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Research article

Response of soil health indicators to dung, urine and mineral fertilizer application in temperate pastures

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ABSTRACT

Healthy soils are key to sustainability and food security. In temperate grasslands, not many studies have focused on soil health comparisons between contrasting pasture systems under different management strategies and treatment applications (e.g. manures and inorganic fertilisers). The aim of this study was to assess the responses of soil health indicators to dung, urine and inorganic N fertiliser in three temperate swards: permanent pasture not ploughed for at least 20 years (PP), high sugar ryegrass with white clover targeted at 30% coverage reseeded in 2013 (WC), and high sugar ryegrass reseeded in 2014 (HG). This study was conducted on the North Wyke Farm Platform (UK) from April 2017 to October 2017. Soil health indicators including soil organic carbon (SOC, measured by loss of ignition and elemental analyser), dissolved organic carbon (DOC), total nitrogen (TN), C:N ratio, soil C and N bulk isotopes, pH, bulk density (BD), aggregate stability, ergosterol concentration (as a proxy for fungi biomass), and earthworms (abundance, mass and density) were measured and analysed before and after application of dung and N fertilizer, urine and N fertiliser, and only N fertiliser. The highest SOC, TN, DOC, ergosterol concentration and earthworms as well as the lowest BD were found in PP, likely due to the lack of ploughing. Differences among treatments were observed due to the application of dung, resulting in an improvement in chemical indicators of soil health after 50 days of its application. Ergosterol concentration was significantly higher before treatment applications than at the end of the experiment. No changes were detected in BD and aggregate stability after treatment applications. We conclude that not enough time had passed for the soil to recover after the ploughing and reseeding of the permanent pasture, independently of the sward composition (HG or WC). Our results highlight the strong influence of the soil management legacy in temperate pasture and the positive effects of dung application on soil health over the short term. In addition, we point out the relevance of using standardised methods to report soil health indicators and some methodological limitations.

1. Introduction

Most terrestrial-based nutrients consumed by humans are produced by soils. Therefore, healthy soils determine important sustainability issues such as food insecurity and poverty (Montanarella et al., 2015; Kopittke et al., 2022). The link between soil health, sustainability and food security is key to achieving the United Nation's (UN's) Sustainable Development Goals, particularly tackling poverty, ending hunger and land degradation, and promoting health, well-being, responsible production and carbon sequestration (Keesstra et al., 2016). As a consequence, the quality of soils around the globe influences national development and, thus, regional policies (Rojas et al., 2016; Stroud, 2019; Visser et al., 2019).

In agricultural systems, a soil health assessment is especially relevant for feeding an ever-increasing population. Projections estimate that 95% of human food relies on healthy soils for food security (Montanarella et al., 2015; Sarkar et al., 2020; Williams et al., 2020). As a case exemplar, animal-sourced foods grown on extensive unmanaged lands will typically produce lower yields than food grown on well-managed land, of which soil is arguably the most important factor (Dick et al.,

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2015).

The first step of soil health assessment, a term often used interchangeably with soil quality, is a selection of physical, chemical and biological soil properties as indicators relevant to soil functions due to soil health cannot be directly measured (Drobnik et al., 2018; Janzen et al., 2021; Rinot et al., 2019). Soil functions include food and biomass production, nutrient cycling and water filtering, carbon sequestration, biodiversity hosting and heritage support which can be inferred through measuring texture, bulk density, pH, soil organic carbon (SOC), macro and micronutrients, and earthworms, among other popular parameters (Drobnik et al., 2018; Hermans et al., 2021). These measurements need to be accurate and reliable in order to report and verify changes over time as well as to allow comparisons across land uses and different managements. For instance, in clayey soils, the loss of ignition method can overestimate the soil organic matter (SOM). Furthermore, it is not recommended to use the conversion factor from SOM to SOC calculation to avoid adding significant bias for reporting an accurate SOC content (Jensen et al., 2018; Nelson and Sommers, 1982). In this sense, many studies have suggested that the SOC should be determined using an elemental analyser, and when only SOM is available, developing specific equations for calculating the SOC instead of applying the general conversion factor (FAO, 2019; Nelson and Sommers, 1982; Orgiazzi et al., 2018).

Soil health indicators are influenced by a variety of factors that, individually or interactionally, give rise to complex processes with the soil medium itself (Adhikari and Hartemink, 2016). Many soil health studies have focused on arable soils or on a few soil parameters and do not typically include different grassland soils and management interventions (Byrnes et al., 2018; Idowu et al., 2008; Lai and Kumar, 2020; O'Neill et al., 2021), even though grasslands cover ~ 40% of the terrestrial surface (Blair et al., 2014). Soil type, climate, grazing management (strategy and intensity), and pasture composition are among the main factors that can impact grazing effects on soil (Byrnes et al., 2018). However, their combined effects, for instance, manure and fertiliser applications under different pasture systems on soil health indicators, are still far from being understood (Dahal et al., 2021).

Given the importance of soil health as defined above, this study was conducted on the North Wyke Farm Platform (NWFP), a National Capability where the major hypothesis is that re-seeding of permanent grassland with varieties that potentially increase C sequestration, such as high sugar grass species and legumes, can provide more sustainable grassland systems. In this study, we specifically focused on assessing the aforementioned soil health indicators as they represent essential criteria to our understanding of the complexity of soil functionality to optimise grazed grassland management to maximise sustainability at a regional scale (Byrnes et al., 2018; Lai and Kumar, 2020).

The overarching aim was to assess soil health indicators responses to dung, urine and inorganic N fertilizer in three pastures or swards, which were historically grazed until the experiment started, under temperate climate. The pasture types included were PP, permanent pasture (*Lolium perenne* L.); HS, the same permanent pasture that had been converted to a high sugar ryegrass (*Lolium perenne* cv. AberMagic); and WC, the permanent pasture converted to a high sugar ryegrass mixed with clover (*Trifolium repens* L.). We hypothesize that: (i) the dynamics of soil health indicators in the three swards are mainly influenced by the previous land management; (ii) in the short term (months), dung, urine and inorganic fertiliser application impact mainly chemical and biological soil parameters but not physical soil properties.

2. Material and methods

2.1. Study area

This study was conducted on the NWFP, a farm-scale experimental system established as a National Capability at Rothamsted Research in the southwest of England in 2010 (Orr et al., 2016). The region has a temperate maritime climate, with a historical mean annual rainfall of 1033 mm, and an annual average temperature ranging from 6.8 (low) to 13.4 (high) °C. Daily maximum and minimum temperature, as well as rainfall data, were recorded from April to October from the meteorological station situated on site (Fig. 1). According to the British soil series, soils included in this experiment are classified as Hallsworth and Halstow series (Harrod and Hogan, 2008), both described as Stagnic Vertic Cambisol by the IUSS Working Group WRB (2015). Soils are non-calcareous and characterized by a high clay content (22–36%, depending on the horizon), minor drainable pores, and very slow hydraulic conductivity (Harrod and Hogan, 2008).

The NWFP comprises three pasture-based livestock farming systems (referred to as pasture systems from now on), each consisting of five catchments over 21 ha that maintain the same grazing intensity (30 weaned beef cattle, and 75 ewes and their lambs each). The cattle are born and reared in an adjacent but separate cow-calf enterprise until weaning. Then, they are randomly assigned to the three pasture systems or swards following a covariate-based constrained randomisation process to assure a balance for breed, gender, and sire among the three groups. At six months of age, the calves are housed for the winter period until the following April, when cattle are turned out to pasture and rotated around the catchments of each sward. In other words, the cattle from each system only consume feed, i.e., silage, or roughage produced from the same system. The grazing season generally lasts six months, from April to October with silage produced from each sward providing forage for the winter months, with typically two to three cuts per season. Manure generated during the housing period by each herd is applied to the fields in their associated system after each silage cut.

