

## DISTINCT SPATIAL DEPENDENCY OF CARBON DISTRIBUTION BETWEEN SOIL POOLS IN GRASSLAND SOIL

### DEPENDENCIA ESPACIAL CARACTERÍSTICA DE LA DISTRIBUCIÓN ENTRE FRACCIONES DE CARBONO EN SUELO DE PRADERA

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#### ABSTRACT

Grassland soils play a key role in climate change and food security, and carbon (C) and nitrogen (N) mineralization is central to this. Although there are a number of mathematical models available to estimate C and N mineralization, they do not encompass the variability of the process and there is uncertainty in their predictions. The input parameters of the SOMA model (Soil Organic Matter "A") have been conceptualized and validated to predict mineralization in arable soils. The objective of this research was to measure the spatial dependence of the input parameters in order to further obtain spatial predictions of mineralisation in a grassland system. A nested design was applied using sampling intervals of 30 m, 10 m, 1 m, and 0.12 m as sources of variation. From each sampling point a soil sample was taken (0-23 cm) and physical sequential fractionation was applied to obtain the free light fraction (FLF) and intra-aggregate light fraction (IALF). The C and N contents in the fractions were measured by mass spectrometry, and the results analysed by residual maximum likelihood (REML) to obtain components of variance at each stage, and then accumulated to plot the approach to a variogram. Both fractions showed spatial dependence at the finest scales measured, and the general pattern was different from that in an arable site. The recommended soil sampling interval where C and N mineralization predictions would be spatially distributed according to the correlation of input light fractions parameters of SOMA is 0.5m.

**Key words:** Carbon, nitrogen, organic matter, fractionation, nested sampling, variogram.

#### RESUMEN

Existe un grado de incertidumbre en las decisiones agronómicas y ambientales relacionadas con el ciclaje de carbono (C) y nitrógeno (N) basadas en modelos de estimación, y en particular en suelos de praderas por su rol en alimentación humana y en el cambio climático. El modelo SOMA (Soil Organic Matter "A") ha sido parametrizado para obtener predicciones de mineralización de C y N, y sus parámetros de entrada no son solo conceptuales sino que también han sido validados. Sin embargo se desconoce la variabilidad espacial de sus parámetros de entrada, por lo que el objetivo de este estudio fue medir la dependencia espacial de estos. Para ello se diseñó un muestreo espacial anidado

considerando intervalos de muestreo de 30 m, 10 m, 1 m, y 0,12 m como fuentes de variación. Desde cada punto de muestreo se obtuvieron muestras de suelo (0-23 cm) a las que se aplicó fraccionamiento físico para aislar en secuencia la fracción liviana libre (FLF) y la fracción liviana intra-agregados (IALF). Los contenidos de FLF-C(N) e IALF-C(N) fueron medidos a través de espectrometría de masa, y los resultados se analizaron mediante máxima probabilidad residual (REML) para obtener los componentes de varianza a cada escala, los que fueron acumulados para graficar aproximaciones al variograma. Ambas fracciones mostraron tener dependencia espacial con los intervalos de distancia más cortos, y en general el patrón fue distinto de aquel estudiado en suelo arable. El intervalo recomendado en función de la correlación espacial entre las fracciones es de 0,5 m, donde las predicciones de mineralización de C y N quedarían distribuidas espacialmente según input variables de SOMA.

**Palabras clave:** Mineralización, carbono, nitrógeno, fraccionamiento, suelo, muestreo anidado, variograma.

## INTRODUCTION

The contribution of grassland and pasture systems to agricultural production and their active role in climate change has been widely acknowledged (Dick et al., 2015). Unless large amounts of nitrogen (N) fertiliser are applied, nutrient cycling in grassland and pastures is mostly controlled by the turnover of soil organic matter (SOM), particularly through the mineralization process in which N, phosphorus (P) and sulphur (S) become available. Robust predictions of carbon (C) and N mineralisation are required to support crucial decisions that have to be taken by farmers, environmental scientists, and policy makers, regarding agricultural management for the present and future scenarios of global warm-

ing (Nel and Cooper, 2009; Ferrarini et al., 2014). The understanding of mineralisation in pastures and grassland soils has progressed in the last years, but knowledge of its variability in space and time is still limited, making estimations and predictions of this process and its outcome difficult and uncertain.

Mineralisation is modelled in the SOM model 'SOMA' (Soil Organic Matter "A") (Sohi, 2001; Fig. 1) and related to SOM fractions that differ in their physical location in the soil, chemical composition, and so their reactivity, meeting the conditions required for a better understanding not only of the C but the N cycle as well. The model has been parameterised with laboratory data to describe the decomposition of organic matter added to various soil types in a series

