

*Supporting information*

**The composition of antibiotic resistance genes is not affected by grazing but is determined by microorganisms in grassland soils**

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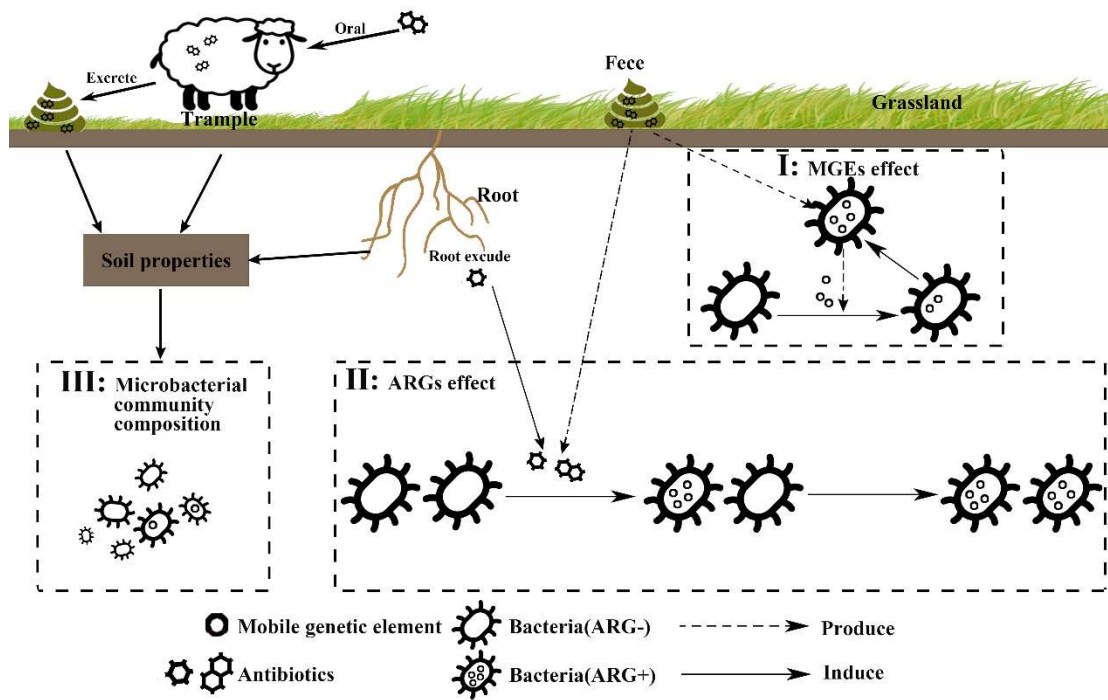
