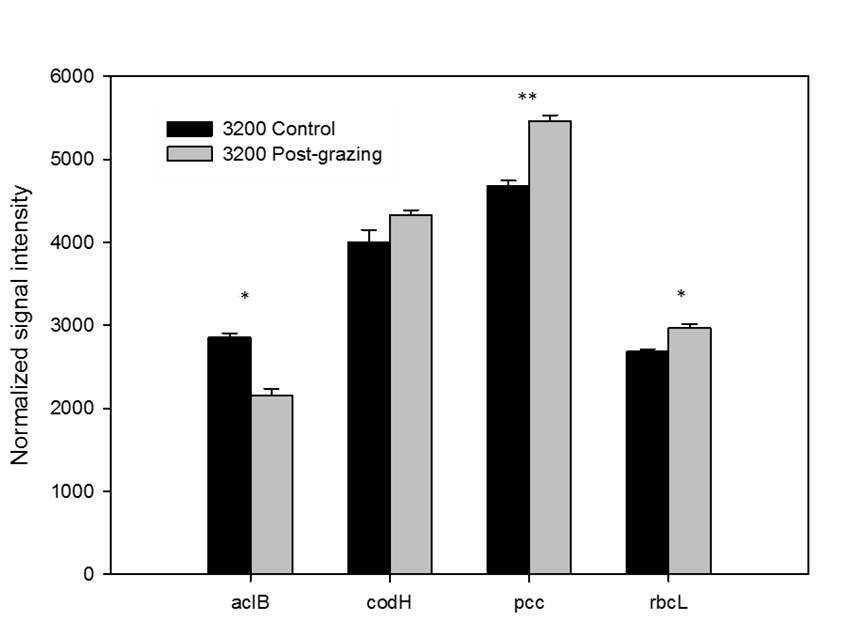
|  |  |  |
| --- | --- | --- |
| **Gene category** | **Number of detected genes** | **Number of detected probes** |
| **Carbon cycling** | **41** | **11 034** |
| Acetogenesis | 1 | 52 |
| Methane cycling | 3 | 251 |
| Carbon fixation | 4 | 1077 |
| Carbon degradation | 33 | 4759 |
| Cellulose | 4 | 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**

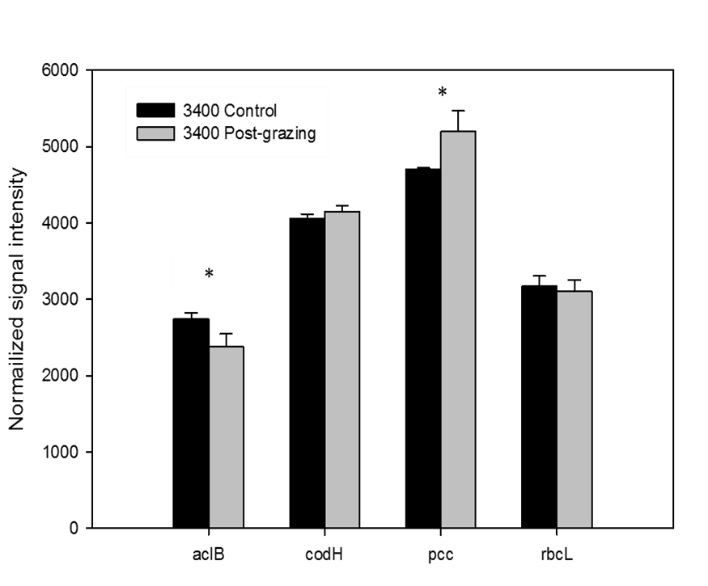
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**Figure S1.** Dissimilarity of functional gene compositions between control and post-grazing grassland samples based on Bray-Curtis coefficients. Significance was examined by ANOVA: ‘\*’ *P*<0.05.

(A)

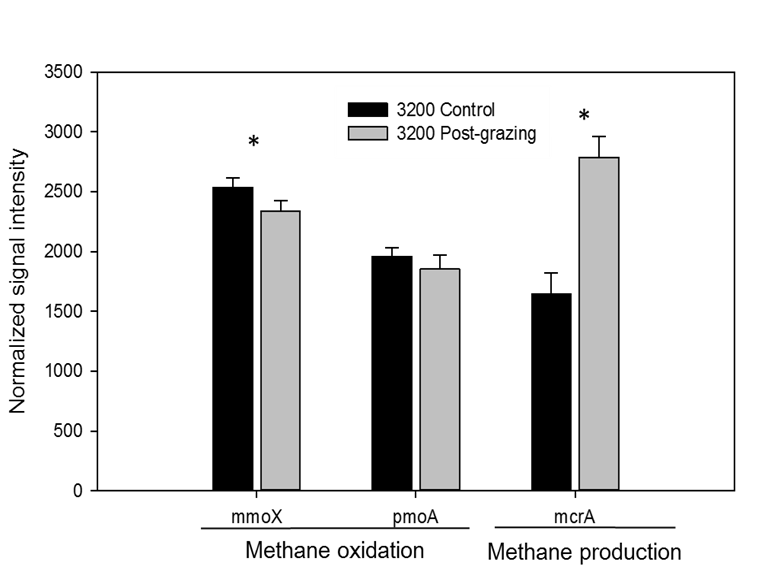


(B)

