

Supplementary material

Table S1. Effects of different planting regimes on the abundance of nitrogen-cycling functional genes ($n = 3$). Values are mean \pm standard deviation.

| Pathway | Gene | F | W | PWWM | CWWM |
|-----------------|------|----------------------|----------------------|---------------------|----------------------|
| Nitrification | amoA | 130.7 \pm 11.6 b | 174.7 \pm 18.3 a | 173.3 \pm 8.7 a | 157.3 \pm 7.1 ab |
| | amoC | 124.7 \pm 7.3 b | 184 \pm 7.6 a | 174 \pm 9.2 a | 170 \pm 2 a |
| | hao | 9.3 \pm 1.3 b | 35.3 \pm 7.4 a | 35.3 \pm 7.9 a | 40 \pm 5.3 a |
| | nir | 406 \pm 31.7 b | 519.3 \pm 8.4 a | 443.3 \pm 7.1 b | 459.3 \pm 17.7 ab |
| Denitrification | narG | 836.7 \pm 37 a | 1016.7 \pm 54.1 a | 1031.3 \pm 71.7 a | 980 \pm 69.6 a |
| | nirK | 1316.7 \pm 74.4 b | 1441.3 \pm 55.3 ab | 1575.3 \pm 27.4 a | 1518.7 \pm 57.6 a |
| | nirS | 57.3 \pm 8.1 a | 39.3 \pm 11.8 a | 44.7 \pm 2.4 a | 56 \pm 2 a |
| | norB | 714.7 \pm 18.6 b | 753.3 \pm 20.8 ab | 858.7 \pm 20.3 a | 788 \pm 69.4 ab |
| DNRA | nosZ | 353.3 \pm 13 a | 306 \pm 37.6 a | 366 \pm 10.1 a | 346.7 \pm 12.3 a |
| | napA | 440.7 \pm 47.9 a | 427.3 \pm 14.3 a | 416.7 \pm 48.9 a | 452.7 \pm 7.4 a |
| | nrfA | 543.3 \pm 56.4 a | 538.7 \pm 36 a | 545.3 \pm 22.6 a | 568 \pm 38.6 a |
| ANR | nirA | 714 \pm 45.5 a | 730.7 \pm 10.9 a | 808.7 \pm 14.7 a | 733.3 \pm 48.1 a |
| | nirB | 1728 \pm 47.8 b | 2135.3 \pm 34.1 a | 2043.3 \pm 32 a | 2160.7 \pm 108.7 a |
| | nasA | 1149.3 \pm 53.1 b | 1351.3 \pm 29 a | 1362.7 \pm 35.6 a | 1473.3 \pm 34.6 a |
| Ammonification | nasB | 99.3 \pm 10.7 bc | 127.3 \pm 5.8 ab | 154.7 \pm 10.5 a | 88.0 \pm 7.2 c |
| | ureC | 2337.3 \pm 125.3 b | 2700 \pm 119.3 a | 2703.3 \pm 44.5 a | 2709.3 \pm 83.7 a |
| | gdh | 1720.7 \pm 70.7 a | 1906.7 \pm 75.1 a | 1960.7 \pm 16.2 a | 2004.7 \pm 134.4 a |

Note: Values within the same row followed by different letters indicate significant differences ($p < 0.05$, ANOVA, Tukey HSD). DNRA, Dissimilatory nitrogen reduction to ammonia; ANR, Assimilatory nitrogen reduction. F, fallow; W, wheat monoculture; PWWM, pea-wheat-wheat-millet rotation; CWWM, corn-wheat-wheat-millet rotation.

Table S2. Effects of different planting regimes on pH, BD and SWC ($n = 3$). Values are mean \pm standard deviation.

| Regimes | pH | BD g·cm ⁻³ | SWC % |
|---------|-------------------|-----------------------|--------------------|
| F | 8.4 \pm 0.11 a | 1.19 \pm 0.01 a | 17.91 \pm 0.54 a |
| W | 8.6 \pm 0.02 a | 1.08 \pm 0.01 b | 17.78 \pm 0.28 a |
| CWWM | 8.39 \pm 0.09 a | 1.07 \pm 0.02 b | 17.33 \pm 0.31 a |
| PWWM | 8.49 \pm 0.04 a | 1.1 \pm 0.01 b | 16.83 \pm 0.36 a |

Note: F, fallow; W, wheat monoculture; PWWM, pea-wheat-wheat-millet rotation; CWWM, corn-wheat-wheat-millet rotation. Values within the same column followed by different letters indicate significant differences ($p < 0.05$, ANOVA, Tukey HSD).

