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Fu, Q., Abadie, M., Blaud, A., Carswell, A. M., Misselbrook, T. H., Clark, I. M. and Hirsch, P. R. 2019. Effects of urease and nitrification inhibitors on soil N, nitrifier abundance and activity in a sandy loam soil . *Biology And Fertility Of Soils*.

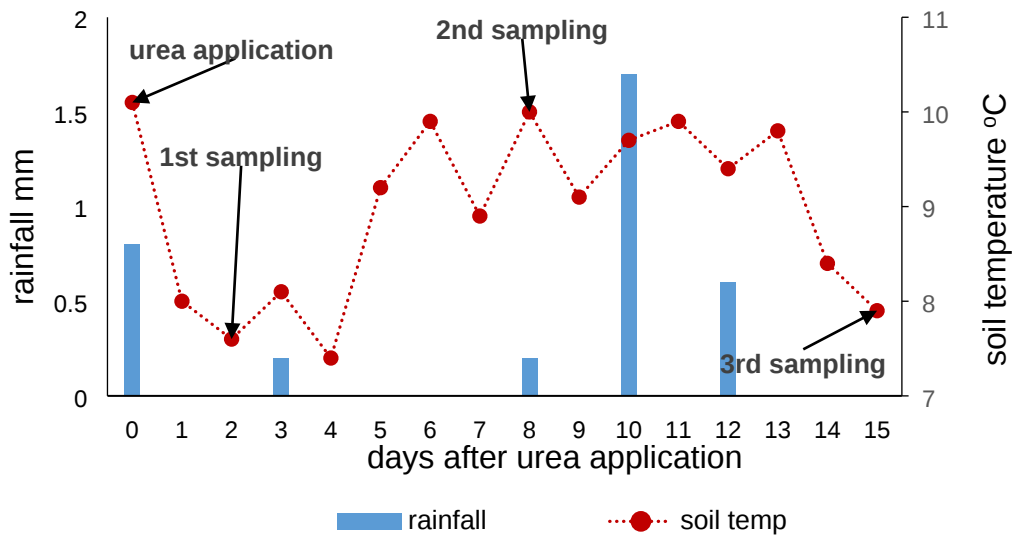
The publisher's version can be accessed at:

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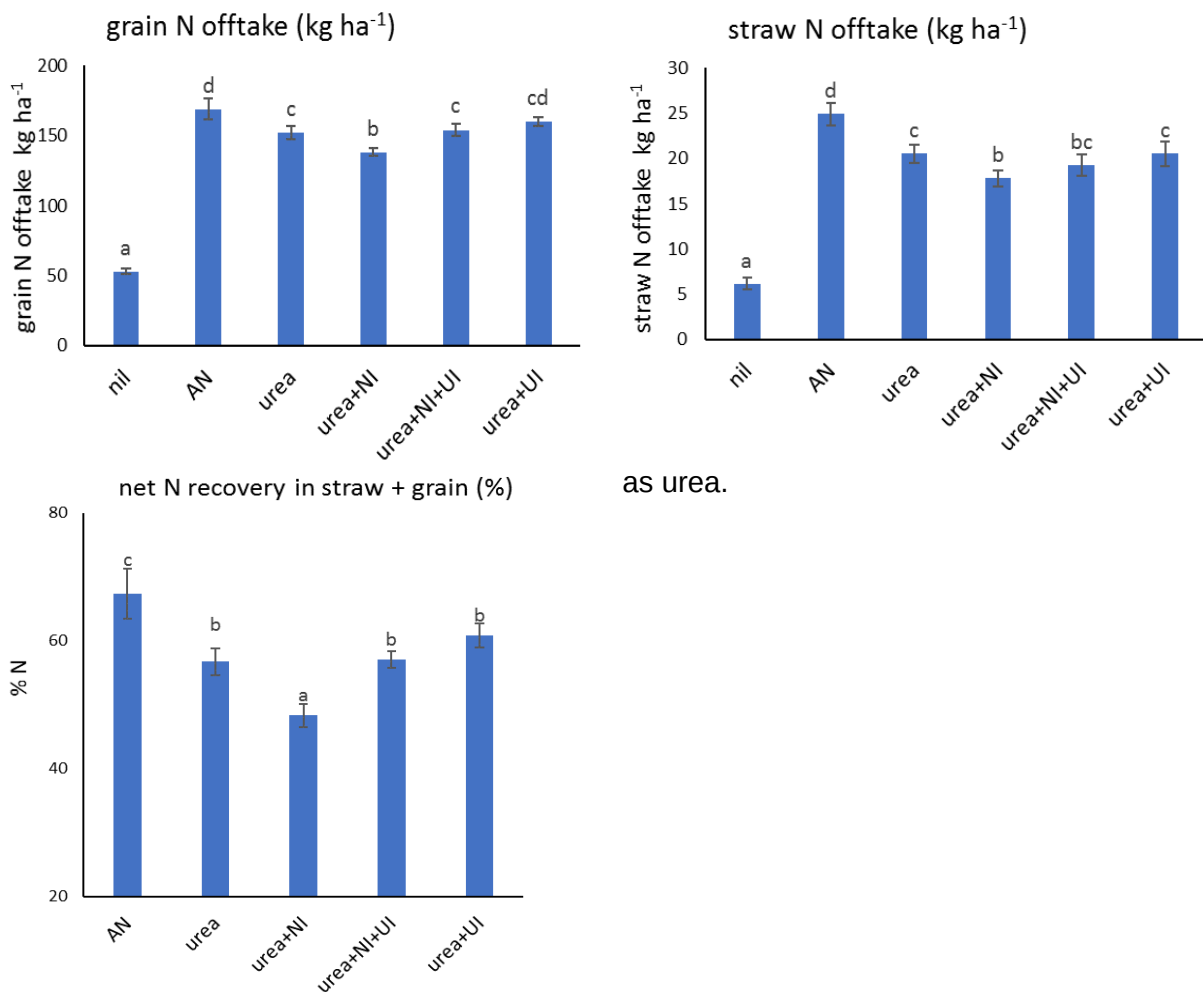
The output can be accessed at: <https://repository.rothamsted.ac.uk/item/96z51/effects-of-urease-and-nitrification-inhibitors-on-soil-n-nitrifier-abundance-and-activity-in-a-sandy-loam-soil>.