For this study, carried out during the grazing season of 2017, one field from each pasture system (Table 1) was selected according to field size and animal movement: (1) permanent pasture (PP), composed mainly of perennial ryegrass (*Lolium perenne* L.) with some unsown grass, legume and forb species (See Orr et al., 2016 for complete botanical composition); (2) high sugar perennial ryegrass monoculture (HS) (*Lolium perenne* cv. AberMagic); and (3) a species mixture (WC) of white clover (*Trifolium repens* L.) and the perennial ryegrass variety seeded in HS to provide 30% cover of the legume.

The three fields were managed as permanent pasture (PP) until 2013 when step-wise changes were applied in two of them to establish the HS and WC treatments (Takahashi et al., 2018). The WC treatment was established in July 2013, and HS in July 2014. PP was considered as control to allow the comparison between treatments over time (Orr et al., 2016). Prior to this sward transitional conversion, the permanent pasture in the three fields had not been ploughed for at least 20 years. The farm management plan established the inorganic fertilisation for PP and HS following a standard rate of 40 kg N ha $^{-1}$ per application. Inorganic fertiliser is generally not used in WC because of the white clover role in N fixation.

2.2. Experimental design

The experiment was established according to a split-plot design. On each field, three experimental blocks (15 m × 1.5 m) were established 50 m from each other in an equilateral triangular pattern in Spring 2017). We treated blocks as replication of pasture type since the NWFP is an unreplicated system-based approach that was established with equivalent starting conditions (Orr et al., 2016).

These blocks were fenced while animals were grazing the fields but otherwise managed similarly to the rest of the pasture. Each block was further divided into six plots (2.5 m × 1.5 m) positioned along a contour and randomly assigned to either a treatment (2 treatments) or control (2 controls). Therefore, four plots of each block were included in this study (Fig. S1). In total, we obtained 3 pasture types x 3 blocks per pasture type x 4 plots per block x 1 treatment or control per plot = 36 plots.

Treatments were defined as dung (D) and urine (U) and the controls were defined as inorganic N fertiliser (N_fert) and no N fertiliser (0 N).

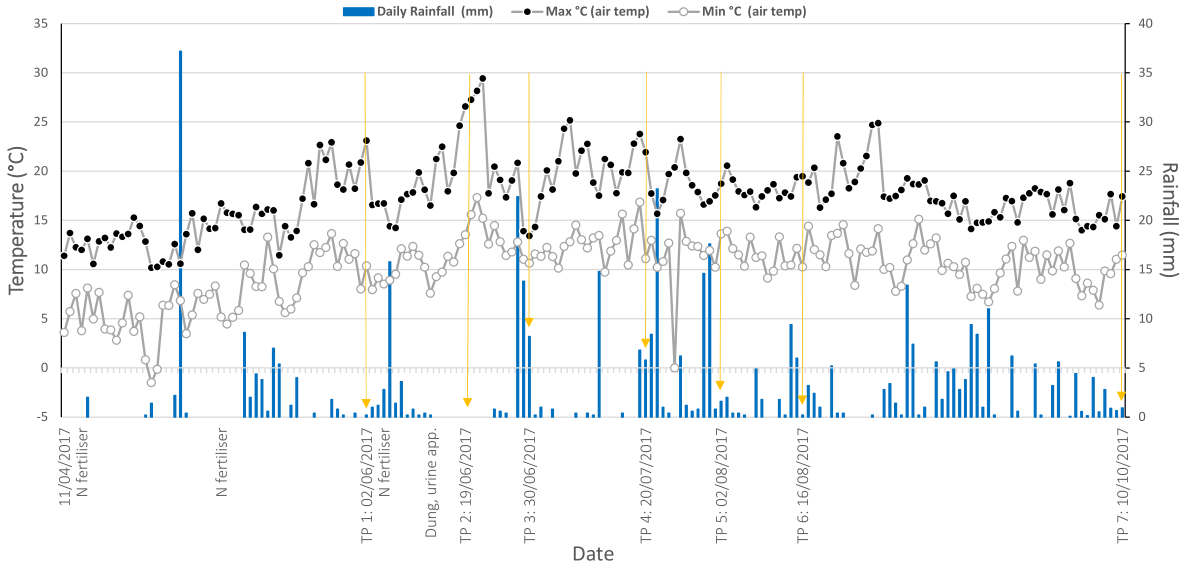

Fig. 1. Daily rainfall, maximum and minimum daily temperature measured during the experiment at North Wyke meteorological station. Date of soil sampling (timepoints 1 to 7, TP1-TP7) and N-applications are marked on x-axis. Arrows indicate soil sampling dates.

Table 1
Pasture system and soil properties (Mean ± SE) of each field in the uppermost 10 cm of the soil measured in July 2016 (Orr et al., 2016). SOC = soil organic carbon (%); TN = total nitrogen (%); BD = bulk density (g cm^{-3}).

Field	Pasture	SOC (%)	TN (%)	pH	BD (g cm^{-3})
Poor Field	HS	3.88 ± 0.22	0.41 ± 0.02	5.74 ± 0.03	1.08 ± 0.03
Orchard Dean South	PP	6.02 ± 0.17	0.61 ± 0.02	5.63 ± 0.04	0.86 ± 0.03
Higher Wyke Moore	WC	3.87 ± 0.15	0.40 ± 0.01	5.47 ± 0.03	0.98 ± 0.02

HS = high sugar grass monoculture; PP = permanent pasture; WC = white clover and high sugar grass mix.

Rates of dung and urine (see manures collection in 2.3.) were applied to D and U plots on June 13, 2017 (Fig. 1 and Fig. S1) equivalent to values of returned material during a single deposition event (20 kg m^{-2} and 5 l m^{-2} for dung and urine respectively) (Cardenas et al., 2016). Inorganic N was applied in the form of ammonium nitrate three times during the grazing season at a rate of 40 kg N ha^{-1} per application on N_fert plots (Fig. 1 and Fig. S1). In PP and HS, the treatment plots (D and U) received the same rate of inorganic N fertiliser as the N_fert control. N fertiliser was not applied in WC plots, therefore, two 0 N controls were included for this sward. Details on the experimental design were described previously in McAuliffe et al. (2020).

2.3. Dung and urine collection

Fresh dung deposits on pastures were identified, collected, and homogenized for each pasture type to ensure there was no cross-contamination using a cement mixer. Urine came from steers, which was collected in the cattle handling facility, and from heifers encouraged to urinate via vulva stimulation. Both urine and dung were collected separately from cattle grazing in each sward to maintain closed nutrient cycles, they were processed, and properly stored in sealed containers at 4 °C until the day of the application (McAuliffe et al., 2020).

2.4. Forage

Herbage samples were collected by cutting from a designated 50 cm × 50 cm area in each plot different from soil sampling area at two timepoints during the experiment that lasted 169 days, once before dung and urine application, and once 58 days after treatment application (Fig. S1).

