INRAE (UMR PEGASE and SAS) and the Livestock Institute (IDELE) contributions to the CCCfarming project

- Final Report -

Project: Climate Care Cattle Farming Systems - CCCfarming

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Introduction

The main objective of the Climate Care Cattle Farming Systems (CCCFarming) was to develop cattle farming systems having as low greenhouse gases (GHG) and ammonia (NH_3) emissions as possible but with no detrimental consequences on social and production aspects.
To do so several actions have been carried out under the six work packages (WP) presented in the Figure 1. The Livestock Institute (Institut de l'Elevage – IDELE) contribution has been extensively developed in a report named "FinalReport_CCCFg_IDELE-INRAE(SAS)_Nov2023". **The present report has the objective to present the joint contributions of INRAE PEGASE, INRAE SAS and IDELE, mainly regarding WP2 (for which INRAE PEGASE was WP leader) and WP4. The contributions of INRAE PEGASE to WP5 and WP6 are also mentioned.**

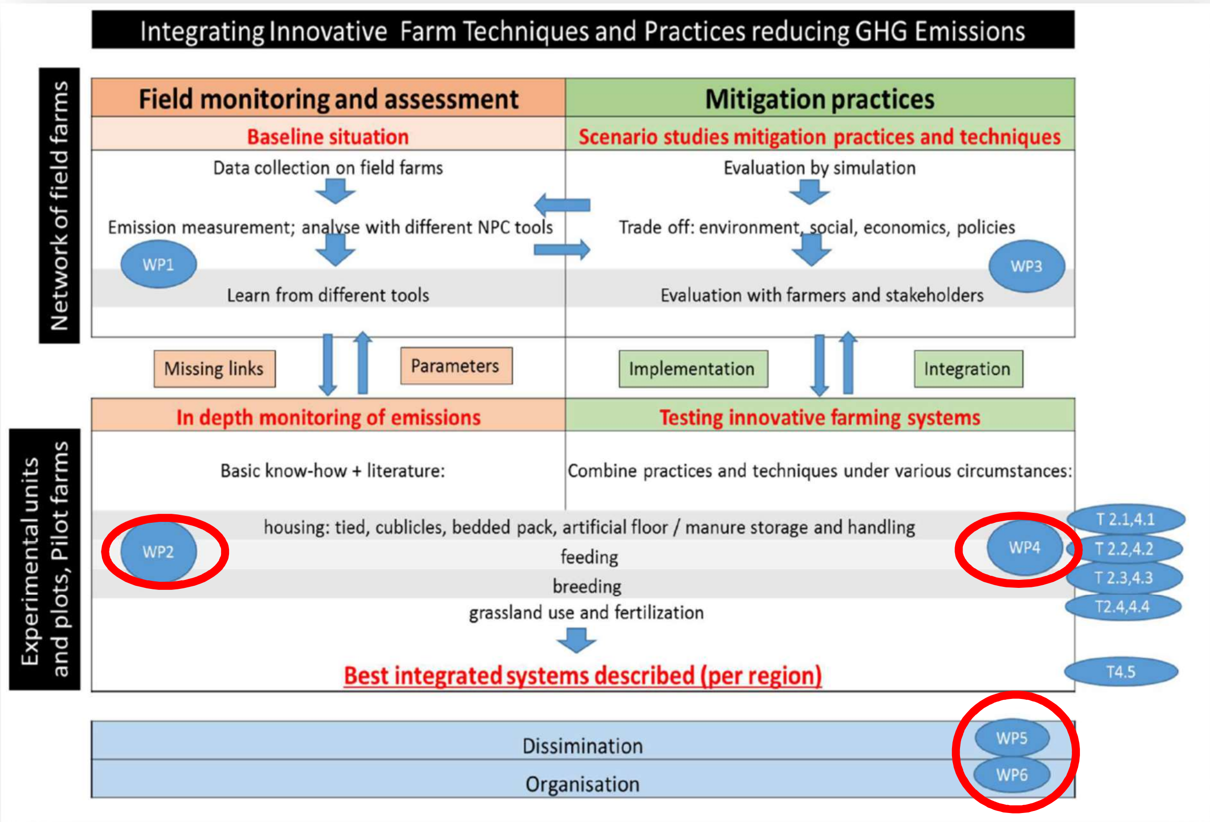


Figure 1 : Overview of the CCCFarming project and joint contributions from INRAE PEGASE, INRAE SAS and IDELE described in this report (red circles)

1. WP2 – In depth monitoring and research

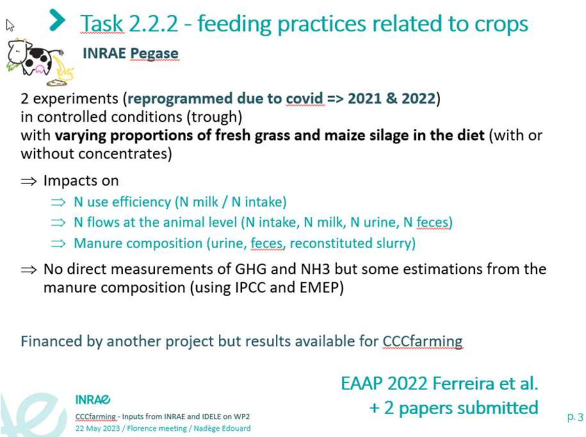
1.1. WP2.2.2: Study and monitor novel feeding practices related to crops

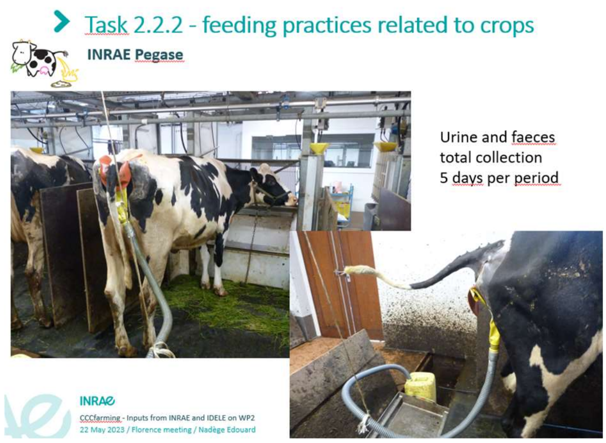
Combining fresh herbage and maize silage in dairy cows' diets is a common practice on farms, but the nutritional and environmental consequences are still poorly known. Two experiments (not financed by the CCCfarming project) were conducted at the INRAE experimental farm of Méjusseaume (Le Rheu, France; https://doi.org/10.15454/yk9q-pf68) to explore the effects of different proportions of fresh herbage (0 to 100%) in a maize silage-based diet, with or without soya bean meal. For the same dietary crude protein (CP) concentration (130 g/kg DM), feed intake and milk yield decreased as the proportion of fresh herbage increased. However, without soya bean meal and with a low N concentration in herbage, feed intake and milk yield increased strongly as the proportion of fresh herbage increased, due to the high CP deficit in diets rich in maize silage (< 110 g/kg DM).

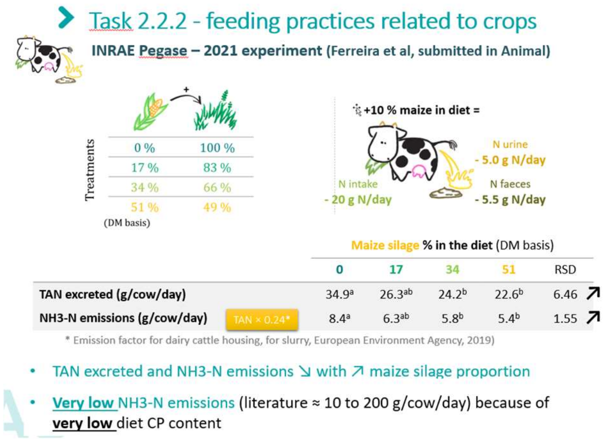
The experiments and their results were described in:

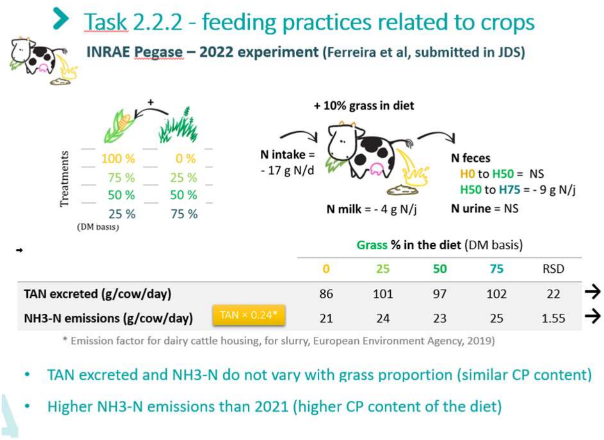
- One paper published in the Animal Journal: Ferreira M, Delagarde R, Edouard N, 2023. Nitrogen balance in dairy cows fed low-nitrogen diets based on various proportions of fresh grass and maize silage. Animal 17, 100976. https://doi.org/10.1016/j.animal.2023.100976 (see Annex 1)
- One paper under review for Animal Science Journal: Ferreira M, Delagarde R, Edouard N, 2023. Effects of replacing corn silage and soybean meal with an increasing percentage of fresh herbage on dairy cow nitrogen use efficiency and flows. Animal Science Journal.
- One oral communication given at EAAP in 2022: Ferreira M, Delagarde R, Edouard E, 2022. Nitrogen excretion and ammonia emissions in dairy cows fed low-N fresh grass and maize silage. 73th Annual meeting of the European federation of animal science (EAAP), Porto, Portugal. pp.315. https://hal.inrae.fr/hal-03938337 (see Annex 2)

Some extra results regarding total ammonia nitrogen (TAN) and ammonia nitrogen (NH3-N) emissions potential (based on emission factors) were also presented during a CCC Farming seminar in Florence (Italy) in May 2023 and are reported below:









1.2. WP2.4.1: Study and monitor grazing and fertilization practices and techniques

This task was based on continuous measurements conducted on a grazed and fertilised pasture at the INRAE experimental farm of Méjusseaume (https://doi.org/10.15454/yk9q-pf68).

Some of the objectives were fully achieved while some are still incomplete today due to equipment failure:

a) Field-scale measurements of NH_3, N_2O, CO_2 surface-atmosphere fluxes during grazing.
☑ **DONE**

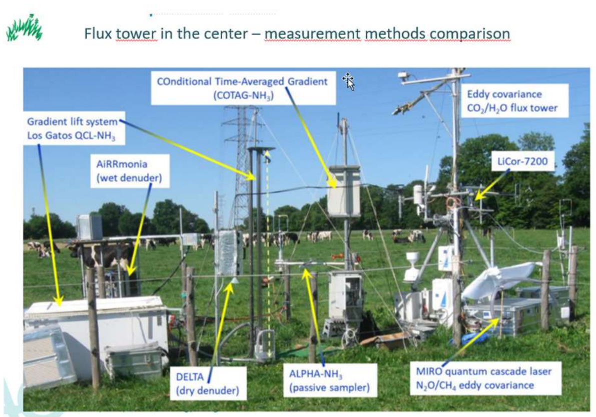
b) Assess spatial variability in N_2O emissions from « fast-box » intensive campaigns.
☑ ☒ **Incomplete**

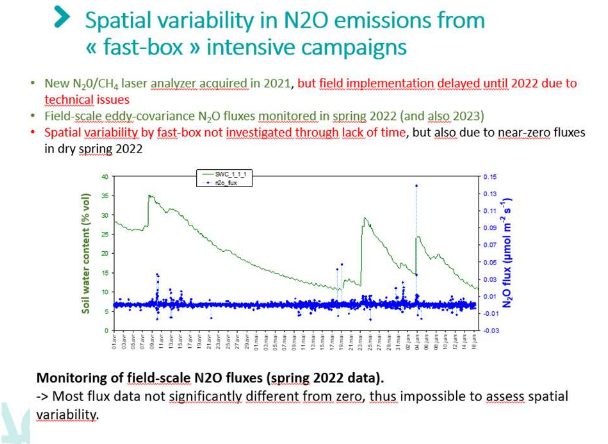
c) Estimate annual scale C and N fluxes from flux tower & other measurements
☑ **DONE** (soil carbon sequestration, N-budget)

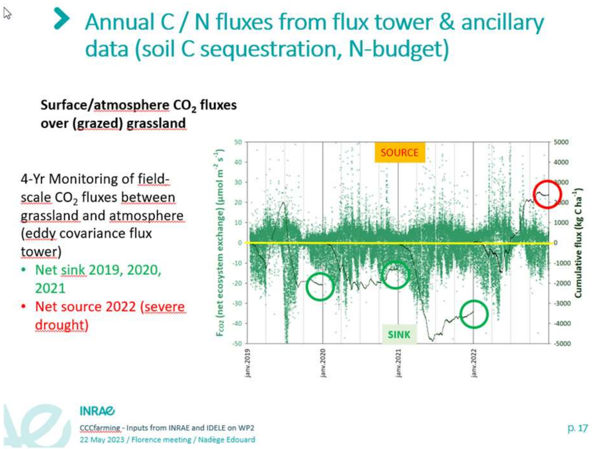
The experiments and their results were described in:

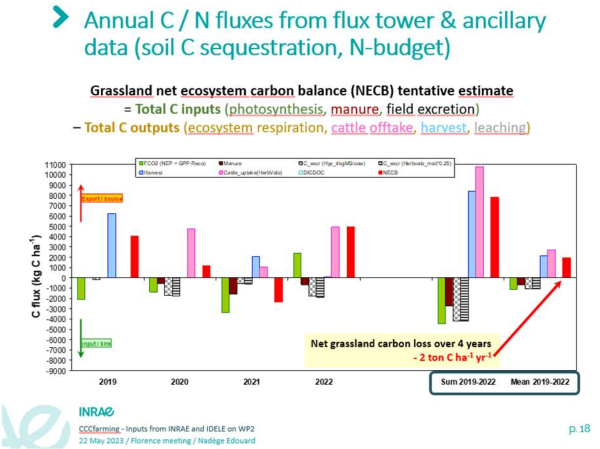
➢ One oral communication at the World Meteorological Organization in 2022: Chris Flechard, Yannick Fauvel, Adrien Rémy Delagarde, Anne Isabelle Graux, Nadège Edouard, 2022. Surface/atmosphere exchange of NH3 above managed grassland - Long term low cost monitoring and short term intensive campaigns WMO GAW, SAG TAD, Geneva, 05 October 2022 (see Annex 3).

Some extra results regarding N2O emissions and C-N annual fluxes were also presented during a CCC Farming seminar in Florence (Italy) in May 2023 and are reported below:









1.3. WP2.4.3: Perspective of using drones

INRAE was not supposed to participate to this task. However, the UNIFI partner (Italy) in charge of the work contacted Chris Fléchard (INRAE SAS) and Nadège Edouard (INRAE PEGASE) to discuss about the possibility of testing their drone system in the field, at the INRAE experimental farm, and to organise a cross-reference ground and in-flight measures with the equipment managed by INRAE-SAS.

Several discussions and virtual meetings occurred during 2022. However, the idea was abandoned because:

- measuring gases in the field with the drone multisensor system (and comparing measurements with the gas analyzers in the field), even when animals are present in high density, would be challenging.

- only comparing the results from the flights over the whole farm with estimates from emission factors would be somehow informative but maybe not the best experimental option.

2. WP4 - Testing innovative farming systems

2.1. WP4.1 and WP4.2: Testing of feeding (including grazing), housing and manure handling practices on emissions

Remark: The experiment described below was initially proposed to fulfil the work proposed in WP2.1.1, WP2.1.2, WP2.2.2 and WP2.4.1 respectively. However, given the systemic nature of the approach, this experimentation was more logically proposed as a deliverable for WP4.

Context and objective of the study:

In order to achieve agronomic, zootechnical, economic and environmental multi-performance, dairy farms must make better use of food resources, allow better efficiency in the use of nutrients by animals, reduce the use of inputs and reduce their environmental impacts associated in particular with nitrogen losses. To achieve this, dairy farming systems can rely on grazed grass, which turns out to be a balanced food resource of good nutritional value, not in competition with human food resources, and which also allows a controlled feed cost and contributes to animal welfare. When the livestock system allows it, grazing can also be considered as a strategy to reduce ammonia emissions compared to animals exclusively in buildings, while playing a role in the looping of CNP cycles and contributing to the provision of ecosystem services.

In this context, we more specifically focused on feeding periods and / or feeding strategies combining grazed and conserved forages in dairy systems and their consequences on environmental impacts. Indeed, the diet of dairy animals relies heavily on grazed grass. However, at certain times of the year, and for different reasons (food transition, insufficient grass resources, breeder's strategy), conserved forages and concentrates can be distributed in addition to pasture. Although there is now a great deal of knowledge on the use of nitrogen by animals for rations based either on conserved forages or on grazed herbage, few studies have focused on the consequences of their association on nitrogen flows at the animal level and manure composition. In addition, in these situations, the animals divide their time between the pasture, where their droppings fall directly on the ground, and the building, where the effluents must be managed (evacuation of the building, storage, possible treatment, spreading) involving various impacts on the environment.

Through setting up of experimental tests, the project aimed to acquired data and new knowledge on these mixed systems associating grazed and conserved forages by comparison with mono-forage feeding strategies. The consequences of these contrasting situations on the composition of effluents was studied to assess their contribution to environmental impacts. The gaseous emissions (ammonia and greenhouse gases) from different systems, whether or not combining grazing and housing in buildings were compared on the different emission items (building, storage, grazed plot).

Experimental approach:

2 continuous trials, lasting 3 months, were conducted in spring and autumn 2022 to compare 3 contrasting management methods:

- Housing: full-time housing (cubicles and slatted floors), feeding based on the distribution of conserved forage (maize silage) and concentrates (soya meal), manure deposited in the building and stored in uncovered outdoor pits.

- Grazing: full-time grazing excluding milking (i.e. around 20 h/day), feed based on grazed grass, with no concentrates, manure deposited on the plot.

- Mixed management: grazing during the day only (8 hours between the 2 milkings) and housing in the barn at night with distribution of conserved forage (maize silage) and concentrates (soya meal), manure deposited partly in the barn and stored in uncovered outdoor pits and partly on the field.

In the barn, the groups of animals were housed in experimental pens (with mechanical ventilation) for individualised feeding and monitoring of intake of forages and concentrates. Manure management was carried out at group level, with daily scraping of and storage in a dedicated pits for each pen, allowing measurement of gaseous emissions (NH_3, N_2O, CO_2, CH_4) for each type of management in the building and in the storage of manure. Animal performance and emissions (NH_3, N_2O) were also monitored at pasture for the exclusively grazed and day-grazed groups.

The experiments and some of their results were described in:

- One oral communication given at EAAP 2023: Nadege Edouard, Xavier Vergé, Christophe Flechard, Yannick Fauvel, Adrien Jacotot, 2023. Gas emissions (building, storage, pasture) of dairy systems combining or not grazing and housing. 74th Annual meeting of the European federation of animal science (EAAP), Lyon, France (see Annex 4)

3. WP5 - Dissemination & Communication

Nadège Edouard (INRAE PEGASE), Xavier Vergé (IDELE) and Katja Klumpp (INRAE UREP) were asked to organise, with their INRAE and IDELE colleagues, a presentation for the virtual seminar organised by the CCC Farming project leaders in April 2021 on "Visionary aspects of dealing with C in dairy systems and C storage" (see Annex 5).

The same authors proposed an oral communication at EAAP 2022, focusing on "Nutrient circularity: the role of dairy systems and a solution for GreenHouse Gas and NH3 mitigation" (see Annex 6).

As mentioned previously, WP2 and WP4 were presented at EAAP 2022 (see Annexes 2 and 4).

Nadège Edouard (INRAE PEGASE) was also asked by the project leaders to be chairwoman of 2 sessions of the CCC Farming project at EAAP, one in 2022 and one in 2023.

Some local/national presentations of the project were organised in the meantime (ex: the RMT MAELE web seminar in May 2023 presented by Nadège Edouard).

4. WP6 - Project management

In the document describing the work planned in the CCC Farming project, it was proposed to put in place a management team that would deal with daily decision making through regular phone and skype meetings each month. It would be composed of the scientific coordinator (WR), a technical assistant **and a representative from INRA/IDELE (Nadège Edouard)** and SRUC,.

Nadège Edouard participated to such meetings during the first year, together with Abele Kuipers, Paul Galama (both from WR) and Bob Rees (SRUC). However, the management team was not maintained in the long term.

As WP2 leader, Nadège Edouard was also asked (but only once, at the end of 2021) to send updates of the work done in WP2 and the progress of deliveries. Due to Covid, most of the deliveries were delayed and new dates were proposed.

Finally, the INRAE and IDELE team was asked to organise a CCC Farming seminar in Rennes during the project. Due to Covid, the two first attempts were cancelled. The seminar was finally held on the 06-07-08 of June 2022. The program proposed was based on results presentations during meetings and a visit of the INRAE experimental farm. Unfortunately, Nadège Edouard could not be present due to her own Covid situation. Christophe Fléchard, Paul Robin and some students could help in the organisation and everything went well!

ANNEX 1:

Ferreira M, Delagarde R, Edouard N, 2023. Nitrogen balance in dairy cows fed low-nitrogen diets based on various proportions of fresh grass and maize silage. Animal 17, 100976. https://doi.org/10.1016/j.animal.2023.100976

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Nitrogen balance in dairy cows fed low-nitrogen diets based on various proportions of fresh grass and maize silage

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ABSTRACT

To ensure sustainable and efficient production, dairy farms must reduce their environmental impacts and nitrogen losses, which are sources of pollution, while increasing their feed self-sufficiency. Grass-based dairy systems, frequently combine fresh grass with maize silage when grass is scarce or during dietary transitions. However, the effects of combining fresh grass and maize silage on cow performance and N excretion are poorly known. This study aimed to quantify the effects of increasing the proportion of maize silage in a fresh grass diet on cow N flows and metabolism, in the context of grass-based dairy systems. Four proportions of maize silage in a fresh grass diet (objectives of 0, 17, 34 and 51% DM of maize silage) were investigated. The experiment was performed in a 4 × 3 Latin square design using eight lactating cows during three 3-week periods. DM intake (**DMI**), milk yield, faeces and urine outputs, and their N concentrations were measured for each cow. The fresh grass CP concentration was lower than planned (106 ± 13.0 g/kg DM). This resulted in very low dietary CP concentration, which decreased from 108 to 86 g/kg DM when maize silage in the diet increased from 0 to 51% DM, respectively. DM intake and milk yield both decreased linearly by 3.3 kg/day from 0 to 51% DM of maize silage in the diet. Thus, N intake decreased linearly by 100 g/day from 0 to 51% DM of maize silage in the diet. The N concentration of milk was highest for the diet with 0% DM of maize silage. Nitrogen excreted in faeces and urine decreased linearly by 29 and 23 g/day, respectively, from 0 to 51% DM of maize silage in the diet. The low dietary N concentration resulted in low ruminal NH_3-N concentrations (8 mg/L, on average) and urinary urea excretion (down to 8% urea N in urinary N). Increasing the proportion of maize silage in an unusually low-N grass diet, without protein-rich concentrates, induced highly N-deficient diets with minimal N losses in faeces and urine but large and unsustainable decreases in DMI and milk yield.

Implications

In dairy systems, farmers frequently offer maize silage to grazing cows during dietary transitions or when grass is scarce. Grass nitrogen concentration can vary greatly, and combining grass with maize silage may result in nitrogen-deficient diets. This study provides new data on nitrogen balance in lactating cows fed fresh grass and maize silage in situations of high nitrogen deficit but usual energy supply. This study highlights the farmers' difficulty in anticipating periods of poor grass quality, and the importance of finding a trade-off between decreasing nitrogen losses and maintaining dairy performance.

