Interactions of fertilisation and crop productivity on soil nitrogen cycle microbiome and gas emissions

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Supplementary Materials

Table S1: Field activities and their timings in the field.

Field operation	**Date**	**Wheat**	**Barley**	**Sorghum**
Sowing date		May 6th		May 18th
Organic fertilisation	April 28th			*N0 – 40 t/ha of (231.2* kg N ha^{-1}*)* *N80 – 40 t/ha of (231.2* kg N ha^{-1}*)* *N160 – 40 t/ha of (231.2* kg N ha^{-1}*)*
Mineral fertilisation	May 5th	N80 – 80 kg N ha^{-1} N160 – 120 kg N ha^{-1} N		
	May 6th			N80 – 80 kg N ha^{-1} N160 – 160 kg N ha^{-1}
	June 10th	N160 – 40 kg N ha^{-1}		
Herbicide application	June 7th	Sekator OD: 0.1 l/ha		
	June 17th	Tomahawk 200 EC: 0.35 l/ha MCPA: 1.5 l/ha Orius 250 EW: 1 l/ha Cerone: 0.5 l/ha		Tomahawk 200 EC: 0.35 l/ha MCPA: 1.5 l/ha
Harvest date		August 18th	August 16th	September 27th

Table S2: Primers used in qPCR, their concentrations and qPCR programs.

Marker gene	Primer	Primer concentration (µM)	Reference	qPCR program
bacterial 16S rRNA	Bact517F	0,6	Liu *et al*., 2007	95°C 10 min; 35 cycles: 95°C 30 s, 60°C 45 s; 72°C 45 s
	Bact1028R		Dethlefsen *et al*., 2008	
archaeal 16S rRNA	Arc519F	0,6	Espenberg *et al*., 2016	95°C 10 min; 45 cycles: 95°C 15 s, 56°C 30 s; 72°C 30 s
	Arch910R			
nirK	nirK876	0,8	Hallin ja Lindgren, 1999	95°C 10 min; 45 cycles: 95°C 15 s, 58°C 30 s; 72°C 30s, 80°C 30 s[a]
	nirK1040			
nirS	nirSCd3af	0,8	Kandeler *et al*., 2006	95°C 10 min; 45 cycles: 95°C 15 s, 55°C 30 s; 72°C 30s, 80°C 30 s[a]
	nirSR3cd			
nosZI	nosZ2F	0,8	Henry *et al*., 2006	95°C 10 min; 45 cycles: 95°C 15 s, 60°C 30 s, 72°C 30 s, 80°C 30 s[a]
	nosZ2R			
nosZII	nosZIIF	1.2	Jones *et al*., 2013	95°C 10 min; 45 cycles: 95°C 30 s, 54°C 45 s, 72°C 45 s, 80°C 45 s[a]
	nosZIIR			
bacterial *amoA*	amoA-1F	0,8	Rotthauwe *et al*., 1997	95°C 10 min; 45 cycles: 95°C 30 s, 57°C 45 s, 72°C 45 s
	amoA-2R			
archaeal *amoA*	CrenamoA 23F	0.8	Tourna *et al*., 2008	95°C 10 min; 45 cycles: 95°C 30 s, 55°C 45 s, 72°C 45 s
	CrenamoA 616R			
comammox *amoA*	comamoA AF	1.2	Wang *et al*., 2018	95 °C 10 min; 40 cycles: 95 °C 15 s, 55 °C 30 s, 72 °C 30 s
	comamoA SR			
nrfA	nrfAF2awMOD	1.2	Cannhenryon *et al*., 2019	95 °C 10 min; 45 cycles: 95 °C 15 s, 56 °C 30 s, 72 °C 30 s
	nrfAR1MOD			

Table S3: Partial N budget of the sites (kg N ha^{-1}).

