Virtual Joint Centre for Improved Nitrogen Agronomy
Supporting documentation for data

© Photo by A.R. Sánchez-Rodríguez: overview of the digestate winter wheat experiment where you can see the isotopomer, automatic and manual chambers for N_2O emissions, wind tunnels for NH_3 volatilisation and different colors for the wheat (yellowish-control and lower N rates, more green for the highest N doses) at Henfaes farm in North Wales, UK. Photo: first week of May 2017.

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1. Document outline

2. Information about the project

With the CINAg project we propose to meet the needs of China and the UK to optimise farm practices and soil management to make more effective use of different nitrogen sources and reduce losses of Nr to the environment, thus mitigating against environmental impacts associated with this loss.

The project objectives are:

1. Develop novel indicators of N use efficiency (NUE) and to combine these with other new real-time physical and chemical metrics to obtain holistic metrics of soil health and quality which informs farm practices and soil management to deliver improved agronomic NUE and sustainable crop production.
2. Use these indicators and other emerging knowledge to test and develop on field experiments and farm platforms, farm systems that permit the sustainable intensification of agriculture.
3. Translate these developments to Chinese farmers using the proven 'Science and Technology Backyard' programme developed by CAU and CAAS that is moving farmers out of poverty and enriching whole communities.

The Project was structured into the following work packages to address the above objectives

1. WP1. Improved fundamental understanding of N cycling
2. WP2. Harnessing novel N technologies
3. WP3. Improved agronomic practices
4. WP4. Predictive capacity and knowledge exchange

3. Information on experimental farm platforms and cross-UK sites

3.1 Locations of farm platforms and cross-UK sites

Fertiliser experiments were conducted at four farm platforms across the United Kingdom (UK) in 2016 and 2017 (Figure 1). Table 1 gives information about locations and experiments carried out at the four farm platforms. Table 2 presents general information on soil type, temperature and rainfall.

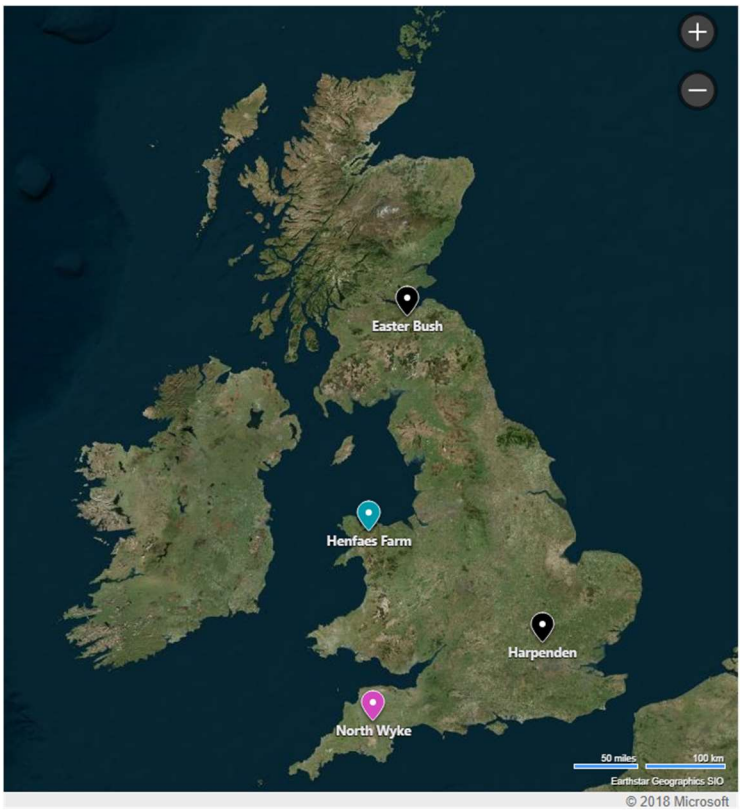


Figure 1: Locations of the four farm platforms used for experimental work: North Wyke and Harpenden are operated by Rothamsted Research. North Wyke is located in Devon and Harpenden in Herefordshire. Henfaes farm is operated by Bangor University in North Wales, and Easter Bush by the Centre for Ecology & Hydrology by Edinburgh.

Table 1: General information for the four the experimental platforms: Site name and abbreviation, coordinates of the experiments at each site, and the type of fertiliser used. At Easter Bush, the experimental fields are frequently used for experiments.

Site	Site name	Coordinates	Experiments	fertiliser	Field name
		50.778625, -3.926043	Grass trial 2016	Inorganic fertiliser	Beacon field
NW	North Wyke	50.793893, -3.952632	Winter wheat 2017	Digestate	Skittle Alley
HA	Harpenden	51.804414, -0.362838	Grass/Winter Wheat	Inorganic fertiliser	Highfield
		51.812611, -0.378003	Grass/Winter Wheat		Fosters
		51.803969, -0.372667	Grass		Park Grass
		51.810224, -0.375246	Grass/Winter Wheat		Broadbalk
	Woburn	52.009033, -0.607586	Winter Wheat		Horsepool
HF	Henfaes Farm	53.239000, -4.014611	Grass trial 2016 Winter wheat 2017	Inorganic fertiliser Digestate	
EB	Easter Bush	55.866161, -3.2081401	Grass trial 2016 Grass trial 2017	Inorganic fertiliser Inorganic fertiliser	Engineers field Upper Joiner Field

Table 2: General site information for the four experimental farm platforms North Wyke (NW), Harpenden (HA), Henfaes (HF) and Easter Bush (EB). BFI = base flow index, MAT = mean annual temperature, MAP = mean annual precipitation.

Site	Soil type	Texture	MAT (°C)	MAP (mm)
NW	1. Grass trial: Stagni-vertic Cambisol, often waterlogged 2. Winter wheat trial: Free-draining Dystric Cambisol	1. Clay loam/silty clay loam 2. Stony loam brown earth	minima = 6.9 maxima = 13.8	1107
HA	free draining Chromic Luvisol (or Alisol)	Clay loam to silty clay loam over clay-with flints overlying chalk	mean of the min = 5.2 mean of the max = 13.1 mean annual = 9.1	685.5
HF	free-draining Eutric Cambisol	sandy clay loam	mean of the min = 8.8 mean of the max = 15.2	1060
EB	imperfectly-drained Eutric Cambisol	Clay loam	Not assessed	Not assessed

Sampling across the UK in 2018 was conducted at nine sites. At each site, at least two different land uses were present (Table 3). Two sites were located in Scotland, three sites in Wales and four sites in England (Figure 2).

Figure 2: The nine sites across the UK used for soil sampling and aboveground biomass measurements. At each site, at least two different land uses were present (for details see Table 3).

Table 3: Land uses sampled at the nine sites across the UK.

Site	Land uses
Parsonage Down	pristine & cattle grazed intensively grazed wheat cultivated
Silwood	GL unlimed no nutrients GL unlimed with NPK
Harpenden	Highfield: Arable wheat NPK Highfield: GL Fosters: Arable rotation Fosters: Reseeded GL Fosters: GL/arable rotation NPK Park Grass: GL limed pH7 Park Grass: GL, limed, pH5 Broadbalk: GL mown Broadbalk: wheat + limed + NKMg
Wymondham	Permanent pasture + low density sheep Floristically enhanced grass margin Arable continuous rotation
Plynlimon	Acid GL/heathland lightly sheep grazed Semi-improved GL, heavily sheep grazed Reseeded GL, heavily sheep grazed different areas: Acid GL sheep grazed (1) Acid GL sheep grazed (2) Acid GL sheep grazed (3)
Abergwyngregyn	Semi-improved GL & sheep GL & sheep Acid GL
Newborough	Sand dunes grazed Sand dunes grazing exclosures Sand dunes grazed, 53 years
Easter Bush	GL sheep grazed GL un-grazed GL for silate un-grazed Arable crops oilseed rape Arable crops wheat
Kirkton	improved permanent pasture Reseeded GL Poor-sem- improved GL

© Photos by E. Fitos: Site in South west England (left: Parsonage Down) and Scotland (right: Kirkton).

3.2 Meteorological data at the four farm platforms

At the **NW and HF sites**, daily rainfall, and daily mean, minimum and maximum temperatures were recorded from weather stations located at HF and NW. Water filled pore space was calculated using soil bulk density and soil moisture readings from 10 (HF) and 5 (NW) SDI-12 soil moisture sensors (Acclima Inc., USA) at 2.5 cm depth.

At the **EB site**, soil temperature and water filled pore space (WFPS %) were measured using handheld probes (31/162/0, Brannan,UK & Hydrosense II, Campbell Scientific, UT, US) simultaneously when chamber measurements were carried out. Long term meteorological and soil measurements were recorded at the permanent Easter Bush measurement station which was situated at the edge of the Engineer's Field. This station provided measurements of air temperature (1.8 m), soil temperature (0.3 m depth) and rainfall (tipping bucket) at 30 min intervals throughout the measurement campaigns.

4. Experimental information

Two multi-platform experiments were carried out in 2016 and 2017, respectively. In 2016 inorganic fertiliser trials were performed on grassland across the NW, HF and EB sites. In 2017 a digestate trial was performed on winter wheat at NW and HF. In 2017, additional grassland trials were performed at EB and HA.