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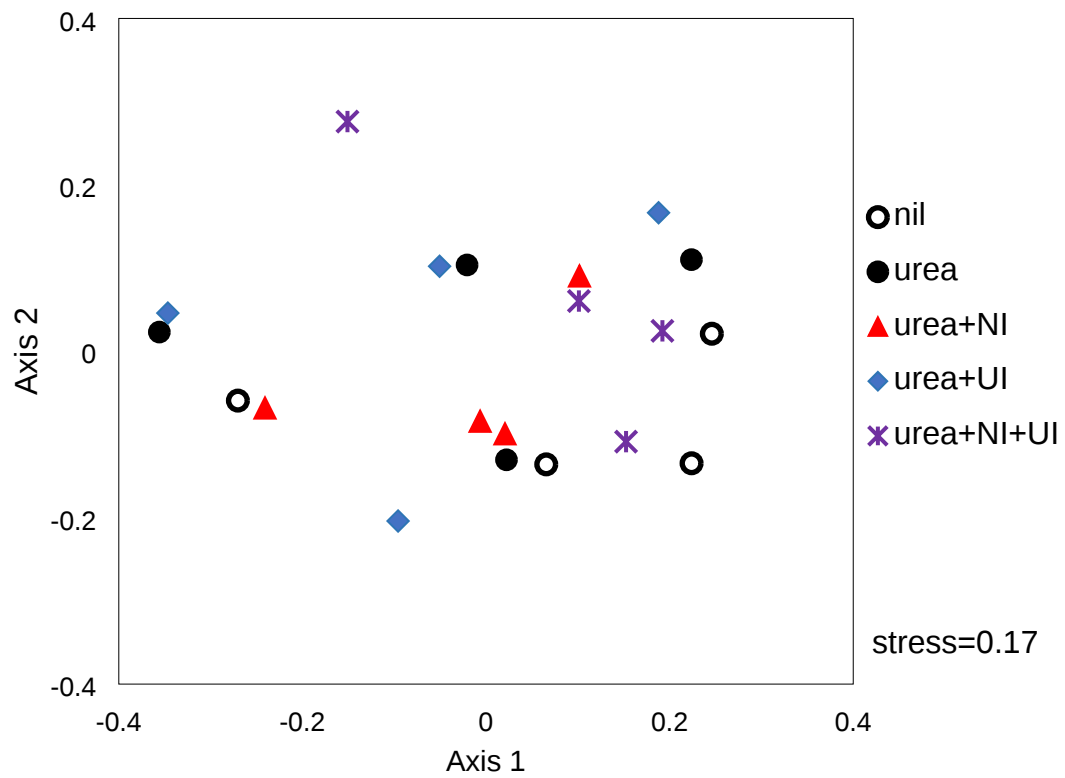
Supplementary Fig. 1. Daily measurements at Woburn of soil temperature (10 cm depth) and rainfall after urea application.



