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Field-scale heterogeneity overrides management impacts following conversion to no-till within an arable system

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ABSTRACT

Crop establishment in no-till arable systems benefits from soil health conducive for growth. Combined with the incorporation of crop residues and manures, no-till can influence soil organic carbon (SOC) and organic matter (SOM) dynamics, crop productivity and nutrient cycling. These processes are shaped by spatial and temporal factors including associated microbial activity. This study aimed to investigate the interaction between microbial and soil physicochemical properties during the transition from full-inversion to no-till soil management. Assessments were conducted over a two-year period and included a combination of soil microbial assays (microbial biomass carbon and nitrogen with physicochemical analyses, SOC, SOM, textural class, pH, gravimetric water content, and macronutrients). 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 and N-fertiliser), Green Manure (GM, *Raphanus sativus* and *Vicia* sp. mix) and Standard Practice (SP = Control, N-fertiliser only). 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. The results were influenced by spatial (i.e. where the same was collected from) and temporal (i.e. the time at which the same was collected) variations that were not always linked to management practices. This study demonstrated that the quantification of SOC and SOM were poor predictors of change in management practices over two years, while microbial biomass responded quickly to the incorporation of FYM. SOC and SOM were affected by soil texture, but not significantly by inputs, and were associated with extractable Ca^{2+} and total-N. Diachronic studies increase our understanding of biological and physicochemical dynamics in response to short-term change in soil management practices. This study emphasises the impact of soil texture within a single heterogenous field, and how it affects management outcomes. It highlights the importance of considering spatial differences to develop effective and sustainable agricultural solutions.

1. Introduction

Soils are critical to life, playing a central role in agricultural systems and ecosystem service provision 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 (Denef et al., 2002; Six et al., 2004). Its management has often been 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 (Arneth et al., 2019; Riddle, 2019; Evans et al., 2020). These practices have led to loss of SOM, and thus SOC, and biodiversity, caused soil erosion, impoverished soil structure, and weakened water infiltration capacity (COP21, 2015; Lal, 2016; Arneth et al., 2019; Riddle, 2019; Evans et al., 2020). A change to agricultural practices which preserve soils, a non-renewable resource at timeframes relevant to agriculture, and prevents further degradation is urgently required to ensure the sustainability of crop production (Lal, 1997; Tsiafouli et al., 2015; Science 20, 2018; Searchinger et al., 2019; Arnold et al., 2020; Defra, 2020).

There has been increasing focus on identifying optimum management practices to reduce costs whilst improving soil health, producing

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economically viable yields, and preventing further expansion of agricultural land (NFU, 2019; Searchinger et al., 2019; CCC, 2020). Adoption of no-till (i.e. zero tillage with direct drilling) in arable systems, a cultivation that minimises soil disturbance, has been promoted as a practice in soil protection to increase soil organic matter and build carbon (C) stocks, to prevent loss of structure, compaction and nutrient leaching, and to reduce establishment costs (Lal, 1997; Decker et al., 2009; Tsiafouli et al., 2015; 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 currently managed as no-till (Allison, 2015; Alskaf et al., 2020). Part of the reason for poor uptake is the yield penalties that are often reported during the first years of conversion (Pittelkow et al., 2015).

Manures are frequently applied in arable systems and interest in sowing cover crops has intensified in recent years; both approaches are used for building soil health (Roesch-Mcnally et al., 2018; Abdalla et al., 2019; Lin et al., 2019; Storr et al., 2019). Farmyard manures (FYMs) are applied on 51 % of surveyed British farms (1500 farms >20 ha in size), 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 soil degradation (Roesch-Mcnally et al., 2018; Storr et al., 2019). However, 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.

No-till can reduce decomposition rates of crop residues by approximately 10–20 %, with functions such as of organic matter associated nutrient cycling being reduced too (Lupwayi et al., 2004; Janzen, 2006). Temporal patterns influence microbial communities, and dynamic changes can be rapidly triggered through resource addition such as C or N-rich 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). 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).

There is a disparity in studies investigating no-till arising from the specifics of each experiment such as longevity of the experiment and crop diversity, with some showing increased C stocks and others not (Luo et al., 2010; Virto et al., 2012; Powlson et al., 2014; Valboa et al., 2015; Meurer et al., 2018). Moreover, considering farming is a business requiring profits to sustain it and reinvest, 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 aimed to investigate impacts associated with the application of N-fertiliser alone, and in combination with farmyard manure, and the sowing of cover crops for use as green manure, on SOC/SOM dynamics and crop yield response after the transition from full inversion tillage to no-till. Both SOM and SOC were measured for comparability with other studies because SOM is usually more accessible to farmers, while SOC is regarded as a more reliable measurement (Pribyl, 2010; Abram, 2020). Furthermore, quantifying SOM by a conversion factor of SOC can lead to under or overestimations because management and inherent soil properties influence measurements (Pribyl, 2010). Two field experiments were established on arable land previously under a full inversion tillage regime to test the following hypotheses:

1. The heterogeneity of the field, as measured by the different soil textures, has a greater impact on SOC, SOM, yields, and nutrients than the type of input.
2. The nutrient concentrations of soil changes with the type of organic amendments.
3. The effect of incorporating farmyard manure on microbial biomass, SOC/SOM, and macronutrients is measurable over the two-year period.
4. Cover crops will cause increase in microbial biomass, SOC, and SOM.

2. Materials and methods

2.1. Experimental design

The completely randomised experiments (Fig. 2) 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 (Fig. 1, Table 2). Experiment-1 (area of 2 ha) consisted of three treatments: Farmyard Manure (FYM), Green Manure (GM) and Farmers' Standard Practice (SP = Control). Experiment-2 (area of 0.3 ha) was conducted on a sloping side of a field and excluded the FYM treatment because it lacked space for the spreader to turn. 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, at a seed ratio of 50:50, as a green manure (GM) for the next crop season. All inputs were applied in accordance with the Nutrient Management Guide (RB209) which offers guidance on best practice, and it is widely used nationally by UK practitioners. The control plots (Table 1) were managed as per farmer standard practice (SP, i.e. using synthetic N at maximum rates permissible under RB209 (Defra, 2010)). 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 treatment) which were direct drilled across all plots in both experiments. Refer to Table 1 for detailed field record.