2.5. Soil health indicators sampling

We selected a widely considered set of soil parameters linked to soil functions, such as biomass production, climate regulation, nutrient and water cycling: bulk density (BD), aggregates, pH, soil organic carbon (SOC), total nitrogen (TN), and earthworms (Adhikari and Hartemink, 2016; Bünemann et al., 2018; Drobnik et al., 2018; Lehmann et al., 2020). Dissolved organic carbon (DOC) was included because it is considered a primary form of labile C in terrestrial ecosystems and used as an index of C availability (Guo et al., 2020). Similarly, we included in our assessment ergosterol concentration as a proxy of fungal biomass in grasslands (Frac et al., 2018; Rousk et al., 2011) and bulked soil isotopes in order to understand carbon and nutrient transformation processes in temperate grazing grasslands (Dungait et al., 2009).

A soil sample from the topsoil (0–10 cm) was randomly taken in each plot for chemical soil health indicators analysis at seven timepoints during the experiment: 11 days before treatment (dung and urine) application, and 6, 17, 37, 50, 58, 64 and 119 days after treatment application (Fig. S1). In total, 36 soil samples per timepoint were obtained (3 blocks x 4 plots x 3 sward). Soil samples for bulk density determination (BD, g cm^{-3}) were taken at the end of the experiment in each plot using the cylinder method (Blake and Hartge, 1986). The soil aggregates were extracted from the soil cores collected for BD once this property was determined.

Subsamples for ergosterol analysis were taken from the soil samples collected in timepoints 1 and 7 (Fig. S1). Earthworms were collected from a 20 cm × 20 cm x 20 cm soil pit in each plot at the end of the study (Bone et al., 2012). The soil from each pit was hand-sorted for 10 min and earthworms were separated into adults (with a well-developed clitellum) and juveniles to record the number of individuals (N) and mass (g) for each pit. Earthworm density (N m^{-2}) and mass per area (g m^{-2}) were calculated according to Stroud (2019).

2.6. Laboratory analysis

Dung and herbage samples were analysed for total carbon (TC, %), total nitrogen (TN, %), and C and N bulk isotope abundance (‰) using a

Carlo Erba NA 1500 analyser. Previously, 15 mg for dung and 3 mg for herbage ground oven-dried material was weighed into a foil capsule. The C and N isotope abundances were expressed in delta units ($\delta^{13}C$ and $\delta^{15}N$, ‰) relative to the international PDB limestone standard (Balesdent and Mariotti, 1996). Urine samples were diluted 50-fold (0.5 ml urine was made up to 25 ml with ultra-pure Milli-Q water), and then analysed for TN and TC contents using a Shimadzu Total Organic Carbon Analyser TOC-L Series.

Soil organic matter (SOM, % dry matter) was estimated by the loss on ignition (LOI) method. The Carlo Erba elemental analyser was also used to analyse TC (%), TN (%), and isotope abundance in the soil in timepoints 1,5, and 7. Due to the absence of carbonates in soils of NWFP (mean pH = 5.96), TC (%) in soil was assumed as SOC (ISO, 1995). Hot water extraction was carried out to determine the dissolved organic carbon (DOC, mg g^{-1}) concentration in soil samples. Soil pH was measured at a soil:water ratio of 1:2.5 (w/w).

The mean weight density (MWD, mm) was used as an index of soil resistance to disintegration. MWD was determined by wet aggregate stability on oven-dried aggregates (Kemper and Rosenau, 1986). For soil loss (% dry mass), the percentage of soil lost through a 50 μm mesh was calculated. This value was inversely proportional to water-stable aggregates (WSA) (Horrocks et al., 2019).

Ergosterol concentration was determined in soil samples following the extraction method described by Rousk and Bååth (2007).

2.7. Statistical analysis

Differences between pasture types in dung and urine composition were tested using one-way ANOVA. Dung isotope abundances were log-transformed.

Our experimental design followed a split plot design where pasture types were applied at block level and treatments were applied to plots within blocks. Preliminary differences in soil pasture systems before applying dung and urine (timepoint 1) were tested using two-way ANOVA with block (Pasture x Treatment). For these analyses, the treatment factor for each plot was re-coded as "fertilised" and "not-fertilised" to include the N fertilisation in PP and HS before dung and urine application.

Linear mixed models (LMMs) fitted with restricted maximum likelihood (REML) were performed to investigate the effects of pasture systems on forage composition and soil health indicators over time. Where there were multiple samples taken over time, the effect of time was tested at the within plot level. For forage composition analysis, Pasture x Treatment x Cut event were selected as fixed factors and the random factors were Block/Plot. Similarly, for exploring physical, chemical, and biological soil health indicators, the fixed effects were Pasture x Treatment x Timepoint, with Block/Plot as random factors. Normality and homoscedasticity were checked by exploring the residual plots of each model. The least significant difference (LSD) at the 5% level was calculated to further explore differences between mean values where evidence of a statistically significant difference was found. Errors are expressed as the standard error of the means (SEM). Correlation analyses and scatterplots were conducted to explore relationships between variables at the end of the experiment.

A linear regression was performed to obtain the relationship between SOC determined by the elemental analyser and SOM by LOI to calculate the SOC content for the timepoints 2, 3, 4, and 6 in order to correct a potential over estimation of SOC in NWFP clay soil. The stocks of SOC (Mg C ha^{-1}) for the topsoil (0–10 cm) at the end of the experiment were calculated using the BD after removing both the weight and volume of coarse particles according to Poeplau et al. (2017).

Statistical analyses were carried out with GenStat® for Windows (20th version, VSN International, 2020) and R (4.0.3 version, R Development Core Team, 2020).

3. Results

3.1. Dung, urine and pasture chemical characteristics

Pasture type had significant effect on dung and urine chemical composition (Table 2). The WC pasture showed significantly lower dung TC, TN, $\delta^{13}C$ and $\delta^{15}N$, while no differences were found between HS and PP. HS and PP had the mean lowest and highest urine TC, TN and C:N, respectively. Regarding pasture characteristics, significantly lower TC in the plots treated with D and U and N_fert was detected only in cut 2 (Table 3; Table S1). Before treatment application, TN in forage was higher in PP and lower in WC (Table 3; Table S1). Differences between D, U, and N_fert and 0 N were detected in PP and HS. After treatment application, TN decreased in HS and PP but increased in WC (Table 3; Table S1). Although the highest TN concentration in forage was found in the D treatment, no significant differences were detected between D and U treatments. As expected, WC showed the highest C:N ratio and PP the lowest before treatment application (Table 3; Table S1). Forage in D treatment showed a significantly lower C:N ratio compared to U and 0 N in HS, and N_fert and 0 N in PP after treatment application (Table 3; Table S1). In the first herbage cut event, with lower $\delta^{13}C$ than after treatment application, significant differences were found among pastures (WC < PP < HS; Table 3; Table S1). However, PP showed the lowest $\delta^{13}C$ and no differences were detected between WC and HS in cut 2 (Table 3). The highest $\delta^{13}C$ was found in the second cut event in the D treatment. Significantly lower $\delta^{15}N$ values occurred in WC regarding PP and HS after treatment application (Table 3; Table S1). Herbage in N_fert showed the highest mean $\delta^{15}N$ followed by D (Table 3). The lowest mean value was found in 0 N, although high heterogeneity was found in each pasture in treatments (Table 3).

3.2. Baseline soil health indicators in the three swards before treatments application (timepoint 1)

In preliminary two-way ANOVAs analysis before D and U

Table 2
Means (±SE) of total carbon (TC), total nitrogen (TN), C:N ratio, and isotope ratios ($\delta^{13}C$ and $\delta^{15}N$, ‰) in dung and urine for the different pasture types. Differences between mean values tested by ANOVA (LSD = Average least significant difference at 5% level).