	Barley			Sorghum			Sorghum with manure			Wheat		
	N0	N80	N160	N0	N80	N160	N0	N80	N160	N0	N80	N160
N added with manure + mineral fertilizer	0	80	160	0	80	160	231.2	311.2	391.2	0	80	160
N in plant biomass	18.17	46.06	90.14	11.63	21.02	51.73	44.82	60.48	106.34	26.39	93.56	128.74
Change of soil N content	−50.09	−40.38	104.40	−18.41	−64.38	−222.79	−163.11	−159.34	−198.82	−116.62	−273.28	−214.64
N losses (Methodology S4)	31.93	74.31	−34.54	6.78	123.36	331.06	349.49	410.06	483.68	90.24	259.72	245.89
N_2O-N emissions	0.08 ± 0.02	0.16 ± 0.02	0.31 ± 0.03	0.09 ± 0.04	0.09 ± 0.03	0.34 ± 0.03	0.18 ± 0.04	0.34 ± 0.06	0.32 ± 0.02	0.10 ± 0.01	0.22 ± 0.04	0.45 ± 0.06
N_2-N emissions	1.59 ± 0.14	2.29 ± 0.33	7.12 ± 0.78	3.38 ± 0.87	2.52 ± 0.49	6.66 ± 2.11	3.93 ± 0.51	4.59 ± 0.80	4.47 ± 0.50	1.81 ± 0.48	2.64 ± 0.53	7.69 ± 2.58

Table S4: Spearman correlation coefficients between soil total carbon, total nitrogen, NH_4^+-N, NO_3^--N, total dry weight biomass and N in total dry weight biomass. Significance is indicated as *** – 0.001; ** – 0.01; * – 0.05; ns – not significant.

	Total carbon	Total nitrogen	NO_3^--N	NH_4^+-N	Total biomass	N in total biomass
Total carbon	1	0.91***	ns	ns	0.49*	0.35*
Total nitrogen	0.91***	1	0.50*	ns	0.59**	0.58**
NO_3^--N	ns	0.50*	1	ns	0.78***	0.84***
NH_4^+-N	ns	ns	ns	1	ns	ns
Total biomass	0.49*	0.59**	0.78***	ns	1	0.83***
N in total biomass	0.35*	0.58**	0.84***	ns	0.83***	1

Table S5: Spearman correlation coefficients between gene copies abundance and N_2O-N emissions. Significance is indicated as *** – 0.001; ** – 0.01; * – 0.05; ns – not significant.

Gene parameter	N_2O				
	All	Barley	Sorghum	Sorghum with manure	Wheat
bacterial 16S rRNA	ns	ns	ns	ns	ns
archaeal 16S rRNA	0.18*	ns	ns	ns	ns
nirK	ns	ns	ns	ns	ns
nirS	0.19*	0.58***	ns	ns	ns
nosZI	ns	ns	ns	ns	ns
nosZII	ns	ns	ns	0.41*	-0.46**
bacterial *amoA*	ns	ns	ns	ns	-0.40*
archaeal *amoA*	-0.15*	ns	ns	ns	ns
comammox *amoA*	ns	ns	-0.47**	ns	ns
nrfA	ns	ns	ns	ns	ns

Table S6: Spearman correlation coefficients between moisture and gene copies abundance and N_2O-N emissions. Significance is indicated as *** – 0.001; ** – 0.01; * – 0.05; ns – not significant.

Gene parameter/N_2O-N	Moisture				
	All	Barley	Sorghum	Sorghum with manure	Wheat
bacterial 16S rRNA	ns	ns	0.55***	ns	ns
archaeal 16S rRNA	ns	ns	ns	ns	ns
nirK	ns	ns	ns	ns	ns
nirS	0.29***	0.54***	ns	0.56***	ns
nosZI	ns	ns	ns	ns	ns
nosZII	ns	ns	ns	ns	ns
bacterial *amoA*	ns	ns	ns	ns	ns
archaeal *amoA*	ns	−0.38*	ns	ns	ns
comammox *amoA*	ns	ns	−0.50**	ns	ns
nrfA	ns	ns	0.44**	ns	ns
N_2O-N	ns	ns	ns	ns	ns

