10.1071/SR21097

Soil Research

Supplementary Material

Effects of alpine meadow degradation on nitrifying and denitrifying microbial communities, and N2O emissions on the Tibetan Plateau

Lu Zhang[A,B], *Xiangtao Wang*[C], *Jie Wang*[D], *Qian Wan*[D], *Lirong Liao*[A,B], *Guobin Liu*[A,D], *and Chao Zhang*[A,D,*]

[A]State Key Laboratory of Soil Erosion and Dryland Farming on the Loess Plateau, Institute of Soil and Water Conservation, Chinese Academy of Sciences and Ministry of Water Resources, Yangling 712100, PR China.

[B]University of Chinese Academy of Sciences, Beijing 100049, PR China.

[C]Department of Animal Sciences, Xizang Agriculture and Animal Husbandry College, Linzhi 860000, PR China.

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

[*]Correspondence to: Chao Zhang Institute of Soil and Water Conservation, Chinese Academy of Sciences and Ministry of Water Resources, Yangling 712100, PR China Email: zhangchaolynn@163.com

Table S1. Primer pairs and thermal cycling conditions used for real-time quantitative PCR (qPCR) and amplicon sequencing.

Gene	Encoded protein	Primer sequence	Sequence (5'-3')	Thermal profile	Reference
amoA-AOA	ammonia monooxygenase α subunit (Archaea)	Arch-amoAF	STAATGGTCTGGCTTAGACG	95°C/5min; 35 cycles of 95°C/60s, 58°C/30s, 72°C/60s	(Francis *et al.* 2005)
		Arch-amoAR	GCGGCCATCCATCTGTATGT		
amoA-AOB	ammonia monooxygenase α subunit (Bacteria)	amoA-1F	GGGGTTTCTACTGGTGGT	95°C/5min; 35 cycles of 95°C/60s, 60°C/30s, 72°C/60s	(Rotthauwe *et al.* 1997)
		amoA-2R	CCCCTCKGSAAAGCCTTCTTC		
nirS	nitrite reductase	Cd3aF	GTSAACGTSAAGGARACSGG	95°C/5min; 35 cycles of 95°C/60s, 58°C/30s, 72°C/60s	(Palmer *et al.* 2012)
		R3CdR	GASTTCGGRTGSGTCTTGA		
nirK	copper-containing nitrite reductase	FlaCu	ATCATGGTSCTGCCGCG	95°C/5min; 35 cycles of 95°C/60s, 60°C/30s, 72°C/60s	(Hallin and Lindgren 1999)
		R3Cu	GCCTCGATCAGRTTRTGGTT		
nosZ	cytochrome cd1-containing nitrite reductase	nosZ-1F	CGYTGTTCMTCGACAGCCAG	95°C/5min; 35 cycles of 95°C/60s, 58°C/30s, 72°C/60s	(Henry *et al.* 2006)
		nosZ-1622R	CGSACCTTSTTGCCSTYGCG		

Table S2. Geographical characteristic and plant composition of study sites. ND, non-degraded meadow; LD, lightly degraded meadow; MD, moderately degraded meadow; SD, severely degraded meadow.

Degradation level	Latitude (N)	Longitude (E)	Altitude (m)	Dominant Species
ND	30°45′19″	91°03′23″	4456	*Kobresia pygmaea* Clarke., *Stipa capillacea* Keng., *Stipa purpurea* Griseb., *Potentilla saundersiana* Royle., *Kobresia humilis* (C. A. Mey ex Trauvt.) *Sergievskaya*.
LD	30°43′48″	91°07′41″	4389	*Stipa capillacea* Keng., *Carex montis-everestii* Hillebr., *Potentilla saundersiana* Royle., *Potentilla bifurca* L.
MD	30°49′31″	91°09′17″	4350	*Leontopodium nanum* Hand.-Mazz., *Pleurospermum hedinii* Diels., *Artemisia wellbyi* Hemsl. et Pears. Ex Deasy
SD	30°52′15″	91°12′32″	4348	*Leontopodium nanum* Hand.-Mazz., *Artemisia wellbyi* Hemsl. et Pears. ex Deasy

Table S3. Vegetation characteristics in four meadows. Values are means ± standard error (n = 5). ND, non-degraded meadow; LD, lightly degraded meadow; MD, moderately degraded meadow; SD, severely degraded meadow. MDI, meadow degradation index.