4.1 Inorganic fertiliser experiments (grass trials) - 2016 (and 2017 for EB)

The NW, HF and EB sites were managed as a three-cut silage system with a total N-application of 240 kg nitrogen per ha. A complete randomized block design was applied at NW and HF (see appendix), and EB with four control (C) plots and four replicates for each fertiliser treatment:

1) Urea only (U, N-content ~ 46%) - urea pellets
2) Urea and urea inhibitor (IU), urea pellets with coating of powdered urease inhibitor (Agrotain®)
3) Ammonium-nitrate (AN, N-content ~36%) - nitrate pellets: Nitram, $NH_4^+NO_3^-$
4) Control plots

All 16 plots received P, K, and S in accordance with national fertiliser guidelines as published by Defra 2010. All fertilisers were added manually and applications rates and dates are detailed in the Appendix in Tables A1 and A2.

At the **NW and HF sites** all plots were divided into three sub-plots:

1) 4 m x 2 m subplot designated for NH_3 emission measurements
2) 2 m x 2 m subplot designated for soil sampling and N_2O emission measurements
3) 6 m x 2 m subplot designated for biomass harvesting for yield and yield quality measurements.

At the **EB site in 2016**, all plots were 2 m x 8 m with 0.5 m spacing between them

1) a 1 m x 1 m subplot designated for biomass harvesting for yield and yield quality measurements

At the **EB site in 2017**, plots were arranged in a square grid of an area 20 m x 20 m. There was not spacing between the treatment plots. Treatments were assigned randomly. The experimental design for the **HA site in 2017** is detailed elsewhere.

© Photo by A. Carswell: Fertiliser application at North Wyke.

4.2 Digestate experiment (winter wheat) -2017

A complete randomized block design was implemented at the **NW and HF sites** (see Appendix Figures A1 and A2) with five control plots and five replicates for each digestate treatment:

1) Digestate only,
2) Digestate and nitrification inhibitor (DMPP - 2 litre per ha),
3) Acidified digestate acidified in 1 m^3 tanks
4) Acidified digestate with nitrification inhibitor (DMPP - 2 litre per ha)
5) Control plots


© Photos by A. Carswell: Digestate application to winter wheat (left) and treatment differences (right) at North Wyke.

All 25 plots were divided into two subplots:

1) Harvest subplot: NW 4.5 x 2 m and HF 6.5 x 1.2 m
2) Sampling subplot: NW 4.5 x 2 m and HF 7.5 x 1.2 m

All treatments were investigated as part of WP2, whereas the control treatment, digestate only and the acidified digestate with nitrification inhibitor were sampled for WP1 (n = 15 plots).

The target application rate was 190 kg nitrogen per ha as digestate, but application rates varied in the field. The digestate was manually band-spread in parallel with the crop rows at a rate of 40 m^3 per ha using 20 litre capacity watering cans. Digestate was applied on 20th March 2017 at NW, and on 19th April at HF.

4.3 UK wide sampling - 2018

In 2018, sites with different land uses and soil types were visited across the UK (Figure 2, Table 3). Some sites that were part of the UGRASS project (https://www.soilsecurity.org/u-grass/) were re-visited sites for soil sampling and the measurement of aboveground biomass productivity.

5. Protocols and data processing

Sampling protocols and sample dates differ slightly for the measurements taken for WP1 and WP2. If so, sampling protocols below were clearly separated into measurements carried out for WP1 and WP2 respectively. All treatment plots were sampled the same day within a site. Sampling days varied across site due to different weather conditions at each site and the effect this had on plant growth. Crops were harvested after a full growing season as advised by the farm managers.

5.1 Yields (herbage production and quality) and biomass production

Measured by Rothamsted Research and Bangor University for NW and HF, respectively. Measured by CEH Edinburgh for EB; measured by CEH Bangor for 2018 sampling.
Units: Dry matter yields - yield of dry grass in tonnes per hectare, acid detergent fibre - g nitrogen per kg, total mineral content - g per kg, protein content - g per kg, metabolisable energy - MJ per kg, Neutral detergent fibre - MJ per kg, D value (digestibility metric) - percent.

Inorganic fertiliser (grass) experiment (2016). Three silage cuts were performed at each site (see dates in Appendix Table A1). At the **HF site** a 1.27 m strip was cut down the centre of each harvest subplot to a residual height of 5 cm. The cut herbage was collected, weighed and total fresh weight was determined. Representative sub-samples of approximately 200 g were collected, dried at 80°C for 72 h, and their dry weight was determined. This measure was used to upscale dry weight of yield to total dry yields, and to convert yield dry matter to a m^2 basis.

Dried herbage samples were analysed for herbage quality, including crude protein (CP), metabolisable energy (*ME*), non-digestible fibre, acid digestible fibre and dry matter (DM) using near infra-red spectrometry (NIRs; by Sciantec Analytical Laboratories, Stockbridge Technology Centre, York, UK). Digestibility (*D*, %) was determined according to Beever et al. (2000) as:

$$D = ME / 0.16$$

At the **NW site**, a 1.5 m strip was cut down the centre of each harvest subplot to a residual height of 5 cm and the fresh herbage weighed immediately using a Haldrup small plot harvester. Representative subsamples of approximately 2 kg were taken manually and analysed for the same herbage quality analyses as described for HF (also via NIRs; by Trouw Nutrition GB, Blenheim House, Ashbourne, UK).

At the **EB site in 2016**, a 1 m^2 subplot was cut using sheers and the fresh yield was recorded. Subsamples (200 grams) were then taken from each plot for the analysis of dry matter content, *ME*, CP, modified acid detergent (MAD), digestability (D value), total carbon and total nitrogen content at SRUC Analytical Services (Midlothian, UK). The same herbage parameters were measured at the EB site in 2017 but the harvest was carried out using a small harvester with on-board weighing capabilities (Haldrup F-55). A total area of 30 m^2 was harvested. Each of the plots was harvested and above-ground biomass was dried at 60 ^oC for 24 hours and both fresh and dry weights were recorded.

Digestate (winter wheat) experiment (2017). One harvest was performed at each site at the end of the experiment. At the **HF site** quadrats of 0.4 x 0.4 m were harvested 2 cm above the ground for all plots. Grain and straw were separated by hand and the fresh weight was determined.

At the **NW site**, a Sampo small-plot combine harvester was used to harvest the wheat, separating the grain and straw, which were weighed. A sub-sample from each plot was used to determine grain weight and dry matter biomass.


© Photos by A. Carswell: Grass harvest (left) and winter wheat harvest (right) at North Wyke.

UK wide sampling (2018). Annual plant biomass production was assessed using grazing exclusion cages for actively grazed sites (Table 3). Silwood, Harpenden and Easter Bush are experiments and no exclusion cages were needed. Biomass estimates were provided by partners. Sites were visited in spring, and re-visited in late summer/autumn. The vegetation phase of the grass was particularly short in 2018 due to warm and dry weather.

Before cages (60 cm x 60 cm x 60 cm) were randomly deployed across fields, vegetation was cut back to the ground with gardening scissors. Cages were anchored in the soil using at least four tent pegs, one in each corner. Unfortunately, anchoring in many cases for was not strong enough and cages were moved or destroyed by animals in the field and windy conditions. At the boggy Plynlimon site, moss meshes were deployed to assess moss growth over one year. For a more thorough biomass assessment in grasslands (and other ecosystems) we refer to the supporting documentation of Smart et al. (2017).

In late summer/autumn, vegetation of the 60 cm x 60 cm area was cut inside the cage area. Biomass was dried at 65°C and dry biomass was determined. Biomass production was expressed as g dry biomass per m^2 per year.

5.2 Wheat N content, N offtake and N use efficiency

Measured by Rothamsted Research and Bangor University for NW and HF, respectively. Measured by CEH Bangor for EB.
Units: Herbage nitrogen content - kg nitrogen per hectare, nitrogen offtake - kg nitrogen per hectare, nitrogen use efficiency - percent.

For the **NW and HF sites in 2016**, N content in the grass was calculated from the crude protein results using a factor of 6.25. The value of 6.25 is a standard value and is the multiplication factor the contracted labs used to gain crude protein content from their total N analyses, this value was used to back-convert to total N content (http://www.fao.org/docrep/006/y5022e/y5022e03.htm). Herbage N offtake (*N-off*) was then calculated as:

$$N\text{-}off = N_{content} \times yield$$

where $N_{content}$ is the nitrogen content of the grass as a percentage and *yield* is the grass offtake from each plot (kg dry matter ha^{-1}).

For the **NW and HF sites in 2017**, grain and plant production were estimated from the 'harvest area' of each plot at the end of the experiment. At the **HF site**, wheat plants from three 0.4 × 0.4 m quadrats were harvested 2 cm above the ground and grain and straw were separated by hand and weighed. At the **NW site**, a Sampo small-plot combine harvester was used to harvest the wheat, separating the grain and straw, which were weighed.

A sub-sample from each plot was used to determine grain and straw moisture. Total N was analysed using a TruSpec® analyser (Leco Corp., St Joseph, MI) from ground oven-dried plant tissue (80 °C, 24 h); N offtake by the total crop was calculated by multiplying the N content of the grain and the straw by the grain and straw yield, respectively. Thousand-grain weight (TGW) was determined by weighing 1,000 oven-dried grains. Grain yield, straw yield and TGW are reported at 85 % dry matter.

Nitrogen Use Efficiency of the crop (total for grain and straw, NUE_c) and grain (NUE_g) were calculated as:

$$NUE_c = (N_t - N_c) / N_{applied} \times 100,$$

$$NUE_g = (N_t - N_c) / N_{applied} \times 100,$$

where N_t is the crop and grain N offtake from N (digestate or NH_4NO_3) treatment plots, N_c is the mean crop and grain N offtake from the control plots and $N_{applied}$ is the N fertiliser applied to the plots.

