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SOIL ORGANIC CARBON STOCK AND FRACTIONAL DISTRIBUTION IN UPLAND GRASSLANDS

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ABSTRACT

Upland grassland soils are an important terrestrial carbon (C) store and provide vital ecosystem services such as climate regulation. The C stocks in these soils are subject to changes due to management activities. In this study, we compared soil organic C (SOC) stocks and fractions under traditional hay meadow and conventional (silage or permanent) pasture management regimes at two upland grassland locations within the Yorkshire Dales, northern England, United Kingdom. Stocks of SOC in the top 15 cm of the grassland soils were determined from bulk soil samples and in soil fractions after a combination of physical and chemical fractionation. Results showed that these upland grasslands stored significant amounts of organic C in the top 15 cm of their soils, ranging from 58.93 ± 3.50 to 100.69 ± 8.64 Mg ha⁻¹. Overall, there was little contrast between the sites except that soil C stock was significantly higher under the Nidderdale silage pasture that received the highest nitrogen (N) inputs, possibly due to enhanced vegetation growth and subsequent litter return to the soil. No effect of soil parent material was observed on soil C stock due to liming-induced increase in the pH of Nidderdale soils and neither management

activities nor soil parent materials affected the distribution of soil C into different fractions. In all sites, about 70% of the SOC stock was protected in stable soil fractions, specifically in the mineral soil mass, indicating a potential for the grasslands to contribute in climate change mitigation. However, due to the rapid turnover of the labile C pool and especially in a changing climate, it is recommended that further research be carried out in order to understand the turnover rates of labile C pools and whether management activities alter the distribution between the decomposition products. This will help to better understand whether rapid turnover of labile C will negate the likely benefits of stable C pools to climate change mitigation.

KEYWORDS: Soil carbon dynamics; carbon fractions; grassland management; upland soils; soil carbon stock

1. Introduction

Grasslands store a significant amount (~90%) of its sequestered carbon (C) in the soil (Ajtay et al., 1979), and often have higher soil C stock relative to other vegetation types in a given climate regime (IPCC, 2003). Management of grasslands can range from extensive practices such as no/minimal chemical inputs and occasional grazing to intensive practices such as regular chemical additions including lime, nitrogen (N), phosphorus (P) and potassium (K) containing fertilizers, and year-round grazing (Tiemeyer et al., 2016). Generally, these management activities have been shown to influence soil C dynamics (Soussana et al., 2007) but the exact effects of each management practice on C stock are not fully understood. For example, variable results have been reported in a number of studies that investigated how soil C respond to different management activities such as grazing intensity (Burrows et al., 2012; Han et al., 2008; Schuman et al., 1999), liming (Mijangos et al., 2010; Sochorova et al., 2016; Wang et al., 2016), and fertilizer application (Fornara et al., 2013; He et al., 2013; Neff et al., 2002). These studies reported either an increase, a decrease or no change in soil C stock due to management activities. The inconsistencies in these results may be due to differences in soil types, specific management activities and duration of management regimes as they change over time, for example, in response to financial incentives such as agri-environmental schemes.

An important area of current research interest is understanding the factors controlling soil C stock and its stability in response to grassland management (e.g. Ward et al., 2016). This is because storage of soil C in long-term pools is necessary for climate change mitigation and requires stabilization in forms that are less susceptible to loss (Adkins et al., 2016; Lal and Kimble, 1997; O'Brien and Jastrow, 2013). The stabilization mechanisms may be through: 1) biochemical formation of organic C (OC) compounds with molecular structures that make them

relatively more resistant to decomposition, 2) interaction of OC with soil mineral particles particularly clay and silt, and 3) occlusion of OC within soil aggregates (Breulmann et al., 2016; Sollins et al., 1996). In recent years, the idea of soil OC (SOC) possessing recalcitrant properties which make it stable in the soil has been challenged (Amelung et al., 2008; Lehman and Kleber, 2015; Schmidt et al., 2011) and the key mechanism of stabilization now favoured by researchers is physical protection of SOC from microbes regardless of its chemical structure (Dungait et al., 2012; Kleber et al., 2011). Also, the most important physical mechanism for stabilizing soil C is its association with reactive soil mineral particles because access by microbes and enzymes is limited (Conant et al., 2011; Mikutta et al., 2006). This has recently been corroborated by the results of a meta-analysis of 376 published laboratory incubation data, where clay content was found to be the most crucial regulator of SOC decomposition (Xu et al., 2016).

SOC is usually fractionated into functional pools such as labile (or active) and stable (or passive) pools, with different turnover rates or mean residence times (ranging from days to centuries and millennia), and this often serves as a relative measure of stability (Breulmann et al., 2016; Cambardella and Elliot, 1992; Roscoe and Machado, 2002; Von Lutzow et al., 2007). The active C pools such as particulate OC (POC), dissolved OC (DOC) and microbial biomass C (MBC) have the fastest turnover and are more sensitive to land management than the stable pools (Alvarez and Alvarez, 2016; deFigueiredo et al., 2010; Van Leeuwen et al., 2015). It is therefore important to study not just the changes in total SOC in response to environmental change, which requires long term monitoring to be able to detect, but the partitioning and dynamics of the various soil C pools. This would improve understanding of land management effects on SOC even in the short term.

In the UK, the greater number of studies on the response of soil C to management activities have focused on lowland grasslands (e.g. Clegg, 2006; Clegg et al., 2003; Hopkins et al., 2009, 2011), with results showing either a decrease, an increase or no change in soil C stock. However, a recent report based on a study of 180 permanent grasslands (mostly lowlands) in the UK, revealed a decrease in soil C stock with increasing management intensity (Ward et al., 2016). This study also suggested that soil parent material might have influenced the response of soil C to management activities (Ward et al., 2016) but this has not fully been investigated for UK grasslands. The effect of soil parent material is possible because there is a likely interaction between inherent soil characteristics and management activities that subsequently influences soil C dynamics. For example, in northern Spain, Mijangos et al. (2010) studied the effects of liming on soil characteristics and plant productivity in calcareous and siliceous grasslands; they found that liming significantly increased soil pH and microbial activity in the siliceous grassland compared to the calcareous grassland. Soil acidity is important for C dynamics because C tends to accumulate in the soil under acidic conditions, due to the predominance of fungi with high biomass and restricted microbial decomposition of organic materials (Alexander, 1977; Bardgett et al., 1993). This is also evident in the report of the UK Countryside Survey of 2007, where acid grassland soils store $82 \text{ t ha}^{-1} \text{ C}$ compared to neutral grassland soils which contain $61 \text{ t ha}^{-1} \text{ C}$ within 15 cm of the soil surface (Carey et al., 2008). Leifeld et al. (2013) also found that a one pH unit of acidification resulted in 22 to 86% increase in mean residence times of organic C pools.