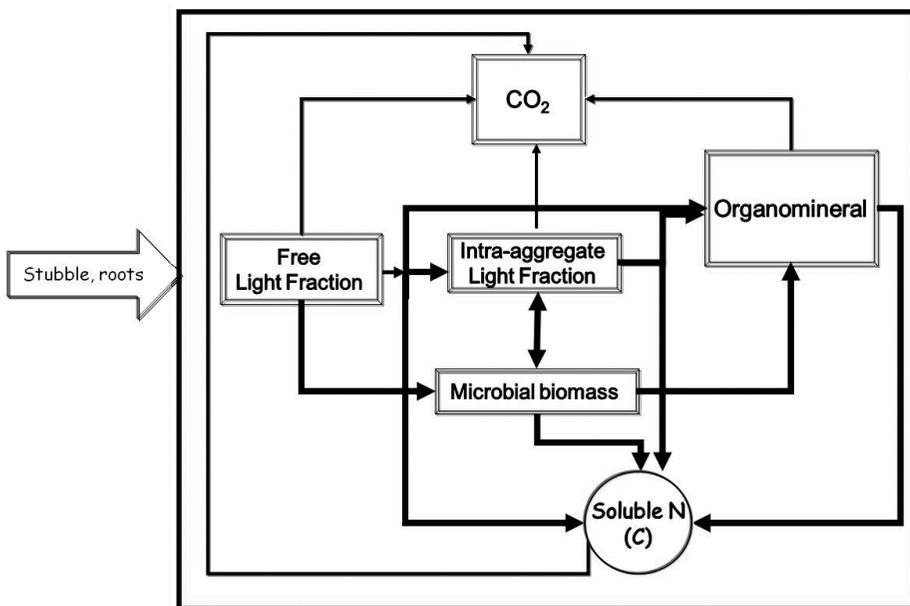


Fig. 1. Soil organic fractions and fluxes of carbon and nitrogen defined in SOMA model.

Fig. 1. Fracciones de la materia orgánica y flujos de carbono y nitrógeno definidas por el modelo SOMA.

of soil incubation experiments, where rates of N mineralisation have been measured and the output variables validated (Sohi, 2001). SOMA is therefore one of very few models in which the pools can be directly measured and their associated fluxes inferred.

The input variables of SOMA can be obtained by soil analysis in a sequential analytical procedure in which the C and N content of the free light fraction (FLF), intra-aggregate light fraction (IALF), and heavy fraction (HF) are isolated and quantified (Sohi, 2001). Previous research has shown that light fractions differ in chemical composition: the FLF comprises mainly O-alkyl groups, whilst the IALF contains alkyl groups representing lipids and waxes, suggesting a more transformed organic matter such as microbial debris and remains of the most recalcitrant plant material (Lopez-Capel et al., 2005). Incorporation of the intra-aggregate pool in the model improves predictions, suggesting that a pool sequestering organic matter into soil micro-aggregates is important in the mineralization process (Gabrielle et al., 2002). Light fractions have been shown to be important components of agricultural ecosystems reflecting, for example, changes in land use not apparent in measurements of total C content of the soil (Lopez-Capel et al., 2005). Light fractions have been used as soil quality indicators, and correlated with other labile fractions of C and N, such as potentially mineralizable N (PAN) (Haynes, 2005). In contrast, the heavy fraction, an organo-mineral fraction, is a more stabilised form of SOM, containing less mineralizable C, and obtaining an accurate chemical composition of this fraction has proved difficult (Poirier et al., 2005). Overall, due to its poor correlation with soil respiration HF is regarded as a major sink for C in the soil (Tan et al., 2007).

Other models for the simulation of C and N turnover are available (Rodrigo et al., 1997; Cerri et al., 2002), but provide less information about the process. The measurable compartments of SOMA and the defined status of soil N demand allow the fate of C and N to be tracked. This can improve our understanding of the decomposition processes and the relationship between C and N in soil. For example, by applying SOMA, the hypothesis that the light OM fractions are more likely to be related to N supply in the field than the total SOM may be tested, because these fractions have specific biological functions represented by decomposition rates in C and N turnover in the model (Sohi, 2001). Thus, the rationale of this research is that the labile fractions, FLF and IALF play a fundamental role in the mineralization process.

In particular, N mineralization has been shown to be highly variable, as are most of the availability indexes linked to mineralization, and spatial variability is a key part of this (Baxter, 2002). Research to predict N mineralization that encompasses spatial variability is limited, especially in grassland soils, and our hypothesis postulates that measurable input parameters of SOMA sampled according their spatial dependence will provide spatial C and N mineralization predictions and so this research is focused on measuring the spatial dependence of the C and N contents of the light fractions.

Central to understanding the spatial variability of N mineralization is an appropriate sampling strategy, and a key component of this is the interval of sampling. Ideally, that sampling interval should allow autocorrelated soil properties or processes within an area to be assessed without over/under sampling. Furthermore, the sampling interval of distance is particular to the scale of the study since soil properties and processes vary at different spatial scales, from millimetres to kilometres, and at any point the obtained value is a function of the position within the space. This is, the variation of a soil property in the environment is the result of the interaction of several processes and factors, each of them having and individual scale of variation (Webster and Oliver, 2007).

Relationships between different degrees of the scale of variation are difficult to establish but important, so that the results of research can be put into practical applications (Jarvis et al., 1996; Pringle et al., 2008). Reay et al. (2009) reported N<sub>2</sub>O emissions at the field, farm, and catchments scales, finding that the thresholds published by the IPCC (Intergovernmental Panel on Climatic Change) were underestimating the indirect N<sub>2</sub>O emissions at the field scale. This inconsistency represents the gap between policies and actual natural processes, and scale-modelling tries to resolve it. Therefore, knowing the spatial dependency of a soil property at a particular scale is key to study its spatial variability.

Autocorrelation is revealed in the variogram, where the spatial dependence of a soil variable is described, according to the interval of sampling at which soil variables are autocorrelated. This paper reports a study of the spatial dependence of the C and N content in the light organic fractions defined in SOMA at the field scale in a grassland site, in order to recommend an appropriate sampling interval for further application of SOMA in grassland systems, and to better understand and predict C and N cycling in these systems, which occupy 40% of the land area of the earth.

## MATERIALS AND METHODS

The experimental site was located in Higher Wyke Moor field (50°45'46" N, 3°53'57" W) at North Wyke Research Farm (Devon, Southwest of England), part of Rothamsted Research. Geologically, the site is within the Carboniferous Crackington Formation, which comprises clay shales with thin subsidiary sandstone bands. The soil is classified as Typic Haplaquept and detailed description of the soil series is provided in Table 1.

The field can be described as permanent pasture, having not been reseeded for the last 33 years; since 2004 the field management has been consistent (Table 2). The herbage consists of only Gramineae species with no presence of Leguminosae species.

