Supplementary Material

Microbial genes highlight different trends in short term for N cycling in historical alpine pastures

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Table S1. ANOVA and post-hoc test based on permutation test analyzing the abundance of 16S, nifH, nirK, nosZ, archaeal amoA (AOA) and eubacterial amoA (AOB) genes.

	16S				*nifH*				*nirK*			
	Df	Sum Sq	Pr(Prob)		Df	Sum Sq	Pr(Prob)		Df	Sum Sq	Pr(Prob)	
grazing	1	0.08	0.75		1	0.025	0.7451		1	0.21	0.51	
sampling time	3	0.67	0.09	.	3	8.33	<2e-16	***	3	4.48	0.030	*
grazing:sampling time	3	0.08	0.77		3	0.15	0.9433		3	0.15	0.99	
pasture area	2	0.80	0.10		2	0.90	0.4533		2	0.85	0.47	
grazing:pasture area	2	0.30	0.21		2	0.010	1		2	0.21	0.96	
Sampling time:pasture area	6	0.18	1.00		6	2.12	0.632		6	1.49	0.67	
Residuals	54	7.68			54	21.62			54	25.99		

	nosZ				*amoA* AOA				*amoA* AOB			
	Df	Sum Sq	Pr(Prob)		Df	Sum Sq	Pr(Prob)		Df	Sum Sq	Pr(Prob)	
grazing	1	5.27	0.027	*	1	0.79	0.56		1	0.93	0.58	
sampling time	3	5.83	0.14		3	4.94	0.60		3	8.84	0.58	
grazing:sampling time	3	0.69	1		3	3.07	0.95		3	2.83	0.73	
pasture area	2	9.18	0.0088	**	2	3.21	0.74		2	9.93	0.19	
grazing:pasture area	2	2.69	0.16		2	0.12	0.90		2	14.16	0.12	
Sampling time:pasture area	6	5.95	0.33		6	10.69	0.93		6	7.23	0.91	
Residuals	54	51.60			54	191.07			54	182.97		

nosZ			*nifH*			*nirK*		
Pasture area	means	groups	Sampling time	means	groups	Sampling time	means	groups
Juribello	4.72	ab	T1	4.71	a	T1	4.97	ab
Vallazza	4.08	b	T2	5.02	a	T2	5.27	ab
Venegiota	4.91	a	T3	4.15	b	T3	4.77	b
			T4	4.95	a	T4	5.41	a
Grazing	means	groups						
Yes	4.84	a						
No	4.30	b						

Optimal number of clusters
Silhouette method

Average silhouette width plotted against Number of clusters k (1–8).
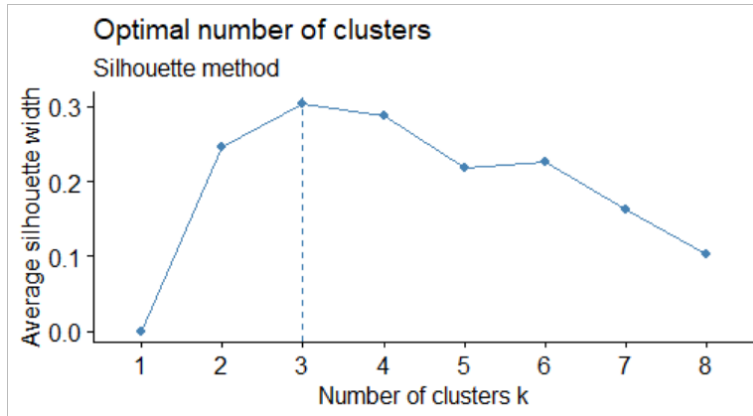
Fig. S1. Optimal number of clusters among sampling sites estimated with the Silhouette method.

Corrplot correlation matrix (lower triangle, Kendall rank's coefficient):

