

TITLE: Field's Spatial Variation Influenced Outcomes more so than N-fertiliser, FYM, Cover Crops or Their Legacy Effects Following Conversion to a No-till Arable System

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Field's Spatial Variation Influenced Outcomes more so than N-fertiliser, FYM, Cover Crops or Their Legacy Effects Following Conversion to a No-till Arable System

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1 Abstract

No-till in agricultural arable systems is a practice that offers benefits to soil health. Combined with methods such as the incorporation of crop residues and manures, no-till can influence the dynamics of soil organic carbon (SOC) and organic matter (SOM), crop productivity and nutrient status. These turnovers are shaped by spatial and temporal factors and associated microbial mineralisation processes. There is a lack of diachronic large-scale field studies that include baseline data and seasonal variations in arable systems. This study aimed to investigate the interaction between microbial and soil physicochemical properties as they evolved over time during the transition from full-inversion to no-till. It utilised a combination of soil microbial assays (microbial biomass carbon (MBC) and nitrogen (MBN) with bio-physicochemical analyses (SOC and SOM quantification, textural class, pH, gravimetric water content (GWC), and macronutrients to assess soil over a period of two-years. Two experiments were established within the same four-hectare field, one on a relatively level area (Experiment-1) and another on a slope (Experiment-2). Experiment-1 treatments consisted of Farmyard Manure (FYM), Green Manure (GM) and Standard Practice (SP = Control). Experiment-2 was a repeat of Experiment-1, but without the FYM treatment. Soil was sampled twice per crop season, in Spring and Autumn, in Experiment-1, and in Autumn only in Experiment-2. Diachronic analyses were applied to quantify changes in SOC and SOM, MBC and MBN, and associated physicochemical properties. The results from shifting to a no-till system were influenced by spatial and temporal variations that were not always linked to management practices. This two-year study demonstrated that the quantification of SOC and SOM were poor predictors of change in management practices over the timeframe of the study, but that microbial biomass responded quickly to the incorporation of FYM. SOC and SOM were affected by texture class, but not significantly by inputs, and associated with extractable Ca^{2+} and %tN. This study demonstrated that diachronic studies increase our understanding of SOC, SOM, MBC and MBN dynamics and the impacts of short-term impacts of change in soil management practices. Furthermore, spatial variation within one

field was found to lead to different outcomes and a better predictor in response to those management practices.

2 Introduction

Soils are critical to life, playing a central role in agricultural systems, ecosystem services, whilst being a habitat for a remarkable diversity and abundance of life (Orgiazzi *et al.*, 2016). Soil is a dynamic environment, and its biological and physicochemical aspects infuse it with various degrees of functionality. Soil is commonly thought of as a “Black Box” because of the challenge in untangling interactions between the different functional components to understand the mechanisms behind processes (Denef *et al.*, 2002; Six *et al.*, 2004). This means that historically, soil management was results based, with little concern of the underlying mechanisms or impacts if soil management interventions supported or enhanced yields.

Agricultural practices such as full inversion tillage, removal of crop residues and periods of fallow have been linked to the degradation of soils, threatening their productivity and sustainable use (Riddle, 1988; Arneth *et al.*, 2019; Evans *et al.*, 2020). Some practices have led to loss of SOM, thus SOC, and biodiversity, caused soil erosion, poor structure and weakened water infiltration capacity (Riddle, 1988; COP21, 2015; Lal, 2016; Arneth *et al.*, 2019; Arnold *et al.*, 2020; Evans *et al.*, 2020). A change in agricultural practices which preserve global soils and prevent further degradation is urgently required to ensure the sustainability of future crop production (Lal, 1997; Tsiafouli *et al.*, 2015; Science 20, 2018; Searchinger *et al.*, 2019; Arnold *et al.*, 2020). There has been increasing focus on identifying optimum management practices to reduce fertiliser inputs whilst improving soil health (NFU, 2019; Searchinger *et al.*, 2019; CCC, 2020).

Adoption of no-till (i.e. zero tillage or direct drilling) in an arable system, a cultivation that minimises soil disturbance, has been promoted as a practice in soil protection which increases soil organic matter and builds carbon stocks (C), prevents loss of structure, compaction and nutrient leaching (Lal, 1997; Tsiafouli *et al.*, 2015; Science 20, 2018; Arneth *et al.*, 2019; Searchinger *et al.*, 2019; Arnold *et al.*, 2020; Evans *et al.*, 2020; No-Till.UK, 2020). It has been estimated that only 3-7% of the agricultural land in the UK is being managed as no-till (Allison, 2015; Alskaf *et al.*, 2020). Part of the reason for poor uptake is to do with yield penalties that are often reported during the first years of conversion (Pittlekow *et al.*, 2015).

Two commonly used approaches for building soil health, in addition to no-till, are the application of manures and the use of cover crops (Roesch-Mcnally *et al.*, 2018; Abdalla *et al.*, 2019; Lin *et al.*, 2019; Storr *et al.*, 2019). Manures are frequently used as soil organic amendments in agricultural arable systems, and interest in sowing cover crops has intensified (Roesch-Mcnally *et al.*, 2018; Storr *et al.*, 2019). Farmyard manures (FYM) are applied on 51% of surveyed British farms, of which the greatest volume originates from beef and dairy farms (National Statistics, 2019). Cover cropping is also increasingly promoted as beneficial to soil and the environment through their preventive and/or restorative role against the degradation of soil structure (Roesch-Mcnally *et al.*, 2018; Storr *et al.*, 2019). These inputs can vary in their nutrient composition. For example, the N-content of manures can vary from 6 kg N t⁻¹ to 30 kg N t⁻¹ depending on whether they are from cattle or poultry (Defra, 2018). Cover crops also vary in their N-content and their C:N ratios influence the effects of soil priming that impact nutrient stoichiometry (Liu *et al.*, 2020). For example, legume crops can typically have C:N ratios of 8 – 15 : 1, whereas cereal crop residues can be as high as 80:1 (Silgram and Harrison, 1998; USDA-NRCS, 2011; Schrumpf *et al.*, 2013). Therefore, the characteristics of the soil amendment along with the inherent characteristics of soil is one main factor that controls nutrient dynamics.

Persistence of C in soils is influenced by microbial activity, and the rate it decomposes is influenced by rhizospheric properties such as organic matter, moisture, and temperature. These interactions render complex feedback loops, across scales, affecting microbial processes like enzyme production and CO₂ efflux (Min *et al.*, 2018). Carbon stocks change continuously depending on organic C inputs (e.g. manures and crop residues) and the decomposition rate varies with the accessibility of microorganisms (Gunina and Kuzyakov, 2015). Soil organic matter is a complex and dynamic substance where its functions are maintained through continuous turnover (Six *et al.*, 2004; Janzen, 2006). However, organic matter turnover releases CO₂ through microbial respiration, which impacts how much C is stored in soils (Janzen, 2006).

No-till can reduce decomposition rates by approximately 10-20%, with functions such as of organic matter associated nutrient cycling being reduced too, although not halted completely (Lupwayi *et al.*, 2004; Janzen, 2006). Moreover, temporal patterns influence microbial communities, and dynamic changes can be rapidly triggered through resource addition such as N-inputs. Microbial decomposition is further influenced by physicochemical properties of soil, such as texture, temperature, and water content (Schmidt *et al.*, 2011; Kallenbach *et al.*, 2016; Sierra *et al.*, 2018). It has been argued that these properties determine the persistence of SOC/SOM more so than the chemical properties of the inputs (Schmidt *et al.*, 2011).

Carbon sequestration is a soil function that has received media attention as a solution to mitigate climate change through reducing atmospheric greenhouse gas concentrations (Amelung *et al.*, 2020; Gewin, 2020). A Google search on 02/07/2022 using the sentence “soil carbon sequestration to mitigate climate change”, returned 24,700 results indicating the high level of interest that is present for this topic. Increasing soil C stocks and/or stopping its decline remains central to some governmental goals in achieving agricultural sustainability (COP21, 2015; Defra, 2020). This is the main aim of the “Soil carbon 4 per mille” initiative, which aspires to increase stocks of soil organic matter by 0.4% per year on a global scale. The goal is to offset anthropogenic greenhouse gas emissions, help achieve Net Zero C emissions and limit warming to 1.5°C or keep it below 2°C (Minasny *et al.*, 2017; Hausfather and Forster, 2021). The authors suggest agricultural soils with low SOC have greater potential to increase C stocks. However, there is some controversy as to whether it is possible to achieve such an increase, which works against measures to reduce fertiliser use whilst maintaining profitable yields (van Groenigen *et al.*, 2017). Soils’ potential to sequester atmospheric carbon has been highlighted as often being exaggerated, which could consequently mask benefits associated with the protection of soils (Hernanz *et al.*, 2009; Amundson and Biardeau, 2018; Searchinger *et al.*, 2019).

There is a disparity of results from studies investigating no-till, with some showing increased C stocks and others not. This disparity arises from the specifics of each experiment such as crop diversity and frequency of crop rotation (Luo *et al.*, 2010; Virto *et al.*, 2012; Powlson *et al.*, 2014; Valboa *et al.*, 2015; Parthasarathi *et al.*, 2016; Meurer *et al.*, 2018; Mary *et al.*, 2020). Moreover, considering farming is a business it is important to understand potential causes of yield penalties if they occur and soil macronutrients dynamics in no-till systems (Watts *et al.*, 2006; Storr *et al.*, 2019). Therefore, this study investigated impacts of the application of N-fertiliser alone, and in combination with FYM, and the sowing of cover crops, subsequently acting as green on SOC/SOM dynamics under no-till, considering microbial biomass, physicochemical properties and crop productivity in a large scale, two-crop-years field experiment. Both SOM and SOC have been measured because the former is usually more accessible to farmers, but it has been suggested that a better approach is to use the latter (Abram, 2020). Two field experiments were established in the UK on arable land that was historically under a full inversion tillage. It was hypothesised that:

1. The effect of incorporating farmyard manure on SOC, SOM, and macronutrients is measurable over two-year period.
2. Cover crops will cause increase in microbial biomass, SOC and SOM.
3. The nutrient status of soil changes with the type of organic amendments.

