10.1071/SR23133

Soil Research

Supplementary Material

Alpine meadow degradation decreases soil P availability by altering *phoD*-harbouring bacterial diversity

Yanuo Zou[A,B], *Xiangtao Wang*[C], *Jie Wang*[D], *Lu Zhang*[A,B], *Lirong Liao*[A,B], *Guobin Liu*[A,B], *Zilin Song*[E], *and Chao Zhang*[A,B,*]

[A]State Key Laboratory of Soil Erosion and Dryland Farming on the Loess Plateau, Northwest A&F University, Shaanxi, 712100, P. R. China.

[B]Institute of Soil and Water Conservation, Chinese Academy of Science, Shaanxi, 712100, P. R. China.

[C]College of Animal Science, Tibet Agriculture and Animal Husbandry University, Nyingchi, 860000, P. R. China.

[D]College of Forestry, Guizhou University, Guiyang, 550025, P. R. China.

[E]College of Natural Resources and Environment, Northwest A&F University, Shaanxi, 712100, P. R. China.

[*]Correspondence to: Chao Zhang State Key Laboratory of Soil Erosion and Dryland Farming on the Loess Plateau, Northwest A&F University, Shaanxi, 712100, P. R. China Email: zhangchao1985@nwafu.edu.cn

supplementary materials

Method of calculation of $TER_{C:N}$ and $TER_{C:P}$:

$$TER_{C:N} = (BG/NAG)B_{C:N}/n_o, \tag{1}$$

$$TER_{C:P} = (BG/ALP)B_{C:P}/po, \tag{2}$$

where BG/NAG is the ecoenzymatic activity ratio for β-1,4-glucosidase and β-1,4-N-acetylglucosaminidase, BG/ALP is the ecoenzymatic ratio for β-1,4-glucosidase, and ALP, $B_{C:N}$ and $B_{C:P}$ are the C:N or C:P ratios of the microbial biomass, respectively, and p_0 and n_0 are dimensionless normalization constants for N and P, respectively. The normalization constants p_0 and n_0 are the intercepts in the standardized major axis regression plots for $\log_e$(BG) vs. $\log_e$(NAG) and $\log_e$(BG) vs. $\log_e$(ALP), respectively. For a more detailed analysis of the derivations of these equations, refer to Sinsabaugh *et al.* (2009).

In this study, $TER_{C:N}$ and $TER_{C:P}$ was calculated to determine whether P is a limiting nutrient element for microbial growth, which was widely used in nutrient stoichiometry studies (Tapia-Torres *et al*. 2015; Montiel-González *et al*. 2017; Cui *et al*. 2018; Xiao *et al*. 2020). According to Sterner and Elser (2002), If the C: N or C: P ratio of the organic matter being consumed is greater than the TER for that element, it suggests nutrient limitation.

Cui, Y, Fang, L, Guo, X, Wang, X, Zhang, Y, Li, P, Zhang, X (2018) Ecoenzymatic stoichiometry and microbial nutrient limitation in rhizosphere soil in the arid area of the northern Loess Plateau, China. *Soil Biology and Biochemistry* **116** 11-21. https://doi.org/10.1016/j.soilbio.2017.09.025

Montiel-González, C, Tapia-Torres, Y, Souza, V, García-Oliva, F (2017) The response of soil microbial communities to variation in annual precipitation depends on soil nutritional status in an oligotrophic desert. *PeerJ* **5** 10.7717/peerj.4007

Spohn, M, Kuzyakov, Y (2013) Distribution of microbial- and root-derived phosphatase activities in the rhizosphere depending on P availability and C allocation – Coupling soil zymography with 14C imaging. *Soil Biology and Biochemistry* **67** 106-113. https://doi.org/10.1016/j.soilbio.2013.08.015

Sterner, J.J. Elser, R.W. (2002) Ecological Stoichiometry: the Biology of Elements from Molecules to the Biosphere, Princeton University Press, Princeton. 1-43

