Supplemental materials

Soil pH modulates microbial nitrogen cycling under integrated soil-crop system management across agroecosystems

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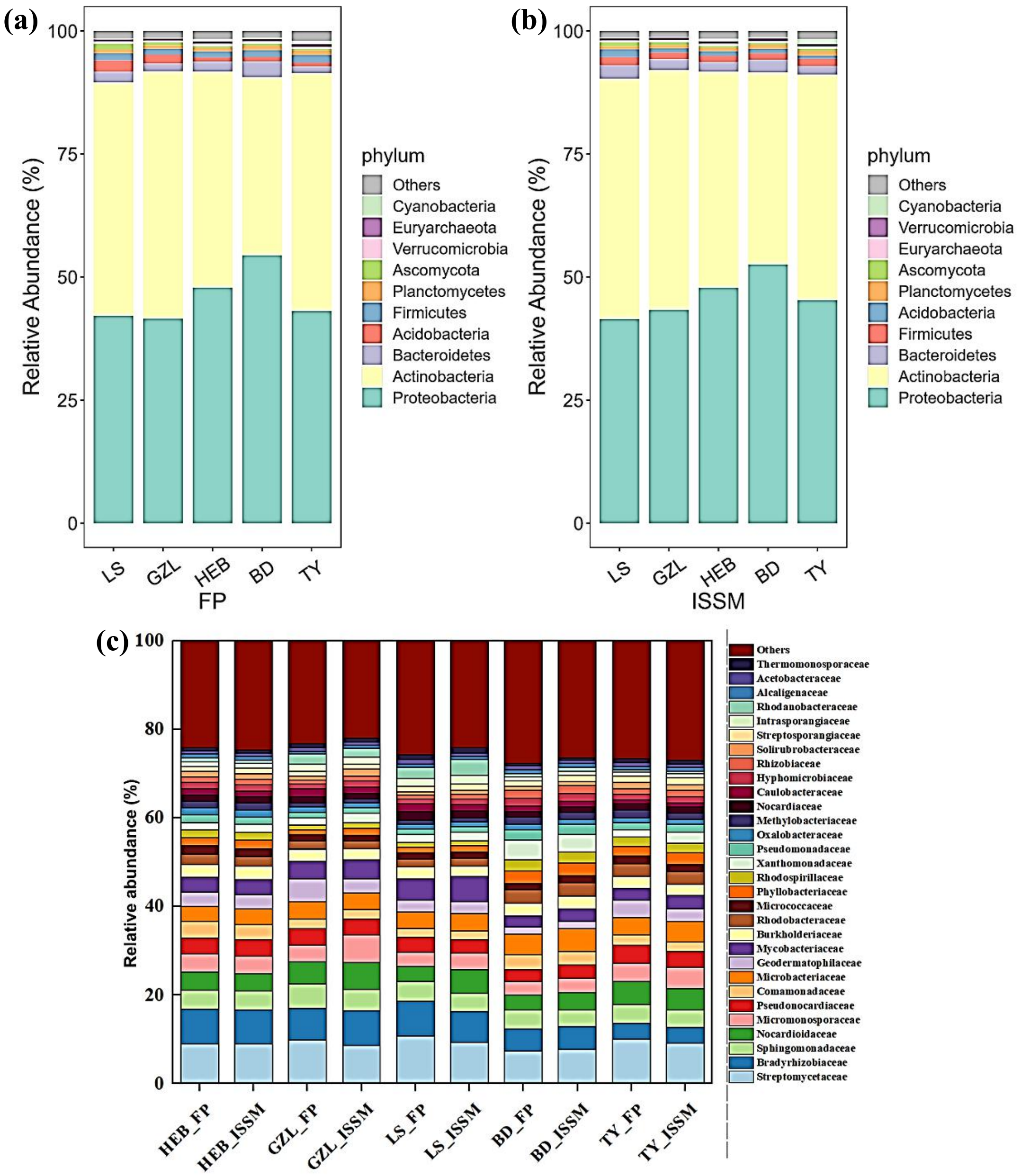

Fig. S1. Phylum- and family-level composition of soil microbial community structure under different fertilization treatments across sites with varying pH. Panels (a) and (b) show the phylum-level composition of soil microbial communities under Farmland Practice (FP) and Integrated Soil-System Management (ISSM) fertilization, respectively, while panel (c) presents the family-level composition under both FP and ISSM treatments.

