**Table S3. Absolute abundances of *nifH*, *amoA*, *nirK*, *nirS*, *nosZ*,and 16S rRNA genes as well as relative abundances of *nifH*, *amoA*, *nirK*, *nirS*,and *nosZ* genes in relation to agricultural season, nitrogen fertilization, grass species, and their combinations.** Different lowercase letters indicate significant differences between treatment levels within groups. The letters were only shown in groups that have significant effects. Means were compared using Least Significant Difference (LSD) tests (α = 0.05). *nifH* data is from Hu et al., 2021c. *amoA* data is from Hu et al., 2021b.

|  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Factor† | *nifH* | *amoA* | *nirK* | *nirS* | *nosZ* | 16S rRNA  | *nifH* | *amoA* | *nirK* | *nirS* | *nosZ* |
| Log(gene copies g-1 dry weight soil) | Log(functional gene copies/16S rRNA gene copies) |
| April (G) | 6.61±0.07b | 6.01±0.14b | 7.21±0.07b | 7.27±0.06b | 7.30±0.06b | 9.65±0.05b | -3.05±0.04b | -3.65±0.10b | -2.44±0.03b | -2.39±0.04b | -2.35±0.02a |
| June (H1) | 6.17±0.06c | 5.27±0.10c | 6.93±0.05c | 7.12±0.04c | 7.00±0.04c | 9.53±0.04c | -3.36±0.05c | -4.26±0.10c | -2.59±0.03c | -2.41±0.04b | -2.52±0.03b |
| August (H2) | 7.04±0.04a | 6.93±0.09a | 7.59±0.03a | 7.74±0.04a | 7.55±0.03a | 9.86±0.03a | -2.82±0.04a | -2.93±0.10a | -2.27±0.04a | -2.12±0.03a | -2.31±0.03a |
| 0N | 6.65±0.10 | 5.68±0.20b | 7.23±0.09 | 7.38±0.08 | 7.29±0.07 | 9.69±0.05 | -3.05±0.08 | -4.01±0.17b | -2.46±0.05 | -2.31±0.05 | -2.41±0.04 |
| 67N | 6.57±0.11 | 6.17±0.18a | 7.25±0.10 | 7.41±0.09 | 7.28±0.07 | 9.69±0.06 | -3.12±0.06 | -3.51±0.13a | -2.44±0.05 | -2.28±0.05 | -2.40±0.03 |
| 202N | 6.60±0.09 | 6.35±0.18a | 7.25±0.07 | 7.33±0.07 | 7.29±0.07 | 9.66±0.05 | -3.06±0.07 | -3.31±0.15a | -2.40±0.03 | -2.33±0.05 | -2.37±0.03 |
| SG | 6.69±0.08 | 6.21±0.16a | 7.32±0.06a | 7.46±0.06a | 7.32±0.06 | 9.73±0.04 | -3.03±0.05 | -3.51±0.13a | -2.41±0.04 | -2.26±0.04a | -2.40±0.03 |
| BB | 6.52±0.09 | 5.92±0.16b | 7.17±0.07b | 7.29±0.07b | 7.25±0.06 | 9.63±0.04 | -3.11±0.06 | -3.71±0.14b | -2.46±0.04 | -2.35±0.03b | -2.38±0.02 |
| 0N-SG | 6.67±0.15 | 5.90±0.29 | 7.26±0.12 | 7.38±0.10b | 7.25±0.10 | 9.71±0.07 | -3.03±0.12 | -3.81±0.25 | -2.44±0.08 | -2.33±0.08 | -2.46±0.07 |
| 67N-SG | 6.74±0.13 | 6.35±0.25 | 7.43±0.10 | 7.62±0.10a | 7.40±0.10 | 9.80±0.08 | -3.06±0.07 | -3.45±0.19 | -2.37±0.06 | -2.18±0.05 | -2.40±0.05 |
| 202N-SG | 6.67±0.13 | 6.39±0.27 | 7.26±0.11 | 7.38±0.10b | 7.32±0.10 | 9.67±0.07 | -3.00±0.10 | -3.29±0.23 | -2.41±0.06 | -2.29±0.08 | -2.35±0.05 |
| 0N-BB | 6.62±0.15 | 5.47±0.27 | 7.20±0.13 | 7.39±0.12b | 7.33±0.10 | 9.68±0.08 | -3.07±0.10 | -4.22±0.22 | -2.48±0.07 | -2.30±0.07 | -2.36±0.04 |