Tapia-Torres, Y, Elser, JJ, Souza, V, García-Oliva, F (2015) Ecoenzymatic stoichiometry at the extremes: How microbes cope in an ultra-oligotrophic desert soil. *Soil Biology and Biochemistry* **87** 34-42. https://doi.org/10.1016/j.soilbio.2015.04.007

Xiao, L, Liu, G, Li, P, Li, Q, Xue, S (2020) Ecoenzymatic stoichiometry and microbial nutrient limitation during secondary succession of natural grassland on the Loess Plateau, China. *Soil and Tillage Research* **200** 104605. https://doi.org/10.1016/j.still.2020.104605

Table S1 Soil physicochemical properties in four meadows along the degradation gradient

Parameters	ND	LD	MD	SD	F	P
NO_3^--N ($mg\cdot kg^{-1}$)	13.05±1.07a	6.74±0.21b	5.32±0.64bc	3.69±0.47c	36.98	<0.001
NH_4^+-N ($mg\cdot kg^{-1}$)	6.97±0.73a	3.49±0.35b	3.72±0.36b	3.290.45b	12.40	<0.001
Total nitrogen ($g\cdot kg^{-1}$)	2.42±0.19a	1.77±0.05b	1.30±0.02c	1.06±0.09c	29.08	<0.001
Dissolved organic carbon ($mg\cdot kg^{-1}$)	664.22±13.47a	496.34±9.92b	427.84±9.56c	327.48±7.73d	186.25	<0.001
Dissolved organic nitrogen ($mg\cdot kg^{-1}$)	195.66±7.65a	147.46±8.19b	96.10±4.50c	62.46±4.14d	83.83	<0.001
Soil organic carbon ($g\cdot kg^{-1}$)	33.51±3.49a	19.46±0.61b	13.57±0.41c	10.94±0.88c	30.02	<0.001
Available potassium ($mg\cdot kg^{-1}$)	116.96±11.19a	106.23±4.48a	95.52±10.38a	95.98±6.54a	1.39	0.280
pH	6.43±0.04a	6.65±0.03b	6.78±0.02bc	6.90±0.07c	21.14	<0.001
Soil moisture (%)	23.35±1.19a	22.88±0.55a	18.37±0.57b	15.50±0.33c	26.25	<0.001
C/N	13.73±0.45a	11.01±0.11b	10.46±0.23b	10.37±0.13b	34.74	<0.001

Values are means ± standard error (n = 5). Different letters indicate significant differences between different treatments (Duncan's test, $P < 0.05$). ND: non-degraded meadow; LD: lightly degraded meadow; MD: moderately degraded meadow; SD: severe degraded meadow, C/N: the ratio of soil organic carbon and total nitrogen.

Table S2 Plant properties in four meadows along the degradation gradient

Parameters	ND	LD	MD	SD	*F*	*P*
Richness	8.20±1.10a	7.40±1.67a	12.20±0.84b	8.20±0.84a	17.38	<0.001
Plant Shannon index	0.94±0.08a	1.42±0.20b	1.77±0.10c	1.64±0.17c	31.64	<0.001
Above ground biomass	178.38±14.27a	253.28±33.61b	167.35±23.80a	45.77±28.26c	54.69	<0.001
Below ground biomass	894.05±49.55a	866.25±58.96a	593.86±114.11b	158.98±89.58c	86.17	<0.001
Biomass	1072.43±62.38a	1119.53±76.38a	761.21±134.49b	204.75±113.69c	87.01	<0.001
Coverage	94.20±2.77a	83.00±3.47b	74.00±2.35c	39.40±4.05d	268.67	<0.001

Values are means ± standard error (n = 5). Different letters indicate significant differences between different treatments (Duncan's test, $P < 0.05$). ND: non-degraded meadow; LD: lightly degraded meadow; MD: moderately degraded meadow; SD: severe degraded meadow.