The soil was sampled to 23 cm depth so that the measurements of soil organic matter parameters were comparable with those from arable sites. After the sampling, the soil samples were kept at 4°C and then sieved to pass 6 mm.

Spatial sampling of the soil was done according to a nested design (Webster and Oliver, 2007; Lark, 2011). In nested sampling a population is organized into a hierarchical structure of nested classes. For example, one might sample randomly selected locations within randomly selected fields within randomly selected farms within randomly selected districts as part of a study to understand sources of variation in soil properties at national scale. A hierarchical analysis of variance then

partitions the variance of the measured values of soil properties into components associated with each level of the nested classification (between districts, between farms within districts, etc). In spatially nested sampling the hierarchical structure controls distances between sample points (Webster and Oliver, 1990). One may sample the soil at randomly (or systematically) selected main stations. Within each main station one then chooses a pair of stations at stage 2, some specified distance apart (although the direction of the line joining the stations may be randomized); this is then repeated for successively shorter distances. The model of the variance for observations in a case with four nested scales within each main station is given by:

$$Z_{ijkl} = \mu + A_i + B_{ij} + C_{ijk} + \varepsilon_{ijkl}$$

where  $Z_{ijkl}$  is the value of the  $l$ th unit in the  $k$ th class at stage 3, in the  $j$ th class at stage 2, and in the  $i$ th mainstation;  $\mu$  is the mean value;  $A_i$  is the difference between  $\mu$  and the mean of the  $i$ th main station;  $B_{ij}$  is the difference between the mean at the  $j$ th value in the  $i$ th mainstation and the mainstation mean, and so on. The terms  $A$ ,  $B$ ,  $C$  and  $\varepsilon$  are assumed to be independent random variables with mean zero and each with a variance. It is the variance associated with each scale of the nested scheme that we want to estimate because this is informative about the importance of sources of soil variation that operate at different spatial scales.

**Table 1. Hallsworth soil series description for pasture soil.**

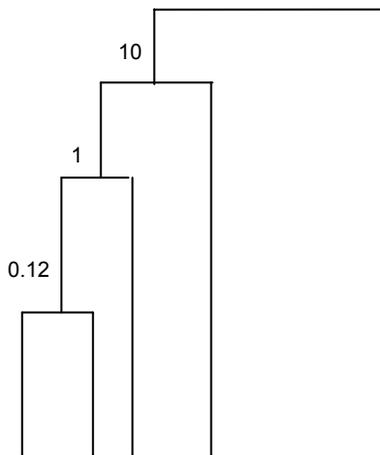
**Tabla 1. Descripción de la serie de suelo Hallsworth de pradera.**

Horizon (cm)	Description
0-15 Ah	Dark grey (10 YR 4/1 - 3/1) humose silt loam; spongy, very fibrous with abundant coarse <i>Molinia</i> roots; high organic matter; moist; brown staining on roots, organic matter carried down prominent structure faces into horizon 3; sharp boundary.
15-20 Eg1	Grey (10 YR 6/1) silty clay with strong brown (7.5 YR. 5/6 - 5/8) pipes around roots and red-rusty accumulations on old roots; large subangular blocky tending to prismatic; fissured, tenacious; moderate organic matter; marked reduction in root density, many dead roots; moist; this thin horizon may be the humus-stained top of the horizon below; clear boundary.
20-32 Eg2	White (10 YR 8/1) silty clay to clay with reddish yellow (7.5 YR 6/8) pipes to root channels; prismatic structure, not strongly fissured, tenacious; active roots not numerous, many dead roots; moist to wet; merging boundary.
32+ Bg	White (10 YR 8/1) clay with reddish yellow (7.5 YR 6/8) around roots and abundant blotching of 10 YR 6/8, brownish yellow; occasional stones; prismatic structure, fissured, tenacious; few roots.

**Table 2. Field management at Higher Wyke Moor site since 2004, United Kingdom.**  
**Tabla 2. Manejo aplicado al sitio de estudio Wyke Moor desde el año 2004, Reino Unido.**

Time of the year	Activity	Details
Mar	Fertilisation (NPK)	106-56-75 kg ha <sup>-1</sup>
May/ Jun	Silage cutting	25 t ha <sup>-1</sup>
Jun- Jul	Dung application	
Jul - Oct	Grazing	Suckler cattle
Nov - Dec	Grazing	Sheep flock

An unbalanced nested sampling scheme (Webster and Oliver, 1990; Lark, 2005) was applied at Higher Wyke Moor site, the topology of which is shown in Fig. 2. This is a hierarchical sampling scheme with eighteen main points located on a regular 30 × 30-m grid, and nested sample points separated from the main station in a set of steps of fixed decreasing length in a random direction, as represented in Fig. 3, for the FLF-C results. As well as FLF-C (N), the IALF (C, N) and total soil C and N contents were measured in each sample. The substations were taken subsequently in random directions at 10 m, 1 m, and 0.12 m apart from the main station in the grid (actual directions not shown in Fig. 3). These distances were selected to span a scale range of interest in roughly equal steps on a logarithmic scale. The directions were chosen as random numbers between 0 and 360, allocated in the field by a compass. The total sample size was 72.