For the **EB site**, total N in the harvested crop was analysed at SAC services (Midlothian) using the Kjedahl digestion method. After oven drying sub-samples of the crop at 80 °C for 24 hours, NUE was calculated as the mean total N content in the harvested crop from four plots, minus the mean total nitrogen content of the control plots, divided by the total N applied as fertiliser (same equation as above).

5.3 Sampling of greenhouse gases: Ammonia and nitrous oxide

Measured by Rothamsted Research and Bangor University for NW and HF, respectively.
Measured by CEH Bangor for EB.
Units: Ammonia emission rate - kg NH_3-N per hectare per day, nitrous oxide - µg N_2O-N per m^2 per hour.

Ammonia emissions. Ammonia volatilization was measured using a system of small wind tunnels (Misselbrook et al. 2005) at the **NW and HF sites.** For the 2016 inorganic fertiliser trials at HF and NW wind tunnel constructions were run continuously for three weeks following each inorganic fertiliser application, with 0.02 M H_3PO_4 acid traps (100 mL) changed daily. Wind tunnels were moved daily to one of three positions, with a return to position one on the fourth day. This was carried out to minimize the impact of the tunnel canopy on the plot area. For the digestate experiment (2017), traps were changed three times at NW and twice at HF on the first day after digestate application, because high rates of ammonia volatilization were expected. Following the first day the acid traps were changed daily until the end of the 7-day sampling period. Wind tunnels remained in the same position over the 7-day period for the digestate experiment. Ammonia fluxes from the measurement area (F_{NH3}, µg m^{-2} s^{-1}) were determined for each measurement period using:

$$F_{NH3} = (C_o - C_i)\, v/t$$

where C_o and C_i are the NH_3-N concentrations (µg NH_3-N m^{-3}) at the tunnel outlet and inlet, respectively, and v is the air volume (m^3) drawn through the wind tunnel over the sampling period (t, s).

© Picture by A. Carswell: Wind tunnels for measuring ammonia emissions on winter wheat at North Wyke.

During the inorganic fertiliser experiments at the **NW site**, NH_3 emissions were measured from the AN, IU and U treatments (n = 4), whereas at HF, measurements were conducted from the U and IU treatments only (n = 3 for the first N application, n = 4 for the second and third N applications).

For both the inorganic fertiliser and the digestate experiments, the acid trap samples were taken from the field, topped up to 100 mL with deionized H_2O and a subsample was taken and stored at 4°C (NW) or -18°C (HF) prior to colorimetric analysis (Mulvaney 1996) for the inorganic fertiliser experiment in 2016.

For the inorganic fertiliser experiments, cumulative NH_3 emissions were calculated for each plot for the three N-application periods using the area under a curve function "cumtrapz()" from the "pracma" package (Borchers 2016) in R (version 3.3.2; R Core Team, 2016).

For the digestate experiment (2017), N-loss through NH_3 volatilization was expressed as a percentage of the total N applied for each treatment to normalize for the different N application rates.

At the **EB site**, NH_3 fluxes were only measured as part of the grassland trial in 2017. Fluxes of NH_3 were derived using the FIDES inverse dispersion model (Loubet et al. 2017). The basis of the model is the solution of the advection-diffusion equation by Philip 1959, assuming power law profiles for the wind speed (U(z)) and the vertical diffusity (K_z(z)). Furthermore, the model assumes no chemical reactions in the atmosphere over the time scales relevant in our case, and that roughness length, wind speed and vertical and lateral diffusivity are spatially homogenous. More details on the dispersion model is given in Huang (1979). Following these assumptions, as expressed by

$$X_{model}\,(x,y,z) = X_{bgd} + \int_{all\ x_s\ and\ y_S} S(x_s,y_s,z_s\,)D(x_s,y_s,z_s\,|x,y,z),$$

the model assumes that the atmospheric NH_3 concentration (X in µg NH_3 m^{-3}) in a given point is the sum of the background concentration (X_{bgd} in µg NH_3 m^{-3}) unaffected by the sources, and the influence of the sources. Latter is equal to all the source strengths per unit surface (S in µg NH_3 m^{-2} s^{-1}) in the locations (x_s, y_s, z_s) multiplied by the dispersion function $D(x_s,y_s,z_s|x,y,z)$ in s m^{-1} which expresses the contribution of each source to each receptor

point in which the concentration c is considered. The meaning of $D(x_s, y_s, z_s|x, y, z)$ can be viewed simply as the concentration at location (x,y,z) for a source of unit strength at location (x_s, y_s, z_s).

In order to calculate S, D was computed by the model, and both *X* and X_{bgd} were measured. To calculate D, the description of Philips (1959) was followed using the equations:

$$U(z) = az^p \text{ and } K_z(z) = bz^n$$

Here, the values of a, b, p and n are derived from a linear regression between ln(U), ln(K_z) and ln(z), over the height range 2 × z_0 to 20 m, using U(z) and K_z(z) estimated based on the Monin-Obukhov similarity theory (see, e.g. Kaimal & Finnigan, 1994), where z_0 denotes the roughness length.

$$D(X, Y, z) = 1/(\sigma_y \sqrt{2\pi})\, exp(-\ ^2/(2\sigma\_y^2)) \times (zz_s{}^{(1-n)/2})/baX \times exp(-(a(z^\alpha + z_s{}^\alpha))/(ba^2 X)) \times I_{-v}((2a(zz_s)^{\alpha/2})/(ba^2 X))$$

where $X = (x - x_s)\, sin(WD) - (y - y_s)\, cos(WD)$, and $Y = (x - x_s) cos(WD) - (y - y_s)\, sin(WD)$, where WD is the wind direction; $\alpha = 2 + p - n$, $v = (1 - n)/\alpha$, and I_{-v} is the modified Bessel function of the first kind of order $-v$. Finally, C_y and m are parameters taken from Sutton (1932) in the equation:

$$\sigma_y = (1/\sqrt{2})\, C_y\, x^{(2-m/2)}$$

Wind data were recorded by two sonic anemometers (IRGASON, Campbell Scientific, UT, USA) which were positioned at the north east and south west sides of the plots, 30 m from the borders of the plots in alignment with the two wind predominant wind directions. The anemometers measured 3D wind components at 10 Hz. Following Loubet et al. (2001), the source height was tuned to z_s = 1.01 z_0 + d, where d is the displacement height, in order to insure best comparison with Lagrangian Stochastic models and experiments (see also Loubet at al. 2010). The dispersion model embedded in FIDES is essentially similar to the Kormann and Meixner (2001) footprint model, except for the retrieval of the a, b, p, n parameters which are here inferred by fitting the wind speed and diffusivity profiles over a height range 0.2-20 m while in Kormann and Meixner (2001) it was computed by forcing the profiles at a reference height. The FIDES model was shown to behave similarly to a Lagrangian Stochastic model in Loubet et al. (2018).

For the concentration measurements, Alpha passive air samplers (Tang et al., 2001) were used. These samplers are small hollow plastic tubes (27 mm ID) with a PTFE membrane which allows air to pass through. Inside there is a layer of filter paper coated in citric acid which traps atmospheric NH_3 and hold it in place within the sampler. This method enabled us to measure cumulative NH_3 concentrations at a fixed point, integrated over a certain period of time (t) several hours or days can be determined. To observe χ_{meas}, duplicate samplers were positioned at the centre of the 16 treatment plots (20 m by 20 m) at heights of 30 cm and 50 cm. In order to measure $\chi_{bgd,}$ samplers were installed in triplicate at the four edges of the experimental grid, 30 m away from the plots. Samplers were placed immediately before fertilisation and removed/replaced 0.25, 1, 2, 3, 7 and 14 days after fertilisation. Samplers were stored at 4 °C after collection before extraction by deionised water and analysis using Ammonia Flow Injection Analysis (AMFIA, CEH Edinburgh, UK).

The source strength S of each plot was computed by least square optimisation of means against mod, using the linear model function lm in R (package stats, R version 3.2.3), as described in details in Loubet et al. (2017). The method was shown to be slightly negatively biased (-16% on average) under moderately oceanic meteorological situations.

Nitrous oxide emissions. At the **NW site**, N_2O emissions were measured on each plot (*n* = 4), using static manual chambers. At the HF site, a combination of static manual and static automatic chambers (combined with an Isotopic N_2O Analyser, Los Gatos Research Inc. San Jose, CA, USA) was used. Following the first N application at HF, fluxes from the control treatment were measured using static manual chambers, whereas static automatic chambers were used for all other treatments (*n* = 4 for each treatment). For the second and third N-applications at HF, only static automatic chambers were used across all treatments. Measurement replication was thus reduced to three of the four experimental blocks (*n* = 3 for each treatment).

Where manual chambers were used, chambers (50 × 50 × 30 cm) were inserted into slots cut so that they projected 15 to 20 cm above the soil surface. Soil was packed around the outside of the chambers to ensure an air-tight seal. Chambers were installed two weeks prior to the start of the experiment, removed prior to silage/herbage cutting events and replaced immediately after. Following fertiliser application, N_2O sampling from the manual chambers was performed three times weekly for the first and second weeks, twice weekly for the third and fourth weeks, and once weekly thereafter. Sampling was carried out between 10:00 and 12:00 am using the protocol of de Klein and Harvey (2012). On each sampling occasion, lids were placed on the chambers and remained in place for 40 minutes with headspace samples taken at 0, 20 and 40 minutes from each chamber. Gas samples were analysed for N_2O concentration using a Perkin Elmer 580 Gas Chromatograph (linked to a TurboMatrix 110 headspace autosampler).