Acidic soil conditions prevail in the UK's upland grasslands (grasslands mostly above 300 m altitude) primarily due to base-poor geology and high leaching associated with cool and wet climate (Floate, 1977; Holden et al., 2006). Elevated C stocks in these wet, acidic upland soils is

therefore to be expected. Yet, to the best of our knowledge, the dynamics of SOC in the mineral soils of the British upland grasslands remains poorly understood. These upland grasslands are mostly used for livestock production (Leifeld and Fuhrer, 2009; Medina-Roldan et al., 2012; Stevens et al., 2008) under management options that typically are influenced by environmental stewardship schemes, which aim to protect and enhance biodiversity. The interaction between parent material and management activity on soil C stock and stability has received relatively little research attention, and yet there are potentially important implications for development of new agri-environmental stewardship schemes and climate mitigation strategies. Therefore, the aim of this research is to assess the stock and fractions of SOC in the mineral soils of upland grasslands in northern England under: 1) traditional hay meadow and conventional pasture management regimes, and 2) soils of siliceous stone and limestone parent materials. We hypothesize that 1) SOC stock will be higher in soils under traditional hay meadow with intermittent grazing compared to soils under conventional pasture, 2) SOC stock will be lower in soils developed on limestone compared to soils that are poorly drained and developed on base-poor parent material due to likely effects of acidity and higher moisture content on organic matter decomposition, and 3) greater proportions of SOC stocks will be protected in stable forms due to high mineral contents of the soils at all the locations investigated.

2. Methodology

2.1 Study area

Two locations within the Yorkshire Dales, an upland area of the Pennines in Northern England, UK, were selected for this study; one in Nidderdale (54°09'N, 01°53'W) and the other in Ribblesdale (54°05', 02°16'W). Both locations are at an elevation of approximately 300 m and a

distance of about 20 km apart. These two locations were chosen because of their contrasting soil parent material (siliceous stones and limestone, respectively), which we expected to affect soil properties differently and how the soils would respond to management activities. The two locations are characterized by shallow soils, Stagnohumic gley at Nidderdale and Brown earth at Ribblesdale, with maximum depth to compacted layer being 20 cm. Mean annual temperature is 7.4°C and mean annual rainfall is 1550 mm, based on 1981 to 2010 average. At each location, there are two contrasting grassland management regimes: traditional hay meadow managed under an agri-environment scheme and conventional pasture managed for silage (Nidderdale) or permanent grass (Ribblesdale), making a total of four sites. The traditional hay meadows under agri-environment schemes are managed with the aim of restoring, protecting and enhancing biodiversity. At the time of this study, hay meadow management typically involved avoiding inputs of inorganic fertilizer, no cutting before July, no grazing during spring and early summer, and re-seeding with a wildflower mix where necessary (Natural England, 2010). Hay meadow management was very similar at the two locations. In contrast to the hay meadows, the management regime of conventional pasture was quite different at the two locations, and at the Nidderdale site was very similar to the hay meadow except that the field managed for silage received inorganic N and additional inputs of P and K in poultry manure, and had recently been reseeded with ryegrass (Table 1). At the Ribblesdale location, the permanent pasture received no organic manure inputs or fertilizer and was not cut, but was continuously grazed by sheep (Table 1). The Nidderdale site had been limed to raise soil pH three years before sampling.

2.2 Experimental design, soil sampling and analysis

As multiple fields with the two contrasting management regimes and soil types could not be found at each location, we established five 25 m² replicate plots within single fields. The plots were part of an ongoing study to monitor gaseous land-atmosphere C fluxes. Soil samples were collected in May and June 2016. Within each plot, soil samples (0 – 15 cm depth) were randomly collected from five points with a soil auger (5 cm diameter) and bulked into a composite sample, giving five replicate samples per site at each location. One additional augered sample was collected from each 25 m² plot to determine bulk density of the 15 cm core depth (N = 5 per management). Samples were transported and stored at 4°C prior to analysis at the School of Geography, University of Leeds, UK.

Soil samples were analyzed using standard laboratory techniques. A small proportion of each of the fresh soil samples was used for the determination of moisture content and available nitrogen (ammonium – nitrogen, NH₄-N; and nitrate – nitrogen, NO₃-N) within three days of sample collection. Moisture content was determined by measuring the weight loss after drying the fresh soil samples in an oven at 105°C for 12 hours. Available N was extracted from the fresh soil samples with 1M potassium chloride (KCl) and analyzed within 24 hours using a Skalar SAN++ auto analyzer. Bulk density was determined by drying core samples in an oven overnight and dividing the weight of dry soil by the volume of the core occupied by the soil after correction for stoniness (percentage of rock fragments greater than 2 mm in diameter; Poeplau et al., 2017).

$$\text{Bulk density (g cm}^{-3}\text{)} = \frac{\text{Mass of dry soil} - \text{Mass of stones}}{\text{Volume of soil} - \left(\frac{\text{Mass of stones}}{\text{Density of stones (2.6 g cm}^{-3}\text{)}} \right)} \quad 1$$

The remaining soil samples were air dried and passed through a 2 mm sieve, after visible roots and other plant materials were removed. The sieved samples (< 2 mm) were used for the determination of all other soil physico-chemical properties. Soil pH was measured in a soil suspension of 1:2.5 soil-water ratio (Robertson et al., 1999). Total phosphorus (P) was determined using a Skalar San ++ continuous flow auto analyzer, after the soil samples were digested using sulphuric acid and hydrogen peroxide (Tiessen and Moir, 1993). Total C and N were measured by combustion in an elemental analyzer (Vario Micro Cube), after the soil samples were first ball milled and passed through a 0.50 mm sieve. The total C values were used as SOC because the soil samples were free from carbonates when tested with hydrochloric acid (HCl). Exchangeable basic cations (calcium, Ca; magnesium, Mg; potassium, K; and sodium, Na) and aluminium (Al) were extracted with 1 M ammonium acetate (Allen, 1989) and analyzed using a Thermo Scientific iCAP 7600 Duo ICP-OES Analyzer. For particle size analysis, soil samples were dispersed using 5% sodium hexametaphosphate and passed through 0.53 mm sieve to separate the sand particles; the clay fraction was then determined by pipette method and the silt fraction calculated by subtraction (Van Reeuwijk, 2002). SOC stocks ($Mg\ ha^{-1}$) were calculated from values of SOC concentration and bulk density using the relation:

$$SOC\ stock\ (Mg\ ha^{-1}) = C \times BD \times D \times (1 - stone\ fraction) \quad 2$$

Where C = SOC concentration (%), BD = bulk density ($g\ cm^{-3}$), D = soil depth (cm). As the soil bulk density of the four sites differed, we performed a soil mass correction by calculating the mass of soil within 0 – 15 cm depth of the site with the highest bulk density and calculating the stock of SOC in the equivalent soil mass in the other sites (Lee et al., 2009).