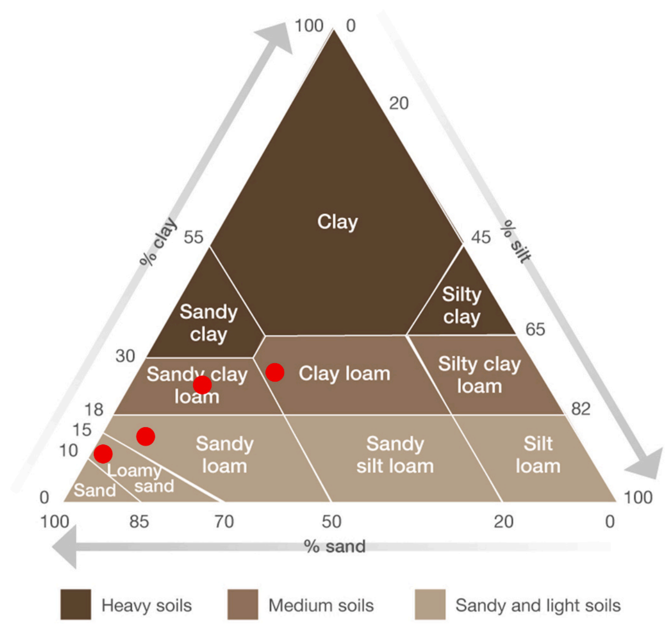

Fig. 1. Soil texture triangle based on the class intervals of the Soil Survey of England and Wales. Red circles represent identified textures. Available at: https://ahdb.org.uk/knowledge-library/how-to-determine-soil-texture. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

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 × 6 m) plots	14/04/2017	Spring wheat, *Triticum aestivum*, 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 × 6 m) plots	14/04/2017	Spring wheat, *Triticum aestivum*, 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 × 6 m) plots	14/04/2017	Fodder radish (*Raphanus sativus*) and vetch (*Vicia* sp.)	29 kg ha^{-1}, seed ratio of 50:50	Weaving GD3001T Direct Disc Drill												
Experiment 2, N-fert treatment	9 x (24 × 6 m) plots	14/04/2017	Spring wheat, *Triticum aestivum*, 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 × 6 m) plots	14/04/2017	Fodder radish (*Raphanus sativus*) and vetch (*Vicia* sp.)	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 × 6 m) plots	18/10/2017	Winter oats (*Avena sativa* 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

2.2. Soil sampling and processing

Experiments were established on 7 Apr 2017 and the soil sampling baseline of Experiment-1 was conducted on 3 May 2017. Soil was collected from the topsoil because any quantifiable change would likely occur here within this two-year study. Aboveground biomass sampling took place on 20 Sep. 2017 for both experiments. Post-harvest soil sampling was done on 12 Oct. 2017 for Experiment-1 and on 15 Nov. 2017 for Experiment-2. Subsequent crop season had spring sampling of Experiment-1 on 30 Apr. 2018, and in autumn on 2 Oct. 2018 of Experiment-1 and 1 Nov. 2018 of Experiment-2. There was no possible access to Experiment-2 in spring.

2.2.1. Experiment-1

Sampling points were selected using a random number table (Rand Corporation, 1955), the random numbers used to determine the distance to travel starting from west side of Experiment-1 and north side of Experiment-2. Twenty soil cores were collected from each sampling point within 1 m radius using an auger (10 cm depth * 4.5 cm diameter). Plots, 6 × 200 m, were sampled individually, at two sampling points, with one composite sample of 20 cores produced per sample point, a total of 40 soil cores per plot, and a total of 36 soil samples. 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 in a shaded area 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 kept refrigerated at 4 °C until analysis with microbial assays performed within 10 days of sample collection (Wang et al., 2021).

2.2.2. Experiment-2

Each plot, 6 × 24 m, 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, totalling 8 and 9 samples from the GM and SP treatments, respectively.

2.3. 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 and sand 63–2000 μm (Tan, 2005). Soil organic carbon (SOC) was measured using a Leco SC-144DR Carbon/Sulfur Analyser at 840 °C (Leco Corporation; USA). Quantification of pH was done in diH_2O solution (1:5, soil:water ratio) (Tan, 2005) and read on a pH meter (Jenway 3510, UK).

The soil nutrient concentrations were 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. In Experiment-1, nutrients were 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. 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 (USA) spectrophotometer at 880 nm (Tan, 2005).

2.4. 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 (Vance et al., 1987; Brookes, 2001), using two 10 g aliquots of fresh soil from each composite sample. 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 et al., 2004).

2.5. 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 (Fig. 8). Cash crop parameters were measured twice during this two-year study, after harvest in autumn of both Experiments 1 and 2 (Fig. 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 (Auburn, USA). 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).

Cover crop, *Raphanus sativus* and *Vicia* sp., aboveground biomass was sampled 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).

2.6. Statistical analysis

Statistical analyses were conducted in R-Studio (R version 4.0.3 (2020-10-10 ucrt) – "Bunny-Wunnies Freak Out"; R version 4.1.0 (2021-05-18 ucrt) – "Camp Pontanezen"; R version 4.2.1 (2022-06-23 ucrt) – "Funny-Looking Kid"; R Core Team), and the packages "correlation", "corrplot", "tidyverse", "ggpubr", "rstatix", "rcompanion", "Hmisc", and "psych" (Wickham et al., 2019; Kassambara, 2020; Mangiafico, 2021; Wei et al., 2021; Harrell Jr, 2022; Makowski et al., 2022; Revelle, 2022).