© Photos by A. Carswell (left) and A.R Sánchez-Rodríguez (right): Static N_2O chamber at North Wyke (left) and automated N_2O chamber at Henfaes farm (right).

At the **HF site**, (see above for description of manual static chamber methodology) the automatic chambers were installed two weeks prior to the start of the experiment, the chamber bases were inserted into the soil and the chambers (50 cm × 50 cm × 20 cm) attached to the bases at surface height to ensure an air-tight seal. Closing and opening of the chambers was controlled by pneumatic actuators. The chambers closed sequentially for a 30 minute period, during which the chamber-headspace was sampled via a sampling port at a rate of 1 litre per minute. Samples were delivered to an Isotopic N_2O Analyser and N_2O concentrations were recorded at 0.1 Hz for each 30 minute sampling period. The first 30 seconds of data from each sampling period was removed from calculations to account for the dead volume in the sample lines. Accuracy of the N_2O analysis was checked weekly using certified N_2O standards.

Hourly N_2O fluxes (μg N_2O-N m^{-2} h^{-1}) were calculated using linear regression, with the assumption of linearity. Calculations on the automatic chamber data set were made using the

lm() function in R (version 3.3.2., R Core Team 2016). The manual chamber data set were calculated according to de Klein and Harvey (2012; Excel, Office 2016) as:

$$F_{N2O} = H(C_t - Ct_0)/t$$

where *H* is the ratio of chamber volume to soil surface area (m^3 to m^{-2}), C_t is the concentration of N_2O within the chamber at the time (*t*) of sampling and C_{t0} is the N_2O concentration measured at 0 minutes, measured after the chamber had been sealed. Cumulative N_2O emissions were calculated for each plot for the three N-application periods using the area under a curve function "cumtrapz()" from the "pracma" package (Borchers 2016) in R (R Core Team 2016).

For the digestate experiment (2017), N-loss as N_2O was expressed as a percentage of the total N applied for each treatment after subtracting the cumulative N_2O emissions from the control plots to normalize for the different N application rates.

At the **EB site**, N_2O fluxes were measured during the length of the growing season using static chambers. The chambers consisted of a cylindrical polyvinyl chloride (PVC) plastic pipe of 38 cm inner diameter (ID) and 22 cm height fitted with sealed lid and a flange at the base. The chambers were placed onto a plastic flanged collar that had been inserted on average 5 cm into the soil to form a seal in the soil. A layer of draught sealant material held in place by four strong gripping clips formed an airtight seal between the chamber and the collar for the duration of the flux measurement. Chambers were closed for 60 minutes, during which time four gas samples were collected via a syringe and a three-way tap fitted to the lid, at times 0, 20, 40 and 60 minutes. Gas samples were stored in 20 mL glass vials which were flushed with 100 mL of air from the syringe using a double needle. Samples were analysed using gas chromatography (7890B GC system fitted with an electron capture detector, Agilent Technologies, UK). Measurements were carried out daily for two weeks after fertiliser additions, then every second day for a further two to four weeks. Measurements were made only on working days (Monday to Friday) between 09:00 and 15:00 GMT. Fluxes were calculated as:

$$F = dC/dt * \rho V/A$$

where F is the gas flux from the soil (nmol m^{-2} s^{-1}), d*C*/d*t* is the rate of change in the concentration in time in nmol mol^{-1} s^{-1} estimated by linear regression, ρ is the density of air in mol m^{-3}, V is the volume of the chamber in cubic meters and A is the ground area enclosed by the chamber in square meters.

Cumulative fluxes over the experimental periods (25 days) were calculated using a Bayesian approach, taking into account the lognormal distribution of spatial samples and the lognormal peak-and-decay pattern in time (Levy et al. 2017). Based on the assumption that at a given time, N_2O fluxes, F, are typically lognormally-distributed in space, the probability density is given by:

$$f(F) = 1/(\sqrt{((2\pi))}\ \sigma_{log}\ F) exp(-((log(F) - \mu_{log})^2/(2{\sigma_{log}}^2)))$$

where μ_{log} and σ_{log} are the location and scale parameters, equivalent to the mean and standard deviation of the log-transformed variate. The mean of the distribution is given by:

$$\mu = exp(\mu_{log} + 0.5{\sigma_{log}}^2)$$

Following a fertiliser addition event, the course of N_2O flux is expected to rise to a peak, then decay exponentially. This pattern in time is also well described by the lognormal equation:

$$\mu_t = 1/(\sqrt{((2\pi))}\ kt)exp(-((log(t) - \Delta)^2/(2k^2)))\ * N_{in}\,\Omega$$

where μ_t is the spatial mean of the N_2O flux at time t, Δ and k are analogues for the location and scale parameters, and with the additional term N_{in} is the fertiliser nitrogen input and Ω is the fraction of this which is emitted as N_2O as *t* tends toward infinity. Δ can be interpreted as the natural logarithm of the delay between fertiliser application and peak flux; k is a decay rate term. Equation 4 is an intrinsic function in all statistical software, and can be encoded as:

$$\mu_t\ =\ dlnorm(t, \Delta, k)\ *\ N_{in}\Omega$$

So, at time t following fertiliser addition, the mean flux is given by equation 4 or 5, at which time the N_2O flux has a distribution:

$$F\ \sim\ lnN\left(\mu_{log,t}, {\sigma_{log}}^2\right), \text{ where } \mu_{log,t} = log(\mu_t)\ -\ 0.5{\sigma_{log}}^2$$

The parameters μ, μ_{log} and σ_{log} were estimated using the Markov Chain Monte Carlo (MCMC) method with Gibbs sampling (Gelman, 2013). This was implemented using the freely available JAGS software (Plummer, 2016). The prior distribution for omega was based on the data collated by Stehfest (2006). The prior distributions for Delta and k were based on the dynamics of the DNDC model (Li 1992, as described in Levy et al. 2017). To obtain the cumulative flux at time t, we use the standard lognormal cumulative distribution function:

$$F_{cum,t}\ = \Phi\ ((ln\ \ -\ \Delta)/k)\ *\ N_{in}\Omega$$

where Φ is the cumulative distribution function of the standard normal distribution. This equation is also an intrinsic function in JAGS, so can be encoded simply as:

$$F_{cum,t}\ =\ plnorm(t, \Delta, k)\ *\ N_{in}\Omega$$

5.4 Soil sampling

WP1: Soil sampling was carried out by teams at each farm platform using a standard volumetric soil corer for sampling topsoil cores (0-15 cm, by 5 cm diameter), and any auger for 15-30 cm and below 30 cm soil depth. **In 2016**, soil cores were taken in control plots before the fertiliser treatments were initiated (T0), before the first harvest (T1), and before the final harvest (T2) (Table 4). **In 2017**, soils were taken shortly after the digestate treatments were initiated (T1) and before the final harvest (T2) (Table 4). **In 2018**, five soil samples were taken from the topsoil 0-15 cm at each site and for each land use (Table 5).

Table 4: Soil sampling dates for the grass trial in 2016 and the digestate trial in 2017.

Sampling dates 2016

Time	Site	Date
T0	NW	18/04/2016
	HF	25/04/2016
	EB	19/05/2016
T1	NW	13/06/2016
	HF	20/06/2016
	EB	29/08/2016
T2	NW	26/09/2016
	HF	26/09/2016
	EB	29/09/2016

Sampling dates 2017

Time	Site	Soil sampling date
T1	NW	18/04/2017
	HF	08/05/2017
T2	NW	26/07/2017
	NH	07/08/2017

© Pictures by E. Fitos (left & right) and D. Chadwick (middle): Soil sampling at Rothamsted Resarch (2018), Henfaes farm (2017) and Kirkton (2018).

Table 5: Biomass harvest dates were planned to be at plant peak biomass. In 2018, the summer was very dry and the plant growth was accelerated. This meant that harvest times had to be moved forward to capture plant peak biomass. If sampling took more than one day, the first day is indicated.

Site	**Soil sampling & veg. cut**	**Plant biomass harvest**	**Harvests carried out by:**
Parsonage Down	02 May	NA	no harvest
Silwood	01 May	Aug	Imperial College
Harpenden	06 Jun	TBD	Rothamsted Research
Wymondham	22 Mar	07 Sep	CEH Bangor
Plynlimon	04 Apr	08 Aug	CEH, moss meshes remained in the field
Abergwyngregyn	28 Mar	13 Aug	CEH Bangor
Newborough	21 Mar	28 Aug	CEH Bangor
Easter Bush	22 May	TBD	CEH Edinburgh
Kirkton	24 May	16 Aug	CEH Bangor

WP2: At both the **NW and HF sites in 2016** soil was sampled three times weekly for the first two weeks following N fertiliser application, then twice weekly for the following two weeks, and once weekly thereafter. From each plot a minimum of 6 soil samples were taken using an auger (of 1 or 2.5 cm i.d. at HF and NW respectively) to a depth of 10 cm. The cores were bulked and transported to the laboratory for storage at 4°C or -18°C in the dark at NW and HF respectively. Soils were subsequently analysed for NH_4^+ and NO_3^- using the colorimetric methods of Mulvaney (1996; for NH_4^+) and Miranda et al. (2001; for NO_3^-).

At the **NW and HF sites in 2017**, for the first three months, soil was sampled from the sampling area of each plot three times per week for the first two weeks after digestate application, two times per week for the next two weeks, followed by weekly sampling thereafter. Subsequently, soil samples were taken once per month until the end of the experiment. On each occasion, eight soil samples were taken per plot for 0-15 cm depth and pooled to provide one representative sample per plot. At the **NW site**, soil was sampled proportionally from within and between the digestate bands. At the **HF site**, soil was sampled randomly, as there were no distinct digestate bands. Soil samples were stored at 4 °C and in the dark prior to analyses.