Introduction

To ensure sustainability, dairy farms must increase their feed self-sufficiency while reducing their negative environmental impacts. Ruminant production is frequently highlighted as a contributor to the emission of greenhouse gases and pollutants such as NO_3^- and NH_3 (Lesschen et al., 2011; European Environment Agency, 2019), while using resources which could be consumed as human food. One way to address this challenge is to include more forage in dairy cow diets, like fresh grass, whether grazed or not. Fresh grass is a low-cost feed produced on-farm, with a good nutritive value for lactating cows, especially for nitrogen supply when compared to conserved forages (INRA, 2018; Delaby et al., 2020). However, the availability and composition of fresh grass vary throughout the year. Thus, when grass is scarce and during dietary transitions, farmers frequently supplement grazed grass with other feeds. Grass-based dairy systems, such as organic farms, that use few concentrates, commonly combine fresh grass

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with conserved forages, such as maize silage, in cow diets. Fresh grass is most of the time rich in protein and degradable N in contrast to maize silage (INRA, 2018). Thus, combining fresh grass and maize silage could help balance the nutritional value of diets. French technical guidelines recommend not supplementing fresh grass-based diets with N-rich concentrate when maize silage is less than 50% of the diet's DM (French Livestock Institute, 2010). Effects of this combination of forages on cow N balance and metabolism are not well known compared to those of full-time grazing or a total mixed ration with only conserved forages and concentrates.

The present study aimed to determine to what extent increasing the proportion of maize silage in a fresh grass diet can decrease N excretion without compromising dairy performance in the context of grass-based dairy systems. We hypothesised that increasing the proportion of maize silage in fresh grass diets could decrease N excretion in manure by decreasing dietary N concentration (Castillo et al., 2000; Huhtanen and Hristov, 2009; Spanghero and Kowalski, 2021), with little impact on dairy performance, by maintaining the dietary energy concentration and increasing urea recycling (Reynolds and Kristensen, 2008; Edouard et al., 2016; 2019). To this end, we quantified the effects of increasing the proportion of maize silage in a fresh grass diet with no N-rich supplements on cow N balance and metabolism. Fresh grass N concentration was lower than initially planned. This allowed us to focus on the effect of highly N-deficient diets on cow performance and N use.

Material and methods

Treatments, experimental design and cows

The study was performed at the INRAE PEGASE experimental dairy farm of Méjusseaume (Le Rheu, France, https://doi.org/10.15454/yk9q-pf68) from 12 April to 19 June 2021. Four treatments with an increasing proportion of maize silage in a fresh grass diet were investigated: treatments MS0, MS17, MS34 and MS51 corresponded to objectives of 0, 17, 34 and 51% DM of maize silage, respectively. These proportions were chosen to create regular intervals between treatments until half of the diet's DM was composed of maize silage (French Livestock Institute, 2010). Treatments were tested during three experimental periods according to two 4 × 3 Latin squares (one for primiparous and one for multiparous) balanced for potential carry-over effects. The study was limited to three periods to avoid a shortage of grass availability in late spring. Each period consisted of 13 days for adaptation to the treatment and 6 days for measurements (as in Kristensen et al., 2010; Cantalapiedra-Hijar et al., 2014).

The experiment was performed with eight ruminally cannulated Holstein cows (four primiparous and four multiparous) with two cows per treatment within each period. At the beginning of the experiment, cows were in mid-lactation (166 ± 40 days in milk) and had a mean BW of 601 ± 83.1 kg. During the pre-experimental period from 29 March to 7 April, cows were individually fed the MS34 diet *ad libitum*, and DM intake (**DMI**) and milk yield were 16.0 ± 2.26 and 22.2 ± 4.78 kg/day, respectively. Fresh grass was offered after the morning milking and then at 0930, 1130 and 1600 h, and maize silage was offered only after the afternoon milking, to mimic daytime grazing followed by spending all night indoors.

The grass came from the same paddock, managed to offer grass at the same vegetative stage throughout the experiment, by cutting regularly specific areas 25–35 days prior to be fed to the cows. The grassland was sown with a mixture of grasses (16 kg/ha of *Lolium perenne* L., Trybal cultivar; 8 kg/ha of *L. perenne* L., Ibisal cultivar; and 8 kg/ha of *Festuca arundinacea* Schred., Philona cultivar) in September 2018. The grassland received 30 kg N/ha as ammonium nitrate in March 2021 and after each grass harvest (i.e. ca. every 30–40 days). The botanical composition of the grass was determined on one day during each measurement period from a representative sample of 1 kg of freshly cut grass. On a DM basis, it contained a mean of 96.1 ± 2.8% grasses (mainly *L. perenne*) and 3.9 ± 2.8% other species, none of them legumes.

Housing and feeding management

Cows were housed in tie stalls in two temperature-controlled and mechanically ventilated rooms throughout the entire experiment. Cows were milked twice a day in the rooms. They could see, smell and hear each other during the experiment. Cows were fed *ad libitum* on a 24-h basis in individual troughs and had unlimited access to water and a salt lick. Two feeding management rules were followed throughout the experiment. First, total refusals for the entire day had to exceed but remain close to 10% of the offered diet; thus, at least one forage was offered *ad libitum*. The second rule was to ensure that the diet ingested contained the required proportion of maize silage. This involved adjusting the amounts of forage offered each day depending on the daily DM concentration and individual intake of each forage. For example, if the proportion of maize silage in the diet was too low one day, maize silage was fed *ad libitum* the next day and fresh grass was restricted.

Fresh grass was cut once daily at 0800 h at 6 cm from the ground using a mechanical mower with a cutter bar (Haldrup GmbH, Ilshofen, Germany) and was immediately offered at the trough or conserved in a cool room at 4 °C until the next feeding. The fresh grass offered was spread over four feedings, at 0800, 0930, 1130 and 1600 h, and refusals were removed at 1745 h for all treatments, except for MS0. The first two grass feedings were the most important as they followed the cows' natural feeding behaviour. Cows fed the MS0 diet also received fresh grass for the night at 1800 h (a fifth meal). For MS17, MS34 and MS51, maize silage was fed once per day at 1800 h immediately after removing grass refusals; maize silage refusals were removed at 0745 h the following morning. Thus, for MS17, MS34 and MS51, cows had 10 h per day to eat fresh grass and 14 h per day to eat maize silage. For the MS0, cows had access to grass all day long.

Forage characteristics and intake calculations

Quantities of forages offered and refused were weighed daily and samples were dried in a ventilated oven for 48 h at 60 °C to measure their DM concentrations and calculate the daily DMI of each forage for each cow. To this end, a 1 kg sample of each forage offered and refused per cow was dried daily. The DM concentration of maize silage was corrected by considering the volatilisation of fermentation products in the oven during drying. To this end, NH_3, volatile fatty acids, alcohols and lactic acid in a frozen sample (−20 °C) of maize silage were analysed, and volatilisation was then calculated using the equation of Dulphy et al. (1975). The volatilisation correction factor was 26 g/kg DM. Chemical analyses (organic matter (**OM**), N, NDF, ADF and ADL) were performed per period on pooled lyophilised daily samples of fresh grass and maize silage offered during the six measurement days. Similarly, chemical analyses of grass and maize silage refusals were performed per period on pooled lyophilised daily samples per cow on the same days. Nutrient intake (g/day) was calculated individually from amounts of nutrients offered and refused, thus considering the potential feed preferences of each cow:

$$\text{Nutrient intake} = \sum\nolimits_{n}^{1} [\text{Nutrient offered} - \text{Nutrient refused}]$$

with n the number of forages in the diet (n = 1 for MS0, 2 for the other diets), nutrient offered (g/day) the amount of forage offered (kg DM/day) multiplied by its nutrient concentration (g/kg DM), and nutrient refused (g/day) the amount of forage refused (kg DM/day) multiplied by its nutrient concentration (g/kg DM).

The net energy for lactation (**UFL**, Unité Fourragère Lait, equivalent to the net energy for the lactation, 1 UFL = 7.37 MJ of net energy/kg DM; INRA, 2018) concentration, **PDI** (protein digestible in the small intestine, equivalent to the metabolisable protein, INRA, 2018) concentration and rumen protein balance (**RPB**, CP intake minus the non-ammonia CP flowing from the duodenum (INRA, 2018)) of each forage were calculated from its chemical composition and the INRA 2018 feeding system (PrevAlim® software, https://www.inration-ruminal.fr/en/; INRA, 2018). The UFL and PDI concentrations, UFL and PDI supplies, and RPB of the entire diet were then calculated from the initial forage nutritional values and the DMI of each feed, considering digestive interactions, using the INRA 2018 feeding system (INRAtion® software, https://www.inration-ruminal.fr/en/; INRA, 2018).

Milk yield and composition

Cows were milked twice a day, and individual milk yield was recorded daily. True protein and fat concentrations were measured in fresh milk at each milking from days 15 to 19. For each cow, N concentration was measured in a 50 mL sample of fresh milk taken from pooled morning and afternoon milk once a week (day 17). A subsample of this pool was then ultra-filtered and frozen at −20 °C for later analysis of urea concentration.

Faeces and urine output, sampling and digestibility calculation

Faeces and urine output were determined individually by collecting all faeces and urine during a 5-day period (days 15–19). Faeces were collected in a gutter behind the cow and regularly transferred to a closed bucket. Total amounts were weighed and sampled daily (2% of the faecal output). Half of the sample was dried in a ventilated oven for 72 h at 60 °C to determine the faecal DM concentration, and the other half was frozen and lyophilised for chemical analyses (OM, N, NDF and ADF) of pooled samples per cow and period. The whole-tract digestibility of nutrients was calculated from the amount of each component ingested (intake, kg/day) and excreted in faeces (faecal output, kg/day):

$$\text{Digestibility} = (\text{intake} - \text{faecal output})/\text{intake}$$

The nutrient intake and faecal output used to calculate whole-tract digestibility are given in Supplementary Table S1.

To collect urine separately from faeces, cows were equipped with a harness that held a tube around the vulva to drain urine into a plastic container. Urine was immediately acidified in the container with 500 mL of 20% H_2SO_4 to prevent NH_3 volatilisation. Urine was weighed and sampled daily (1% of the urine output). Samples were pooled per cow and period and frozen before analysing N, urea, allantoin, uric acid and creatinine. Daily samples were successively stored in the same container at −20 °C.

Rumen fermentation and plasma metabolites

Ruminal pH and NH_3 concentration kinetics were determined on day 18 based on 10 sampling times during the day. Basal concentrations were determined at 0745 and 1745 h (before the first morning feeding of 0800 h and the evening feeding of 1800 h, respectively). The other samples were taken 1, 2, 3 and 5 h after these feedings (0900, 1000, 1100 and 1300 h, respectively, for morning grass feeding and 1900, 2000, 2100 and 2300 h, respectively, for evening silage or grass feeding). At each time, 50 mL of rumen fluid was sampled in the ventral sac via the cannula. The pH was immediately measured. Rumen fluid was then filtered through six layers of muslin and frozen at −20 °C (4 mL of rumen fluid in 4 mL of 20% NaCl preservative). The weighted means of ruminal pH and NH_3 concentration for the entire day were calculated based on the sampling times and intervals.

Blood urea was determined from blood sampled before the first morning feeding and the evening feeding (0745 and 1745 h, respectively) and then 3 h later (1100 and 2100 h, respectively) on day 17. Blood was sampled via the caudal vein and centrifuged (2 000g at 4 °C for 15 min), and the plasma was then frozen at −20 °C.

Chemical analyses

The lyophilised offered forages, refused forages and faeces were ground (0.8 mm) to analyse OM, fibre and N. The OM concentration was measured by ashing in a muffle furnace at 550 °C for 8 h (AOAC, 1990). Fibre concentrations (NDF, ADF and ADL) were determined sequentially with a Fibersac extraction unit (Ankom Technology, Fairport, NY, USA) (AOAC, 1990; Van Soest et al., 1991). The pepsin-cellulase digestibility of dried feeds was determined according to Aufrère and Michalet-Doreau (1988). The N concentrations of the offered and refused forages, faeces, urine and milk were analysed using the Dumas method (Leco, Saint Joseph, MI, USA) (AOAC, 1990). Urine, milk and plasma urea concentrations were analysed based on an enzymatic and colourimetric reaction assessed using a multi-parameter analyser (KONE Instruments 200 Corporation, Espoo, Finland). True protein and fat concentrations in the milk were measured by mid-infrared spectrophotometry (Milkoscan, Foss Electric, Hillerød, Denmark). The ruminal NH_3-N concentration was determined using the Berthelot colourimetric reaction method (KONE Instruments 200 Corporation, Espoo, Finland) (Gordon et al., 1978). The urinary concentrations of allantoin, uric acid and creatinine were analysed by high-performance liquid chromatography (HPLC Alliance, Waters Corporation, Milford, MA, USA) (George et al., 2006).

Calculation of unaccounted-for N

Unaccounted-for N was calculated as N intake minus the N exported in milk, N excreted in faeces and urine (g N/day), and retained N (g N/day). The retained N (g N/day) was estimated from the UFL balance (UFL/day, calculated as the dietary UFL supply minus cow UFL needs for lactation, gestation, growth and maintenance), assuming that 6 g N/UFL was retained by protein accretion or mobilised when the UFL balance was positive or negative, respectively (INRA, 2018).

Statistical analyses

One cow was removed from the analysis due to an unexplained deterioration of its health at the end of the first experimental period. Data were averaged per cow and per period (n = 21 statistical units) and analysed using the following mixed model (SAS, 2020; PROC MIXED):

$$Y_{ijk} = \mu + \text{Treatment}_i + \text{Period}_j + \text{Cow}_k + e_{ijk}$$

with Y_{ijk} the analysed variable; μ the overall mean; Treatment_i the fixed effect of the proportion of maize silage in the diet (3 df); Period_j the fixed effect of the experimental period (2 df); Cow_k the random effect of the cow and e_{ijk} the residual error term.

Linear and quadratic responses to the proportion of maize silage in the diet were determined using orthogonal contrasts.

Results

Feed and diet compositions, intake and digestibility

The mean chemical composition of maize silage lay within normal ranges (INRA, 2018) (Table 1). The mean fresh grass CP concentration, PDI concentration and RBP were very low (106, 80 and −28 g/kg DM, respectively), while the mean grass energy value was normal, with low mean NDF and ADF concentrations. The proportion of maize silage in the ingested diet followed a regular interval among diets, as intended (Table 2). As planned, the amount of DM refused was ca. 15% of the DM offered in each diet. Cows were less likely to refuse fresh grass than maize silage. To obtain the expected proportion of maize silage in the diet, the supply of fresh grass had to be restricted to an increasing degree as the proportion of silage in the diet increased. Thus, fresh grass refusals were near zero for the MS51 diet.

Total DMI decreased linearly by 3.3 kg/day from MS0 to MS51 ($P < 0.01$). The dietary DM and OM concentrations increased linearly as the proportion of maize silage in the diet increased ($P < 0.05$), while the dietary CP concentration decreased linearly by 22 g/kg DM from MS0 to MS51 ($P < 0.01$). Dietary NDF and ADF concentrations did not differ significantly among diets. Dietary UFL and PDI concentrations decreased linearly as the proportion of maize silage increased ($P < 0.01$). Dietary RPB was negative for all diets. Dietary RPB and the dietary PDI:UFL ratio decreased linearly as the proportion of maize silage in the diet increased ($P < 0.01$). The whole-tract digestibilities of DM, OM, NDF and ADF decreased linearly as the proportion of maize silage in the diet increased ($P < 0.05$).

Milk yield, milk composition and nitrogen partitioning

Milk yield decreased linearly by 3.3 kg/day from MS0 to MS51 ($P < 0.01$; Table 3). Milk fat concentration tended to vary quadratically among the diets and was lowest for MS17 and highest for MS51 ($P = 0.08$). Milk protein concentration of the MS0 diet was higher than those of the other three diets (quadratic effect: $P < 0.01$).

Nitrogen intake decreased linearly by 100 g/day from MS0 to MS51 ($P < 0.01$; Table 3). Milk N of the MS0 diet was greater than those of the other three diets (quadratic effect: $P < 0.05$). Faecal and urinary N decreased linearly by 29 and 23 g/day from MS0 to MS51, respectively ($P < 0.01$). Diet did not influence urine output. The unaccounted-for N was negative for all diets and tended to decrease linearly as the proportion of maize silage in the diet increased ($P = 0.08$).

Rumen fermentation, urea and non-urea nitrogen metabolites

The mean ruminal pH increased linearly as the proportion of maize silage in the diet increased ($P < 0.01$; Table 4). The basal ruminal pH at 0745 h for the MS0 diet was lower than those for the other three diets (linear effect: $P < 0.01$; Fig. 1a). At 0900 h, just after the first grass feeding, the ruminal pH for the MS34 and MS51 diets was higher than that for the MS0 diet (linear effect: $P < 0.01$). At 1745 h, just before feeding maize silage, the ruminal pH was highest for the MS51 diet, intermediate for the MS34 and MS0 diets, and lowest for the MS17 diet (quadratic effect: $P < 0.01$).

The mean ruminal NH_3-N concentration was not influenced by the diet (8.2 ± 3.87 mg/L). The basal ruminal NH_3-N concentration at 0745 h for MS51 tended to be higher than those for the three other diets (linear effect: $P = 0.08$; Fig. 1b). Ruminal NH_3-N concentrations at 1900 and 2000 h for the MS51 diet were higher than those for the other three diets (quadratic effect: $P < 0.05$ and $P = 0.07$ for 1900 and 2000 h, respectively). At 2300 h, the ruminal NH_3-N concentration for the MS34 diet was higher than those for the other three diets (quadratic effect: $P = 0.06$).

The mean plasma urea concentration decreased linearly by 18.2 mg/L from MS0 to MS51 ($P < 0.01$; Table 4). Milk and urinary urea concentrations tended to decrease linearly as the proportion of maize silage increased ($P = 0.08$). Urinary urea N excretion decreased linearly by 2.3 g/day from MS0 to MS51 ($P < 0.01$). The urinary creatinine and urea N proportion in total urinary N were not influenced by the diet. Urinary allantoin and uric acid decreased linearly as the proportion of maize silage in the diet increased ($P < 0.01$).

Discussion

Highly nitrogen-deficient diets

All diets had CP concentrations (86–108 g/kg DM) much lower than those usually recommended for dairy cows (140–160 g/kg DM, INRAE, 2018) and reported in the literature (from 100 to more than 250 g/kg DM, Huhtanen and Hristov, 2009), mainly due to the unexpectedly low-CP concentration in the fresh grass. A relatively low mean temperature in spring 2021 (11.9 °C vs 13.4 °C during the same period from 1991 to 2020, AgroClim INRAE, INRAE CLIMATIK platform, 2022) likely decreased N mineralisation in the soil and thus N availability for grass growth (Miller and Geisseler, 2018). Under these conditions, the mineral N fertilisation was certainly insufficient to ensure correct plant nutrition and grass N uptake (Peyraud et al., 1997).

Such conditions may occur in dairy farms and result in low-N diets, while farmers generally do not have the opportunity to mea-

Table 1
Chemical composition and nutritional value of forages offered to dairy cows.

	Maize silage		Fresh grass	
Component	Mean	SD	Mean	SD
DM, g/kg fresh weight	329	4.1	230	37.8
OM, g/kg DM	956	0.6	933	4.5
CP, g/kg DM	64	1.5	106	13.0
NDF, g/kg DM	501	11.1	489	71.7
ADF, g/kg DM	274	7.3	252	41.7
ADL, g/kg DM	21	0.6	17	4.8
Nutritional value				
UFL/kg DM	0.88	0.008	1.00	0.079
PDI, g/kg DM	57	0.4	80	2.9
RPB, g/kg DM	−41	1.2	−28	10.3

Abbreviations: OM = organic matter; PDI = protein digestible in the small intestine; RPB = rumen protein balance, CP intake minus non-ammonia CP flowing from the duodenum; UFL = unité fourragère lait (1 UFL = 7.37 MJ of net energy for lactation).
Means and SD for six samples per forage.

Table 2
Effects of increasing the proportion of maize silage in a fresh grass diet on dairy cow intake, diet composition and digestibility.

Variable	Treatment[1]				RSD	P-value	
	MS0	MS17	MS34	MS51		Linear	Quadratic
Feed intake							
DM intake, kg/day	15.6[a]	13.9[b]	13.4[bc]	12.3[c]	0.99	<0.001	0.550
Fresh grass intake, kg DM/day	15.6[a]	11.4[b]	8.9[c]	6.2[d]	0.93	<0.001	0.126
Maize silage intake, kg DM/day	0[a]	2.5[b]	4.5[c]	6.1[d]	0.53	<0.001	0.118
Fresh grass in the diet, % DM	100[a]	82.2[b]	66.1[c]	51.1[d]	1.12	<0.001	0.021
Maize silage in the diet, % DM	0[a]	17.8[b]	33.9[c]	48.9[d]	1.12	<0.001	0.021
Total DM refused, % DM offered	17.5	14.4	13.5	16.2	3.72	0.526	0.118
Fresh grass refused, % DM grass offered	17.5[a]	14.5[a]	10.7[ab]	4.0[b]	5.00	0.002	0.425
Maize silage refused, % DM silage offered	0[a]	11.8[b]	17.3[bc]	24.7[c]	5.94	<0.001	0.428
Diet chemical composition							
DM, g/kg fresh weight	228[a]	242[ab]	253[ab]	265[b]	22.6	0.021	0.924
OM, g/kg DM	933[a]	937[b]	942[c]	944[c]	2.8	<0.001	0.540
CP, g/kg DM	108[a]	101[b]	93[c]	86[d]	4.7	<0.001	0.998
NDF, g/kg DM	475	485	488	491	23.5	0.303	0.771
ADF, g/kg DM	245	254	256	261	14.1	0.116	0.772
Diet nutritional value							
UFL/kg DM	0.997[a]	0.969[b]	0.937[c]	0.910[d]	0.0157	<0.001	0.922
PDI, g/kg DM	80[a]	76[b]	73[c]	69[d]	1.2	<0.001	0.561
PDI/UFL, g/UFL	81[a]	79[b]	78[bc]	76[c]	1.5	<0.001	0.713
RPB, g/kg DM	−27[a]	−29[ab]	−32[bc]	−34[c]	2.3	<0.001	0.845
Whole-tract digestibility, g/g							
DM	0.733[a]	0.724[ab]	0.703[ab]	0.692[b]	0.0249	0.020	0.897
OM	0.751[a]	0.740[ab]	0.717[b]	0.705[b]	0.0232	0.007	0.950
NDF	0.694[a]	0.672[a]	0.620[b]	0.585[b]	0.0339	<0.001	0.708
ADF	0.710[a]	0.686[a]	0.633[b]	0.598[b]	0.0334	<0.001	0.731

Abbreviations: OM = organic matter; PDI = protein digestible in the small intestine; RPB = rumen protein balance, CP intake minus non-ammonia CP flowing from the duodenum; UFL = unité fourragère lait (1 UFL = 7.37 MJ of net energy for lactation).

[1] Treatments MS0, MS17, MS34 and MS51 correspond to objectives of 0, 17, 34 and 51% DM of maize silage in a fresh grass diet, respectively. In a given row, adjusted means with different superscript letters differ significantly between treatments ($P < 0.05$).

Table 3
Effects of increasing the proportion of maize silage in a fresh grass diet on dairy cow milk yield and composition, and nitrogen partitioning.