4. Soil textural heterogeneity due to spatial variation has a greater impact on soil properties than inputs.
5. Yield penalties will be observed but the extend depends on soil heterogeneity.

3. Materials and Methods

3.1 Experimental Design

The experiments were established at Norbury Park, Staffordshire, United Kingdom (52°48'20.9"N, 2°17'49.9"W). Textural class of the field varied from clay loam to sandy loam. Experiment-1 consisted of three treatments: Farmyard Manure (FYM), Green Manure (GM) and Farmers' Standard Practice (SP = Control). Experiment-2 was on a sloping side of the field and excluded the FYM treatment. Spring wheat, *Triticum aestivum*, var. Mulika (with Beret Gold seed dressing) was direct drilled on all plots except GM plots. Fodder radish (*Raphanus sativus*) and vetch (*Vicia sp.*) were also direct drilled, seed ratio of 50:50, to act as green manure (GM) for the next crop season. The control plots were managed as per farmer standard practice (SP, i.e. using synthetic N at maximum rates permissible under RB209). Nitrogen fertiliser was applied too in the FYM plots to equalise total N inputs. The following crop season had winter oats (*Avena sativa* var. Mascani with Beret Gold seed dressing) which were direct drilled across all plots in both experiments. Refer to Table 1 for detailed field record.

3.2 Soil Sampling and Processing

Baseline soil sampling of Experiment-1 was conducted on 03/05/2017. Aboveground biomass sampling took place on 20/09/2017 for both experiments. Post-harvest soil sampling was done on 12/10/2017 for Experiment-1 and on 15/11/2017 for Experiment-2. Subsequent crop season had spring sampling of Experiment-1 on 30/04/2018, and in autumn soil on 02/10/2018 of Experiment-1 and 01/11/2018 of Experiment-2.

3.2.1 Experiment-1

Sampling points were selected using a random number table (Rand Corporation, 1955) to determine how far down each plot to sample. Twenty soil cores were collected from each sampling point using an auger (10 cm depth * 4.5 cm diameter). Plots were sampled individually, at two sampling points, with one composite sample of 20 cores produced per sample point, and a total of 40 soil cores per plot. Sampling was conducted at least one meter away from plot boundaries at all points. Soil samples were kept in a press-grip plastic bag in a cooler box whilst in the field and subsequently in a fridge overnight at 4°C. Stones

and plant residues were removed from soil before homogenising samples by sieving (4 mm mesh) and hand mixing fresh soil samples. Subsamples were prepared for microbial biomass and physicochemical analyses. All subsamples were and kept refrigerated at 4°C until analysis with microbial assays performed within 10 days of sample collection (Wang *et al.*, 2021).

3.2.2 Experiment-2

Each plot was sampled individually using the zig-zag sampling pattern (Krebs, 2014). Twenty soil cores were collected in total per plot using an auger (10 cm depth * 4.5 cm diameter). There were five sampling points from which four subsamples were collected to produce one composite sample per plot.

3.3 Soil Characteristics

Fresh homogenised soil samples were dried at 105°C for 48 h and gravimetric water content (GWC) determined. Soil organic matter (SOM) was estimated from loss on ignition, at 550°C for 4 h on oven-dried soils (Tan, 2005). Fresh soil was air dried at 30°C, ground to pass 2 mm mesh sieve and homogenised for all subsequent analyses. Soil texture was determined using the pipette method based on oven-dry weight. Particle sizes were categorised as: Clay <2 µm, Silt 2–20 µm, Sand 63–2000 µm (Tan, 2005). Soil organic carbon (SOC) was measured using the Leco SC-144DR Carbon/Sulfur Analyser at 840°C (Leco Corporation; USA). Quantification of pH was done in diH₂O solution (1:5, soil:water ratio) (Tan, 2005) and read on a pH meter (Jenway 3510, UK).

3.4 Crop Analysis

Parameters associated with crop productivity such as grain yield, thousand grain weight (TGW), protein content of grain and biomass and cover crop aboveground biomass were quantified. Cash crop parameters were measured twice during this two-year study, after harvest in autumn of both Experiments 1 and 2. Grain of *Triticum aestivum*, var. Mulika (spring wheat) and *Avena sativa* var. Mascani (winter oats) were directly bagged in the field during harvest using a plot harvester for each plot and subsequently weighed to calculate yields per replicate (t ha⁻¹). Three sub-samples of grain were collected using a 0.5 l jug to sample from the grain bags. Grain was dried at 60°C and moisture content determined (w/w, %) using a DICKEY-john GAC[®] 2500-UGMA grain analysis computer. Afterwards, Thousand Grain Weight (TGW, g) was quantified by randomly collecting and weighing 100 grains and repeating the process five times for each dried subsample. The mean weight was then used

to estimate the weight of a thousand grains. All reported yields (t ha^{-1}) and TGW values (g) were standardised to 14.5% moisture content (Mulvaney and Devkota, 2020).

Aboveground biomass was sampled for the cover crop, *Raphanus sativus* and *Vicia* sp., using the quadrat method. On each GM plot, three quadrats of 1 m^2 were placed randomly within each plot, 2 m away from the edge of each plot, and used to cut whole plants 10 cm from the ground. The plants were dried at 60°C and aboveground biomass quantified (t ha^{-1}). The cereals grain and cover crop biomass were separately ground to 0.5 mm. Total N (tN%) analysis was conducted by the dry combustion method (950°C) using Leco FP528 (EVISA, EU). Protein content was calculated by using the conversion factor of 6.25, which assumes that 16% of protein content is nitrogen (Tomé *et al.*, 2019).

3.5 Chemical Analysis

The soil nutrient status was determined by measuring the standard macronutrients required for plant growth: phosphorus (Olsen-P), extractable potassium (K^+) and magnesium (Mg^{2+}). In addition, extractable calcium (Ca^{2+}), total nitrogen (%tN) and total sulfur (%tS) were also measured alongside pH and gravimetric water content (GWC). In Experiment-1, the nutrient status of soil was measured four times, twice per crop season, once in Spring and again in Autumn. In Experiment-2, soil samples were collected once per crop season after harvest in autumn. The macronutrients analysed were selected for their role as the major nutrients required for crop growth and which are measured routinely in accordance with standard agricultural procedure (Lines-Kelly, 1992; Defra, 2010). Additionally, soil's gravimetric water content (GWC) and pH values were quantified because they highly influence the solubility and availability of nutrients (Rowell, 1994). Extractable- K^+ , Mg^{2+} and Ca^{2+} ions were extracted with 1 M NH_4NO_3 (MAFF/ADAS, 1986). Their fractions were analysed by Atomic Absorption Spectrometry (AAS). Phosphorus was extracted using the Olsen-P method in 0.5 M NaHCO_3 solution adjusted to pH 8.5 at 20°C . Absorbance of the final blue complex concentration was read using Jenway 6305 UV/Vis spectrophotometer at 880 nm (Tan, 2005).

Table 1: Field record of both experiments

		Field Information				Manufactured Fertilisers				Organic Manures				Crop Protection			
Field Name	Field Area	Drilling Date	Crop	Rate	Drill Type	Date	Type	Nutrients (%)	Rate	Date	Manure Type	Rate (t ha ⁻¹)	N Content (%)	Method of Application	Date	Active Ingredient	Rate
Both experiments	2.5 ha					07/04/2017	LimeX70 (CaCO ₃ precipitate)	52% Ca, 1% P, 0.7% Mg, 0.6% S	12.5 Mg ha ⁻¹								
Experiment 1, FYM + N-fert treatment	6 x (200 x 6 m) plots	14/04/2017	Spring wheat, <i>Triticum aestivum</i> , var. Mulika (with Beret Gold seed dressing)	150 kg ha ⁻¹	Weaving GD3001T Direct Disc Drill	04/05/2017	Nitram	34.5% N	150 kg ha ⁻¹	13/04/2017	Cattle FYM	40 Mg ha ⁻¹	2.2	Surface spread and incorporated by discing	20/09/2017	Ally Max SX and Duplosan	42 g ha ⁻¹ and 1 L ha ⁻¹ in water 200-240 L ha ⁻¹
Experiment 1, N-fert treatment	6 x (200 x 6 m) plots	14/04/2017	Spring wheat, <i>Triticum aestivum</i> , var. Mulika (with Beret Gold seed dressing)	150 kg ha ⁻¹	Weaving GD3001T Direct Disc Drill	04/05/2017	Nitram	34.5% N	125 kg ha ⁻¹						20/09/2017	Ally Max SX and Duplosan	42 g ha ⁻¹ and 1 L ha ⁻¹ in water 200-240 L ha ⁻¹
Experiment 1, Cover Crops treatment	6 x (200 x 6 m) plots	14/04/2017	Fodder radish (<i>Raphanus sativus</i>) and vetch (<i>Vicia sp.</i>)	29 kg ha ⁻¹ , seed ratio of 50:50	Weaving GD3001T Direct Disc Drill												
Experiment 2, N-fert treatment	9 x (24 x 6 m) plots	14/04/2017	Spring wheat, <i>Triticum aestivum</i> , var. Mulika (with Beret Gold seed dressing)	150 kg ha ⁻¹	Weaving GD3001T Direct Disc Drill	04/05/2017	Nitram	34.5% N	125 kg ha ⁻¹						20/09/2017	Ally Max SX and Duplosan	42 g ha ⁻¹ and 1 L ha ⁻¹ in water 200-240 L ha ⁻¹
Experiment 2, Cover Crops treatment	8 x (24 x 6 m) plots	14/04/2017	Fodder radish (<i>Raphanus sativus</i>) and vetch (<i>Vicia sp.</i>)	29 kg ha ⁻¹ , seed ratio of 50:50	Weaving GD3001T Direct Disc Drill												
Experiment 1 and 2, FYM + N-fert, N-fert, and Cover Crops treatments	18 x (200 x 6 m) plots	18/10/2017	Winter oats (<i>Avena sativa</i> var. Mascani with Beret Gold seed dressing)	160 kg ha ⁻¹	Weaving GD3001T Direct Disc Drill	09/11/2017	Muriate of potash	60% KCl	100 kg ha ⁻¹						16/10/2017 and 23/10/2017	RoundUp Bioactive GL	1.5 L ha ⁻¹ in 200 L of water
Experiment 1 and 2, FYM + N-fert, N-fert, and Cover Crops treatments															26/10/2017	Slug pellets (3% metaldehyde)	7 kg ha ⁻¹
Experiment 1 and 2, FYM + N-fert, N-fert, and Cover Crops treatments						07/05/2018	Calcium nitrate fertiliser	15.5% N + 26.3% Ca ²⁺ O ²⁻	100 Kg ha ⁻¹						19/04/2018	Nevada, Dow AgroScience	1.0 L ha ⁻¹ , 200 L of water ha ⁻¹