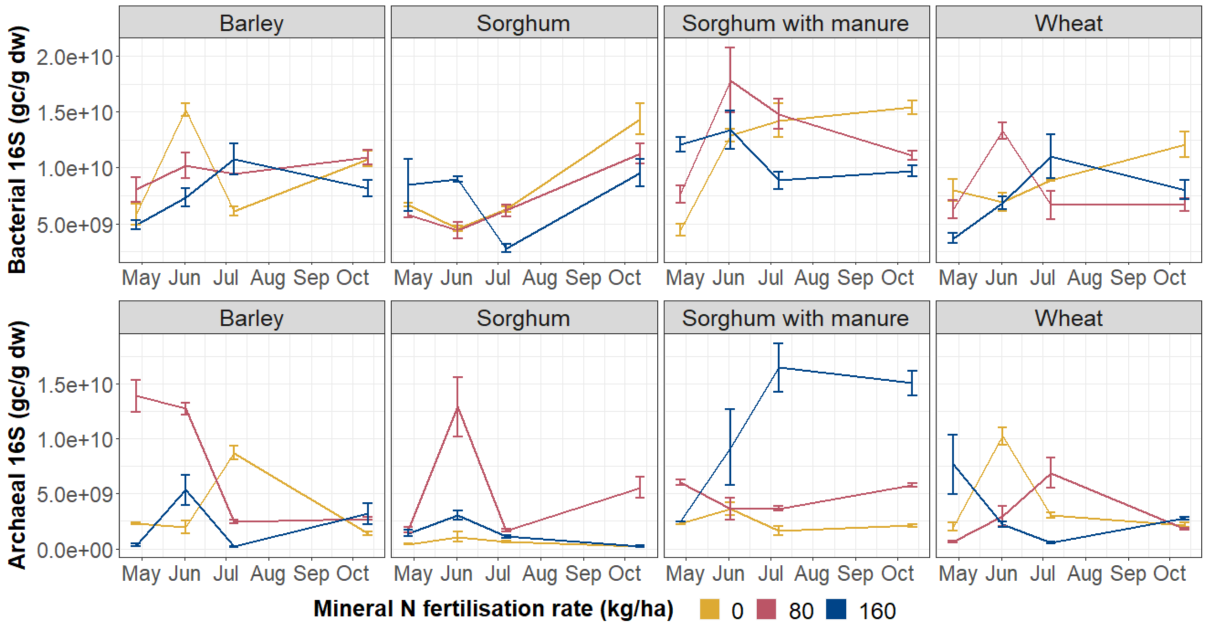

Figure S1: Abundances of bacterial and archaeal 16S rRNA genes according to crops and fertilisation rates during the study period.

