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Supplemental information

Root exudates enhanced rhizobacteria complexity and microbial carbon metabolism of toxic plants

Wenyin Wang, Tianhua Jia, Tianyun Qi, Shanshan Li, A. Allan Degen, Jin Han, Yanfu Bai, Tao Zhang, Shuai Qi, Mei Huang, Zihao Li, Jianxin Jiao, and Zhanhuan Shang

Supplemental Figures Legends

Figure S1. Sampling sites (a), stages of grassland degradation (b) and some sampled plants (c). Related to Figure 1.

Figure S2. The relative abundances of phyla in the bacterial community ($n \geq 3$). L, lightly degraded grassland; M, moderately degraded grassland; H, heavily degraded grassland; S, degraded sown grassland. Nr, the bulk soil, namely non-rhizosphere soil. Al, *Ajuga lupulina*; Ef, *Euphorbia fischeriana*; Sg, *Sphallerocarpus gracilis*; Oo, *Oxytropis ochrocephala*; Mk, *Morina kokonorica*; Pk, *Pedicularis kansuensis*; An, *Artemisia nanschanica*; Ad, *Artemisia dubia*; Ap, *Aconitum pendulum*; Lv, *Ligularia virgaurea*; En, *Elymus nutans*; Kp, *Kobresia pygmaea*. Related to Figure 2.

Figure S3. The contents of $\text{NO}_3\text{-N}$ and $\text{NH}_4\text{-N}$ in bulk soil ($n = 3$, $P < 0.05$, One-Way ANOVA) (a). Values are means \pm S.E.M. of triplicate observations. L, lightly degraded grassland; M, moderately degraded grassland; H, heavily degraded grassland; S, degraded sown grassland. Nr, non-rhizosphere soil. Redundancy analysis (RDA) of the relationship between soil physical and chemical properties and the Bray-Curtis distances in bacterial communities (b). Dots indicate individual rhizospheres of toxic plants. The arrow length and direction correspond to the variance that could be explained by the individual soil physical and chemical properties. Insert: loadings (RDA axis values) for the individual soil physical and chemical properties ($P < 0.05$). Black and gray colors of the bars indicate negative and positive values, respectively. TN, total nitrogen; TP, total phosphorus; TOC, total organic carbon; AP, available phosphorus; NO_3 , nitrate nitrogen; NH_4 , ammonium nitrogen; WC, water content. Related to Figure 2.

Figure S4. Correlations between main ecological clusters and soil chemical properties. TOC, soil total organic carbon; TN, soil total nitrogen; TP, soil total phosphorus; DON, soil dissolved organic nitrogen; AP, soil total phosphorous; AK, soil available potassium; Na, soil sodium. Related to Figure 3.

Figure S5. Clustering results displayed as heatmap (distance measure using Euclidean distance, clustering algorithm using ward.D). L, lightly degraded grassland; M, moderately degraded grassland; H, heavily degraded grassland; S, degraded sown grassland. Mk, *Morina kokonorica*; Pk, *Pedicularis kansuensis*; Lv, *Ligularia virgaurea*; En, *Elymus nutans*; Kp,

Kobresia pygmaea. H, M, L in S-Lv represented high, middle and low density of *Ligularia virgaurea* in degraded sown grassland. Related to Figure 4.

Figure S6. Correlation heatmap between microbial module eigengene and root exudates in non-toxic plants. Asterisks indicate Spearman's correlations between rhizosphere bacterial modules and root exudates (*, $P < 0.05$; **, $P < 0.01$). Related to Figure 4.

Figure S7. Relationship between microbial module eigengene and soil multi-functionality. Gray indicates confidence intervals. Related to Figure 4.

Figure S8. Correlations between phyla of microbial taxa in ME12 and up-regulated compounds in non-toxic plants (a) and toxic plants (b). *, $P < 0.05$; **, $P < 0.01$ (Spearman's correlation) . Related to Figure 4.

Figure S9. AWCD growth curves of soil microbes in different levels of grassland degradation (n = 3) (a), and principal component analysis (PCA) of carbon source utilization of soil microbial communities (b). In panel a, Values are means \pm S.E.M. of triplicate observations. L, lightly degraded grassland; M, moderately degraded grassland; H, heavily degraded grassland; S, degraded sown grassland. Nr, bulk soil, that is, non-rhizosphere soil. Al, *Ajuga lupulina*; Ef, *Euphorbia fischeriana*; Sg, *Sphallerocarpus gracilis*; Oo, *Oxytropis ochrocephala*; Mk, *Morina kokonorica*; Pk, *Pedicularis kansuensis*; An, *Artemisia nanschanica*; Ad, *Artemisia dubia*; Ap, *Aconitum pendulum*; Lv, *Ligularia virgaurea*. Related to Figure 5.

Figure S10. The networks of bacterial OTUs (a) and root exudation (b) in the rhizosphere of toxic plants. Threshold = 0.2, $P < 0.05$; n, number of OTUs in different color modules. Correlations between OTUs (c) or exudates (d) and plant species in each module. L, lightly degraded grassland; M, moderately degraded grassland; H, heavily degraded grassland; S, degraded sown grassland. Pk, *Pedicularis kansuensis*; En, *Elymus nutans*; Lv, *Ligularia virgaurea*; Mk, *Morina kokonorica*; Ap, *Aconitum pendulum*. LD and MD were the low and middle densities, respectively, of *Ligularia virgaurea* in degraded sown grassland. Related to Figure 3.