	ND	LD	MD	SD
Total coverage	94.20 ± 1.24	83.00 ± 1.55	74.00 ± 1.05	39.40 ± 1.81
The proportion of the grassland productivity (%)	96.70 ± 3.30	69.20 ± 1.88	46.8 ± 1.98	22.8 ± 1.07
The proportion of the plants (%)	65.60 ± 2.84	60.80 ± 3.54	40.20 ± 4.53	26.40 ± 1.03
The height of the plants(cm)	35.20 ±1.93	24.80 ±1.71	21.00 ± 1.41	7.00 ± 0.71
MDI	5.97 ± 1.03	23.21 ± 1.41	36.06 ± 0.89	59.67 ± 0.82

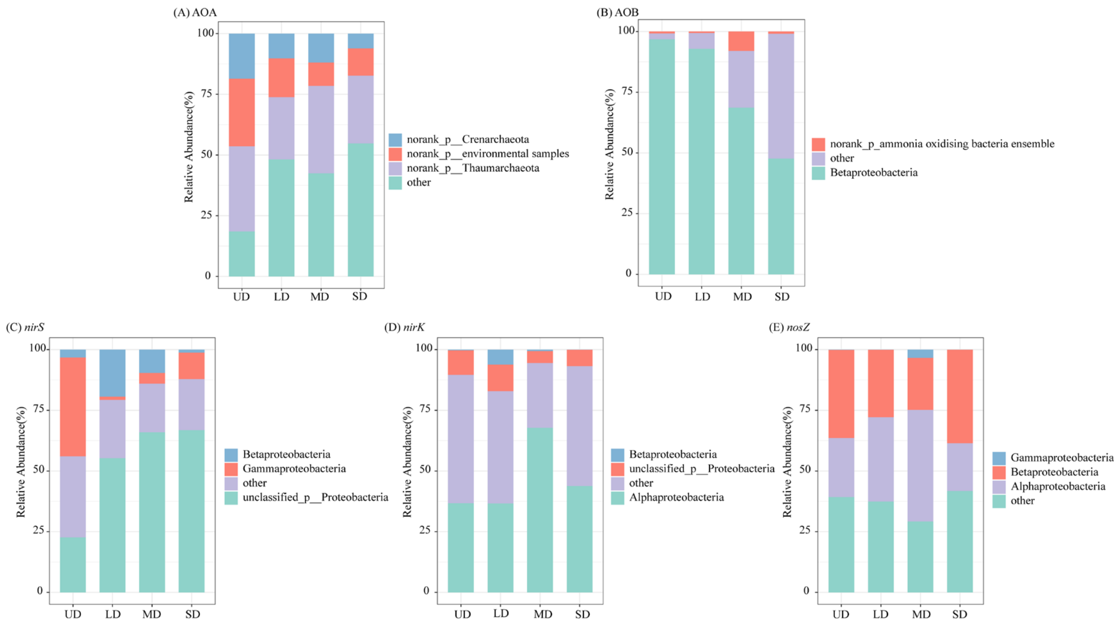

Fig. S1. Community composition of (A) AOA, (B) AOB, (C) *nirS*, (D) *nirK*, (E) *nosZ* in four meadows at the class level. ND, non-degraded meadow; LD, lightly degraded meadow; MD, moderately degraded meadow; SD, severely degraded meadow.