3.6 Quantification of Microbial Biomass Chloroform Fumigation

Microbial biomass carbon and nitrogen were analysed using the chloroform fumigation direct extraction method for all fresh soil samples (see Chapter 2) (Vance, Brookes and Jenkinson, 1987), (Brookes, 2001). Two 10 g aliquots of fresh soil were subsampled from each composite sample. One subsample was kept refrigerated at 4°C until extraction with the lid tightly fitted. The other subsample was fumigated in a desiccator containing ~50 ml ethanol-free chloroform and anti-bump crystals for 48 h in the dark. Total soluble soil carbon was extracted from both samples in 0.5 M K₂SO₄ by horizontal shaking for 1 h and filtered through Whatmann 42 ash-less filter paper. Microbial biomass carbon (MBC) and nitrogen (MBN) were quantified on a TOC (Analytik Jena AG TOC/TN, U.K.) and results calculated by subtracting the fumigated by the non-fumigated samples using the formulae:

$$MBC = \frac{(F_C - nF_C)}{K_C} \qquad MBN = \frac{(F_N - nF_N)}{K_N}$$

Where: F = fumigated sample, nF = non-fumigated sample, K = constant ($K_C = 0.45$, $K_N = 0.54$) (Jenkinson, Brookes and Powlson, 2004).

3.7 Statistical Analysis

Statistical analyses were conducted in R-Studio (R version 4.1.0 (2021-05-18) "Camp Pontanezen" (R Core Team, 2021)), and the packages "correlation", "corrplot", "tidyverse", "ggpubr", "rstatix", "rcompanion", "Hmisc", and "psych" (Wickham *et al.*, 2019; Kassambara, 2020, 2021; Mangiafico, 2021; Wei *et al.*, 2021; Harrell Jr, 2022; Makowski, Dominique Wiernik *et al.*, 2022; Revelle, 2022).

Raw data was visualised using boxplots, and normality of data was tested using the Shapiro-Wilk test and QQ-plots. Tukey's ladder transformation of data (Zuur *et al.*, 2009) was used where data did not satisfy the necessary assumptions of linear regression. The transformed variables were: GWC in May-2018 and MBC with the transformation applied = x^λ ; P in October-2017 with the transformation applied = $\log(x)$; P in May-2017, %tN, %tS, Ca in October-2017, %tS, P in May and October-2018, Mg in November-2017, Clay, Silt, Mg in November-2018 with the transformation applied = $-1 * x^\lambda$.

Linear regression models were run with continuous response variables (i.e. SOC, SOM, MBC, MBN, tN, tS, GWC, pH, P, K, Mg, Ca, grain yield, TGW, grain and cover crop protein content or cover crop biomass), which were the target variables, with treatments (categorical variable: FYM, GM, SP) as the explanatory variables for each temporal observation (May, October or November 2017-2018), and/or with soil texture included (clay, silt and sand). Relationships between response variables and both the interaction and main effects of treatment and sampling period with soil texture were assessed using factorial ANOVA

models. Tukey's Honest Significant Difference *post-hoc* tests (Tukey HSD) were computed on significant models ($p \leq 0.05$) to perform multiple pairwise-comparisons between the group means. Results are presented as means \pm standard error unless indicated otherwise. Factorial ANOVA regression models were also run with SOC as the continuous response variable with treatment (categorical variable: FYM, GM, SP) as the explanatory variable for each temporal observation (May, October, or November 2017-2018). The other explanatory variables added were MBC and MBN, Ca^{2+} and tN, pH and GWC or soil texture. Tukey Honest Significant Difference *post-hoc* tests were computed on significant models ($p \leq 0.05$) for all individual comparisons. The Pearson correlation test was applied between pairs of variables, i.e. SOC, SOM, tN, tS, GWC, pH, Olsen-P, K^+ , Mg^{2+} , Ca^{2+} , Clay, Silt and Sand. The p-value was adjusted to the Holm method (1979) and set to significant level at >0.05 . Stronger correlations near -1 or 1, and a value of $r = \emptyset$ indicates independence of variables (Table 2).

Table 2: Interpretation of the Pearson's correlation coefficient (Akoglu, 2018).

Strength of Association	Coefficient, r	
	Positive	Negative
None	0	0
Weak	0.1 to 0.3	-0.1 to -0.3
Moderate	0.3 to 0.6	-0.3 to -0.6
Strong	0.6 to 1.0	-0.6 to -1.0

4 Results

4.1 Soil Properties

Soil Texture

Most soil types in Experiment-1 were classified in the categories of sandy loam (48.4%) and sandy clay loam (45.2%). Clay loam soil made up 4.0% and loamy sand 2.4% of the soil types. Soil type of Experiment-2 was dominated by loamy sand soil (74%) and the remaining 26% was categorised as sandy loam soil (Table 3).

Table 3: Soil texture classes identified in each experiment.

Texture Class	Experiment-1 (%)	Experiment-2 (%)
Loamy Sand	2.4	73.5
Sandy Loam	48.4	26.5
Sandy Clay Loam	45.2	0.0
Clay Loam	4.0	0.0

pH and GWC

May-2017 was the only period that exhibited significant pH differences between the SP treatment and FYM ($p = 0.01$) or GM ($p = 0.003$) of Experiment-1. No significant differences in pH were observed in Experiment-2 between treatments over time, (Nov-2017 $p = 0.8$ and Nov-2018 $p = 0.2$) (Table 4). No significant differences were observed between treatments in soil gravimetric water content (%GWC) in either Experiment-1 and 2.

Table 4: Values of soil pH on a temporal scale and respective \pm standard deviation of the mean for both Experiment 1 and 2 (E1 $n = 6$; E2 SP $n = 9$, GM $n = 8$).

Treatment	Experiment-1				Experiment-2	
	May-17	Oct-17	May-18	Oct-18	Nov-17	Nov-18
SP	7.0* (± 0.12)	6.6 (± 0.19)	6.8 (± 0.26)	6.6 (± 0.36)	6.2 (± 0.24)	6.2 (± 0.28)
FYM	6.8 (± 0.09)	6.6 (± 0.07)	6.8 (± 0.28)	6.6 (± 0.23)		
GM	6.7 (± 0.22)	6.8 (± 0.38)	6.9 (± 0.17)	6.7 (± 0.28)	6.2 (± 0.26)	6.4 (± 0.36)

Macronutrients in Soil

Total nitrogen supply was shown to increase soon after FYM (FYM characteristics: OM = 71%, %tN = 2.2%) was spread and incorporated in May-2017 (Fig. 1) but was only marginally non-significant ($p = 0.06$). The yields obtained in the FYM treatment were comparable to those in the SP treatment which received N-fertiliser alone (Fig. 1). While the GM treatment did not receive N-fertiliser in 2017 it achieved equivalent concentrations of %tN in October-2017, showing an increase from 0.18 to 0.21%. This result was analogous to

SP where %tN increased from 0.18 to 0.22%. Both GM and SP started at the same %tN level, but the different inputs delivered similar results.

An increase in Olsen-P in the SP and FYM treatments of Experiment-1 sampled in May-2018 and October-2018 was observed, but an increase in the Olsen-P in the GM treatment was only seen in October-2018 (Fig. 1). It was found that %tN effect on Olsen-P was temporal, i.e. observed in October 2017 ($p = 0.03$) and 2018 ($p = 0.04$), with a lag period from N inputs, and related to spatial variation as it was only observed with the results of Experiment-1.

The FYM treatment of Experiment-1 and GM (*R. sativus* and *Vicia sp.* mix cover crop) of Experiment-2 offered a supply of potassium (K^+). In Experiment-1, an average of around 50% more exchangeable- K^+ was measured in the FYM ($p = 0.01$) treatment than in the SP or GM treatments in May-2017, three weeks after incorporating FYM (Fig. 1). This effect continued to be significantly higher in October 2017 ($p = 0.01$) and 2018 ($p = 0.002$). The effect from the cover crop was only observed in the GM treatment of Experiment-2 in November-2018 ($p = 0.001$).

Exchangeable-Mg ($\text{mg Mg}^{2+} \text{ l}^{-1}$) was only significantly different between SP and GM in November-2018 ($p = 0.04$) of Experiment-2 (Fig. 1). The higher concentration of exchangeable- Ca^{2+} across all treatments in May-2017, in comparison with other periods, likely occurred in response to liming (Fig. 1). Ca^{2+} concentrations returned to previous levels from October-2017 onwards. The effect of FYM on sulfur (%tS) concentration in soil was observed in October-2018, but it was quite varied as shown by the standard error of the mean (Fig. 1). Fresh cattle FYM, as applied in this study, can be a source of sulfur because it typically averages $2.4 \text{ kg SO}_3 \text{ t}^{-1}$ (Defra, 2010).