		Pasture			ANOVA results
		HS	PP	WC	
Dung	TC (% dry matter)	42.5 (0.36)	42.71 (0.32)	40.93 (0.33)	$F_{2,6}$ = 8.47, p-value = 0.018, LSD = 1.16
	TN (% dry matter)	3.46 (0.03)	3.36 (0.03)	3.17 (0.03)	$F_{2,6}$ = 19.90, p-value = 0.002, LSD = 0.11
	C:N	12.25 (0.15)	12.69 (0.13)	12.90 (0.14)	$F_{2,6}$ = 5.06, p-value = 0.052, LSD = 0.49
	$\delta^{13}C$ (‰)	−32.04 (0.13)	−32.21 (0.11)	−32.70 (0.12)	$F_{2,6}$ = 7.96, p-value = 0.021, LSD = 0.41
	$\delta^{15}N$ (‰)	7.89 (0.35)	7.08 (0.31)	6.12 (0.33)	$F_{2,6}$ = 8.50, p-value = 0.018, LSD = 1.14
Urine	TC (mg L^{-1})	4522.00 (108.00)	7294.00 (74.00)	6051.00 (136.00)	$F_{2,6}$ = 171.73, p-value <0.001, LSD = 377
	TN (mg L^{-1})	1733.00 (20.00)	3311.00 (32.00)	1958.00 (34.00)	$F_{2,6}$ = 857.13, p-value <0.001, LSD = 100.91
	C:N	2.20 (0.04)	2.61 (0.03)	3.32 (0.03)	$F_{2,6}$ = 243.95, p-value <0.001, LSD = 0.13

HS = high sugar grass monoculture; PP = permanent pasture; WC = white clover and high sugar grass mix.

Table 3
Predicted means (±SEM) of forage total carbon concentration (TC, %), total nitrogen concentration (TN, %), C:N ratio, and isotope ratios ($\delta^{13}C$ and $\delta^{15}N$, ‰) for each pasture, treatment, and cut event. Forage was cut on May 25, 2017 (cut 1 = before dung and urine application), and on August 10, 2017 (cut 2 = 56 days after dung and urine application).

		HS				PP				WC			
		0 N	N_fert	D	U	0 N	N_fert	D	U	0 N	N_fert	D	U
TC	Cut 1	41.92 (0.37)	42.31 (0.31)	42.54 (0.31)	42.44 (0.31)	42.28 (0.31)	42.49 (0.31)	42.47 (0.31)	42.71 (0.31)	42.20 (0.26)	*	42.23 (0.31)	42.21 (0.31)
	Cut 2	41.94 (0.51)	40.91 (0.37)	41.13 (0.37)	41.52 (0.37)	42.42 (0.28)	41.41 (0.31)	41.00 (0.51)	41.73 (0.31)	41.58 (0.24)	*	41.23 (0.31)	41.18 (0.31)
TN	Cut 1	2.08 (0.19)	2.32 (0.16)	2.80 (0.16)	2.66 (0.16)	2.53 (0.16)	3.23 (0.16)	3.00 (0.16)	3.04 (0.16)	2.11 (0.12)	*	2.15 (0.16)	2.14 (0.16)
	Cut 2	1.61 (0.27)	2.03 (0.19)	2.24 (0.19)	1.70 (0.19)	1.85 (0.19)	2.23 (0.15)	3.08 (0.26)	2.67 (0.16)	2.48 (0.11)	*	2.69 (0.16)	2.45 (0.16)
C:N	Cut 1	20.31 (1.35)	18.29 (1.11)	15.18 (1.11)	16.27 (1.11)	17.01 (1.09)	13.39 (1.09)	14.00 (1.09)	13.85 (1.07)	20.08 (0.85)	*	19.72 (1.11)	19.90 (1.11)
	Cut 2	26.05 (1.86)	20.17 (1.33)	18.31 (1.33)	24.62 (1.33)	22.89 (0.95)	18.73 (1.08)	13.56 (1.84)	15.76 (1.09)	16.87 (0.78)	*	16.01 (1.11)	16.85 (1.11)
$\delta^{13}C$	Cut 1	−30.93 (0.23)	−30.89 (0.19)	−30.77 (0.19)	−30.57 (0.19)	−31.32 (0.19)	−30.93 (0.19)	−31.30 (0.19)	−31.09 (0.19)	−31.54 (0.14)	*	−31.55 (0.19)	−31.64 (0.19)
	Cut 2	−30.29 (0.32)	−30.39 (0.23)	−29.79 (0.23)	−29.65 (0.23)	−30.61 (0.23)	−30.67 (0.19)	−29.94 (0.32)	−30.24 (0.19)	−30.38 (0.13)	*	−29.45 (0.19)	−30.43 (0.19)
$\delta^{15}N$	Cut 1	6.10 (0.64)	5.36 (0.53)	5.03 (0.53)	4.84 (0.53)	4.51 (0.49)	5.13 (0.49)	4.90 (0.49)	4.87 (0.46)	4.70 (0.38)	*	4.80 (0.53)	4.27 (0.53)
	Cut 2	4.43 (0.81)	4.36 (0.60)	3.38 (0.60)	3.37 (0.60)	3.25 (0.43)	4.44 (0.47)	4.79 (0.77)	3.48 (0.49)	1.89 (0.36)	*	4.10 (0.53)	2.08 (0.53)

HS = high sugar grass monoculture; PP = permanent pasture; WC = white clover and high sugar grass mix. 0 N = Control with no amendments; N_fert = control with nitrogen fertiliser; D = dung; U = urine.