At the **EB site**, soil cores were sampled in 2016 and 2017 from a distance of approximately 2 m from the static gas chambers (within the appropriate experimental plot) each time N_2O flux measurements were made. Cores were 3 cm in diameter and 10 cm in depth. Samples were stored at -18 °C until further processing up to three months later. Potassium Chloride (KCl) solution (50 mL, 1 mol L^{-1}) was used to extract Nr (in the form of NH_4^+ and NO_3^-) from the samples (15 grams of wet soil). Having added the 1 M KCl solution to the samples, they were subsequently mixed on an orbital shaker for 60 mins before the solution was filtered using 2.5 µm filter paper (Fisherbrand, US) and stored at -18 °C until analysis, up to three months later. A further 10 g of mixed soil was dried to provide the dry soil ratio of each soil sample.

5.4.1 Soil N metric: ammonium, nitrate, amino acids, peptides, mineralisable N

Measured by Bangor University.
Units: Ammonium, nitrate, amino acids, peptides mineralisable N - all in mg N per kg dry soil.

A sub-sample of 5 g fresh soil was gently mixed and used to determine mineral N measures: a 0.5 M K_2SO_4 solution was used in a 1:5 soil:extractant ratio (w:v) shaking at 150 rev min^{-1} for 30 minutes and then centrifuged at 10,000 *g* for 10 minutes. The supernatant was stored at −20 °C prior to analyses.

Ammonium in the supernatant was determined colorimetrically using the salicylate method of Mulvaney (1996) and **nitrate** using vanadium chloride according to Miranda et al. (2001) in a Powerwave XS plate reader (Bio Tek Instruments Inc., Winooski, VT).

Amino acids and peptides were extracted using OPA-MET reagent (containing a mix of *o*-phthaldialdehyde dissolved in methanol, b-mercaptoethanol and borate buffer-pH 9.5) which was added in a 1:10 sample:reagent ratio (Jones et al., 2002). The samples used for soluble peptides and proteins were centrifuged at 10,000 *g* for 10 minutes, and then 100 µL of each sample was mixed with 100 µL of concentrated HCl, the O_2 replaced with N_2 in a O_2-free atmosphere, heated for 16 hours at 105 °C, and, finally, 200 µL 6M NaOH added to each sample when they returned to ambient temperature (modified from Bremmer, 1950). Standards of glycine were used to make the calibration curve in both cases. Amino acids and peptides were measured in the same supernatant stored at −20°C using a Cary Eclipse Fluorescence Spectrophotometer with a ProStar Solvent Delivery Module (Varian, USA).

Mineralisable N was determined after anaerobic incubation according to Keeney (1982) using 5 g of soil and calculating the differences in NH_4^+ between the initial concentrations and the concentrations after 7 days of anaerobic incubation.

Data were compiled in Microsoft Excel files. All measurements were on a fresh soil-basis. Based on the soil moisture content of each sample, data were converted to nitrogen content per grams dry soil.

5.4.2 Soil dissolved organic C and total dissolved N (DOC and TDN)

Measured by Bangor University.
Units: DOC - mg carbon per kg dry soil, TDN - mg N per kg dry soil.

DOC and TDN were measured in the supernatant of the K_2SO_4 extract (see section on soil nitrogen metric above), using a Multi N/C 2100/2100 analyser (AnalytikJena AG, Jena, Germany). DON was calculated by subtracting NH_4^+ and NO_3^- from the TDN value.

Data were compiled in Microsoft Excel files. All measurements are based on a fresh soil basis. Based on the soil moisture content of each sample, data were converted to nitrogen content per grams dry soil.

Data were compiled in Microsoft Excel files. All measurements were on a fresh-soil basis. Based on the soil moisture content of each sample, data were converted to DOC and TDN per grams dry soil.

5.4.3 Soil microbial biomass C and N (MBC and MBN)

Measured by Bangor University in 2016 and 2017, measured by CEH Bangor in 2018.
Units: MBC - mg carbon per kg dry soil, MBN - mg N per kg dry soil.

MBC and MBN were measured at Bangor University and CEH Bangor using the same method. MBC and MBN were measured on 5 grams of soil that were kept for 7 days in a desiccator with chloroform (to kill the microorganisms). The difference between the DOC and DON after 7 days incubation (as explained above) and the initial values DOC and DON value (calculated as in the previous paragraph) were used to calculate MBC and MBN using the correction factors 0.45 for MBC and 0.54 for MBN.

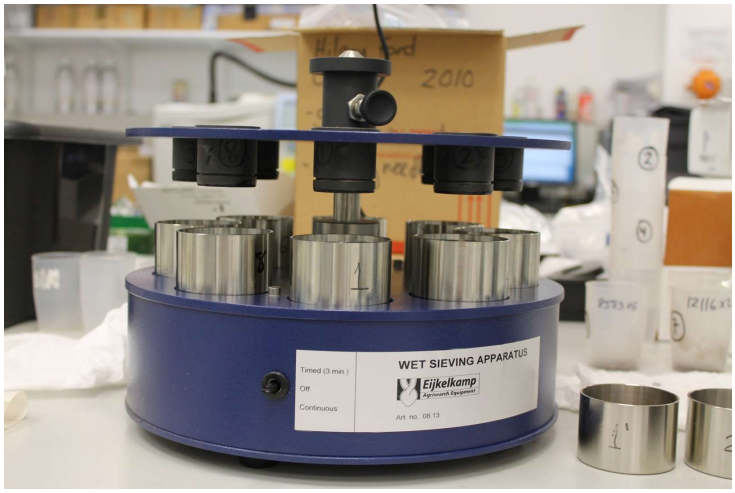
QA/QC: two laboratory standards (BS1 and BS3) and random replicates were run within each batch. Data were compiled in Microsoft Excel files and data were checked by another member of staff before released to the project manager.

5.4.4 Aggregate size distribution

Measured by CEH Bangor.
Units: Aggregates size distribution - percent.

Aggregate size distribution was measured using about 1 g of air-dry and sieved soil through a stack of 2 mm and 1 mm sieves, the fraction collected into the 1 mm sieve was introduced into a Beckman-Coulter LS 13320 laser diffraction particle size analyser and measured for the size distribution.

QA/QC: each sample measurement was replicated, and two laboratory standards (BS1 and BS3) were run with each batch. Data were compiled in Microsoft Excel files and data were checked by another member of staff before being released to the project manager.



Aggregate size distribution measured using a wet sieving apparatus.

5.4.5 Soil texture (sand, silt, clay)

Measured by CEH Bangor.
Units: sand, silt, clay in percent; add up to 100%.

Soil texture was measured at CEH Bangor. Particle size distribution was measured using a Beckman-Coulter LS 13320 laser diffraction particle size analyser. Soil was manually coned and quartered 0.5 g from the soil to get a subsample, removed organic matter using H_2O_2 and transferred the sample into a 250 mL bottle. Then, 5 mL of 5% Calgon were added and the solution was shaken overnight. The entire content of the bottle was introduced to the laser analyser for measuring particles size distribution.

QA/QC: two laboratory standards (BS1 and BS3) were run with each batch, random replicates were run, and soils of standard sizes were run to check for accuracy and precision. The sand fraction was collected and compared to the amount of sand calculated. BS1 and BS3 have been analysed by the traditional gravimetric method and serve as external evaluation for the performance of the laser technique. Data were compiled in Microsoft Excel files and data were checked by another member of staff before released to the project manager.

5.4.6 Soil moisture and soil organic matter content (SOM)

Measured by CEH Bangor.
Unit: SOM in percent.

WP1: Soil water content and SOM were measured at CEH Bangor. SOM was measured as loss-on-ignition (LOI). Soil was initially dried at 25ºC for 14 days. Then, a 10 g subsample was weighed into a crucible and put into an oven at 105ºC for 24 hours, the weight loss provides the soil water content. The same crucible with the dry soil was then introduced into a Carbolite furnace at 375 ºC for 16 hours, this second weight loss is quantified and reported as SOM. Soil water content and SOM were recorded in percent.

QA/QC: two laboratory standards (BS1 and BS3) and random replicates were run in each batch. Data were compiled in Microsoft Excel files and data were checked by another member of staff before data to the project manager.

5.4.7 Soil pH and electrical conductivity (EC)

Measured at CEH Bangor for WP1 and by Bangor University and Rothamsted Research for WP2.
Units: pH is unitless, EC - micro Siemens per metre.

WP1: In a beaker, 25 mL deionised water was added to 10 g of field moist soil; the solution was rested for 30 minutes to allow the solution to reach an equilibration. The solution was stirred at time=0 and 15 minutes. pH was measured using a Corning 220 pH meter, VWR combination electrode 662-1805. EC was measured after the pH measurement in the same beaker using a Jenway 4510 with a 662-1805 electrode. pH in $CaCl_2$ was measured after EC following the addition of 2 mL of 0.125 molar $CaCl_2$.

QA/QC: Before the measurement of each batch, the pH is calibrated with pH 4 and pH 7, and the EC electrode in these solutions. The two laboratory standards (BS1 and BS3) are checked for accuracy of measurements. Data were compiled in Microsoft Excel files and data were checked by another member of staff before released to the project manager.

WP2: pH and EC were determined in a 1:2.5 (w/v) soil:distilled water suspension with standard electrodes using a Model 209 pH meter (Hanna Instruments Ltd., Leighton Buzzard, UK) and a Jenway 4520 conductivity meter (Cole-Palmer Ltd., Stone, UK).