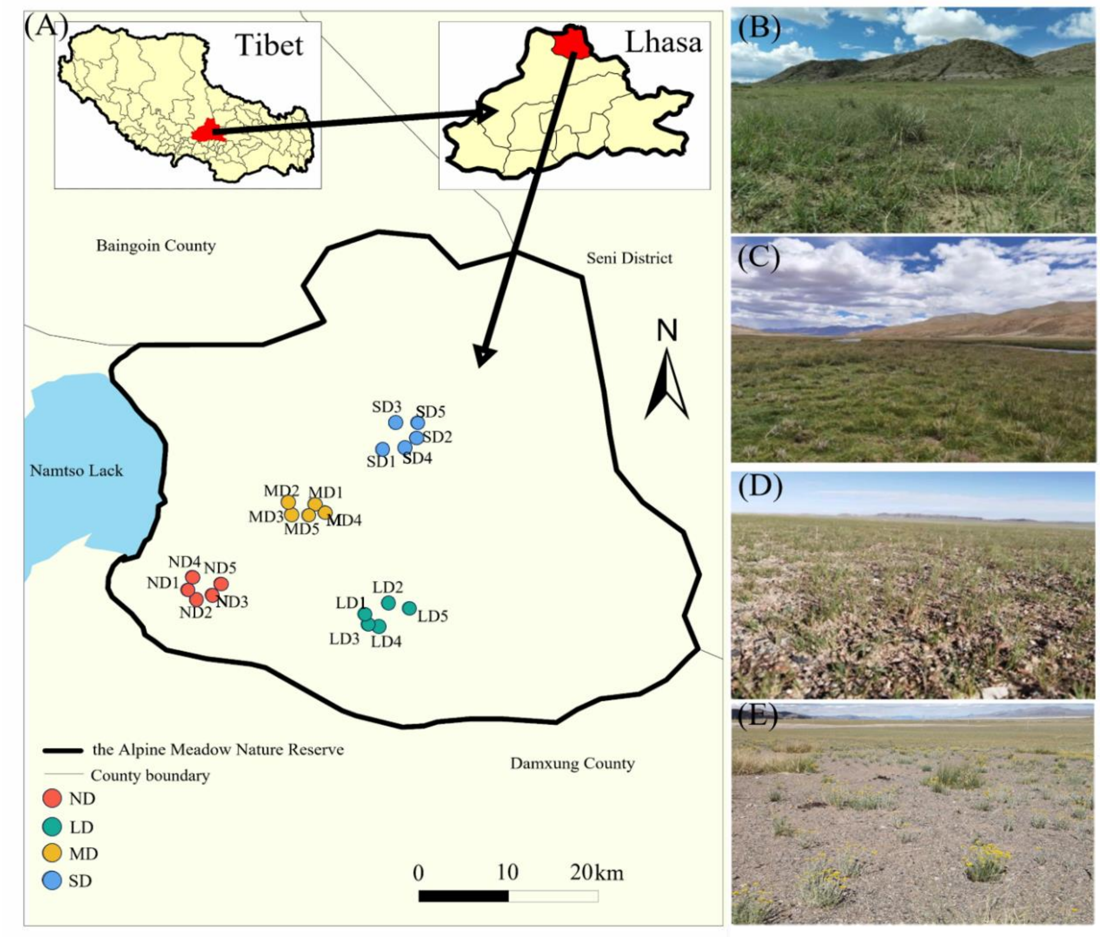

Fig. S1 Sampling sites in the Alpine Meadow nature Reserve (A) and the landscape of ND: non-degraded Meadow (B), LD: lightly degraded meadows (C), MD: moderately degraded meadows (D) and SD: severely degraded meadows (E).