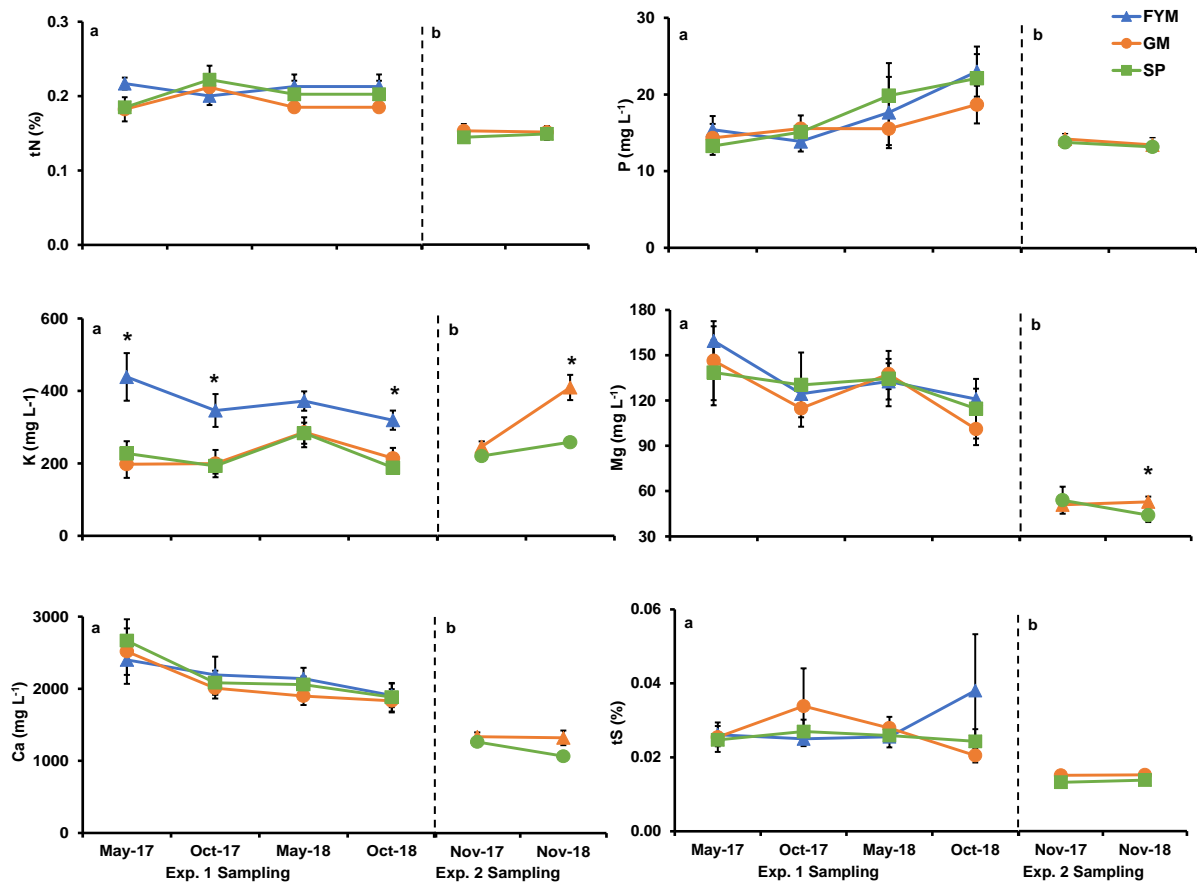


Figure 1: Experiment 1 and 2 (a and b) mean results of soil % total nitrogen (%tN), phosphorus (mg Olsen-P L⁻¹), potassium (mg K⁺ L⁻¹) and magnesium (mg Mg²⁺ L⁻¹), extractable calcium (mg Ca²⁺ L⁻¹) and total sulfur (%tS). Four sampling sessions (May and October 2017-2018) were conducted on Experiment-1 (a), and two sampling sessions (November 2017-2018) on Experiment-2 (b). Treatments: Farmyard manure (FYM in blue); Green manure (GM in orange); Standard practice (SP in green). Bars represent \pm standard error of the means. Asterisk (*) show significant differences between treatments. Experiment-1 n = 6; Experiment-2 GM n = 8, SP n = 9.

4.2 Microbial Biomass

Experiment-1

A factorial ANOVA model was applied to investigate the effect of treatments over time on MBC ($\mu\text{g g}^{-1}$) (Fig. 2). There was a significant interaction between the different treatments and sampling period ($p = 0.03$). The relationship between treatments and sampling periods was compared to identify where the differences occurred using Tukey's Honest Significance Difference test. MBC was significantly higher in the FYM May-2017 treatments in comparison to GM (May-Oct 2017-2018 $p < 0.001$, $p = 0.01$, $p = 0.01$ and $p < 0.001$) and SP (May-Oct 2017-2018 $p = 0.01$, $p = 0.001$, $p = 0.005$, $p < 0.001$) and FYM (Oct-2018 $p < 0.001$). No significant interaction effect was observed between treatment and sampling period for the amount of MBN ($\mu\text{g g}^{-1}$) ($p = 0.5$) (Fig. 2). There was, however, an effect observed from treatments ($p = 0.001$) or sampling periods ($p < 0.001$). In May-2017, MBN was significantly higher in FYM in Oct-May 2017-2018 than in the GM treatment ($p = 0.004$, $p = 0.004$ respectively) or SP ($p = 0.004$, $p = 0.004$ respectively). It was also lower in the GM Oct-2018 treatment than in FYM Oct-2017 ($p = 0.02$) and May-2018 ($p = 0.02$).

Experiment-2

A significant response by MBC ($p = 0.03$) or MBN ($p = 0.04$) to treatments and sampling period were identified when tested using factorial ANOVA models (Fig. 2).

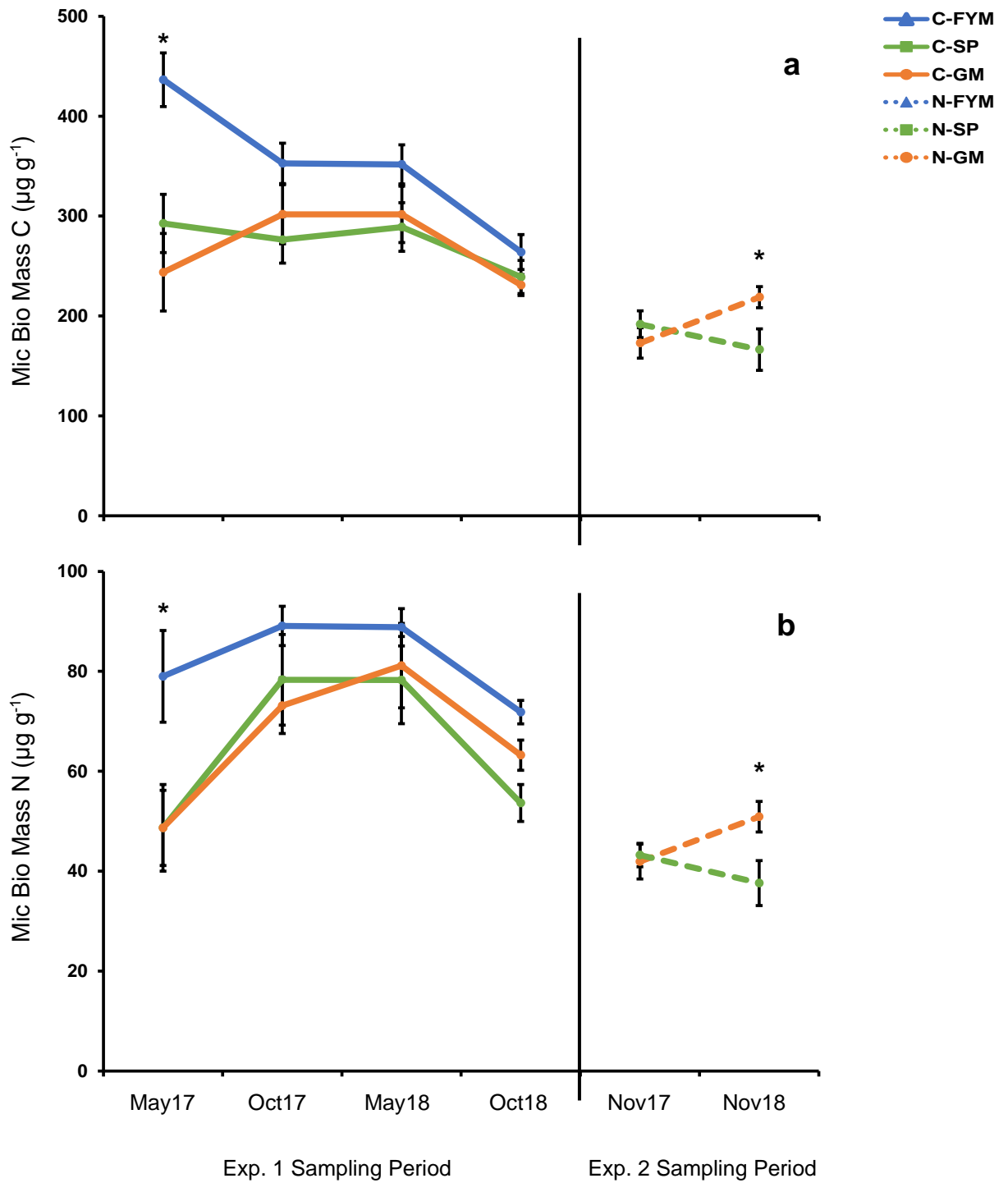


Figure 2: **a**) Microbial biomass **C**arbon ($\mu\text{g Mic C g}^{-1}$) and **b**) **N**itrogen ($\mu\text{g Mic N g}^{-1}$ of soil) change over time from the start of Experiment-1 (solid lines) in May 2017 to completion in October 2018, and Experiment-2 (dotted lines) in November 2017 and 2018. Experiment-1 treatments: FYM = Farmyard Manure, $n = 6$; SP = Standard Practice, $n = 6$; GM = Green Manure, $n = 6$; \pm standard error. Experiment-2 treatments: SP = Standard Practice, $n = 9$; GM = Green Manure, $n = 8$; \pm standard error. Asterisks symbolise significant differences between groups that were identified from Tukey HSD *post-hoc* test.