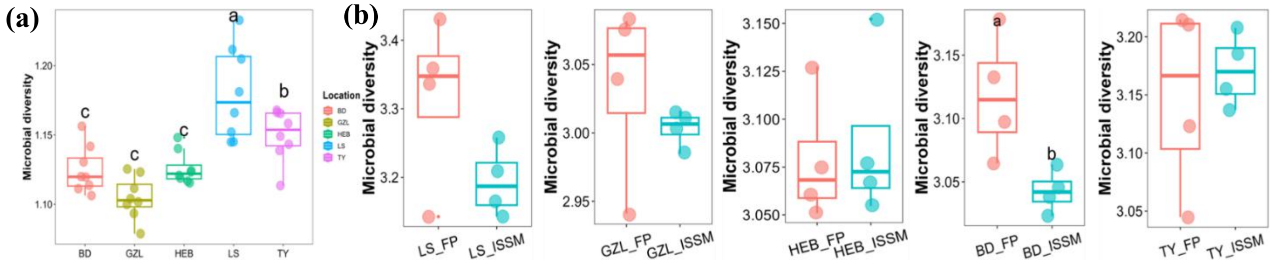

Fig. S2. Shannon Index of microbial alpha diversity across sampling sites and fertilization treatments. (a) Microbial alpha diversity across the five sampling sites, analyzed by combining the alpha diversity values from FP and ISSM treatments at each site. (b) Comparative analysis of microbial alpha diversity between FP and ISSM treatments at each sampling site based on four biological replicates: LS_FP vs. LS_ISSM, GZL_FP vs. GZL_ISSM, HEB_FP vs. HEB_ISSM, BD_FP vs. BD_ISSM, and TY_FP vs. TY_ISSM. Detailed information on the five sampling sites is provided in Table S1.