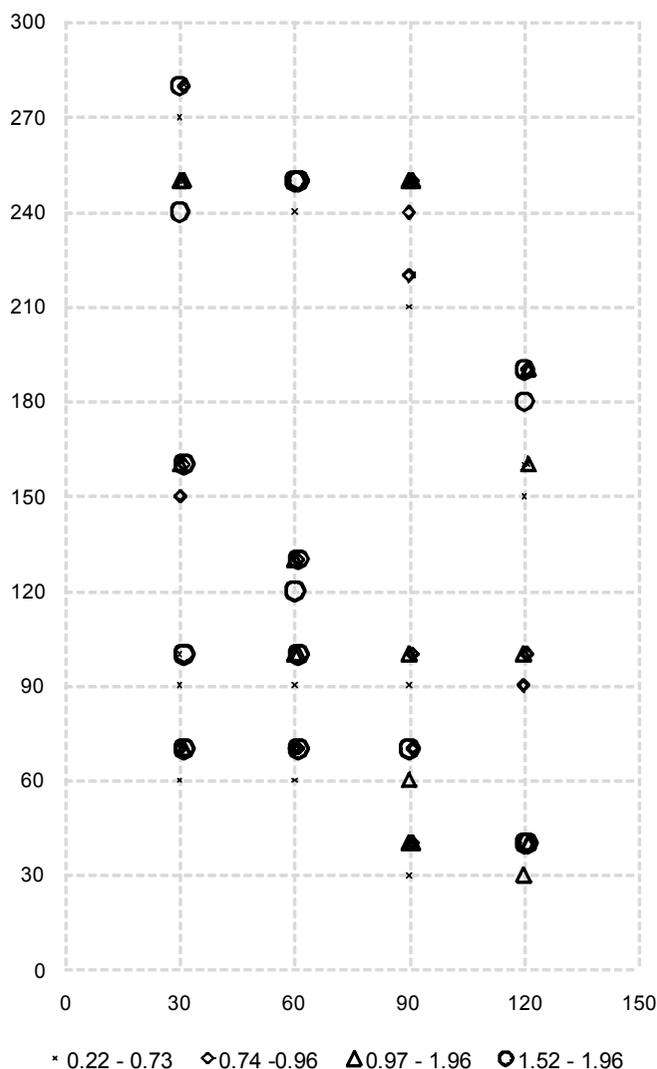
**Fig. 2. Topology of the unbalanced version of the nested sampling applied to this study. The vertical lines represent sampled points.**

**Fig. 2. Esquema del diseño anidado desbalanceado aplicado a este estudio. Las líneas verticales representan los puntos de muestreo.**

To measure C and N in the whole soil, a subsample of 3 g soil was dried at 40°C. The sample was milled to a powder in a disc mill, and 20 mg used to determine C and N content in an elemental analyser linked to a mass spectrometer (ANCA 2020 Europa Scientific, Crewe, United Kingdom). A sequential physical fractionation of the SOM was used to obtain the labile input variables of the SOMA model (Sohi, 2001). Gravimetric soil moisture (average 18%) was measured before fractionation so that its dilution of the density separation medium could be counteracted. FLF was obtained by adding 90 mL concentrated sodium iodide (NaI), prepared to a density of 1.820 g cm<sup>-3</sup> to 15 g of soil, followed by centrifugation (8000 g for 30 min) after gentle swirling for 30 s. The organic matter floating in the NaI solution was captured by vacuum filtration onto pre-weighed glass fibre filters of 42 mm diameter. The FLF obtained on the filter was rinsed with deionised water to remove remaining NaI. The filter containing FLF was dried in a Petri dish in an oven at 40°C and the combined filter-fraction weight was recorded. The recovered NaI solution was added back to the soil sample after FLF was isolated and sonication applied at 750 J g<sup>-1</sup>. This treatment breaks down stable aggregates in the soil, releasing organic matter within them. After re-suspension a second centrifugation enabled the IALF to be isolated in the same way as described for FLF.

Filters and light fractions were milled to powder and then prepared for analysis for C and N content by mass spectrometer as described above.

A descriptive statistical analysis was made for C and N content in the soil and light fractions, and components of variance for each variable were found using the residual maximum likelihood (REML) procedure in the Genstat 9th edition statistical software package (Payne, 2006), restricted to return non-negative estimates. Additionally, covariance components and correlation coefficients, as the covariance divided by the root of the product of the two corresponding variance components, each stage were also calculated. Ac-



**Fig. 3. Spatial distribution of carbon content in the free light organic matter fraction (mg FLF-C g<sup>-1</sup>) in the grassland field, sampled using an unbalanced nested design for 30, 10, 1 and 0.12 m. Abscise (m) represents E/W and ordinate (m) N/S.**

**Fig. 3. Distribución espacial del contenido de carbono en la fracción liviana libre de la materia orgánica (mg FLF-C g<sup>-1</sup>) en el suelo de pradera, muestreado usando un diseño anidado desbalanceado de 30, 10, 1, y 0,12m. La abscisa (m) representa la dirección E/O y la ordenada (m) la dirección N/S**

cumulated variance components obtained from nested analysis were plotted against the sampling distance as this has showed to provide an approximation to the variogram of a random variable (Miesch, 1975).

## RESULTS

### Descriptive statistics of soil organic matter variables at Higher Wyke Moor

The summary statistics for total C and N content in the soil, in the organic matter fractions,

and their C:N ratios are shown in Table 3. Most of the variables could be regarded as normally distributed, with similar mean and median values, skew coefficients within the range of [-1, 1] and octile skew coefficients within the range [-0.2, 0.2]. The C:N ratio of the IALF data was log-transformed to achieve the required conditions.

As expected, total soil C and N contents were much larger than those in the light fractions (Fig. 4), and the data dispersion of C and N contents, i.e. the standard deviation, followed the order of soil > FLF > IALF (Table 3); the FLF showed a larg-

er range (1.74 mg C g<sup>-1</sup>; 0.11 mg N g<sup>-1</sup>) than the IALF (1.09 mg C g<sup>-1</sup>; 0.06 mg N g<sup>-1</sup>). Total C in the soil was large compared to that in an arable soil in the United Kingdom, within the range of 11.6 to 23.10 mg C g<sup>-1</sup> (Córdova et al., 2012), and smaller than the range found for grassland soils in Yorkshire, UK (Bhatti et al., 2014) and Ireland (53 mg C g<sup>-1</sup>) (McGrath and Zhang, 2003). The average C:N ratios were soil (8.52) < FLF (15.52) < IALF (19.81) (Table 1).