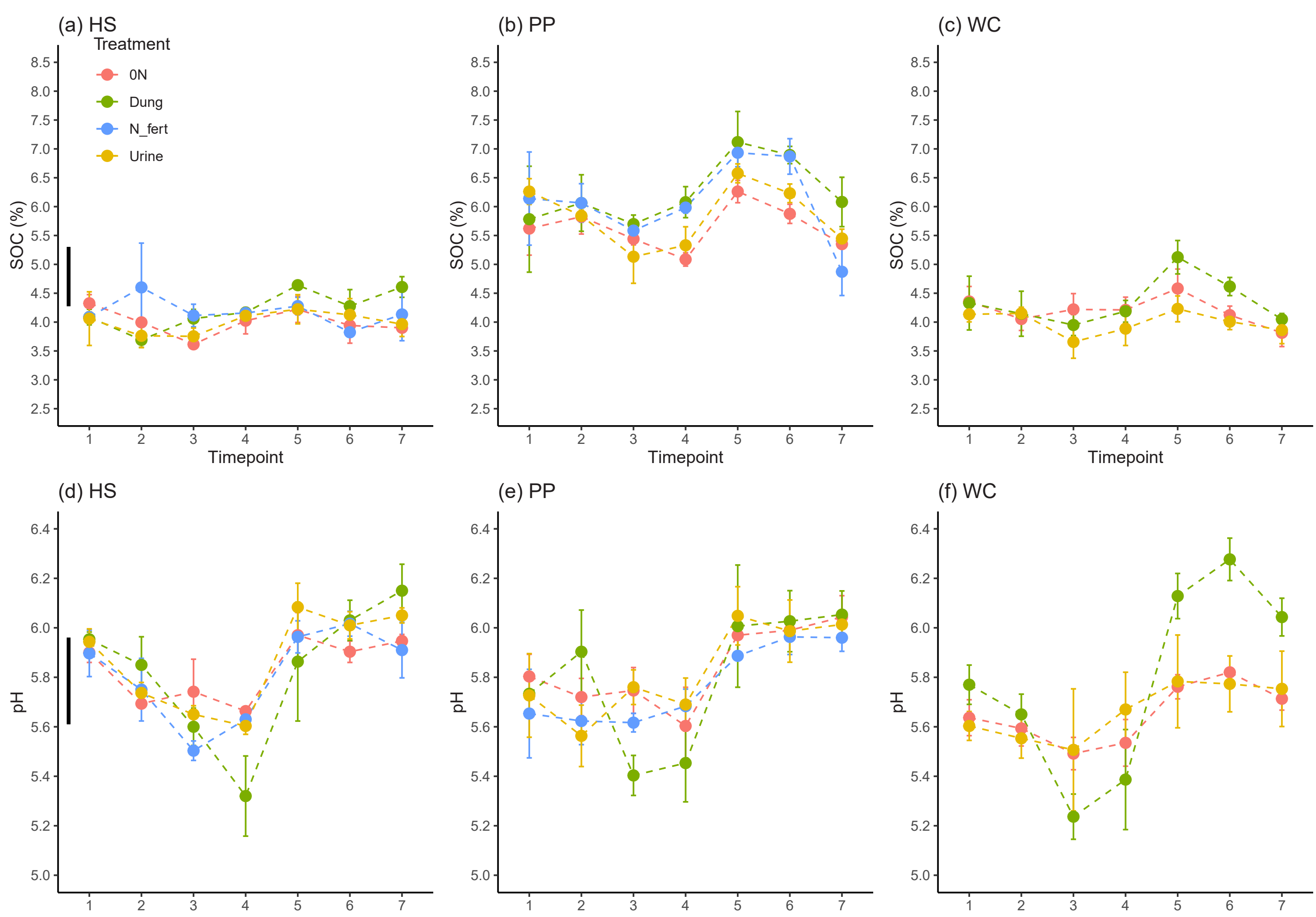

Fig. 2. Changes in soil organic carbon concentration (SOC, %) and pH in each pasture system over the experiment (mean ± SEM). Pasture systems: HS = high sugar grass monoculture; PP = permanent pasture; WC = white clover/high sugar grass mix. 0 N = Control with no amendments; D = dung; N_fert = control with nitrogen fertiliser; U = urine. Average LSD (5%) is represented by the black segment.

applications, PP showed significantly higher SOC, TN, $\delta^{13}C$, DOC, and ergosterol concentration than WC and HS (Table S2, Fig. S2). In addition, HS showed the highest pH. No differences among pastures were found neither in C:N ratio nor $\delta^{15}N$.

3.3. Soil health indicators in the three swards before treatments application over time

3.3.1. Chemical soil health indicators

The equation obtained to report SOC (%) for soils at NWFP was:

SOC = −0.23374 + 0.45268*SOM (R^2 adjusted = 0.84), where:

SOC = soil organic carbon determined by a Carlo Erba analyser (%), and SOM = soil organic matter estimated by LOI (%).

The highest SOC was found in PP before and after treatment application (Fig. 2a–c). Significant differences between treatments and controls were not always identified (Fig. 2a–c; Table S3). In general, D showed higher SOC content after treatment application, and U did not show differences regarding the controls, especially 0 N. In PP, SOC in the D treatment was only significantly higher than N_fert at the end of the experiment (Fig. 2b). Changes in SOC over time in the different pasture types were not always statistically significant, but in general, SOC increased after timepoint 3 to timepoint 5 in all treatments and decreased after timepoint 5 in HS and PP (Fig. 2a and b). In WC, the highest SOC was found in timepoint 5 (Fig. 2c). Subsequently, SOC fell to the same values as at timepoint 1.

At the end of the experiment, we did not find significant differences on SOC stocks (Table S3). The SOC stocks were 29.19 ± 2.17 Mg C ha^{-1} in WC, 32.95 ± 2.17 Mg C ha^{-1} in HS, and 35.27 ± 2.17 Mg C ha^{-1} in PP. The 0 N (30.88 ± 1.69 Mg C ha^{-1}) and D (35.32 ± 1.80 Mg C ha^{-1}) treatments showed the lowest and the highest SOC values, respectively.

DOC showed consistently higher concentration in PP than HS and WC (Fig. 3a–c; Table S3), and an identical trend to SOC (Fig. 2a–c, Fig. 4a-c). After treatment application, DOC in D treatment was generally higher than the rest of the treatments.

The TN results showed similar dynamics to SOC (%), but treatment was not statistically significant (Table S3). Significant changes between timepoint 1 and 7 were not found in TN (Fig. 3d-e).

Only the timepoint effect was significant for C:N ratio, which was highest at timepoint 5 (Fig. 3g-i, Table S3).

A significant $\delta^{13}C$ decrease between timepoint 1 and 5 was found in PP (Fig. 4b; Table S3). At the end of the experiment, the D treatment showed the lowest negative values while the highest values were found in 0 N (Fig. 4a–c; Table S3).

Different pastures and treatments displayed significant influence on $\delta^{15}N$ (Table S3) WC had the lowest values and the D treatment had the highest regarding U and 0 N, respectively (Fig. 4d–f). No differences were found between PP and HS pasture systems.

Timepoints, the interaction pasture and timepoint, and the interaction treatment and timepoint showed evidence of effects on pH. The main differences were found between timepoints 2-3-4 versus timepoints 5-6-7 (Fig. 3d-f; Table S3). A pH decrease was detected until timepoint 4, followed by a significant increase (Fig. 2d–f). The higher pH