QA/QC: Two laboratory standards and random replicates were run each time. Data were compiled in Microsoft Excel files and data were checked by another member of staff before data being released to the project manager.

5.4.8 Soil base cations (Na, K, Ca and Mg)

Measured by CEH Lancaster.
Units: mg per kg dry soil.

Two grams of sieved soil were weighed into acid-washed LDPE extraction bottles and 50 mL of 1 molar Ammonium Acetate at pH 7 was added. The samples were placed on an end-over-end shaker for 1 hour at 30 revolutions per minute. The samples were filtered using Whatman no. 44 filters which were pre-rinsed with ultra-pure water. The first 5 mL of the filtrates were discarded and a subsequent 45 mL aliquote collected in acid washed tubes and stored at 4°C prior to measurement. The base cations Na, K, Ca and Mg were measured on a 10 mL filtrate by ICP-OES using matched calibration standards (Perkin Elmer Optima 7300 DV).

QA/QC: Blanks and two reference soils were extracted using the same method and measured alongside the samples. The reference soils SR2 and SR3 were locally sampled glacial drift and locally sampled agricultural soil, respectively. Data were compiled in Batch97 (laboratory LIMS) with the concentration of base cations in the soil extract being converted to mg/kg dry weight soil, and data were checked by the laboratory technical manager before being released to the project manager.

5.4.9 Total soil C and N

Prepared by CEH Bangor and Lancaster.
Measured by CEH Lancaster for WP1, at Bangor University and Rothamsted Research for WP2.
Units: C and N - percent.

WP1 carbon and nitrogen: Ball milled soil samples were oven dried at 105°C (± 5°C) for a minimum of 3 hours, cooled and sealed prior to weighing. 20 mg of soil were weighed into a tin cup on a 6-place micro-balance. An Elementar Vario EL was used to measure **total soil C and N** and works on the principle of oxidative combustion followed by thermal conductivity detection.

QA/QC: All calculations were done by the instrument software and results expressed in % notation. The instrument's calibration is checked on use using a working standard (Acetanilide) with concentrations of 71.1% total C and 10.4 % total N and the data corrected (factored) against these values. Two of these standards are analysed at the beginning of every run, with every 10 samples and again at the end of a run. At least two reference soils were analysed with each batch at intervals of every 20 samples.

WP2: Total soil C and N were determined on 0.05 and 0.10 grams of ground soil using a TruSpec® analyser (Leco Corp., St Joseph, MI) and ground oven-dried soil (105 °C, 24 h).

QA/QC: Soil samples with known C and N concentration were analysed every 10 to 12 samples. Data were compiled in Microsoft Excel files.

5.4.10 Total phosphorus

Measured by CEH Lancaster.
Unit: total P - mg P per kg dry soil.

Total phosphorus was digested with a H_2O_2/H_2SO_4 based digestion reagent mix (along with selenium and lithium sulphate) in soil samples and subsequent colorimetric analysis carried out on a Seal discrete analyser. Soils were air dried and ground to 2 mm, 0.36 g of soil was weighed into boiling tubes and 4.4 mL of digestion mix was added. Sufficient blanks are run without sample to allow for standards and controls to be prepared later in the same matrix as the samples.

The boiling tubes were then placed in a block digester and the temperature stepped to 250°C and held for 15 minutes to allow the mixture to stabilise and then further stepped to 400°C where the temperature is maintained for 2 hours to complete the digestion.

Once digested the samples, reference samples and blanks are allowed to cool, then diluted to a total volume of 50 mL using ultrapure water. After allowing the precipitate to settle overnight, the supernatant is collected and diluted a further 5 times and then measured colourimetrically using a SEAL AQ2 discrete analyser. The samples were mixed in heated discrete reaction segments with acidic ammonium molybdate and potassium antimony tartrate to form a complex with phosphate. This complex is reduced with ascorbic acid to develop a molybdenum blue colour which is measured for absorbance at 880 nm.

QA/QC: References soil samples are handled and measured the same way as the samples. Blanks are run to allow for standards and controls to be non-contaminated. Calibrations are run using standards prepared from blank matrix digested in the same manner as the samples with control standards (also prepared in matrix) analysed every ten samples to check precision throughout the run. Blanks are run every 20 samples with certified reference samples (prepared in matrix). Data is extracted into LIMS where the data is processed, blank corrected, moisture corrected and reported as P in mg/kg.

5.4.11 Permanganate oxidisable C (POXC)

Measured by CEH Bangor.
Units: POXC - mg carbon per kg dry soil.

POXC was measured on manually quarter 2.5 grams of air dried soil following the method described in Weil et al. 2003. Deionized water (18 mL) were added to each soil sample with 2 mL of 0.2 molar $KMnO_4$. Samples were vigorously shaken for 2 seconds, then shaken at 240 oscillations per minute for 2 minutes. Soils were allowed to settle in the tubes in a dark and cool place for exactly 10 minutes. Then 0.5 mL of the supernatant and 49.5 mL of deionised water were transferred into another tube. This step has to be done quickly as the permanganate will continue to react with the soil. Sample solutions were transferred to 96-well plates and measured at 550 nm. Blank samples (only deionised water) and standards were shaken and rested in the same way as the soil samples and transferred to the same 96-well plate. The more POXC in the sample, the lower the absorbance.

QA/QC: two reference soils (BS1 and BS3) and blanks were included in each batch which were processed in the same way as the samples. A standard calibration curve allows for calculating the amount of POXC in the sample solution. Data were compiled in Microsoft Excel files and data were checked by another member of staff before released to the project manager.

© Photos by S. Reinsch: Colorimetric measurement of permanganate oxidisable C from soils.

5.4.12 Citric acid extractable P, acetic acid extractable P, Olsen-P

Measured: **citric acid extractable phosphorus** (CEH Bangor) and **Olsen-P** (CEH Lancaster) were for WP1. **Acetic acid extractable phosphorus** was measured by Bangor University for WP2.

Units: all measures - mg phosphorus per kg dry soil.

Olsen-P was measured using two grams of sieved, air dried soil were extracted in 40 mL Olsen's reagent (0.5 M $NaHCO_3$ at pH 8.5) for 30 minutes in a mechanical end over end shaker. The sample was then filtered through a Whatman 44 filter paper to separate the soil and the filtrate; the filtrate was kept for Olsen-P analysis.

The analysis is performed on a Seal Analytical AA3 segmented flow analyser. The samples are mixed in the flow channel with acidic ammonium molybdate and potassium antimony tartrate to form a complex with phosphate. This complex is reduced with ascorbic acid to develop a molybdenum blue colour. The reaction is temperature controlled to ensure uniform colour development. The developed colour is measured at 880 nm.

QA/QC: The data is processed by the instrument software, exported and placed in an Excel file. The mean of two extraction blanks is used to correct the data set. The data is then extracted to LIMS which calculates the final concentration in mg-P per kg soil, corrected for moisture content. Two quality control reference samples and a duplicate sample were run every 25 samples to ensure data quality. The calibration range of this method is 0-5 mg-P per Litre.

Citric acid extractable P was measured using 10 mM citrate acid which extracted the active inorganic P pool sorbed to clay particles or weakly bound in inorganic precipitates (DeLuca et al. 2015). This method emulates organic acid release by plants and microorganisms. Briefly, 0.5 g of fresh soil was added to 15 mL tubes and 10 mL of 10 mM citric acid were added. Samples then shaken for 3 hours at ~ 200 rev per minute. Samples then centrifuged at 3200 *g* for 30 minutes. An aliquot of the supernatant was removed and stored for max of 3 days at 4°C prior to analysis.

Samples were then analysed for P by colorimetry using Malachite Green Method (Ohno and Zibilske (1991). Colored samples were transferred to a 96-well plate: 50 µL of reagent were added in a well, followed by 200 µL of sample/standard, then the plate was left to develop a green colour for one hour. The absorbance was read at 630 nm using a Biotek PowerWave XS microplate spectrophotometer. A standard curve regression was used to convert absorbance readings into concentrations of P.

QA/QC: Blanks and two reference standards (BS1 and BS3) were run on each 96-well plate as well as a standard curve. Individual samples were re-run when the measured values were outside the range of the standard curve. Data were compiled in Microsoft Excel files and data were checked by another member of staff before released to the project manager.

WP2: Acetic acid extractable P was used as a proxy for plant-available P, determined after extracting the field moist soil with 0.5 M acetic acid (1:5 w/v, 200 rev min^{-1} for 1 h) by the molybdate blue method (Murphy and Riley, 1962) following centrifugation (10,000 *g*, 10 min).

QA/QC: Standards for the calibration curve were prepared using a 1000 mg P L^{-1} commercial solution and random replicates were run each time. Data were compiled in Microsoft Excel files and data were checked by another member of staff before data were released to the project manager.

5.4.13 DNA, N genes

Measured: **DNA** extractions were performed by Rothamsted Research Harpenden. Extracts were analysed for nitrogen genes at Rothamsted Research in Harpenden. Extracts were also sent to CEH Wallingford for analyses of OTUs.
Units: qPCR data in gene copy number per gram dry soil

DNA extractions: Soil community DNA was extracted from 0.25 gams soil using the MoBio DNA PowerSoil DNA isolation kit (Mo Bio Laboratories, Inc. Carlsbad, CA), following manufacturers protocol. Extracted DNA was quantified by fluorometer Qubit® 2.0 dsDNA BR Assay Kit (Thermo Fisher Scientific) and quality checked by nanodrop (Thermo Fisher Scientific).

qPCR (followed protocol as detailed in De Sosa et al. 2018)

Microbial nitrogen cycling gene abundance was investigated by quantitative-PCR (qPCR) targeting specific genes or genetic regions. Bacterial and archaeal communities were targeted via the 16S rRNA genes, while the fungal community abundance was targeted by the ITS region. The different communities involved in soil nitrogen cycling were investigated:

- nitrogen fixation (nifH gene);
- nitrification by targeting the ammonia oxidising bacteria (AOB) and archaea (AOA) (amoA gene), and
- denitrifiers via the nitrite reductase (nirK and nirS genes) and the nitrous oxide reductase (nosZ genes clade I and II)
- The ability to degrade urea was also assessed by targeting the ureC gene.