There are no other reports of light fraction C and N content values from grassland ecosystems, made using this particular physical density fractionation (Sohi, 2001). However, the results from the sampled site here are comparable to those from the 14-year old permanent pasture analysed by Accoe et al. (2004). In comparison, the FLF at Higher Wyke Moor site was just half that of the labile organic matter found by Peigne et al. (2009), defined as the substrate ready to be used by microorganisms, but larger than the FLF reported for arable soils (Córdova et al., 2012).

Little difference was found between the sizes of the FLF compared to IALF as a proportion of total C, and their C:N ratios were also similar. The small amounts of C in the light fractions compared to the total soil C may be due to the C from the large humified root litter accumulation and the large rhizosphere system under grassland, as observed in the top 15 cm depth in Higher Wyke Moor field (Table 1). Chemical characterisation of root-rich surface soil in permanent grassland has revealed the input and the depth distribution of fresh organic substrates and also the physical protection of readily mineralized organic matter as well, which make it distinctive from an arable system (Nierop et al., 2001).

### Spatial dependence and spatial dependence correlation of light fractions

The results of the REML estimation of the scale-specific variance components for FLF are shown on Table 4 as an example. The total variance of FLF-C was 0.19 mg C g soil<sup>-1</sup>; 61% of this was accounted for by the shortest scale of sampling, 0.12 m. Additional contributions to the total variance were recorded at 1 m and 10 m (about 12 and 27% of the variance, respectively), where the total variance was reached. An approached variogram was plotted for all variables by building up the components of the variance over the lag distances (Fig. 5 and 6), where the spatial dependence is shown at the point at which the range distance reaches the total variance.

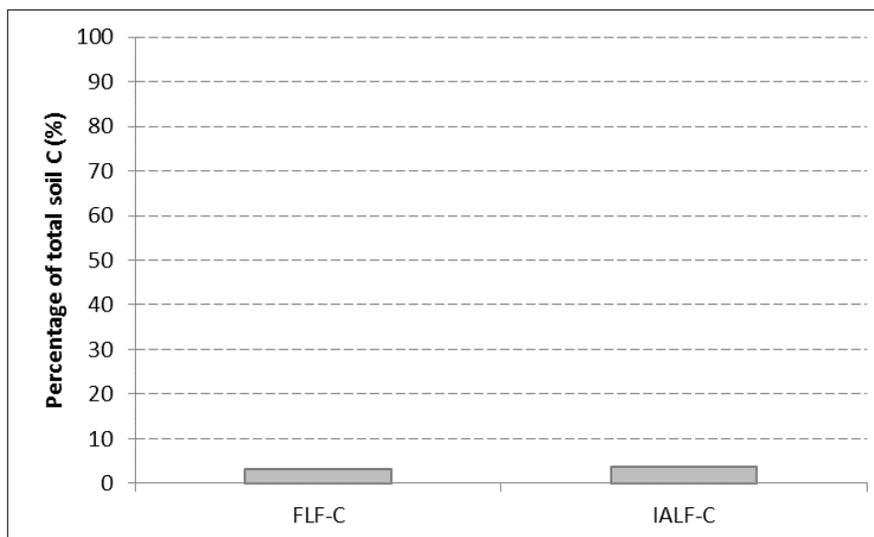
The accumulated variance plots of soil C and N had similar shapes, with little or no variance over distances of 10 m, and about 60% of the variance was explained by distances < 10 m (Fig. 5a and 5b). In both cases there was a substantial variance component between main stations. Almost all the within-mainstation variance for soil C:N ratio was found at the shortest distance and the small contribution from 1 and 10 m distance added little to the total variance (Fig. 5c). For all variables shown in Fig. 5 the largest component of variance was between mainstations, and the next-largest at the finest scale.

The accumulated variance plots for C and N contents in the light fractions are shown in Fig. 6. The plot for FLF-C shows that there were substantial components of variance at all scales up to and including 10 m, but no additional variance between mainstations. The finest scale dominates, contributing 61% of the variance (Fig. 6a). The plot for IALF-C shows that 49%, and 25% of the

**Table 3. Summary statistics for soil carbon and nitrogen content, and soil organic fractions measured in an unbalanced nested sampling design in a grassland site. Higher Wyke Moor, Devon, United Kingdom.**

**Tabla 3. Resumen estadístico del contenido de carbono y nitrógeno del suelo y de las fracciones orgánicas, medidos en un diseño de muestreo anidado desbalanceado en un suelo de pradera. Higher Wyke Moor, Reino Unido.**

	Soil C	FLF-C	IALF-C	Soil N	FLF-N	IALF-N	Soil C:N	FLF C:N	IALF C:N	Log IALF C:N
	----- mg g <sup>-1</sup> -----									
Mean	30.92	1.01	1.11	3.63	0.07	0.06	8.52	15.52	19.81	1.30
Median	30.81	0.96	1.08	3.63	0.06	0.06	8.55	15.34	19.55	1.29
Minimum	21.80	0.22	0.77	2.60	0.01	0.04	7.78	11.56	16.72	1.22
Maximum	41.35	1.96	1.86	4.77	0.12	0.10	9.05	19.05	25.12	1.40
St. deviation	3.51	0.43	0.22	0.38	0.03	0.01	0.29	1.51	1.66	0.04
Skewness	0.15	0.29	0.75	0.05	0.15	0.77	-0.52	0.32	0.67	0.46
Kurtosis	0.46	-0.60	0.43	0.45	-0.86	0.33	-0.09	-0.05	0.19	-0.16
Octile skew	-0.01	0.14	0.16	-0.11	0.04	0.18	-0.14	0.16	0.24	0.20