Variable	Treatment[1]				RSD	P-value	
	MS0	MS17	MS34	MS51		Linear	Quadratic
Milk yield, kg/day	16.4[a]	14.6[ab]	13.3[b]	13.1[b]	1.27	0.002	0.222
Corrected milk yield[2], kg/day	16.8[a]	14.6[b]	13.6[b]	13.5[b]	1.08	<0.001	0.074
Milk fat concentration, g/kg	42.2[ab]	40.4[a]	42.0[ab]	43.8[b]	1.91	0.135	0.076
Milk true protein concentration, g/kg	32.6[a]	30.6[b]	30.5[b]	31.1[b]	0.70	0.011	0.003
N partitioning, g/day							
Intake	267[a]	223[b]	201[b]	167[c]	17.0	<0.001	0.547
Milk	90[a]	75[b]	69[b]	68[b]	5.9	<0.001	0.037
Faeces	103[a]	89[b]	86[b]	74[c]	7.2	<0.001	0.864
Urine	68[a]	60[ab]	53[b]	45[c]	5.5	<0.001	0.836
Unaccounted-for N[3]	−5	−8	−10	−14	7.0	0.078	0.959
Urine output, kg/day	19.6	20.9	19.5	18.1	4.48	0.527	0.550

Abbreviations: UFL = unité fourragère lait (1 UFL = 7.37 MJ of net energy for lactation).

[1] Treatments MS0, MS17, MS34 and MS51 correspond to objectives of 0, 17, 34 and 51% DM of maize silage in a fresh grass diet, respectively. In a given row, adjusted means with different superscript letters differ significantly between treatments ($P < 0.05$).

[2] Milk yield corrected for standard milk with concentrations of 40 g/kg fat and 31 g/kg protein (INRA, 2018).

[3] Calculated from N intake, in milk, in faeces, in urine and corrected from an estimate of retained or mobilised N. Retained or mobilised N was estimated from the balance of net energy for lactation (UFL balance), assuming 6 g N retained per UFL (INRA, 2018).

sure the grass N concentration regularly. The present study tested an unexplored range of dietary CP concentrations for lactating dairy cows, enabling to better predict the detrimental effects of low-N diets on dairy performance over a short period. This enabled investigating effects of extreme N deficit in relatively high-energy diets, confirmed by the negative RPB (−30 g/kg DM) and the extremely low milk and plasma urea concentrations (27.6 and 37.2 mg/L, respectively). In particular, the mean ruminal NH_3-N concentration (8 mg/L) was lower than typical concentrations for cows fed fresh grass diets (67–275 mg/L for diets with 140–225 g CP/kg DM, Van Vuuren et al., 1993; Delagarde et al., 2008) and even lower than those of low-N fresh grass diets in the literature (11–28 mg/L for diets with 106–113 g CP/kg DM, Peyraud et al., 1997; Delagarde et al., 1999; Rojen et al., 2008). Ruminal pH did not fall below 5.9 at any time, which is uncommon for fresh grass diets, which generally cause it to decrease to at least 5.6 (Kolver et al., 1998; Delagarde et al., 1999; 2008). These ruminal pH and NH_3-N concentrations clearly indicate that microbial activity decreased with such a high dietary N deficit. For most of the measurement hours, the ruminal NH_3-N concentration lay below the critical threshold of 20 mg/L mentioned by Clark et al. (1992) as the minimum for optimal cellulolytic activity in dairy cows. Ruminal NH_3-N concentration varied slightly throughout the day, with a postprandial peak that did not exceed 25 mg/L above the basal level, while it usually reaches up to 80–260 mg/L in dairy cows fed a fresh grass diet with a standard CP concentration (130–

Table 4
Effects of increasing the proportion of maize silage in fresh grass diet on dairy cow ruminal pH and NH_3-N, urea in plasma, milk, urine and non-urea urinary N components.

Variable	Treatment[1]				RSD	*P*-value	
	MS0	MS17	MS34	MS51		Linear	Quadratic
Ruminal pH[2]	6.23[a]	6.28[ab]	6.41[bc]	6.49[c]	0.090	<0.001	0.694
Ruminal NH_3-N concentration[2], mg/L	9.01	7.79	6.53	9.44	3.866	0.997	0.259
Urea concentration, mg/L							
Plasma[3]	45.3[a]	43.4[a]	32.8[ab]	27.1[b]	9.18	0.006	0.657
Milk	29.5[ab]	34.5[a]	27.7[ab]	18.7[b]	9.82	0.072	0.144
Urine	590	562	471	396	165.0	0.079	0.782
Urinary urea N, g/day	5.09[a]	5.56[a]	4.02[ab]	2.84[b]	1.20	0.010	0.213
Urinary urea N, % of urinary N	7.48	9.45	7.76	6.48	2.067	0.300	0.163
Urinary allantoin, g/day	34.1[a]	29.2[ab]	27.6[b]	20.6[c]	3.65	<0.001	0.556
Urinary uric acid, g/day	4.4[a]	3.4[b]	3.4[b]	2.0[c]	0.67	<0.001	0.498
Urinary creatinine, g/day	12.6	12.3	12.7	11.3	1.48	0.261	0.423

[1] Treatments MS0, MS17, MS34 and MS51 correspond to objectives of 0, 17, 34 and 51% DM of maize silage in a fresh grass diet, respectively. In a given row, adjusted means with different superscript letters differ significantly between treatments ($P < 0.05$).
[2] Weighted means of ruminal pH and NH_3-N concentration for the entire day based on the sampling times and intervals between the 10 sampling times (0745, 0900, 1000, 1100, 1300, 1745, 1900, 2000, 2100 and 2300 h).
[3] Mean of four plasma sampling times (0745, 1100, 1745 and 2100 h).

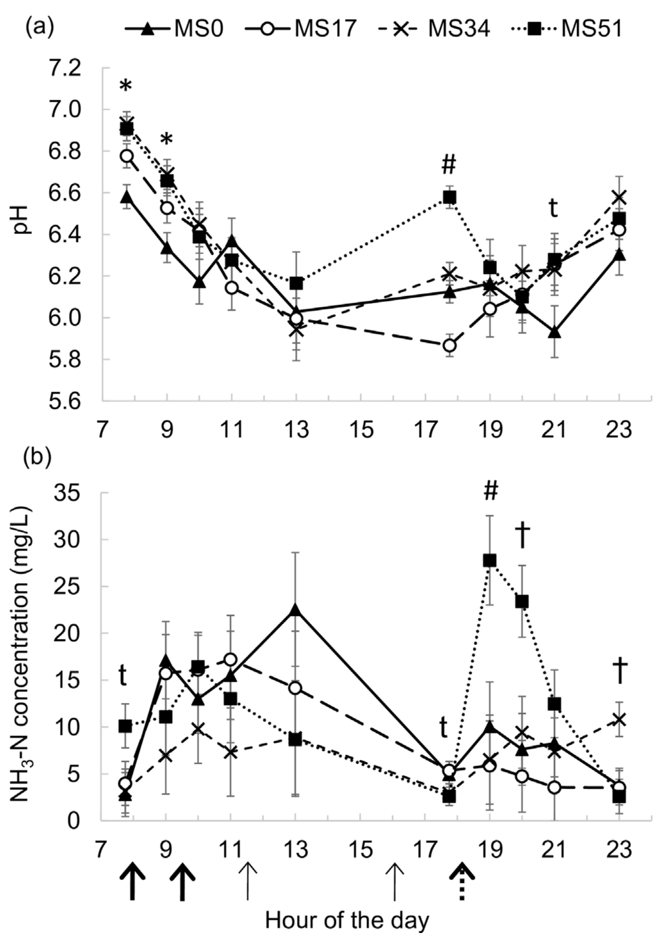

Fig. 1. Effect of increasing the proportion of maize silage in a fresh grass diet on dairy cow (a) ruminal pH and (b) NH_3-N concentration per hour. Treatments MS0, MS17, MS34 and MS51 correspond to objectives of 0, 17, 34 and 51% DM of maize silage in a fresh grass diet, respectively; * = linear effect ($P < 0.05$); t = linear trend ($P < 0.10$); # = quadratic effect ($P < 0.05$); † = quadratic trend ($P < 0.10$); vertical bars = SEM; bold arrows = main fresh grass feedings; fine arrows = supplementary fresh grass feedings; dashed arrow = maize silage feeding (except for MS0: grass feeding).

185 g/kg DM, Van Vuuren et al., 1993; Kolver et al., 1998; Delagarde et al., 2008; Ribeiro Filho et al., 2012). This clearly illustrates the chronic shortage of degradable N in the diet, which was immediately used by micro-organisms in the rumen.

Intake, digestion and milk yield

The large decrease in voluntary DMI as the proportion of maize silage in the diet increased was due to the fact that cows limited their intake of maize silage despite being fed *ad libitum* (strong increase in maize silage refusals). Achieving the planned proportion of maize silage in the diet required strongly restricting the fresh grass supply. The decrease in DMI was unlikely to be due to differences in feeding management between diets, as the cows that ate the least were not those that had the shortest access to the diet. Cows fed the MS51 diet had 14 h each day to eat the maize silage, which is much longer than the time required to eat 6 kg DM of maize silage, given its high intake rate (5–6 kg DM/h; Le Liboux and Peyraud, 1998 and 1999). We assume that the cows' greater preference for fresh grass rather than maize silage was likely enhanced by the low-CP and metabolisable protein concentrations of the diets, which is known to decrease voluntary intake (Faverdin et al., 2003; INRA, 2018). A deficit in degradable N may have decreased microbial activity, fibre ruminal digestion and slowed down passage rate (Köster et al., 1996). A decrease in protein in the intestine may also have directly affected voluntary intake by regulating appetite (Faverdin et al., 2003). The loss of 1.4 kg/day of DMI observed in the present study for every 10 g CP/kg DM decrease in the diet was in complete agreement with Bryant and Donnelly (1974), who observed the same loss of DMI (1.5 kg/day for every 10 g CP/kg DM decrease) for dairy cows fed a combination of fresh grass and maize silage, with dietary CP concentrations similar to those in the present study (94–114 g/kg DM). They even showed that cows stopped eating when their diets consisted entirely of maize silage fed *ad libitum*. The decrease in DMI related to that of dietary CP concentration in our study was greater than that reported in the literature, which ranged from 0 to 0.5 kg/day for dairy cows fed diets ranging from 106 to 173 g CP/kg DM (Peyraud et al., 1997; Kristensen et al., 2010; Yang et al., 2022). Adverse effects on voluntary intake are likely to increase as the CP concentration of the diet decreases, particularly below 100 g CP/kg DM (Rico-Gómez and Faverdin, 2001; Faverdin et al., 2003), which is extremely low for lactating dairy cows.

The decrease in dietary OM and fibre digestibilities as the proportion of maize silage in the diet increased was expected and consistent with the specific fibre digestibility of each forage, as fibre in maize silage is much less digestible (0.51 and 0.45 for NDF and ADF, respectively; INRA, 2018) than that in fresh grass (0.69 and 0.71 for NDF and ADF, respectively, in treatment MS0). The decrease in dietary CP concentration and RPB also likely decreased dietary OM digestibility, but only marginally, as all four diets were deficient in degradable N, with low and similar ruminal NH_3-N concentrations, thus limiting microbial synthesis (INRA, 2018).

The loss of DMI was likely the main cause of the decrease in faecal N as the proportion of maize silage in the diet increased (Castillo et al., 2000). Faecal N output had a range similar to those of other experiments with dairy cows fed low-N diets (Susmel et al., 1995; Peyraud et al., 1997; Rojen et al., 2008). The decrease of 9 g N/day for every 1 kg loss of DMI in the present study is consistent with the decrease in faecal N observed in several studies (Hindrichsen et al., 2006; Yang et al., 2022), which ranged from 6-13 g/day for every 1 kg loss of DMI.

The large decreases in DMI, N intake, diet digestibility and thus energy supply, as the proportion of maize silage in the diet increased were the main factors responsible for the decrease in milk yield and milk protein concentration (Coulon and Rémond, 1991). These decreases in DMI and milk yield as the proportion of maize silage increased in a fresh grass diet agree with the results of Bryant and Donnelly (1974). The decrease in milk yield, milk N concentration and N exported in milk were similar to those observed by Susmel et al. (1995) and Cantalapiedra-Hijar et al. (2014).

In this study, the N use efficiency (N in milk in g/day divided by N intake in g/day) of the MS51 diet was particularly high (41%), and such a high value has rarely been reported in the literature. However, given the severe restrictions on DMI and milk production, such high efficiency is not sustainable, as it requires highly N-deficient diets that strongly disrupt rumen function and can endanger cow health in the medium-to-long term.

Nitrogen metabolism, urea and urinary nitrogen

The urinary N and urinary urea N outputs were low, which is consistent with previous experiments with dairy cows fed low-N diets (Susmel et al., 1995; Peyraud et al., 1997; Cantalapiedra-Hijar et al., 2014). Susmel et al. (1995) observed a urinary urea N (4.7 g/day) similar to that in the present study (4.4 g/day) in dairy cows fed a diet with 94 g CP/kg DM. The low urinary urea concentration resulted from the low plasma urea concentration (37 mg/L), which was lower than those reported in the literature for dairy cows fed a slightly higher dietary CP concentration (76 mg/L with 120 g CP/kg DM) (Cantalapiedra-Hijar et al., 2014). A decrease in the clearance rate of urea (volume of blood cleared per unit time (L/h); urinary urea excretion (g/day) divided by the plasma urea concentration (g/L)) can also explain the low concentrations of urinary urea N, as the latter is known to decrease as dietary CP concentration decreases (Kristensen et al., 2010). In the present study, clearance rate was lower than those in the literature for lactating dairy cows fed higher dietary CP concentrations (11 L/h vs 20–41 L/h with 120–180 g CP/kg DM, according to Kristensen et al. (2010) and Edouard et al. (2016)). The extreme N deficit likely resulted in renal regulation being responsible for the low clearance rate of urea. Eriksson and Valtonen (1982) observed active regulation of urea filtration and reabsorption in the kidneys, which decreased the clearance rate in goats fed extremely low-CP straw-based diets (<20 g/kg DM).

The decrease in urinary N as the proportion of maize silage in the diet increased was primarily due to the decrease in N intake (Spanghero and Kowalski, 2021). However, this decrease was much smaller than those in previous studies which investigated wider ranges of dietary CP concentrations (2 g/day vs 5–9 g/day for every 10 g/day decrease in N intake for Susmel et al. (1995), Peyraud et al. (1997) and Cantalapiedra-Hijar et al. (2014)). The proportion of urea N in urinary N was particularly low in our study (8%). The literature indicates that this proportion is correlated with the dietary protein supply and CP concentration (Dijkstra et al., 2013). It can reach 80% in dairy cows when dietary CP concentration is high (>175 g/kg DM) and decreases to ca. 40% when CP concentration is ca. 120 g/kg DM (Edouard et al., 2016; 2019). It can even decrease to 14% for low-CP diets (<110 g/kg DM) (Susmel et al., 1995; Peyraud et al., 1997). Although a proportion of 8% of urea N in urinary N has never been reported in the literature for lactating dairy cows, it is consistent with the variability previously described and the extremely low dietary CP concentrations observed in the present study. Thus, the main variable component of urinary N – urea N – was small, even for the diet with the highest CP concentration (MS0), which explained the small decrease in urinary N in situations of extreme N deficit (MS51). This suggests that a minimum threshold of urinary N was reached for such low-N intake, likely due to a large and non-compressible part of endogenous N in urinary N (Castillo et al., 2000). According to the literature, calves fed a highly N-deficient diet that was not compatible with production excreted little urinary N, which was composed almost entirely of endogenous N and varied proportionally to their BW (Swanson, 1977). In the present study, mean endogenous urinary N can be estimated as 30 g/day (0.05 $\times$ BW, INRA, 2018), representing up to 50% of the urinary N.

The urinary urea N decreased by only 2.3 g/day from MS0 to MS51, while urinary N decreased 10 times as much ($-$23 g/day). This suggests a large decrease in non-urea N in urine. Indeed, allantoin and uric acid decreased as the proportion of maize silage increased, in accordance with the decrease in dietary CP concentration (Bristow et al., 1992; Susmel et al., 1995). The decrease in purine derivatives indicates a decrease in microbial protein synthesis in the rumen (Dijkstra et al., 2013). For dairy cows fed fresh grass, Peyraud et al. (1997) showed that despite the large decrease in dietary CP concentration (150 to 106 g/kg DM), the microbial N flow entering the duodenum remained constant (237 g/day), which highlights compensation of the low dietary N by urea recycling in the rumen. However, in the present study, urea recycling was likely restricted by the low plasma urea concentration, as suggested by Peyraud et al. (1997), due to the extreme dietary N deficit.

The present study showed cow metabolic adaptations over a short period that conserve urea and increase its recycling. Although these low-N diets minimise N excretion in manure, such a high N deficit is most likely not sustainable in the medium-to-long term as it does not ensure good rumen function or lactation support and may degrade cow health. These long-term effects of high diet N deficit require further investigation.

Conclusion

Increasing the proportion of maize silage from 0 to 51% DM in a low-N fresh grass diet resulted in very low dietary N concentrations (106 to 86 g CP/kg DM, respectively), with negative RPB (down to $-$30 g/kg DM) causing a decrease of 3.3 kg/day in DMI and milk yield. In these N-deficit situations with highly digestible diets, most of the ingested N was used, and the N excreted in faeces and urine was minimal, especially for the diet composed of half fresh grass and half maize silage (74 and 45 g/day, respectively). The dietary N deficit resulted in low ruminal NH_3-N concentration (8 mg/L), as well as low plasma and milk urea concentrations (28 and 37 mg/L, respectively), which ended in extremely low urinary urea N excretion (4.4 g/day, i.e. 8% of urinary N). This study illustrated the ability of cow metabolism to adapt to a severe N deficit over a short period. However, it also showed that very low-N diets severely decreased intake and dairy production, and altered rumen function. This study underlines the wide variability of the grass CP concentration and the difficulty for farmers in knowing the grass quality offered to cows. These results highlight a trade-off between limiting N losses and maintaining dairy performance, and clearly show that dietary CP concentrations below 100 g/kg DM are not sustainable in the medium-to-long term for lactating dairy cows.

Supplementary material

Supplementary material to this article can be found online at https://doi.org/10.1016/j.animal.2023.100976.

Ethics approval

Experiments were performed in accordance with French and European Union legislation on animal experimentation and animal welfare. All procedures related to the care and management of animals were approved by an animal ethics committee of the French Ministry of Agriculture (approval number: APAFiS #23913-2020020315487805_v2).

Data and model availability statement

None of the data were deposited in an official repository. The data that support the study findings are available from the authors upon request.

Declaration of Generative AI and AI-assisted technologies in the writing process

The authors did not use any artificial intelligence-assisted technologies in the writing process.

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Declaration interest

None.

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ANNEX 2:

Ferreira M, Delagarde R, Edouard E, 2022. Nitrogen excretion and ammonia emissions in dairy cows fed low-N fresh grass and maize silage. 73th Annual meeting of the European federation of animal science (EAAP), Porto, Portugal.

EAAP 2022 - Porto – Session 26: Climate care dairy farming

Nitrogen excretion and ammonia emissions in dairy cows fed low-N fresh grass and maize silage

Manon Ferreira, Rémy Delagarde & Nadège Edouard
PEGASE, INRAE, Institut Agro, Saint-Gilles, France

EMIGRAZE project

Introduction

- **Dairy farm sustainability =**

  ↘ **environmental impacts**
  N losses as water and atmospheric pollution sources
  (Castillo *et al.*, 2000; Lesschen *et al.*, 2011)

  ↗ **feed self-sufficiency**

  } More forage in dairy cow diet

Fresh grass = Low-cost on-farm feed, interesting feeding value, grasslands = environmental services
(European Environment Agency, 2019; Delaby *et al.*, 2020)

BUT availability and composition variable along the year

Frequently associated with conserved forages

Fresh grass + Maize silage → Effects on cow N utilisation and losses ???

What are the effects of increasing maize silage proportion in a fresh grass diet on cow nitrogen excretion, efficiency and NH3 emissions ?

Material & Methods – Dietary treatments and feeding

Four maize silage proportions in a fresh grass (ray-grass) diet

Treatments

0 %	100 %
17 %	83 %
34 %	66 %
51 %	49 %

(DM basis)

7 lactating Holstein cows, Latin square **4 diets** x 3 periods of 3 weeks

WITHOUT concentrate

Individual indoor feeding, in tie stall
Fresh grass cut daily, accessible during the day (8 am to 6 pm)
Maize silage accessible during the night (6 pm to 8 am)

→ **% of each feed**: check daily

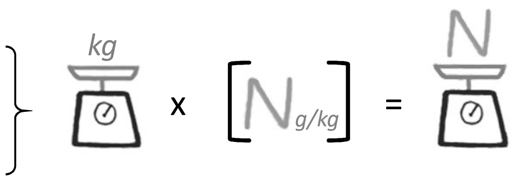
***Ad libitum* feeding** (> 10% of refusals), at least one *ad libitum* feed:

"if the maize proportion in the ingested diet was insufficient, maize silage was *ad libitum* and fresh grass distribution was restricted"

Material & Methods - Measurements

Measurements for each cow:

- Feed intake
- Milk production
- Faeces and urine excretion (total collection)



→ **Nitrogen concentration:** Feeds, refusals, milk, faeces, urine

→ **N intake, N in milk, faecal and urinary N excretion** (g/day)

→ **N efficiency** = N milk (g/day) / N intake (g/day)

Slurry reconstitution: mixing faeces and urine in proportion to their excretions

→ **Total ammonia nitrogen (TAN)**

→ **Potential NH3 emission estimated** from the **TAN excretion x 0.24**

(emission factor for dairy cattle housing, for slurry, EMEP/EEA national inventory guidelines 2019)

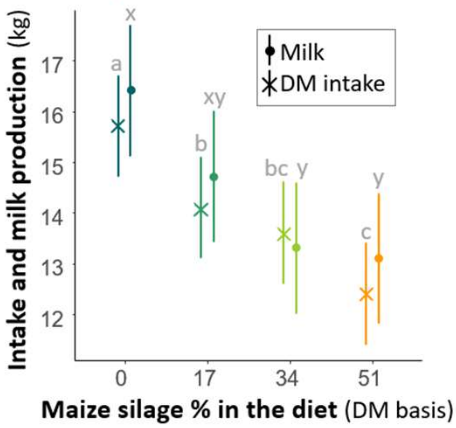
Low to very low-N diets affecting intake and milk production

	Maize silage % in the diet (DM basis)				
	0	**17**	**34**	**51**	RSD
Diet CP concentration (g/kg DM)	107^{a}	99^{b}	92^{c}	85^{d}	4.1
	Low →			**Very low**	

Different letters per row = significant difference with *P*<0.05; RSD= residual standard deviation

Very low grass crude protein (CP) concentration

With increasing % of maize silage without protein-rich concentrate



a, b, c = *P* value intake < 0.05 ; x, y = *P* value milk < 0.05

With ↗ maize silage proportion:

- Intake ↘, due to diet CP concentration↘ (Faverdin *et al.*, 2003)
- Milk ↘, due to intake ↘

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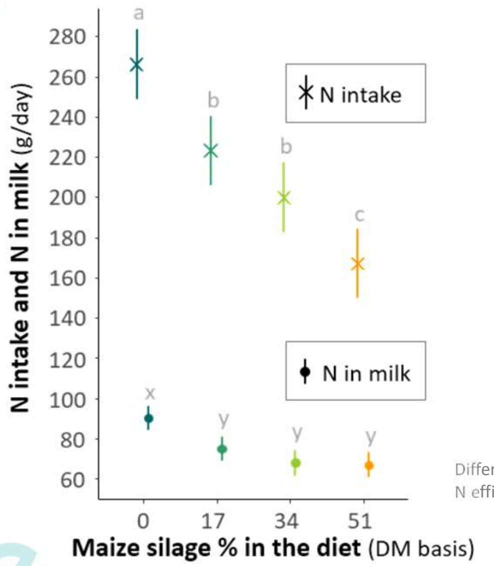
Nitrogen in milk and efficiency



a, b, c = *P* value N intake < 0.05 ; x, y = *P* value N milk < 0.05

With ↗ maize silage proportion:

- N intake ↘
- N milk ↘ quadratically
- N milk ↘ less than N intake

	Maize silage % in the diet (DM basis)				
	0	**17**	**34**	**51**	RSD
N efficiency (%)	33^{a}	33^{a}	34^{a}	40^{b} **+ 6%**	2.9

Different letters per row = significant difference with *P*<0.05; RSD= residual standard deviation
N efficiency = N milk / N intake

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Nitrogen excretion and partition

	Maize silage % in the diet (DM basis)					
	0	17	34	51	RSD	
Faecal N (g/day)	103[a]	90[b]	87[b]	75[c]	7.2	↘
Urinary N (g/day)	68[a]	60[b]	53[b]	44[c]	5.5	↘
N excreted (g/day)	171[a]	150[b]	140[b]	119[c]	8.0	↘
Urinary N as % of N excreted	40	40	38	38	3.0	=

Different letters per row = significative difference with $P<0.05$; RSD= residual standard deviation

+ 10% of maize silage in the diet =

N intake
- 20 g N/day

Urinary N
- 4.8 g N/day

Faecal N
- 5.4 g N/day

With ↗ maize silage proportion:

- Faecal N ↘, due to DM intake ↘
- Urinary N ↘, due to N intake ↘

(Castillo *et al.*, 2000; Peyraud and Delaby, 2006; Spanghero and Kowalski, 2021)

Total ammonia N (TAN) excretion and NH3 emission

		Maize silage % in the diet (DM basis)					
		0	17	34	51	RSD	
TAN excreted (g/cow/day)		34.9[a]	26.3[ab]	24.2[b]	22.6[b]	6.46	↘
NH3-N emissions (g/cow/day)	TAN × 0.24*	8.4[a]	6.3[ab]	5.8[b]	5.4[b]	1.55	↘

Different letters per row = significative difference with $P<0.05$; RSD= residual standard deviation
* Emission factor for dairy cattle housing, for slurry, European Environment Agency, 2019)

- TAN excreted and NH3-N emissions ↘ with ↗ maize silage proportion
- Very low NH3-N emissions (emission range ≈ 10 to 210 g/cow/day) (Hristov *et al.*, 2011; Bougouin *et al.*, 2016)
- TAN excreted in slurry ≈ **20% of N excreted** in faeces + urine
  EMEP/EAA estimates TAN in slurry as **60% of N** in faeces + urine

→ Overestimation of TAN by EMEP/EEA methodology for low-N diet ?