4.3 SOC and SOM Response to Treatments

The same pattern could be observed with both SOC and SOM over time (Fig. 3) as expected, and analysis of variance (ANOVA) confirmed the lack of interaction effect with either variable in relation to treatment too. There was no statistical interaction between Experiment-1 treatment and sampling period on either SOC or SOM ($p = 0.8$, $p = 1.0$, respectively). As in Experiment-1, there were no significant treatment effects within the first year of Experiment-2 (2017) nor were there detectable legacy effects (2018) on the proportion of both SOC ($p = 0.7$) or SOM ($p = 0.8$). Therefore, the decision to use only the SOC variable from now on was taken.

4.4 SOC and Interaction Effects

Experiment-1

ANOVA results indicated that a change in microbial biomass carbon (MBC) would significantly affect the concentration of SOC ($p = 0.001$). So would the interaction between treatments, sampling period and MB-Nitrogen (MBN) ($p = 0.04$). HSD *post-hoc* tests were performed in multiple pairwise-comparison between the means of the groups and no significant pairwise comparisons were identified.

The model looking at the interaction effect between treatment and sampling period with or without calcium (Ca^{2+}) or total nitrogen (tN) identified a significant interaction with Ca^{2+} ($p < 0.001$) and with the interaction between tN and treatment ($p = 0.03$) or sampling period ($p = 0.03$). Once again, no significant pairwise comparisons were computed.

Another model showed that the interaction between treatments and sampling periods with gravimetric water content (GWC, $p = 0.003$) or pH ($p = 0.02$) had an association with SOC without any significant pairwise comparisons.

The effects of soil texture were also tested and the amount of sand ($p = 0.02$) or silt ($p = 0.02$) were associated with the concentration of SOC, but not pairwise comparisons showed any significant treatments interaction with or without sampling period.

Experiment-2

No significant interaction effects between treatment, sampling period and MBC ($p = 0.1$) or MBN ($p = 0.5$) on SOC were observed. Similarly, no significant interactions were found when Ca^{2+} ($p = 0.9$) and tN ($p = 0.6$) were added to the model instead of microbial biomass. An association with either Ca^{2+} ($p < 0.001$) or tN ($p < 0.001$) with SOC were however detected. A significant treatment effect ($p = 0.002$) was observed in this complex model that had not been observed with the previous factorial ANOVA model looking at only the effect of

treatment and sampling period. But the interaction between either treatment and Ca^{2+} or tN were not observed.

The effect of soil texture on SOC was associated with either the proportion of clay ($p < 0.001$) or sand ($p < 0.001$) in soil, but not by the interaction effects with treatments and sampling periods ($p = 0.7$, $p = 0.5$ respectively) (Fig. 4). Similarly, no significant interaction effects were computed when the complex ANOVA model replaced soil texture with pH values ($p = 0.9$) and GWC ($p = 0.4$). However, there was a significant response from SOC to pH ($p = 0.01$) or to GWC ($p < 0.001$), but not determined by treatment effect.

4.5 Correlations Between the Pairs

The correlation matrix found many significant associations between pairs both unidirectional and following an opposite trend (Fig. 5). Strong positive correlations were found between the clay and SOM ($r = 0.8$), Ca^{2+} ($r = 0.8$), Mg^{2+} and %tN ($r = 0.7$). Whereas, as the amount of sand increased so do other properties decreased either moderately (i.e. SOC or pH, $r = -0.6$; MBC or MBN or %tS, $r = -0.5$) or strongly (i.e. %tN, $r = 0.6$; SOM or Ca^{2+} or Mg^{2+} , $r = 0.8$).

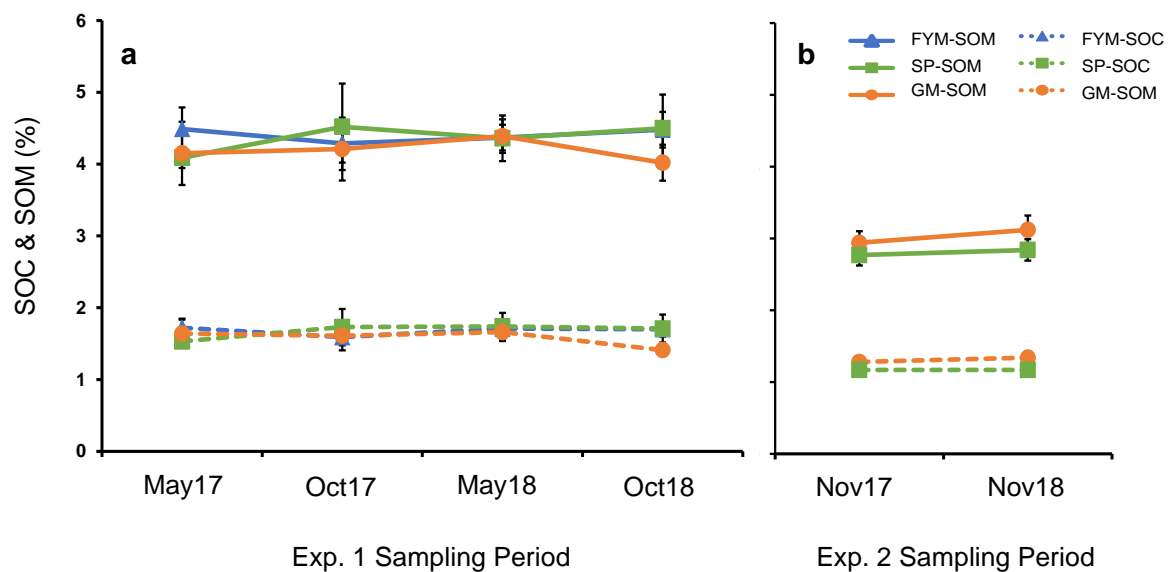


Figure 3: Time series of SOC and SOM results from Experiment 1 (a) and Experiment 2 (b). Soil sampling was done in a) May and October 2017-2018, and in b) November 2017-2018. The treatments were Farmyard Manure (FYM), Green Manure (GM), and Standard Practice (SP). SOC is represented by the dotted lines, and SOM by the full lines. Plot a $n = 6$, Plot b GM $n = 8$, SP $n = 9$, \pm SEM.

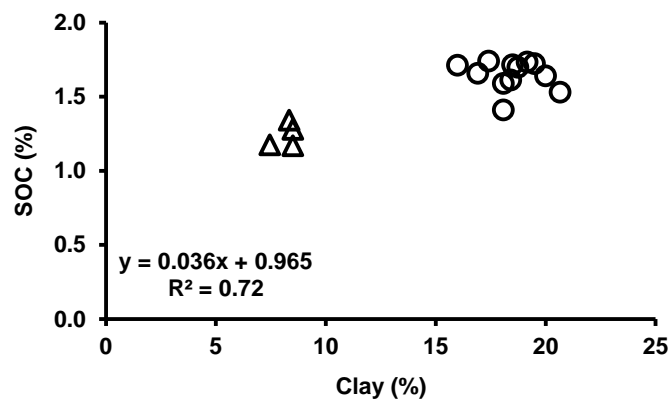


Figure 4: Regression plot indicating the linear relationships between the amount of clay particles in soil texture and soil organic carbon (SOC) using treatment mean data from both Experiment 1 (in circles) and 2 (in triangles) sampled over the two-year experimental period.