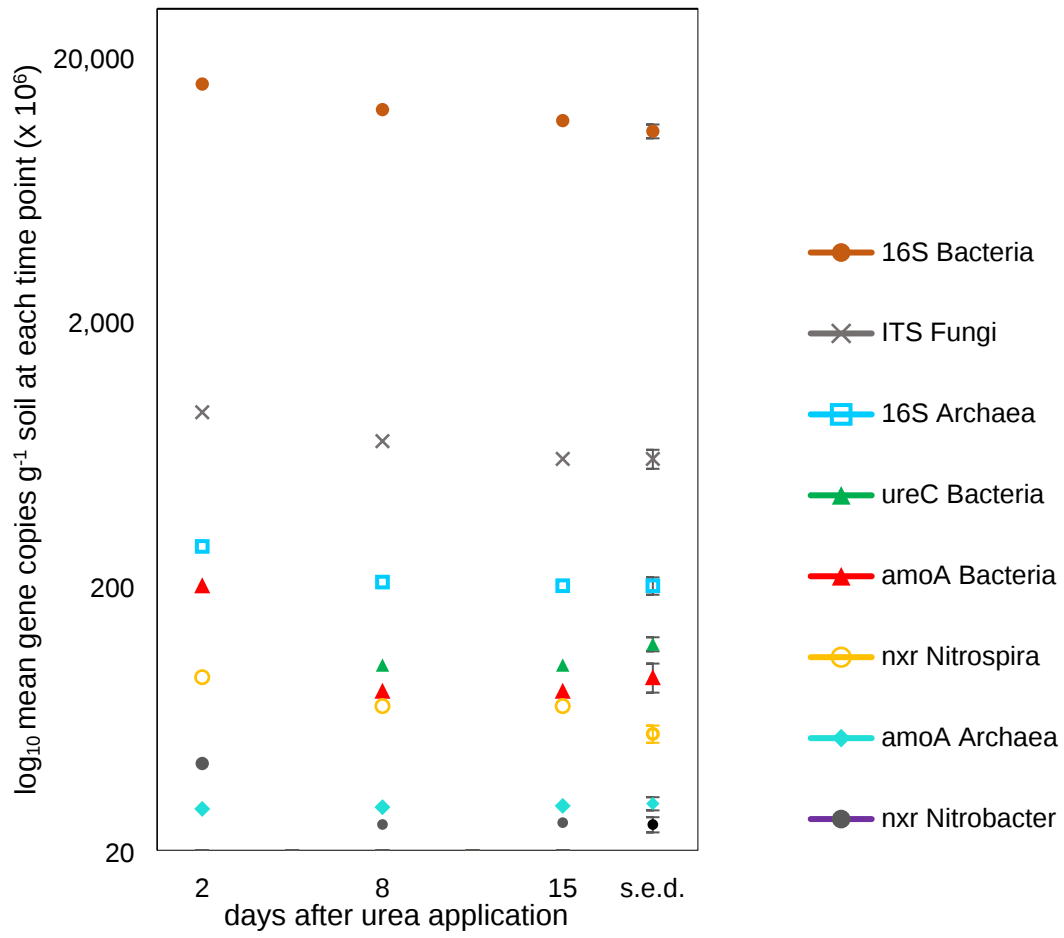
Supplementary Fig. 2. Wheat grain and straw N offtake and net N recovery in all treatment plots (n = 6). Letters above columns denote significantly different means according to Tukey's post-hoc test on ANOVA, $\alpha = 0.05$. AN – ammonium nitrate fertilizer applied at same N rate