The distribution of 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-

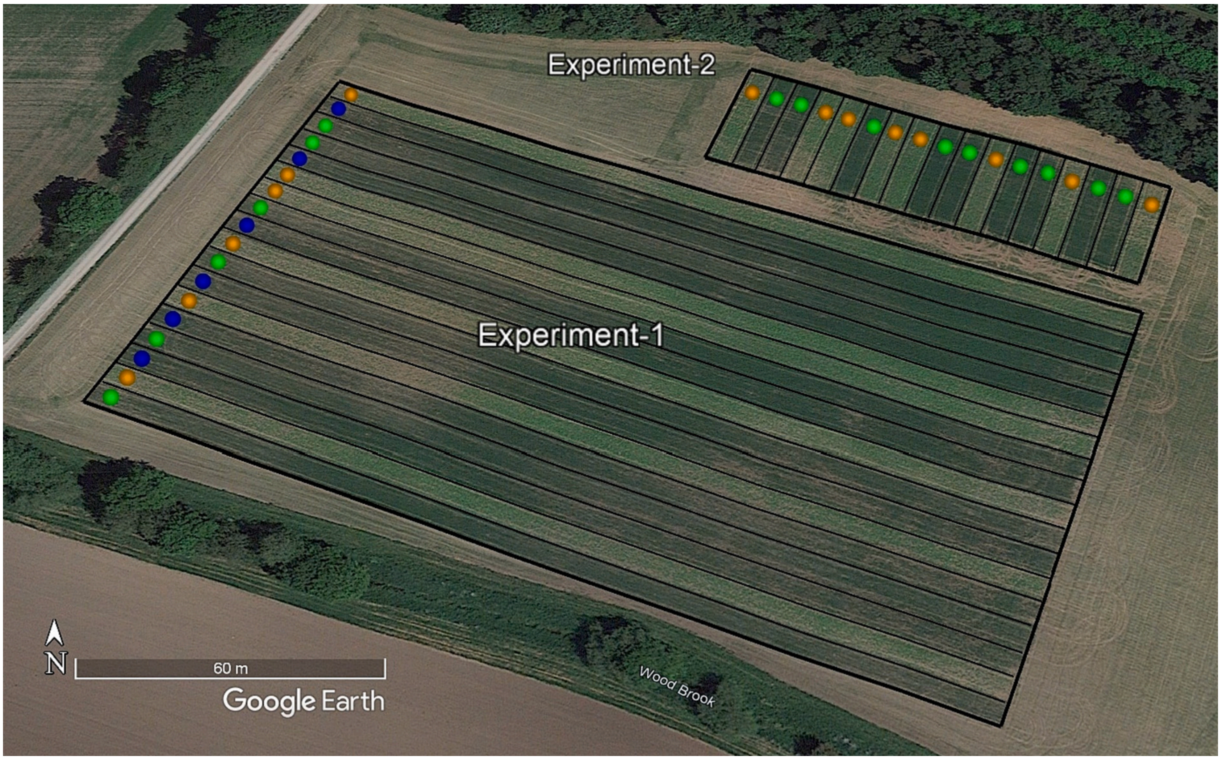

Fig. 2. Completely randomised experimental design: Experiment-1, Standard Practice (SD, green), Farmyard Manure (FYM, blue), Green Manure (GM, orange), $n = 6$, 200 × 6 m/plot; Experiment-2, Standard Practice (SD, green, $n = 9$), Green Manure (GM, orange, $n = 8$), 24 × 6 m/plot. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

2017, %tS, P in May and October-2018, Mg in November-2017, Clay, Silt, Mg in November-2018 with the transformation applied $= -1 * x^{\lambda}$.

Continuous response variables, such as SOC, SOM, MBC, MBN, tN, tS, GWC, pH, Olsen-P, K^+, Mg^{2+}, Ca^{2+}, grain yield, TGW, grain protein, cover crop protein content or cover crop biomass, were used as the target variables in linear regression models. The explanatory variables for each temporal observation (May, October or November 2017–2018) were treatments (categorical variable: FYM, GM, SP). To account for the spatial variability of the site, the models were extended to include soil texture (clay, silt, and sand) as the second explanatory factor.

Factorial ANOVA was used to analyse the relationships between response variables and both the interaction and main effects of treatment and sampling period with soil texture. In cases where significant results were obtained ($p < 0.05$), Tukey's Honest Significant Difference *post-hoc* tests (Tukey HSD) were performed. The mean values of the results are presented with their respective standard error, unless otherwise stated.

Factorial ANOVA regression models were also used to analyse the relationship between soil organic carbon (SOC) and different variables. The continuous response variable SOC was regressed with treatment (FYM, GM, SP), as the categorical explanatory variable, for each temporal observation (May, October, or November 2017–2018). Additional explanatory variables such as MBC, MBN, Ca^{2+}, tN, pH, GWC, or soil texture were also included in the models. Tukey Honest Significant Difference post-hoc tests were performed on all significant models ($p < 0.05$) for individual comparisons. The study also used Pearson correlation tests to examine the correlation between pairs of variables including SOC, SOM, tN, tS, GWC, pH, Olsen-P, K^+, Mg^{2+}, Ca^{2+}, clay, silt, and sand. The Holm method (1979) was applied to adjust the *p*-value, which was set at a significant level of <0.05. Stronger correlations near −1 or 1 were considered indicative of a stronger relationship, while a value of r = Ø indicated independence of variables.

3. Results

3.1. Soil properties

3.1.1. Soil texture

Most plots in Experiment-1 were on sandy loam (48.4 %) and sandy clay loam (45.2 %). Clay loam soil made up 4.0 % and loamy sand 2.4 % of the types of soil present. Experiment-2 was dominated by loamy sand soil (73.5 %) and the remaining 26.5 % was sandy loam (Table 2).

3.1.2. pH and GWC

May-2017 was the only period that exhibited significant pH differences between SP and FYM ($p = 0.01$) or GM ($p = 0.003$) in 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 3). No significant differences were observed in soil gravimetric water content (%GWC) in either Experiment-1 or 2.

3.1.3. Macronutrients in soil

Total nitrogen increased soon after FYM (FYM characteristics: OM = 71 %, %tN = 2.2 %) was spread and incorporated in May-2017 (Fig. 3) although this was marginally non-statistically significant ($p = 0.06$). Yields obtained in FYM were comparable to SP, which received synthetic N-fertiliser alone (i.e. no manure; Fig. 3). While the GM treatment did

Table 2
Soil texture classes identified in each experiment investigating the effects of N-fertiliser, FYM and cover crops.

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

Table 3
Mean soil pH values ± standard deviation for Experiment 1 and 2 (E1 $n = 6$; E2 SP $n = 9$, GM $n = 8$) investigating the effects of N-fertiliser, FYM and cover crops.

	Experiment-1				Experiment-2	
Treatment	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)

not receive N-fertiliser in 2017 it exhibited 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 %.

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. 3). 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 in Experiment-1.

FYM in Experiment-1 and GM (*R. sativus* and *Vicia* sp. mix cover crop) in Experiment-2 resulted in increased potassium (K^+) concentration. In Experiment-1, 50 % more extractable-K^+ (mg K^+ L^{-1}) was measured in the FYM (p = 0.01) treatment than in the SP or GM treatments in May-2017, on average, 3 weeks after incorporating FYM (Fig. 3). This concentration 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 GM in Experiment-2 in November-2018 (p = 0.001).

Extractable-Mg^{2+} (mg Mg^{2+} L^{-1}) was only significantly different between SP and GM in November-2018 (p = 0.04) in Experiment-2 (Fig. 3). The concentration of extractable-Ca^{2+} was higher across all treatments in May-2017 in comparison with other periods (Fig. 3). A notable effect of FYM on sulfur (%tS) concentration in soil was observed in October-2018, results were highly variable and not statistically significant (Fig. 3).