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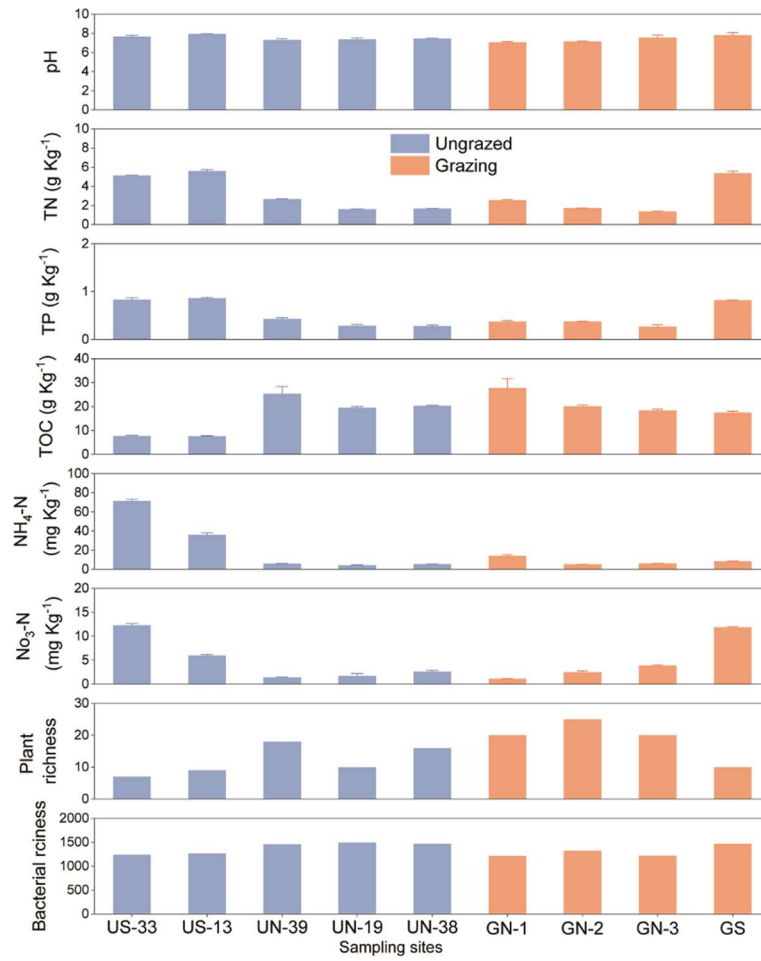
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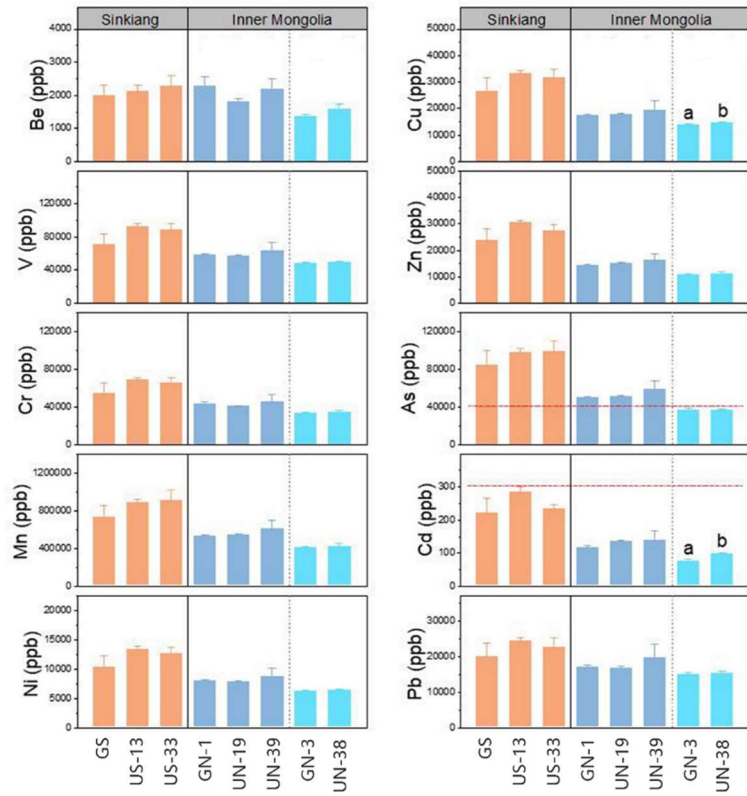
**Fig. S1** Processes and mechanisms underlying impacts of grazing on antibiotic resistance genes (ARGs).