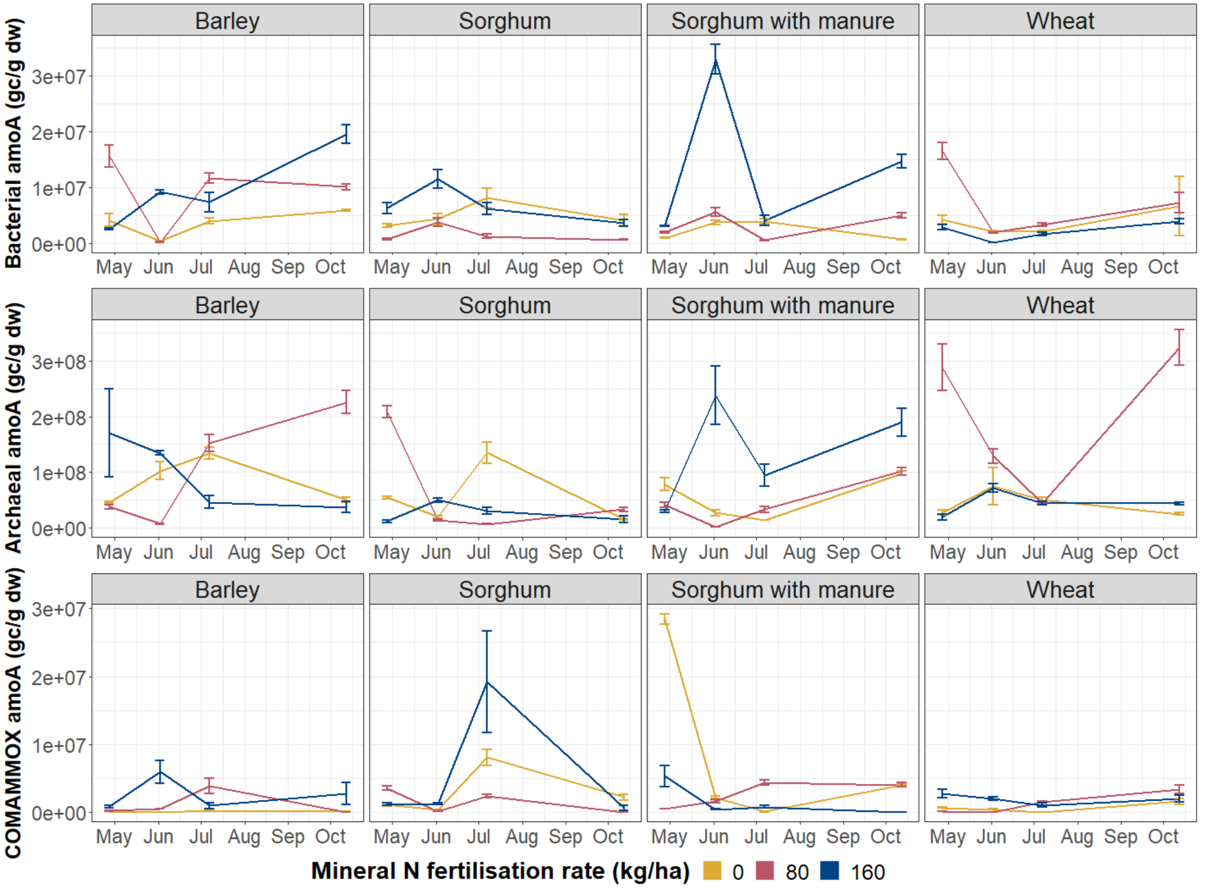

Figure S2: Abundances of bacterial, archaeal and comammox *amoA* genes according to crops and fertilisation rates during the study period.

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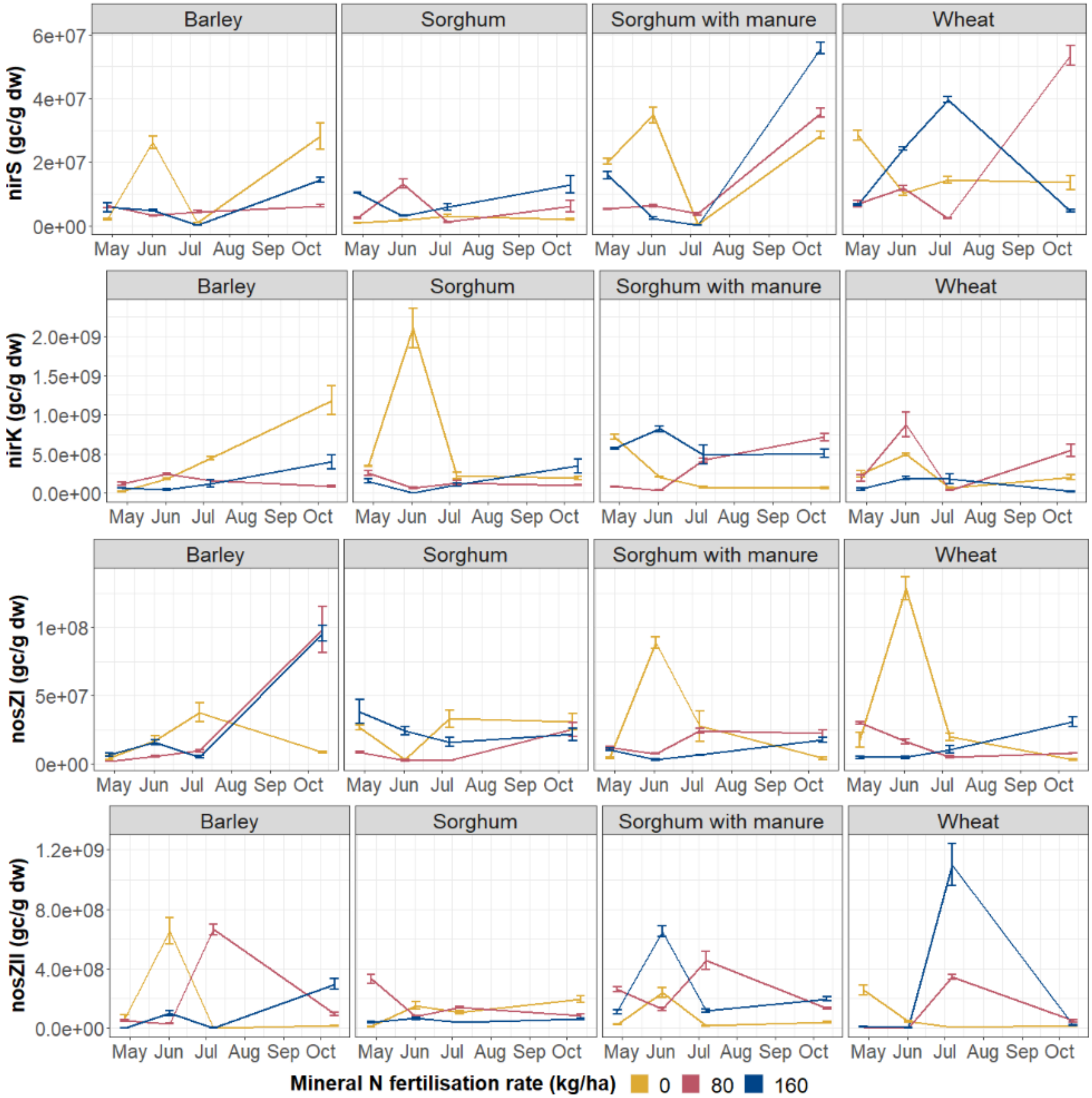