| 67N-BB | 6.40±0.17 | 5.99±0.25 | 7.07±0.14 | 7.19±0.12c | 7.17±0.10 | 9.57±0.07 | -3.17±0.11 | -3.58±0.19 | -2.50±0.08 | -2.38±0.06 | -2.41±0.05 |
| 202N-BB | 6.53±0.13 | 6.31±0.27 | 7.24±0.09 | 7.28±0.10bc | 7.25±0.09 | 9.64±0.07 | -3.11±0.09 | -3.33±0.21 | -2.40±0.04 | -2.36±0.05 | -2.39±0.03 |
| Apr-0N | 6.66±0.12 | 5.72±0.26 | 7.19±0.11 | 7.26±0.07 | 7.28±0.11 | 9.64±0.09 | -2.98±0.07 | -3.92±0.20 | -2.45±0.04 | -2.37±0.05 | -2.36±0.04 |
| Apr-67N | 6.54±0.15 | 6.02±0.21 | 7.15±0.16 | 7.26±0.16 | 7.26±0.11 | 9.61±0.09 | -3.07±0.08 | -3.59±0.15 | -2.46±0.08 | -2.34±0.08 | -2.35±0.04 |
| Apr-202N | 6.62±0.09 | 6.29±0.22 | 7.30±0.11 | 7.27±0.08 | 7.37±0.11 | 9.71±0.09 | -3.09±0.07 | -3.43±0.15 | -2.41±0.05 | -2.44±0.06 | -2.34±0.04 |
| Jun-0N | 6.21±0.12 | 4.83±0.18 | 6.91±0.11 | 7.12±0.07 | 7.03±0.09 | 9.59±0.10 | -3.38±0.12 | -4.76±0.15 | -2.67±0.06 | -2.47±0.09 | -2.56±0.07 |
| Jun-67N | 6.12±0.13 | 5.45±0.09 | 6.93±0.10 | 7.15±0.10 | 6.99±0.06 | 9.51±0.06 | -3.40±0.08 | -4.06±0.09 | -2.58±0.06 | -2.36±0.08 | -2.52±0.04 |
| Jun-202N | 6.18±0.10 | 5.52±0.04 | 6.95±0.04 | 7.07±0.07 | 6.98±0.03 | 9.48±0.05 | -3.29±0.08 | -3.96±0.05 | -2.52±0.04 | -2.41±0.06 | -2.50±0.03 |
| Aug-0N | 7.07±0.07 | 6.50±0.13 | 7.60±0.06 | 7.76±0.05 | 7.55±0.06 | 9.86±0.03 | -2.79±0.05 | -3.36±0.16 | -2.26±0.08 | -2.09±0.03 | -2.31±0.05 |
| Aug-67N | 7.06±0.06 | 7.05±0.11 | 7.67±0.05 | 7.81±0.08 | 7.6±0.06 | 9.94±0.06 | -2.88±0.05 | -2.89±0.09 | -2.27±0.07 | -2.14±0.06 | -2.34±0.07 |
| Aug-202N | 7.00±0.06 | 7.24±0.03 | 7.51±0.06 | 7.65±0.07 | 7.51±0.06 | 9.78±0.06 | -2.78±0.08 | -2.54±0.08 | -2.27±0.04 | -2.13±0.06 | -2.26±0.04 |
| Apr-SG | 6.72±0.10 | 6.28±0.16 | 7.34±0.09 | 7.39±0.08 | 7.41±0.09 | 9.75±0.07 | -3.03±0.05 | -3.48±0.12 | -2.42±0.05 | -2.36±0.05 | -2.34±0.04 |
| Apr-BB | 6.49±0.08 | 5.74±0.19 | 7.09±0.09 | 7.14±0.07 | 7.19±0.07 | 9.55±0.05 | -3.06±0.07 | -3.81±0.16 | -2.46±0.05 | -2.41±0.05 | -2.36±0.02 |
| Jun-SG | 6.27±0.07 | 5.33±0.14 | 7.00±0.04 | 7.20±0.05 | 7.00±0.04 | 9.55±0.05 | -3.28±0.09 | -4.22±0.14 | -2.55±0.04 | -2.35±0.08 | -2.55±0.04 |
| Jun-BB | 6.07±0.10 | 5.20±0.15 | 6.87±0.09 | 7.03±0.06 | 7.00±0.06 | 9.50±0.07 | -3.43±0.05 | -4.30±0.16 | -2.63±0.05 | -2.47±0.02 | -2.50±0.03 |
| Aug-SG | 7.09±0.04 | 7.03±0.11 | 7.62±0.06 | 7.79±0.06 | 7.55±0.05 | 9.87±0.06 | -2.79±0.04 | -2.84±0.12 | -2.26±0.07 | -2.08±0.04 | -2.32±0.06 |
| Aug-BB | 7.00±0.06 | 6.82±0.15 | 7.56±0.04 | 7.69±0.05 | 7.55±0.05 | 9.85±0.04 | -2.85±0.06 | -3.02±0.17 | -2.28±0.03 | -2.16±0.04 | -2.29±0.02 |
| Apr-0N-SG | 6.74±0.24 | 6.10±0.23 | 7.27±0.18 | 7.31±0.14 | 7.35±0.20 | 9.71±0.17 | -2.96±0.09 | -3.60±0.09 | -2.44±0.04 | -2.40±0.04 | -2.36±0.04 |
| Apr-67N-SG | 6.79±0.11 | 6.36±0.28 | 7.44±0.15 | 7.57±0.12 | 7.46±0.13 | 9.79±0.09 | -3.00±0.04 | -3.43±0.24 | -2.35±0.11 | -2.22±0.07 | -2.33±0.09 |