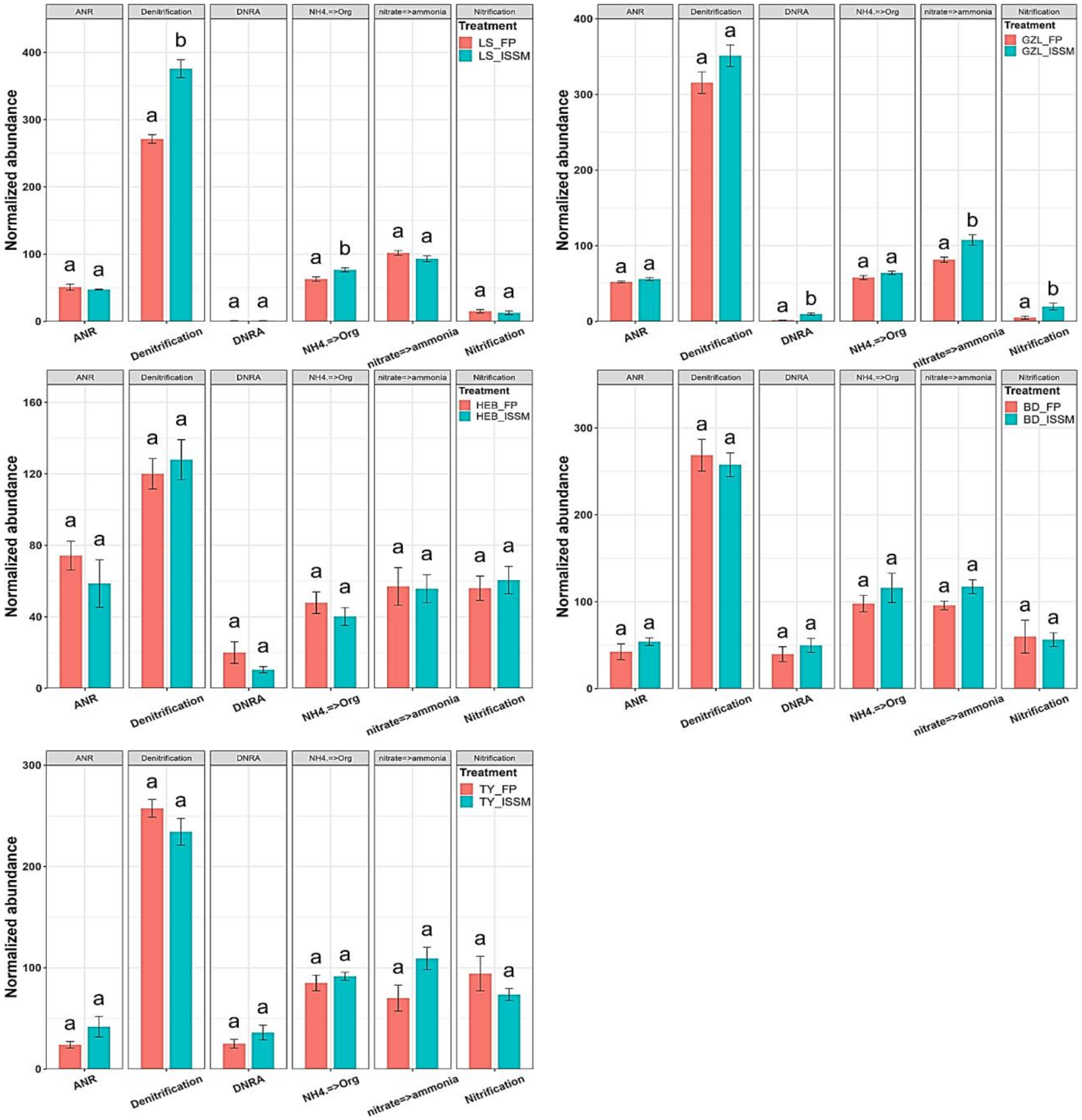

Fig. S3. Normalized collective abundance of genes pathway involved in soil nitrogen-cycling and nutrients conversion across sampling sites and fertilization treatments. Bar plots illustrate the normalized collective abundance of pathway genes associated with soil nitrogen cycling processes across five sampling sites and two fertilization treatments (FP and ISSM). Sampling sites and treatments are distinguished by soil pH differences: LS_FP vs. LS_ISSM (pH 4.8 vs. 4.9), GZL_FP vs. GZL_ISSM (pH 5.0 vs. 5.4), HEB_FP vs. HEB_ISSM (pH 6.3 vs. 6.4), BD_FP vs. BD_ISSM (pH 7.5 vs. 7.4), and TY_FP vs. TY_ISSM (pH 8.0 vs. 7.7). Pathway abundances within each treatment were calculated based on four biological replicates, with genes contributing to each pathway detailed in Fig. 4. Different lowercase letters indicate significant differences between treatments ($P_{FDR} < 0.05$).