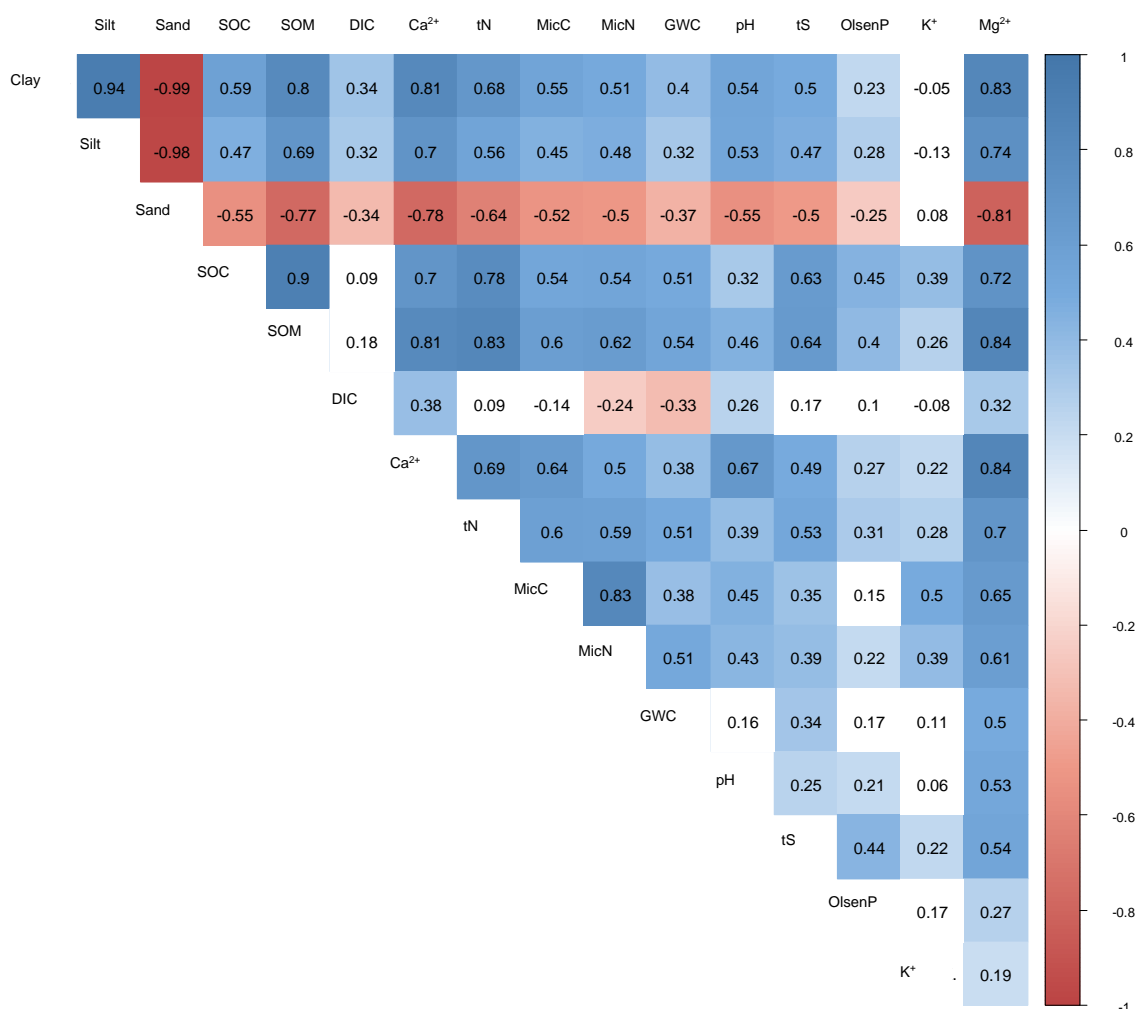


Figure 5: Correlogram of correlation coefficients for pairs of variables from both Experiment 1 and 2. Positive correlations are displayed in blue and negative correlations in red. The intensity of the colour is proportional to the correlation coefficient. The stronger the correlation by being nearer to $r = -1$ or $r = 1$, the darker the boxes are. The white boxes symbolise no significant quantifiable correlation between the pairs.

4.6 Cereals

Experiment-1

The average spring wheat 2017 yield was 1.2 t ha^{-1} ($\pm 0.2 \text{ SD}$) in both the SP and FYM treatments. Wheat yields in the UK in 2017 were on average 8.5 t ha^{-1} (Defra, 2017), There were no significant differences in yield ($p = 0.4$) (Fig. 6), TGW ($p = 0.9$) or grain protein ($p = 0.6$) between the SP and FYM 2017 treatments. In 2018, the yields of winter oats, TGW and grain protein were not significantly different either between the SP and FYM ($p = 1.0$, $p = 0.4$, $p = 0.1$, respectively) or GM treatments ($p = 0.3$, 0.5 , 0.3 , respectively). The average yield was 5.1 t ha^{-1} on both SP ($\pm 0.5 \text{ SD}$) and FYM ($\pm 0.7 \text{ SD}$) treatments, and 5.4 t ha^{-1} ($\pm 0.8 \text{ SD}$) in the GM treatment, which was equivalent to the 2018 yearly UK average of 5.0 t ha^{-1} (Defra, 2019).

Experiment-2

Harvest 2018 resulted in significantly greater yields of winter oats ($p = 0.01$) and respective grain protein ($p = 0.02$) in the GM treatment in comparison with SP (Fig. 6). However, yields on this slope side of the field were much lower than the UK average; SP = 2.1 t ha^{-1} and GM = 2.5 t ha^{-1} versus UK average = 5.0 t ha^{-1} . TGW was significantly lower in the GM ($p < 0.001$) treatment in comparison with SP with a reduction of 4.4 g (± 0.1) on average.

4.7 Cover crop

Experiment-1 and 2

The aboveground biomass of the cover crops (*R. sativus* and *Vicia sp. mix*) was greater in Experiment-2 than in Experiment-1 (Fig. 6). However, the difference was marginally non-significant ($p = 0.06$) and may have been caused by differences in soil properties.

No significant difference in protein content of the cover crop aboveground biomass was identified, with results ranging from $24\% \pm 0.3$ in Experiment-1 and $23\% \pm 0.2$ in Experiment-2.

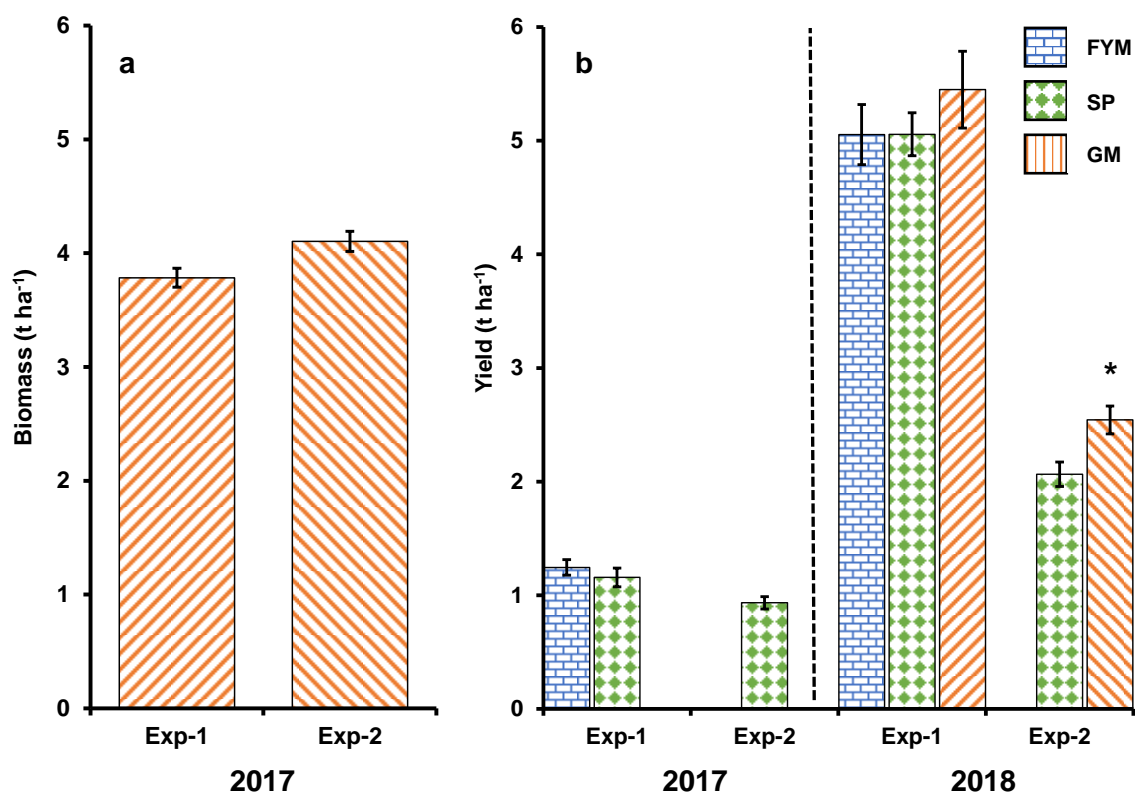


Figure 6: **a)** Aboveground biomass ($t\ ha^{-1}$) of *Raphanus sativus* and *Vicia* sp. cover crop mix from both Experiments 1 and 2 (Exp-1 and Exp-2) in 2017 crop season ($n = 6$ and $n = 8$, respectively), and **b)** grain yields for both experiments of two crop seasons, 2017 and 2018. In 2017, Spring wheat (*Triticum aestivum*) was only drilled in the Standard Practice (SP) and Farmyard Manure (FYM) Exp-1 treatments, and in Exp-2 SP treatment; a cover crop was drilled in the GM plots – hence the missing columns there. Winter oats (*Avena sativa*) were direct drilled in 2017 and harvested in Summer 2018 (Exp-1 and Exp-2) on all treatment plots. Columns show means (E1 $n=6$; E2, SP $n = 9$, GM $n = 8$); Error bars show \pm standard error of the mean. Asterisk (*) symbolises significant difference ($p < 0.05$) between treatments.

4.5 Discussion

Focus has been increasing on identifying optimum management practices to increase soil carbon stocks, reduce fertiliser inputs and associated greenhouse gas emissions, (NFU, 2019; Searchinger *et al.*, 2019; Amelung *et al.*, 2020; CCC, 2020). Thus, understanding the short-term interaction between inputs to soil in a no-till arable system on SOC and SOM, along with microbial biomass and soil properties such as Extractable Ca^{2+} (a key factor in SOM stabilisation (Rowley *et al.*, 2018)) and other cations, total-Nitrogen (%tN), pH, gravimetric water content (GWC) and soil texture is key to developing best management practices. The results presented here demonstrated that the type of organic material input to soil (i.e. FYM or cover crops), at the amounts used for this study (Experiment-1: 8.9 Mg OM ha⁻¹ for FYM and 3.8 Mg OM ha⁻¹ for 50:50 cover crops (CC); Experiment-2: 4.1 Mg OM ha⁻¹ for CC) did not significantly impact SOC but did impact microbial biomass and soil nutrient status over a two-year period. Soil characteristics (i.e. %clay, %sand or %silt) were often a better predictor of SOC and MBC differences than the type of organic amendment. This result supports the findings of other studies such as Schmidt *et al.* (2011) and Lehmann and Kleber (2015) who reported that ecosystem properties such as conditions for enzymatic accessibility were more effective predictors of organic matter turnover than the chemical properties of the organic amendments. Moreover, spatial and temporal variations impacted treatment outcomes as observed with the variable response of the nutrient status of soil. In fact, other studies have also confirmed that organic amendments added to soils are mineralised fairly quickly influencing the availability of nutrients for crop uptake (Watts *et al.*, 2006; Gan *et al.*, 2020; Bullock *et al.*, 2021; Berthelin *et al.*, 2022). Fertiliser costs can be prohibitive to buy but are essential to produce profitable yields and ensure food security. FYM and/or cover crops could be a viable method in ensuring food productivity whilst aiding the reduction of synthetic fertilisers use helping prevent or reduce environmental pollution. The results presented here provide an arable system case study on the short-term effects of conversion to no-till under three different management practices, namely FYM, cover crops and N-fertiliser alone.