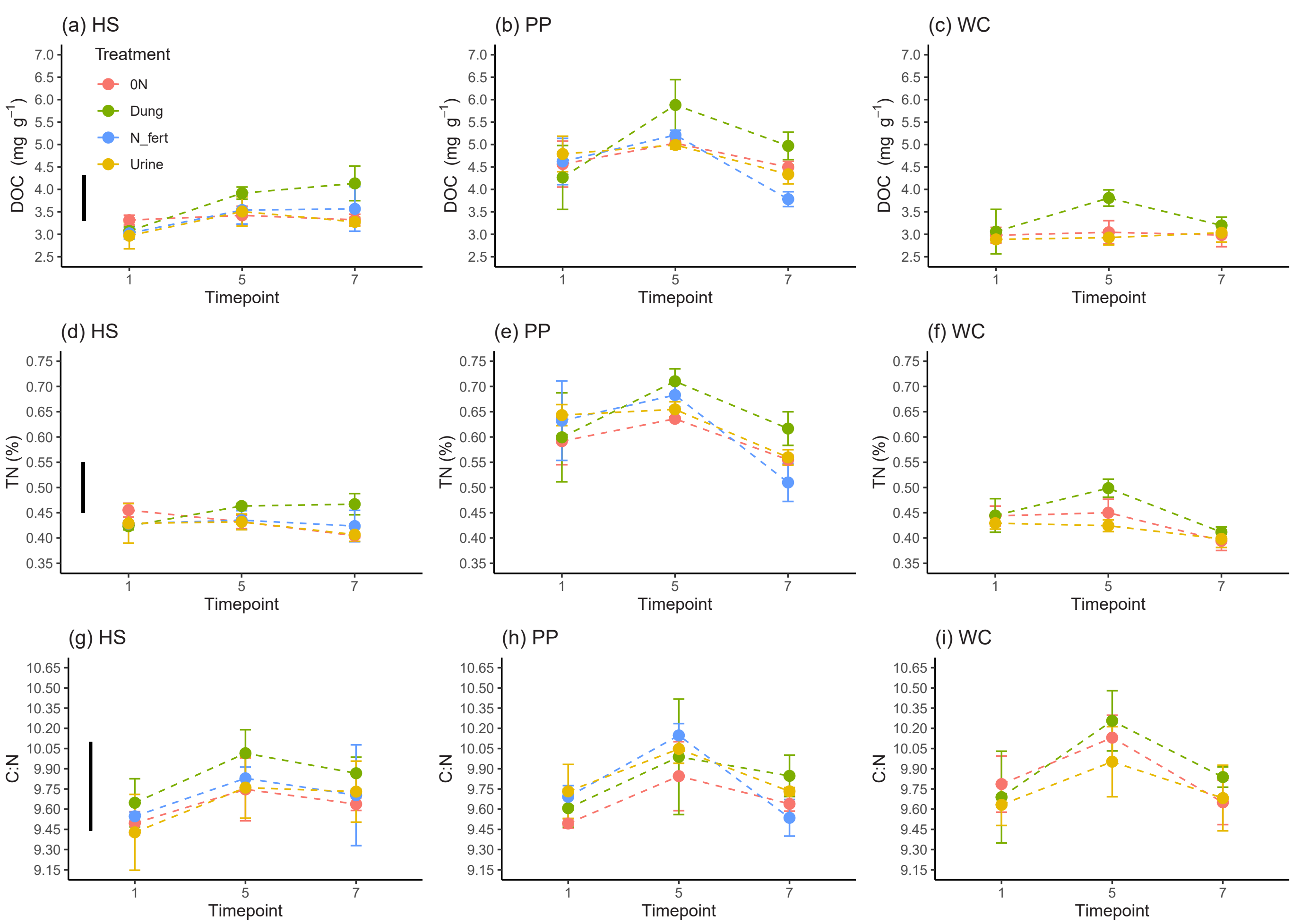

Fig. 3. Changes in soil dissolved organic carbon (DOC, mg g^{-1}) soil total nitrogen (TN, %) and C:N in each pasture system over the experiment (mean ± SEM). Pasture systems: HS = high sugar grass monoculture; PP = permanent pasture; WC = white clover/high sugar grass mix. 0 N = Control with no amendments; D = dung; N_fert = control with nitrogen fertiliser; U = urine. Average LSD (5%) is represented by the black segment.

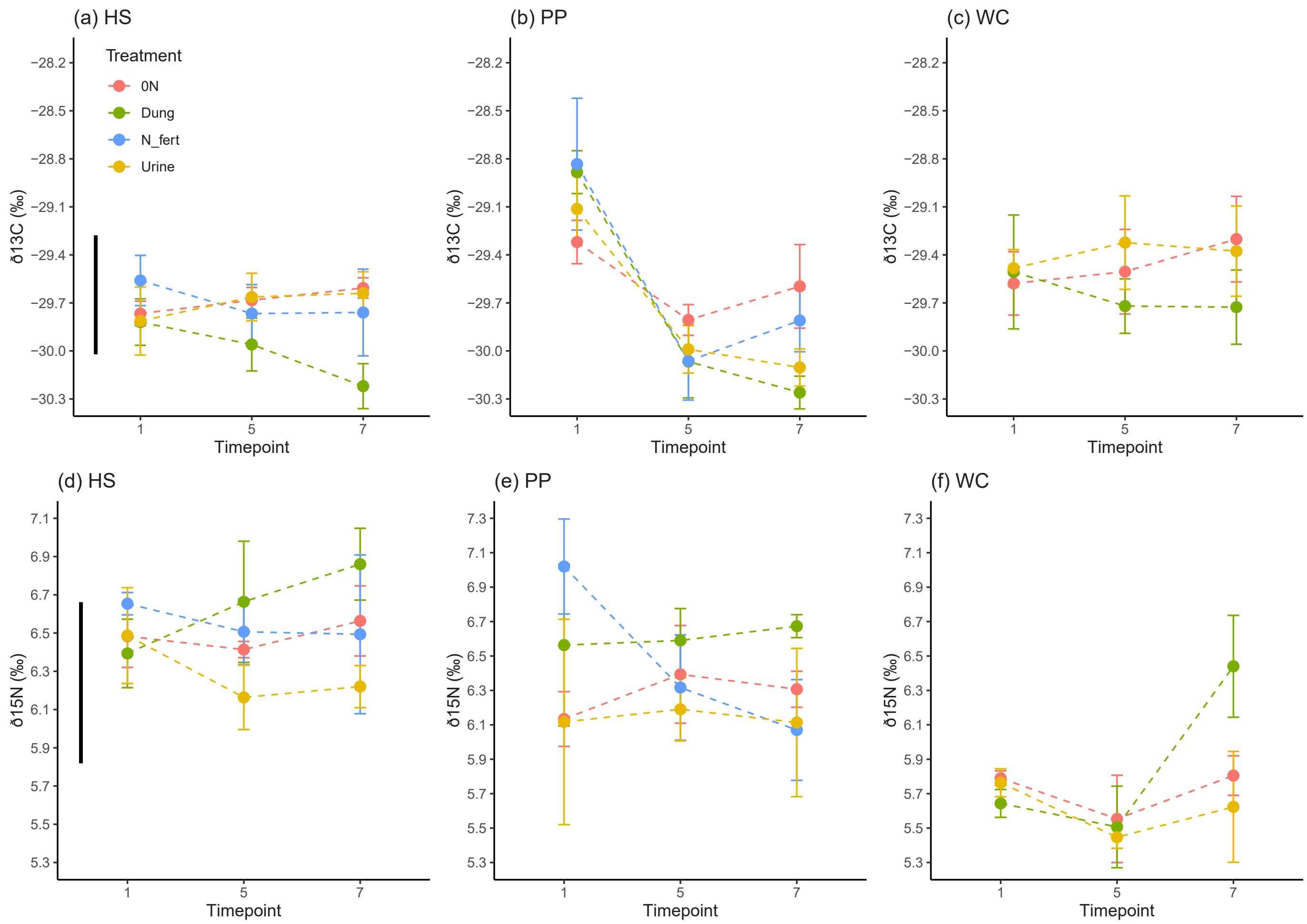

Fig. 4. Changes in soil $\delta^{13}C$ and $\delta^{15}N$ abundance (mean ± SEM). Pasture systems: HS = high sugar grass monoculture; PP = permanent pasture; WC = white clover/ high sugar grass mix. 0 N = Control with no amendments; D = dung; N_fert = control with nitrogen fertiliser; U = urine. Average LSD (5%) is represented by the black segment.

fluctuations were obtained in HS and WC pastures (Fig. 2d, f), and in the D treatment (Fig. 2d–f).

3.3.2. *Physical soil health indicators*

At the end of the experiment, the pasture system showed a significant effect on BD (Table S3). The PP pasture showed lower BD (0.66 ± 0.01 g cm^{-3}) than HS (0.80 ± 0.01 g cm^{-3}). No significant differences were found between HS and WC (0.75 ± 0.01 g cm^{-3}), and between PP and WD. The lowest BD values were found in the 0 N treatment in PP (0.60 ± 0.04 g cm^{-3}), and the highest BD was found in HS, and in D and U treatments (0.80 ± 0.05 and 0.80 ± 0.04 g cm^{-3}, respectively), although treatments showed no significant evidence of effects on BD.

