*Supplement of*

# N dynamics during a 3-year crop rotation fertilized with digestates and cattle effluents

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# Supplementary material 1: meteorological conditions

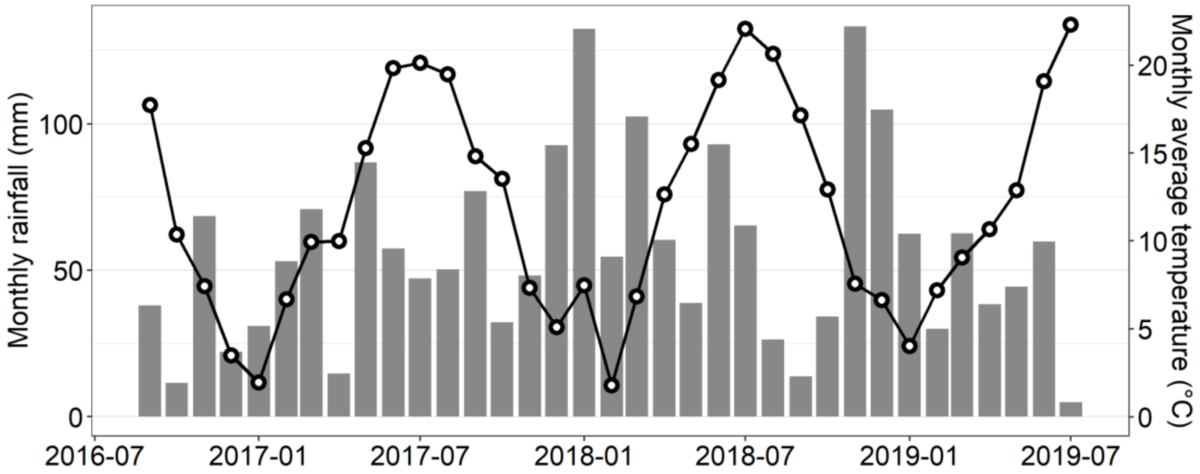


**Figure S1.1: Monthly average air temperature (line, °C), and rainfall (bars, mm) during the field experiment.**

# Supplementary material 2: Map of the experimental design

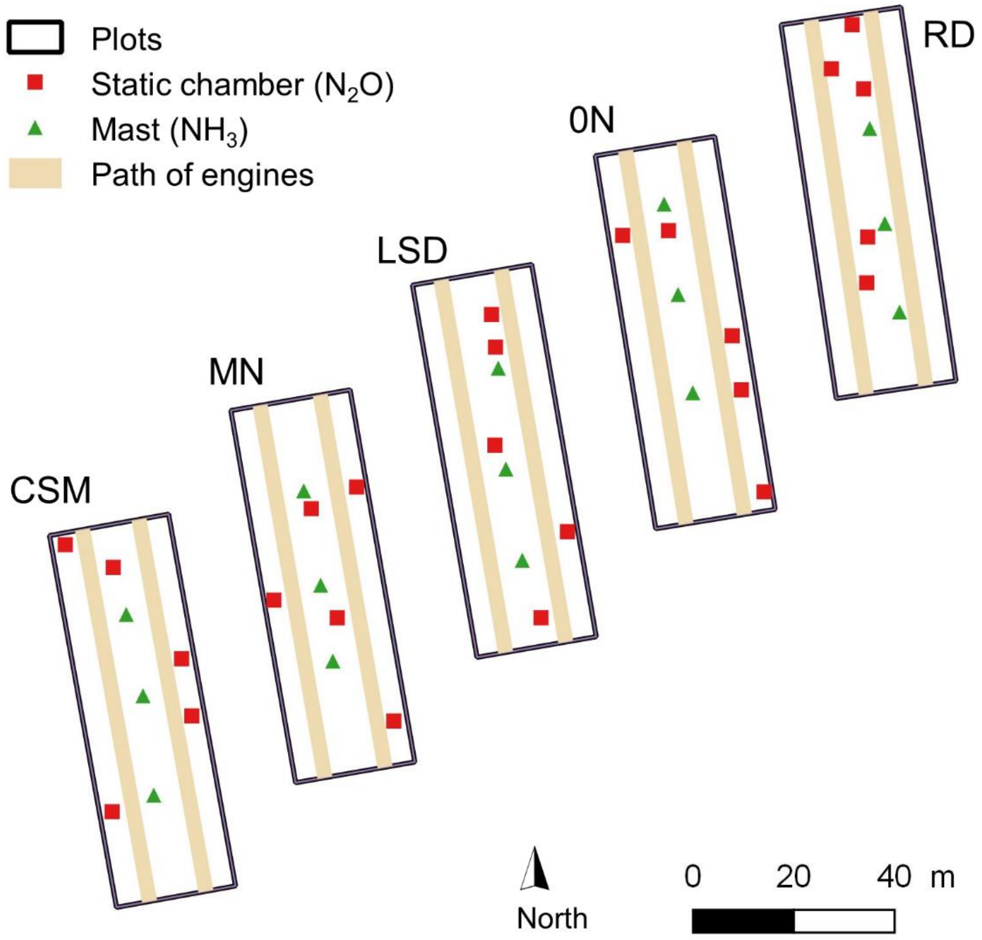


**Figure S2.1 – Map of the experiment design. The five treatments consisted in: (MN) fertilization with only mineral N solution in late winter and spring, and synthetic mineral P fertilizer when needed; (CSM) fertilization with cattle farmyard manure in summer and cattle slurry in late winter and spring; (RD) application of raw digestate (without phase separation) in both summer and late winter and spring; (LSD) application of solid digestate in summer and liquid digestate in late winter and spring; and (0N) no fertilization.**

# Supplementary material 3: Crop management

**Table S3.1 – Technical management of the crops.**

| Crop | Date | Action |
|---|---|---|
| 2016 – 2017 Wheat | 26 October 2016 and 27 October 2016 | Soil tillage: disc harrow (15 cm), |
| | 27 October 2016 | Dish harrow (10 cm), followed by wheat sowing (cultivar Syllon, 260 gr m$^{-2}$) |
| | 22 November 2016 | Herbicide |
| | 22 March 2017 | Fertilization (mineral fertilizers or EOMs) |
| | 19 April 2017 | Fertilization (mineral fertilizers or EOMs) |
| | 04 May 2017 | Herbicide |
| | 15 May 2017 | Fungicide |
| | 20 July 2017 | Grain harvest |
| | 23 July 2017 | Straw harvest |
| 2017 – 2018 Rapeseed | 02 August 2017 | Application of EOMs |
| | 03 August2017 | Burying and soil tillage: ploughing (20 cm), disc harrow (10 cm), roller (2 cm) |
| | 21 August 2017 | Rapeseed sowing (cultivar Fernando, 40 gr m$^{-2}$) |
| | 21 August 2017 | Herbicide |
| | 25 October 2017 | Insecticide |
| | 21 March 2018 | Fertilization (mineral fertilizers or EOMs) |
| | 23 March 2018 | Fungicide and insecticide |
| | 04 July 2018 | Harvest |
| 2018 – 2019 Wheat | 19 September 2018 | Application of EOMs |
| | 20/09/2018 | Burying: disc harrow (10 cm) |
| | 24 October 2018 | Soil tillage: disc harrow (10 cm), followed by wheat sowing (cultivar Descartes, 300 gr m$^{-2}$) |
| | 26 October 2018 | Herbicide |
| | 19 February 2019 | Fertilization (mineral fertilizers or EOMs) |
| | 12 March 2019 | Fertilization (mineral fertilizers or EOMs) |
| | 26 April 2019 | Herbicide |
| | 01 May 2019 | Fertilization (MN and CSM treatments only, ammonium nitrate) |