2.2.1 SOC fractionation

SOC fractionation was carried out following the method of Zimmermann et al. (2007) and the standardization of some of its stages by Poeplau et al. (2013). A calibrated ultrasonic probe with an output energy of 22 J ml^{-1} was used to disperse 30 g of soil sample ($< 2 \text{ mm}$) in 150 ml of deionized water. The dispersion time was 177 s in order to maintain an effective fixed amount of power (20 W) (Poeplau et al., 2013). The dispersed suspension was transferred into a $63 \text{ }\mu\text{m}$ aperture sieve bags clamped to a wet sieve shaker (this made the sieving process easier and quicker). The suspension was wet sieved until the rinsing water was clear. In order to avoid the effect of the amount of sieving water on dissolved OC (DOC), a minimum of 2000 ml of water was used for wet sieving (Poeplau et al., 2013). The sand and stable aggregates (S+A) ($> 63 \text{ }\mu\text{m}$) together with particulate organic matter (POM) retained on the sieve, was dried at 40°C and weighed. The suspension ($< 63 \text{ }\mu\text{m}$) was filtered through a $0.45 \text{ }\mu\text{m}$ aperture nylon sieve and the material greater than $0.45 \text{ }\mu\text{m}$, silt and clay (s+c), was dried at 40°C and weighed. Filtration through the $0.45 \text{ }\mu\text{m}$ aperture nylon sieve was accomplished using centrifuge. The $0.45 \text{ }\mu\text{m}$ sieve was placed over a 1200 ml centrifuge containers and slightly pushed into the container to create a depression where the suspension ($< 63 \text{ }\mu\text{m}$) was poured. The container was covered and then centrifuged at 1000 g for 5 minutes. This gave a very clear separation. An aliquot of the filtrate ($< 0.45 \text{ }\mu\text{m}$) was analyzed for DOC by thermal oxidation with a liquid analyzer (Analytik Jena Multi N/C 2100).

The S+A was stirred in sodium polytungstate at a density of 1.8 g cm^{-3} in order to separate POM. The mixture was centrifuged at 1000 g for 15 minutes and the light fraction (POM) decanted. The two fractions (S+A and POM) were washed with deionized water to remove all traces of sodium polytungstate, dried at 40°C and weighed.

A chemically resistant soil organic carbon (rSOC) fraction was extracted from the s+c fraction by sodium hypochlorate (NaOCl) oxidation. One gram (1 g) of s+c fraction was oxidized for 18 hours at 25°C in a water bath with 50 ml of 6% NaOCl adjusted to pH 8 with HCl. The oxidation residue was centrifuged at 1000 g for 15 minutes, decanted, washed with deionized water and centrifuged further. The oxidation process was repeated twice.

After the fractionation, the C and N contents of S+A, s+c, POM and rSOC, were measured by combustion with an elemental analyzer (Vario Micro Cube). The C fractions were further grouped into three classes: the labile pool (POM and DOC), the physically protected pool (S+A and s+c – rSOC) and chemically resistant pool (rSOC) (Figure 1). In order to assess the relative sizes of C fractions, the C content in the measured mass of each fractional pool was calculated. This enabled us to determine the percentage recovery of C. The OC contained in the different fractional pools were then expressed as a percentage of the total SOC of the bulk soil. This revealed what proportion of the grasslands' total SOC was distributed in the different fractional pools.

2.3 Statistical analysis

The distributions of all the data to be analyzed were assessed using Shapiro-Wilk normality test. As all the data were normally distributed ($p > 0.05$), they were subjected to parametric tests. One-way analysis of variance (ANOVA) was used to compare the means of soil parameters between the four sites. Homogeneity of variance was assured by Levene's Test and post hoc multiple comparison (Tukey HSD) was used to separate means of soil parameters that differed significantly between fields.

3. Results

Results of selected soil physical and chemical properties including SOC and its fractions are presented in Tables 2 to 4. There was no significant difference ($p > 0.05$) in some soil attributes such as clay content, exchangeable aluminium (Al^{3+}), exchangeable calcium (Ca^{2+}) and exchangeable magnesium (Mg^{2+}) between the two locations (Table 2). Nidderdale soils contained higher exchangeable potassium (K^+), nitrate – nitrogen ($\text{NO}_3\text{-N}$) and sand with the textural class being sandy loam, and had lower silt content, stoniness and ammonium nitrogen ($\text{NH}_4\text{-N}$) compared to the Ribblesdale soils which were characterized by a loam texture. In Ribblesdale, there was no significant difference in pH, exchangeable sodium (Na^+), total phosphorus (P), total nitrogen and $\text{NH}_4\text{-N}$ between the soils under hay meadow and permanent pasture. On the other hand, the hay meadow soil in Nidderdale had significantly higher pH and significantly lower exchangeable Na^+ , total P and total N than the silage pasture soil in Nidderdale and the two fields in Ribblesdale (Table 2). The hay meadow soils in the two locations had higher bulk density compared to the conventional pasture soils, however, this was only statistically significant at Nidderdale.

The SOC stock in all the sites investigated ranged from 58.93 ± 3.50 to 100.69 ± 8.6 Mg ha^{-1} , and was significantly higher ($p < 0.05$) in the Nidderdale silage pasture (100.69 ± 8.6 Mg ha^{-1}) than in the Nidderdale hay meadow (58.93 ± 3.50 Mg ha^{-1}) and both sites at Ribblesdale (64.58 ± 6.29 to 68.74 ± 3.15 Mg ha^{-1}) (Table 3). The Ribblesdale sites had similar C/N values which were significantly lower than those in the Nidderdale sites, and the soils of the silage pasture in Nidderdale had the highest C/N (14.62 ± 0.21).