Table S3. Effects of distinct planting regimes on the relative abundance of species related to key functional genes ($n = 3$). Values are mean \pm standard deviation.

| Phylum | F | W | PWWM | CWWM |
|-----------------------------|--------------------|--------------------|---------------------|--------------------|
| p__Proteobacteria | 31.12 \pm 1.12 a | 27.24 \pm 2.68 b | 28.59 \pm 1.44 ab | 31.33 \pm 1.04 a |
| p__Actinobacteria | 22 \pm 1.52 ab | 22.84 \pm 1.46 a | 22.89 \pm 1.11 a | 19.92 \pm 0.67 b |
| p__Firmicutes | 9.91 \pm 1.39 a | 11.29 \pm 0.55 a | 10.66 \pm 0.51 a | 10.29 \pm 1.75 a |
| p__Thaumarchaeota | 10.13 \pm 0.6 a | 10.97 \pm 1.18 a | 11.03 \pm 0.21 a | 10.54 \pm 0.7 a |
| p__Cyanobacteria | 9.53 \pm 0.61 a | 8.97 \pm 0.45 a | 9.3 \pm 1.04 a | 10.37 \pm 1.38 a |
| p__Acidobacteria | 6.26 \pm 0.51 a | 7.48 \pm 0.15 a | 6.67 \pm 0.58 a | 6.64 \pm 1.49 a |
| p__unclassified_d__Bacteria | 2.9 \pm 0.3 a | 3.41 \pm 0.6 a | 3.23 \pm 0.59 a | 3.67 \pm 0.08 a |
| p__Bacteroidetes | 2.13 \pm 0.26 a | 2.01 \pm 0.3 a | 2.08 \pm 0.17 a | 1.88 \pm 0.41 a |
| p__Chloroflexi | 1.52 \pm 0.46 a | 1.28 \pm 0.21 a | 1.39 \pm 0.25 a | 1.23 \pm 0.28 a |
| p__Verrucomicrobia | 1.54 \pm 0.37 a | 1.46 \pm 0.55 a | 1.46 \pm 0.34 a | 1.18 \pm 0.39 a |
| p__Planctomycetes | 0.83 \pm 0.13 a | 1.06 \pm 0.19 a | 0.91 \pm 0.38 a | 1.1 \pm 0.15 a |

Note: Values within the same row followed by different letters indicate significant differences ($p < 0.05$, ANOVA, ANOVA, Tukey HSD). F, fallow; W, wheat monoculture; PWWM, pea-wheat-wheat-millet rotation; CWWM, corn-wheat-wheat-millet rotation. The top 10 species with the highest relative abundance in each regime were selected from phylum levels.