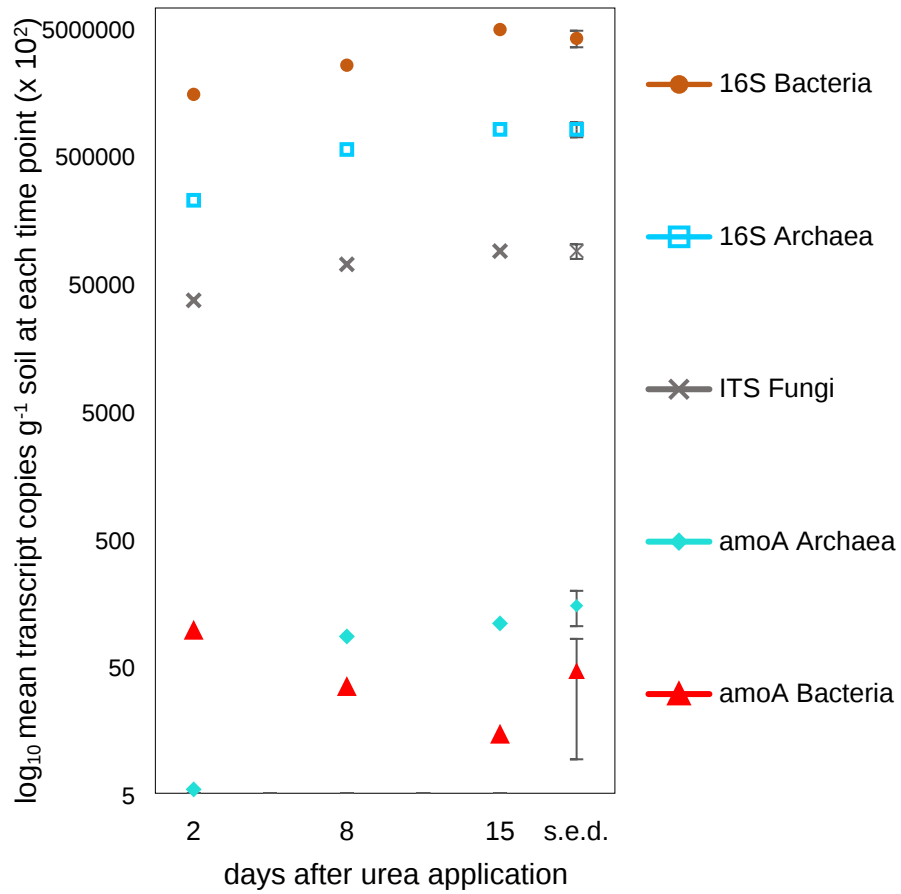
Supplementary Fig. 3. NMDS plot, Bray-Curtis matrix, for 16S rRNA amplicon diversity (2000 most abundant OTU) in all treatments 2 d after urea application.



Supplementary Fig. 4. Mean abundance for all treatments of genes at each time point. All genes were significantly less abundant at 15 days compared to two days after urea addition (Tukey's post-hoc $\alpha = 0.05$) except *nxr Nitrospira* and *amoA Archaea* (NSD).



Supplementary Fig. 5. Mean abundance for all treatments of transcripts at each time point. All transcripts were significantly more abundant at 15 days compared to two days after urea addition except *amo* Bacteria which was significantly less abundant (Tukey's post-hoc $\alpha = 0.05$).



Supplementary Table 1. PCR efficiency of primer sets

| | Efficiency | r² | slope | y-int |
|-------------------------------|-------------------|----------------------|--------------|--------------|
| 16S Bacteria | 84.8% | 0.995 | -3.749 | 36.359 |
| ITS Fungi | 87.30% | 0.998 | -3.669 | 35.258 |
| 16S Archaea | 100.10% | 0.995 | -3.319 | 33.365 |
| <i>ureC</i> Bacteria | 95.40% | 0.997 | -3.437 | 31.779 |
| <i>amoA</i> Bacteria | 82.20% | 0.997 | -3.837 | 35.908 |
| <i>nxr</i> Nitrospira | 92.70% | 0.999 | -3.511 | 31.665 |
| <i>amoA</i> Archaea | 86% | 0.997 | -3.709 | 33.318 |
| <i>nxr</i> Nitrobacter | 93.30% | 0.999 | -3.494 | 33.457 |