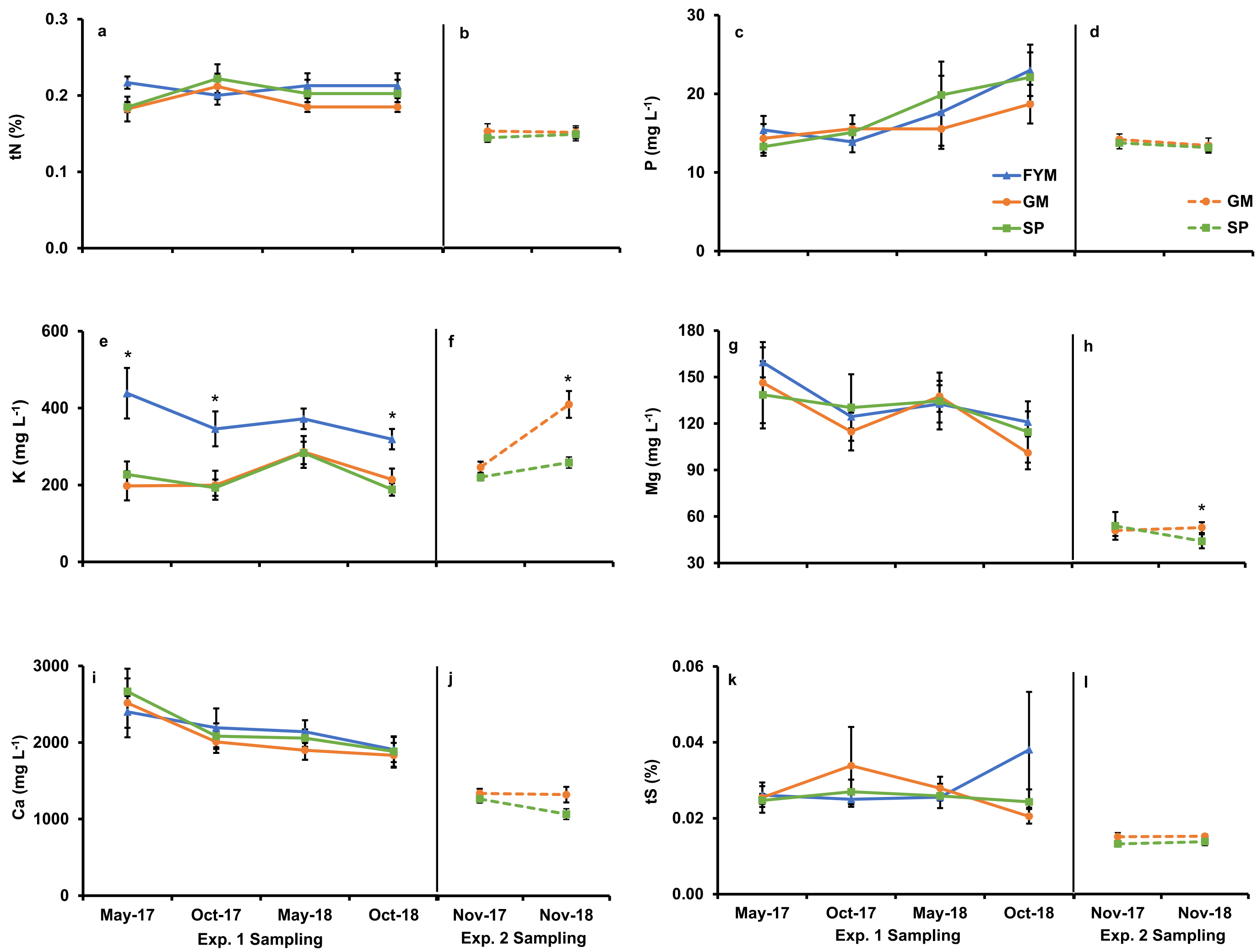

Fig. 3. Experiment 1 (solid lines) and 2 (dotted lines) mean results of soil % total nitrogen (%tN, a and b), phosphorus (mg Olsen-P L^{-1}, c and d), potassium (mg K^+ L^{-1}, e and f) and magnesium (mg Mg^{2+} L^{-1}, g and h), extractable calcium (mg Ca^{2+} L^{-1}, i and j) and total sulfur (%tS, k and l). Four sampling sessions (May and October 2017–2018) were conducted for Experiment-1 (a, c, e, g, i and k), and two sampling sessions (November 2017–2018) for Experiment-2 (b, d, f, h, j and l). Treatments: Farmyard manure (FYM in blue); Green manure (GM in orange); Standard practice (SP in green). Lines show means; bars show ± standard error of the means. Asterisk (*) show significant differences between treatments. Experiment-1 n = 6; Experiment-2 GM n = 8, SP n = 9. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

3.2. Microbial biomass

3.2.1. Experiment-1

There was a significant interaction between the different treatments and sampling period ($p = 0.03$; Fig. 4). 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 marginally significant interaction effect was observed between treatment and sampling period for MBN (μg g^{-1}) ($p = 0.5$) (Fig. 4). 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).

3.2.2. Experiment-2

A significant response in MBC (p = 0.03) and MBN (p = 0.04) to treatments and sampling period were observed in November-2018 (Fig. 4). Both were significantly higher in the GM treatment than SP.

3.3. SOC and SOM response to treatments

The same pattern was observed with both SOC and SOM over time (Fig. 5) with no significant interaction effect with either variable. There was no statistical interaction between Experiment-1 treatment and sampling period for either SOC or SOM ($p = 0.8$, $p = 1.0$, respectively). There were no significant treatment effects within the first year of Experiment-2 (2017) nor were detectable legacy effects (2018) on the proportion of either SOC ($p = 0.7$) or SOM (p = 0.8). Therefore, the decision to use only the SOC variable going forward was taken.

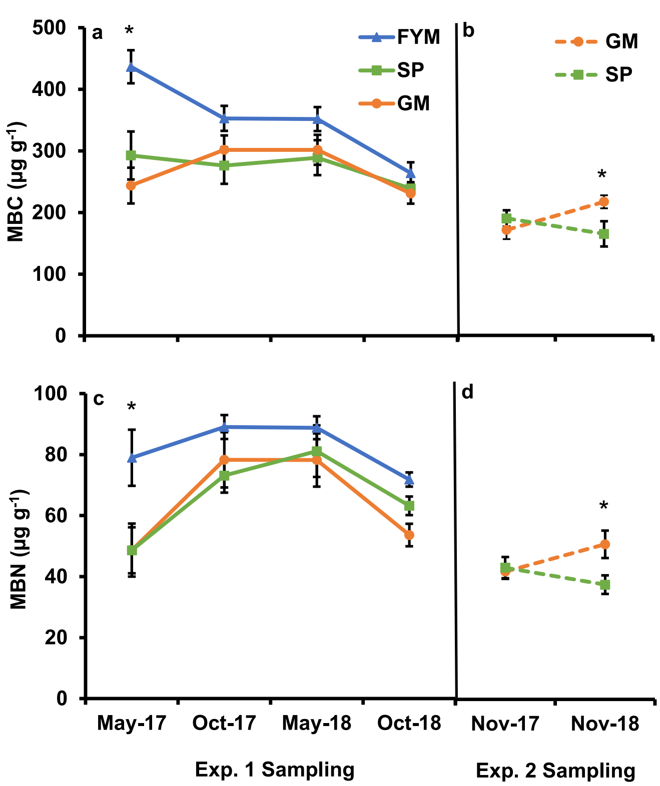

Fig. 4. a and b) Microbial biomass Carbon (μg Mic C g^{-1}) and c and d) Nitrogen (μg Mic N g^{-1} of soil) change over time from the start of Experiment-1 (a and c, solid lines) in May 2017 to completion in October 2018, and Experiment-2 (b and d, dotted lines) in November 2017 and 2018. Experiment-1 treatments: FYM = Farmyard Manure, n = 6; SP = Standard Practice, n = 6; GM = Green Manure, n = 6; ± standard error. Experiment-2 treatments: SP = Standard Practice, n = 9; GM = Green Manure, n = 8; ± standard error of the mean. Asterisks show significant differences between treatments.