Quantitative-PCR amplifications were performed in 10 µL volumes containing 5 µL of QuantiFast (Qiagen, Manchester, UK), 2.8 µL of nuclease-free water (Severn Biotech, Kidderminster, UK), 0.1 µL of each primer (1 µM) and 2 µL of template DNA at 5 ng μL^{-1}, using a CFX384 Touch® Real-Time PCR Detection System (Bio-Rad, Hemel Hempstead, UK).

QA/QC: Standards for each molecular target were obtained using a 10 fold serial dilution of PCR products amplified from an environmental reference DNA (also used as positive control) and purified by gel extraction using the Wizard® SV Gel and PCR Clean Up System (Promega, Southampton, UK) following the manufacturer's instruction and quantified by fluorometer Qubit® 2.0 dsDNA BR Assay Kit (Thermo FisherScientific).

Standard curve template DNA and the negative/positive controls were amplified in triplicate. Amplification conditions for all qPCR assays consisted in 2 steps: first denaturation at 95°C for 5 minutes followed by 40 cycles at 95°C for 10 seconds and 60°C for 30 seconds that included annealing, elongation and reading. Each amplification was followed by melting curve (increase in temperature from 60°C to 95°C, with a reading every 0.5°C) to assess the specificity of each assay.

The efficiency of the qPCR varied between 85.2% and 95.9% (except nozII 74.4%, amplicon 746 bp long), and R^2 between 0.987 and 0.999. The melting curves showed specificity for all the genes, except as expected for the fungal ITS, that showed the amplification of products of different lengths,due to the variability in length of the ITS region between different fungal taxa (Manter and Vivanco, 2007).

5.4.14 Microbial diversity indices

Measured by CEH Wallingford.
Units: Shannon's Index, Richness, Bacterial Phylum level frequency (percentage of total)

Molecular analyses of soil DNA: Extracted DNA was supplied from Rothamsted Research in Harpenden (see 5.4.13) for amplicon sequencing at the facilities of the Molecular Ecology Group, Centre for Ecology and Hydrology, Wallingford. <50 µL volumes of DNA extract were supplied in 96-well plate format, these were subject to:

- 16S rRNA amplicon sequencing as per Kozich 2013 (Development of a dual-index sequencing strategy and curation pipeline for analyzing amplicon sequence data on the MiSeq Illumina sequencing platform), utilising V3-V4 region primers
- ITS rRNA amplicon sequencing employing the Kozich 2013 strategy and utilizing ITS primers by Ihrmark 2012 (New primers to amplify the fungal ITS2 region--evaluation by 454-sequencing of artificial and natural communities)

Amplicons were sequenced on Illumina MiSeq platform with V3 chemistry. Resulting sequences were demultiplexed using Illumina Basespace. Bacterial 16S amplicons were processed using in-house developed pipelines for quality filtering, merging and taxonomic assignment (GreenGenes database) of operational taxonomic units. Fungal ITS amplicons were processed using the R package Dada2 for quality filtering, merging and taxonomic assignment (UNITE database) of Actual Sequence Variants. R package Vegan was used to analyse amplicon sequences. Bacterial and fungal Shannon's Index diversity scores for each sample were produced from rarefied data using 'diversity' function. Species richness for each sample were similarly produced using 'specnumber' function.

QA/QC: Amplicon specific PCRs were undertaken for each sample along with negative controls. All samples including negative controls were normalised using Invetrogen SequalPrep normalisation kit before sequencing. Samples which produced read numbers below 2000 were discarded before rarefication and further analysis.

5.4.15 Soil water infiltration

Measured by CEH Bangor.
Units: mL.

Soil water infiltration was measured in the field in spring and summer 2018 using a Mini Disk Infiltrometer (METER group, Inc. USA), a compact infiltration measuring device with a disk radius of 2.25 cm. At each site, where possible, nine measurements were conducted, at three different tensions (0.5 cm, 1 cm and 3 cm). Measurements were taken along a transect avoiding field or boundary edges, transitions between different types of vegetation or land use and from the same slope gradient; or in the case of small experimental plots (i.e Silwood, Harpenden) using an appropriately spaced 'W' pattern sampling design. Vegetation was carefully trimmed away to reveal the soil surface and a thin layer of sand applied to ensure good contact between the disk and the soil surface. The raw data was then entered into the Decagon mini-disk infiltrometer Excel macro to calculate hydraulic conductivity, with further processing and graphical work conducted in R.

© Photos by S. Reinsch: Soil water infiltration measurements at Newborough Warren (left), Plynlimon (middle) and Wymondham (strawberry field) in spring 2018.

5.4.16 Soil water release curves

Measured by CEH Bangor.

Units: Volumetric soil water content in m^3 per m^3; hydraulic conductivity in cm per day.

Soil water release curves were measured on 250 cm^3 soil cores, 0–5 cm deep, taken from different land uses across the UK in 2018. Water release curves were determined using the laboratory evaporation method using a hyprop22 (UMS, Munchen, Germany). The very dry end of the water retention curve was measured on samples using a WP4 (Decagon devices, Pullman, Washington, USA) (Tuller and Or 2005). Hydraulic conductivity was determined at low suctions (1–6 cm) with the hyprop and at high suctions (30–800 cm) in the field using a mini-disk infiltrometer (ETER group, Inc. USA). Moisture release curve data was modelled using HYPRO-FIT software (UMS, Munchen, Germany) to determine hydraulic parameters using the Mualem-Durner bimodal soil water retention curve (Durner 1994) and the Peters and Durner hydraulic conductivity model (Peters and Durner 2008).

6. More information

Project webpage: www.rothamsted.ac.uk/international/china/cinag

Publications derived from the datasets described here:

Carswell, A., Shaw, R., Hunt, J., Sánchez-Rodríguez, A.R., Saunders, K., Cotton, J., Hill, P.W., Chadwick, D.R., Jones, D.L. and Misselbrook, T.H., 2018. Assessing the benefits and wider costs of different N fertilisers for grassland agriculture. *Archives of Agronomy and Soil Science*, pp.1-15. https://doi.org/10.1080/03650340.2018.1519251

Sánchez-Rodríguez, A.R., Carswell, A., Shaw, R., Hunt, J., Saunders, K., Cotton, J., Chadwick, D.R., Jones, D. and Misselbrook, T., 2018. Advanced processing of food waste based digestate for mitigating nitrogen losses in a winter wheat crop. *Frontiers in Sustainable Food Systems*, *2*, p.35. https://doi.org/10.3389/fsufs.2018.00035

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8. Appendix

8.1 Inorganic fertiliser Experiment – 2016

Table A1: Fertiliser application rates and harvest dates (cuts) for the **inorganic fertiliser experiments** (2016) for Henfaes farm (HF) and North Wyke (NW).

Henfaes Farm and North Wyke	HF date	NW date	**Fertiliser application rate (kg-N ha^{-1})**				
			N (all treatments)	K (applied as K_2O)	S (applied as SO_3)	P (applied as P_2O_5)	
						HF	NW
Application 1	05/05/2016	23/03/2016	90	66.4	40	8.72	17.4
Cut 1	07/06/2016	16/05/2016					
Application 2	13/06/2016	19/05/2016	90	74.7	40	0	10.9
Cut 2	19/07/2016	05/07/2016					
Application 3	25/07/2016	08/07/2016	60	66.4	40	0	6.54
Cut 3	15/09/2016	24/08/2016					

Table A2: Fertiliser application rates and harvest dates (cuts) for the **inorganic fertiliser experiment** at the Easter Bush site in 2016. Only N was added.

Easter Bush	Date	Fertiliser rate (kg-N ha^{-1})
Application 1	13/06/2016	70
Cut 1	15/07/2016	
Application 2	27/07/2016	70
Cut 2	03/10/2016	

Table A3: Fertiliser application rates and harvest dates (cuts) for the inorganic fertiliser experiment at the Easter Bush site in 2017. Only N was added.

Easter Bush	Date	Fertiliser rate (kg-N ha^{-1})
Application 1	13/03/2017	70
Cut 1	25/05/2017	
Application 2	12/06/2017	70
Cut 2	19/07/2017	
Application 3	07/08/2017	70
Cut 3	15/09/2017	

8.2 Digestate Experiment – 2017

Pre-treatment information for digestate experiment at the NW and HF sites:
Pre-treatment information: *Triticum aestivum* (var. KWS Siskin) was drilled on 10th October 2016 with a row spacing of 0.1 m at the **NW and HF sites**. Prior to this, the fields were ploughed to 15 cm depth and limed to increase the soil pH. Phosphorus (P) and potassium (K) were applied during the same week of sowing. Kieserite ($MgSO_4 \cdot H_2O$) was applied in March 2017 at both sites. Application rates were based on routine soil analyses and national fertiliser guidelines (Defra, 2010) so that these elements were non-limiting. Herbicides at both sites, and insecticides and fungicides only at the NW site were also applied according to manufacturers' recommendations.