The percentage recovery after fractionation of the soil into five pools (S+A, s+c – rSOC, rSOC, POM and DOC) were 91 – 98% of the total soil mass and 61 – 81% of total SOC (Tables 3 and 4). The average contribution of the different soil fractions to the recovered soil mass was as follows: S+A fraction (78.62%), s+c fraction (16.62%) and POM fraction (0.20%) whereas the average concentration of C in the different fractions was in the decreasing order of POM (38.74%) > s+c – rSOC (4.24%) > S+A (3.38%) > rSOC (2.39%) > DOC (0.03%) (Table 4). The percentage of SOC stock that resided in the S+A fraction was the highest (an average of 48.0%) of all the fractions and at all the four sites studied; the percentage of the SOC stock in the other fractions were in the decreasing order: s+c – rSOC (12.0%) > rSOC (7.0%) > POM (1.4%) > DOC (0.4%) (Table 3). The C/N values were highest in the POM fraction and decreased in the order of s+c – rSOC > S+A > rSOC. Across all the locations and management regimes studied, there was no significant difference in the percentage of SOC stock residing in s+c – rSOC, rSOC and POM. The Ribblesdale permanent pasture had the highest percentage of SOC stock (62.40%) in the S+A fraction, and the Nidderdale silage pasture had the lowest SOC stock in this fraction (38.08%). The percentage of SOC stock in DOC fraction was generally higher in Nidderdale than in Ribblesdale, but only in Nidderdale hay meadow was the difference statistically significant.

The results of grouping the SOC pools into three classes, based on their relative stability in the soil and the mechanism of storage are shown in Figure 2. Across all the sites studied, the distribution of SOC stock was an average of 60.31% in physically protected pool, 6.98% in chemically protected pool and 1.77% in labile pool. About 30.94% of the bulk soil OC stock was not recovered during the fractionation process. The proportion of SOC stock in chemically protected and labile pools did not differ significantly between the sites studied. On the other

hand, a significantly higher percentage of SOC stock (73.12%) was physically protected in the permanent pasture in Ribblesdale than in the silage pasture in Nidderdale (52.49%). There was no difference in physically protected SOC between hay meadows.

4. Discussion

The range in OC stock of soils (0-15 cm) reported here (58.93 ± 3.50 to 100.69 ± 8.6 Mg ha⁻¹) is similar to the range previously reported for grasslands in Britain. For example, Bradley et al. (2005) reported a mean surface (0-30 cm depth) SOC stock of 80 Mg ha⁻¹ for pasture lands in England, and Carey et al. (2008) reported an average of 69 (61 to 82) Mg ha⁻¹ C stock for British grassland soils (0 – 15 cm) in the Countryside Survey of 2007. Similarly, Ward et al. (2016) reported mean surface (0-20 cm) SOC stocks of 82.6 Mg ha⁻¹ and 84.7 Mg ha⁻¹ for 60 intensively managed and 60 extensively managed grasslands in England. This indicates that the upland grasslands in both Nidderdale and Ribblesdale are important C stores with implication for delivering various ecosystem services including climate change mitigation.

4.1 Effects of grassland management and soil parent material on soil organic C stocks

The Nidderdale site under silage pasture had a significantly higher SOC stock (100.69 ± 8.64 Mg ha⁻¹) compared to other sites. This did not support our first hypothesis which expected a greater C stock in hay meadow fields under agri-environmental schemes with intermittent grazing.

However, at our field sites, the differences in stocking rates that are likely to affect vegetation removal and input of organic materials to the soil, and which ultimately determine the C balance of the ecosystem, were relatively small. The key management difference between the sites that could explain the greater SOC stock in the Nidderdale silage pasture was the addition of inorganic N fertilizers (50 kg N/ha/year). Firstly, increased forage productivity due to fertilizer

application might have led to a greater return of plant litter to the soil of the Nidderdale silage pasture, despite offtake via cutting for silage, compared to the other sites with lower or no fertilizer inputs. It is also likely that there was a greater belowground biomass at the Nidderdale silage pasture due to N-induced increase in the network of roots. A number of studies have shown that added N stimulates vegetation growth and the return of biomass litter to the soil, and subsequently enhances soil C storage (Amman et al., 2009; Fornara et al., 2013; Liang and Balsler, 2012; Song et al., 2014; Yue et al., 2016). In addition, the significantly higher C/N (14.62 ± 0.21) in the Nidderdale silage pasture may limit the rate of decomposition of organic matter, thereby resulting in the accumulation of C. This was shown by Hassink (1994) who found a negative correlation between the rate of organic matter mineralization and C/N values ranging from 10 to 18 in grassland soils. A high C/N is generally associated with low quality of organic matter which renders it more resistant to microbial decomposition in the soil (Berg and Laskowski, 2006; Yue et al., 2016).

Contrary to our second hypothesis, there was no significant difference in SOC stocks between the Nidderdale soils ($59 - 101 \text{ Mg ha}^{-1}$) with base-poor parent material and the Ribblesdale soils ($65 - 69 \text{ Mg ha}^{-1}$) with limestone parent material, although the range was greater at Nidderdale possibly due to N fertilizer addition. The lack of difference between locations could be attributed to similar soil pH levels, most likely as a consequence of liming of the Nidderdale sites.

Although the hay meadow field in Nidderdale had a significantly higher pH compared to all the other sites ($p < 0.05$), the soils at all sites were moderately acidic with pH values ranging from 5.64 to 6.06; this is similar to an average pH of 6.06 reported for improved grasslands in Britain in 1998 (Carey et al., 2008). At this pH range, the soil's exchange complex is mostly saturated with Ca^{2+} and Mg^{2+} while Al^{3+} concentration is reduced (Paradelo et al., 2015). This is consistent

with the concentrations of exchangeable polyvalent cations observed in our two locations: high Ca^{2+} and Mg^{2+} , and low Al^{3+} . The concentration of Al^{3+} in the soil is often associated with soil C stability. For example, Haynes and Naidu (1998) explained that at a lower soil pH than observed in our sites, and with higher Al^{3+} concentration, acid soils are normally flocculated because of the attraction between Al^{3+} and negatively charged clay surfaces, as well as the adsorption of OM. Liming raises pH, causing the Al^{3+} ions to precipitate and leaving a net negative charge on the soil surfaces. The resultant dominance of repulsive forces disperses the soil particles, thereby releasing adsorbed OM and exposing it to microbial decomposition. This was perhaps the case in the limed Nidderdale sites, resulting in the reduction of soil C to the levels observed at the Ribblesdale sites. The result obtained in this study therefore indicates that soil management activities such as liming has the capacity to change any effects of parent material on soil chemistry and thus C dynamics. This needs to be considered in soil management and climate mitigation strategies so as to minimize soil C loss.