3.4. SOC and interaction effects

3.4.1. Experiment-1

A change in microbial biomass carbon (MBC) could significantly affect the concentration of SOC (p = 0.001). The interaction between treatments, sampling period and MB-Nitrogen (MBN) was also significant (p = 0.04). However, no significant pairwise comparisons were identified. Treatment and sampling period, with or without calcium (Ca^{2+}) or total nitrogen (tN), exhibited a significant interaction with Ca^{2+} ($p < 0.001$) and with the interaction between tN and treatment (p = 0.03) and sampling period (p = 0.03). Once again, no significant pairwise comparisons were computed. Treatment and sampling periods showed a significant interaction between gravimetric water content (GWC, $p = 0.003$) or pH (p = 0.02) with SOC but no pairwise comparisons were significant. 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 no pairwise comparisons showed any significant treatments interaction with or without sampling period.

3.4.2. 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. Significant associations between either Ca^{2+} ($p < 0.001$) or tN (p < 0.001) with SOC were detected. A significant treatment effect ($p = 0.002$) was observed but the interaction between either treatment and Ca^{2+} or tN were not.

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. 6). Similarly, no significant interaction effects were computed between 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.

3.5. Correlations

Correlations between the pairs were conducted to look at the relationships involving soil texture (sand, silt, and clay, i.e. spatial variation) and SOC, SOM, major ions (tN, Olsen-P, K^{+}, Ca^{2+}, Mg^{2+}, and tS), pH, and GWC. The correlation matrix found many significant associations between pairs both unidirectional and following an opposite trend (Fig. 7). Strong positive correlations were found between the clay and % tN ($r = 0.7$), and SOM, Ca^{2+} or Mg^{2+} (all $r = 0.8$). Whereas, as the amount of sand increased so do other properties decreased either moderately (i.e. MBC or MBN or %tS, $r = -0.5$) or strongly (i.e. SOC, % tN or pH, $r = -0.6$; SOM, Ca^{2+} or Mg^{2+}, $r = -0.8$).

3.6. Cereals

3.6.1. Experiment-1

The average spring wheat 2017 yield was 1.2 t ha^{-1} (± 0.2 SD) in both the SP and FYM treatments. There were no significant differences in yield (p = 0.4) (Fig. 8), 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 (± 0.5 SD) and FYM (± 0.7 SD) treatments, and 5.4 t ha^{-1} (± 0.8 SD) in the GM treatment.

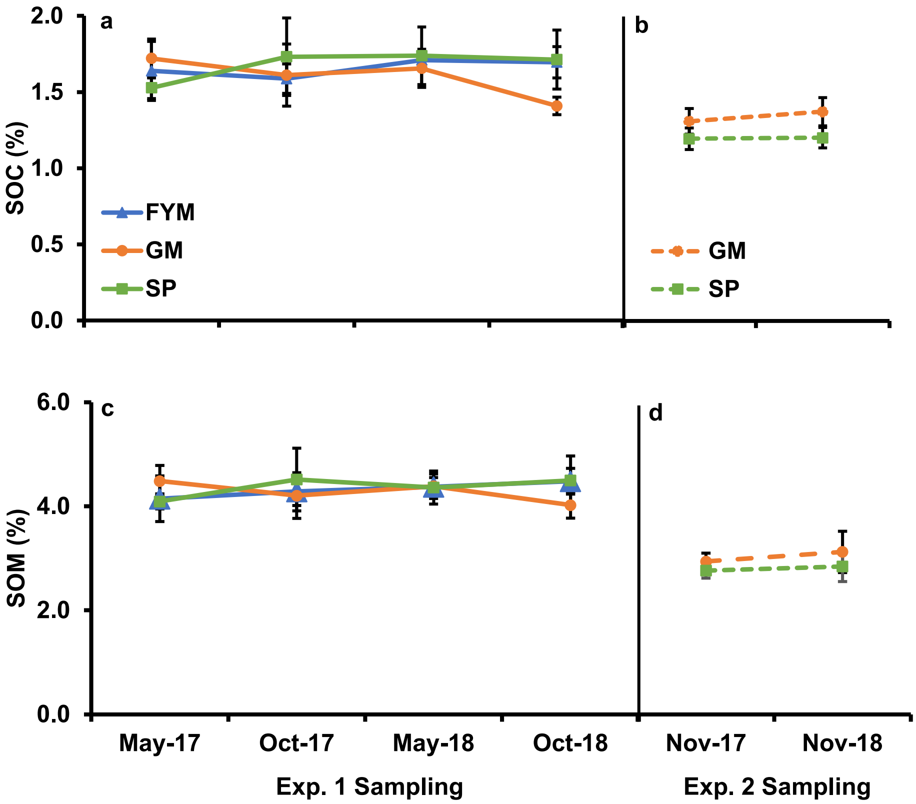

Fig. 5. Time series of SOC and SOM results from Experiment 1 (a and c) and 2 (b and d). Soil sampling was done in May and October 2017–2018 (a and c, n = 6), and in November 2017–2018 (b and d, GM n = 8, SP n = 9). The treatments were Farmyard Manure (FYM), Green Manure (GM), and Standard Practice (SP). SOC is represented by dotted lines, and SOM by full lines. ± standard error of the mean. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

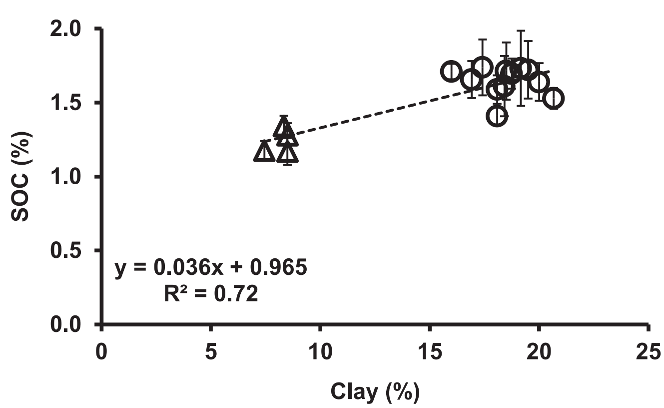

Fig. 6. Regression plot indicating the linear relationships between the amount of clay particles in soil texture and soil organic carbon (SOC ± SEM) using treatment mean data from both Experiments 1 (in circles) and 2 (in triangles) sampled over the two-year experimental period. Treatments of Experiment-1 were replicated six times each. Experiment-2: SP n = 9; GM *n* = 8. Significant level at $p < 0.05$.