Figure S11. Network analysis of positive correlations between bacterial communities and root exudate modules ($P < 0.05$) (A). Circles represent OTUs, diamonds represent root exudates, and the colors correspond to module colors. For OTUs, the size of the shapes

represent the relative abundance, and the relative content for root exudates. a-e are the networks of M-Lv, S-Pk, L-Lv, H-Lv and S-Lv. Related to Figure 3.

Supplemental Tables

Table S1. Microbial community assembly fitting parameters. Related to Figure 1.

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Table S5. Operational taxonomic units (OTUs) numbers of soil dominant taxa at the phyla level within dominant phylotypes in Module #0, Module #1 and Module #2. Related to Figure 3.

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Table S7. Summary and abbreviations of experimental samples. Related to Figure 2.

Table S1. Microbial community assembly fitting parameters

Sample	Environment	Niche theory		Neutral theory
		AIC (Log-normal)	R ²	N _m
Toxic plants_2017	Rhizosphere	660276	0.858	28705.5 (26408*1.087)
Bulk soil	Bulk soil	37452	0.779	31399.1 (26408*1.189)
H-Ap	Rhizosphere	37418	0.254	35730.0 (26408*1.353)
M-Ap	Rhizosphere	37715	0.260	39664.8 (26408*1.502)
H-Al	Rhizosphere	36222	0.258	39612.0 (26408*1.500)
H-Ad	Rhizosphere	36890	0.276	38978.2 (26408*1.476)
H-An	Rhizosphere	36329	0.235	34937.8 (26408*1.323)
H-Ef	Rhizosphere	36233	0.263	40615.5 (26408*1.538)
M-Ef	Rhizosphere	38643	0.207	37895.5 (26408*1.435)
H-Lv	Rhizosphere	37883	0.260	38529.3 (26408*1.459)
M-Lv	Rhizosphere	38305	0.264	40509.9 (26408*1.534)
S-Lv	Rhizosphere	35240	0.297	41328.5 (26408*1.565)
H-Mk	Rhizosphere	36251	0.293	37552.2 (26408*1.422)
M-Mk	Rhizosphere	41813	0.267	41328.5 (26408*1.540)
L-Mk	Rhizosphere	36329	0.248	37552.2 (26408*1.508)
S-Oo	Rhizosphere	36378	0.265	40668.3 (26408*1.537)
S-Pk	Rhizosphere	37048	0.264	39823.3 (26408*1.529)
S-Sg	Rhizosphere	37452	0.235	40589.1 (26408*1.339)
Toxic plants_2018	Rhizosphere	488658	0.769	40377.8 (103157*0.617)
Non-toxic plants	Rhizosphere	147489	0.690	35360.3 (103157*1.576)
S-H-Lv	Rhizosphere	66878	0.399	63647.9 (103157*2.116)
S-M-Lv	Rhizosphere	58196	0.404	162575.4 (103157*1.808)
S-L-Lv	Rhizosphere	52042	0.395	218280.2 (103157*1.312)
S-PK	Rhizosphere	73296	0.423	186507.9 (103157*2.202)
H-Lv	Rhizosphere	45718	0.362	135342.0 (103157*0.764)
L-MK	Rhizosphere	70541	0.469	192903.6 (103157*1.87)
L-Lv	Rhizosphere	47229	0.429	58902.6 (103157*0.571)
M-Ap	Rhizosphere	72906	0.445	223334.9 (103157*2.165)
M-Lv	Rhizosphere	45098	0.430	101403.3 (103157*0.983)
S-En	Rhizosphere	71328	0.403	225913.8 (103157*2.190)
L-Kp	Rhizosphere	76428	0.477	206830.0(103157*2.005)

Note: L, lightly degraded grassland; M, moderately degraded grassland; H, heavily degraded grassland; S, degraded sown grassland. Nr, non-rhizosphere soil. Al, *Ajuga lupulina*; Ef, *Euphorbia fischeriana*; Sg, *Sphallerocarpus gracilis*; Oo, *Oxytropis ochrocephala*; Mk, *Morina kokonorica*; Pk, *Pedicularis kansuensis*; An, *Artemisia nanschanica*; Ad, *Artemisia dubia*; Ap, *Aconitum pendulum*; Lv, *Ligularia virgaurea*; En, *Elymus nutans*; Kp, *Kobresia pygmaea*. AIC, Akaike Information Criterion; R², the fit to model; N_m, the community size times immigration.