| | 03 May 2019 | Fungicide |
| | 07 May 2019 | Fungicide |
| | 22 July 2019 | Grain harvest |
| | 23 July 2019 | Straw harvest |

# Supplementary material 4: Detailed method for incubations

The soil used in the experiments was sampled once in summer (incubations of manure, raw digestate, solid digestate) and once in winter (incubations of liquid digestate and slurry), in the field experiment on the 0N control treatment. The soil was sieved fresh to a size of 4 mm. The field capacity of the sieved soil was measured with a Richards press (30,990 Pa) and was 25% w/w. Soil was stored in a closed container at 5°C before being used.

Raw digestate, solid digestate, and cattle manure were sampled on 19th of September 2018 during amendment. Cattle slurry and liquid digestate were sampled on 12th of March 2019 during fertilization. Solid digestate and cattle manure were chopped by hand for homogenization. The EOMs were stored at 4°C in plastic containers at most two months before the incubations.

The EOMs were incubated fresh, in non-limiting conditions for moisture, SMN, and temperature. They were mixed homogeneously into 500 g of soil in the proportion of 2000 mg of C per kg of dry soil (mgC. $kgDS^{-1}$), i.e. 16.6, 20.5, 77.1, 106.1, and 145.1 g FW kg $DS^{-1}$ (mass of Fresh Weight per mass of dried soil) for cattle manure, solid digestate, raw digestate, liquid digestate, and cattle slurry, respectively. After soil mixing, $KNO_3$ solution (farmyard manure) or deionized water (other EOMs) was added to the mixture to reach a minimum SMN content of 35 $mgN_{min}$ $kgDS^{-1}$, and a soil moisture of 25% w/w (weight/weight), corresponding to soil moisture at field capacity. The addition of the liquid digestate and slurry to the soil provided large amounts of water, which had been considered in the incubation experiments to achieve a common soil moisture level in all treatments. Because of the large amount of water linked to organic matter in the EOMs, the DM content of the EOMs is not relevant for this adjustment of soil moisture. Thus, we used a “moistening power” of an EOM as the quantity of water significantly humidifying soils in microcosm experiments. It was determined by weighing free water after the centrifugation of the EOMs for 10 min at 614 × g (3 replicates). This method was previously described on the same soil by Moinard et al. (2021). Soil moisture was maintained constant during the incubation, with regular additions of deionized water. Mixtures were incubated in darkness at 28 ± 1 °C for 175 days.