3.6.2. 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. 8). 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}, with a reduction in the mean TGW of 4.4 g on average in the GM ($p < 0.001$).

3.7. Cover crop

3.7.1. 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. 8). However, the difference was marginally non-significant (*p* = 0.06). No significant difference in protein content of the cover crop aboveground biomass was identified, with results ranging from 24 % ±0.3 in Experiment-1 and 23 % ±0.2 in Experiment-2.

4. 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, combined with microbial biomass and soil properties such extractable Ca^{2+} (a key factor in SOM stabilisation; Rowley et al., 2018) and other ions, total-Nitrogen (%tN), pH, gravimetric water content (GWC) and soil texture is key to developing best management practices (Lines-Kelly, 1992; Defra, 2010). This study demonstrates that the type of soil organic amendments, FYM or cover crops, at the amounts used (Experiment-1: 8.9 Mg OM ha^{-1} for FYM and 3.8 Mg OM ha^{-1} for 50:50 cover crops; Experiment-2: 4.1 Mg OM ha^{-1} for CC) did not significantly impact SOC but did impact microbial biomass and soil nutrient concentrations over a two-year period. Response to treatments was influenced by spatial and temporal variations as observed in soil nutrient response. 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.

4.1. SOC response

Increases in SOC are expected to be substantial on sites where initial C stocks are low (Six and Jastrow, 2002; Hernanz et al., 2009; Virto et al., 2012; Amelung et al., 2020). However, soil C storage is limited and influenced by its textural properties, in particular clay and silt content (Six and Jastrow, 2002; Schweizer et al., 2021). Reduced tillage, retaining of crop residues/stubble, crop rotations, sowing of N-fixing

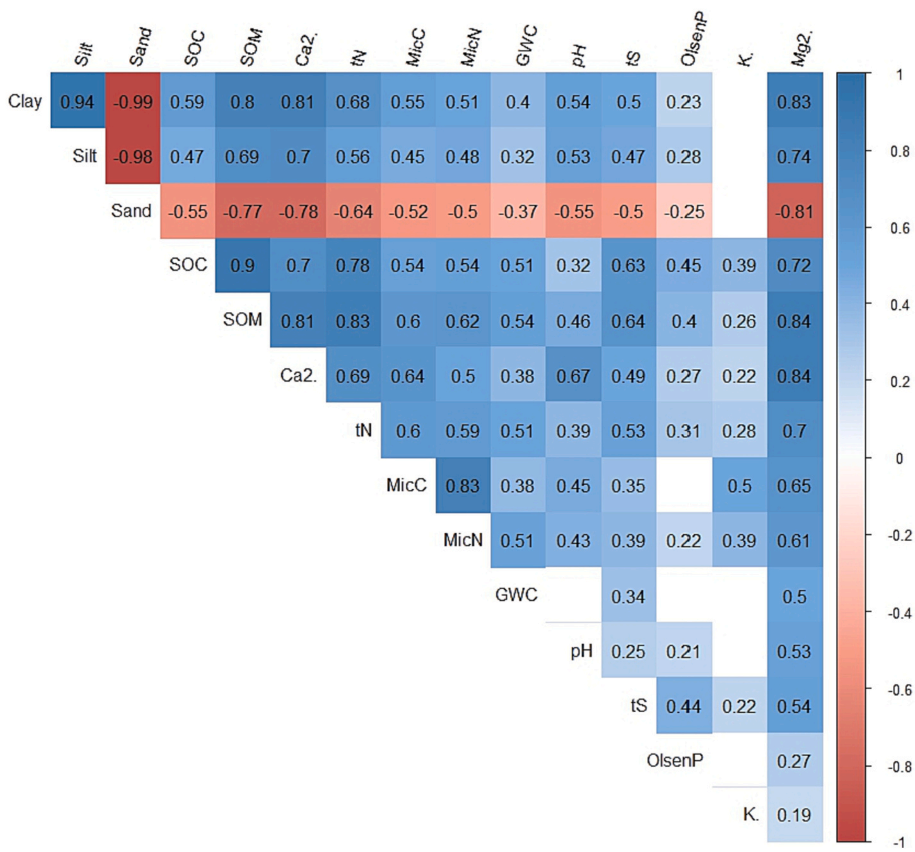

Fig. 7. Correlogram of correlation coefficients for pairs of variables from both Experiment 1 and 2. Positive correlations are displayed in blue and negative correlations in red. The intensity of the colour is proportional to the correlation coefficient. The stronger the correlation by being nearer to $r = -1$ or $r = 1$, the darker the boxes are. The white boxes symbolise no significant quantifiable correlation between the pairs. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

plants, and incorporating manures are recommended for their potential to increase soil C stocks (Mutegi et al., 2011; Amundson and Biardeau, 2018; Abdalla et al., 2019; Baveye et al., 2020). Still, these practices do not always lead to increased C, as was observed by Buysse et al. (2013), Powlson et al. (2014), and Mary et al. (2020). In this study, no measurable effect of FYM, cover crops or N-fertiliser on SOC were observed. Soil characteristics (i.e. %clay, %sand or %silt) were often better predictors of SOC and MBC differences than organic amendments. 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 topsoil in no-till systems (Minasny et al., 2017). The level of soil disturbance, i.e. tilling or not, could have no impact on SOC stocks as seen in long-term experimental sites when considering 40 cm depth soil layer (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 (Mary et al., 2020; Valboa et al., 2015). There are, however, other benefits that can be incentivised by these practices like increased microbial abundance associated with ecosystem resilience (Zuber and Villamil, 2016), increased earthworm abundance (Briones and Schmidt, 2017) and improved soil structure (Ayoubi et al., 2012; Buysse et al., 2013). Other factors influence SOC, such as microbial-SOC dynamics, land-management, precipitation, geology, and temperature, and these should be considered to better understand the mechanisms behind changes in SOC stocks (Powlson et al., 2011).

4.2. Microbial biomass

Microbial biomass quantification provides early insights into changes in management practices where other changes such as of 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). Our results here are in line with those reported by Brookes (2001), Gan et al. (2020) and Liang et al. (2022). Regular organic inputs such as FYM introduces C into the system, improves soil structure and water holding capacity, and it offers a readily available source of labile organic matter that triggers processes such as microbial decomposition, nutrient mineralisation and immobilisation to occur (Brookes, 2001; Powlson et al., 2011; Gan et al., 2020; Liang et al., 2022). Conditions for enzymatic accessibility can be better predictors of organic matter turnover than its chemical properties (Schmidt et al., 2011; Lehmann and Kleber, 2015). The response to organic amendments can rapidly dissipate if inputs are too sporadic because they can be mineralised quickly, influencing nutrient availability for crop uptake (Watts et al., 2006; Gan et al., 2020; Bullock et al., 2021; Berthelin et al., 2022).