Table S2. The physico-chemical properties of soil

Habitat	Samples	pH	TOC (g/kg)	TN (g/kg)	TP (g/kg)	AP (mg/kg)
Lightly degraded grassland (L)	Bulk soil	6.55	75.0 bA	2.69	0.46	11.1A
	Mk	6.95	110.3 a	3.72	0.44	21.4
	Bulk soil	7.18	50.6 cC	2.23 c	0.51	3.9 cB
	Al	7.39	65.9 abc	2.66 ab	0.58	8.5 b
Moderately degraded grassland (M)	Ef	7.13	84.1 a	3.22 abc	0.56	8.9 b
	Mk	7.54	76.6 ab	2.95 ab	0.64	16.0 a
	Ap	7.63	68.9 abc	2.94 a	0.53	8.5 b
	Lv	7.44	62.1 bc	2.50 bc	0.64	7.5 bc
	Bulk soil	7.22	64.0AB	2.48 bc	0.38	9.9 cA
	Al	7.39	63.9	2.24 c	0.51	13.7 bc
	Ef	7.20	77.1	3.11 ab	0.56	9.3 c
Heavily degraded grassland (H)	Sg	7.22	70.7	2.75 abc	0.47	15.7 bc
	Mk	7.53	82.5	3.10 ab	0.43	17.9 bc
	An	7.38	57.0	2.35 c	0.45	19.2 b
	Ad	7.08	99.3	2.72 abc	0.59	30.2 a
	Pk	7.61	56.8	2.47 bc	0.42	11.0bc
	Lv	7.12	80.9	3.29 a	0.48	15.7 bc
	Bulk soil	7.27	57.5 bBC	2.48 b	0.57	4.6 cB
	Sg	7.45	90.4 a	3.60 a	0.69	12.9 ab
Degraded sown grassland (S)	Oo	7.00	67.5 b	2.99 ab	0.58	10.9 abc
	Pk	7.29	86.9 a	3.73 a	0.67	18.2 a
	Lv	7.37	65.3 b	2.75 b	0.60	8.3 bc

Note: TN, soil total nitrogen; TP, soil total phosphorus; TOC, soil total organic carbon; AP, soil available phosphorus. Values are means of bulk soil (n = 3) and rhizosphere soil (n = 3) of each plant in different habitat. Values with different lowercase letters within a column represent are significantly from each other; capital letters represent significant difference among the bulk soils in different levels of grassland degradation ($P < 0.05$, one way ANOVA). Al, *Ajuga lupulina*; Ef, *Euphorbia fischeriana*; Sg, *Sphallerocarpus gracilis*; Oo, *Oxytropis ochrocephala*; Mk, *Morina kokonorica*; Pk, *Pedicularis kansuensis*; An, *Artemisia nanschanica*; Ad, *Artemisia dubia*; Ap, *Aconitum pendulum*; Lv, *Ligularia virgaurea*.

Table S3. *T*-tests of soil physicochemical properties in different habitats

Habitat	Property	Bulk soil	Rhizosphere soil	<i>P</i> value
L	pH	6.55±0.17	6.95±0.06	0.09
L	TOC (g/kg)	75±4.8	110.3±11.83	0.05
L	TN (g/kg)	2.69±0.07	3.72±0.48	0.10
L	TP (g/kg)	0.46±0.04	0.44±0.06	0.78
L	DON (mg/kg)	382±20.7	459±51.4	0.24
L	AP (mg/kg)	11.1±1.24	21.4±4.67	0.10
L	AK (mg/kg)	150±28.9	217±48.4	0.30
L	ANa (mg/kg)	15.7±1.76	23.3±6.74	0.33
M	pH	7.18±0.18	7.42±0.08	0.24
M	TOC (g/kg)	50.7±3.03	71.5±3.22	0.01
M	TN (g/kg)	2.23±0.09	2.85±0.1	0.02
M	TP (g/kg)	0.51±0.04	0.59±0.02	0.18
M	DON (mg/kg)	288±26.7	323±15.01	0.34
M	AP (mg/kg)	3.88±0.35	9.9±0.98	0.02
M	AK (mg/kg)	113±24	194±18.38	0.05
M	ANa (mg/kg)	15±1.73	15.3±1.09	0.92
H	pH	7.22±0.18	7.32±0.06	0.59
H	TOC (g/kg)	64±2.73	73.5±4.23	0.08
H	TN (g/kg)	2.48±0.18	2.75±0.1	0.36
H	TP (g/kg)	0.38±0.1	0.49±0.02	0.16
H	DON (mg/kg)	298±26.2	336±15.34	0.40
H	AP (mg/kg)	9.86±0.53	16.59±1.49	0.00
H	AK (mg/kg)	90±14	232.1±35.07	0.00
H	ANa (mg/kg)	17.7±2.19	14.1±1.22	0.33
S	pH	7.27±0.27	7.28±0.1	0.97
S	TOC (g/kg)	57.5±3.78	77.5±3.91	0.03
S	TN (g/kg)	2.48±0.18	3.26±0.16	0.04
S	TP (g/kg)	0.57±0.03	0.63±0.03	0.24
S	DON (mg/kg)	339±42.4	394±39.99	0.52
S	AP (mg/kg)	4.61±0.39	12.56±1.6	0.00
S	AK (mg/kg)	130±15.3	296±56.32	0.02
S	ANa (mg/kg)	14.7±1.2	14.3±1.85	0.92

Note: L, lightly degraded grassland; M, moderately degraded grassland; H, heavily degraded grassland; S, degraded sown grassland. TOC, soil total organic carbon; TN, total nitrogen; total phosphorous (TP), available phosphorous (AP), nitrate nitrogen (NO₃-N), ammonium nitrogen (NH₄-N), Dissolved organic nitrogen (DON), available potassium (AK), available sodium (ANa). Bold values indicate a significant difference between bulk soil and rhizosphere. Values are means ± SD of bulk soil (n = 3) and rhizosphere soil (n = 3 in L, n= 15 in M, n = 21 in H and n = 12 in S).

