

Supplementary material

Table S1. Primers and amplification conditions for the quantification of nitrifying and denitrifying bacterial indicator genes in the soil samples collected from the grazing experiment.

Organism or group	Target gene	Primer	Primer sequence (5'-3') [†]	Tm [‡] (°C)	Product size (bp) [§]	Assay type
All bacteria	16s rRNA	Forward	ATGGCTGTCGTCAGCT	58	337	Taqman
		Reverse	ACGGGCGGTGTGTAC			
		Probe	CAACGAGCGCAACCC			
Ammonia-oxidizing bacteria	<i>amoA</i>	Forward	TCGGTAGCYGACTACACSGG	56	204	SYBR
		Reverse	CYTTKACRTAGTAGAAAGCGG			
Nitrate reducing bacteria	<i>narG</i>	Forward	GAYTTCCGCATGAGYAC	60	69	SYBR
		Reverse	TTYTCGTACCAGGTGGC			
Nitrite reducing bacteria	<i>nirK</i>	Forward	ATCATGGTSCTGCCGCG	57	472	SYBR
		Reverse	GCCTCGATCAGRTTGTGGTT			
Nitrous oxide reducing bacteria	<i>nosZ</i>	Forward	CGYTGTTCMTCGACAGCCAG	53	200	SYBR
		Reverse	CGGTCCTTGGAGAACTTG			

*Tm is the annealing temperature at which the PCR assay was performed

[†] Probe sequences each contained a 5' FAM fluorophore and 3' Black Hole Quencher combination for use in probe-based 5' nuclease assays.

Probe concentration of 100nM; primer concentration of 600nM

[‡] Tm. (°C) is the annealing temperature of the PCR reaction.

[§] Product size refers to the expected amplification product size in nucleotide base pairs (bp).

Assay type refers to the type of PCR assay used: TaqMan® and SYBR® are quantitative, real-time PCR assays run on the Bio-rad CFX 96 Real-time PCR Detection System (Bio-Rad, Hercules, CA).

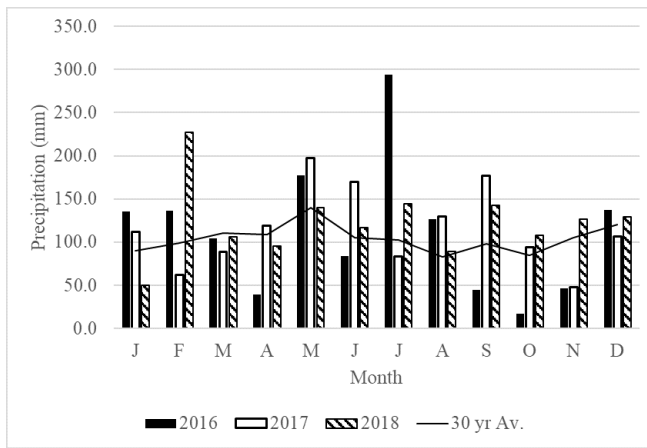
Table S2. Summary of step-up multiple linear regression between abundance of nitrogen cycling functional genes and soil properties (n=48).

Gene	Explanatory variables	r ²	n
16S RNA gene			
TF	soil OM, NH ₄ -N	0.15*	48
WS/WGS	soil pH, OM	0.12*	92
amoA gene			
TF	soil OM, pH, NO ₃ -N	0.22*	48
WS/WGS	soil pH, OM	0.35**	92
narG gene			
TF	soil OM, pH, NO ₃ -N	0.35**	48
WS/WGS	soil pH, OM	0.25**	92
nirK gene			
TF	soil OM, NH ₄ -N	0.30**	48
WS/WGS	soil pH, NH ₄ -N	0.35**	92
nosZ gene			
TF	soil OM, NH ₄	0.29**	48
WS/WGS	soil pH, NH ₄ -N	0.40**	92

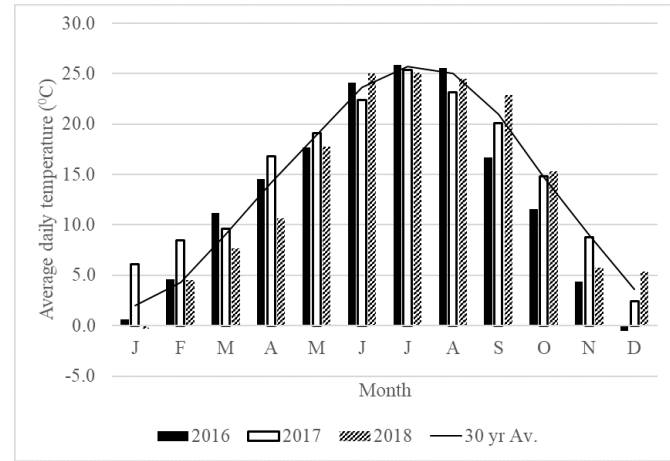
* Model significant (p < 0.05) ** Model significant (p < 0.001)

Table S3. Semi-variogram parameters of total and nitrogen cycling bacteria functional genes and mean soil pH (n = 36).

Bacterial gene/ Soil properties	Range (m)	Sill	Nugget	RMSE	Variogram model
16S rRNA	161.6	0.51	0.77	0.30	Gaussian
amoA	163.1	0.19	0.59	0.19	Spherical
narG	157.6	0.10	0.54	0.31	Gaussian
nirK	174.4	0.39	0.69	0.33	Spherical
nosZ	141.8	0.41	0.70	0.32	Spherical
Soil pH	186.8	0.24	0.61	0.31	Spherical



(a)



(b)

Figure S1. Mean monthly precipitations (a) and temperatures (b) of the study site during the experimental period.