**Table S4. Absolute abundances of *nifH*, *amoA*, *nirK*, *nirS*, *nosZ*,and 16S rRNA gene transcripts as well as relative transcript abundances of *nifH*, *amoA*, *nirK*, *nirS*,and *nosZ* 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 transcript copies g-1 dry weight soil) | | | | | | | Log(functional gene transcript copies/16S rRNA copies) | | | | | |
| April (G) | 3.80±0.12a | 3.46±0.11b | | 4.48±0.09b | 4.89±0.11a | 4.90±0.04b | 11.23±0.03 | -7.44±0.11a | -7.77±0.11b | -6.76±0.09b | -6.35±0.10 | -6.34±0.04 | |
| June (H1) | 4.04±0.10a | 3.88±0.11a | | 4.89±0.06a | 4.85±0.12a | 5.11±0.04a | 11.33±0.06 | -7.19±0.14a | -7.36±0.16a | -6.35±0.11a | -6.39±0.14 | -6.12±0.11 | |
| August (H2) | 3.02±0.17b | 2.95±0.16c | | 4.33±0.07b | 4.40±0.17b | 4.79±0.03c | 11.06±0.08 | -7.97±0.12b | -8.04±0.15b | -6.66±0.07b | -6.69±0.13 | -6.20±0.08 | |
| 0N | 3.63±0.18 | 3.09±0.18b | | 4.55±0.09 | 4.66±0.15ab | 4.91±0.05b | 11.18±0.07 | -7.55±0.14 | -8.09±0.14b | -6.63±0.07 | -6.52±0.11ab | -6.27±0.06 | |
| 67N | 3.81±0.16 | 3.64±0.15a | | 4.65±0.11 | 4.98±0.13a | 5.02±0.05a | 11.26±0.06 | -7.36±0.17 | -7.53±0.18a | -6.53±0.14 | -6.20±0.12a | -6.15±0.11 | |
| 202N | 3.45±0.15 | 3.60±0.10a | | 4.51±0.08 | 4.51±0.13b | 4.88±0.04b | 11.18±0.06 | -7.67±0.11 | -7.52±0.09a | -6.60±0.08 | -6.71±0.13b | -6.24±0.06 | |
| SG | 3.63±0.15 | 3.49±0.11 | | 4.53±0.07 | 4.78±0.13 | 4.91±0.03 | 11.21±0.05 | -7.54±0.11 | -7.68±0.09 | -6.64±0.06 | -6.45±0.11 | -6.26±0.05 | |
| BB | 3.63±0.12 | 3.39±0.14 | | 4.61±0.08 | 4.65±0.10 | 4.97±0.05 | 11.21±0.05 | -7.51±0.12 | -7.76±0.15 | -6.54±0.10 | -6.49±0.10 | -6.18±0.08 | |
| 0N-SG | 3.59±0.27 | 3.29±0.22 | | 4.53±0.11 | 4.61±0.24 | 4.87±0.05 | 11.16±0.09 | -7.57±0.21 | -7.87±0.15 | -6.63±0.06 | -6.55±0.18 | -6.29±0.06 | |
| 67N-SG | 3.85±0.20 | 3.63±0.17 | | 4.59±0.12 | 5.11±0.16 | 4.99±0.05 | 11.30±0.08 | -7.45±0.18 | -7.67±0.15 | -6.71±0.11 | -6.19±0.16 | -6.31±0.07 | |
| 202N-SG | 3.46±0.29 | 3.56±0.18 | | 4.47±0.15 | 4.63±0.25 | 4.87±0.07 | 11.18±0.09 | -7.59±0.21 | -7.49±0.17 | -6.58±0.14 | -6.62±0.23 | -6.19±0.11 | |
| 0N-BB | 3.67±0.25 | 2.89±0.27 | | 4.56±0.14 | 4.70±0.18 | 4.95±0.08 | 11.20±0.12 | -7.53±0.18 | -8.31±0.23 | -6.64±0.13 | -6.50±0.14 | -6.25±0.11 | |
| 67N-BB | 3.78±0.26 | 3.66±0.25 | | 4.70±0.18 | 4.84±0.20 | 5.05±0.10 | 11.22±0.09 | -7.27±0.29 | -7.39±0.32 | -6.35±0.25 | -6.21±0.20 | -6.00±0.21 | |
| 202N-BB | 3.43±0.10 | 3.64±0.07 | | 4.56±0.09 | 4.38±0.08 | 4.89±0.04 | 11.19±0.08 | -7.79±0.06 | -7.54±0.06 | -6.61±0.06 | -6.81±0.10 | -6.29±0.06 | |