Our study has shown that although grasslands may be subjected to different management regimes such as traditional hay meadow under agri-environment scheme and conventional permanent pasture and silage pasture, in practical terms the stocking rates, vegetation removal and indirect fertilization via animal droppings may be similar and application of inorganic and organic fertilizers may have comparable effects, thereby causing similar effects on the soil ecosystem. The continuous low intensity grazing at the Ribblesdale permanent pasture possibly offsets the lack of organic manure inputs, thereby explaining why there was no significant difference in SOC stock between the hay meadow and the permanent pasture field at this location. It is therefore necessary for future grassland management policies to specify the exact combination of management activities that define a management regime if soil C accrual is one

of the target benefits. This will ensure that the efficacy of agri-environment policy for multiple environmental benefits can be adequately assessed across sites and geographical regions.

4.2 Effects of grassland management and soil parent material on soil C fractions

Consistent with our third hypothesis, a greater proportion (67.29%) of SOC stock was protected in relatively stable forms, with physically protected pools associated with soil mineral fractions accounting for 60.31%. This is somewhat lower than has been found for mineral soils elsewhere. For instance, Leifeld and Fuhrer (2009) studied C distribution within the surface (0-20 cm) of some clay loamy soils under pasture and hay meadow management in Switzerland; they found only less than 20% of the SOC in the POM fraction whereas over 80% of the C was associated with the mineral fractions. This is despite slightly higher SOC contents (10%) compared to soils studied here (4.7 – 8.0% C). Similarly, in a silty loam (73% silt and 16% clay) grassland in Germany with high fertilizer inputs (160 kg N ha⁻¹ year⁻¹) and cut four times a year, John et al. (2005) found that 86-91% of SOC was associated with the mineral soil fraction.

The distribution of SOC stock in all other soil fractions, except sand and stable aggregate fraction (S+A), was similar across the four sites we studied. The SOC stock in S+A was similar between the two management regimes in each location, and in the hay meadows of the two locations but was greater in the permanent pasture of Ribblesdale than in the silage pasture of Nidderdale. This indicates an effect of soil aggregate stability rather than management regime or soil parent material. The permanent pasture in Ribblesdale with a higher C stock in its S+A fraction had lower sand and higher clay content compared to other sites. Intuitively, one would expect a lower C stock in the S+A fraction of the Ribblesdale permanent pasture due to lower sand content, however, the stable aggregate component of the fraction which is a function of

finer particles such as clay with greater surface area, is known to have a high capacity for C occlusion (Hassink and Whitmore, 1997). The relatively higher clay contents in the Ribblesdale permanent pasture might have induced a greater soil aggregate stability thereby leading to a higher SOC stock to be occluded in their S+A fraction. This is corroborated in a number of studies where soil aggregate stability was shown to increase with increasing clay contents and decreasing sand contents (Aziz and Karim, 2016; Lado et al., 2004), resulting in an increase in SOC stock (Le Bissonnais and Arrouays, 1997; Arrouays et al., 2006). Our study therefore highlights the need to always consider the particle size distribution of soils irrespective of their parent materials and site management activities when fractional distribution of soil C is being studied.

4.3 The significance of soil C distribution to environmental change

In all the sites studied, about 60% of SOC stock is stored in physically protected pools. This indicates that the mineral soils of both Nidderdale and Ribblesdale grasslands have large stocks of stable SOC with potentially long mean residence times important for climate change mitigation. For example, Leifeld and Fuhrer (2009) reported mean residence times of 210 to 795 years for physically protected C pools compared to 5.5 to 11.5 years for labile soil C pools in a Swiss grassland under hay meadow and pasture management regimes. The apparent beneficial effects to climate change mitigation of the grasslands we studied may however be negated depending on the fate of the labile C pool. Although only about 2% of the SOC stock in our study is present in labile pools, it is likely that the proportion of SOC stock that were not recovered after fractionation (19 to 39%) was mostly labile. We suspect that labile C, particularly the POM fraction was lost during the wet sieving and decantation stages of the fractionation process, at the following steps: 1) during wet sieving, suspended POM could have escaped from

the sieve bags due to agitation caused by the mechanical shaker; 2) when the sodium polytungstate suspension was being decanted from the S+A fraction, and 3) when sodium polytungstate was being washed off the POM and S+A fractions. An additional likely source of C loss is the recovery of s+c fraction after filtering the suspension ($< 63 \mu\text{m}$) through a $0.45 \mu\text{m}$ aperture nylon sieve. This is possible as very fine clay particles containing OC could be retained on the sieve. Despite its lower fractional mass (0.2% of soil mass compared to 94.8% of mineral soil mass recovered), the labile C fraction particularly POM had a significantly higher OC concentration (39%) than the mineral fraction (7%). It is therefore possible that if the labile fraction accounts for the 5% loss in soil mass, it could contain about 20% of SOC stock lost during fractionation. The dynamics of this labile C will be critical to the climate system when regional and global scales are considered.

The general view that the labile C pool is highly vulnerable to environmental change is due to its short mean residence time (Leifeld and Fuhrer, 2009), hence it is considered to be affected by management activities such as grazing and fertilizer application (Cao et al., 2013; Riggs et al., 2015). In our study, despite the inorganic fertilizer-induced higher SOC stock in the Nidderdale silage pasture, there was no significant difference in the distribution of the labile C fractions between the Nidderdale silage pasture and the other three fields. This could mean that the benefits of the higher SOC stock in the Nidderdale silage pasture may be short-term. In a wider study of UK grasslands, Ward et al. (2016) found that labile SOC in the surface soil (to 7.5 cm depth) decreased with increasing management intensity, whereas the stable pools remained unaffected. In the Ward et al. (2016) study no differentiation was made between upland and lowland grassland sites, and it is possible that the management effect on labile C is less pronounced in upland environments such as in this study where climatic conditions are less

favourable for productivity. This has implications for a changing climate, and may mean that rapid C turnover in upland sites is relatively robust to subtle changes in management.