MGEs: Mobile genetic elements.



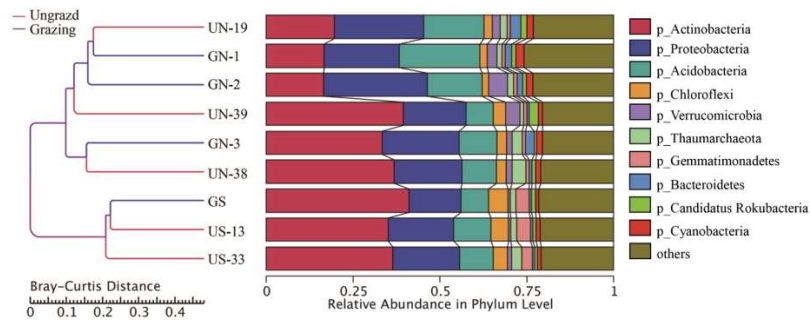
**Fig. S2** Soil pH, content of total nitrogen (TN), total phosphorus (TP), total organic carbon (TOC),

NH<sub>4</sub>-N, NO<sub>3</sub>-N, plant richness, and bacterial richness in each sample site. Values are mean ± SE.

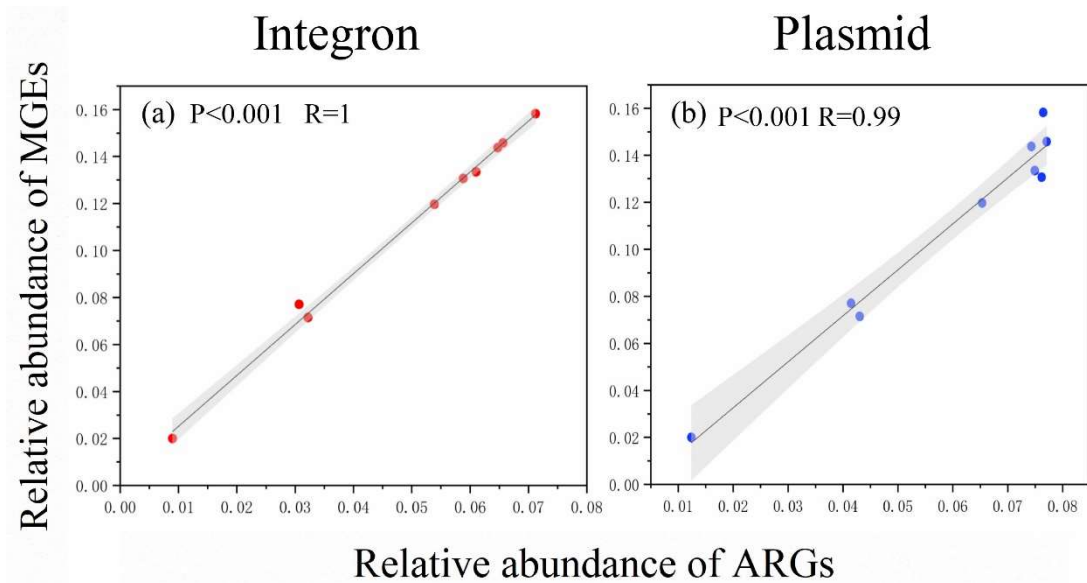


**Fig. S3** The content of metallic elements in each sample site. The red dashed lines represent the risk screening value. Values are mean  $\pm$  SE. Different letters indicate significant differences ( $P \leq 0.05$ ) between sample sites.





**Fig. S6.** The bacterial community compositions and relative abundance at the phylum level in each sample sites. 39-, 38-, and 19-year ungrazed (UN-39, UN-38 and UN-19) and corresponding long-term grazing grasslands (GN-1, GN-3 and GN-2) in the Inner Mongolia. 33- and 13-year ungrazed (US-33 and US-13) and corresponding long-term grazing grassland (GS) in Sinkiang.



**Fig. S7** The relationships between the relative abundance of total antibiotic resistance gene (ARGs) and mobile genetic elements (MGEs) in integrons (a) or plasmids (b).

**Table S1** Information about the locations, dominant species and management pattern of the sample sites.

| <b>Site ID</b> | <b>Location</b> | <b>Dominant species</b> | <b>Management pattern</b> |
|----------------|-----------------|-------------------------|---------------------------|
| UN-39          | Inner Mongolia  | <i>Leymus chinensis</i> | 39-year Ungrazed          |
| UN-38          | Inner Mongolia  | <i>Stipa grandis</i>    | 38-year Ungrazed          |
| UN-19          | Inner Mongolia  | <i>Leymus chinensis</i> | 19-year Ungrazed          |
| GN-1           | Inner Mongolia  | <i>Leymus chinensis</i> | Long-term Grazing         |
| GN-2           | Inner Mongolia  | <i>Leymus chinensis</i> | Long-term Grazing         |
| GN-3           | Inner Mongolia  | <i>Stipa grandis</i>    | Long-term Grazing         |
| US-33          | Sinkiang        | <i>Stipa purpuea</i>    | 33-year Ungrazed          |
| US-13          | Sinkiang        | <i>Stipa purpuea</i>    | 13-year Ungrazed          |
| GS             | Sinkiang        | <i>Festuca ovina</i>    | Long-term Grazing         |

**Table S2** 12 unique antibiotic resistance genes (ARGs) in the GN-3 in Inner Mongolia Plateau.

| <b>ARGs</b>                  | <b>The relative abundance<br/>(<math>\times 10^{-6}</math>)<sup>a</sup></b> |
|------------------------------|-----------------------------------------------------------------------------|
| vanJ                         | 7.04                                                                        |
| EXO_beta-lactamase           | 6.82                                                                        |
| gimA                         | 6.40                                                                        |
| mgtA                         | 5.71                                                                        |
| APH3-Ia                      | 5.65                                                                        |
| AAC3-Ib                      | 2.69                                                                        |
| LRA-8                        | 1.85                                                                        |
| Propionibacterium_acnes_gyrA | 1.41                                                                        |
| RlmAII                       | 1.39                                                                        |
| dfrG                         | 1.30                                                                        |
| OXA-62                       | 1.26                                                                        |
| Campylobacter_jejuni_gyrA    | 1.22                                                                        |

<sup>a</sup>Relative abundance is calculated as the total number of ARG divided by the number of genes predicted per sampled site



**Table S3** Results of mantel correlations between the antibiotic resistance genes (ARGs) richness and heavy metals.

|    | $R^2$ | $P$ -value |
|----|-------|------------|
| B  | 0.13  | 0.18       |
| V  | 0.24  | 0.10       |
| Cr | 0.22  | 0.11       |
| Mn | 0.16  | 0.16       |
| Ni | 0.21  | 0.12       |
| Cu | 0.12  | 0.19       |
| Zn | 0.11  | 0.20       |
| As | 0.10  | 0.22       |
| Cd | 0.04  | 0.29       |
| Pd | 0.26  | 0.10       |