Table S4. Comparison of the relative abundances of the dominant taxa among different habitats in Module #0, Module #1 and Module #2

	Habitat	Mode	Bulk soil	Rhizosphere soil	<i>P</i> value
Bacteria	L	Module #0	0.75±0.02	0.77±0.06	0.8
Bacteria	L	Module #1	1.36±0.19	1.59±0.14	0.37
Bacteria	L	Module #2	1.88±0.41	1.51±0.06	0.46
Bacteria	M	Module #0	0.52±0.03	0.47±0.04	0.67
Bacteria	M	Module #1	0.94±0.06	1.06±0.05	0.27
Bacteria	M	Module #2	2.78±0.41	1.85±0.09	0.002
Bacteria	H	Module #0	0.66±0.04	0.8±0.06	0.44
Bacteria	H	Module #1	1.91±0.06	1.72±0.2	0.39
Bacteria	H	Module #2	2.03±0.45	1.93±0.27	0.9
Bacteria	S	Module #0	0.82±0.05	1.18±0.28	0.53
Bacteria	S	Module #1	1.43±0.15	2.22±0.5	0.46
Bacteria	S	Module #2	2.3±0.26	1.58±0.14	0.04
Fungi	L	Module #0	0.87±0.39	1.54±0.29	0.25
Fungi	L	Module #1	0.19±0.13	0.61±0.13	0.08
Fungi	L	Module #2	0.41±0.13	0.56±0.05	0.34
Fungi	M	Module #0	1.33±0.55	0.97±0.24	0.54
Fungi	M	Module #1	0.5±0.16	0.81±0.21	0.53
Fungi	M	Module #2	1.33±0.71	1.08±0.23	0.68
Fungi	H	Module #0	1.07±0.26	1.82±0.27	0.35
Fungi	H	Module #1	1.04±0.26	1.3±0.24	0.72
Fungi	H	Module #2	0.82±0.36	2.13±1.12	0.69
Fungi	S	Module #0	1.04±0.28	4.28±2.09	0.46
Fungi	S	Module #1	0.72±0.31	3.13±2.21	0.61
Fungi	S	Module #2	1.03±0.35	0.41±0.06	0.01

Note: BS, bulk soil; RS, rhizosphere soil. Values are means ± SD of bulk soil (n = 3) and rhizosphere soil (n = 3 in L, n= 15 in M, n = 21 in H and n = 12 in S).

Table S5. Operational taxonomic units (OTUs) numbers of soil dominant taxa at the phyla level within dominant phylotypes in Module #0, Module #1 and Module #2

Phylum	Dominant phylotypes		
	from module #0	from module #1	from module #2
Acidobacteria	2	0	8
Actinobacteria	2	3	0
Armatimonadetes	1	0	0
Bacteroidetes	4	0	0
Chloroflexi	2	1	6
Cyanobacteria	2	2	1
Bacteria	0	0	1
Gemmatimonadetes	1	1	5
Latescibacteria	0	0	3
Nitrospirae	0	0	4
Omnitrophica	0	0	1
Planctomycetes	3	12	6
Proteobacteria	10	10	11
Verrucomicrobia	0	0	2
Fungi	17	7	9
Basidiomycota	1	6	1

Table S6. Comparison of the relative abundances of the dominant taxa between toxic plants and non-toxic plants in ME12