There is therefore a need to further investigate the fate of the labile C pool in our sites because it is the most active soil C pool. A number of questions that need to be answered are: 1) What are the turnover rates of C in the labile pools? 2) What are the products of turnover of the labile C pools? Providing answers to these questions is important because similar distributions and sizes of the labile C pools in our sites do not necessarily imply the same dynamics. Similar pool sizes may be due to different C flux patterns such as high input – high output of C or low input – low output of C in the system. The pattern of C flux and the nature of the output such as carbon dioxide (CO₂), methane (CH₄) or DOC are particularly important as they could affect climate feedbacks, and have other environmental effects. For example, under high input – high output scenario where the major output is CO₂, the radiative effects of high efflux of CO₂ into the atmosphere may be balanced by the positive physiological and fertilization effects on vegetation, which enhances soil C accumulation (Bala et al., 2013). On the other hand, if the major output is CH₄ under the same high input – high output scenario, there is a greater risk of an increased radiative effects. This is because the capacity of CH₄ to absorb infrared radiation as a greenhouse gas is 20 to 30 times more efficient than CO₂ (Mer and Roger, 2001). High DOC outputs from the upland grasslands may be costly for water treatment companies if the DOC ends up in river networks that are drinking water sources, and have consequences for aquatic ecosystems (Chapman et al., 2010). Conducting C flux studies in the upland grasslands investigated in this study will therefore help to improve understanding of the dynamics of C in their soils as well as potential implications for climate change.

5. Conclusion

The upland grasslands in both Nidderdale and Ribblesdale stored a significant amount of C in their soils which ranged from 58.93 ± 3.50 to 100.69 ± 8.64 Mg ha⁻¹. There was little contrast between sites except that the only field that received inorganic N fertilizer (Nidderdale silage pasture) had a significantly higher SOC stock than the other three fields studied. The activities that constitute grassland management regimes need to be considered carefully in agri-environment policies in order to be able to assess their net benefits to the ecosystem. No effects of parent material was observed on soil C stock due to liming-induced increase in the pH of Nidderdale soils, and neither management activities nor parent materials affected the distribution of soil C into different fractions. About 70% of the SOC stock was protected within the stable soil fractions in all the sites studied, indicating a potential for the grasslands to contribute in climate change mitigation. However, there is need to further study the dynamics of the labile SOC fraction which has short residence time and could negate the beneficial effects of the protected C pools in a changing climate. Further studies should focus particularly on the turnover rates of the labile C pools as well as the outputs from the turnover such as CO₂, CH₄ and DOC. This is expected to help in better understanding of not just the dynamics of the labile soil C pools but also the implication of such dynamics for climate change.

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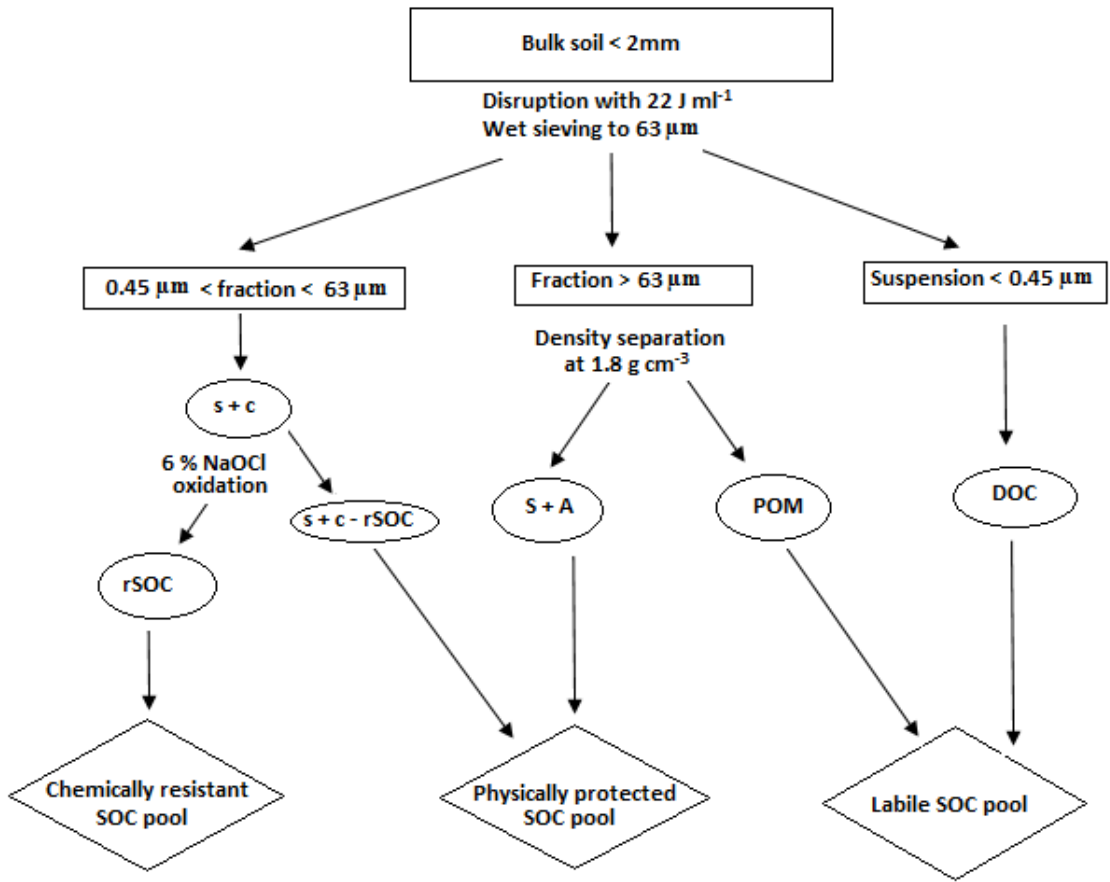


Figure 1: Flow chart showing SOC fractionation stages and their products (modified from Zimmermann et al., 2007); DOC = dissolved organic carbon, POM = particulate organic matter, S+A = sand and stable aggregates, and s+c = silt and clay.