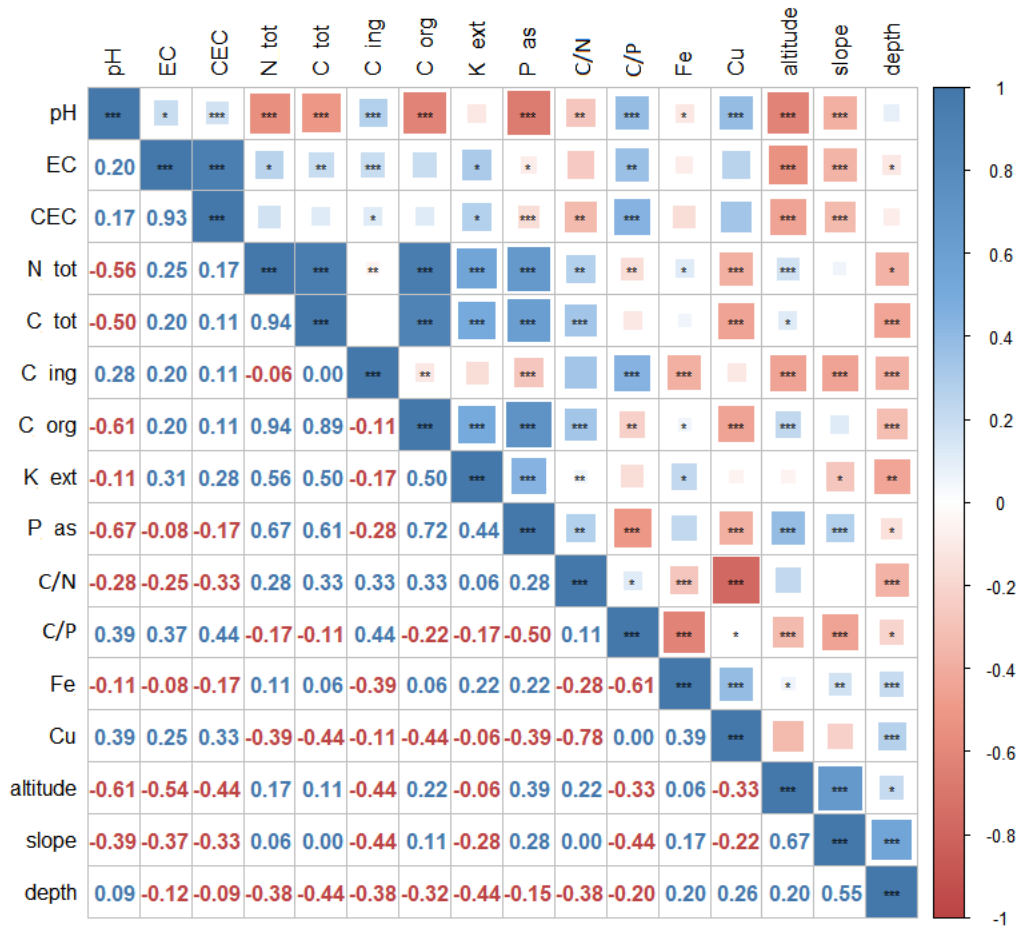|	pH	EC	CEC	N tot	C tot	C ing	C org	K ext	P as	C/N	C/P	Fe	Cu	altitude	slope	depth
pH																
EC	0.20															
CEC	0.17	0.93														
N tot	-0.56	0.25	0.17													
C tot	-0.50	0.20	0.11	0.94												
C ing	0.28	0.20	0.11	-0.06	0.00											
C org	-0.61	0.20	0.11	0.94	0.89	-0.11										
K ext	-0.11	0.31	0.28	0.56	0.50	-0.17	0.50									
P as	-0.67	-0.08	-0.17	0.67	0.61	-0.28	0.72	0.44								
C/N	-0.28	-0.25	-0.33	0.28	0.33	0.33	0.33	0.06	0.28							
C/P	0.39	0.37	0.44	-0.17	-0.11	0.44	-0.22	-0.17	-0.50	0.11						
Fe	-0.11	-0.08	-0.17	0.11	0.06	-0.39	0.06	0.22	0.22	-0.28	-0.61					
Cu	0.39	0.25	0.33	-0.39	-0.44	-0.11	-0.44	-0.06	-0.39	-0.78	0.00	0.39				
altitude	-0.61	-0.54	-0.44	0.17	0.11	-0.44	0.22	-0.06	0.39	0.22	-0.33	0.06	-0.33			
slope	-0.39	-0.37	-0.33	0.06	0.00	-0.44	0.11	-0.28	0.28	0.00	-0.44	0.17	-0.22	0.67		
depth	0.09	-0.12	-0.09	-0.38	-0.44	-0.38	-0.32	-0.44	-0.15	-0.38	-0.20	0.20	0.26	0.20	0.55	

Fig. S2. Corrplot of pedological and morphological variables based on Kendall rank's coefficient coefficient ($* = P < 0.05$; $** = P < 0.01$; $*** = P < 0.001$).