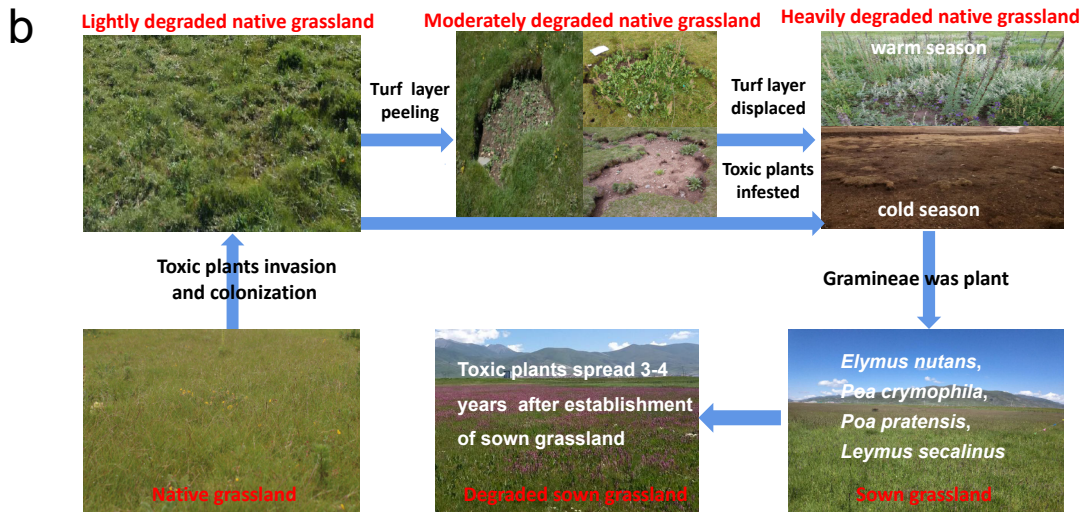
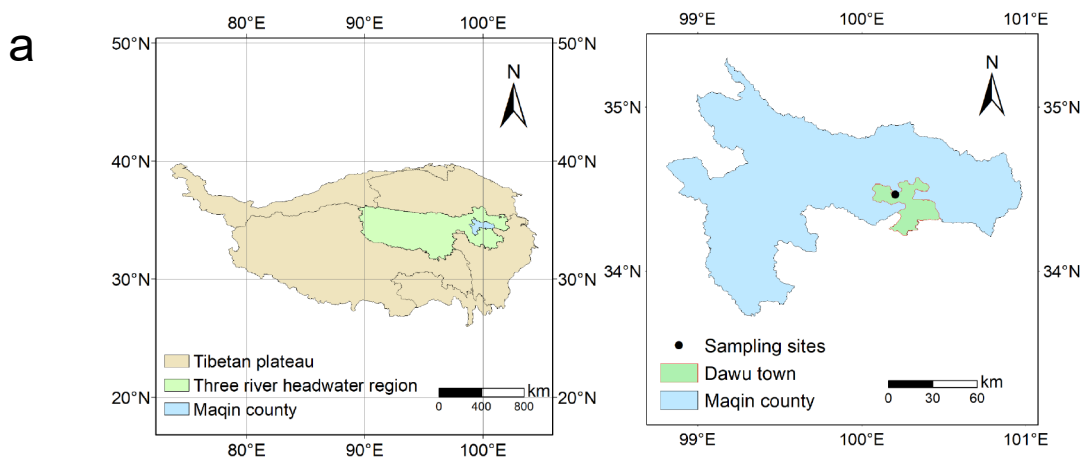
	Taxa	Toxic plants	Non-toxic plants	<i>P</i> value
Bacteria	p_Acidobacteria	0.120	0.016	0.000
Bacteria	p_Actinobacteria	0.010	0.009	0.831
Bacteria	p_Armatimonadetes	0.010	0.003	0.013
Bacteria	p_Bacteroidetes	0.014	0.042	0.018
Bacteria	p_Chlorobi	0.003	0.004	0.714
Bacteria	p_Chloroflexi	0.006	0.007	0.074
Bacteria	p_Gemmatimonadetes	0.065	0.032	0.141
Bacteria	p_Planctomycetes	0.093	0.065	0.094
Bacteria	c_Alphaproteobacteria	1.805	0.572	0.000
Bacteria	c_Betaproteobacteria	0.024	0.022	0.600
Bacteria	c_Deltaproteobacteria	0.001	0.002	0.023
Bacteria	c_Gammaproteobacteria	0.038	0.010	0.007
Bacteria	p_Saccharibacteria	0.004	0.003	0.019
Fungi	p_Ascomycota	0.193	0.138	0.358
Fungi	p_Basidiomycota	0.128	0.025	0.005
Fungi	p_Chytridiomycota	0.017	0.001	0.072
Fungi	p_Mortierellomycota	0.341	0.082	0.220

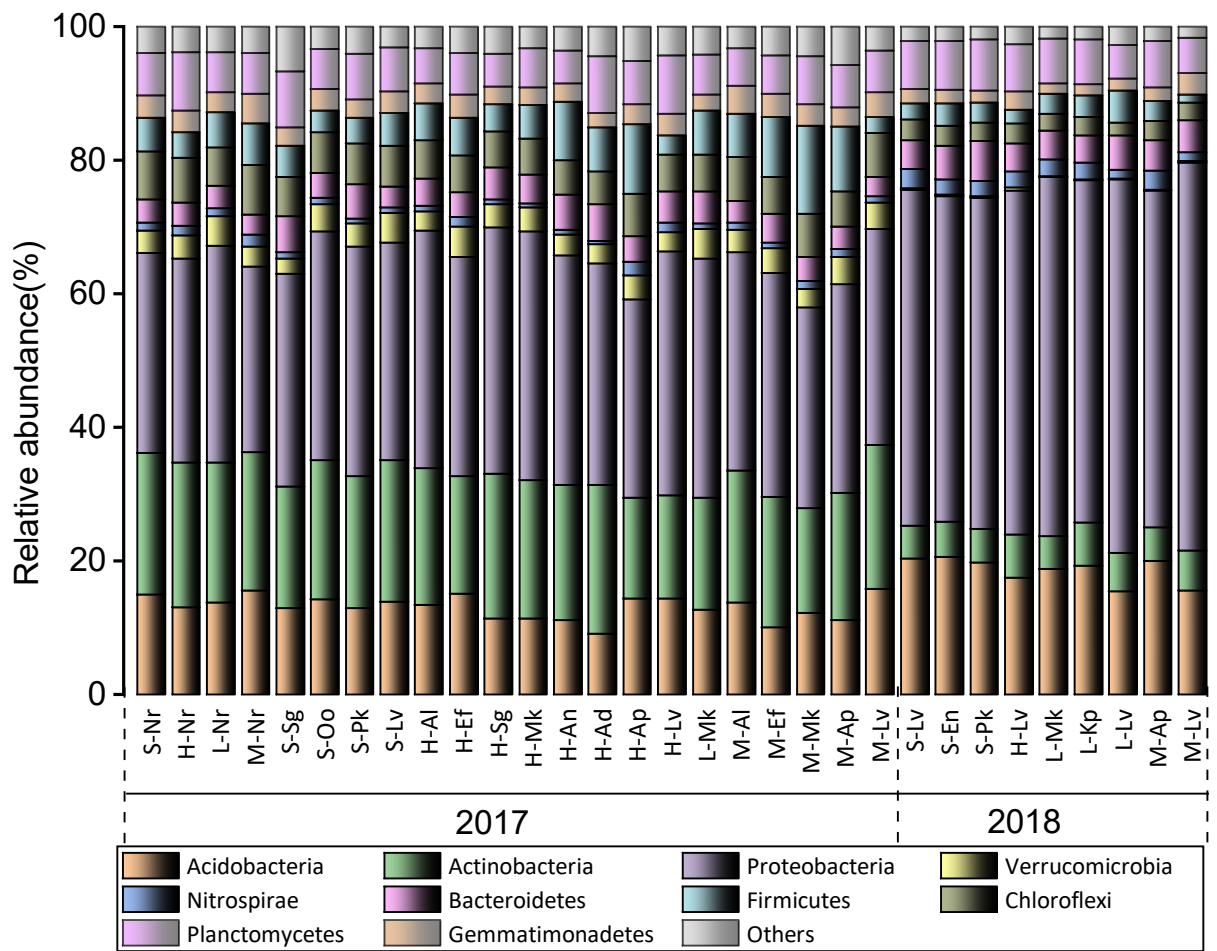
Note: Values are means of toxic plants (n = 15) and non-toxic plants (n = 6). *P* based on Student's t-test.