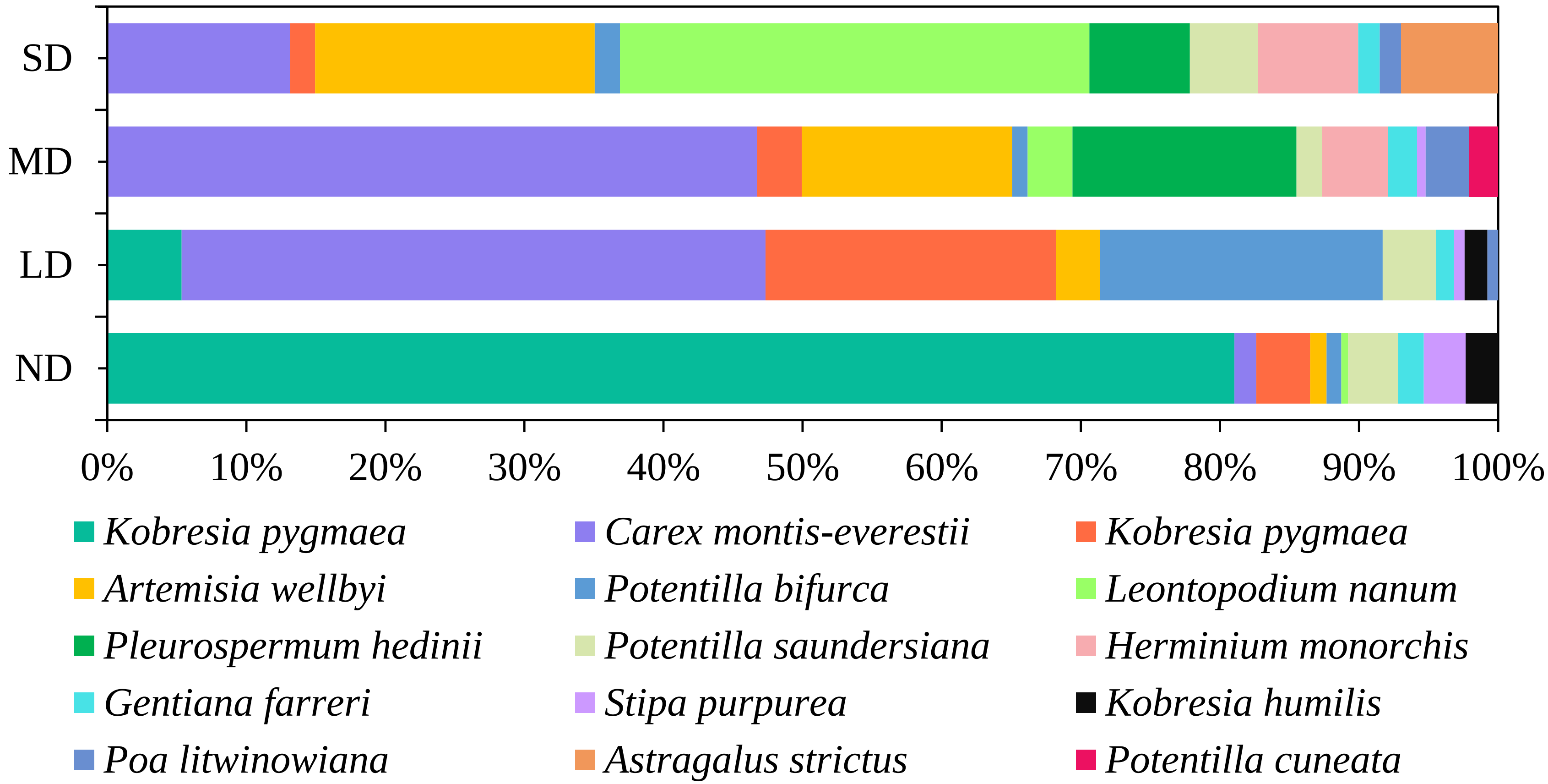

Fig. S2 The dominate vegetations of the four meadows. ND: non-degraded meadow; LD: lightly degraded meadow; MD: moderately degraded meadow; SD: severe degraded meadow.