The following of N mineralization or immobilization used destructive replicates. Four replicates per treatment and per date of measurement were used. Mixture was added in an open 1 L plastic vessel, covered by a perforated plastic film, in order to allow oxygen and air circulation while limiting soil water evaporation. At dates 0, 3, 7, 14, 25, 49, 91, 175 days, SMN was extracted with KCl solution. 120 g of mixture were mixed with 400 mL of KCl solution (1 mol $L^{-1}$), then agitated during 1h. Supernatant is filtrated on glass-fiber filter (pores: 1.2 µm) and frozen at -20°C before dosage. Pure KCl solution were also filtrated and stored on each date to evaluate any exogeneous N contamination (blank). $N\text{-}NH_4^+$ and $N\text{-}NO_3^-$ contents were dosed by colorimetry on a continuous flow analyser (Skalar, The Netherlands). $N\text{-}NH_4^+$ and $N\text{-}NO_3^-$ contents were corrected by subtracting mean $N\text{-}NH_4^+$ and $N\text{-}NO_3^-$ contents in blank sample. At each date, to avoid accounting for initial mineral N, mineralized $N\text{-}NH_4^+$ and $N\text{-}NO_3^-$ contents were computed by subtracting total $N\text{-}NH_4^+$ and $N\text{-}NO_3^-$ contents by their contents at t=0 day in each treatment. Net N mineralization or immobilization was computed by subtracting mineralized $N\text{-}NH_4^+$ and $N\text{-}NO_3^-$ contents in soil by the one in unamended control (soil mineralization). Net N mineralization or immobilization was then expressed in % $N_{org}$ initially present in the EOM. We also computed the available mineral N brought by the EOM, expressed in kg $N_{min}$ in soils per kg FW of EOM brought, taking into account both mineralization/immobilization cited above and initial TAN content in the EOM.

Moinard, V., Redondi, C., Etiévant, V., Savoie, A., Duchene, D., Pelosi, C., Houot, S., Capowiez, Y., 2021. Short- and long-term impacts of anaerobic digestate spreading on earthworms in cropped soils. Applied Soil Ecology 168, 104149. https://doi.org/10.1016/j.apsoil.2021.104149

# Supplementary material 5: Detailed methods for assessing agricultural performance of crops

## Yield uncertainty

The balance that measured grain and straw yield had a precision of ±10 kg. Therefore, the uncertainty of the mass of grain was $\pm\frac{10}{\sqrt{3}} = 5.8$ kg for 1800 $m^2$ (rectangular probability distribution, type B evaluation of uncertainty). It was then scaled in t $ha^{-1}$.

## Computing of N uptake

In each subplot, we computed two intermediary variables. The N concentration in the whole aboveground crop ($[N]_{aerial}$, gN $kgDM^{-1}$), including grain and straw, was computed with Eq S5.1:

$$[N]_{aerial} = ([N]_{grain} \,.\, m_{grain} + [N]_{straw} \,.\, m_{straw}) / (m_{grain} + m_{straw}) \qquad (S5.1)$$

where $[N]_{grain}$ is the N content in grain (gN $kgDM^{-1}$), $m_{grain}$ is the mass of grain in the subplot (kgDM), $[N]_{straw}$ is the N content in straw (gN $kgDM^{-1}$), $m_{straw}$ is the mass of grain in the subplot (kgDM).

The mass ratio of grain to aboveground crop organs ($ratio_{grain:aerial}$) was computed with Eq S5.2:

$$ratio_{grain:aerial} = m_{grain} / (m_{grain} + m_{straw}) \qquad (S5.2)$$

At last, we computed the N uptake by the crop in the whole plot. N export in grain at harvest ($N_{grain}$, kgN $ha^{-1}$) was computed as:

$$N_{grain} = yield \,.\, [N]_{grain} \qquad (S5.3)$$

where yield is grain yield (t DM $ha^{-1}$). Total aboveground N uptake (including straw and grain) ($N_{aerial}$, kgN $ha^{-1}$) was computed as:

$$N_{aerial} = yield \,.\, [N]_{aerial} / ratio_{grain:aerial} \qquad (S5.4)$$

N exported in straw $N_{strawexp}$ (kgN $ha^{-1}$) was computed as:

$$N_{strawexp} = straw\ yield \,.\, [N]_{straw} \qquad (S5.5)$$

Where the straw yield is expressed in t DM $ha^{-1}$ and $[N]_{straw}$ is the concentration of N in straws (gN $kgMS^{-1}$). N returned to soil through aerial crop residues ($N_{returned}$, kgN $ha^{-1}$) were then computed as:

$$N_{returned} = N_{aerial} - N_{strawexp} - N_{grain} \tag{S5.6}$$

Uncertainties on $N_{grain}$ and $N_{uptake}$, $N_{strawexp}$, and $N_{returned}$ were computed using error propagation formulas from yield measurement uncertainty and standard error of $[N]_{grain}$, $[N]_{aerial}$, $[N]_{straw}$, and $ratio_{grain:aerial}$.