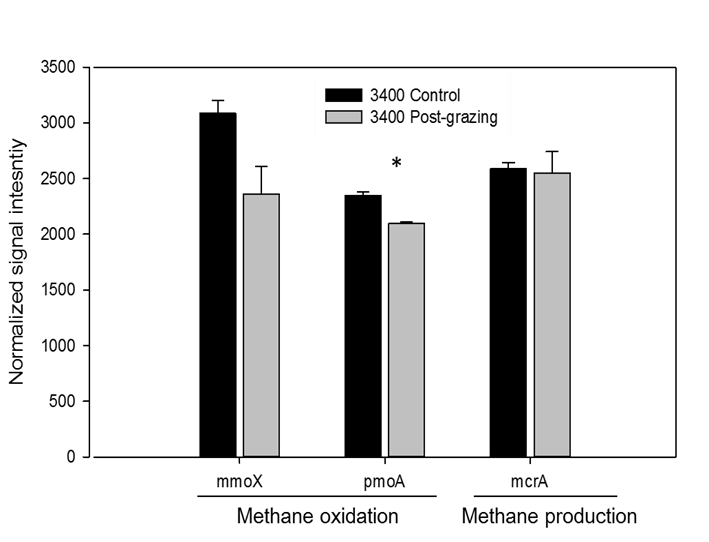
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**Figure S2.** Relative abundances of functional genes associated with C fixation at (A) the 3200-m site and (B) the 3400-m site. The normalized signal intensity for each gene was the sum of signal intensities of detected genes divided by detected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

(A)