4.3. Ca^{2+} effect

Calcium plays an important role in the stabilisation of SOC through cationic bridging and its loss as a result of leaching can reduce soils' productivity potential (Eldor, 2016; 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 (Rowley et al., 2018). Similarly, it has been found that liming led to an increase in SOC accumulation within aggregates perhaps due to increased Ca^{2+} derived from the liming agents (Six et al., 2004; Briedis et al., 2012). This suggests possible mechanisms by which SOC can be increased, for example by ensuring that sufficient Ca^{2+} is available through soil testing or co-application with lime.

4.4. Soil nutrients

The nutrient concentrations of soil benefited from fertilisers and

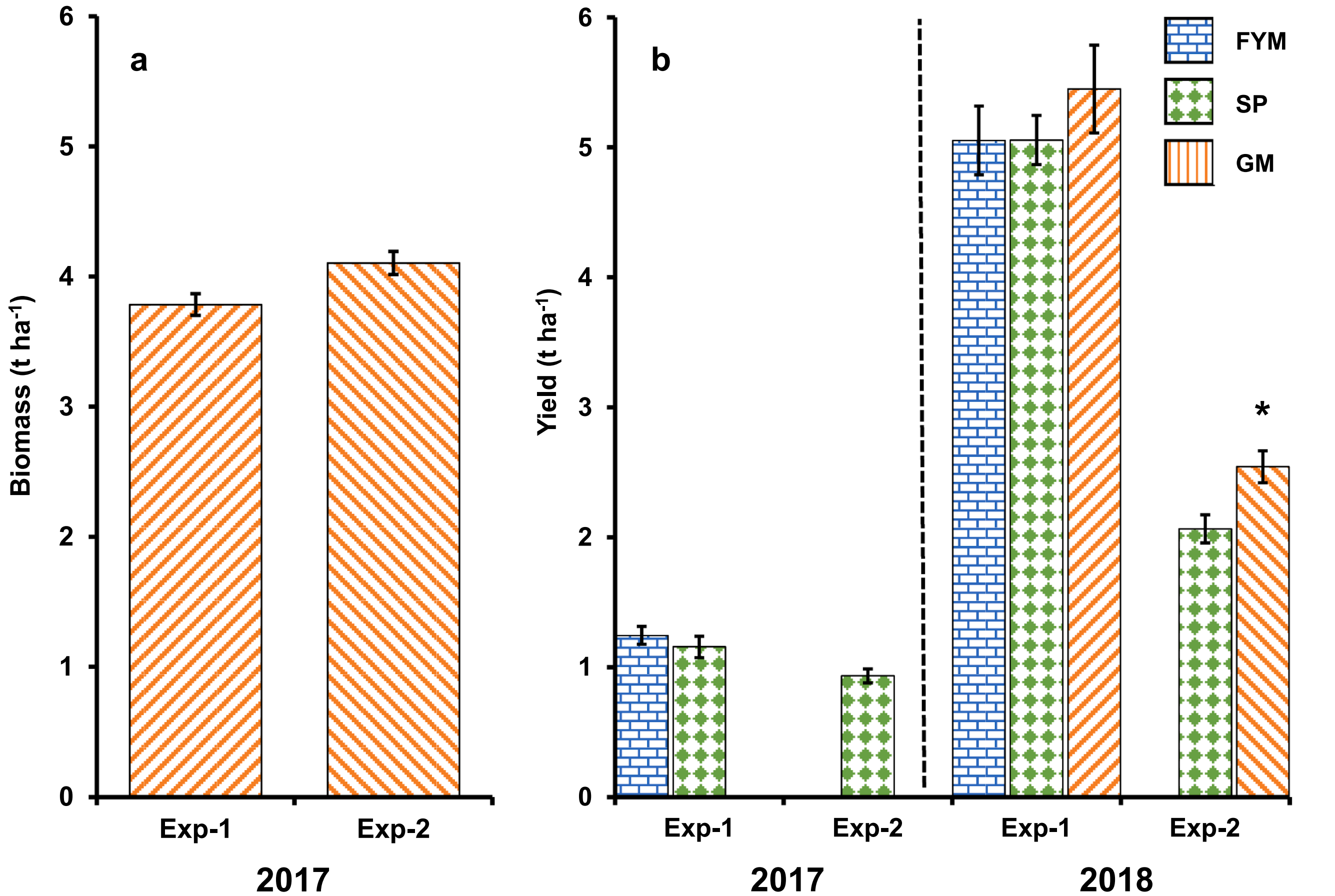

Fig. 8. a) Aboveground biomass (t ha^{-1}) of *Raphanus sativus* and *Vicia* sp. cover crop mix from both Experiments 1 and 2 (Exp-1 and Exp-2) in 2017 crop season (n = 6 and n = 8, respectively), and b) grain yields for both experiments of two crop seasons, 2017 and 2018. In 2017, Spring wheat (*Triticum 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-1and Exp-2) on all treatment plots. Columns show means (E1 n = 6; E2, SP n = 9, GM n = 8); Error bars show ± standard error of the mean. Asterisk (*) symbolises significant difference ($p < 0.05$) between treatments.

manures albeit influenced by spatial and temporal variations. For instance, higher concentrations of extractable-Ca^{2+} in May-2017 as a result of liming. The spreading of Limex70 here, a $CaCO_3$ precipitate, may lead to a cycle of necessary remediations because soil acidification through N-fertilisation can occur with CO_2 being release to the atmosphere and Ca^{2+} leaching (Zamanian et al., 2021). Acidification was not observed, and pH values remained similar across sampling periods.