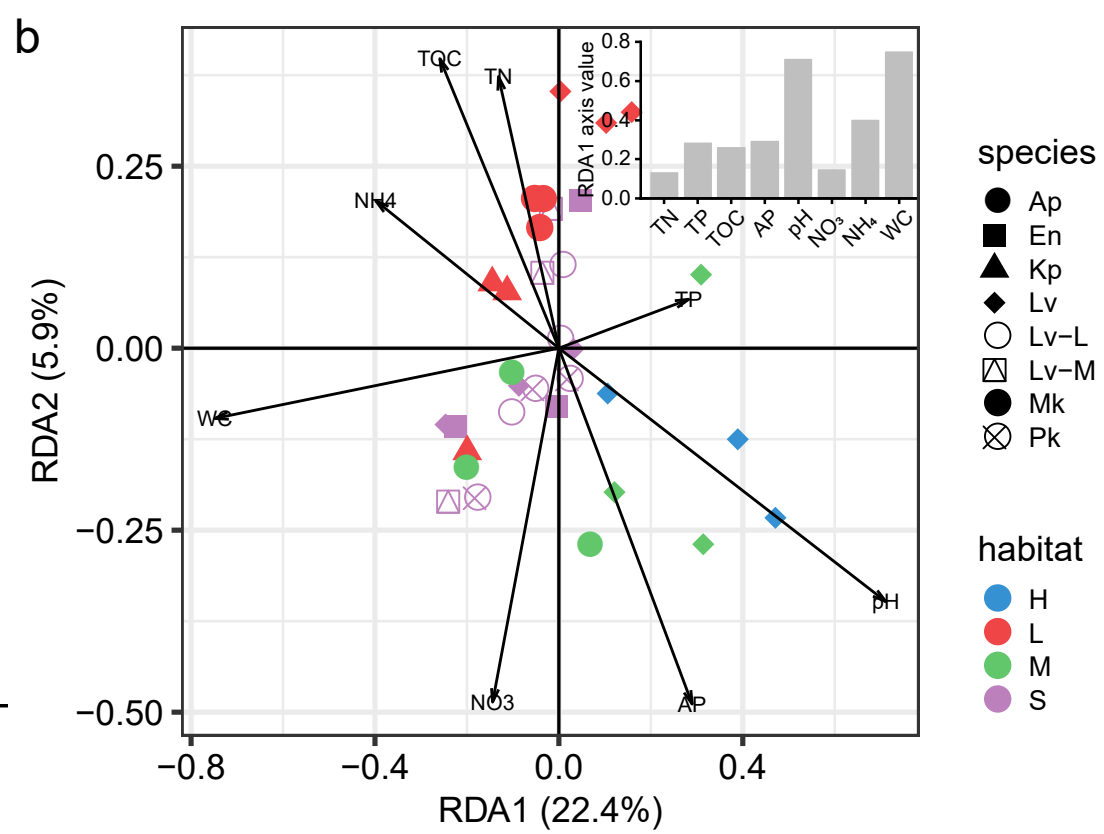
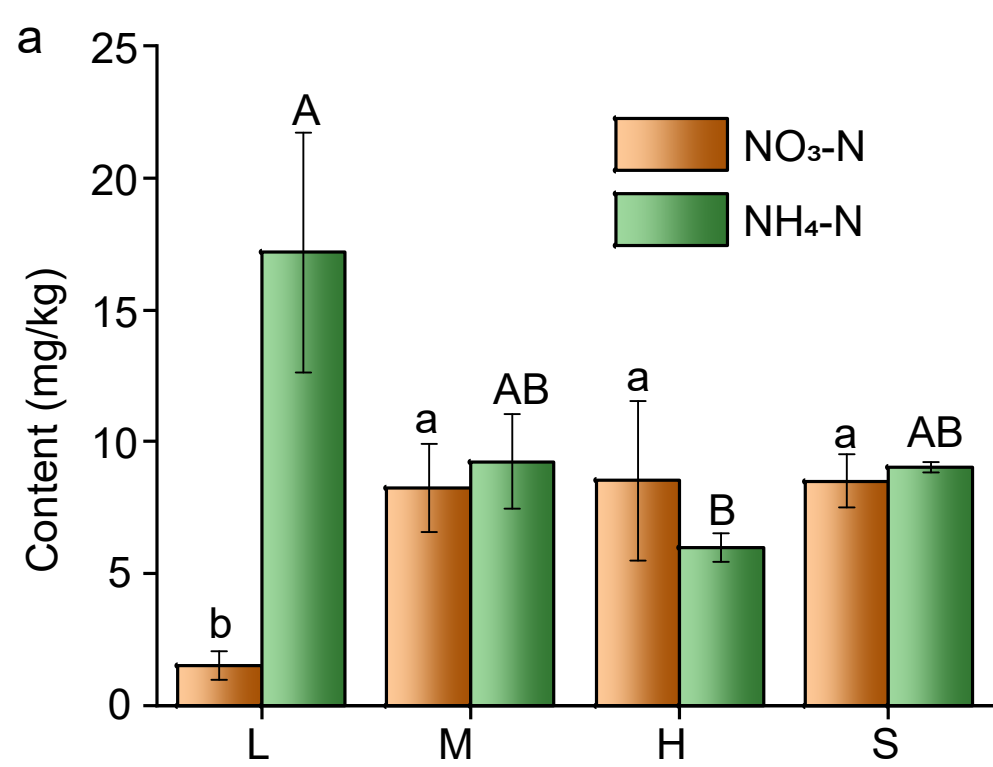
Table S7. Summary and abbreviations of experimental samples