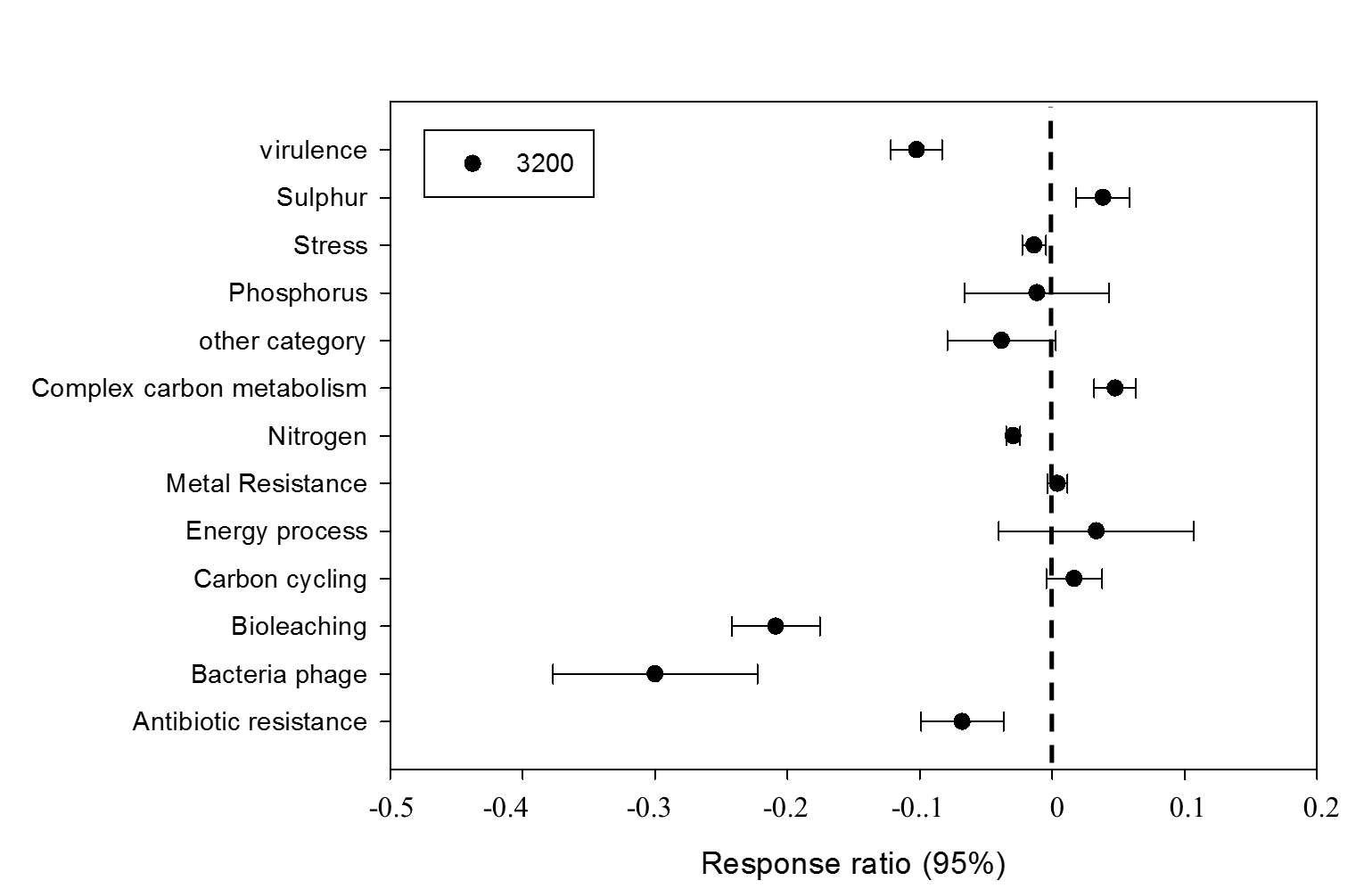


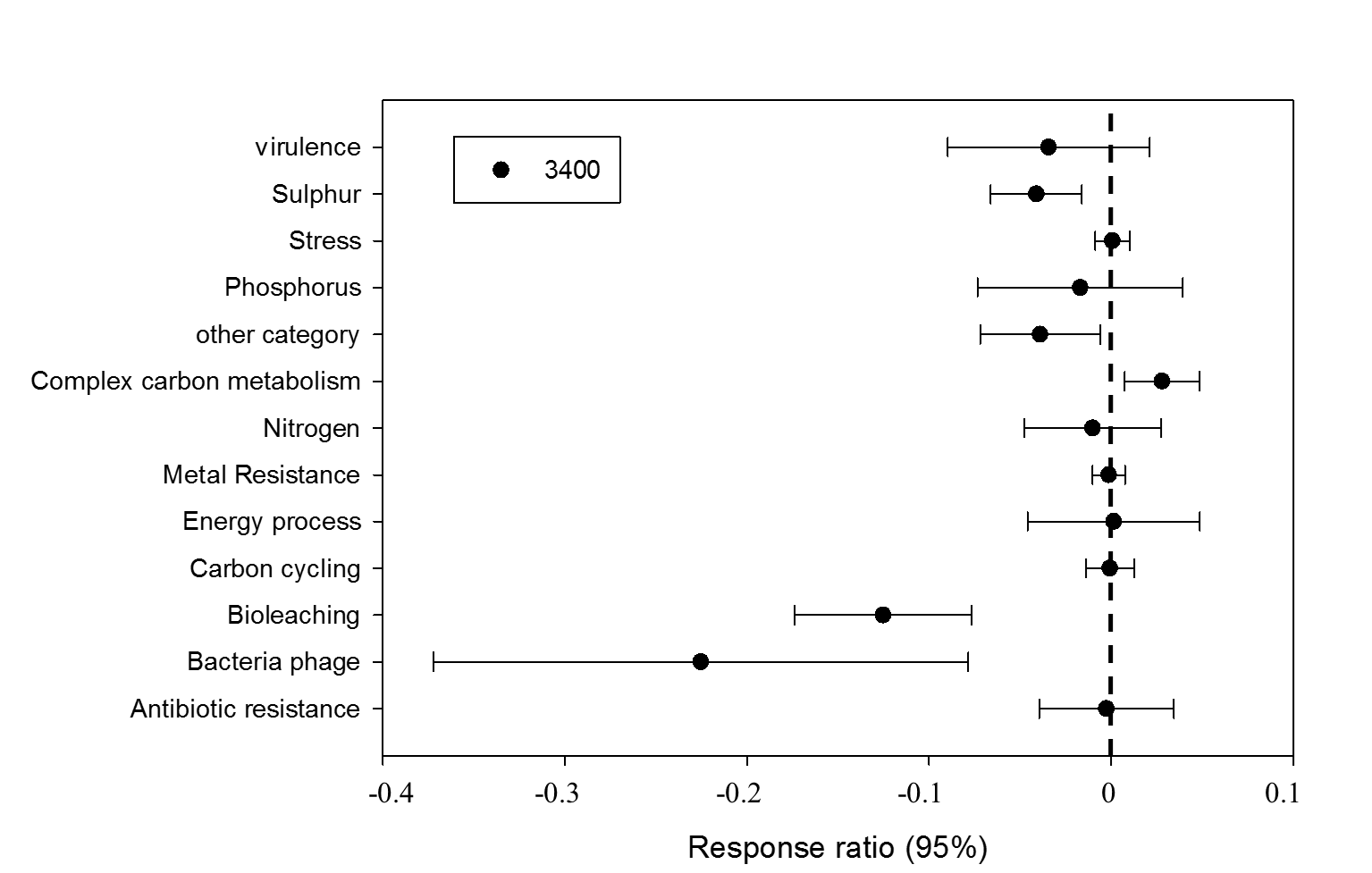
(B)



**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)

**E:\lab\aProjects\Tibetan winter grazing\processed\Results\response ratio_organic remediation 3200.tif**

**E:\lab\aProjects\Tibetan winter grazing\processed\Results\response ratio_organic remediation 3400.tif**

**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.