# Supplementary material 6: Detailed method for SMN analysis

SMN (i.e. $NO_3^-$ and $NH_4^+$ contents in soil) were analyzed by colorimetry after extraction with a KCl solution. 25 g of soil were mixed with 100 mL of KCl solution (1 mol $L^{-1}$), then agitated during 1h. Supernatant is filtrated on glass-fiber filter (pores: 1.2 µm) and frozen at -20°C before dosage. Pure KCl solution were also filtrated and stored on each date to evaluate any exogeneous N contamination (blank). $N\text{-}NH_4^+$ and $N\text{-}NO_3^-$ contents were dosed by colorimetry on a continuous flow analyser (Skalar, The Netherlands). $N\text{-}NH_4^+$ and $N\text{-}NO_3^-$ contents were corrected by subtracting mean $N\text{-}NH_4^+$ and $N\text{-}NO_3^-$ contents in blank sample.

# Supplementary material 7: Detailed method for $N_2O$ emissions

$N_2O$ emissions were measured with the static chamber method (Gu et al., 2013; Jeuffroy et al., 2013). Each plot was equipped with 5 static chambers. The position of each chamber was semi-randomly chosen to be representative of the whole plot surface. They were located more than 1.5 m from the plot border, avoiding wheel tracks. After a spring fertilizer application, $N_2O$ emissions were measured twice a week during 2 weeks, then once a week for one month and once a month during late spring and summer. After a summer fertilizer application, $N_2O$ emissions were measured twice a month, and once a month in late autumn and winter before the next application event. In total, $N_2O$ emissions were measured at 40 dates. The static aluminum chambers measured 50 cm x 50 cm. They were 25 cm high in autumn and winter, and 75 cm high in spring when vegetation was higher. They were buried 10 cm within the soil. At each measurement date, chambers were sealed, and air with accumulated $N_2O$ was sampled at time 0 min, 30 min, 60 min, and 90 min. The measurement of $N_2O$ emissions started between 10 am and 2 pm. $N_2O$ in air samples was analyzed by Gas Chromatography with Electron Capture Detector (Trace GC Ultra, Thermo Scientific). When the $N_2O$ concentration in the chamber was positively correlated with time ($r > 0.9$), there was an accumulation of $N_2O$ and the slope of the regression line was used to compute $N_2O$ emission rate. When the $N_2O$ did not accumulate in the chamber (correlation between $N_2O$ content and time $r < 0.9$), $N_2O$ emissions were set to 0.

$N_2O$ emissions were measured on 40 dates: 14/03/2017, 24/03/2017, 27/03/2017, 31/03/2017, 06/04/2017, 12/04/2017, 21/04/2017, 26/04/2017, 04/05/2017, 10/05/2017, 17/05/2017, 30/08/2017, 15/09/2017, 29/09/2017, 06/10/2017, 07/11/2017, 20/12/2017, 29/01/2018, 23/03/2018, 26/03/2018, 29/03/2018, 06/04/2018, 11/04/2018, 18/04/2018, 24/04/2018, 02/05/2018, 27/09/2018, 17/10/2018, 31/10/2018, 14/11/2018, 18/12/2018, 05/02/2019, 21/02/2019, 07/03/2019, 13/03/2019, 19/03/2019, 28/03/2019, 09/04/2019, 14/05/2019, 12/06/2019.

Gu, J., Nicoullaud, B., Rochette, P., Grossel, A., Hénault, C., Cellier, P., Richard, G., 2013. A regional experiment suggests that soil texture is a major control of N2O emissions from tile-drained winter wheat fields during the fertilization period. Soil Biol Biochem 60, 134–141. https://doi.org/10.1016/j.soilbio.2013.01.029

Jeuffroy, M.H., Baranger, E., Carrouée, B., de Chezelles, E., Gosme, M., Hénault, C., Schneider, A., Cellier, P., 2013. Nitrous oxide emissions from crop rotations including wheat, oilseed rape and dry peas. Biogeosciences 10, 1787–1797. https://doi.org/10.5194/bg-10-1787-2013

# Supplementary material 8: Individual analyses of cattle slurry

**Table S8.1 – Individual analyses of cattle slurries. TS: total solid. FW: Fresh weight. TAN: ammoniacal N. TN: total N.**

| EOM | Application date | TS (%FW) | TAN (g $kgFW^{-1}$) | TN (g $kgFW^{-1}$) | VS (g $kgFW^{-1}$) | pH |
|---|---|---|---|---|---|---|
| Cattle slurry | 22/03/2017 | 1.6 | 0.6 | 1.1 | 11.3 | 7.7 |
| | 19/04/2017 | 0.8 | 0.8 | 1.0 | 4.8 | 7.5 |
| | 21/03/2018 | 7.5 | 1.3 | 3.3 | 56.1 | 6.6 |
| | 19/02/2019 | 4 | 2.0 | 2.7 | 27.6 | 7.5 |
| | 12/03/2019 | 3.2 | 1.1 | 2.2 | 22.1 | 7.9 |

