REPORT TO THE STAPLEDON MEMORIAL TRUST

PERSONAL DATA OF THE FELLOW

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MAIN PURPOSE OF THE FELLOW

The fellowship enabled the fellow to examine how organo-mineral substrates (OMS; formulated by the fellow in Spain) emit greenhouse gases (GHGs) and ammonia (NH_3) when used as fertilizers, with or without plants (ryegrass), and with or without nitrification inhibitors. This project applied a combination of state-of-the-art and conventional techniques for this purpose. Adrian carried out this fellowship in collaboration with Dr Alison Carswell (at Rothamsted Research, North Wyke) due to her experience in GHG and NH_3 emissions in agricultural systems.

DURATION OF THE FELLOWSHIP

Adrian started the fellowship on the 25th of September 2023 and finished it on the 20th of December 2023.

OUTLINE WORK DONE DURING THE FELLOWSHIP

The FILMAWAS (Filters made by waste) project—developed between the Universities of Córdoba (UCO) and Lisbon—aims to elucidate the possibility of valorizing waste as adsorbents for GHGs and NH_3 from slurry stores and their subsequent potential as fertilizers. Dr Alison Carswell has a background in nitrogenous fertilizer research and mitigating nitrogen (N) losses in agricultural systems. At Rothamsted, the main mechanisms inhibiting nitrification, particularly for the current experiment, was examined to optimize their use as fertilizers by adding the nitrification inhibitor dicyandiamide (DCD).

Experimental design

The study was divided into two main experiments: (1) Formulation of filters for slurry gas capturing, carried out in the University of Cordoba (UCO, Spain), and (2) use of the filter as an OMS, performed at Rothamsted Research (North Wyke, United Kingdom) under this fellowship.

The first experiment was developed at UCO to ensure research feasibility of the whole study in time. Different filters (Annex 1) were formulated to capture GHG and NH_3 from stored slurries, enriching them with N (an essential plant macronutrient) in the process. The experiment had 4 treatments —Control, Filter 1, 2 and 3— and each treatment had three biological replicates and three technical replicates (4 × 3 × 3 = 36 experimental units). Each technical replicate is formed by a 50 mL beaker filled with pig slurry (20 g) and covered with a fabric, where combined residues were deposited (Figure 1). Once only the dried slurry remained in the bottom of the beaker, the total N retained in the filters and the one remaining in the dried slurry were analyzed [total N, nitrate (NO_3) and ammonium (NH_4)], and the filters material was kept in plastic bags at room temperature prior to being sent to Rothamsted Research.

Figure 1. The experimental units used to test the filter combinations for capturing GHGs and NH_3 emissions.

The second experiment was developed with the filters from the UCO, which were applied as OMS to an acid grassland soil local to North Wyke. Greenhouse gases and NH_3 emissions were then monitored following application. Three different factors were analyzed: (1) sown/not sown (with ryegrass) soil; (2) original OMS/N-enriched OMS; (3) with/without nitrification inhibitors (Table 1). The combination of factors (2 × 2 × 2) plus two controls —C and C´ (for sown and not sown)— made a total of ten treatments, each with four replicates (10 × 4 = 40 pots). This experiment was split into two experimental runs, firstly with the sown soils and then with the not sown soils, being both monitored for 6 weeks.

Table 1. Description of the controls (C and C') and treatments (T) evaluated and the variables used: sowing (S), nitrogen enrichment (N-Enriched), nitrification inhibitor (NI). Absence of a variable is indicated by "0" and presence of a variable is indicated by "1".

	C	**T1**	**T2**	**T3**	**T4**	**C´**	**T5**	**T6**	**T7**	**T8**
Sowing	0	0	0	0	0	1	1	1	1	1
OMS	0	1	1	0	0	0	1	1	0	0
N-Enriched	0	0	0	1	1	0	0	0	1	1
NI	0	0	1	0	1	0	0	1	0	1

Soil characterization

The soil used was a Dystric Cambisol which was under permanent pasture, collected from the North Wyke research site. The soils was air dried and passed through a 2 mm sieve prior to use. In this experiment, each pot was filled with 150 g dry weight soil (10 treatments × 4 replicates × 0.15 kg). The amount of water (mL) needed to keep soil at 60% of Water Filled Pore Space (WFPS) was calculated according to this formula:

$$(1)\ V = (WFPS/100) * V_{soil} * (1-(Db/Dp))$$

Where V is the ml of water that must be added to the soil to keep this at 60% $WFPS$, V_{soil} is the volume of the 150 g of soil, Db is the bulk density and Dp is the particle density.