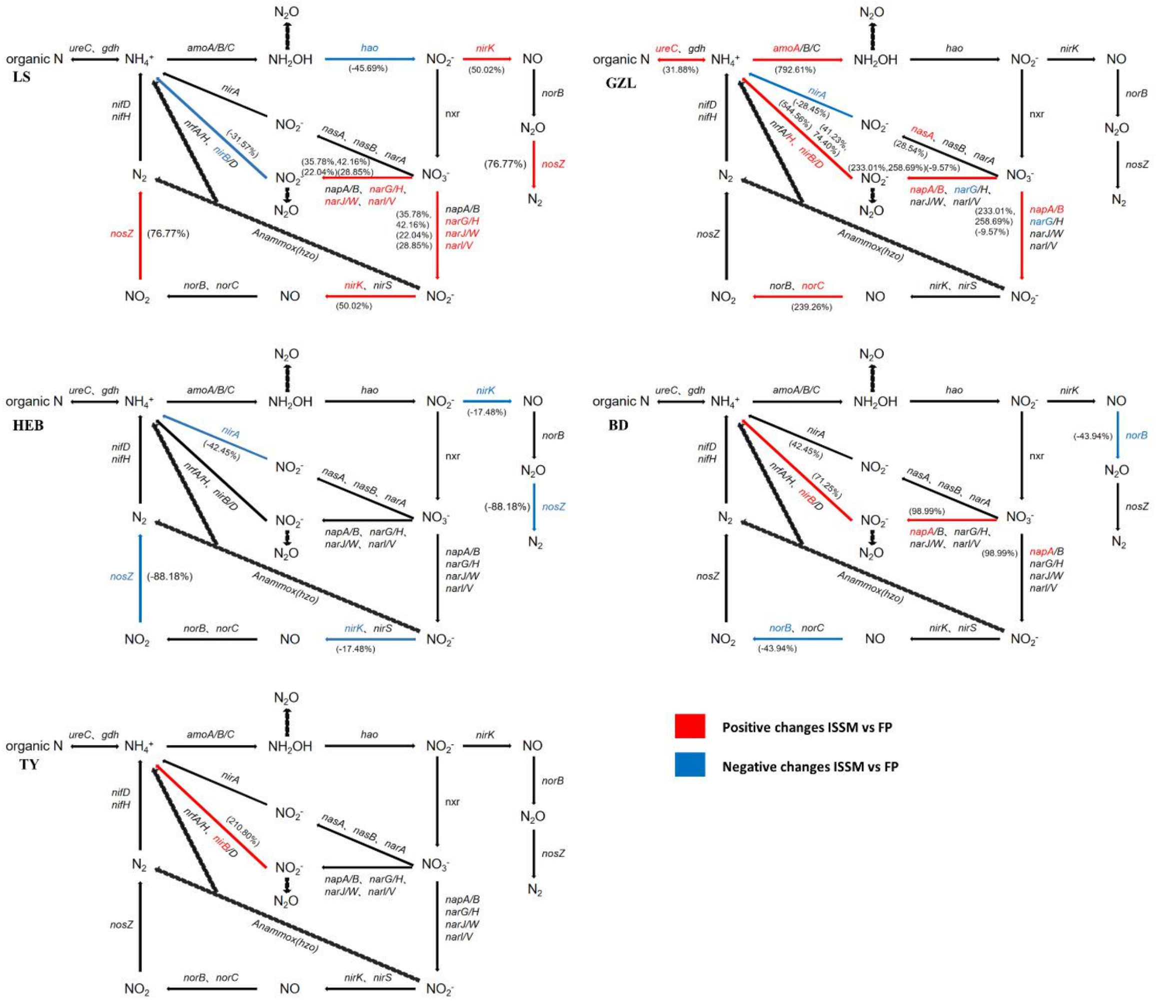

Fig. S4. Effect of fertilization treatments on metagenomic abundance of genes involved in soil nitrogen cycling across sampling sites. The figure shows the impact of the two fertilization treatments (ISSM vs. FP) on the metagenomic abundance of nitrogen-cycling genes at each of the five sampling sites (HEB, GZL, TY, LS, BD; Table S1). The abundance data are based on four biological replicates per treatment and site. Genes with significantly different abundances between the ISSM and FP treatments are highlighted in red (higher abundance under ISSM) and blue (lower abundance under ISSM). The percentage values next to pathways represent the contribution of the color-coded genes to the total pathway abundance. The exact changes in relative metagenomic abundance of each nitrogen-cycling gene between treatments at each site are shown in Fig. 4.