Pasture and treatment did not show significant effects on MWD and Soil Loss, both indicators used for assessing aggregate stability (Table 4, Table S3).

3.3.3. *Biological soil health indicators*

Ergosterol concentration at timepoint 1 was significantly higher before applying the treatments than at the end of the experiment (Table 5).

The earthworms survey showed a greater predominance of juvenile earthworms in total abundance (98.7%), and subsequently, in total mass (95.4%) in all treatments. PP had significantly higher earthworm mass and abundance than other pastures (Table 6; Table S3). Both earthworm indicators were significantly higher in PP than in HS, but no differences were found in relation to WC. The D treatment had a higher abundance

Table 4
Predicted means (±SEM) Mean Weight Diameter (MWD, mm) and soil lost through 50 μm mesh on wet sieving (% dry soil mass) for samples collected on the three pasture types for each treatment.

Treatment	MDW			Soil loss		
	HS	PP	WC	HS	PP	WC
0 N	3.07 (0.11)	2.94 (0.11)	2.99 (0.09)	0.34 (0.17)	0.57 (0.17)	0.53 (0.14)
N_fert	2.96 (0.11)	2.81 (0.11)	*	0.62 (0.17)	0.76 (0.17)	*
D	3.09 (0.11)	2.95 (0.11)	3.08 (0.11)	0.39 (0.17)	0.93 (0.17)	0.32 (0.17)
U	3.08 (0.11)	2.79 (0.11)	2.96 (0.11)	0.43 (0.17)	0.62 (0.17)	0.45 (0.17)

HS = high sugar grass monoculture; PP = permanent pasture; WC = white clover and high sugar grass mix. 0 N = Control with no amendments; N_fert = control with nitrogen fertiliser; D = dung; U = urine.

than the N_fert, although no significant differences were detected for 0 N and U treatments.

3.4. *Soil health correlations at the end of the experiment*

The correlation analysis indicated that SOC was significantly positively correlated with TN and DOC and was significantly negatively correlated with BD (Fig. S3). However, no significant correlation was

Table 5
Predicted means of ergosterol concentration (±SEM) (%) for each treatment and in each pasture system. Timepoint 1: before treatment application. Timepoint 7: the end of the experiment, 119 days after treatment application. Approximate least significant difference (5% level) of REML means (LSD) = 0.6083.

Treatment	Pasture	Timepoint	
		1	7
0 N	HS	0.52 (0.19)	0.47 (0.19)
	PP	1.13 (0.19)	0.57 (0.19)
	WC	0.73 (0.15)	0.29 (0.15)
N_fert	HS	0.80 (0.19)	0.28 (0.19)
	PP	1.17 (0.19)	0.39 (0.19)
	WC	*	*
D	HS	0.81 (0.19)	0.39 (0.19)
	PP	1.22 (0.19)	0.52 (0.19)
	WC	0.68 (0.19)	0.41 (0.19)
U	HS	0.76 (0.19)	0.43 (0.19)
	PP	1.23 (0.19)	0.71 (0.19)
	WC	0.79 (0.19)	0.52 (0.19)

HS = high sugar grass monoculture; PP = permanent pasture; WC = white clover and high sugar grass mix. 0 N = Control with no amendments; N_fert = control with nitrogen fertiliser; D = dung; U = urine.

Table 6
Predicted means (±SEM) for earthworm total abundance (N) and total mass (g) per pit and per area (N m^{-2}, g m^{-2}) at the end of the experiment. LSD = Approximate least significant difference (5% level) of REMLS means.

	Total abundance (N) (LSD = 18.660)			Density (N m^{-2}) (LSD = 466.4)		
	HS	PP	WC	HS	PP	WC
0 N	19.67 (5.69)	43.00 (5.69)	33.50 (3.79)	491.7 (142.2)	1075.0 (142.2)	837.5 (94.75)
N_fert	16.33 (5.69)	30.00 (5.69)	*	408.3 (142.2)	750.0 (142.2)	*
D	47.67 (5.69)	65.33 (5.69)	50.00 (5.69)	1191.7 (142.2)	1633.3 (142.2)	1250.0 (142.2)
U	25.00 (3.06)	43.00 (3.06)	38.67 (4.49)	625.0 (142.2)	1075.0 (142.2)	966.7 (142.2)
	Total mass (g) (LSD = 7.605)			Total mass (g m^{-2}) (LSD = 190.1)		
	HS	PP	WC	HS	PP	WC
0 N	3.60 (2.32)	8.32 (2.32)	5.50 (1.66)	90.1 (58.0)	208.0 (58.0)	137.4 (41.5)
N_fert	2.89 (2.32)	9.58 (2.32)	*	72.3 (58.8)	239.5 (58.0)	*
D	10.50 (1.80)	17.07 (2.32)	11.21 (2.32)	262.4 (58.0)	426.7 (58.0)	280.2 (58.0)
U	4.80 (2.32)	9.56 (2.32)	5.99 (2.32)	120.1 (58.0)	239.0 (58.0)	149.7 (58.0)

HS = high sugar grass monoculture; PP = permanent pasture; WC = white clover and high sugar grass mix. 0 N = Control with no amendments; N_fert = control with nitrogen fertiliser; D = dung; U = urine.

found between SOC and MWD. MWD was positively correlated with BD and negatively with the soil loss percentage. Regarding biological indicators, both mass and abundance of earthworms were positively correlated with TN, SOC, and pH, and negatively with BD. Stronger positive correlations were obtained between ergosterol concentration and SOC, TN, DOC.

4. Discussion

4.1. Effects on chemical soil health indicators

It has been postulated that changing the management of grasslands could result in enhanced SOC sequestration, reduced N losses from grazing, and a general improvement of other soil health indicators (Li et al., 2017; Sarkar et al., 2020). However, in our study, PP had higher SOC, TN and DOC concentrations than HS and WC likely due to management legacy effects. The lack of soil disturbance in PP for at least 20 years may explain the high SOC content in this pasture system while ploughing and reseeding 3–4 years prior the experiment in the WC and HS pastures likely affected soil structure and aeration, accelerating the mineralization processes in the soil and decreasing SOC concentrations compared to the PP pasture (Necpálová et al., 2013). Additionally, the differences in soil microbial population composition reported by McAuliffe et al. (2020) before treatment applications reinforced the influence of land management on WC and HS compared to PP. Although our results should not be extrapolated to other fields in the NWFP, some studies have also showed the influence of ploughing and reseeding on sediment loss rates in the same study area (Pulley and Collins, 2020) that could influence soil health indicators.

In our study, differences among treatments were mainly associated with dung application, which resulted in increases in SOC and TN after 50 days (timepoint 5, Figs. 3 and 4), coinciding with heavy rainfall events recorded closely before this timepoint (80 mm from timepoint 4 to timepoint 5, see Fig. 1). Faster incorporation into soil of C derived from dung has previously been observed soon after rainfall events due to enhanced leaching (Dungait et al., 2005). In agreement with our results, other authors have also reported increases in DOC following manure applications prior to wet periods (Jones et al., 2006). Subsequently, a decrease in SOC and TN occurred, and no difference between timepoint 1 and timepoint 7 was observed for the D treatment, suggesting rapid SOM consumption by decomposers and complex interactions between soil processes and weather conditions (Lai and Kumar, 2020; Sarkar et al., 2020; Zhou et al., 2017).