| Apr-202N-SG | 6.63±0.19 | 6.36±0.40 | 7.30±0.21 | 7.30±0.16 | 7.43±0.20 | 9.76±0.15 | -3.14±0.12 | -3.40±0.30 | -2.46±0.09 | -2.47±0.10 | -2.34±0.09 |
| Apr-0N-BB | 6.58±0.05 | 5.33±0.37 | 7.11±0.13 | 7.22±0.07 | 7.21±0.13 | 9.57±0.09 | -2.99±0.13 | -4.24±0.29 | -2.46±0.08 | -2.35±0.09 | -2.36±0.08 |
| Apr-67N-BB | 6.28±0.19 | 5.68±0.18 | 6.86±0.13 | 6.95±0.13 | 7.06±0.04 | 9.43±0.04 | -3.14±0.16 | -3.75±0.15 | -2.56±0.09 | -2.47±0.09 | -2.37±0.02 |
| Apr-202N-BB | 6.61±0.04 | 6.21±0.26 | 7.29±0.14 | 7.24±0.06 | 7.32±0.12 | 9.66±0.11 | -3.05±0.09 | -3.45±0.15 | -2.37±0.03 | -2.42±0.08 | -2.34±0.02 |
| Jun-0N-SG | 6.22±0.14 | 4.91±0.29 | 6.92±0.03 | 7.11±0.08 | 6.93±0.03 | 9.58±0.13 | -3.36±0.26 | -4.67±0.21 | -2.66±0.10 | -2.47±0.19 | -2.65±0.12 |
| Jun-67N-SG | 6.28±0.10 | 5.53±0.13 | 7.11±0.06 | 7.33±0.03 | 7.08±0.09 | 9.56±0.08 | -3.28±0.10 | -4.03±0.19 | -2.45±0.04 | -2.23±0.10 | -2.48±0.02 |
| Jun-202N-SG | 6.32±0.18 | 5.56±0.06 | 6.96±0.09 | 7.16±0.10 | 7.01±0.07 | 9.52±0.09 | -3.20±0.15 | -3.96±0.07 | -2.56±0.03 | -2.36±0.11 | -2.51±0.03 |
| Jun-0N-BB | 6.20±0.24 | 4.76±0.28 | 6.90±0.25 | 7.13±0.14 | 7.14±0.18 | 9.59±0.18 | -3.40±0.10 | -4.84±0.24 | -2.69±0.08 | -2.46±0.04 | -2.46±0.03 |
| Jun-67N-BB | 5.96±0.21 | 5.38±0.15 | 6.75±0.12 | 6.98±0.12 | 6.90±0.04 | 9.47±0.10 | -3.51±0.11 | -4.09±0.08 | -2.72±0.02 | -2.49±0.05 | -2.57±0.07 |
| Jun-202N-BB | 6.05±0.04 | 5.47±0.05 | 6.95±0.02 | 6.98±0.07 | 6.96±0.01 | 9.44±0.05 | -3.39±0.02 | -3.96±0.10 | -2.49±0.07 | -2.46±0.02 | -2.48±0.05 |
| Aug-0N-SG | 7.06±0.09 | 6.68±0.20 | 7.59±0.13 | 7.72±0.05 | 7.47±0.08 | 9.83±0.04 | -2.77±0.07 | -3.15±0.24 | -2.24±0.16 | -2.11±0.01 | -2.36±0.11 |
| Aug-67N-SG | 7.15±0.01 | 7.17±0.02 | 7.73±0.09 | 7.97±0.07 | 7.66±0.08 | 10.06±0.06 | -2.91±0.06 | -2.88±0.07 | -2.33±0.15 | -2.09±0.12 | -2.40±0.14 |
| Aug-202N-SG | 7.05±0.08 | 7.24±0.02 | 7.53±0.10 | 7.69±0.14 | 7.54±0.07 | 9.73±0.07 | -2.68±0.01 | -2.49±0.05 | -2.20±0.03 | -2.05±0.07 | -2.19±0.01 |
| Aug-0N-BB | 7.08±0.12 | 6.31±0.12 | 7.60±0.05 | 7.81±0.10 | 7.63±0.05 | 9.89±0.05 | -2.81±0.07 | -3.57±0.16 | -2.28±0.07 | -2.07±0.06 | -2.26±0.02 |
| Aug-67N-BB | 6.97±0.10 | 6.92±0.21 | 7.61±0.02 | 7.65±0.03 | 7.54±0.07 | 9.83±0.03 | -2.86±0.09 | -2.90±0.19 | -2.22±0.01 | -2.18±0.01 | -2.29±0.04 |
| Aug-202N-BB | 6.94±0.11 | 7.24±0.06 | 7.48±0.09 | 7.61±0.09 | 7.49±0.11 | 9.83±0.12 | -2.89±0.16 | -2.59±0.17 | -2.34±0.06 | -2.21±0.09 | -2.34±0.04 |

† G, grass green up; H1, initial grass harvest; H2, second grass harvest; 0N, no N fertilization; 67N, 67 kg N ha-1 fertilization; 202N, 202 kg N ha-1 fertilization; SG, switchgrass; BB, big bluestem.

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