Figure S3: Abundances of *nirK*, *nirS*, *nosZI* and *nosZII* genes according to crops and fertilisation rates during the study period.

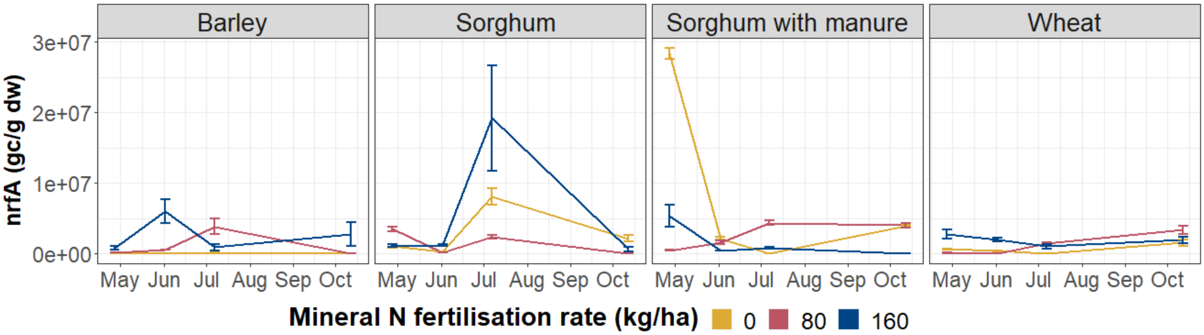

Figure S4: Abundance of *nrfA* gene according to crops and fertilisation rates during the study period.

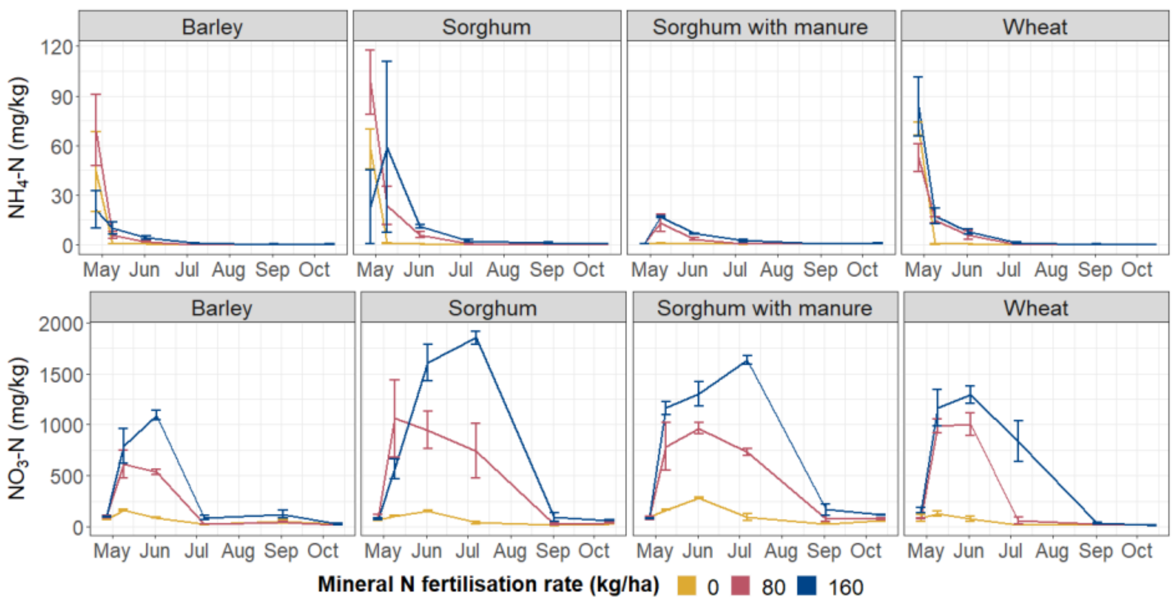

Figure S5: NH_4^+-N (mg/kg) and NO_3^--N (mg/kg) contents of soil according to crops and fertilisation rates during the study period.