Edouard *et al.*, 2019: **TAN in slurry = 40 and 80% of N in faeces + urine**
for diets with low CP concentration (120 g/kg DM) vs high CP concentration (180 g/kg DM)

Conclusion

- ↗ **maize silage %** in unusually low-N grass diets induced **very N-deficient diets** on which **N efficiency was ↗** and **losses** to the environment **were minimal**

  We tested atypical diets for which cow responses were poorly known

- **TAN excreted as % of N excreted** in faeces and urine was **overestimated for very low-N diets** by actual national inventory guidelines.

  This estimation can be improved, considering the protein concentration of the diet

Ferreira M., Delagarde R., and Edouard N., 2022, Nitrogen flows in dairy cows fed various proportions of low-N fresh grass and maize silage, *In: Grassland Science in Europe: Grassland at the heart of sustainable food systems, European Grassland Federation*, 27, 566-568

Thank you for your attention !

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ANNEX 3:

Chris Flechard, Yannick Fauvel, Adrien Rémy Delagarde, Anne Isabelle Graux, Nadège Edouard, 2022. Surface/atmosphere exchange of NH_3 above managed grassland - Long term low cost monitoring and short term intensive campaigns WMO GAW, SAG TAD, Geneva, 05 October 2022

Surface/atmosphere exchange of NH_3 above managed grassland

Long term low-cost monitoring and short-term intensive campaigns

Chris Flechard, Yannick Fauvel, Adrien Jacotot,
Rémy Delagarde, Anne-Isabelle Graux, Nadège Edouard

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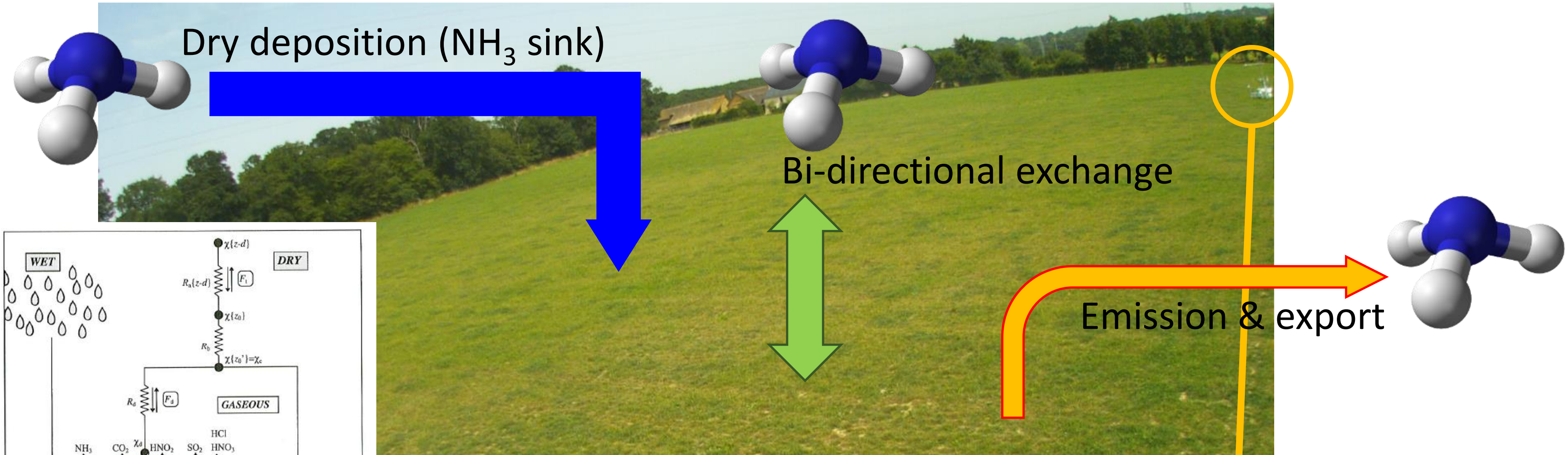
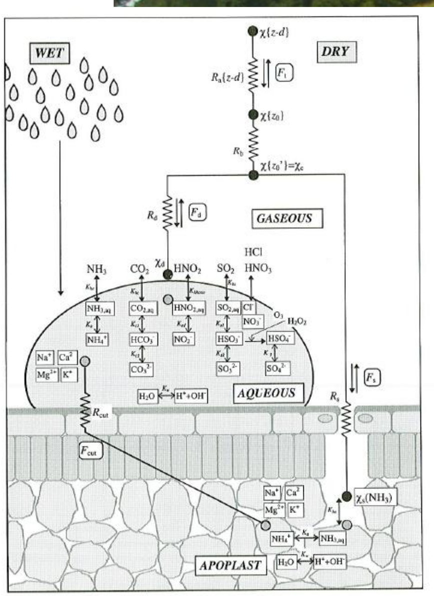
WMO-GAW, SAG-TAD, Geneva, 05 October 2022

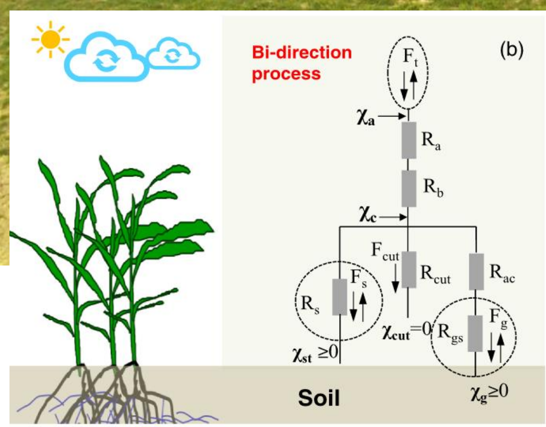
EMIGRAZE

Processes of bi-directional NH_3 exchange over managed grassland: background conditions (~ low ecosystem N status, winter)





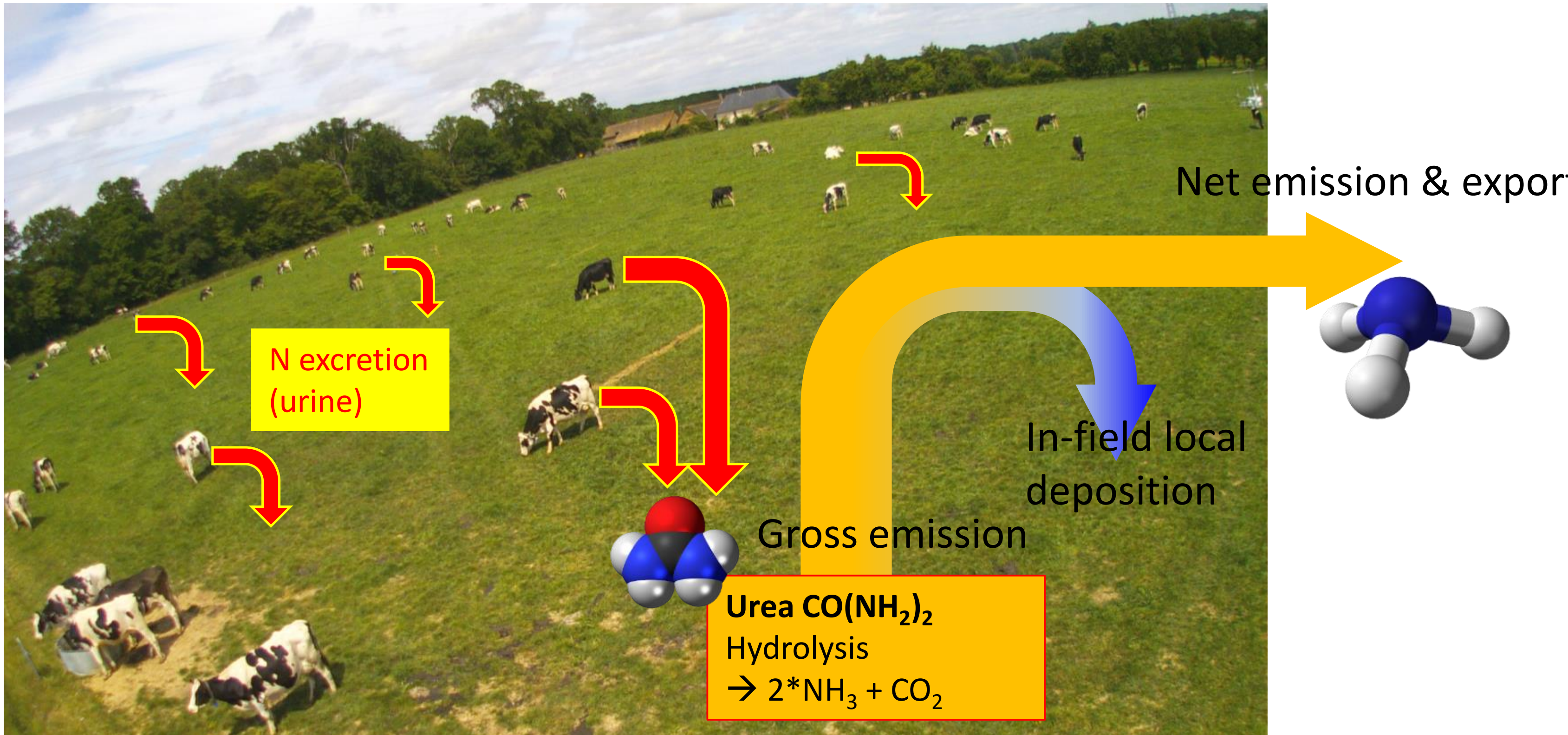
Flechard et al. 1999

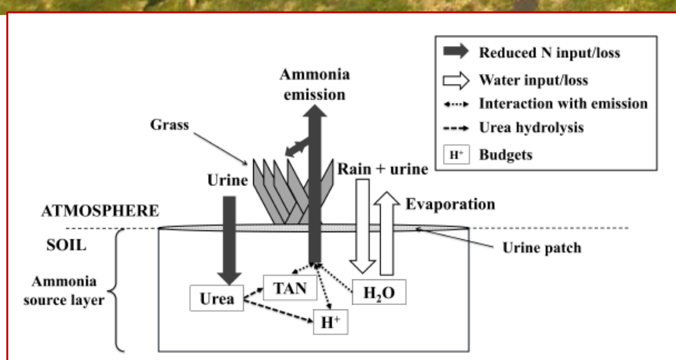


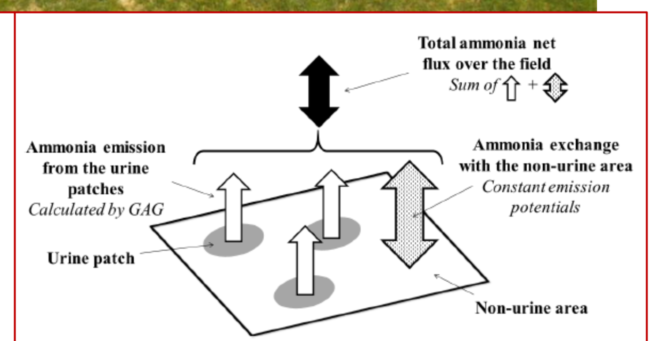
Qi Zhang et al. 2021

SAS INRAE L'INSTITUT agro Rennes Angers

Processes of bi-directional NH_3 exchange over managed grassland : grazing emissions (spring, summer)

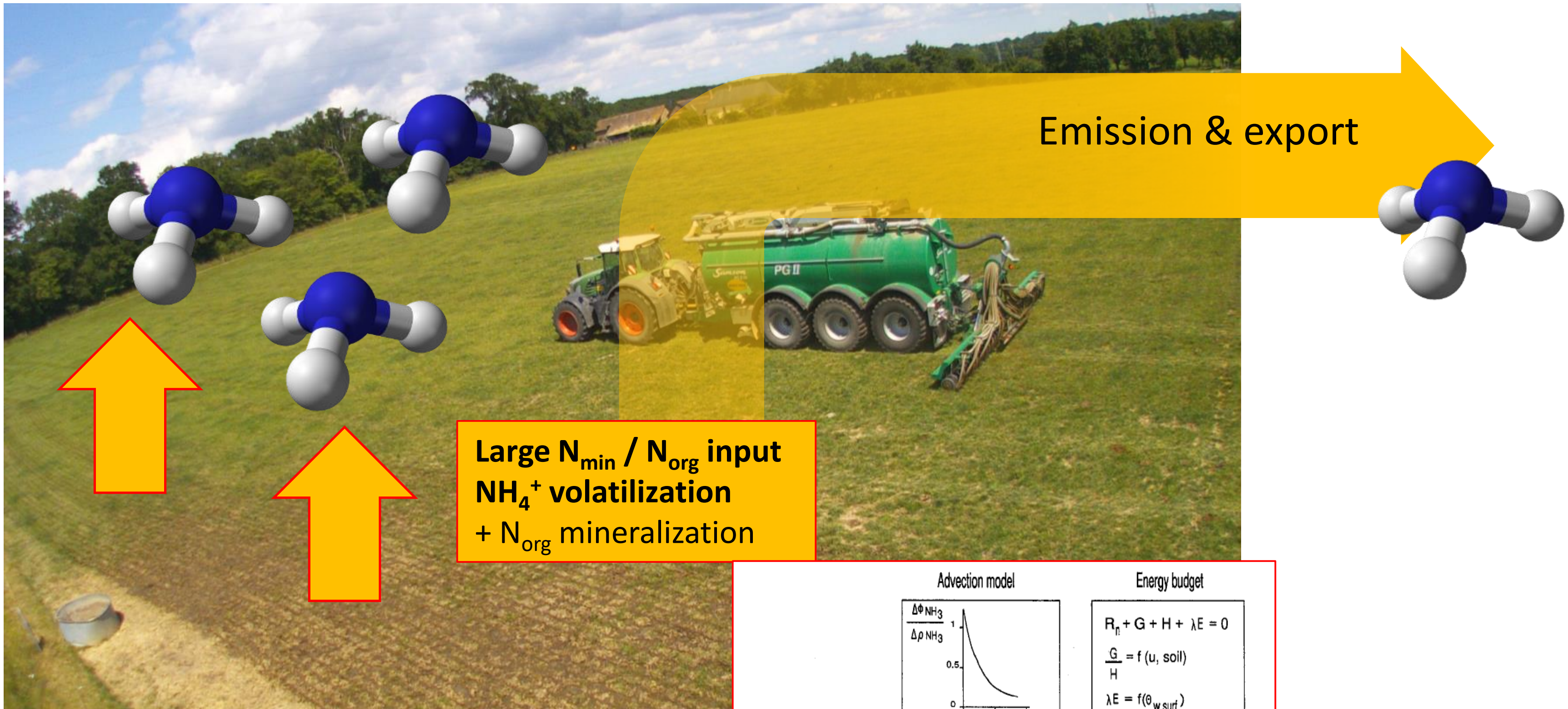






GAG model, Moring et al. 2017

Processes of bi-directional NH_3 exchange over managed grassland : manure/fertilizer application



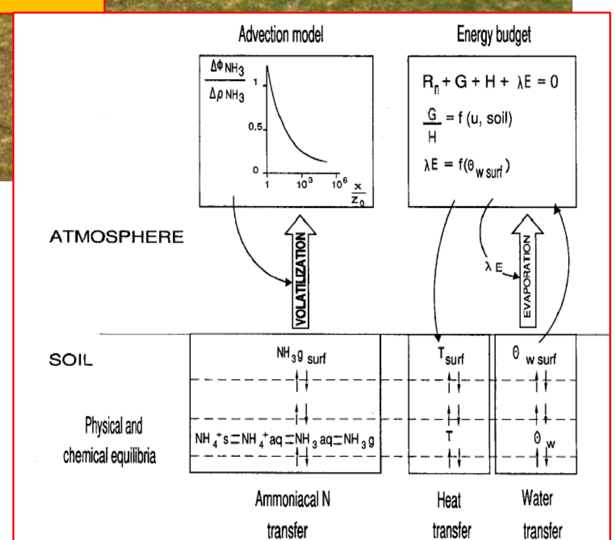


Fig. 1. Diagram of the volatilization model.

VOLT'AIR model
Genermont et al. 1997

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Assessing the NH_3 budget of productive/managed grasslands

Alternating net emission and net deposition phases

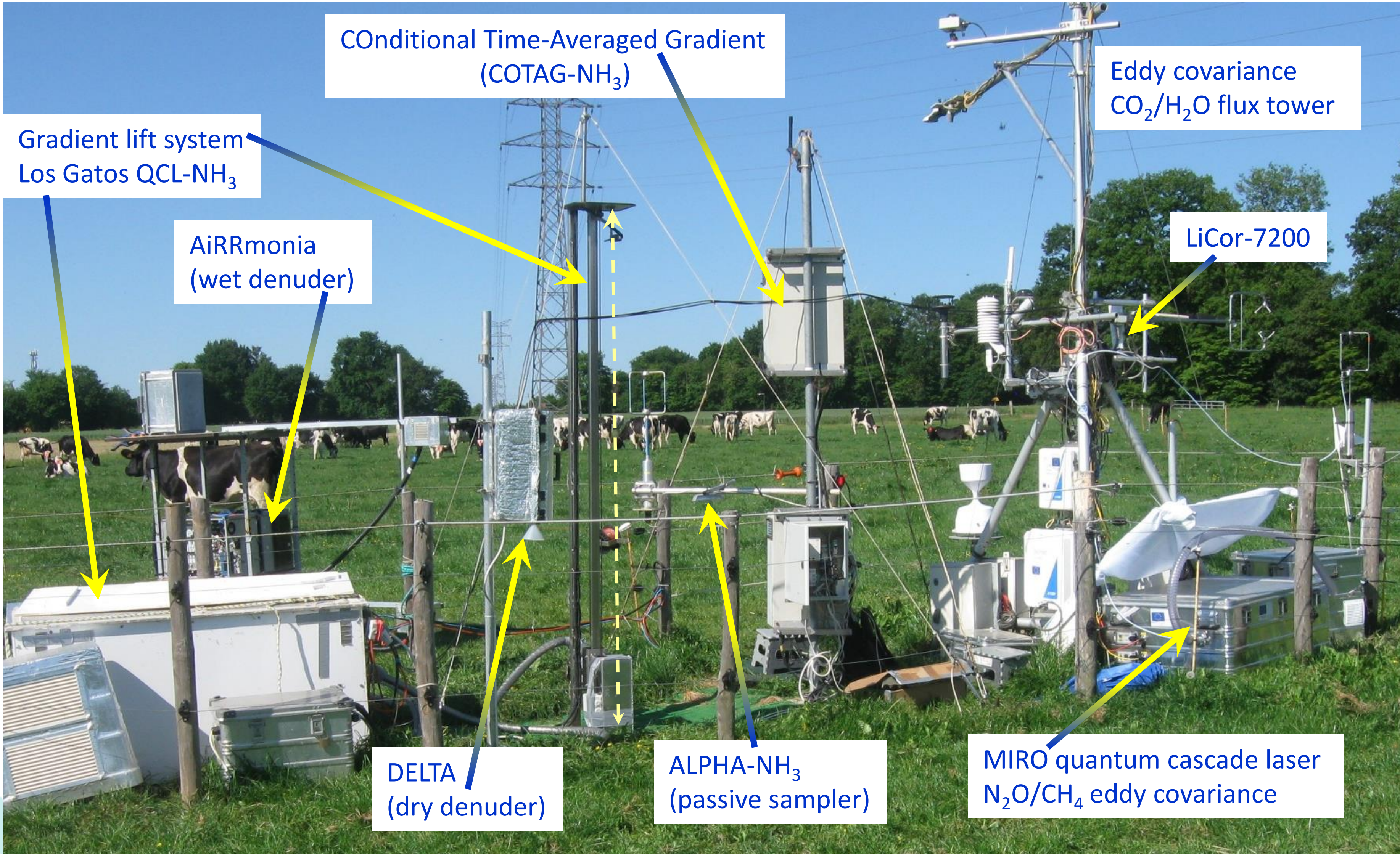
- Short term emission peaks during grazing & fertilization (a few days to weeks)
- Bi-directional transition phase. Flux sign depends on temperature, moisture, ecosystem N status
- Winter half-year: dry deposition prevails
    - **What is the net annual balance?**

Measurement strategy to characterize the NH_3 budget

- **Intensive measurement campaigns** (a few weeks in spring/summer)
    - Short-term (hours-days) response to sudden ecosystem disturbances (grazing, fertilization)
    - High resolution (hourly) flux data for process understanding
    - Use data to develop/parameterize/calibrate emission models