# Supplementary material 9: Rapeseed growth from sowing to February

Aerial biomass and N uptake of rapeseed were evaluated on 3 subplots per treatment (total 3 m$^2$) on 20th of November 2017 and 20th of February 2018 to drive N fertilization, similarly to N uptake at harvest. No significant differences were highlighted between the different treatments.

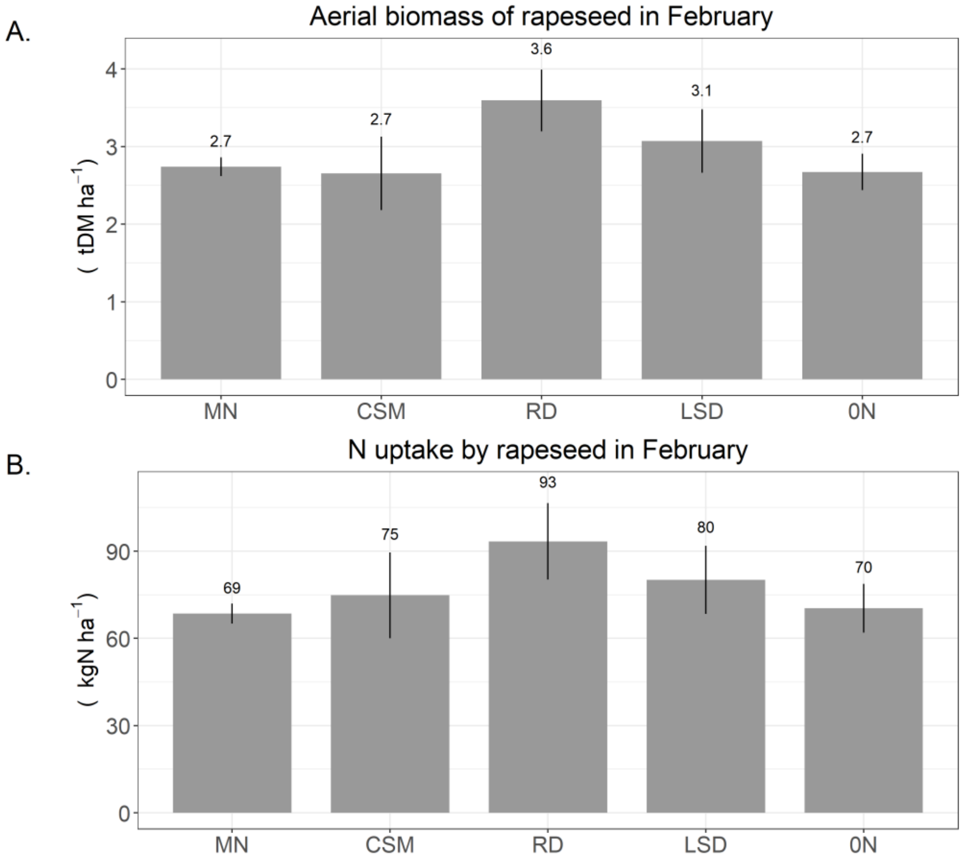


**Figure S9.1: A) Aerial biomass and B) N uptake from rapeseed at the end of winter (February). Error bars show one standard error.**