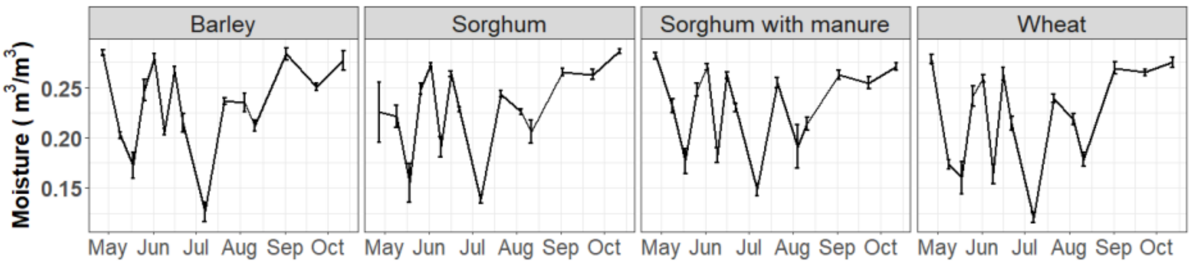

Figure S6: Soil moisture (m^3 /m^3) over the study period according to crop types and treatment.

Supplementary Methodology

<u>Methodology S1: Estimation of the di-nitrogen (N_2) flux from the Daycent model</u>

The N_2 emissions were estimated from the measured N_2O emissions using the N_2:N_2O ratio, which was calculated as proposed in the DAYCENT model (Parton et al., 2001), with the following equation (Del Grosso et al., 2000):

$$R_{N_2/N_2O} = F_r(NO_3/CO_2) \times F_r(\text{WFPS}) \qquad \text{(Eq. 1)}$$

where the factor $F_r(NO_3/CO_2)$ is a function of electron donor to substrate, calculated as:

$$F_r(NO_3/CO_2) = \max\left(0.16\, k_1, k_1 e^{(-0.8\, (C_{NO_3^-}/Flux_{CO_2}))}\right) \qquad \text{(Eq. 2)}$$

and F_r(WFPS) is a factor accounting for the effect of soil water content on the N_2:N_2O ratio, with the water-filled pore space (WFPS):

$$F_r(\text{WFPS}) = 1.4/13^{2.2\, WFPS} \qquad \text{(Eq. 3)}$$

Methodology S2: Nitrogen use efficiency

Nitrogen use efficiency (NUE, kg DM kg^{-1} N^{-1}) was calculated as the biomass yield produced per unit of N applied (Pandey *et al.*, 2001) as follows:

$$NUE = \frac{Treatment\ biomass - Control\ biomass}{Total\ amount\ of\ nitrogen\ applied} \qquad \text{(Eq. 4)}$$

Control biomass is the biomass yield of treatment with mineral fertilisation rate 0. For sorghum with manure amendment plots, control biomass is taken from sorghum without manure amendment plot with mineral N fertilisation rate 0.

Methodology S3: change of soil N content

Change of soil N content was calculated as a difference between the initial soil total N content and final soil total N content (Sainju, 2017).

Total N content in soil was calculated (Sainju, 2017) as following:

$$STN = STN_c \times BD \times T \times 10\ 000$$

STN = Total N content in soil (kg N ha^{-1}), STN_c = Total N concentration in soil (g N kg^{-1}), BD = bulk density (Mg m^{-3}), T=thickness of the soil layer (m), 10 000 = conversion factor.

Methodology S4: N losses

N losses are calculated by substracting N outputs and change of soil N content from N inputs (Sainju *et al.*, 2017; Escuer-Gatius *et al.*, 2022). We consider N deposition, surface run-off and other fluxes neglectable.

$$N\ losses = F_{min.fertiliser} + F_{manure} - harvest - \Delta N_{soil}$$

$F_{min.fertiliser}$ = amount of N added as mineral fertiliser, F_{slurry} = amount of N added as manure, ΔN_{soil} = change of soil N content during the experiment.

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