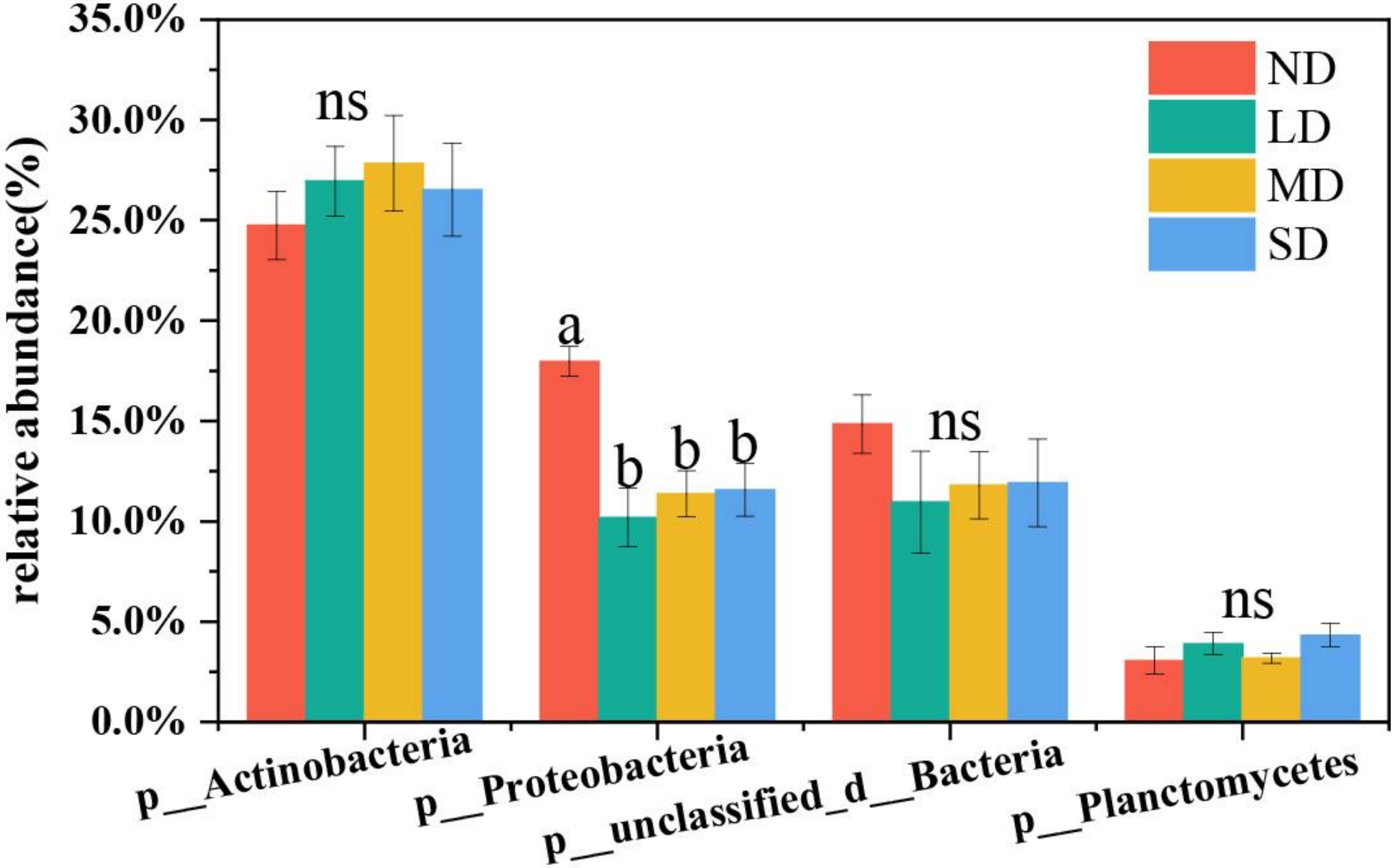

Fig. S3 the composition of *phoD*-harboring bacterial community at phylum level; ND: non-degraded meadow; LD: lightly degraded meadow; MD: moderately degraded meadow; SD: severe degraded meadow.