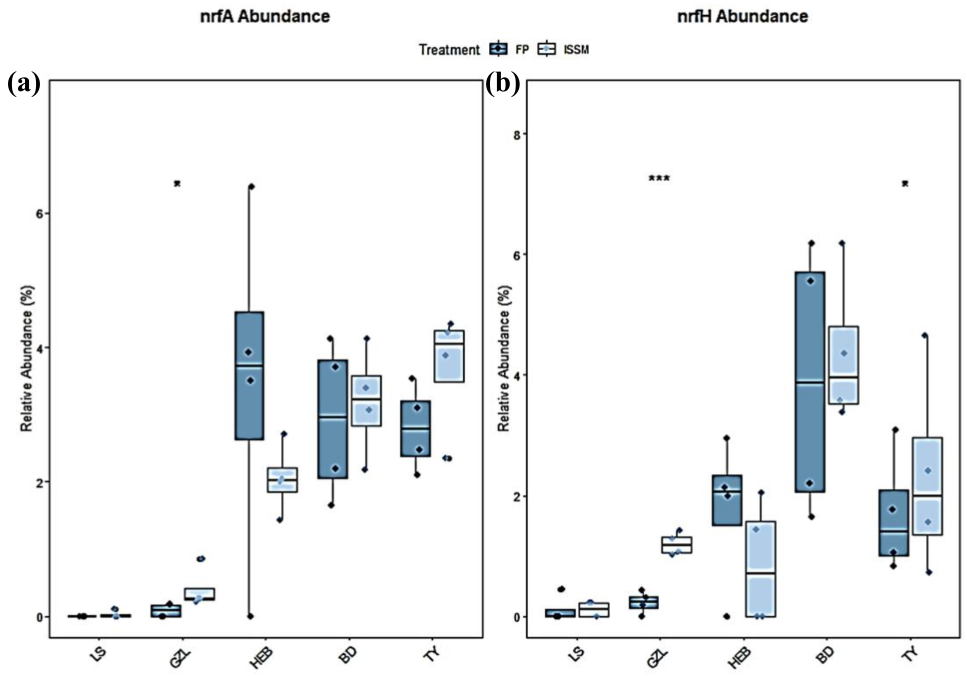

Fig. S5. Relative metagenomic abundance of (a) *nrfA* and (b) *nrfH* genes involved in dissimilatory nitrate reduction to ammonium (DNRA) across five sampling sites (LS, GZL, HEB, BD, TY) under Farmland Practice (FP) and Integrated Soil-System Management (ISSM) treatments. Significance markers indicate: * $p < 0.05$, *** $p < 0.001$ for treatment comparisons.