Soil management practices promoted as having potential to increase soil C stocks are often associated with reduced tillage, retaining of crop residues/stubble, crop rotation, sowing of N-fixing plants, and incorporating manures (Mutegi *et al.*, 2011; Amundson and Biardeau, 2018; Abdalla *et al.*, 2019; Baveye *et al.*, 2020). However, these practices do not always lead to increased C stocks (Buisse *et al.*, 2013; Powlson *et al.*, 2014; Mary *et al.*, 2020), as was observed in this study. It has been suggested that, in the long-term, occasional tillage might be needed to enhance the ability of C stock stratification because gains have been limited to the top 10 cm of soil in no-till systems (Minasny *et al.*, 2017). There are, however, other benefits that can be incentivised by these practices like increased microbial abundance

and diversity associated with ecosystem resilience (Zuber and Villamil, 2016), and other examples outside the scope of this paper such as increased earthworm abundance (Briones and Schmidt, 2017) and improved soil structure (Ayoubi *et al.*, 2012; Buysse *et al.*, 2013).

Increases in SOC are expected to be substantial on sites where initial C stocks are low (Six and Jastrow, 2002; Virto *et al.*, 2012; Amelung *et al.*, 2020). However, soil has limited capacity of storing C which is influenced by its textural properties, in particular clay and silt content (Six and Jastrow, 2002; Schweizer *et al.*, 2021). Studies have found that the level of soil disturbance, i.e. tilling or not, had no impact on SOC stocks in long-term experimental sites when the soil layer was measured to 40 cm depth (Valboa *et al.*, 2015; Meurer *et al.*, 2018; Mary *et al.*, 2020). Stratified sampling showed that C accumulated in the topsoil at 5 cm depth in a no-till study (Mary *et al.*, 2020) and at 10 cm depth in a reduced tillage study (Valboa *et al.*, 2015). This was offset by the lower C stocks being accumulated deeper in comparison with full inversion tillage. SOC stocks were 22.9 t C ha⁻¹ after 47 years in the former no-till treatment with a baseline of 16 t C ha⁻¹ in a silty clay loam soil. These values were analogous to the ones estimated in this study, 22.0 t C ha⁻¹, in Experiment-1 where the soil was classified as sandy clay loam corresponding to both being heavy textured soil types (bulk density = 1.33 g cm⁻³) with clay content above 20% (Rowell, 1994). Whereas in the latter study, in 12 years, it increased from ~13 t C ha⁻¹ to ~16 t C ha⁻¹ in a sandy loam medium soil. Xion *et al.* (2015) found that SOC accumulation was on average 46.6 t C ha⁻¹ (20 cm depth, estimate of 23.3 t C ha⁻¹ in the top 10 cm) in light soils (bulk density = 1.33 g cm⁻³). In the current study, increases in SOC stocks were more likely to be observed on patches with light and medium texture soil classes, but this was not recorded during the two-year study period. Results from other studies suggest that the heterogeneity of soil in the current study may have led to increases being observed in the topsoil (10 cm depth) in the patches with the textural class of loamy sand (17.4 t C ha⁻¹ Experiment-2 estimate) and sandy loam (estimated at 9.9 t C ha⁻¹ in Experiment-2 and 13.2 t C ha⁻¹ in Experiment-1). However, one study contradicts the correlation between soil texture and SOC stocks. The authors reported that SOC increased by 6.7% (30 cm depth) following the conversion from full inversion tillage to no-till, but this was not associated with the proportions of clay, sand or silt (Virto *et al.*, 2012). Other factors in addition to soil texture influence SOC stocks, such as microbial-SOC dynamics, land-management, precipitation and temperature, and these should be considered to better understand the mechanisms behind changes in SOC stocks (Powlson, Whitmore and Goulding, 2011).

A positive effect on SOC from the interaction between treatment, i.e. FYM, and MBC was detected in May-2017 in Experiment-1. In Experiment-2, the effect was also seen but without

a response from the interaction with treatment and/or sampling period. Microbial biomass carbon and nitrogen (MBC and MBN) responded quickly to the incorporation of FYM, and significant greater biomass was observed in comparison with the SP and GM treatments around three weeks after FYM application. These results confirm that microbial biomass quantification provides early insights into changes in management practices where other changes in soil properties such as SOC or SOM may take years to show a significant response to regular organic inputs (Powlson *et al.*, 1987; Brookes, 2001; Erkossa and Stahr, 2015; Heuck *et al.*, 2015). Incorporating FYM into soil offers a readily available source of labile organic matter that triggers processes such as microbial decomposition and nutrient mineralisation and immobilisation to occur (Brookes, 2001; Gan *et al.*, 2020; Liang *et al.*, 2022). Regular organic inputs such as FYM introduces C into the system, and improves soil structure and water holding capacity, which all are conducive to increasing SOC stocks (Powlson, Whitmore and Goulding, 2011). However, benefits to SOC can quickly dissipate due to sporadic inputs over a short period of time as reported by this study (Berthelin *et al.*, 2022).

Calcium plays an important role in the stabilisation of SOC through cationic bridging and its loss through leaching can reduce soils' productivity potential (Eldor, 2016). In agriculture, liming (e.g. spreading of Limex70 a CaCO_3 precipitate) is a management practice done to adjust soil pH levels and to neutralise soil acidity. However, it can lead to a cycle of necessary remediations because acidification of soil through N-fertilisation can occur, with CO_2 being released to the atmosphere and Ca^{2+} leaching (Zamanian *et al.*, 2021). A positive association between Ca^{2+} and SOC was detected in both experiments of this study, possibly linked to Ca-bridging, and with total nitrogen (%tN) too (Rowley *et al.*, 2018). Similarly, studies have found that liming led to an increase in SOC accumulation within aggregates. The increase was dependent on aggregate size, which could be linked to greater concentrations of Ca^{2+} (Six *et al.*, 2004; Briedis *et al.*, 2012). The liming done in April 2017 is likely to have impacted the greater concentrations of extractable Ca^{2+} in soil in that season, but this effect was no longer present in year-2. The high levels of heterogeneity of extractable Ca^{2+} could relate to soil texture, but respiration processes from roots and microbes are potential causes too but are outside the scope of this study.

Adopting no-till along with soil amendments such as fertilisers and manures did benefit the nutrient status of soil albeit influenced by spatial and temporal variations. For example, the greater concentration of exchangeable- Mg^{2+} in Experiment-2 suggests having benefited from cover crops' legacy effect. Magnesium is comparatively mobile in soils in contrast with other cations like K^+ , Ca^{2+} , and NH_4^+ , and forms weaker bonds to soil mineral charges (CEC).

However, a meta-analysis concluded that the concentrations of Mg^{2+} quantified in Experiment-2 of less than 60 mg L^{-1} are not adequate for crop growth (Wang *et al.*, 2020). This finding could help explain the reduced yields of the slope experimental site due in part to Mg deficiency. The higher concentration of exchangeable- Ca^{2+} across all treatments in May-2017, in comparison with other periods, likely occurred in response to liming. In agriculture, liming (here, spreading of Limex70, a $CaCO_3$ precipitate) is done to neutralise soil acidity. However, it can lead to a cycle of necessary remediations because acidification of soil through N-fertilisation can occur with CO_2 being release to the atmosphere and Ca^{2+} leaching (Zamanian *et al.*, 2021). Acidification due to N-fertiliser addition was not observed in this study as shown by the pH values that remained similar across sampling periods.

Total nitrogen supply was shown to increase soon after FYM (FYM characteristics: OM = 71%, %tN = 2.2%) was spread and incorporated in May-2017, these results can be expected as fresh cattle FYM usually averages 1.2 kg t^{-1} (fresh weight) readily available nitrogen (Defra, 2018). The overall use of N-fertiliser has declined in the UK because the N-requirements by the crop is being balanced with other inputs like manures (Defra, 2018). The application of FYM to soil, as it was done in year one, provided a source of readily available nitrogen which can help reduce synthetic N-fertiliser inputs. The GM treatment did not receive N-fertiliser in 2017 but it achieved equivalent concentrations of %tN in October-2017, showing an increase from 0.18 to 0.21% (SP %tN increased from 0.18 to 0.22%). Both GM and SP started at the same %tN level, but the different inputs delivered similar results. The GM result was likely due to the low C:N stoichiometry of the cover crop mix (*Vicia sp.* and *R. sativus* foliage) that are quickly mineralised and subsequently release N (Ketterings *et al.*, 2011; Creamer *et al.*, 2016; Berthelin *et al.*, 2022). Legumes such as *Vicia sp.* form symbiotic relationships with N-fixing bacteria, a desired property when selecting cover crop mixtures because it could offset short-term some N-fertiliser requirements (Kaye *et al.*, 2019).

The Olsen-P increase in the SP and FYM treatments of Experiment-1 sampled in May-October 2018 could have been stimulated by N-inputs, through the addition of N-fertiliser, of which the GM treatment only received in spring 2018 (Widdig *et al.*, 2019; Chen *et al.*, 2020). N-loadings stimulate the activity of soil phosphatase, which catalyses the hydrolysis of P, an effect observable over the initial five-years in fertilised agri-soils which fit with this two-year study (Widdig *et al.*, 2019; Chen *et al.*, 2020). For periods longer than five years, soil bioavailable P can be limited by N-loadings (Widdig *et al.*, 2019; Chen *et al.*, 2020). However, short-term N-loadings can reduce soil pH which can reduce the expression of microbial P-solubilising genes (Dai *et al.*, 2020). Nutrient stoichiometry of N:P is critical for

crop productivity and inputs affect N and P turnover (Chen *et al.*, 2020; Dai *et al.*, 2020). The temporal effect of %tN on Olsen-P, i.e. observed in October 2017, with a lag period from N inputs, related to the field spatial variation. Moreover, the experimental site is known to have been under N-fertilised rotations for over five years before both experiments were established (personal communication with J. Braithwaite, Farm Manager). It could be of interest to determine whether a fallow period, as experienced prior to experimental setup, could reboot/disrupt N-input cycles and if different dynamics are observed under various soil textures.