Supplementary Table 2. PCR efficiency of primer sets in RNA RT-qPCR

| | Efficiency | r² | slope | y-int |
|-----------------------------|-------------------|----------------------|--------------|--------------|
| 16S Bacteria | 78.8% | 0.999 | -3.962 | 36.922 |
| 16S Archaea | 93.40% | 0.995 | -3.491 | 33.852 |
| ITS Fungi | 91.90% | 0.998 | -3.532 | 34.095 |
| <i>amoA</i> Archaea | 90% | 0.997 | -3.601 | 32.279 |
| <i>amoA</i> Bacteria | 90.50% | 0.999 | -3.572 | 34.172 |

| | | | | | | | | | | | | | | | | | | |
|------------------------------------|-----------|-----------------------------------|-----------------------------------|------------------|---------------------|---------------------|--------------------|----------------------|--------------------------|--------------------------|-------------------------------------|--------------------------------------|-----------------------------|-----------------------------|-----------------------------------|-----------------------------|--|--|
| NO₃⁻ | -0.46 | | | | | | | | | | | | | | | | | |
| NH₄⁺ | -0.46 | 0.55 | | | | | | | | | | | | | | | | |
| mineral N | -0.44 | 0.62 | 0.99 | | | | | | | | | | | | | | | |
| 16S Bacteria | -0.02 | 0.21 | 0.18 | 0.19 | | | | | | | | | | | | | | |
| 16S Archaea | 0.08 | 0.17 | 0.12 | 0.13 | 0.76 | | | | | | | | | | | | | |
| ITS Fungi | -0.25 | 0.32 | 0.36 | 0.36 | 0.61 | 0.66 | | | | | | | | | | | | |
| ureC Bacteria | -0.06 | 0.18 | 0.18 | 0.19 | 0.69 | 0.85 | 0.76 | | | | | | | | | | | |
| amoA Bacteria | -0.38 | 0.64 | 0.46 | 0.51 | 0.49 | 0.43 | 0.62 | 0.46 | | | | | | | | | | |
| amoA Archaea | 0.13 | -0.02 | -0.04 | -0.03 | 0.36 | 0.64 | 0.27 | 0.48 | | | | | | | | | | |
| <i>nxrA</i> Nitrospira | -0.06 | 0.06 | 0.13 | 0.13 | 0.70 | 0.81 | 0.75 | 0.82 | 0.46 | 0.59 | | | | | | | | |
| <i>nxrA</i> Nitrobacter | -0.10 | 0.21 | 0.24 | 0.25 | 0.66 | 0.72 | 0.70 | 0.74 | 0.62 | 0.34 | 0.81 | | | | | | | |
| 16S RNA Bacteria | -0.02 | -0.35 | -0.14 | -0.19 | -0.25 | -0.24 | -0.30 | -0.30 | -0.36 | 0.07 | -0.24 | -0.41 | | | | | | |
| 16S RNA Archaea | -0.11 | -0.18 | -0.08 | -0.11 | -0.17 | -0.15 | -0.23 | -0.21 | -0.28 | 0.11 | -0.13 | -0.30 | 0.75 | | | | | |
| ITS RNA Fungi | -0.23 | -0.05 | 0.05 | 0.03 | -0.21 | -0.21 | -0.14 | -0.22 | -0.13 | -0.01 | -0.18 | -0.26 | 0.65 | 0.87 | | | | |
| amoA RNA AOB | -0.41 | 0.37 | 0.25 | 0.27 | 0.28 | 0.25 | 0.30 | 0.29 | 0.39 | 0.04 | 0.15 | 0.33 | -0.29 | -0.23 | -0.15 | | | |
| amoA RNA AOA | -0.19 | -0.04 | 0.00 | -0.01 | -0.06 | -0.06 | -0.09 | -0.11 | -0.22 | 0.13 | -0.08 | -0.24 | 0.61 | 0.91 | 0.84 | -0.09 | | |
| | pH | NO₃⁻ | NH₄⁺ | min N | 16S Bact | 16S Arch | ITS Fun | ureC Bact | amo A AOB | amo A AOA | <i>nxrA</i> <i>spira</i> | <i>nxrA</i> <i>bacter</i> | 16S RNA Bact | 16S RNA Arch | ITS RNA Fung i | amoA RNA AOB | | |

Supplementary Table 3. Spearman's rank correlation (r_s) for all samples, plots and times. Statistically significant correlations ($P < 0.05$) are highlighted yellow; strong positive or negative correlations ($> .6$) are in **bold**.