# Supplementary material 10: Evolution of soil mineral N stocks during the 3-year experiment

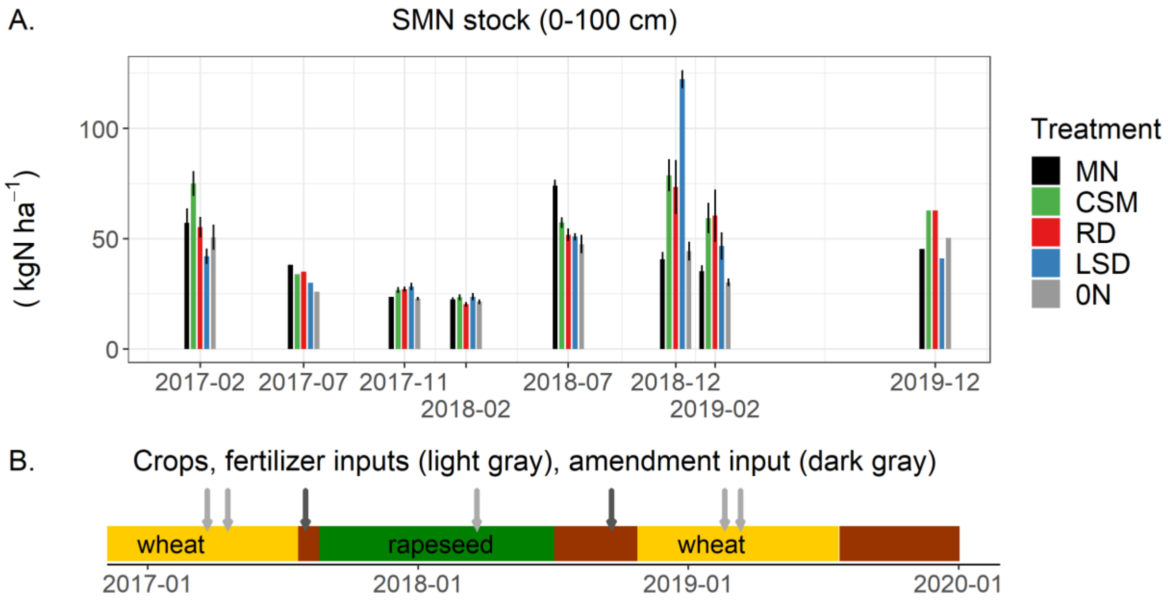


**Figure S10.1: A) Soil mineral N (SMN) stocks (0-100cm), on the same x-scale as B) Crop management. Arrows indicate the fertilization dates in late winter and spring (light gray, MN, CSM, RD, and LSD treatment), and in summer (dark gray, CSM, RD, and LSD treatments).**

# Supplementary material 11: Daily $N_2O$ emissions

At each date, we used a Kruskal-Wallis test to verify whether $N_2O$ emissions were different between treatments. If the treatments had significantly different $N_2O$ emissions, ($p <0 .05$), a post-hoc Dunn's test was used to find which treatment was different from one another ($p < 0.05$). We used the kruskal.test function, and the dunn.test function from the R *dunn.test* package (Dinno, 2017).

Dinno, A., 2017. dunn.test: Dunn's Test of Multiple Comparisons Using Rank Sums. R package version 1.3.5. https://CRAN.R-project.org/package=dunn.test.