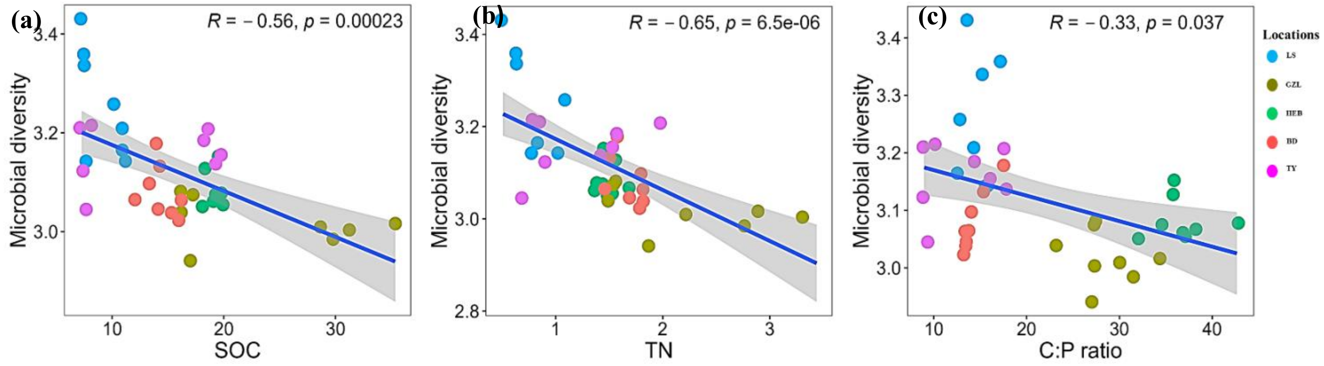

Fig. S6. Correlation analysis of microbial alpha diversity with soil properties across sampling sites and fertilization treatments. The figure presents the correlation between microbial alpha diversity and (a) soil organic carbon (SOC), (b) total nitrogen (TN), and (c) carbon-to-phosphorus (C:P) ratio across the five sampling sites (LS, GZL, HEB, BD, TY; Table S1) and two fertilization treatments (ISSM, FP). In this analysis, data from both fertilization treatments within each sampling site were combined, so each site is represented by eight biological replicate samples.