| Apr-0N | 4.14±0.08 | 3.41±0.22bc | | 4.63±0.16 | 5.13±0.10 | 4.95±0.10 | 11.29±0.04 | -7.16±0.06 | -7.88±0.21bc | -6.67±0.13bc | -6.16±0.09 | -6.35±0.07 | |
| Apr-67N | 3.74±0.27 | 3.53±0.18bc | | 4.50±0.18 | 4.92±0.24 | 4.95±0.07 | 11.23±0.04 | -7.50±0.27 | -7.70±0.19bc | -6.73±0.19bc | -6.31±0.24 | -6.28±0.09 | |
| Apr-202N | 3.53±0.15 | 3.45±0.19bc | | 4.30±0.13 | 4.61±0.13 | 4.81±0.05 | 11.18±0.06 | -7.65±0.15 | -7.73±0.20bc | -6.88±0.13c | -6.57±0.14 | -6.37±0.05 | |
| Jun-0N | 4.03±0.12 | 3.54±0.17bc | | 4.81±0.03 | 4.82±0.12 | 5.06±0.02 | 11.29±0.12 | -7.26±0.15 | -7.75±0.22bc | -6.49±0.12bc | -6.47±0.18 | -6.24±0.12 | |
| Jun-67N | 4.35±0.08 | 4.29±0.12a | | 5.09±0.11 | 5.22±0.15 | 5.26±0.08 | 11.39±0.07 | -6.77±0.25 | -6.84±0.32a | -6.03±0.29a | -5.90±0.19 | -5.87±0.28 | |
| Jun-202N | 3.75±0.20 | 3.82±0.13ab | | 4.77±0.11 | 4.50±0.22 | 5.03±0.05 | 11.30±0.11 | -7.55±0.23 | -7.48±0.14b | -6.52±0.06bc | -6.80±0.24 | -6.26±0.08 | |
| Aug-0N | 2.72±0.23 | 2.32±0.24d | | 4.20±0.13 | 4.02±0.26 | 4.73±0.04 | 10.95±0.15 | -8.24±0.14 | -8.63±0.14d | -6.75±0.09bc | -6.93±0.16 | -6.22±0.12 | |
| Aug-67N | 3.36±0.28 | 3.11±0.17c | | 4.34±0.11 | 4.79±0.26 | 4.86±0.04 | 11.17±0.16 | -7.81±0.21 | -8.05±0.14c | -6.82±0.10bc | -6.37±0.19 | -6.31±0.14 | |
| Aug-202N | 2.99±0.37 | 3.53±0.17bc | | 4.45±0.14 | 4.40±0.35 | 4.77±0.08 | 11.05±0.11 | -7.86±0.20 | -7.33±0.10ab | -6.38±0.09ab | -6.77±0.27 | -6.08±0.15 | |
| Apr-SG | 3.65±0.20 | 3.43±0.15 | | 4.38±0.11 | 4.81±0.17 | 4.85±0.04 | 11.24±0.04 | -7.58±0.19 | -7.81±0.15 | -6.86±0.10 | -6.43±0.16 | -6.39±0.03 | |
| Apr-BB | 3.94±0.12 | 3.50±0.15 | | 4.57±0.15 | 4.96±0.14 | 4.95±0.08 | 11.23±0.04 | -7.29±0.09 | -7.73±0.17 | -6.66±0.14 | -6.27±0.12 | -6.28±0.07 | |
| Jun-SG | 4.12±0.14 | 3.91±0.13 | | 4.87±0.08 | 5.01±0.17 | 5.08±0.04 | 11.32±0.08 | -7.20±0.15 | -7.42±0.12 | -6.46±0.06 | -6.31±0.18 | -6.25±0.05 | |
| Jun-BB | 3.96±0.14 | 3.86±0.18 | | 4.92±0.10 | 4.68±0.15 | 5.15±0.07 | 11.33±0.09 | -7.19±0.24 | -7.29±0.30 | -6.24±0.22 | -6.47±0.23 | -6.00±0.21 | |
| Aug-SG | 3.12±0.30 | 3.15±0.20 | | 4.35±0.10 | 4.53±0.31 | 4.80±0.06 | 11.07±0.11 | -7.83±0.20 | -7.80±0.18 | -6.60±0.11 | -6.61±0.24 | -6.15±0.12 | |
| Aug-BB | 2.91±0.18 | 2.73±0.25 | | 4.30±0.11 | 4.27±0.16 | 4.77±0.02 | 11.04±0.12 | -8.11±0.10 | -8.22±0.22 | -6.70±0.09 | -6.77±0.09 | -6.26±0.10 | |
| Apr-0N-SG | 4.07±0.05 | 3.58±0.17 | | 4.58±0.11 | 5.07±0.05 | 4.86±0.06 | 11.23±0.05 | -7.16±0.07 | -7.65±0.17 | -6.65±0.06 | -6.16±0.10 | -6.37±0.05 | |
| Apr-67N-SG | 3.43±0.49 | 3.32±0.28 | | 4.33±0.18 | 4.73±0.43 | 4.88±0.03 | 11.28±0.05 | -7.85±0.45 | -7.96±0.24 | -6.96±0.14 | -6.55±0.39 | -6.40±0.02 | |