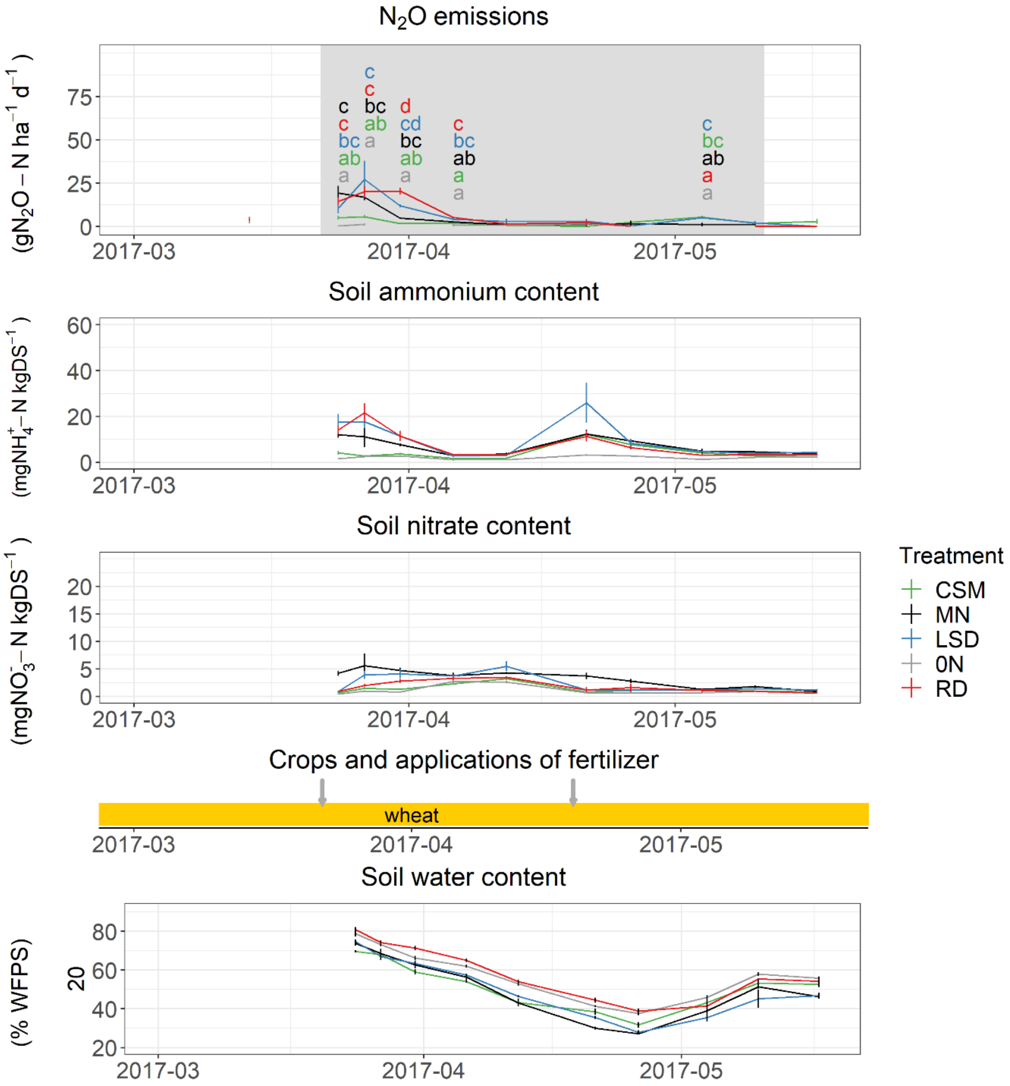
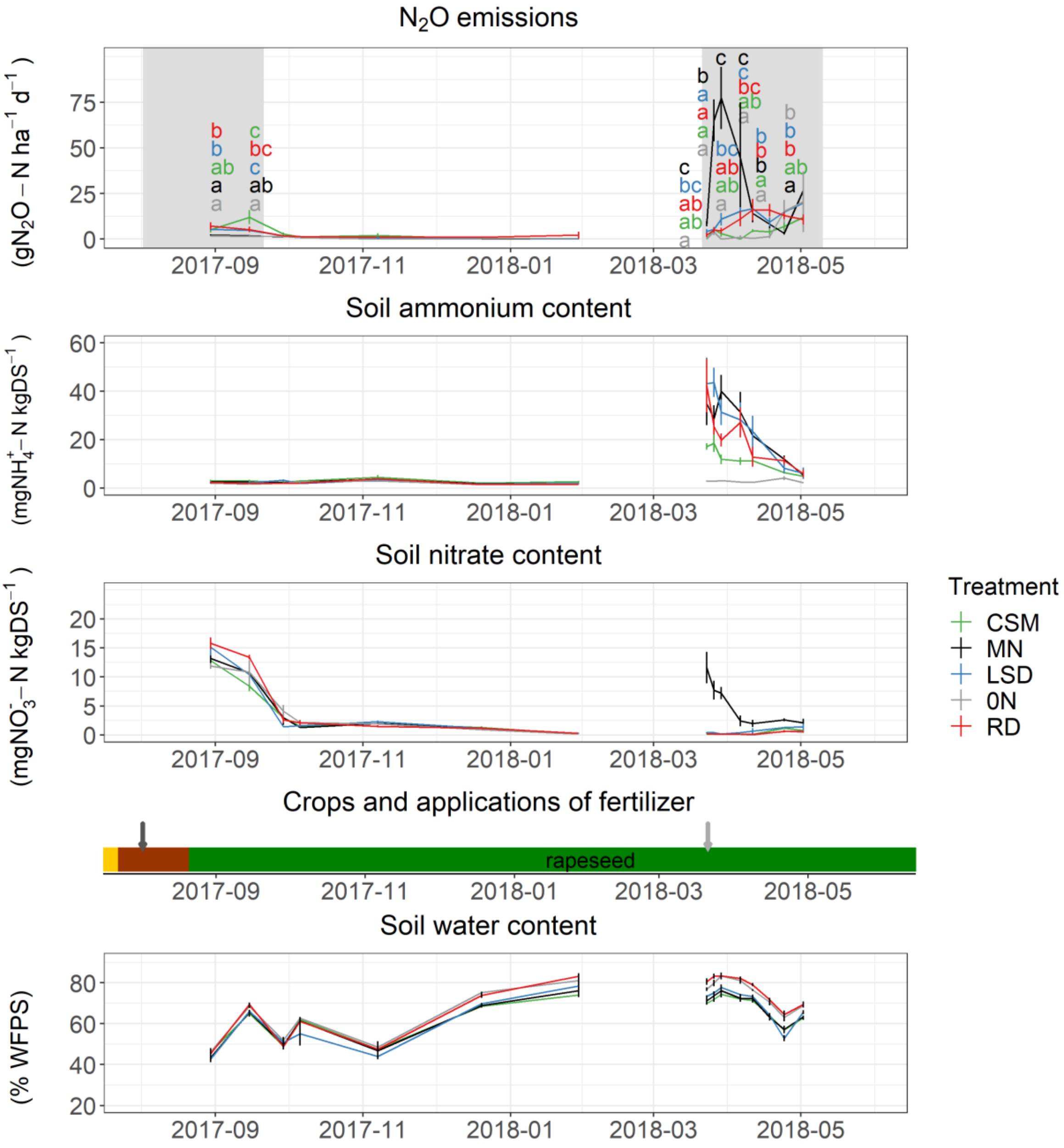