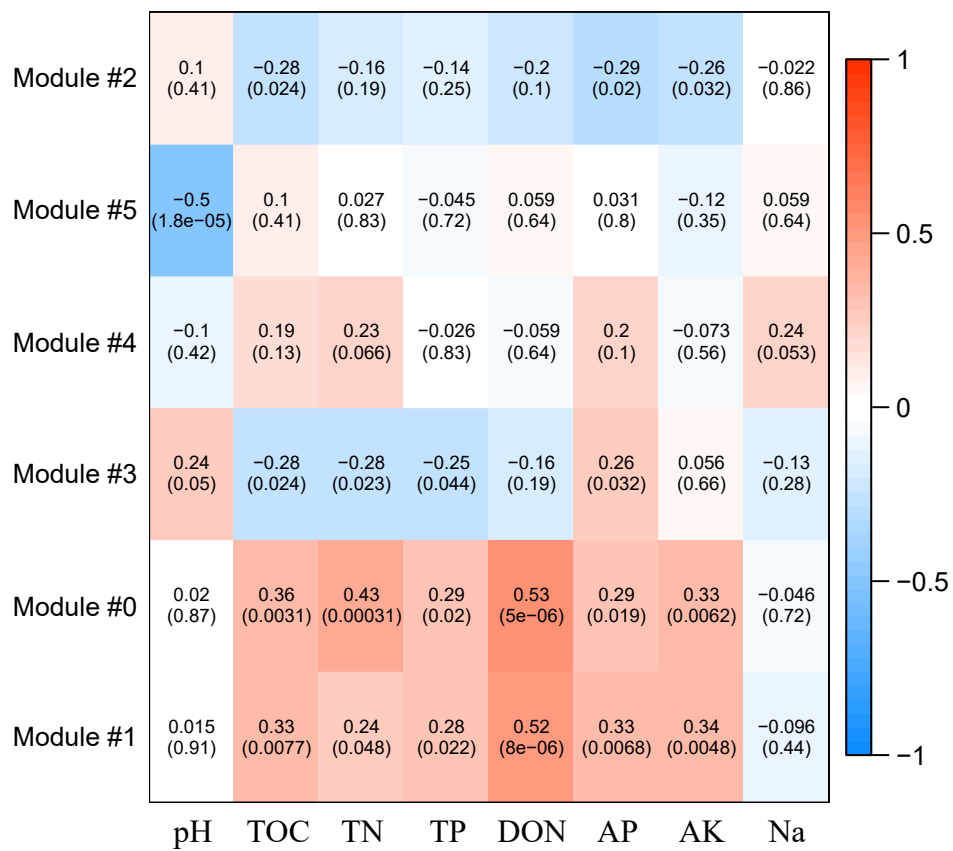
Species	Family	Life cycle	L			M			H			S		
			Freq	2017	2018	Freq	2017	2018	Freq	2017	2018	Freq	2017	2018
<i>Aconitum pendulum</i>	Ranunculaceae	Reproductive				0.9	√	√	0.6	√				
<i>Ajuga lupulina</i>	Labiatae	Reproductive				0.6	√		0.5	√				
<i>Artemisia dubia</i>	Compositae	Reproductive							0.6	√				
<i>Artemisia nanschanica</i>	Compositae	Reproductive							0.7	√				
<i>Elymus nutans</i>	Gramineae	Reproductive										1		√
<i>Euphorbia fischeriana</i>	Euphorbiaceae	Reproductive				0.7	√		0.2	√				
<i>Kobresia pygmaea</i>	Cyperaceae	Reproductive	0.9		√									
<i>Ligularia virgaurea</i>	Compositae	Vegetative	0.2		√	0.7	√	√	0.8		√	0.5	√	√
<i>Morina kokonorica</i>	Dipsacaceae	Reproductive	0.6	√	√	0.9	√		0.5	√				
<i>Oxytropis ochrocephala</i>	Leguminosae	Reproductive										0.8	√	
<i>Pedicularis kansuensis</i>	Scrophulariaceae	Reproductive										0.5	√	√
<i>Sphallerocarpus gracilis</i>	Apiaceae	Reproductive							0.5	√		0.1	√	