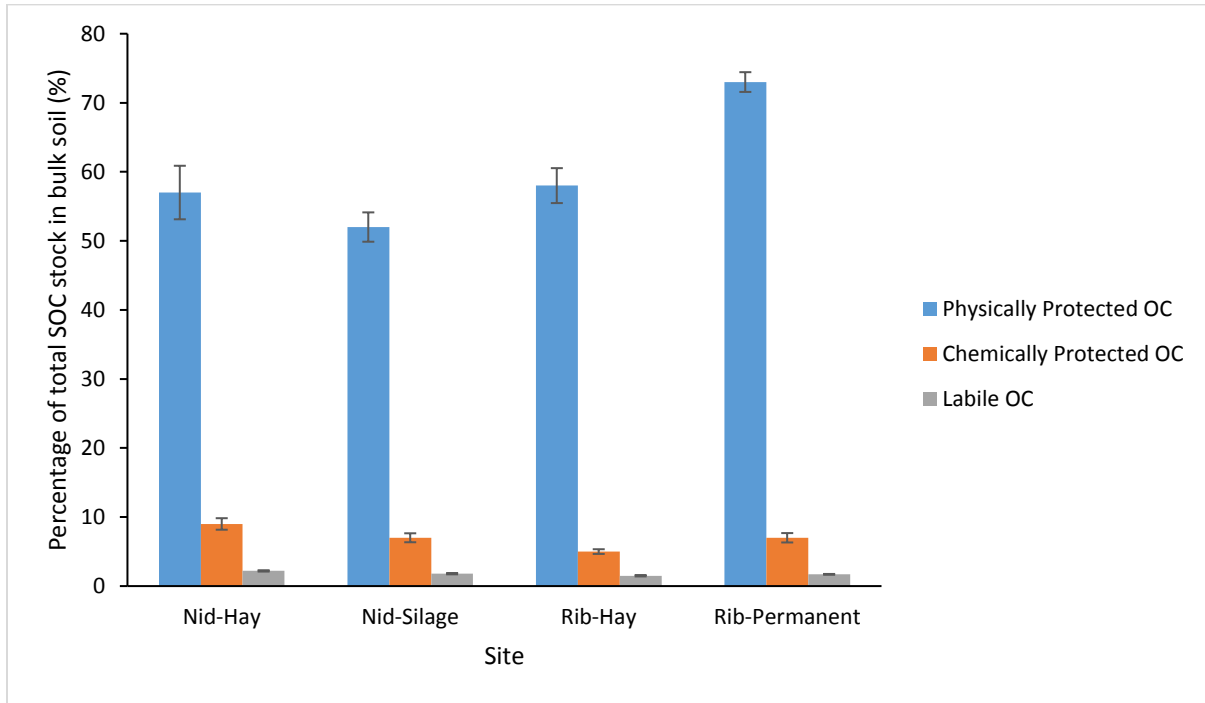


Figure 2: Distribution of SOC stock in three fractional classes: physically protected OC pool (S+A and s+c – rSOC), chemically protected OC pool (rSOC), and labile OC pool (POM and DOC), as a percentage of total SOC stock in bulk soil. About 30% of the total OC stock in bulk soil was not recovered during fractionation. Bars represent mean values \pm standard errors. Nid = Nidderdale and Rib = Ribblesdale.

Table 1: Description of the study area

Site and Management Information				
Site	Nidderdale-Hay	Nidderdale-Silage	Ribblesdale-Hay	Ribblesdale-Permanent
Location	54°09'52"N, 01°53'17"W	54°09'50"N, 01°53'22"W	54°05'34"N, 02°15'55"W	54°05'35"N, 02°16'1"W
Elevation (m)	300	302	300	301
Geology	Clay drift with siliceous stone	Clay drift with siliceous stone	Carboniferous limestone	Carboniferous limestone
Soils	Stagnohumic gley	Stagnohumic gley	Brown earth	Brown earth
Dominant vegetation	<i>Holcus lanatus</i> , <i>Lolium perenne</i> , <i>Ranunculus repens</i> , <i>Trifolium repens</i>	<i>Lolium perenne</i> , <i>Holcus lanatus</i> , <i>Ranunculus repens</i>	<i>Anthoxanthum odoratum</i> - <i>Geratum sylvaticum</i> grassland	<i>Holcus lanatus</i> , <i>Poa trivialis</i> , <i>Festuca rubra</i> , <i>Deschampsia cespitosa</i> , <i>Alopecurus pratensis</i> , <i>Lolium perenne</i>
Management regime	Traditional hay meadow under an agri-environment scheme	Conventional pasture	Traditional hay meadow under an agri-environment scheme	Conventional pasture
Management history	Traditional hay meadow for over 150 years	Traditional hay meadow until about 1960, used as rough pasture between 1960 and 2008, utilized for silage since 2008	Traditional hay meadow for over 25 years	Conventional pasture for over 25 years
Re-seeding	Wild flowers over last six years	Perennial ryegrass broadcast in July 2015	none	none

Inorganic fertilizer application	none	Surface application of 50 kg N ha ⁻¹ year ⁻¹ since 2014 as NH ₄ NO ₃ or (NH ₄) ₂ SO ₄ in early June	None since 1995. Annual dressing of 20 kg N ha ⁻¹ as NPK 20:10:10 from 1980 to 1995	None
Organic manure application	Surface application of 6 t ha ⁻¹ year ⁻¹ of FYM in May.	Surface application of 6 t ha ⁻¹ year ⁻¹ of FYM in May. 100 kg each of both phosphate and potash applied in early June 2016 as burnt poultry manure	Surface application of 4-6 t ha ⁻¹ year ⁻¹ of FYM from 1990 to 2001	None
Liming	Surface application of 5 t ha ⁻¹ Mg limestone in 2010 and 5 t ha ⁻¹ Ca limestone in 2013	Surface application of 5 t ha ⁻¹ Mg limestone in 2010 and 5 t ha ⁻¹ Ca limestone in 2013	None since 1995. There was occasional annual dressing previously	none
Livestock stocking rate/grazing intensity	10-15 sheep ha ⁻¹ in the month of April and from September to the end of December. Higher stocking density in September when the field is used as a holding area	15-20 sheep ha ⁻¹ in the month of April and from September to the end of December	10-15 sheep ha ⁻¹ in the months of April, September and October; intermittent grazing between January and March	5 sheep ha ⁻¹ all year round
Supplementary feeding	None	None	hay	none
Vegetation cutting date	Cut in late July or early August (e.g. 23 rd July 2016) for hay	Cut in July (e.g. 23 rd July 2016) for silage	Cut in mid to late July or early August (e.g. second week of August 2016) for hay	N/A

FYM = farmyard manure, Permanent = permanent grazing, N = nitrogen, P = phosphorus, K = potassium, Ca = calcium, Mg = magnesium, N/A = not applicable

Table 2: Mean values \pm standard error (n=5) of selected soil physico-chemical properties