Organo-mineral substrate (OMS)

The OMS used in this experiment was OMS5, it was formed by a combination of wastes and was either N enriched (OMS5 N-Enriched) or not (OMS5 Original). A total of 1.8 g of OMS (either N-enriched or Original) was applied to each pot simulating an application of 24 t ha^{-1}. The OMS was mixed with the soil before fill the pots.

Plant growth and harvest.

A germination test was conducted on *Lolium perenne* seeds, showing a germination rate higher than 95%. Then, 0.3 g of seeds (136 ± 3 seeds) were placed over the air-dried soil surface that was in turn placed in a 150 ml square pot (8 cm height and 7 cm width), with its base sealed. Plants were grown in a controlled environment room (CER) programmed 12/12 hours light/dark, respectively. They were watered every day with deionized water to keep the soil at 60% WFPS. After 5 weeks from being sown, the number of plants grown were counted, the plants were cut one cm above soil surface, weighed, and washed with deionized water, before drying them in paper envelopes at 55 ºC for 5 days.

Once plants were dried, they were weighed (dry weight, DW), ground, and digested in nitric acid to measure the content of nutrients and other potential toxic elements using ICP-MS. Total N, C and S concentration was measured using a LECO instrument.

Nitrification Inhibitors

The nitrification inhibitor DCD was applied to the mix of soil and OMS. The dose chosen was the highest allowed by the European Union (EU) legislation, 40 g per 1 kg of N applied. The DCD was applied with deionized water, at a rate of 0.80 and 1.04 mg per pot (depending of N-Enriched OMS or not). Thus, soil was watered with 43.3 ml to get the 60% WFPS capacity.

Gas emissions monitoring

Greenhouse gases and NH_3 emissions were monitored for 6 weeks either in sown or not sown soils. Throughout this time two coupled measurements were performed. On one side, NH_3 was measured by dynamic throughflow chambers with acid traps installed at the outlets alone to capture emitted NH_3 (Figure 2), these were running during the dark period (12 hours per day). On the other side, Gasmet and Licor instruments linked to a multiplexer that in turn was connected to three automatic chambers were used to measure CO_2, CH_4, N_2O and NH_3 (Figure 3) during the light period.

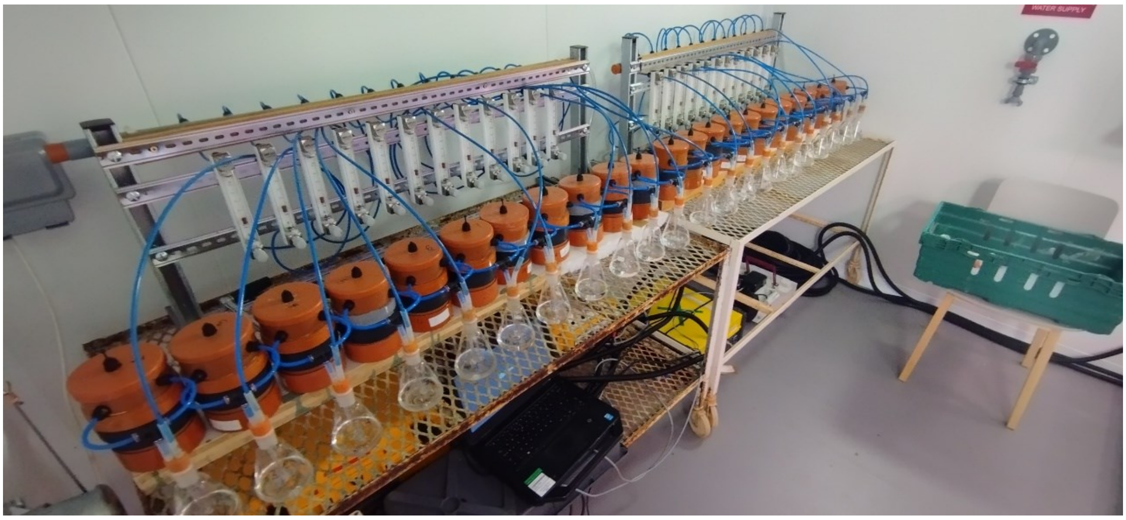

Figure 2. Dynamic air throughflow system used with acid traps fitted at outlets, which captured NH_3 emitted from the OMS and soil. Pots were placed inside the sealed orange chambers during the simulated nighttime period.