The greater concentration of extractable-Mg^{2+} in Experiment-2 suggests legacy effects from cover crops. Magnesium may be comparatively mobile in soils and form weaker bonds to soil mineral charges in comparison with other cations like K^{+}, Ca^{2+}, and NH^{4+} (Litvinovich et al., 2021). However, the concentrations of <60 mg Mg^{2+} L^{-1} can be inadequate for crop growth and Mg^{2+}-deficiency may partially explain reduced sloped site yields (Wang et al., 2020). Plant uptake and release of both Mg^{2+} and K^{+} has been found to be higher under legumes (e.g. *Vicia* sp.) and brassicas (e.g. *R. sativus*) than in cereals, due to their nutrient requirements (Groffman et al., 1987; Cardoso et al., 2013; Wendling et al., 2016). The sandier soil texture of Experiment-2 likely driven cover crop effects in November-2018. For example, Taiwo et al. (2018) reported a positive correlation between fixed K^{+} and %clay, whereas extractable-K^{+} correlated well with %sand. The dry spell of 2018 (Defra, 2019) also possibly aided cation retainment in soil. Sandy soils are prone to leaching following heavy-rainfall, a consideration when using cover crops for slow-release nutrient provision (Groffman et al., 1987). Runoff and leaching risk from sandy and/or sloping land can be minimised if amendments are applied in dryer periods, but nutrient efficiency relies on water transport (Yao et al., 2021). The GM treatment did not receive N-fertiliser in 2017 but it exhibited equivalent %tN concentrations to SP in October-2017. The low C:N stoichiometry of the cover crop mix (*Vicia* sp. and *R. sativus* foliage) means it can be quickly mineralised releasing N (Ketterings et al., 2011; Creamer et al., 2016; Berthelin et al., 2022). This adds further evidence that legumes, such as *Vicia* sp., could offset some short-term N-fertiliser requirements but that it is spatially dependent (Kaye et al., 2019).

An average 50 % more extractable-K^{+} was measured in the FYM treatment of Experiment-1 in May-2017 than in the SP or GM treatments, three weeks after incorporating it. Effects were observable in October 2017 and 2018 too. FYM can provide 7.2 K_2O t^{-1} kg and soil's extractable-K^{+} can increase following application (Defra, 2018; Taiwo et al., 2018). However, the muriate of potash applied in November 2017 could have enhanced legacy results. FYM is also a source of readily available N as observed by the higher %tN in October-2017. The temporal effect of %tN on Olsen-P, as in October-2017 with a lag period from N inputs, related to spatial variation. N-loadings stimulate soil phosphatase which catalyses the hydrolysis of P, a mechanism explaining Olsen-P increase in May–October 2018 in the SP and FYM treatments of Experiment-1 (Widdig et al., 2019; Chen et al., 2020; Dai et al., 2020). FYM (~2.4 kg SO_3 t^{-1}; Defra, 2010) impact on soil %tS was not immediate and quite varied. Microbial communities may not significantly vary across fields, but manures enhance nutrient cycling enzymatic activity, including S-cycling, contributing to bioweathering and mineralisation processes (Bowles et al., 2014; Min et al., 2018; Buckeridge et al., 2020; Finlay et al., 2020). FYM is a source of essential nutrients helping reduce fertiliser inputs and balance crop requirements.

However, regular soil tests should be conducted to avoid nutrient deficiencies or pollution because outcomes are influenced by soil inherent properties (Defra, 2018).

4.5. Crop productivity

The greater *R. sativus* and *Vicia* sp. aboveground biomass in Experiment-2 was likely caused by differences in soil properties. Brassicas, such as *R. sativus,* establish more successfully on soils with pH 5.8–6.2 (AHDB, 2015), thus the pH 6.2 encountered was in line with recommended values. Whereas pH 6.8 of Experiment-1 was higher than the optimum range. Soil texture also influences aboveground biomass, where lighter soils (< 20 % clay) provide better structure for root development and may lead to greater foliage biomass (AHDB, 2015). This was met in Experiment-2, which soil was classified as loamy sand (0–15 % clay) and sandy loam (0–20 % clay) (FAO, 2020). In Experiment-1, four soil textures were identified and 45.2 % of them were on heavier sandy clay loam (20–30 % clay) (FAO, 2020). Other soil properties such as %tN affect crop growth and protein content too. The greater concentration of soil %tN in Experiment-1 resulted in higher N acquisition but not greater aboveground biomass. Soil's inherent properties like texture led to different outcomes, in fact N-uptake may not link to biomass production (Finney et al., 2016).

The *T. aestivum* yields (spring wheat) in the FYM treatment were comparable to those in SP (1.2 t ha^{-1} ± 0.2 SD in both) which received N-fertiliser alone, but Experiment-1 overall yields were considerably lower than national average (6.8 t ha^{-1} UK average in 2017, Defra, 2017). The reasons of reduced yields were not ascertained but there was evidence of leaf scorching, perhaps caused by herbicide application of Ally Max SX (42 g ha^{-1}) and Duplosan (1 L ha^{-1}), and signs of Take-all disease (Thomas, 1986; AHDB, 2010). These factors in combination with conversion to no-till could help explain the low yields. The weather conditions of 2018 negatively impacted crops; ranging from snow cover and heavy rainfall early spring followed by a long hot and dry spell in summer (Defra, 2019). However, yields recovered in 2018 to comparable *A. sativa* (winter oats) national levels (Defra, 2019). The average Experiment-1 yield was 5.1 t ha^{-1} on both SP and FYM treatments, 5.4 t ha^{-1} in GM, and the UK average was 5.0 t ha^{-1} (Defra, 2019). The yields and grain protein in the GM treatment of Experiment-2 implies a response to cover crop residues. Even though there were no soil %tN significant treatment differences, there could have been legacy effects from the N-fixing *Vicia* sp. residues resulting in higher N uptake (Kaye et al., 2019). However, slope side yields were much lower than UK average; SP = 2.1 t ha^{-1} and GM = 2.5 t ha^{-1}.

5. Conclusion

This study investigated the short-term interaction between inputs to soil in a no-till arable system on SOC and SOM, combined with microbial biomass and soil properties such as extractable Ca^{2+} and total-N. The type of soil organic amendments, FYM or *R. sativus* and *Vicia* sp. cover crop mix, did not significantly impact SOC and SOM but did impact MBC and soil nutrient concentrations over a two-year period. The results indicate that spatial heterogeneity in a 2.5 ha area, where soil types vary between sandy loam, sandy clay loam and loamy sand soils, can lead to different results depending on sampling location. This insight is critical when considering setting up experiments to investigate soil C dynamics, and also farm subsidies and C trading based on slowly changing measurements of SOC or SOM. Protecting soil and building C stocks and/or preventing further decline remains a target that should be aimed for.

It was confirmed that MBC 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. FYM and cover crops benefited soil chemical properties that support crop growth such as maintaining a balanced pH, %tN, %S, and extractable K^{+} and Mg^{2+}, although the extent of these varied depending on the spatial and temporal scales of the study. In addition, there were no significant differences in wheat yields observed with reduced synthetic N-fertiliser. However, in the first year after conversion to no-till, the yields were significantly lower than the national average. This could have been caused by the conversion process, crop diseases, and/or scorching. The yields of winter oats in the second year were comparable to national levels in Experiment-1 but not in Experiment-2, indicating spatial variation effects once more. These experiments further highlight the need for long term studies for understanding soil C and nutrients dynamics in sufficient detail to inform policy development.

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.

Data availability

Data will be uploaded to an EU database.

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