**Figure S11.1: N$_2$O emissions, evolution of total ammonium N and nitrate N stocks in the upper soil layer (0 – 20 cm) and evolution of topsoil water content expressed as the water filled pore space during wheat cropping season in 2017. In each subfigure, error bars show one standard error. All subfigures share the same x-axis (time): punctual N$_2$O emissions, topsoil ammonium N content, topsoil nitrate content, crops and fertilization dates, soil water content. Only for N$_2$O emissions, at each date, a Kruskal-Wallis test followed by a post-hoc Dunn's test was realized to test whether the emissions were significantly different from one another. When no differences were highlighted between treatments, no letters are drawn. When treatments are shown different by Kruskal-Wallis test (p-value < 0.05), letters are drawn for each treatment and treatment sharing no letters are significantly different (Dunn's test, p-value <0.05). The periods considered for total emissions were highlighted in gray.**



188

189 **Figure S11.2: $N_2O$ emissions, topsoil mineral N (ammonium and nitrate) content and water**
190 **content (rapeseed 2017-2018). See legend of Figure S13.1 for more details.**

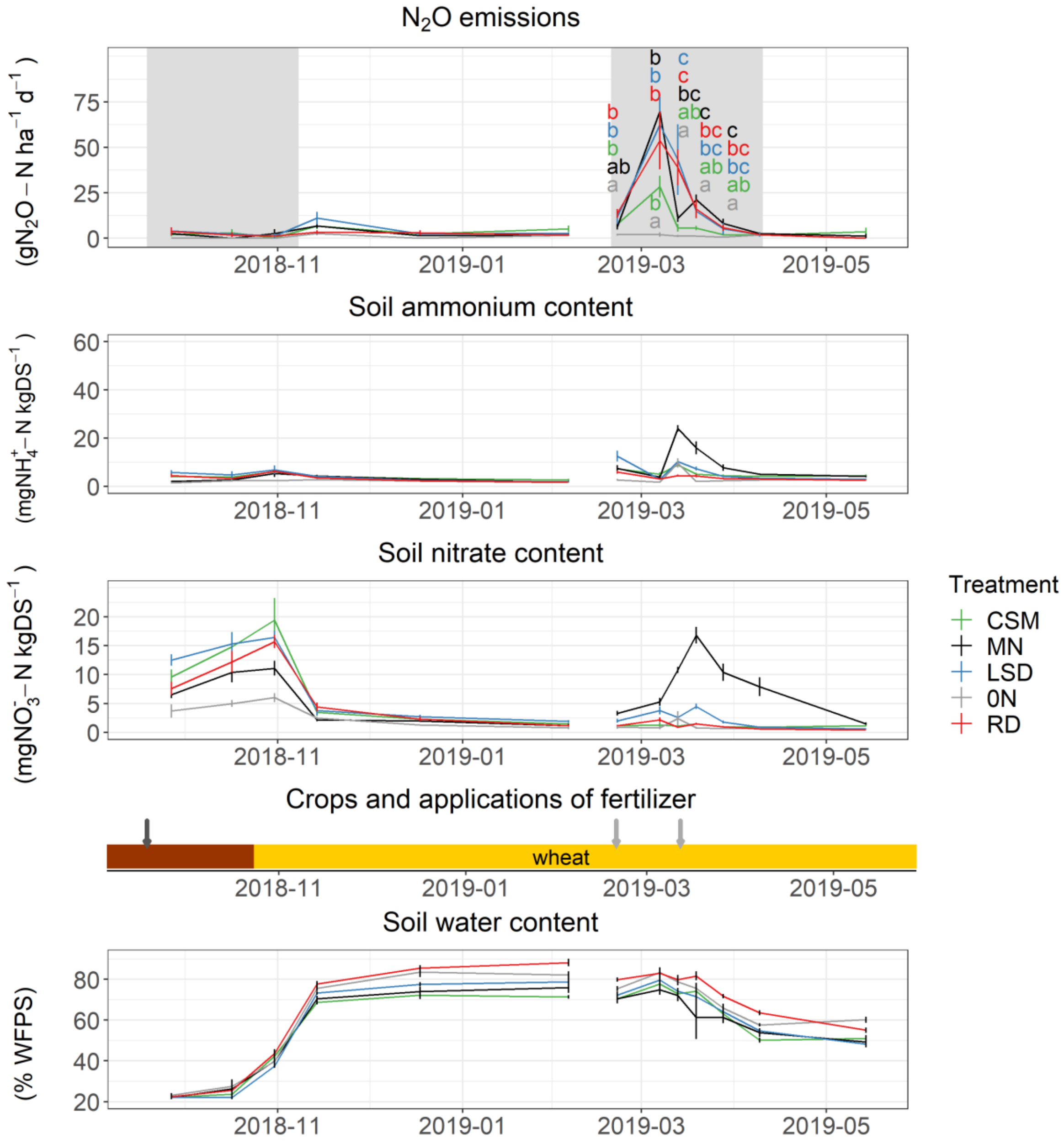


**Figure S11.3: $N_2O$ emissions, topsoil mineral N (ammonium and nitrate) content and water contents (wheat 2018-2019). See legend of Figure S13.1 for more details.**