| Apr-202N-SG | 3.47±0.31 | 3.38±0.40 | | 4.24±0.25 | 4.62±0.30 | 4.80±0.09 | 11.20±0.12 | -7.74±0.32 | -7.83±0.41 | -6.96±0.26 | -6.58±0.32 | -6.40±0.10 | |
| Apr-0N-BB | 4.20±0.15 | 3.24±0.42 | | 4.68±0.33 | 5.19±0.21 | 5.03±0.19 | 11.36±0.04 | -7.15±0.11 | -8.12±0.38 | -6.68±0.29 | -6.17±0.17 | -6.33±0.15 | |
| Apr-67N-BB | 4.04±0.19 | 3.74±0.19 | | 4.67±0.30 | 5.10±0.28 | 5.01±0.14 | 11.18±0.06 | -7.14±0.18 | -7.44±0.22 | -6.51±0.32 | -6.08±0.28 | -6.17±0.17 | |
| Apr-202N-BB | 3.59±0.09 | 3.52±0.09 | | 4.36±0.15 | 4.60±0.03 | 4.81±0.05 | 11.16±0.07 | -7.57±0.02 | -7.64±0.13 | -6.79±0.08 | -6.55±0.05 | -6.35±0.03 | |
| Jun-0N-SG | 4.07±0.22 | 3.74±0.27 | | 4.80±0.07 | 4.88±0.24 | 5.03±0.03 | 11.32±0.11 | -7.25±0.30 | -7.58±0.21 | -6.52±0.14 | -6.43±0.32 | -6.29±0.08 | |
| Jun-67N-SG | 4.33±0.05 | 4.19±0.09 | | 5.00±0.02 | 5.40±0.08 | 5.17±0.01 | 11.41±0.07 | -7.07±0.02 | -7.22±0.16 | -6.40±0.05 | -6.00±0.14 | -6.24±0.07 | |
| Jun-202N-SG | 3.97±0.37 | 3.79±0.26 | | 4.79±0.22 | 4.76±0.39 | 5.02±0.10 | 11.25±0.22 | -7.28±0.42 | -7.45±0.28 | -6.45±0.12 | -6.49±0.42 | -6.22±0.15 | |
| Jun-0N-BB | 4.00±0.13 | 3.35±0.18 | | 4.81±0.02 | 4.75±0.10 | 5.08±0.01 | 11.27±0.24 | -7.27±0.14 | -7.92±0.42 | -6.46±0.22 | -6.51±0.22 | -6.19±0.24 | |
| Jun-67N-BB | 4.37±0.17 | 4.39±0.23 | | 5.18±0.22 | 5.04±0.27 | 5.35±0.15 | 11.37±0.14 | -6.47±0.47 | -6.45±0.58 | -5.66±0.51 | -5.8±0.40 | -5.50±0.51 | |
| Jun-202N-BB | 3.53±0.10 | 3.84±0.13 | | 4.75±0.10 | 4.24±0.18 | 5.04±0.02 | 11.34±0.09 | -7.82±0.04 | -7.51±0.11 | -6.59±0.01 | -7.11±0.14 | -6.31±0.08 | |
| Aug-0N-SG | 2.62±0.38 | 2.55±0.28 | | 4.22±0.23 | 3.88±0.47 | 4.71±0.08 | 10.93±0.22 | -8.31±0.16 | -8.38±0.09 | -6.71±0.07 | -7.05±0.28 | -6.22±0.15 | |
| Aug-67N-SG | 3.79±0.12 | 3.38±0.14 | | 4.45±0.05 | 5.20±0.11 | 4.92±0.01 | 11.21±0.24 | -7.42±0.20 | -7.83±0.16 | -6.76±0.21 | -6.01±0.14 | -6.29±0.23 | |
| Aug-202N-SG | 2.96±0.73 | 3.52±0.34 | | 4.38±0.24 | 4.50±0.69 | 4.77±0.16 | 11.08±0.15 | -7.75±0.44 | -7.18±0.09 | -6.33±0.19 | -6.78±0.58 | -5.94±0.25 | |
| Aug-0N-BB | 2.81±0.35 | 2.09±0.40 | | 4.19±0.18 | 4.16±0.29 | 4.75±0.05 | 10.98±0.25 | -8.16±0.27 | -8.89±0.17 | -6.79±0.17 | -6.82±0.21 | -6.23±0.23 | |
| Aug-67N-BB | 2.92±0.42 | 2.84±0.24 | | 4.24±0.20 | 4.39±0.40 | 4.79±0.05 | 11.12±0.24 | -8.20±0.18 | -8.28±0.14 | -6.89±0.08 | -6.74±0.16 | -6.33±0.20 | |
| Aug-202N-BB | 3.04±0.20 | 3.53±0.06 | | 4.57±0.15 | 4.25±0.01 | 4.78±0.01 | 11.01±0.23 | -7.98±0.03 | -7.48±0.12 | -6.44±0.06 | -6.76±0.15 | -6.23±0.17 | |

† 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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