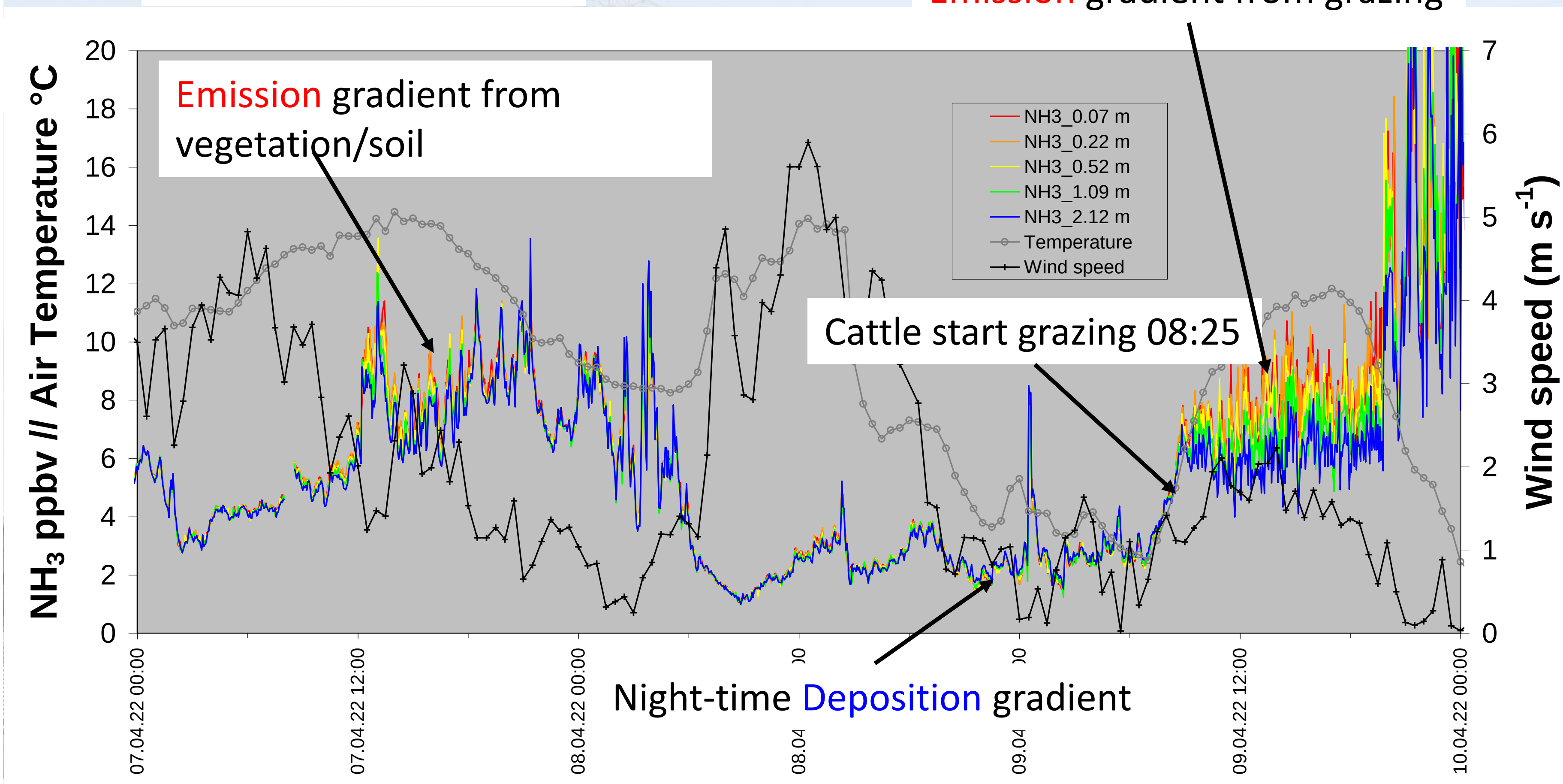
- **Long-term, low-resolution, low-cost flux measurements** (e.g. COTAG)
    - Low maintenance and low frequency allow multi-annual measurements
    - Robust data for long-term budgets, but not adequate for process understanding

Instrumental setup & inter-comparison

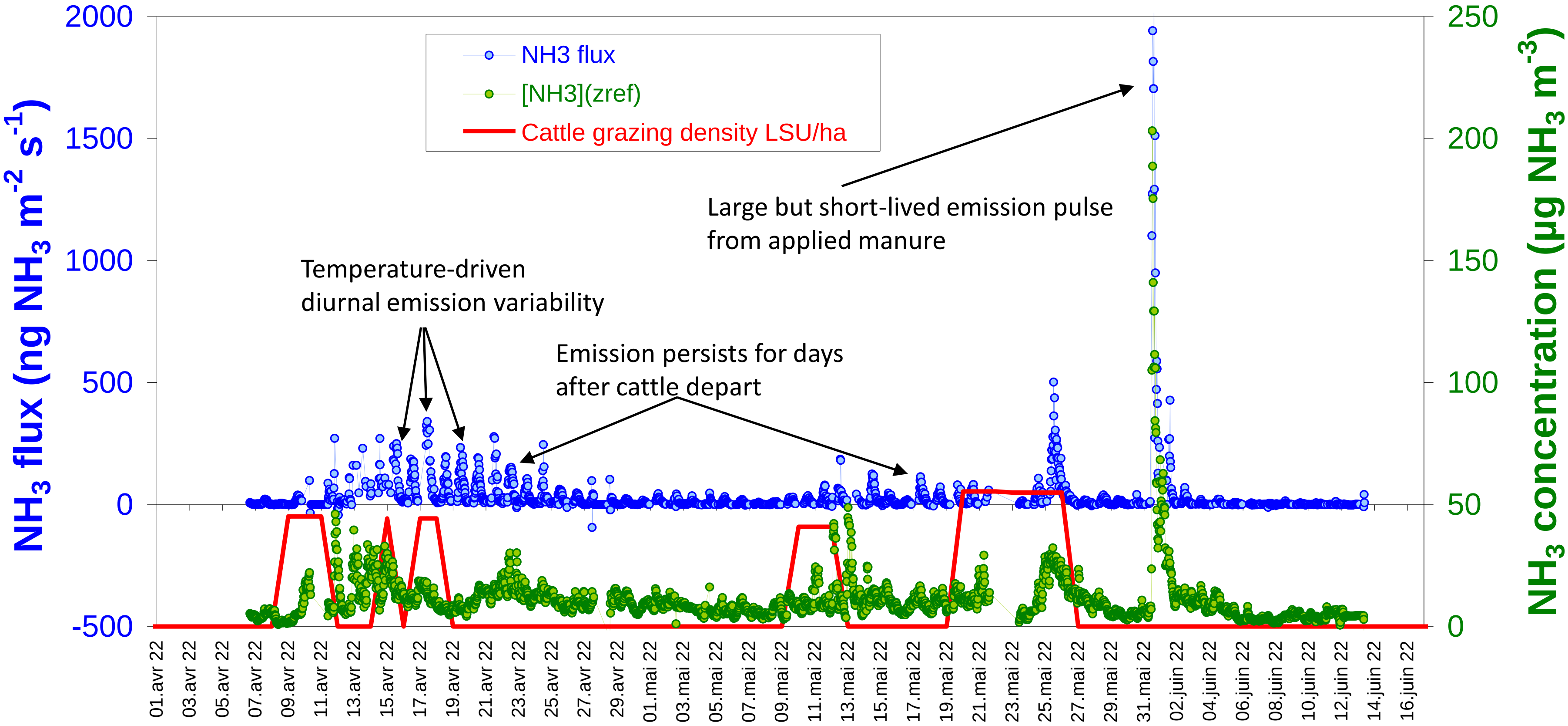


Grazing-related NH_3 emissions: aerodynamic gradient method using Los Gatos quantum cascade laser (QCL) & lift system

$$F_{\chi} = -ku_{*}\frac{\partial \chi}{\partial\left[\ln(z-d) - \psi_{H}\{\zeta\}\right]}$$

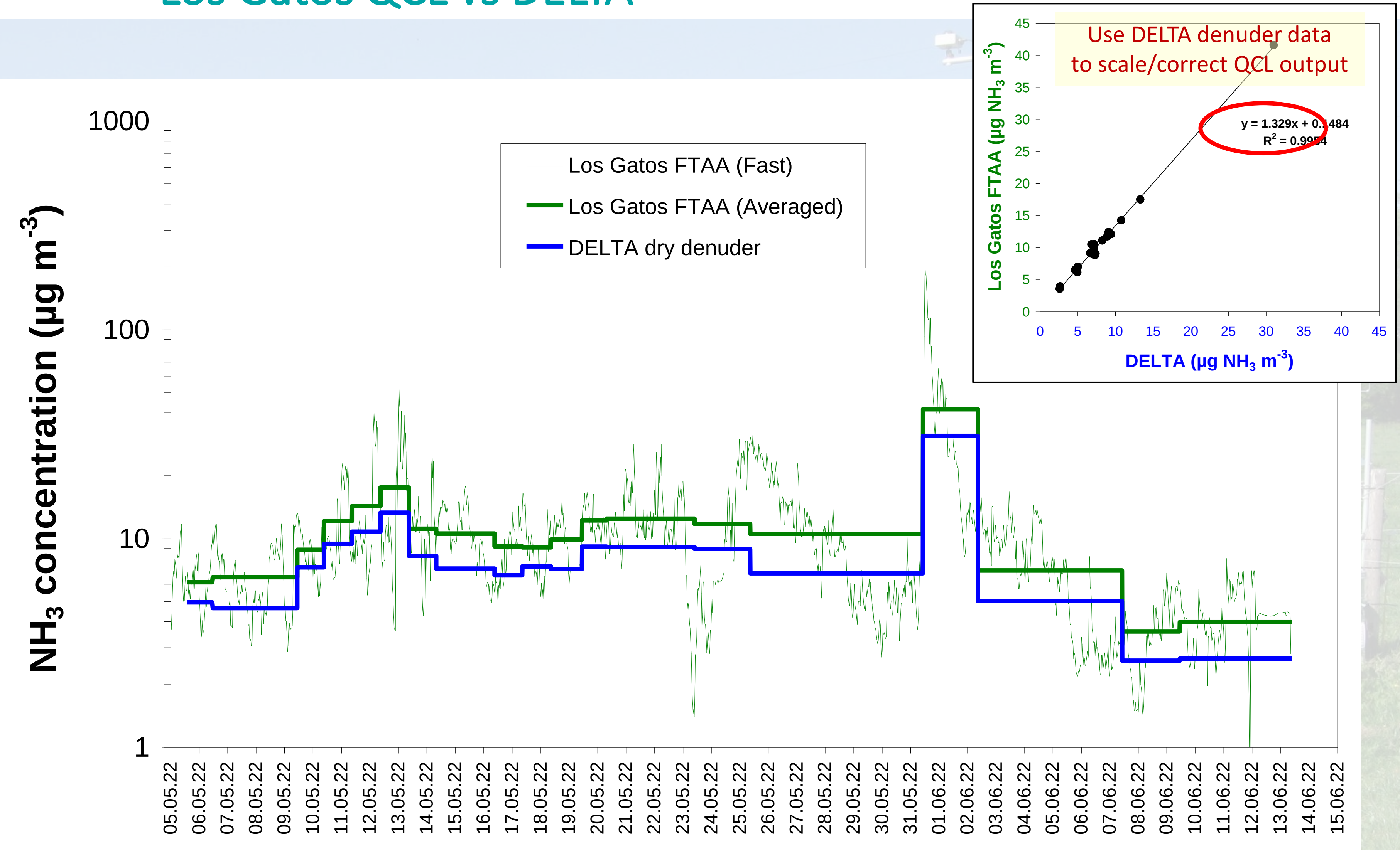


Intensive NH_3 flux campaign Spring 2022 - overview

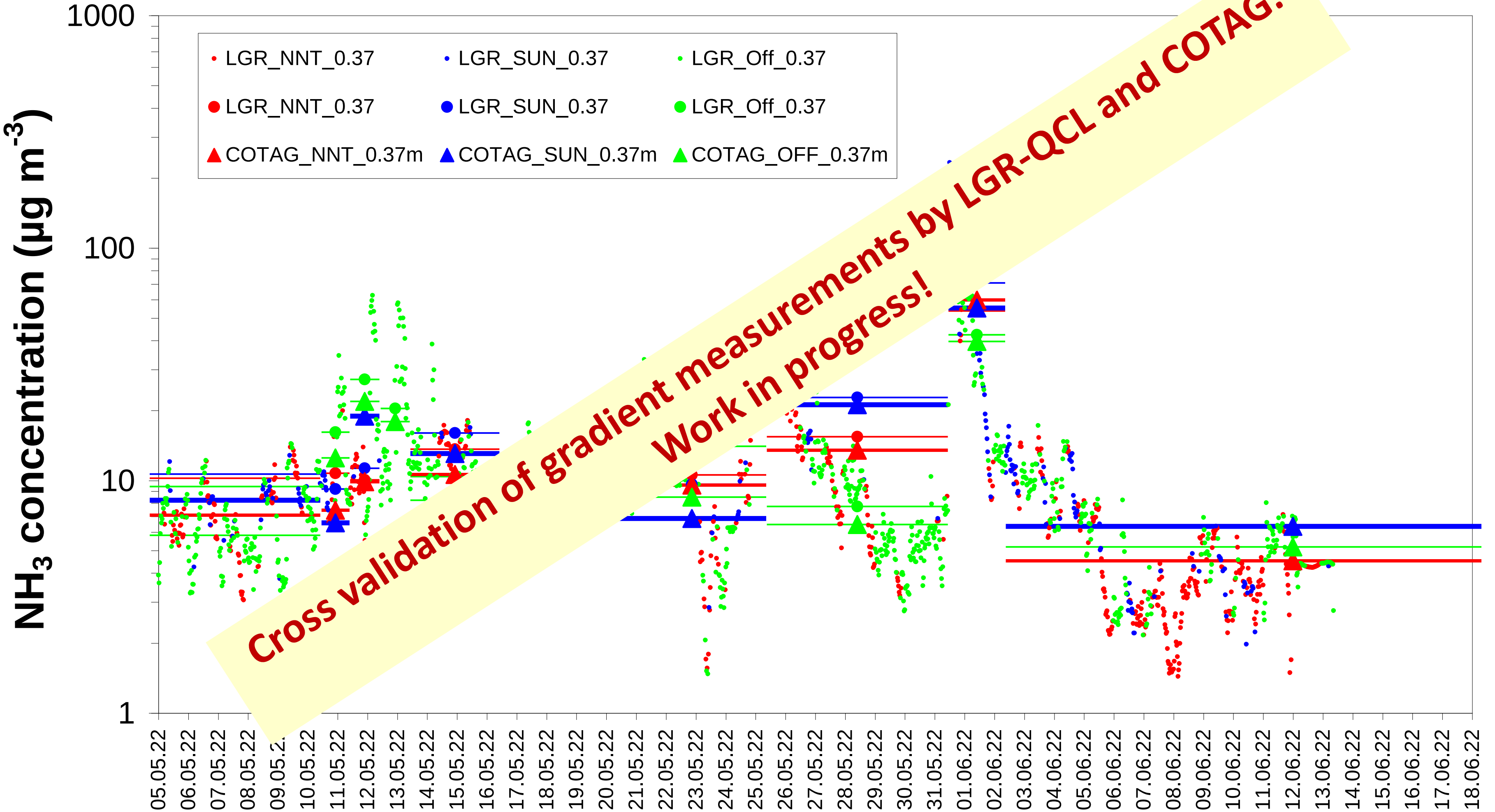


- **Grazing** : moderate but lasting emission fluxes driven by urea hydrolysis on soil surface
- **Organic manure application**: instantaneous release (volatilization) by NH_4^+-rich substrate (phase equilibrium)

NH$_3$ measurement intercomparison: Los Gatos QCL vs DELTA



NH$_3$ concentration & gradient intercomparison: Los Gatos QCL vs COTAG



COTAG stability classes

- NNT: near-neutral
- SUN: slightly unstable
- OFF: very stable or unstable

SAS

Gap-filling of COTAG-derived flux data

COTAG: relaxed aerodynamic gradient method

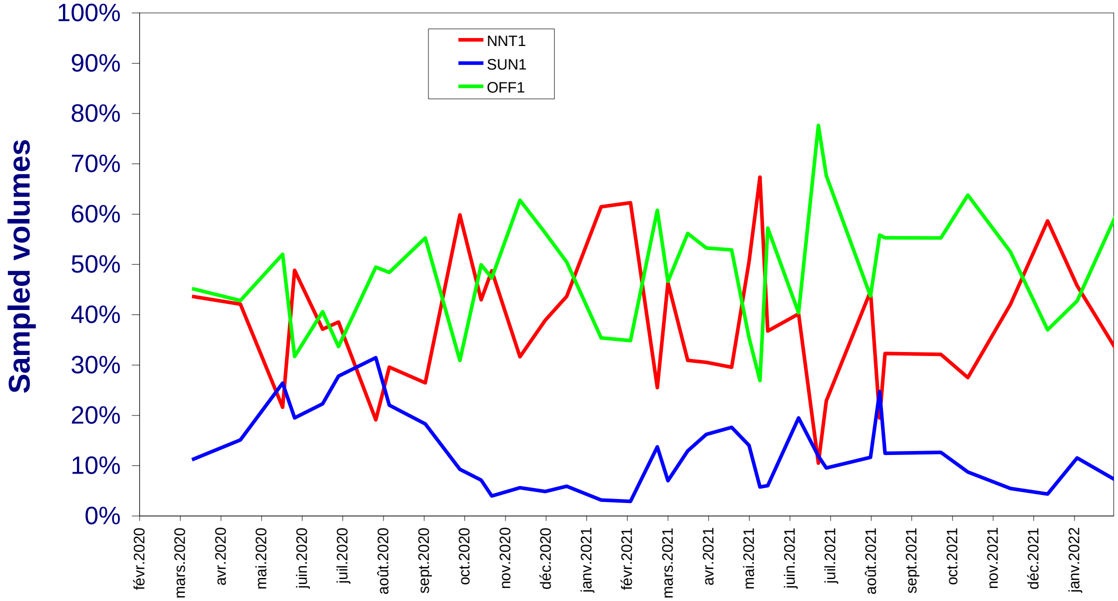
$$F_\chi = -ku_* \frac{\partial \chi}{\partial\left[\ln(z-d) - \psi_H\{\zeta\}\right]}$$

- Turbulence and vertical concentration gradient averaged over several hours or days,
- …but only valid for near-neutral or slightly unstable atmospheric conditions

COTAG stability classes
- **NNT: near-neutral**
- **SUN: slightly unstable**
- **OFF: very stable or unstable**

→ F_{NH3}_NNT & F_{NH3}_SUN measured

→ F_{NH3}_OFF not measured



- To obtain time-integrated total COTAG NH_3 flux, ~**50% of the data** (OFF periods) must be gap-filled
- Develop gap-filling strategy based on inferential modelling and locally-derived ecosystem-scale emission potential ($\Gamma_{surface} = NH_4^+/H^+$)

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C. R. Flechard et al.: The ammonia budget of fertilised grassland

Swiss grassland NH_3 flux study 2006-07

Biogeosciences, 7, 537–556, 2010
www.biogeosciences.net/7/537/2010/

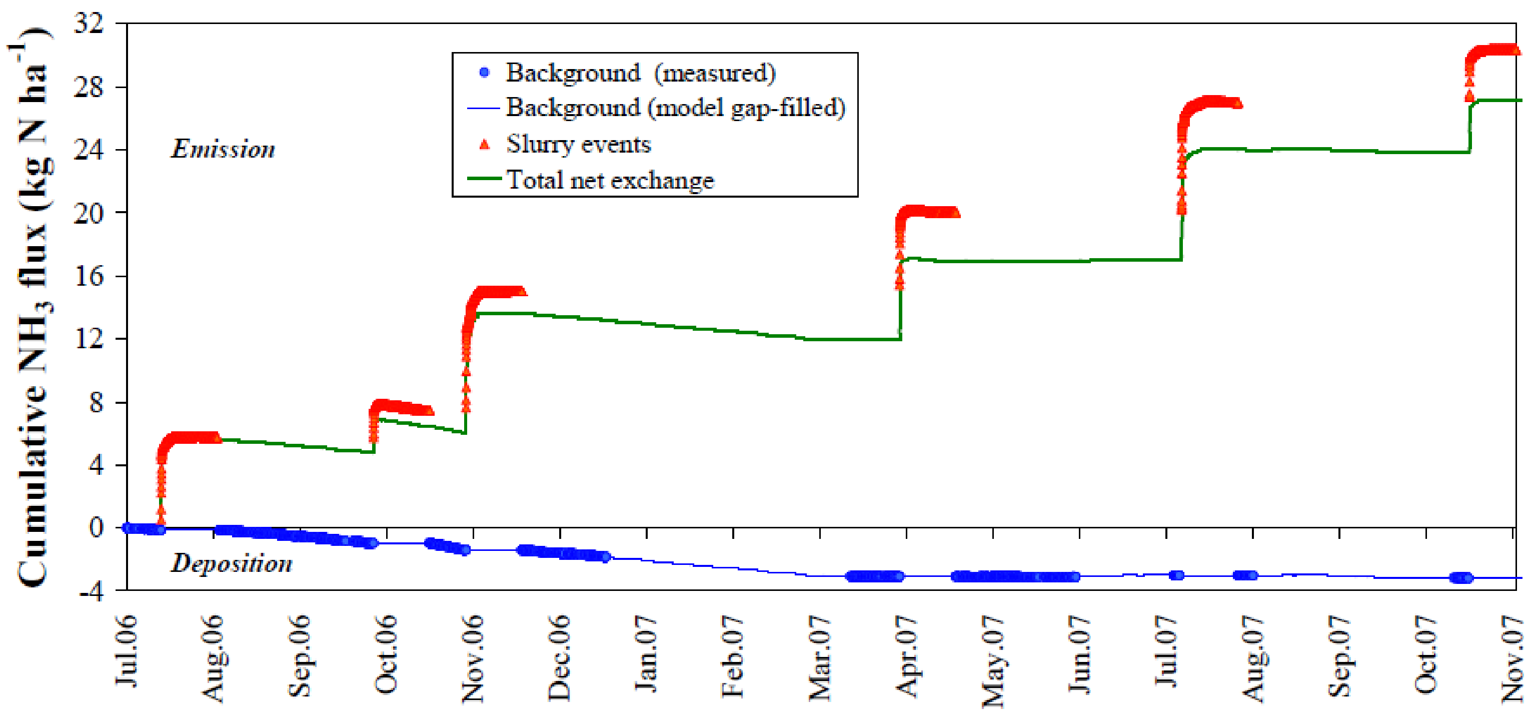

Fig. 6. Cumulative 17-month NH_3 exchange with contributions from background exchange and slurry application events.

Conclusion & take home

Managed grasslands are (generally) net NH_3 sources

- Large emissions by manure applications
- Significant emissions by grazing herbivores (though possibly reduced *per capita* compared with indoor dairy systems)
- Dry deposition / bi-directional exchange in background conditions still relevant for net long term NH_3 budget, and for N-cycling process understanding
- Magnitude and sign of NH_3 flux depends on ecosystem N status and weather

Still too few long-term datasets to support model development

- Field measurements: combine low-cost and high-tech techniques
- Intercomparison of instruments crucial for NH_3 !!

(See Twigg et al. 2022 AMT paper, https://doi.org/10.5194/amt-2022-107)

- Link up ecosystem biogeochemistry and surface-atmosphere exchange.

Inferential modelling requires adequate quantification of surface emission potentials.

- Even COTAG-type methods require good modelling for defensible gap-filling

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Thanks for your attention !