**Fig. 4. Contribution of the carbon content in the light fractions to the total soil carbon measured at Higher Wyke Moor, grassland site (Devon, United Kingdom).**

**Fig. 4. Aportes del carbono en las fracciones livianas de la materia orgánica al carbono total del suelo medido en el sitio de pradera Higher Wyke Moor (Devon, Reino Unido).**

variance occurred at the 0.12-m and 1-m distances, respectively, and 25% of the total variance was between mainstations, with a negligible variance over 10 m (Fig. 6b). Similarities in the spatial structure pattern of the N content measured in the light fractions compared to the C contents were observed, with larger contributions at the finest scale (Fig. 6c, 6d). The accumulated variance plot for the C:N ratio in the FLF was more or less flat with almost all the variance at the finest scale (Fig. 6c). The plot for IALF-C:N resembled that for C and N in the IALF (Fig. 6f).

The spatial correlation between C content in the light fractions and the soil were weak (Table 5). In contrast, at the finest scales the C content in the FLF and IALF were spatially correlated, particularly at the 1-m scale, and no relationship between these two pools was observed at 30 m and 10 m sampling intervals (Table 5).

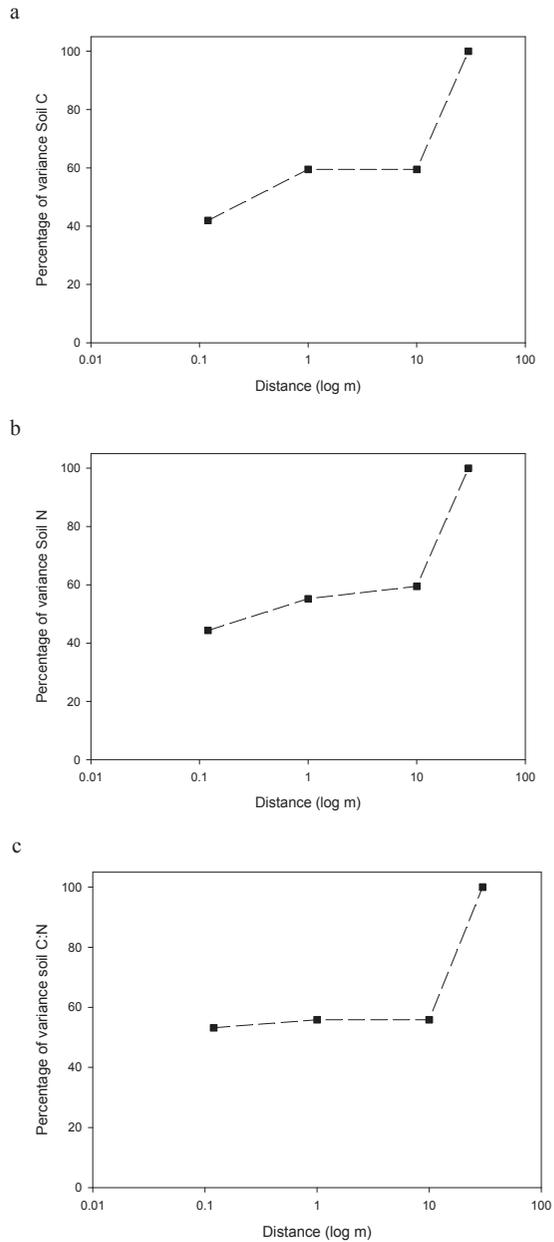
## DISCUSSION

Although the pattern of spatial variation of the light fractions has not been widely explored previously, there are many studies of total soil C contents. For instance, Liu et al. (2006) showed variability in soil C at the regional scale, fitting an exponential model to the variograms and revealing the scale of variation as 632 m. At the field scale, soil C has been shown to vary at > 15 m (Corstanje et al., 2007), and 42 m (Baxter, 2002). In the present study the limit of the

spatial dependence for soil C and N was found to be greater than the coarsest scale considered in this field (> 30 m), perhaps because the total C comprises the individual components (C fractions) likely to vary at specific and different spatial scales, in particular the organo-mineral fraction linked to the mineralogy nature of the soil, interactions of which appear to be at coarse spatial variation.

Particular factors regulating the variability of light fractions in grasslands were not addressed in this study as the rationale was that active sources of variation, such as fresh C inputs (i.e. from plant residues, root biomass, animal deposition), would primarily affect the spatial variability of FLF-C/N, whilst clay content would affect the variability of the IALF-C/N (Sohi et al., 2010). Moreover, a short scale of variation such as that seen in the FLF-C/N can be associated in grasslands with the influence of plant biomass and soil microbial properties, and longer ranges related to agronomic management and topography (Ritz et al., 2004).