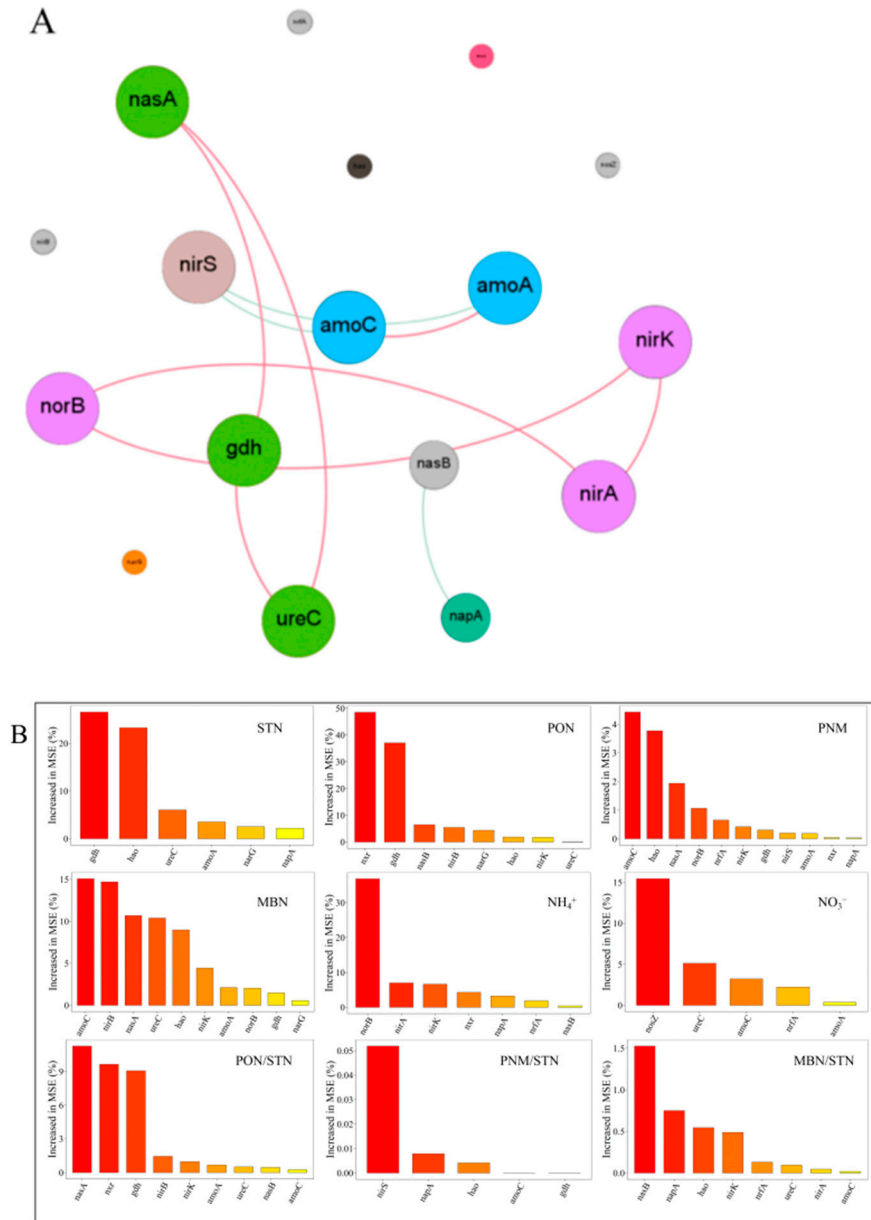


Figure S1. Key functional genes associated with N-cycling processes in different planting regimes. (A) Co-occurrence network of N-cycling functional genes. The circle size represents the degree centrality. (B) Random forest analyses show the significant predictors of functional genes for different nitrogen fractions. MES (%) means the percentage of increase of mean square error. STN, soil total nitrogen; PON, particulate organic nitrogen; PNM, potential nitrogen mineralization; MBN, microbial biomass N; NH₄⁺, ammonium nitrogen; NO₃⁻, nitrate nitrogen; PON/STN, ratio of PON to STN; PNM/STN, ratio of PNM to STN; MBN/STN, ratio of MBN to STN.

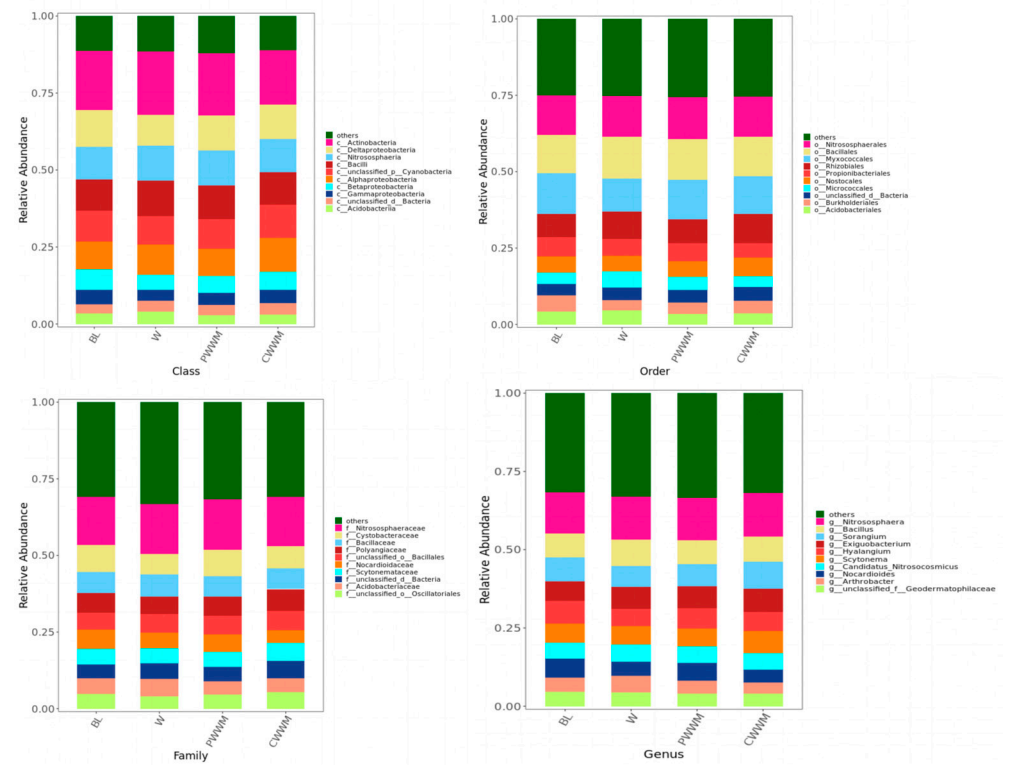


Figure S2. Relative abundance of the other level classification of species related to key functional genes.