ANNEX 4:

Nadege Edouard, Xavier Vergé, Christophe Flechard, Yannick Fauvel, Adrien Jacotot, 2023. Gas emissions (building, storage, pasture) of dairy systems combining or not grazing and housing. 74th Annual meeting of the European federation of animal science (EAAP), Lyon, France

EAAP Lyon, June 2023 - Session: Climate care dairy farming – follow-up

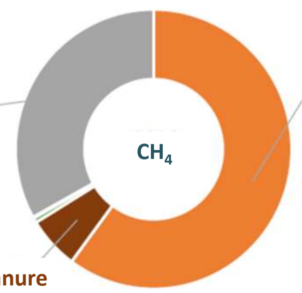
Gas emissions (building, storage, pasture) of dairy systems combining or not grazing and housing

Nadege Edouard, Xavier Vergé, Christophe Flechard, Yannick Fauvel, Adrien Jacotot

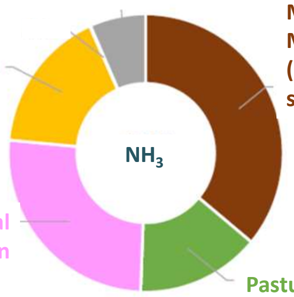
RÉPUBLIQUE FRANÇAISE INRAE INSTITUT DE L'ELEVAGE idele

Livestock contributes to environmental impacts

In France, AGRICULTURE accounts for **67% of CH_4** and **94% of NH_3** total emissions => mainly due to **livestock**



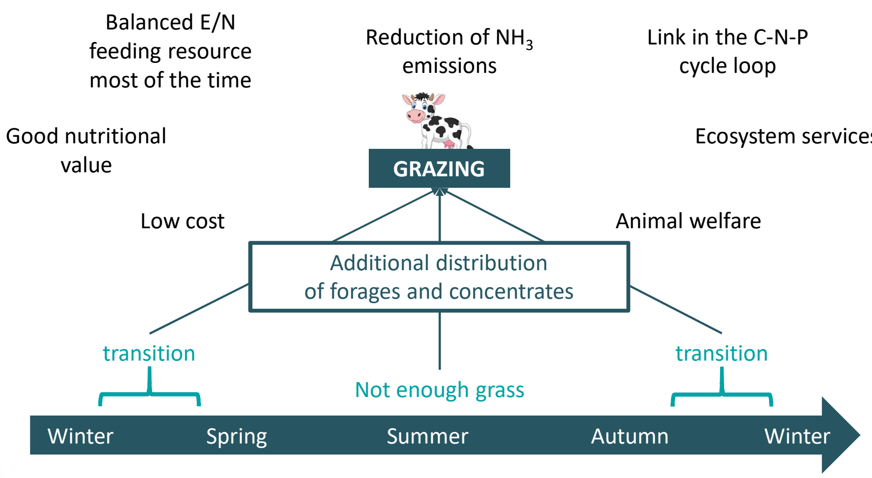
CITEPA 2021



CITEPA 2021

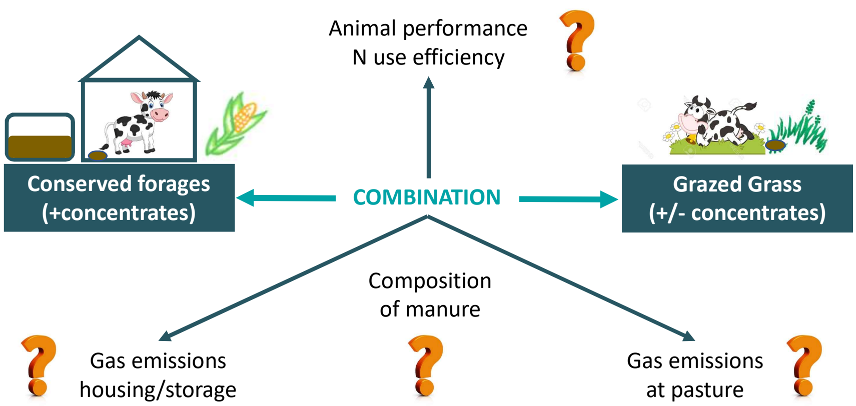
- Climate change
- Water and air pollutions
- Biodiversity losses
- …

Grazing: a lever for greater sustainability



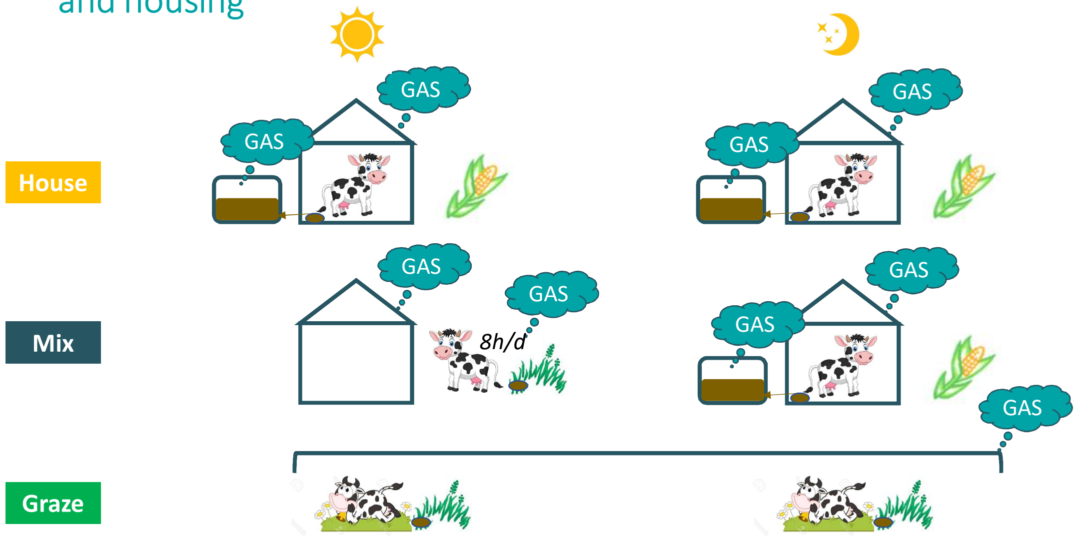
Consequences of combining grazing and forages offered at trough

On GHG and NH_3 emissions



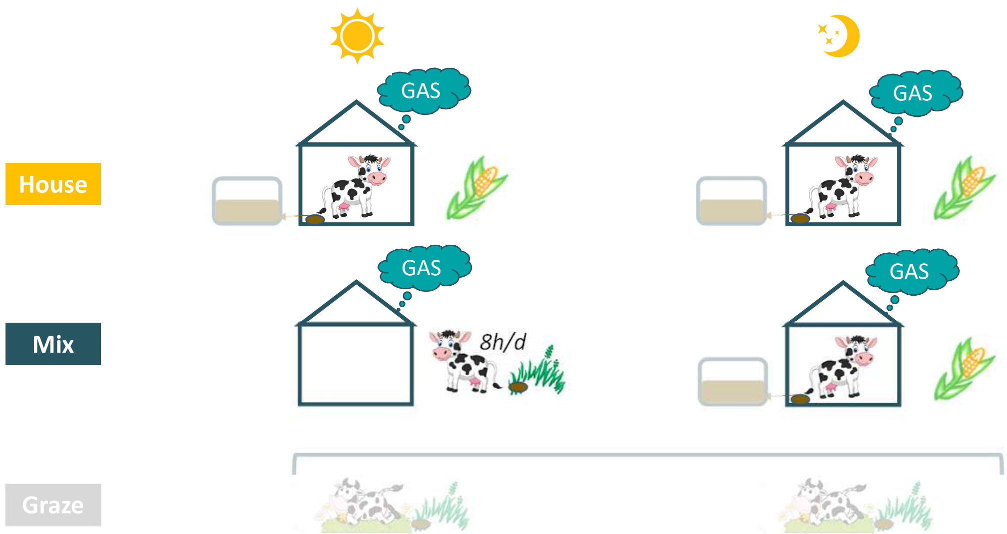
Comparing 3 strategies combining or not grazing and housing

INRAE Dairy Experimental Farm
Rennes, France
https://doi.org/10.15454/yk9q-pf68



2 seasons : Spring and Autumn

Gas emissions at the BARN level



Gas emissions at the BARN level

Experimental design

autumn **House** **Mix** **Graze**

1 group = 3 cows
Measures = 5 last d

	Period 1 3 weeks	Period 2 3 weeks	Period 3 3 weeks
Group 1	House	Mix	House
Group 2	Mix	House	Mix

24h/day **16h/day**

House :
Total Mix Ration ad lib

Mix :
8kgMS TMR in the evening

TMR:
75% maize silage
15% soya meal
10% cereals

Grazing:
Temporary pasture
0,5 à 1ha

8h/day
between milkings

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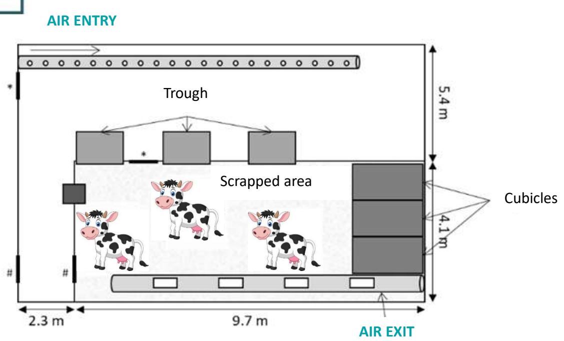
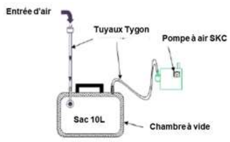
Gas emissions at the BARN level

Dynamic ventilation rooms – free stall barn

autumn **House** **Mix** **Graze**

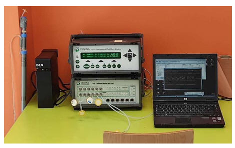


Spot gas sampling in **air entry** and **air exit** at 7:00 before feeding and 18:00 after feeding



Gas concentrations

Analyser INNOVA
CH_4 - N_2O - CO_2



Colorimetric tubes
NH_3

+
DM Intake
Milk Yield
Milk composition
Manure composition
…

INRAE

Gas emissions at the BARN level

autumn **House** **Mix** Graze

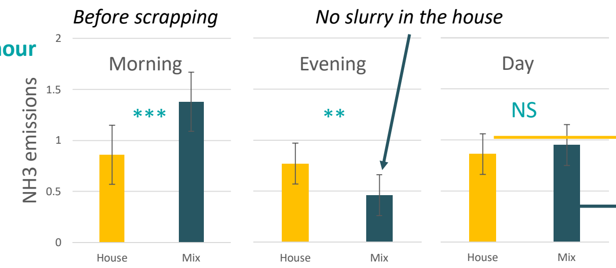
NH_3 emissions

		House		Mix	P-value	RMSE
CP intake	kg/cow/d	3.6	<	4.9	***	0.2
Slurry N content	g/kg	37	<	46	*	0.3

+1.3 kgCP for Mix ↔ Diet CP content: 14% → 22%

despite lower DMI

g/cow/hour



At the BARN and DAY level

x 24h/day => 21 gNH_3/cow/day

x 16h/day => 15 gNH_3/cow/day

Gas emissions of dairy systems combining or not grazing and housing
28th August 2023 / EAAP Annual Meeting / Edouard N et al.

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Gas emissions at the BARN level

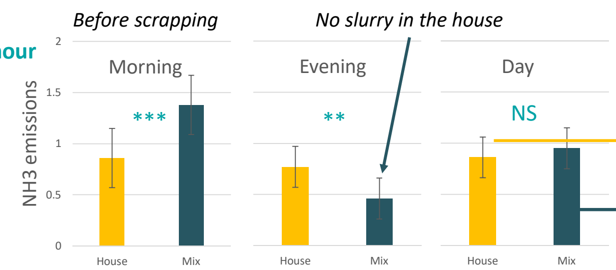
autumn **House** **Mix** Graze

NH_3 emissions

Ammonia emissions in g/cow/hour
are higher for cows eating grass with high CP content
when there is N-rich slurry in the house (morning)
but daily emissions are lower
due to lower amount of slurry in the house

+1.3 kgCP for Mix ↔ Diet CP content: 14% → 22%

g/cow/hour



At the BARN and DAY level

x 24h/day => 21 gNH_3/cow/day

x 16h/day => 15 gNH_3/cow/day

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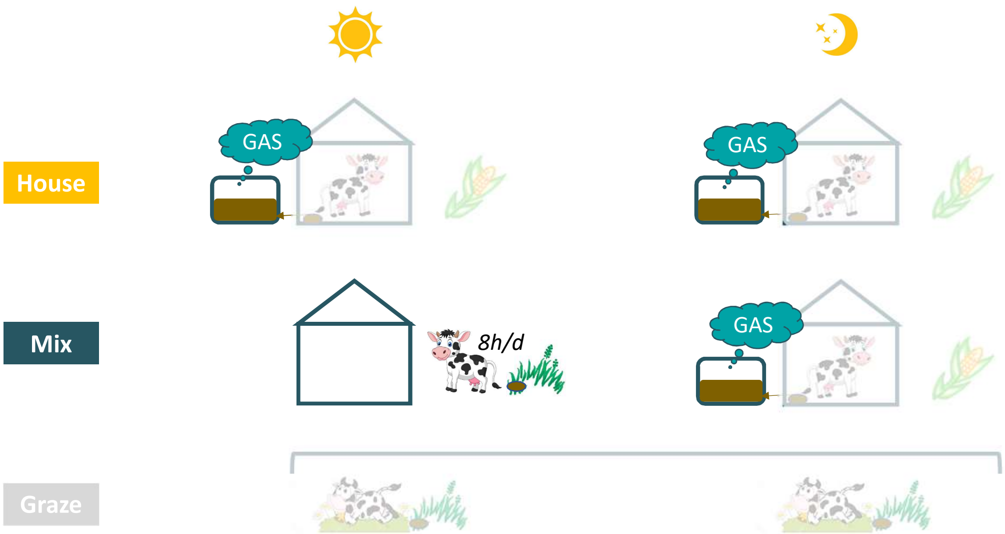
Gas emissions at the STORAGE level



Gas emissions at the STORAGE level

Passive flux chambers

autumn **House** **Mix** **Graze**

- 100 kg slurry / treatment collected in 2 consecutive days during week 3 of the BARN phase
- Homogenized and separated in 4 containers / treatment
- Slurry regularly mixed (2 containers /treatment)
  or not (2 containers /treatment)

Spot gas sampling:
Gas concentrations

Colorimetric tubes

NH_3 - CO_2

Chromatography

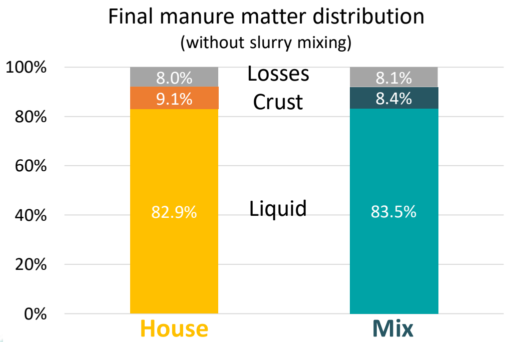
CH_4 - N_2O - CO_2

Cameras

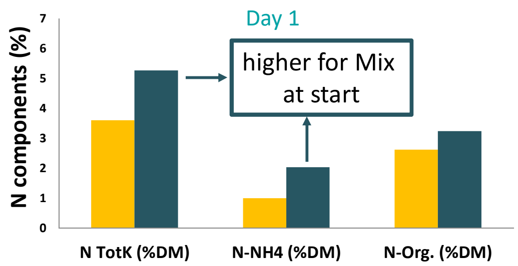
Gas emissions at the STORAGE level

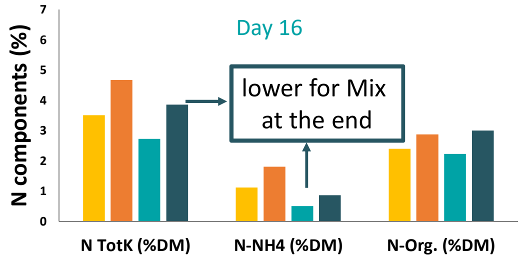
autumn House Mix Graze

Manure characteristics - composition



=> Similar between feeding treatments

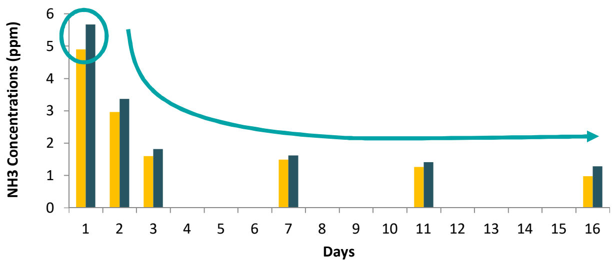




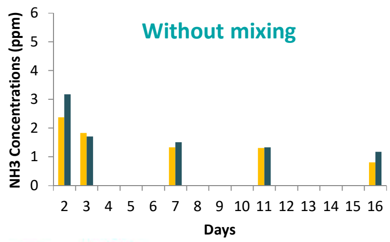
Gas emissions at the STORAGE level

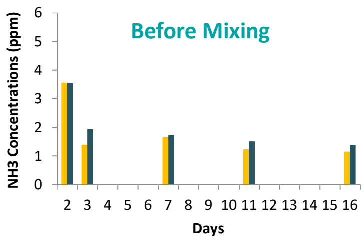
autumn House Mix Graze

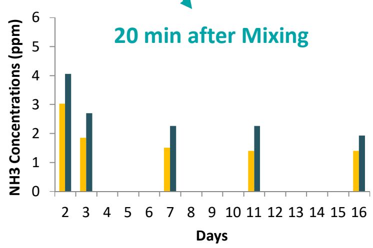
NH_3 volatilisation



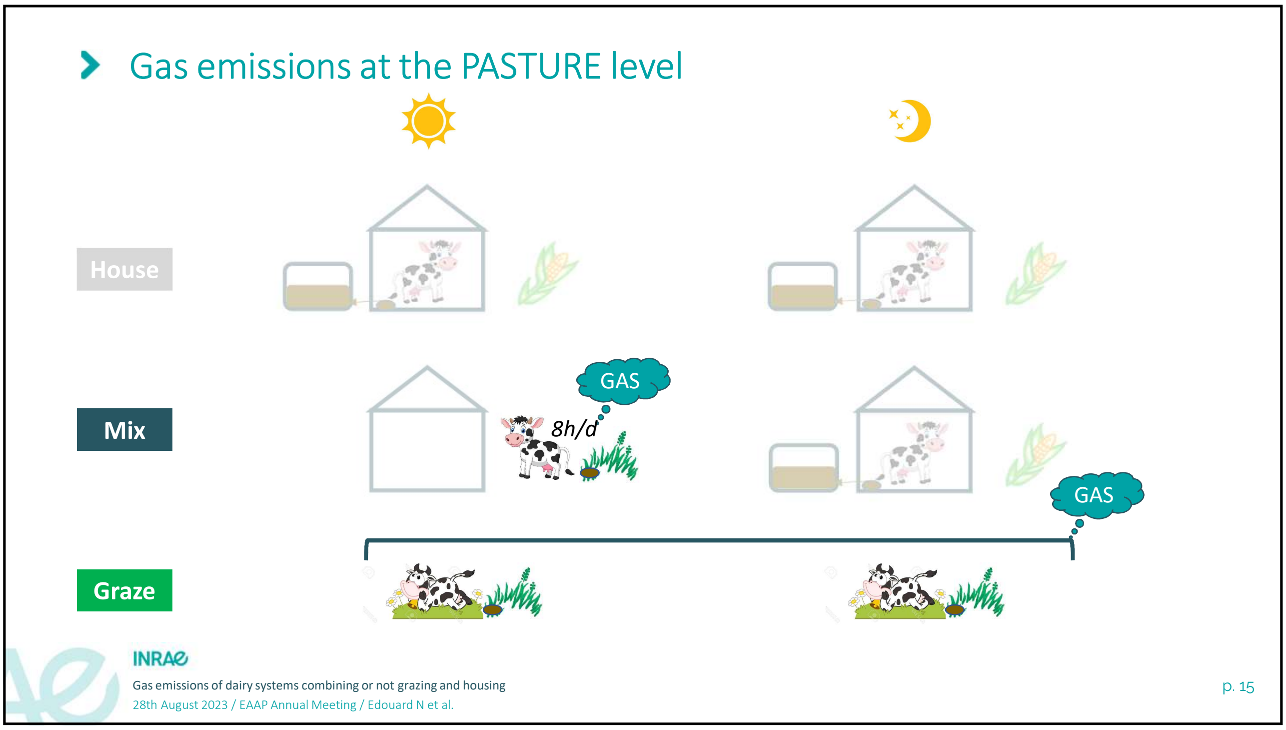
- $[NH_3]$ decreases quite rapidly
- Slightly higher $[NH_3]$ for **Mix** vs **House** on day 1, almost similar thereafter
- Difference more visible just after mixing the manure







MIX: higher NH_3-N in slurry => higher NH_3 volatilisation



Gas emissions at the PASTURE level
House
Mix
Graze
GAS
8h/d
GAS
INRAE
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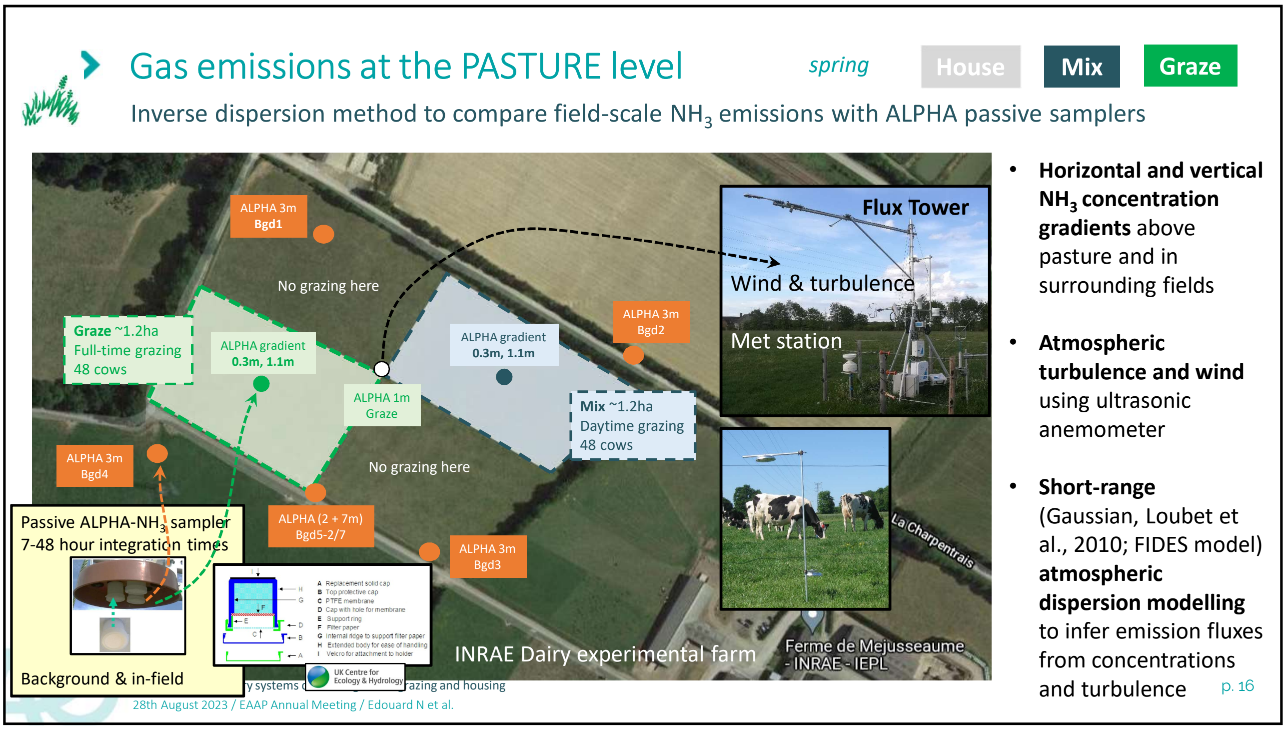