Plot layouts digestate experiments (2017)

Plots and treatments used in WP1 for North Wyke (NW) and Henfaes Farm (HF): C = Control, Dig = digestate, Dig+Acid+NI = Digestate + Acidificatino inhibitor + Nitrification inhibitor.

Plot	NW
18	Dig
20	C
21	Dig+Acid+NI
22	C
23	Dig+Acid+NI
25	Dig
29	Dig
30	Dig+Acid+NI
31	C
32	Dig
33	C
34	Dig+Acid+NI
37	Dig+Acid+NI
39	C
41	Dig

Plot	HF
5	Dig
6	Dig+Acid+NI
9	C
15	Dig
17	C
18	Dig+Acid+NI
21	C
29	Dig
30	Dig+Acid+NI
36	Dig+Acid+NI
39	C
40	Dig
41	Dig+Acid+NI
42	Dig
43	C

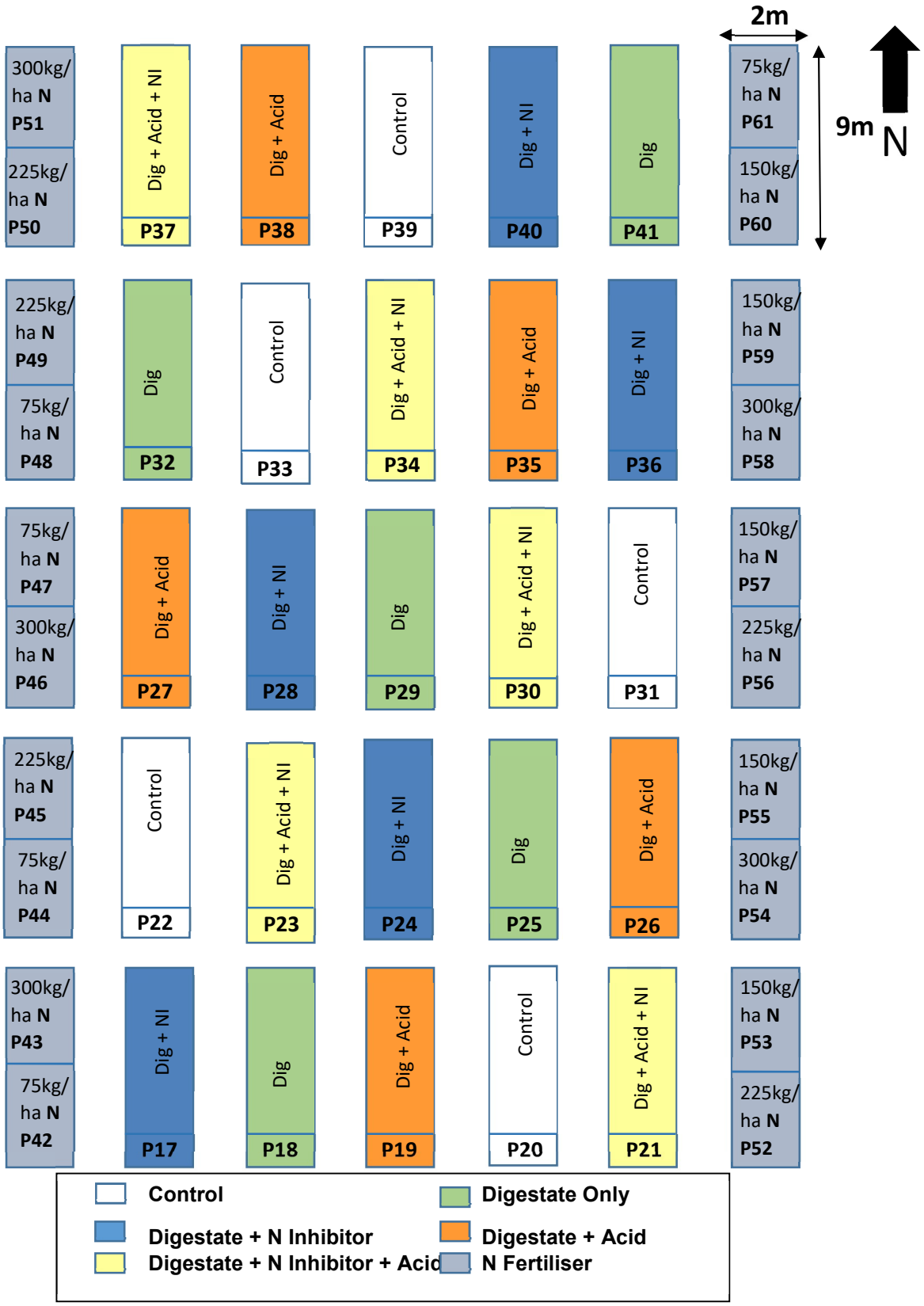

Figure A1: Fully randomized plot design for the digestate experiment in 2017 at North Wyke. N Fertiliser = inorganic fertiliser controls to compare growth of plants in digestate-treated plots to. All plots were sampled in WP2. For WP1 only C, D and D + NI + A were sampled as the biggest differences were anticipated between these treatments. The North Wyke reference for this experiment is NW648 Wheat Trial: Fertiliser & Digestate Harvest Plots.

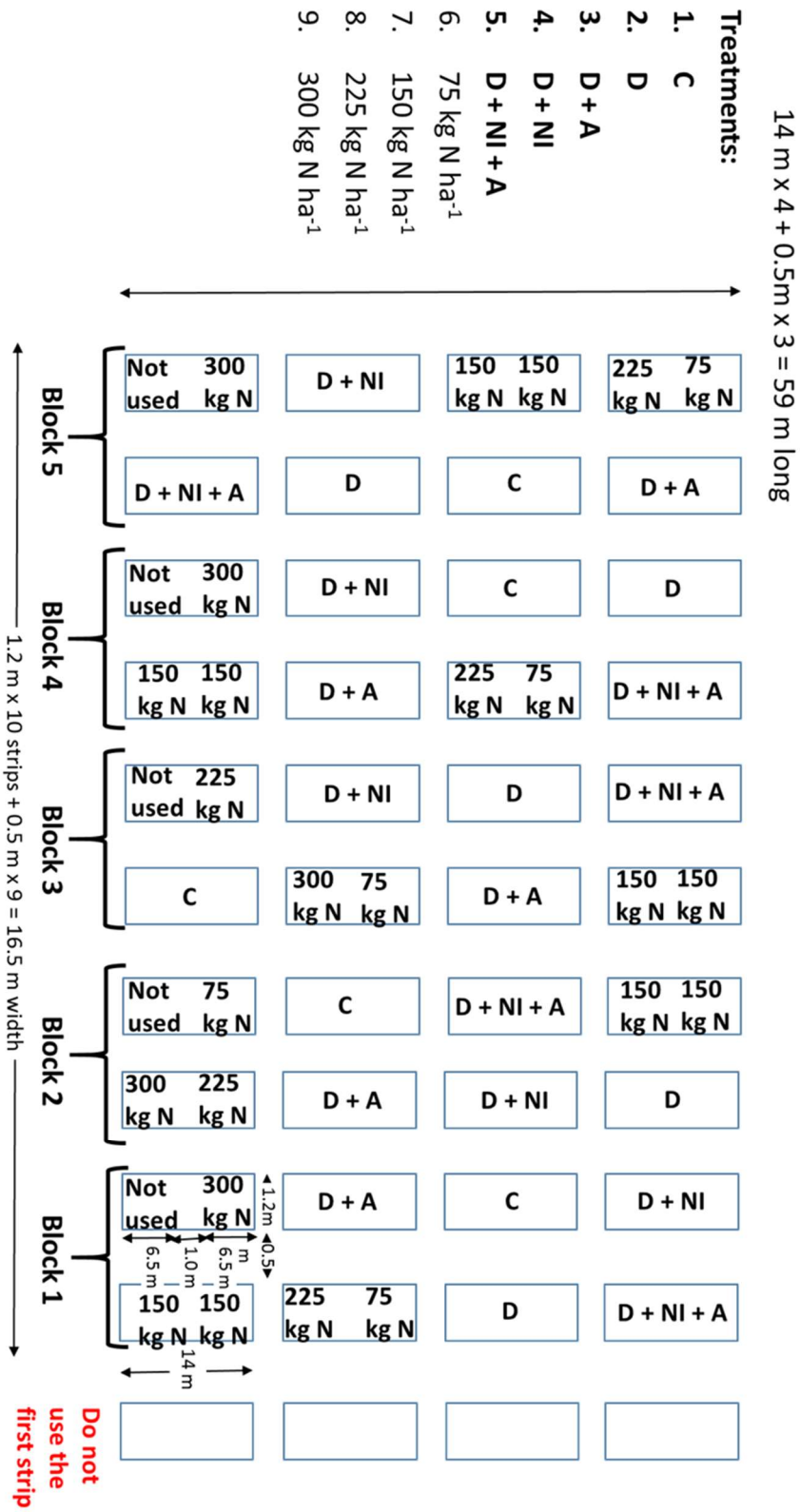

Figure A2: Fully randomized plot design for the digestate experiment in 2017 at Henfaes Farm. C = control (not receiving any fertiliser additions), D = digestate addition, D + A = Digestate + Acid, D + NI = Digestate + N inhibitor, D + NI + A = Digestate + N inhibitor + Acid. Separately, increasing amounts of N fertilisers (75, 150, 225 and 300 kg N ha^{-1}) were controls to compare growth of plants in digestate-treated plots to. All plots were sampled in WP2. For WP1 only C, D and D + NI + A were sampled as the biggest differences were anticipated between these treatments.