Fresh cattle FYM, as applied, can be a source of sulfur because it typically averages 2.4 kg SO₃ t⁻¹ (Defra, 2010). While the impact on soil %tS was not immediately seen in this study and quite varied, this characteristic suggests that FYM could help reduce the use of SO₃ fertiliser. A study reported that microbial communities did not significantly vary across fields, but that the activity of enzymes involved in nutrient cycling, including S-cycling, did differ (Bowles *et al.*, 2014). This difference was mostly triggered by site properties and historical use of manures, thus suggesting that the initial increase in microbial biomass in response to the FYM treatment observed in this study could have led to greater necromass (i.e. soil organic matter consisting of dead microbial materials) later when the substrate was consumed releasing immobilised nutrients (Min *et al.*, 2019). Necromass is an important component of SOM and can quickly bind to soil clay minerals, contributing to bioweathering and mineralisation processes (Buckeridge *et al.*, 2020; Finlay *et al.*, 2020). These processes are important nutrient cycling components that release macronutrients, such as S, needed for crop growth (Finlay *et al.*, 2020). The amount of sulfur fertiliser being applied in the UK has increased by 6% to 34 kg ha⁻¹ since 2013, due to the decline in sulfur deposition from air pollution which used to sufficiently meet crop requirements, but now can leave low in organic matter sandy soils susceptible to S deficiencies (Defra, 2018). Lower %tS was observed in Experiment-2, which had been established on sandier soil and did not have the FYM treatment, and the highly variable sand content of Experiment-1, could help explain how the different texture classes can result in quite varied S concentrations in soil. These results suggest FYM was a source of S along with other essential nutrients, and so it could help reduce synthetic fertiliser inputs and balance crop requirements. However, regular soil tests should be conducted to avoid nutrient deficiencies or excess leading to leaching as responses can be varied, as demonstrated by this study.

The FYM treatment of Experiment-1 and GM (*R. sativus* and *Vicia sp.* mix cover crop) of Experiment-2 offered a supply of potassium (K⁺), with potential in helping reduce fertiliser requirements. The average 50% more exchangeable-K⁺ in the former was measured in the

FYM treatment than in the SP or GM treatments in May-2017, three weeks after incorporating FYM. This effect was still observable in October 2017 and 2018. FYM can provide 7.2 kg K₂O t⁻¹ of available potassium, and it has been demonstrated that its concentration in soil can increase following the application of manures (Defra, 2018; Taiwo *et al.*, 2018). However, the application of muriate of potash fertiliser in November 2017 could have enhanced results. Plant uptake of both K⁺ and Mg²⁺ and subsequent release has been found to be higher under legumes (e.g. *Vicia sp.*) and brassicas (e.g. *R. sativus*) than in cereals, which is linked to differences in nutrient requirements that lead to greater immobilisation by the former two crop groups (Groffman *et al.*, 1987; Cardoso *et al.*, 2013; Wendling *et al.*, 2016). The effect from the cover crop was only observed in the GM treatment of Experiment-2 in November-2018, which suggests that response may have been influenced by soil texture. This agrees with the study of Taiwo *et al.* (2018) who reported that %clay was positively correlated with fixed K⁺, whereas exchangeable-K⁺ correlated well with %sand. However, soluble nutrients like K⁺ can leach quickly from sandy soils if not taken up by plants (Groffman *et al.*, 1987) and this could be a consideration when using cover crops for nutrient provision, which are released slowly and less leachable than K applied as potash. The dry summer months of 2018 (Defra, 2019b) could have aided the concentration of K⁺ to be retained in the soil of Experiment-2 which would otherwise leached following periods of rainfall. These results suggest that risk from nutrient runoff and leaching can be minimised if soil amendments are applied in dryer periods because periods of heavy-rainfall increase nutrient loss, in particular from sandy and/or sloping land (Yao *et al.*, 2021).

The aboveground biomass of the cover crops (*R. sativus* and *Vicia sp.* mix) was greater in Experiment 2 than in Experiment 1, which is likely to have been caused by differences in soil properties. For example, brassicas, such as *Raphanus sativus*, establish more successfully on soils with pH 5.8–6.2 (AHDB, 2015), suggesting that pH 6.2 (±0.26 SD) encountered in Experiment-2 was in line with the recommended values. Whereas pH of Experiment-1 was 6.8 (±0.22-0.38 SD), higher than the optimum range. The texture of soil also impacts aboveground biomass, where lighter soils (< 20% clay) provide better structure for root development and so greater foliage biomass is achieved (AHDB, 2015). This characteristic was met with the soil of Experiment-2, which was classified as loamy sand (0-15% clay) and sandy loam (0-20% clay) (FAO, 2020). In Experiment-1, four texture classes of soil were identified and 45.2% of them were heavier and classified as sandy clay loam (20-30% clay) (FAO, 2020). Soil properties are probable factors influencing the protein content of the cover crop aboveground biomass too, where it was lower in Experiment-2. These results suggest that greater concentration of soil %tN in Experiment-1 resulted in higher N acquisition but did not lead to significantly greater aboveground biomass, and that soil inherent properties like

texture led to differential responses. This is in accordance with the study of Finney *et al.* (2016) who reported N acquisition was not correlated with biomass production.

The *T. aestivum* yields (spring wheat) in the FYM treatment were comparable to those in the SP treatment which received N-fertiliser alone, but overall yields were considerably lower than national average. The causes of the reduced yields achieved in this study could not be ascertain but there was evidence of leaf scorching, wich could have been caused by the application of Ally Max SX (42 g ha⁻¹) and Duplosan (1 l ha⁻¹) herbicides, and there were also signs of Take-all disease (Thomas, 1986; AHDB, 2010). These factors in combination with conversion to no-till could explain results. The weather conditions in 2018 had a negative effect on crop yields and production nationally due to the heavy rainfall experienced in spring followed by a long hot and dry spell in summer (Defra, 2019). However, following the poor production of 2017, yields recovered in 2018 to competitive amounts comparable to national levels (Defra, 2019). The greater yields of *A. sativa* (winter oats) and respective grain protein in Experiment-2 implies a response to cover crop residues in the GM treatment in comparison with SP. Crops utilise macronutrients, such as N, as building blocks for protein synthesis (Morgan and Connolly, 2013). Even though there were no significant differences between treatments in soil %tN, there could have been legacy effects from the N-fixing *Vicia* sp. residues that led to higher N availability and uptake (Kaye *et al.*, 2019). However, the yield on this slope side of the field were much lower than the UK average; SP = 2.1 t ha⁻¹ and GM = 2.5 t ha⁻¹ versus UK average = 5.0 t ha⁻¹.

Limitations of current study

This study was relatively short-term, and it could be improved by having a greater number of replicates and/or a more intensive sampling regime over a longer period, although this was not possible within the constraints of this study. One of the limits of field experiments is that data are often highly variable such that an unrealistic number of samples are likely required to have a high confidence of observing small effect sizes. Heterogeneity of soil within plots was shown to be a bigger factor for some results than treatment effects. This shows the importance of having data from multiple field sites and the weakness of extrapolating from single studies when computing predictive models that might combine data from short- and long-term studies that interject uncertainty.

4.6 Conclusion

This study was relatively short-term, but it has contributed with a monitoring case study that shows how variations in a heterogenous field affect SOC and SOM following changes in

management practices of an arable site, thus slightly reducing the paucity of scientific data. It also showed C capture potential at a local level and how its topography and properties influence the rate of change in soil C using *in situ* direct measurements. It demonstrated that SOC was affected by soil texture and not by the addition of organic amendments over a two-year field experiment. The results suggest that spatial heterogeneity in a two-hectare field, where soil types vary between sandy loam, sandy clay loam and loamy sand soils, can lead to different results depending on sampling location.

Application of FYM and *R. sativus* and *Vicia sp.* cover crop mix did impact soil properties over a short period of time, such as its nutrient status. Furthermore, it confirmed that microbial biomass provides an early indication of changes in management practice where other properties, such as SOC or SOM, may take years to show a significant response to inputs.

Natural field variations can have a significant impact on outcomes and that the results obtained are influenced by both seasonality and spatial variation. This insight is critical when considering farm subsidies and payments based on soil indicators, and C trading based on slowly changing measurements of SOC or SOM, which can vary due to the natural heterogeneities of a field and can be unrelated to management practices such as soil texture.

Baseline data are often missing from large-scale monitoring studies that quantify ecological diachronic change. Initial differences that can be accounted for in terms of management practice are not available, which this study aimed to provide under the conditions used.

Building soil C stocks and/or preventing further decline remains a target that should be aimed for. Carbon stocks provide food for organisms living in or depending on soil processes, it helps stabilise structure and water retention, acts as an ion exchange and has buffering qualities. Protecting and preserving soil by incentivising management practices known to be beneficial without relying solely on biological or physicochemical scoring systems should be the way forward. As such, modelling tools that use soil data to make future projections, support management decisions, and determine farm payment support and carbon trading should be developed with great care, if at all, to not penalise those already making sustainable decisions that might not be quantifiable by the selected parameters.

In conclusion, this study showed that FYM and the *R. sativus* and *Vicia sp.* cover crop mix had an impact on soil chemical properties that are beneficial for crop growth, such as

balanced pH, %tN, %S, and exchangeable K⁺ and Mg²⁺, but that response was influenced by spatial and temporal variation. No significant differences in yields were observed with lower synthetic N-fertiliser inputs. However, yields were significantly lower than national average in the first year following conversion to no-till. The lower yields could be in response to penalties following conversation or in combination with crop disease and/or scorching. The yields of winter oats obtained in year-2 were comparable to national levels in Experiment-1 but not in Experiment-2, showing once again temporal and spatial variation influence on outcomes.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 690142.

The authors would like to thank the supervisory work done by Dr Simon Jeffery and Dr Matthew Back whom have been excluded at this stage of manuscript submission but will be included in following ones.

The authors would like to thank Mr Simon Allen for his agronomy work, Mr Kevin Jones for his demonstration work on analytical techniques, and CERC for their machinery work during crop management field trials.

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