Gas emissions at the PASTURE level
spring
House
Mix
Graze
Inverse dispersion method to compare field-scale NH3 emissions with ALPHA passive samplers
ALPHA 3m Bgd1
No grazing here
Flux Tower
Wind & turbulence
Met station
ALPHA 3m Bgd2
Graze ~1.2ha Full-time grazing 48 cows
ALPHA gradient 0.3m, 1.1m
ALPHA gradient 0.3m, 1.1m
ALPHA 1m Graze
Mix ~1.2ha Daytime grazing 48 cows
ALPHA 3m Bgd4
No grazing here
Passive ALPHA-NH3 sampler 7-48 hour integration times
ALPHA (2 + 7m) Bgd5-2/7
ALPHA 3m Bgd3
La Charpentrais
Background & in-field
UK Centre for Ecology & Hydrology
INRAE Dairy experimental farm
Ferme de Mejusseaume - INRAE - IEPL
Horizontal and vertical NH3 concentration gradients above pasture and in surrounding fields
Atmospheric turbulence and wind using ultrasonic anemometer
Short-range (Gaussian, Loubet et al., 2010; FIDES model) atmospheric dispersion modelling to infer emission fluxes from concentrations and turbulence
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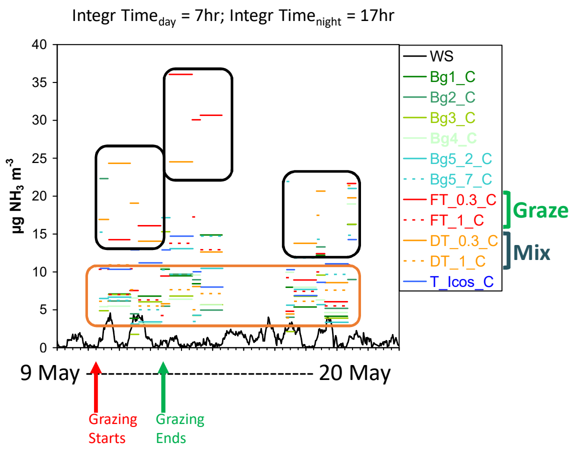
Gas emissions at the PASTURE level

spring House Mix Graze

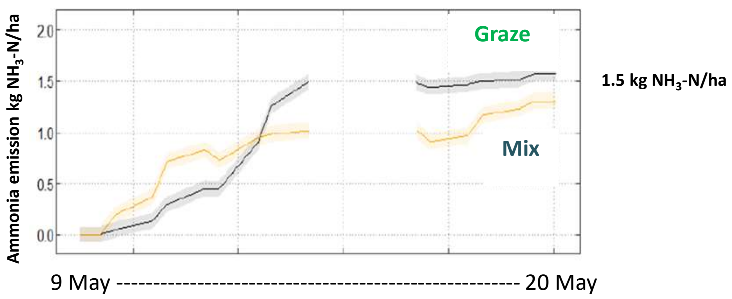
Inverse dispersion method to compare field-scale NH_3 emissions with ALPHA passive samplers

- **NH_3 concentrations** clearly larger within **Graze** and **Mix** paddocks, compared with **surrounding/background** (non-grazed) fields
- Emission gradient peaks for 2 days after end of grazing phase, then almost vanishes after ~one week



Gas emissions at the PASTURE level

Inverse dispersion method to compare field-scale NH_3 emissions with ALPHA passive samplers



- Cumulative emissions in **Graze** apparently tend to be only marginally larger than in **Mix** grazing
- But differences are **not significant** due to large uncertainties in dispersion modelling

Gas emissions at the PASTURE level

spring House Mix Graze

Tentative annual upscaling and grazing-induced emission factors

- With 48 cows/ha, 1.5 kg N cumulative emission over 10 days corresponds to
  => a **daily emission rate of ~ 3.8 g NH_3/cow/d**
- **Similar magnitude with previous NH_3 emission measurements** on the same field using high resolution mini-DOAS NH_3 concentrations and inverse dispersion modelling:
  => Bell et al 2017 (Atmos. Meas. Tech) = ~ ***5.7-6.2 g NH_3/cow/d***
- Assuming 8 months per year of grazing (March-October), this is equivalent to
  => an **annual grazing emission rate of 1 kg NH_3/cow/year**
- The low resolution/low cost diffusion ALPHA sampler & inverse dispersion method provided **realistic estimates**, but is likely **not sensitive enough** to detect differences between **Graze**/**Mix** grazing treatments

Some conclusions and limits

NH_3 emissions

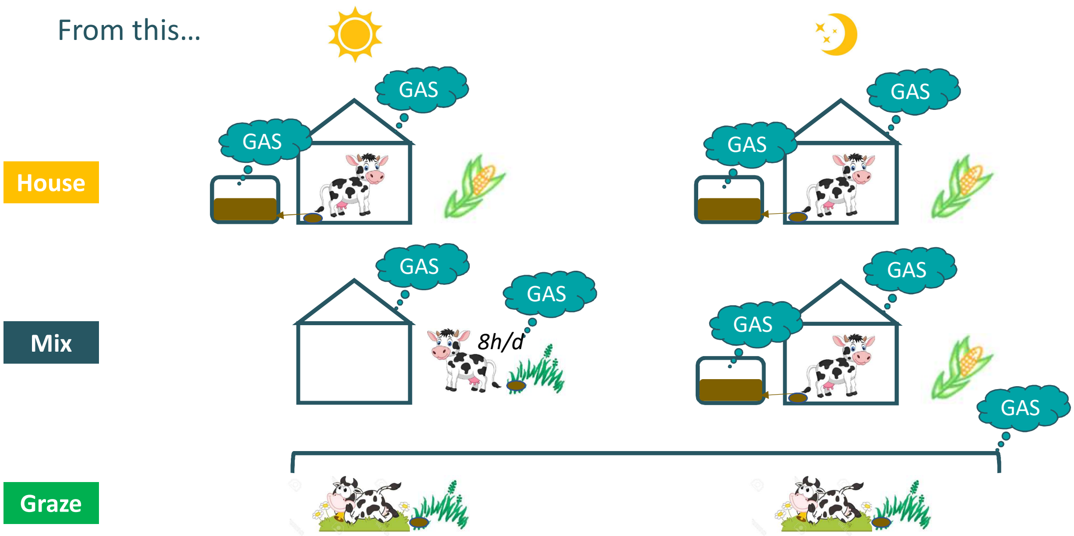
Small amount of slurry in chambers

	BARN level	STORAGE level	PASTURE level
House	*Only 3 cows /treatment…*		*Large uncertainties in modelling*
Mix →	More N in slurry => more g NH_3/hour Less slurry in the house **=> less g NH_3/day**	For the same amount of slurry: slightly higher [NH_3], + with mixing *BUT less slurry produced…* **=> probably less g NH_3/day**	Almost as much **kg NH_3/ha** (NS) *BUT very low daily emissions compared with housing phase (5 times less)*
Graze			

Extrapolation?
Integration?

Need to integrate the whole manure management chain and all gases

From this…



Need to integrate the whole manure management chain and all gases

… to this

THANK YOU FOR YOUR ATTENTION!

Acknowledgements:

ERA-NETs SusAn, FACCE ERA-GAS & ICT AGRI 2018 Joint Call
ANR: Agence Nationale de la recherche (France)
ADEME: Agence de la Transition Ecologique (France)

For more information: www.CCCfarming.eu

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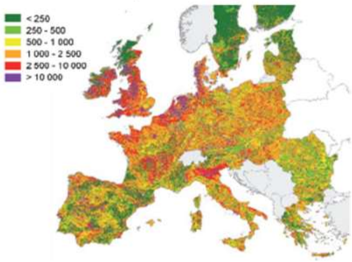
Livestock contributes to environmental impacts

With diverse consequences for **environment**

Climate change (kg CO_2e/kg protein)

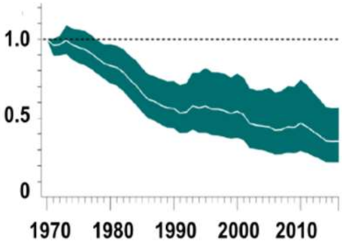
Quelle recherche pour penser l'élevage de demain ?
Shaping the Future of Livestock Farming Through Research

Pollution (Nitrate kg/km²)



Sutton et al., 2011 Leip et al., 2015

Biodiversity losses (LPI)



From WWF

- Dairy farms need to make better use of feed resources, reduce the use of inputs and their environmental impacts, particularly in terms of nitrogen (N) losses.

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ANNEX 5:

J-L Peyraud, C Brocas, K Klumpp, X Vergé, N Edouard, 2021. Visionary aspects of dealing with C in dairy systems and C storage. Climate Care Cattle farming – visionary aspects, April 2021.

Climate Care Cattle farming – visionary aspects

farming

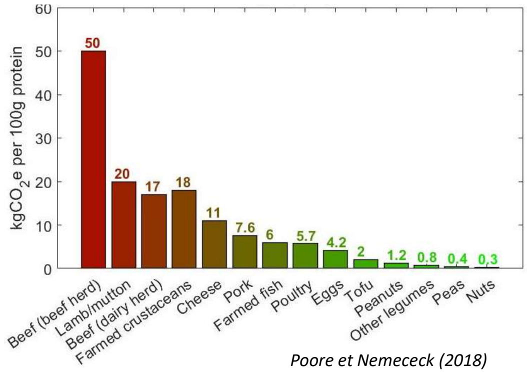
Visionary aspects of dealing with C in dairy systems and C storage

J-L Peyraud, C Brocas, K Klumpp, X Vergé, N Edouard

LCA have consistently shown the impacts of livestock

- **High impact of Animal based products,**
- **The impacts of the lowest-impact animal products exceed average impacts of plant proteins (GHG emissions, eutrophication, acidification and frequently land use),**
- **High variation among both products and producers.**

Kg CO_2-eq / 100 g protéine



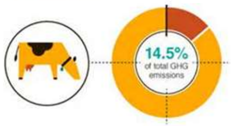
Poore et Nemececk (2018)

- ***Maybe simplistic, but reminds us that we need to find ways of improving the sustainability of livestock farming***

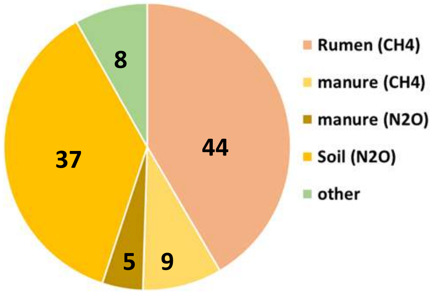
On farm GHG emission of European Livestock sector

Livestock emissions ($Gt\ CO_2$-eq)	
Europe	0.25
World	8.1

Food and Agriculture Organization of the United Nations *(FAO, 2019)*



Sectors	% total
Agriculture	10
Livestock	*6*
Industry	38
Transport	21
Tertiary	12



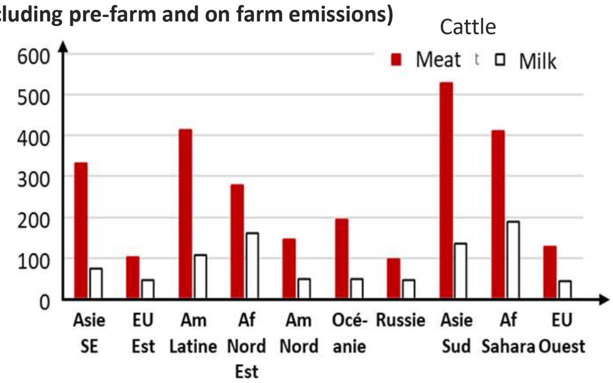
European Environment Agency, (2019) – mean 2003-2018

- **Further emission arise outside of EU. Globally livestock represents 85% of EU Agricultural emission,**
- **Enteric CH_4 and soil N_2O emissions are major issues.**

Emissions intensities of the European livestock sector

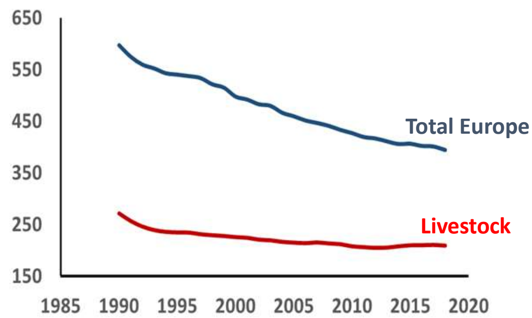
- **EU livestock systems are efficient**
- **But progress is slow compared to other sectors**

Emission (kg eq-CO2 / kg protein)
(including pre-farm and on farm emissions)



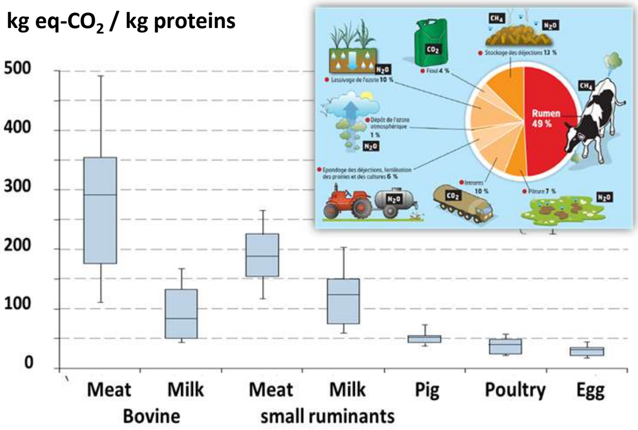
Peyraud and Mc Leod, 2020 (Adapted from FAO data, 2017)

CH_4 (Tg/year)



European Environment Agency, 2019

GHG mitigation options : farm gate approach



From Gerber et al., 2013

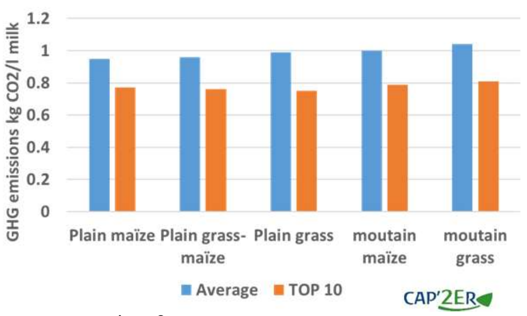
Efficiency	Low emitting animals Feeding practices Herd management Animal health
Resource recycling	Smart use of manure Manure bio-refinery Use of plant by-products
Nature based solutions	Feed (legumes, LULUC) Energy production (manure) Soil C storage

GHG mitigation options at farm gate : the French case

- A 19% gap between average and best performing systems

Maize based dairy systems	Average	Top 10
GHG (kg eq CO_2/L milk)	0.95*	0.77

****0.87 for net emission after considering Soil C sequestration***



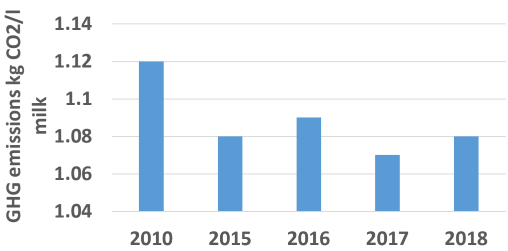
CAP'2ER

Source : CAP'2ER® 2013-2019

- National Strategy of low Carbon : 40% decrease in 2030/1990

The dairy sector is on track…
but stagnation

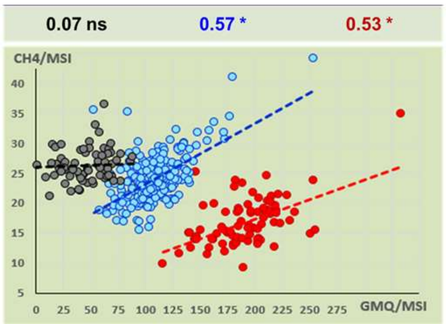
LA FERME LAITIÈRE BAS CARBONE



A perspective of 20% reduction by optimisation of the dairy systems

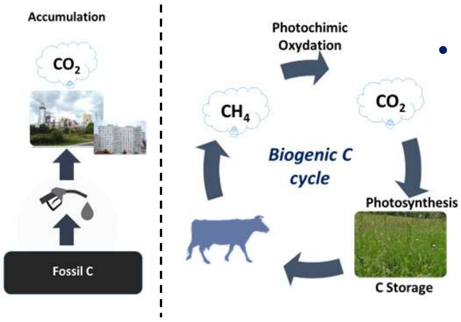
GHG mitigation options: reducing enteric methane (animal genetics and feeding practices)

- It is difficult to reduce enteric methane
- Genetic pathway: Antagonism with digestive efficiency ?
- Feed additives: - 15 to - 30% but high cost and few products are on the market
- Higher forage quality: - 5%, very important in developing countries
- Accounting in the national accounts?



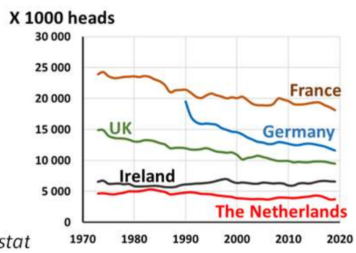
74 génisses (Foin) 252 génises (Ensilage Herbe) 81 taurillons (Pellets)

Is cow methane to blamed for global warming?



- **Fate of CH_4 : the calculation of CO_2-eq misrepresents the role of CH_4 in global warming**
  - CH_4 is part of a natural cycle
  - CH_4 is a short life (10 y) vs CO_2 and N_20 are long live pollutants
  - CH_4 do not accumulate in the atmosphere if the rate of emission is constant or decrease: no additional warming!
  - N_2O and CO_2 accumulate even if the rate of emission decline

- **What consequences?**
  - Reducing CH_4 emissions will have a very important short-term effect (≈storage of C as planting trees): an opportunity for the ruminant to reach climate neutrality
  - Reduce emissions intensity and reduce the number of ruminants (large cattle)



Peyraud, non published, from Eurostat

GHG mitigation options: The national French herd

- **Fewer animals to produce the same amount of milk :**
  - Advancing age at first calving or optimize milk production :
    - - 3% if dairy heifers calving at 24 vs 29 months
    - - 5% if milk prod. increase from 8600 vs 9500 l of milk (but feed/food competition),
- **Produce more meat from the dairy herd** : dual purpose breeds, cross sexing

Calf to beef system :
12 - 14 kg eq-CO_2/kg CE

Young bull from dairy herd :
5 – 7 kg kg eq-CO_2/kg CE

Dollé et al, 2015

- **Substitution :**
    - - 7% Soybean meal substitution by rapeseed meal

GHG (and NH_3) mitigation options: manure management

- **Manure management first target is often NH3 mitigation**

Contribution to FR national emissions *Citepa 2016*		
	Agricultural sector	Cattle sector
Ammonia NH_3	**98 %**	**42 %**
Green House Gases	**17 %**	**11 %**

Housing	Storage	Spreading	Grazing
27 %	**26 %**	**32 %**	**15 %**
51 % (including enteric CH_4)	**10 %**	**8 %**	**7 %**

Best practices for NH_3 mitigation:

Frequency and efficiency of scraping, avoiding urine-faeces mixing
Up to -30 % NH_3
Covering storage tanks
Up to -80 % NH_3
Acidifying manure
Up to -80 % NH_3
Burying manure soon after spreading
From -30% NH_3 24h after spreading
To -90% NH_3 right after spreading

Henning L. et al 2011; Martin et al. 2013; CITEPA 2019

GHG (and NH_3) mitigation options: manure management

- **Some of the best practices for NH_3 mitigation…**
  - Covering storage tanks
- **… also efficient for GHG reduction**

(!) Potential reverse effect:
increase manure t° by 1 or 2°C and then CH_4 emissions

But decrease the volume of liquid manure to spread
by avoiding rain water accumulations
=> mitigate CO_2 emissions by lowering the use of diesel

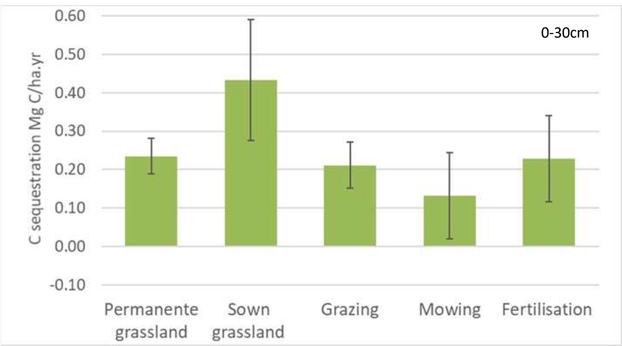
- Better use of organic resource → *Lower the use of chemical fertilizers (↘ N_2O)*

Other practices:

- Methane recovering from covered tanks or from fermenters to replace fossil energies
- Decreasing the storage duration to avoid methane productions
- Empty manure tanks before the warmer season to avoid high level of fermentation

GHG mitigation option: soil C storage

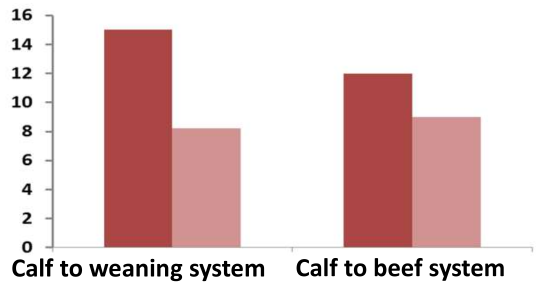
Soil based analyses mean : 230 (±50) kg /ha/year



- **Considerable variations related to climate, management and vegetation type**

EsCo 4p1000, INRAE, Pellerin et al. 2019

C footprint (kg eq CO2/viande)



- **C Sequestration represents compensation in a range of 20 to 60% of gross C footprint**

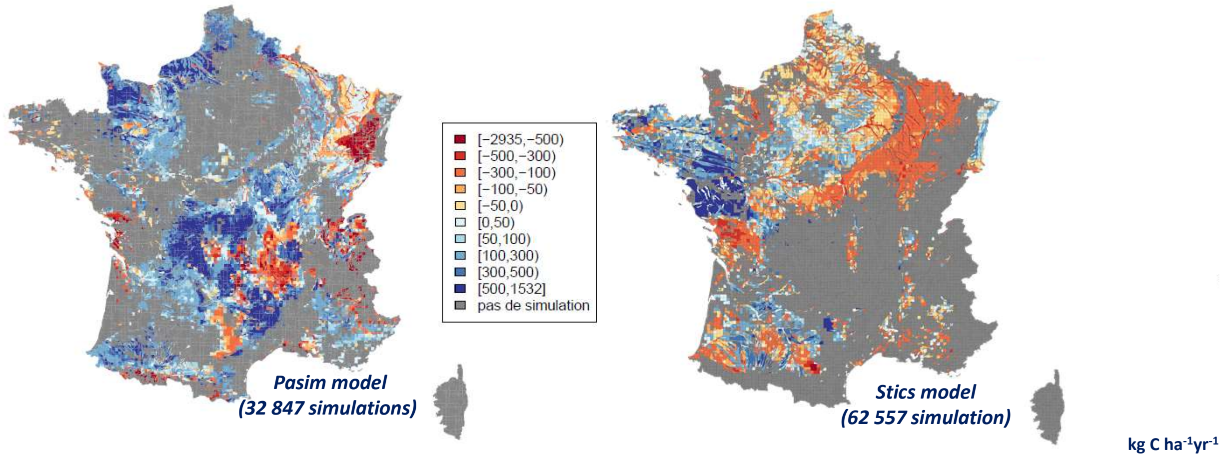
GHG mitigation option: soil C storage

Modelling exercice 1km2 French 4P1000 study (Pellerin et al., 2019)

Sequestration potential (kgC/ha/an)

Permanent grasslands

Cropland & sown Grassland

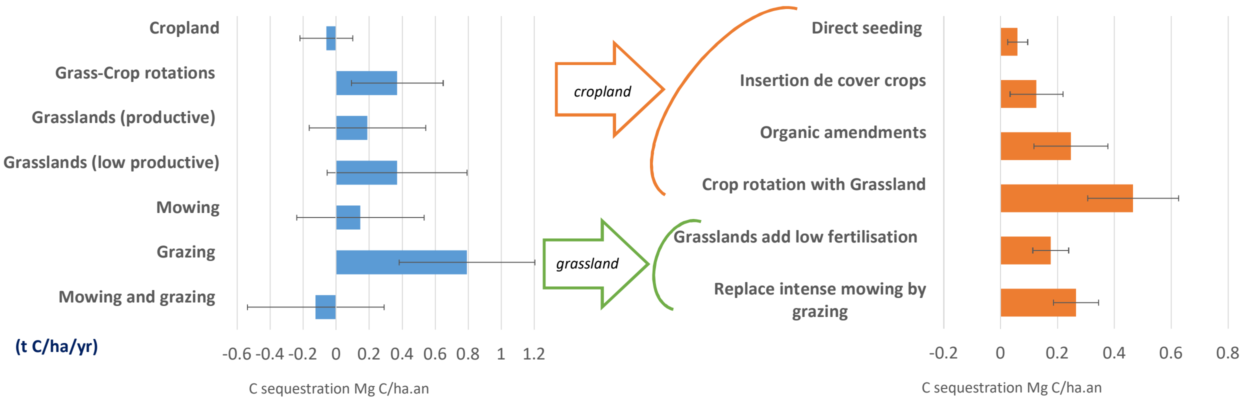


Grassland : +212 (±524) **Cropland: -59 (±160)** **Crop & Grass rotation : +370 (±278)**

GHG mitigation option: soil C storage

Current national C sequestration potential

Additional C sequestration potential for promising levers



Considerable variations related to climate, management and vegetation type

Large potential for a number of levers => need to define regional « Good Management » practices

Modelling exercice French 4P1000 study (Pellerin et al., 2019)

Some conclusions

- Some efforts already led to a reduction of the dairy sector C footprint
- High sequestration potential from grasslands and crop & grass rotations
- Still some room for “best management practices” and mitigation potential
- Practices that should be applied in a systemic perspective (interactions, reverse effects, compensations…)
- To go really further, it will be necessary to reduce the production!