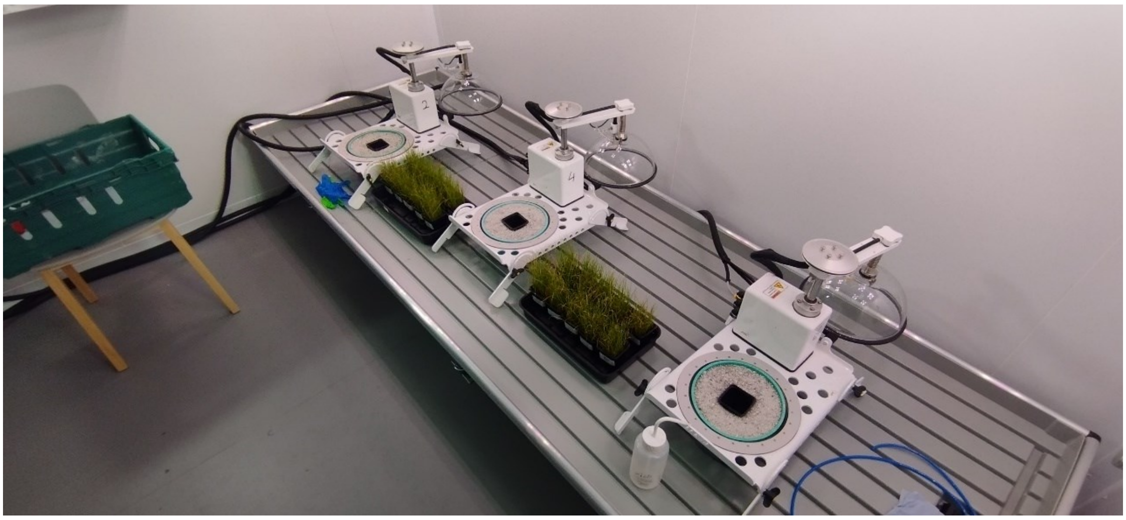

Figure 3. Automated gas measurement chambers formed by a transparent dome and an adapted ring for the pots used in this experiment.

COLLABORATIONS AND RELATIONSHIP BETWEEN RESEARCHERS AND INSTITUTIONS

The fellowship from Stapledon Trust, besides to let scientist test their hypothesis, is something else. This flexible economic support let scientist move to other countries, learn new techniques, teach others, but the key point in this fellowship was enabling scientists to perform experiments physically together, which is essential to design and carry out the most effective, hypothesis testing, experiments. In addition, the network development through getting to know new colleagues, sharing of opinions or working together increases the relevant contribution to the scientific community due to shared knowledge and discussing methodological improvements and results. It should be taken into account that when, state-of-the-art science or new techniques are applied there is little available advice and experience around you and because of that is essential to move temporarily to another research center.

On the other hand, Alison Carswell was an excellent supervisor of Adrian and both create a good network for future collaborations between themselves but also between both institutions. However, Adrian also met other interesting researchers, with whom interesting scientific discussions arose. Among them, Laura Cardenas, William Roberts, Robert Dunn, Liz Dixon and especially Nadine Loick are highlighted. The latter scientist helped Adrian install the automatic chambers and start them running properly. Besides that, and the most important were the discussions about methodology of instruments use, experimental design, and data analysis to obtain the most representative gas results.

Eventually, Adrian has got a great personal and work experience. He has learnt other gas measurements methods and instruments, the importance of different variables in determining each different gas and now he knows better the consequences of applying the N-enriched OMS to the soil, either for ryegrass performance or gas emission.

FOLLOW UP

The collaboration between Alison and Adrian will continue for analyzing the data obtained and with a scientific article intended, but also to try to transfer the new information obtained to the farmers.

Even though no dates are established yet, both researcher agreed to visiting each other again in their respective researching centers to develop further research goals and seek for new synergies between them. This will follow-up with either collaborating in current projects or applying together as partners for other projects calls.

Annex 1: Description of waste combined for creating the filters

OMS2		
Ref	Descripcion	Peso (g)
2022-511	Casca de laranxa moida	1.6
2020-306	Viruta Fe	0.32
2020-916	Lidurma corcho	1.12
2020-276	Compost EMASESA	0.8
2020-55	Nós (Navigator)	1.04
2020-171	Lodos Moura	1.6
2020-279	Cinza Oro de baena(Cinzas Adrian)	0.4
2020-322	Escorias férricas	0.16
2020-324	Virutas de Al	0.96
		8

OMS3		
Ref	Descripcion	Peso (g)
2022-511	Casca de laranxa moida	3.2
2020-916	Lidurma corcho	1.6
2020-276	Compost EMASESA	1.2
2020-55	Nós (Navigator)	0.8
2020-320	Arena inerte con bentonita	0.8
2020-914	Escoria negra ENCE	0.4
		8

OMS5		
2022-511	Laranxa	7
2020-325	Lamas strugal	1