Input of aboveground biomass, root litter and exudates in general make a large contribution to soil C through particulate inputs in grassland ecosystems (Personeni et al., 2005), although the particulate term is technically different from the pools obtained through the fractionation protocol applied in this study. The spatial variability of the FLF-C/N can therefore differ according to soil type and management; i.e. only a nugget



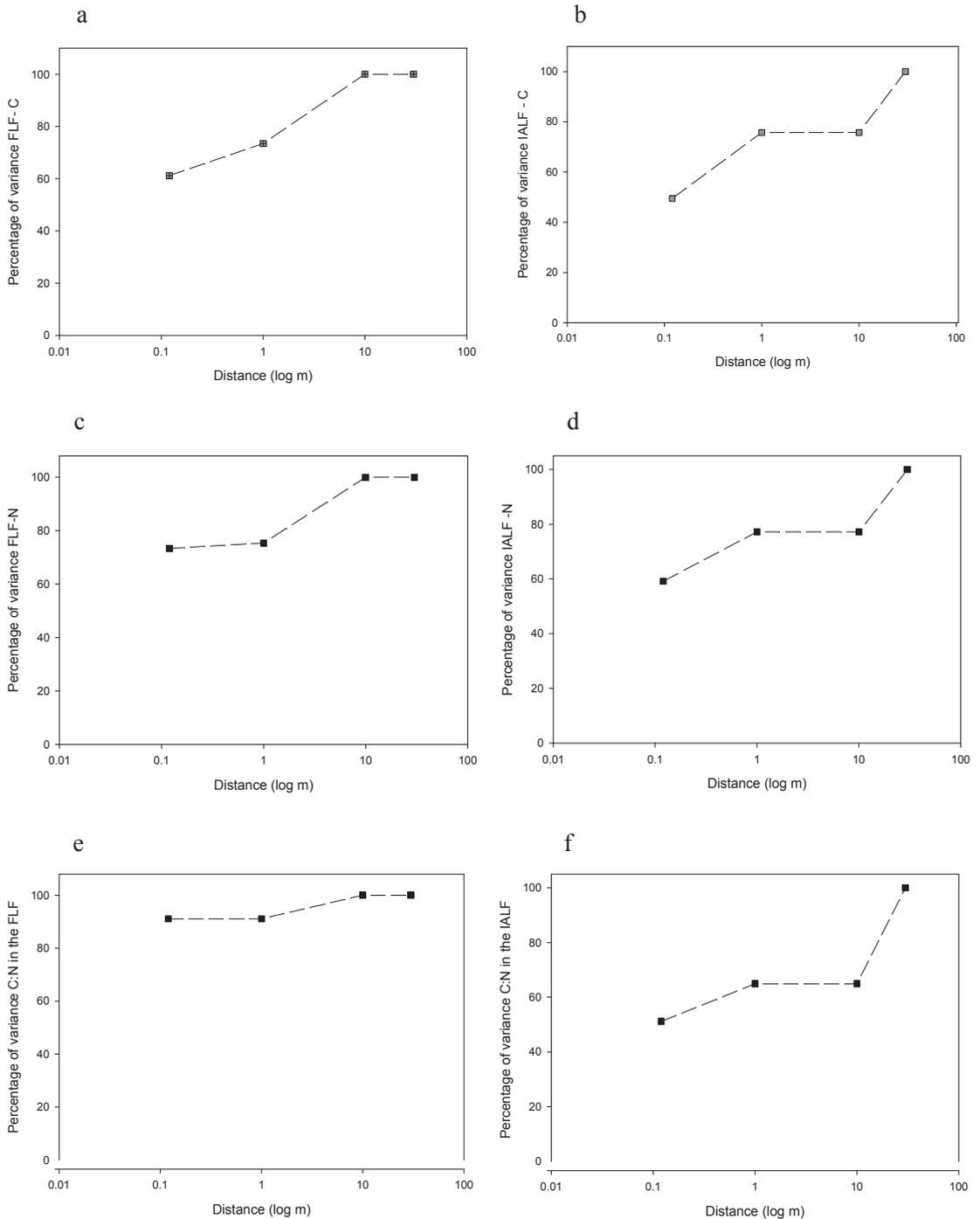
**Fig. 5. Approach to the variograms from the unbalanced nested design showing spatial dependence for soil variables in the grassland site. a) Soil carbon; b) Soil nitrogen; and c) Soil C:N ratio.**

**Fig. 5. Aproximaciones al variograma a través del diseño de muestreo anidado desbalanceado mostrando la dependencia espacial en las variables de suelo de pradera. a) Carbono del suelo; b) Nitrógeno del suelo; y c) Relación C:N del suelo.**

effect was found for FLF-C in a crop rotation site (Córdova et al., 2012) whilst in the grassland soil investigated here, the lag distances, 0.12, 1 and 10 m all contributed the total variance, with 10 m being the limit of spatial dependence. Therefore, a strong source of spatial variation of FLF-C at Higher Wyke Moor site might be aboveground biomass cover, i.e. patchiness, which is common

and distinctive in grazed grassland soils.

Considering the management of a grazed grassland system, it might be expected that the spatial variability of FLF should be more closely associated with the C balance between dung (and fertiliser) additions, and the removal of grass by grazing and cutting. There might be a pattern in animal grazing that causes the variation of FLF



**Fig. 6.** Approach to the variograms for the C and N content, and C:N ratio of two soil organic matter fractions from a nested unbalanced sampling, free light fraction (a, c, e) and intra-aggregate light fraction (b, d, f), respectively.

**Fig. 6.** Aproximaciones al variograma del contenido de C y N, y la relación C:N de dos fracciones de la materia orgánica del suelo a través de un diseño anidado desbalanceado, la fracción liviana libre (a, c, e) y la fracción liviana intra-agregados (b, d, f), respectivamente.