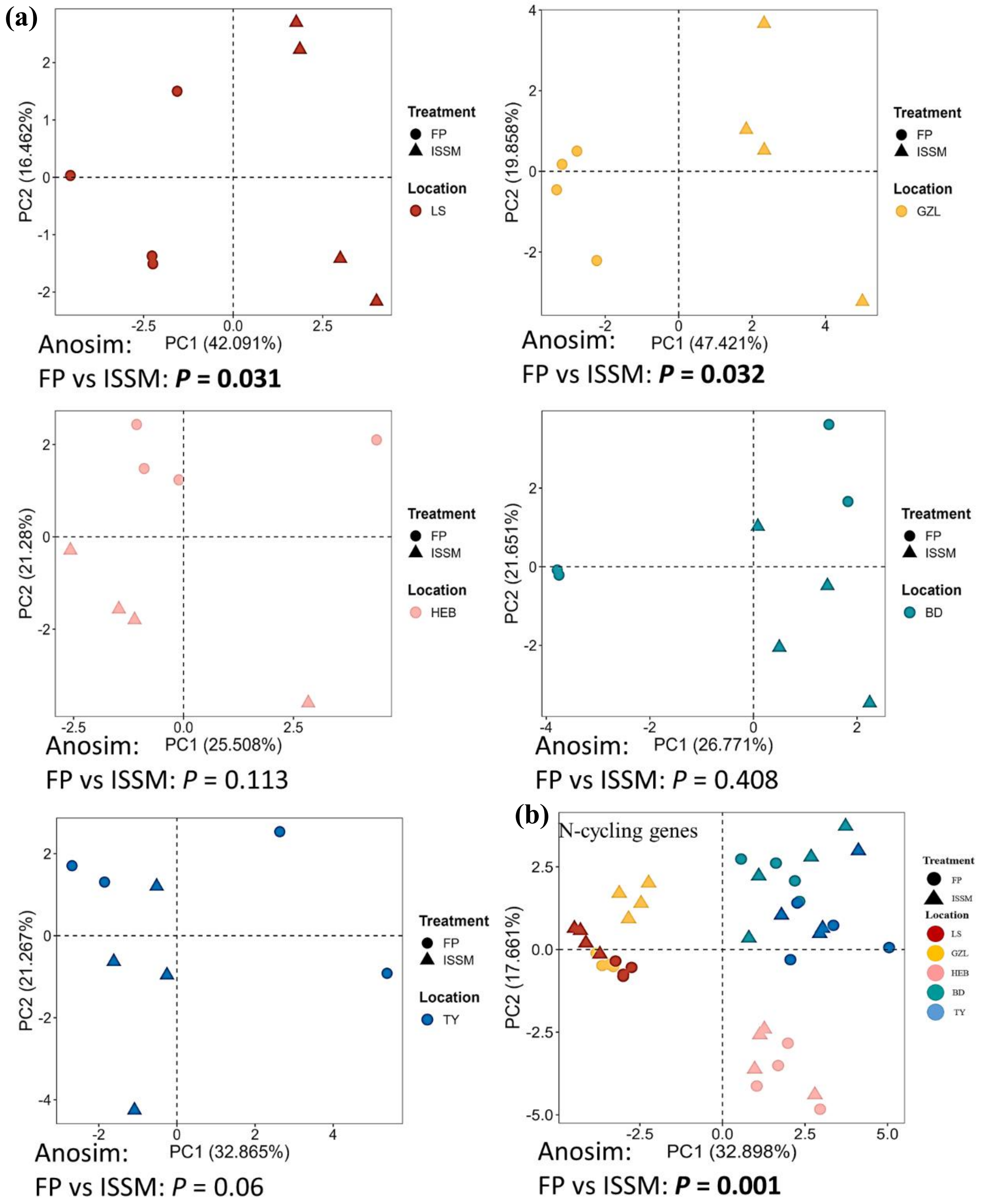

Fig. S7. Principal Component Analysis (PCA) of functional genes of nitrogen-cycling across sampling sites and fertilization treatments. (a) PCA of functional genes in FP vs. ISSM treatments, analyzed separately for each sampling site (based on four biological replicate samples per treatment). (b) Combined PCA of functional genes across both sampling sites and fertilization treatments, resulting in a total of 40 datasets (four biological replicate samples per treatment × two fertilization treatments × five sampling sites).

Table S1. Soil properties of the five study sites (LS, GZL, HEB, BD, TY) under FP and ISSM

Site_Treatment	SOC (g/kg)	TN (g/kg)	TP (g/kg)	TK (mg/g)	AP (mg/kg)	AK (mg/kg)	NO3--N (mg/kg)	NH4+-N (mg/kg)	C:N ratio	pH
LS_FP	7.53^{g}	0.63^{c}	0.49^{e}	5.18^{g}	59.08^{cd}	111.75^{f}	5.56^{de}	5.23^{bc}	12.16^{a}	4.8^{g}
LS_ISSM	10.75^{f}	0.96^{c}	0.78^{cd}	5.30^{g}	125.21^{b}	222.75^{c}	9.55^{c}	9.25^{a}	11.35^{ab}	4.9^{fg}
GZL_FP	16.67^{cd}	1.58^{b}	0.64^{de}	9.34d^{ef}	85.70^{bc}	223^{c}	3.57^{ef}	6.32^{abc}	10.63^{ab}	5.0^{f}
GZL_ISSM	31.18^{a}	2.87^{a}	1.02^{ab}	9.83^{cde}	175.04^{a}	457^{b}	14.77^{b}	5.22^{bc}	11.02^{ab}	5.4^{e}
HEB_FP	18.67^{bc}	1.58^{b}	0.54^{e}	8.54^{f}	42.47^{de}	196.5cd	1.71^{f}	4.47^{bc}	11.81^{ab}	6.3^{d}
HEB_ISSM	19.74^{b}	1.56^{b}	0.51^{e}	8.83^{ef}	42.16^{de}	187.5^{cde}	1.77^{f}	6.38^{abc}	12.73^{a}	6.4^{d}
BD_FP	13.46^{e}	1.49^{b}	0.90^{bc}	10.59^{c}	39.83^{de}	161^{def}	7.92^{cd}	3.34^{c}	9.09^{ab}	7.5^{bc}
BD_ISSM	15.44^{de}	1.73^{b}	1.15^{a}	10.35^{cd}	80.50^{cd}	200.75^{cd}	14.32^{b}	5.64^{abc}	8.97^{b}	7.4^{c}
TY_FP	7.59^{g}	0.82^{c}	0.81^{c}	11.85^{b}	11.73^{e}	133.75^{ef}	2.30^{ef}	6.81^{abc}	9.20^{ab}	8.0^{a}
TY_ISSM	18.97^{bc}	1.64^{b}	1.16^{a}	13.68^{a}	87.64^{bc}	538.5^{a}	19.43^{a}	7.66^{ab}	11.68^{ab}	7.7^{ab}

Soil properties represent mean values based on four replicates. Small letters indicate significant difference between sampling sites.