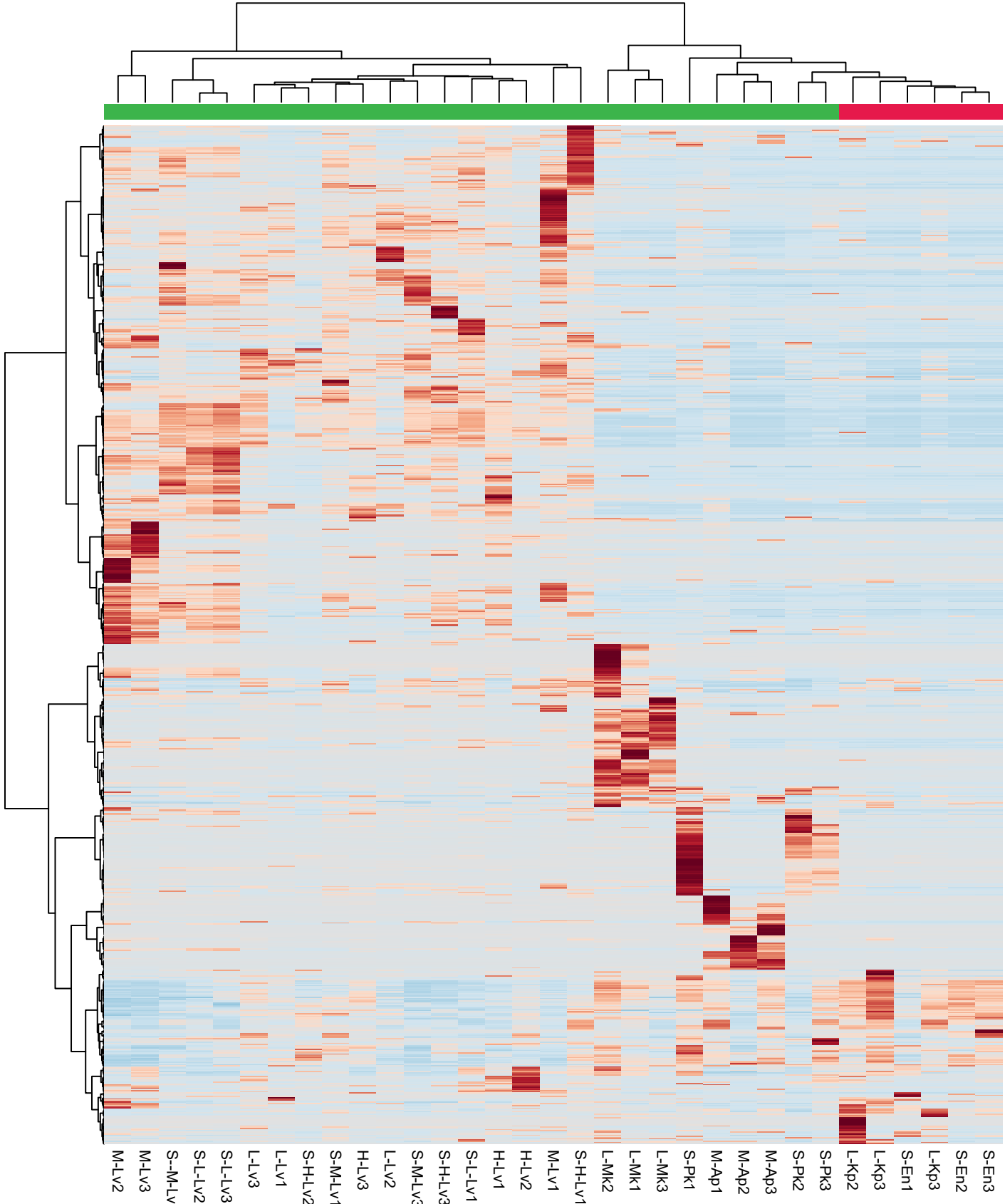
Notes: Freq: the frequency of plant sample survey; √ represented the collected samples. L, lightly degraded grassland; M, moderately degraded grassland; H, heavily degraded grassland; S, degraded sown grassland. *Elymus nutans* and *Kobresia pygmaea* are the non-toxic plants.











class
Non-toxic plants
Toxic plants