**Table 4. Components of variance for the carbon content in the whole soil and light fractions defined in SOMA model from the unbalanced nested sampling at the field scale in a grassland site (Higher Wyke Moor, Devon, United Kingdom).**  
**Tabla 4. Componentes de varianza del contenido de carbono en el suelo y en las fracciones livianas definidas en el modelo SOMA, en un muestreo anidado desbalanceado a escala de campo en un sitio de pradera (Higher Wyke Moor, Reino Unido).**

Stage	Distance	Soil		Free Light Fraction		Intra-Aggregate Light Fraction			
		Component	Accumulated % of total	Component	Accumulated % of total	Component	Accumulated % of total		
1	30 m	5.1280	12.66	0.0000	0.19	100.00	0.012	0.050	100.00
2	10 m	0.0000	7.53	0.0500	0.19	100.00	0.000	0.038	75.73
3	1 m	2.2180	7.53	0.0231	0.14	73.42	0.013	0.038	75.73
4	0.12 m	5.3120	5.31	0.1150	0.12	61.14	0.025	0.025	49.45

**Table 5. Spatial correlation coefficients between soil carbon and organic light fractions defined in SOMA model from the unbalanced nested sampling at the field scale in a grassland site (Higher Wyke Moor, Devon, United Kingdom).**

**Tabla 5. Coeficientes de correlación especial entre el carbono del suelo y las fracciones orgánicas definidas en el modelo SOMA, en un muestreo anidado desbalanceado a escala de campo en un sitio de pradera (Higher Wyke Moor, Reino Unido).**

Stage	Distance	Soil C		Soil C		Free light fraction-C	
		& Free light fraction-C	Correlation	& Intra-aggregate fraction-C	Correlation	& Intra-aggregate fraction-C	Correlation
1	30 m	0.004	-	0.003	0.0119	0.026	-
2	10 m	0.000	-	0.002	-	0.029	-
3	1 m	0.004	0.0177	0.000	0.0000	0.013	0.7436
4	0.12 m	0.005	0.0064	0.001	0.0027	0.008	0.1495

to be 10 m, perhaps due to grass selection made by the cattle and their faeces deposition. When the soil was sampled, the grass in this field was regrowing after being grazed, and so probably had a structure determined by grazing selection and faecal deposition.

The plant cover distribution over a field has been described as the cause of the spatial distribution of total soil C (Don et al., 2007), but it could be applicable to FLF as well. According to a visual observation of the distribution of grass tufts on site, certain locations in the field have more prolific grass growth and so a larger substrate for FLF. Such variable plant growth might be associated with soil conditions and so should be addressed in a further investigation. At the same time, animal faeces can represent an extra source of variation, producing patches of dung at different locations as the grazing animals move across the field. The complexity of this factor was not investigated here but it should be in future research.

As a sampling recommendation for FLF-C and N as defined in SOMA, half of the range is suggested as a reference (Webster et al., 2006). Thus a 5-m sampling interval would fully correlate the FLF and SOMA processes in grassland.

A structured pattern of the spatial dependence was observed in the IALF at Higher Wyke Moor field and, as observed in the FLF a large component of variance was shown at the finest scale, almost 50% of the total variance (Table 3, Fig. 6b). A large proportion (75%) of the total variance was reached at 1 m, so this lag distance can be taken as the critical limit of the spatial dependence. This result contrasts to the spacial dependence of the IALF-C in an arable site (Córdova et al., 2012), where 1 m was the only interval distance that did not contribute to the total variance.

Whilst as a labile fraction, the FLF-C and N spatial dependence might be associated with quality, quantity and distribution of organic residues, the C and N contents of the IALF are released from stable soil aggregates, so the variability of this fraction would be particularly affected by clay content and the mechanical resistance to disruption of the aggregates in grassland (Breulmann et al., 2014). As this permanent grassland soil has not been recently disturbed by ploughing (Table 2), the content of clay in the soil, despite the large organic matter content, might play a main role in the spatial variability of the C and N content in the IALF (Breulmann et al., 2014). Unfortunately, soil clay content was not measured in this study.

Both labile fractions of SOMA showed spatial dependence at the finest scales, but the correlation between them occurs at 1 m. Furthermore, taking

half of this distance, i.e., 0.5 m, would guarantee that the C and N contents of FLF and IALF would be spatially correlated, and thus modelling regarding C and N transformations to predict mineralization for instance, would benefit from soil samples taken at interval distances of 1-m.

The results obtained by the nested survey and the variogram indicated a sampling interval that is appropriate for modelling using SOMA in terms of the correlation between C and N predictions and model variables (light fractions), but that would not be recommended as ordinary soil sampling for farm management, i.e., for fertilizer recommendations. The approximate variograms were useful for characterising the spatial aspects of the variance, but also for demonstrating that a large proportion of the total variance of the light fractions is at the finest scale, which includes any measurement error. A measurement of the experimental error of the light fractions obtained by the physical fractionation procedure is recommended for a better estimation of the unresolved variation.

The application of this type of analysis saved time and resources, as there was no previous information about the spatial scale of variation of the light fractions in grassland soils. For example, Peigne et al. (2009) computed a variogram from a 10-m grid to find the spatial variability of biological soil properties and concluded, after several other geostatistical analyses, that the sample interval was too large to reveal the spatial structure of mineralized C. From the nested analysis here, one scale is selected and then a more complete study is completed.

## CONCLUSIONS

At the grassland site both light fractions were spatially autocorrelated at fine scales, and the C content of FLF showed spatial dependence at 10 m, whilst a shorter range of 1-m dependence was encountered for IALF-C. These results are different from those previously reported in an arable soil for spatial dependence of FLF-C, using the same interval distances to obtain an approach to the variogram, where the spatial dependence was pure nugget. This highlights the influence of different factors underlying the mineralization of C and N between two different agricultural systems, and the necessity of including such spatial dependence of the input parameters of a model. In particular, the input parameters of SOMA model have been not only conceptualized but measured and validated as predictors of the mineralization process. Perhaps further research would benefit of the measurement of the soil clay content as a useful auxiliary variable to better

explain spatial correlations between the input parameters.

The organic fractions of SOMA, measured by physical fractionation, were spatially correlated at the 1-m scale (correlation coefficient = 0.74) and therefore an appropriate sampling interval is 0.5 m to obtain spatial predictions of C and N mineralisation in this grassland field.

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