As is commonly observed, soil TN concentrations covaried with SOC (Necpálová et al., 2013). The higher soil TN in the PP pasture compared with the HS and WC pastures was reflected by the higher TN in PP herbage. However, we could not detect significant differences in TN between treatments ($p = 0.055$). Although this result could indicate a trend, also it could be explained by a dilution effect or due to a statistical artefact. Further studies would be necessary to determine evidence of treatment effects on TN.

The C:N stoichiometry in different compartments of the soil-plant system and at different timepoints can be used as an indicator of nutrient dynamics in pastures. Before treatment application, the low C:N in PP herbage suggested a faster N return to the soil. Some authors have reported that manure with a high C:N ratio could limit decomposition processes whereas a lower C:N ratio, manifested as dung in HS in this study, might result in high N_2O emissions under saturated soil conditions (Jones et al., 2006). McAuliffe et al. (2020) reported the maximum peak of N_2O–N fluxes between timepoint 4 and 5 in D plots. We did, however, observe this dynamic also regarding 0 N: the increase in soil C:N ratio might respond to the interaction between weather conditions, increased SOC and the N uptake by grass, and again, likely linked to gaseous emissions resulting from enhanced denitrification under saturated soil conditions in the D treatment which corresponds to previous, similar studies (Emmett et al., 2010; Jones et al., 2005; Necpálová et al., 2013).

In our study, the varied plant species compositions of the swards are reflected by the differences in forage isotopes (Kriszan et al., 2009). The lack of differences between treatments receiving additions and the 0 N treatment in soil $\delta^{13}C$ suggested that the $\delta^{13}C$ dynamics were not strictly associated with the treatment application but with the timepoint measurements. Forage from the three pasture systems had lower $\delta^{15}N$ values than corresponding soils (Kriszan et al., 2009). The lower $\delta^{15}N$ values in WC soil indicates the potential of legumes to decrease soil $\delta^{15}N$ via N fixation from the atmosphere (Högberg, 1997). The lowest soil $\delta^{15}N$ value occurred in the 0 N treatment under WC, suggesting that a significant source of organic matter and N from dung application could reduce the N fixation by the clover (Kriszan et al., 2009). Similarly, our results showed a tendency of soil $\delta^{15}N$ -depletion after N fertiliser application mainly in PP. However, these results should be interpreted with caution as we were not able to detect a significant effect of the interaction between pasture and treatment factors on forage and soil $\delta^{15}N$, and as Kriszan et al. (2009) reported, the soil $\delta^{15}N$ content is mostly influenced by the N cycling legacy of previous management. Finally, the enrichment in $\delta^{15}N$ at the end of the experiment probably reflected enhanced soil mineralization and denitrification processes in D plots, as previously observed by McAuliffe et al. (2020).

The declining pH during the first few weeks after treatment applications was consistent with nitrification processes in soil (Haynes and Williams, 1993). This is in agreement with the results found by McAuliffe et al. (2020), who detected N_2O emissions driven by nitrification during the relatively dry period of this study (before timepoint 4, Fig. 1). Alternatively, rainfall events after timepoint 4 could have promoted the incorporation of alkaline dung components to the soil, thus increasing soil pH (Dungait et al., 2005; Lai and Kumar, 2020).

4.2. Effects on physical soil health indicators

In our study, the lowest BD occurred in the PP system and can be explained by the absence of ploughing and, consequently, by the high SOC concentration in this pasture system (Blanco-Canqui and Ruis, 2018; Necpálová et al., 2013). The application of fertilisers and manure can reduce BD in grazed grasslands driven by increases in organic matter inputs to the soil (Conant et al., 2017), but there was no evidence of this in our study. The lack of differences between the control and treatments suggested that treatment applications did not result in a significant effect on BD, likely due to the short-term duration of this experiment.

We found contradictory results in aggregate stability indicators. Surprisingly, PP showed the lowest MWD and the highest soil loss, although a statistical difference was not detected among pasture types, contrary to what would be expected considering that WC and HS were ploughed and showed lower SOC (Chenu et al., 2000). Moreover, no correlation was found between aggregate stability and SOC, although there is a lack of evidence of the effect of SOC content on aggregate stability (Abiven et al., 2009). In this sense, Pulley et al. (2021) reported a weak association between SOM and aggregate stability in soils close to our study area. Nevertheless, the wet aggregate stability method remains highly controversial. It has been largely used to assess soil health because of its simplicity and nonspecific equipment requirement (Horrocks et al., 2019), but many consider it to be an artefact due to its dependency on environmental conditions at the time of sampling and soil manipulation intensity, thereby making it a rather crude property to measure in a study such as this (Baveye, 2021).

4.3. Effects on biological soil health indicators

Temporal variation in ergosterol concentration in grassland soils has been linked to seasonal changes in the availability of decomposable substrates for fungi (Linsler et al., 2015; Turgay and Nonaka, 2002). Our values were similar to those reported by (Linsler et al., 2015) for temperate grasslands managed for cattle grazing and silage production, with the minimum ergosterol concentration also recorded in October.

The global database of earthworms in UK grazed pastures has reported similar density and earthworm mass to those we found in WC (Phillips et al., 2021). These values were also in the range of arable fields reported by Stroud (2019). However, we recorded greater earthworm density and mass in PP and WC than in these previous studies. The lack of ploughing and lower BD in PP could explain the higher earthworms values found in our study (Griffiths et al., 2018). The highest earthworm indicators in the D treatment is consistent with previous work in grazed pastures as these organisms are attracted to dung as a food source (Bacher et al., 2018). The higher pH found in the WC pasture under the D treatment could also explain the enhanced earthworm abundance and mass (McCallum et al., 2016).

5. Conclusion

This study highlights the importance of grassland management on indicators of soil health in the mid and short term. Firstly, the non-disturbed soil (PP) showed higher SOC, TN, DOC, and earthworms than the improved pasture systems after 3 and 4 years from the last change in land management (plough and reseeding new swards). We conclude that not enough time had passed for the soil to recover after the ploughing and reseeding of the permanent pasture, independently of the sward composition (HG or WC), indicating a strong influence of the soil management legacy. Secondly, the dynamic of chemical and biological soil health indicators was driven by dung application, not by urine and N-fertiliser treatments. In this sense, dung application improved chemical soil health after 50 days, but its effects disappeared after 119 days of its application. In addition, we reinforce the relevance of using standardised methods to report soil health indicators due to limitations of some methods (e.g., LOI to estimate SOM in clayey soils and aggregate stability).

Author contributions

Carmen Segura: Data curation; Formal analysis; Investigation; Methodology; Software; Visualization; Writing – original draft; Writing – review & editing, Claire Horrocks: Data curation; Investigation; Methodology, Maria Lopez-Aizpun: Writing – review & editing, Martin S. A. Blackwell: Writing – review & editing, Tegan Darch: Investigation; Methodology, Jess Hood: Methodology; Formal analysis; Validation; Writing – review & editing, Kate Le Cocq: Investigation; Writing – review & editing, Graham A. McAuliffe: Writing – review & editing, Michael R. F. Lee: Funding acquisition; Writing – review & editing, Laura Cardenas: Conceptualization; Methodology; Project administration; Resources; Supervision; Writing – review & editing.

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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 made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jenvman.2022.117096.

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