Soil property	Nidderdale-Hay	Nidderdale-Silage	Ribblesdale-Hay	Ribblesdale-Permanent
Sand (%)	65.20 \pm 1.69a	60.60 \pm 1.50a	50.00 \pm 0.84b	49.60 \pm 1.40b
Silt (%)	24.20 \pm 4.51a	29.60 \pm 3.12ab	43.00 \pm 0.45b	36.60 \pm 4.63ab
Clay (%)	10.60 \pm 3.78a	9.80 \pm 2.75a	7.00 \pm 1.22a	13.80 \pm 5.38a
Textural class	Sandy loam	Sandy loam	Loam	Loam
Stoniness (%)	0.77 \pm 0.13a	1.02 \pm 0.18a	0.96 \pm 0.34a	3.9 \pm 1.00b
Bulk density (g cm ⁻³)	0.84 \pm 0.03a	0.63 \pm 0.03b	0.72 \pm 0.03ab	0.66 \pm 0.05b
pH in water	6.06 \pm 0.04a	5.72 \pm 0.12b	5.64 \pm 0.03b	5.81 \pm 0.10ab
Ex-Al (mmol kg ⁻¹)	0.04 \pm 0.01a	0.10 \pm 0.06a	0.13 \pm 0.03a	0.07 \pm 0.03a
Ex-Ca (mmol kg ⁻¹)	41.44 \pm 0.89a	41.62 \pm 1.80a	36.01 \pm 0.47a	37.34 \pm 2.42a
Ex-Mg (mmol kg ⁻¹)	11.16 \pm 0.47a	10.03 \pm 0.42a	9.41 \pm 0.65a	11.89 \pm 1.04a
Ex-K (mmol kg ⁻¹)	1.95 \pm 0.19a	3.44 \pm 0.38b	0.74 \pm 0.09c	0.84 \pm 0.24c
Ex-Na (mmol kg ⁻¹)	0.72 \pm 0.04a	1.45 \pm 0.21b	1.22 \pm 0.06b	1.11 \pm 0.05ab
Total P (mg kg ⁻¹)	928.69 \pm 66.64a	1533.63 \pm 107.86b	1345.67 \pm 46.77b	1438.50 \pm 74.48b
Total N (%)	0.37 \pm 0.02a	0.54 \pm 0.03b	0.55 \pm 0.03b	0.55 \pm 0.04b
NH ₄ -N (mg kg ⁻¹)	1.49 \pm 0.45ab	0.69 \pm 0.09a	2.44 \pm 0.32b	1.61 \pm 0.21ab
NO ₃ -N (mg kg ⁻¹)	1.160 \pm 0.32a	1.23 \pm 0.10a	0.23 \pm 0.06b	0.71 \pm 0.12ab

Ex = exchangeable, Al = aluminium, Ca = calcium, Mg = magnesium, K = potassium, Na = Sodium, P = phosphorus, N = nitrogen, NH₄ = ammonium, NO₃ = nitrate. Row means followed by the same letter are not significantly different ($p > 0.05$) while those followed by different letters differ at $p < 0.05$.

Table 3: SOC and C/N in total soil mass and in soil fractions (mean values \pm standard error, n=5)

Soil fraction	Property	Nidderdale- Hay	Nidderdale- Silage	Ribblesdale- Hay	Ribblesdale- Permanent
Total soil mass	Total SOC (%)	4.70 \pm 0.22a	7.99 \pm 0.49b	5.53 \pm 0.24a	5.37 \pm 0.35a
	SOC stock (Mg/ha)	58.93 \pm 3.50a	100.69 \pm 8.64b	68.74 \pm 3.15a	64.58 \pm 6.29a
	C/N	12.66 \pm 0.16a	14.62 \pm 0.21b	10.04 \pm 0.81c	9.95 \pm 0.22c
S+A	% of total SOC	43.55 \pm 8.11ab	38.08 \pm 5.26a	47.83 \pm 2.79ab	62.40 \pm 2.58b
	C/N	12.33 \pm 0.50a	14.11 \pm 0.32b	9.60 \pm 0.09c	9.57 \pm 0.16c
s+c – rSOC	% of total SOC	13.65 \pm 3.99a	14.41 \pm 4.83a	10.60 \pm 1.49a	10.72 \pm 0.57a
	C/N	9.10 \pm 1.42a	11.24 \pm 1.29a	8.23 \pm 0.27a	8.08 \pm 0.28a
rSOC	% of total SOC	8.94 \pm 1.67a	7.14 \pm 1.30a	4.72 \pm 0.68a	7.10 \pm 1.36a
	C/N	18.60 \pm 1.54a	23.02 \pm 2.02a	12.80 \pm 0.60b	12.83 \pm 0.57b
POM OC	% of total SOC	1.56 \pm 0.18a	1.31 \pm 0.25a	1.28 \pm 0.20a	1.35 \pm 0.13a
	C/N	23.12 \pm 4.45a	22.04 \pm 0.92a	24.13 \pm 0.39a	22.58 \pm 1.82a
DOC	% of total SOC	0.60 \pm 0.03a	0.44 \pm 0.06ab	0.24 \pm 0.03b	0.30 \pm 0.07b
Total SOC recovered (%)		68.30 \pm 8.75a	61.30 \pm 3.65a	64.66 \pm 3.38a	81.86 \pm 2.93a

SOC = soil organic carbon, N = nitrogen, S+A = sand and stable aggregate fraction, rSOC = resistant SOC fraction, s+c – rSOC = silt and clay without the rSOC fraction, POM = particulate organic matter fraction, DOC = dissolved organic fraction. Row means followed by the same letter are not significantly different ($p > 0.05$) while those followed by different letters differ at $p < 0.05$.

Table 4: The organic C contents and soil masses of different soil fractions (mean values \pm standard error, n=5).

Soil fraction	Property	Nidderdale- Hay	Nidderdale- Silage	Ribblesdale- Hay	Ribblesdale- Permanent
S+A	OC content (%)	2.33±0.34a	3.72±0.66ab	3.21±0.14ab	4.24±0.29b
s+c – rSOC	OC content (%)	4.38±1.18a	6.56±1.54a	3.25±0.39a	2.78±0.18a
rSOC	OC content (%)	2.90±0.38ab	3.41±0.40a	1.44±0.18c	1.79±0.29bc
POM OC	OC content (%)	37.38±5.10a	42.10±0.46a	38.36±1.74a	37.12±2.20a
DOC	OC content (%)	0.03±0.001a	0.04±0.005a	0.01±0.001b	0.02±0.003b
S+A	% of total soil mass	83.64±1.15a	80.47±2.51ab	75.57±1.13b	74.79±0.90b
s+c	% of total soil mass	13.92±1.33a	16.27±2.20a	16.47±0.36a	19.80±1.64a
POM	% of total soil mass	0.21±0.03a	0.24±0.04a	0.17±0.02a	0.19±0.02a
Total soil mass recovered (%)		97.77±0.33a	96.97±0.96a	92.23±1.26b	94.77±1.00ab

SOC = soil organic carbon, S+A = sand and stable aggregate fraction, rSOC = resistant SOC fraction, s+c – rSOC = silt and clay without the rSOC fraction, POM = particulate organic matter fraction, DOC = dissolved organic fraction. Row means followed by the same letter are not significantly different ($p > 0.05$) while those followed by different letters differ at $p < 0.05$.