ANNEX 6:

N. Edouard; K. Klumpp; X. Vergé; J.L. Peyraud, 2022. Nutrient circularity: the role of dairy systems and a solution for GreenHouse Gas and NH_3 mitigation. 73th Annual meeting of the European federation of animal science (EAAP), Porto, Portugal.

Nutrient circularity: the role of dairy systems and a solution for GreenHouse Gas and NH_3 mitigation

N. Edouard; K. Klumpp; X. Vergé; J.L. Peyraud

RÉPUBLIQUE FRANÇAISE Liberté Égalité Fraternité · INRAE · INSTITUT DE L'ELEVAGE idele

EAAP 2022 – Session 37
Climate care dairy farming

Livestock contributes to environmental impacts

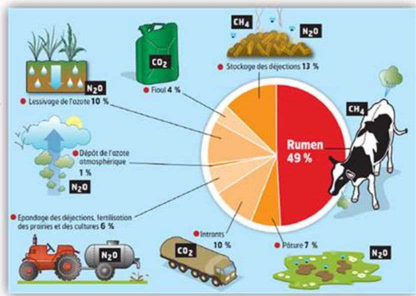
Mainly through CH_4 and N_2O emissions…

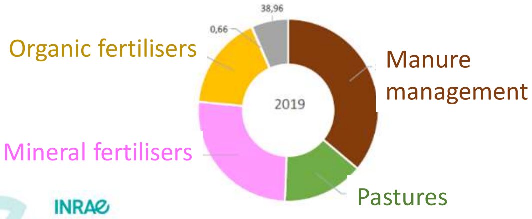
Further emission arise outside EU

=> Globally livestock represents
85% of EU Agric. emission

European Environment Agency, 2019 – mean 2003-2018

Sectors	CO_2eq % total
Agriculture	**10**
Livestock	***6***
Industry	38
Transport	21
Tertiary	12





… But also NH_3 emissions

CITEPA, 2021 (France)

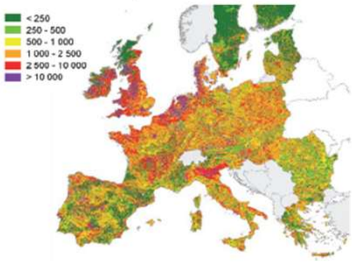
Livestock contributes to environmental impacts

With diverse consequences for environment

Climate change (kg CO_2e/kg protein)

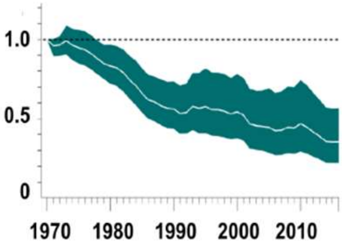
Quelle recherche pour penser l'élevage de demain ?
Shaping the Future of Livestock Farming Through Research

Pollution (Nitrate kg/km²)



Sutton et al., 2011 Leip et al., 2015

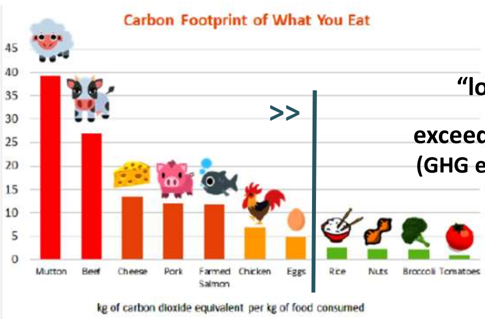
Biodiversity losses (LPI)



From WWF

Livestock contributes to environmental impacts

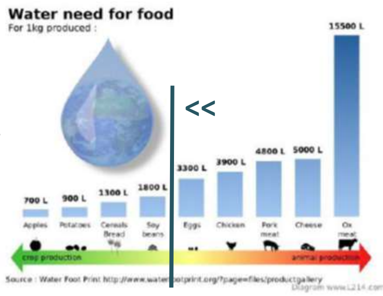
High impact of Animal based products through LCA approaches



Impacts of the

"lowest-impact animal products"

exceed average impacts of plant proteins (GHG emissions, eutrophication, acidification and frequently land use)



"Avoiding meat and dairy is single biggest way to reduce your impact on earth"

The Guardian, 2018

Climate neutrality: could livestock systems be part of the solution?

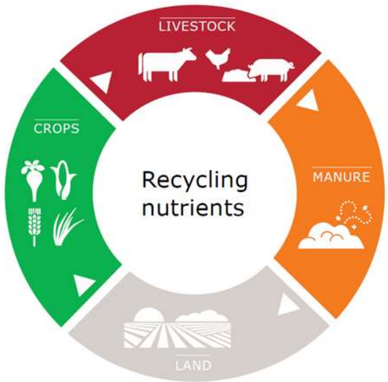
A new paradigm for thinking the future livestock systems

Efficiency, efficiency, efficiency!

Ressource → **Product** → **Waste**

To

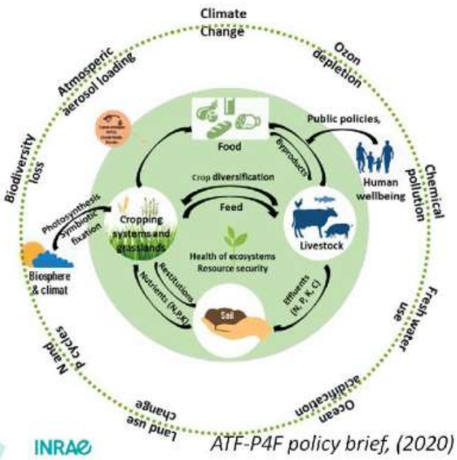
Livestock connects!



Soil is the base!

Climate neutrality: could livestock systems be part of the solution?

Circularity in the agri food systems: Animals are essential



ATF-P4F policy brief, (2020)

- ✓ Move towards Carbon neutral agriculture
- ✓ Adapt to climate change
- ✓ Reduce protein imports
- ✓ Improve land, water, biomass use efficiency
- ✓ Increase biodiversity
- ✓ Improve soil and ecosystem health

Livestock animals are KEYS for circularity

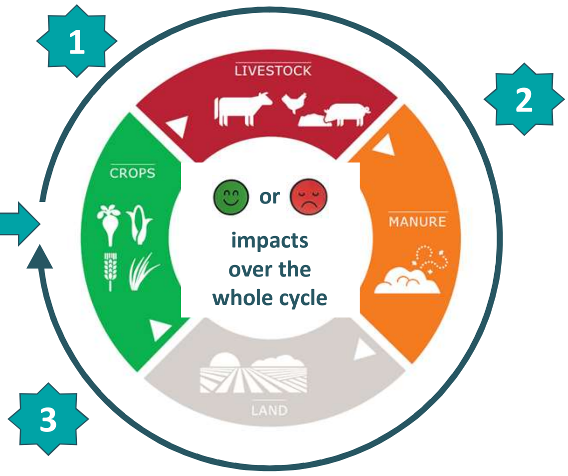
- **Recycling non edible-biomass**
- **Providing nature based organic fertilisers**
- **Enhancing crop diversification**
- **Using grasslands**

Climate neutrality: could livestock systems be part of the solution?

Many levers and mitigation options at the farm level

Efficiency	**Genetics: low emitting animals** **Feeding practices** **Herd management** **Animal health…**
Resource recycling	**Smart use of manure** **Manure bio-refinery** **Use of plant by-products…**
Nature based solutions	**Low input crops** **N fixing plants** **Energy production (manure)** **Soil C storage…**

Cascade of impacts !

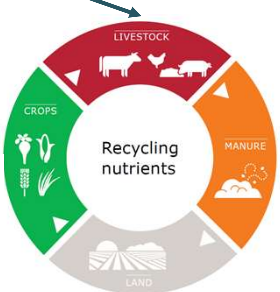


1 Low N and C inputs due to grass based systems

Ruminants are net producers of high quality protein

Introduction of ruminants and pastures (mixed farm system)

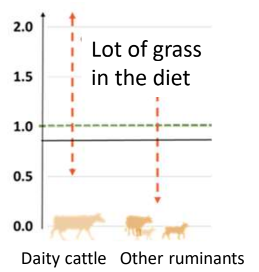
Ruminants can valorise a lot of **diverse/new resources and co-products** with a low C footprint



55 to 90% of feed protein are not edible as human food

Net feed conversion efficiency of livestock

$$\frac{\textit{animal protein (kg)}}{\textit{edible plant protein used as feed (kg)}}$$



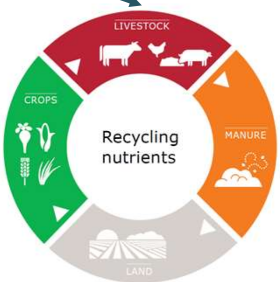
Laisse et al., 2019

1 Low N and C inputs due to grass based systems

Less inputs and better soil health

Introduction of ruminants and pastures (mixed farm system)

Mixed farming systems
↘ the use of pesticides



Grazing
= direct recycling !
Less N input (external purchase)

Nitrate leaching **regulation**

Lower soil erosion (tOM/ha/year)

Eurostat (2011)

1 Low N and C inputs due to grass based systems

+ Resilient crop and animal systems

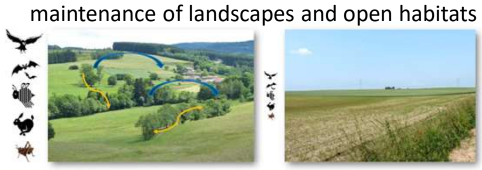
Introduction of ruminants and pastures (mixed farm system)

Promotion of
agro-biodiversity

1 LSU is associated with 90m of hedge

Improved **species diversity** in the rotation (including honey plants) provided sustainable grassland types

Diversification of land use provides the maintenance of landscapes and open habitats



Invertebrates t/ha/year	**3,5**	**0,5**

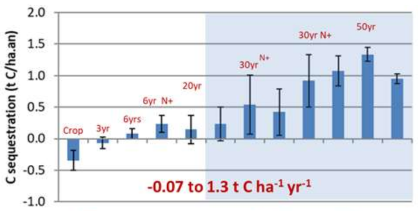
Eurostat, Etude 4 p1000

1 Low N and C inputs due to grass based systems

Systems that promote C storage

Introduction of ruminants and pastures (mixed farm system)

crop → 50y pasture



Temporary-Sown *Permanent*

Louault, Klumpp, Chabbi in prep

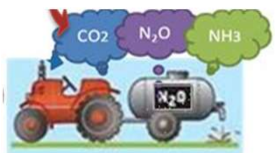
C stock and additionnal C storage

2 Improved manure management due to coverage of liquid storage tank

A more constrasted situation - tradeoffs

Higher manure quality and fertilising level

Higher **leaching** potential



Slurry tank covers

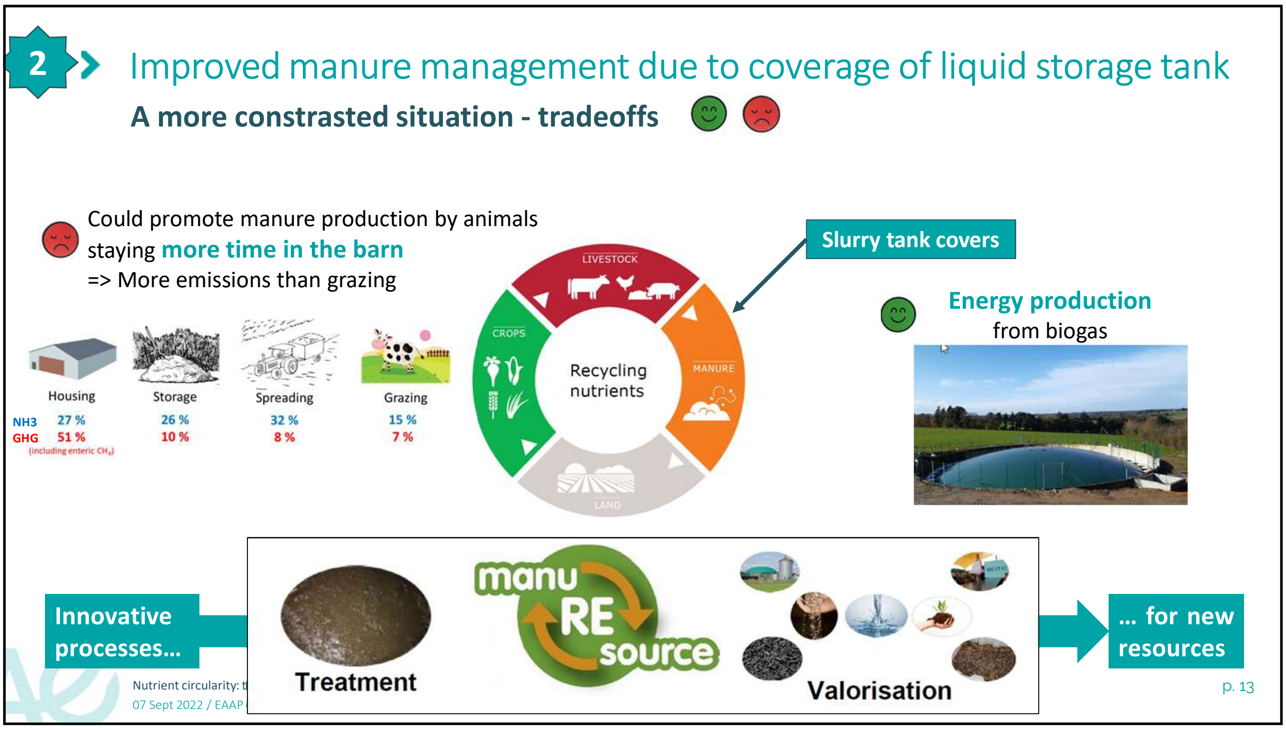
Up to - 80 % NH_3

But + 1-2°C => can lead to more CH_4

Less water dilution

Lower volume to manage and spread

= less fuel needed and associated emissions


2
Improved manure management due to coverage of liquid storage tank
A more constrasted situation - tradeoffs
Could promote manure production by animals staying more time in the barn
=> More emissions than grazing
Housing
Storage
Spreading
Grazing
NH3 27 % 26 % 32 % 15 %
GHG 51 % 10 % 8 % 7 %
(including enteric CH4)
LIVESTOCK
MANURE
LAND
CROPS
Recycling nutrients
Slurry tank covers
Energy production
from biogas
Innovative processes…
Treatment
manu RE source
Valorisation
… for new resources
Nutrient circularity: t
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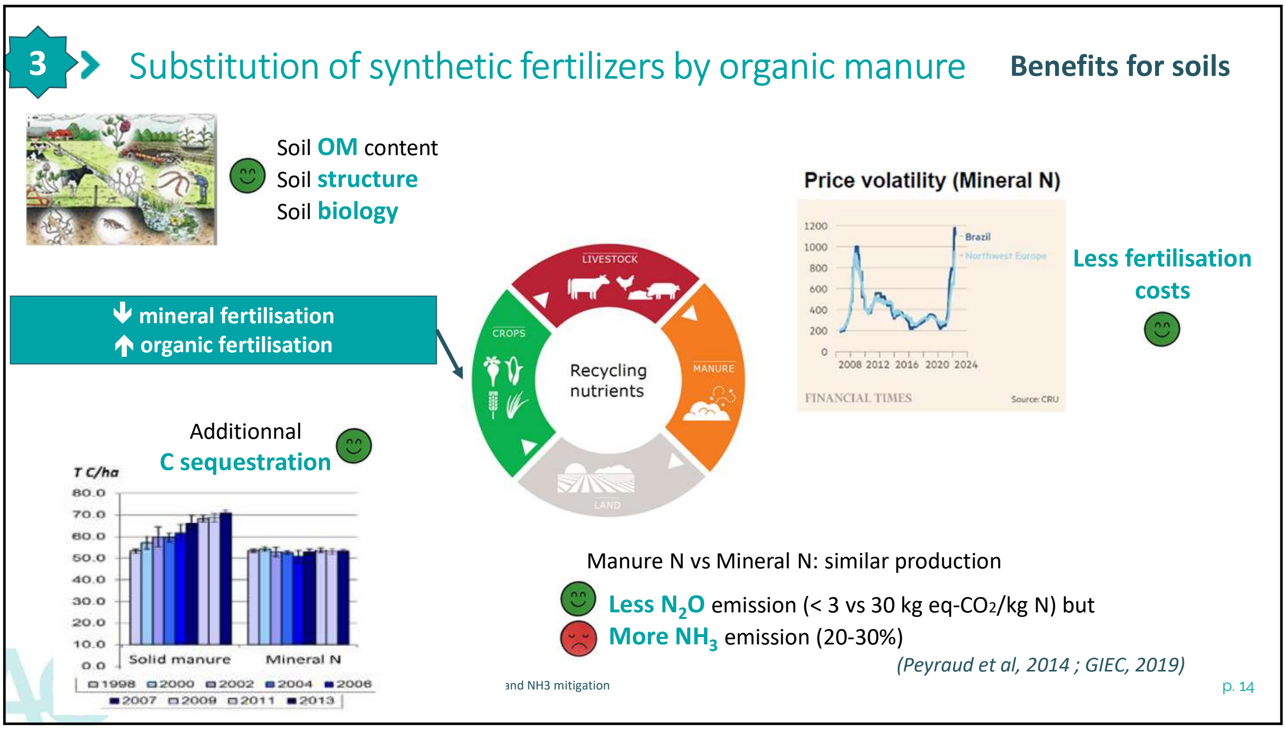

3
Substitution of synthetic fertilizers by organic manure
Benefits for soils
Soil OM content
Soil structure
Soil biology
mineral fertilisation
organic fertilisation
Additionnal
C sequestration
T C/ha
80.0
70.0
60.0
50.0
40.0
30.0
20.0
10.0
0.0
Solid manure
Mineral N
1998 2000 2002 2004 2006
2007 2009 2011 2013
LIVESTOCK
MANURE
LAND
CROPS
Recycling nutrients
Price volatility (Mineral N)
1200
1000
800
600
400
200
0
2008 2012 2016 2020 2024
Brazil
Northwest Europe
FINANCIAL TIMES
Source: CRU
Less fertilisation costs
Manure N vs Mineral N: similar production
Less N2O emission (< 3 vs 30 kg eq-CO2/kg N) but
More NH3 emission (20-30%)
(Peyraud et al, 2014 ; GIEC, 2019)
and NH3 mitigation
p. 14

Still some room for “best management practices” and mitigation potential

EU objectives and strategies

Trade-offs and synergies when applying different mitigation options

	↓ 50% GHG	↑ Energy & Protein autonomy	↓ 50% pesticides	↑ 25% Organic Fert	↑↑ Biodiveristy	↓ 50% anti-microbials
Reducing size of livestock sector	++	++	- ?	- ?	- ?	+
Reconnecting plants and animals						
✓ Livestock efficiency	++	+			- ?	++
✓ Grassland based systems	+	++	++	++	++	
✓ Smart use of manure & substitution to mineral fert.	++	++		++	+	
✓ Crop diversification, N fixing plants	++	++	+ ?	++	+	+

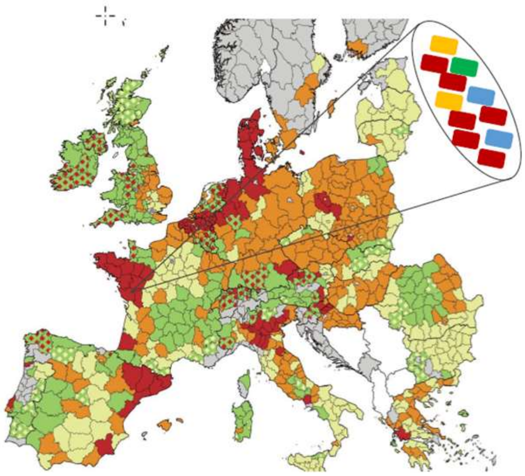
Evaluation in a systemic perspective

Interactions, reverse effects, compensations…

- The livestock production system should be transformed **to integrate circularity**.
- This transition of the livestock system requires **Integrative, Inclusive and Interdisciplinary research** support
- Need to develop **more accurate models to track progress**, in order to assess the **multi-functionality and multi-criteria impacts** of livestock production systems

Does the « best system » exist???

Think Globally, apply locally!



- Low proportion of grassland in agricultural area, high animal density
- High proportion of grassland in agricultural area, high animal density
- High proportion of grassland in agricultural area, medium animal density
- High proportion of grassland in agricultural area, low animal density
- Low proportion of grassland in agricultural area, corps and animals
- Low proportion of grassland in agricultural area, low animal density
- Less than 20% of agricultural area in total area

There is no « one size fits all » optimal solution

We need more diversity!

INRAE

Thank you for your attention

And aknowledments to my co-authors!

J.L. Peyraud

K. Klumpp

X. Vergé