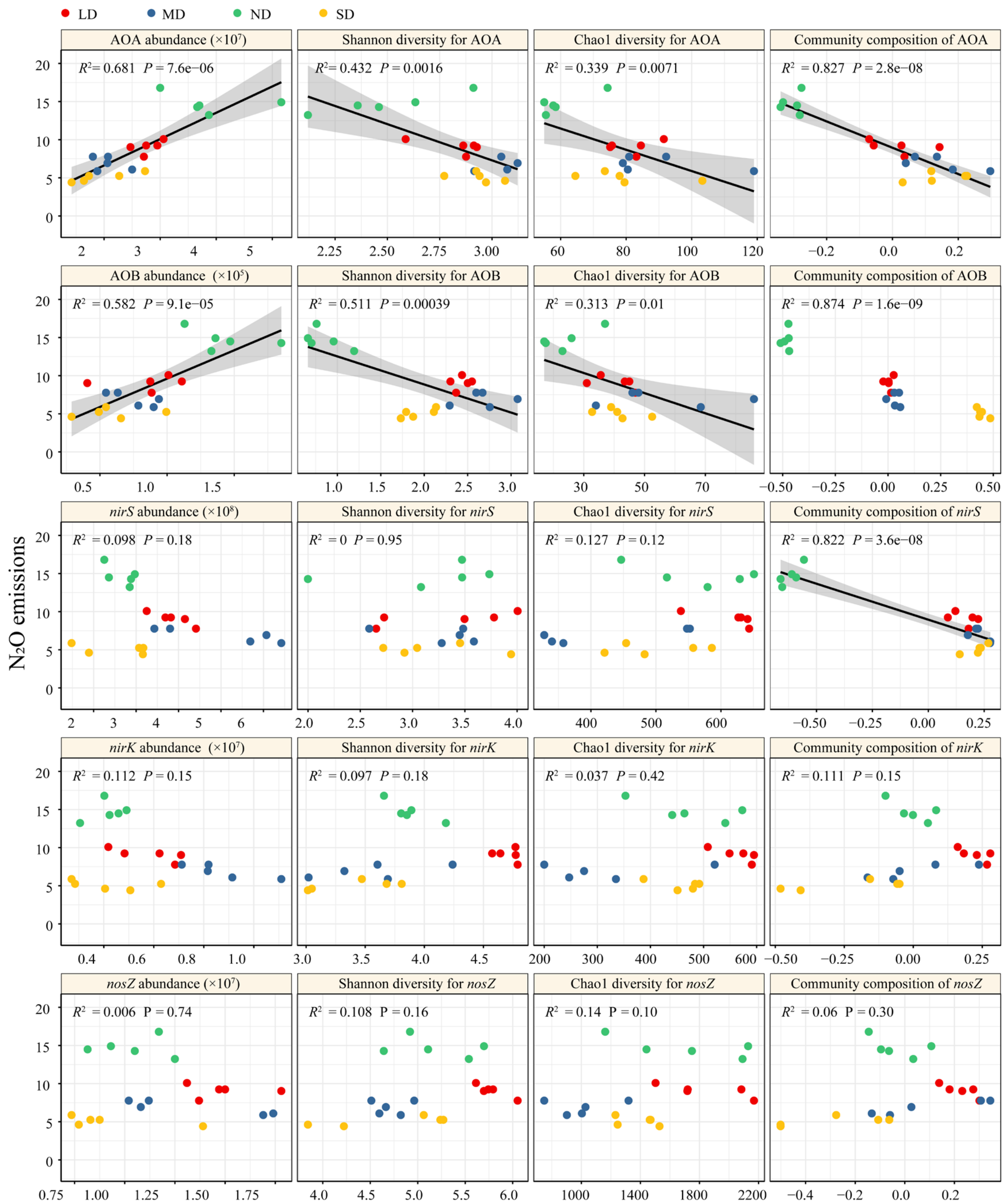

Fig. S2. Ordinary least-squares (OLSRM) regression model shows the association between microbial properties, including abundance, richness, diversity and community composition, and N_2O emission potential. The gray shaded area shows the 95% confidence interval of the fit. ND: non-degraded meadow; LD: lightly degraded meadow; MD: moderately degraded meadow; SD: severely degraded meadow.

References

Francis, CA, Roberts, KJ, Beman, JM, Santoro, AE, Oakley, BB (2005) Ubiquity and diversity of ammonia-oxidizing archaea in water columns and sediments of the ocean. *Proceedings of the National Academy of Sciences of the United States of America* **102** (41), 14683-14688. doi:10.1073/pnas.0506625102

Hallin, S, Lindgren, PE (1999) PCR detection of genes encoding nitrile reductase in denitrifying bacteria. *Applied and Environmental Microbiology* **65** (4), 1652-1657.

Henry, S, Bru, D, Stres, B, Hallet, S, Philippot, L (2006) Quantitative detection of the nosZ gene, encoding nitrous oxide reductase, and comparison of the abundances of 16S rRNA, narG, nirK, and nosZ genes in soils. *Applied and Environmental Microbiology* **72** (8), 5181-5189. doi:10.1128/aem.00231-06

Palmer, K, Biasi, C, Horn, MA (2012) Contrasting denitrifier communities relate to contrasting N2O emission patterns from acidic peat soils in arctic tundra. *Isme Journal* **6** (5), 1058-1077. doi:10.1038/ismej.2011.172

Rotthauwe, JH, Witzel, KP, Liesack, W (1997) The ammonia monooxygenase structural gene amoA as a functional marker: Molecular fine-scale analysis of natural ammonia-oxidizing populations. *Applied and Environmental Microbiology* **63** (12), 4704-4712. doi:10.1128/aem.63.12.4704-4712.1997