

Wetland soil organic matter composition in a Mediterranean semiarid wetland (Las Tablas de Daimiel, Central Spain): Insight into different carbon sequestration pathways

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## ABSTRACT

Wetland soils from a Mediterranean semiarid wetland (Las Tablas de Daimiel, Central Spain) were studied to characterize the organic matter (OM) and determine its origin and transformation. Cross polarization magic angle spinning (CPMAS) <sup>13</sup>C nuclear magnetic resonance (NMR) spectroscopy and mathematical molecular mixing allowed analysis of the organic fraction in terms of six generic components (carbohydrate, protein, lignin, lipid, char and “carbonyl”). Las Tablas is an active carbon sink, with total organic carbon (TOC) content independent of soil OM quality; the TOC content of the upper sediment is  $10.0 \pm 7.8\%$ . The inorganic carbon content is also high ( $5.4 \pm 3.3\%$ ) and is associated mainly with OM of aliphatic character. The OM composition is variable; samples predominantly aliphatic (carbohydrate, lipid and protein) are characteristic of the northern sector, whereas predominantly aromatic samples are typical of the southern Tablas. A strong negative relationship between protein content and lignin content was found, interpreted as a consequence of different proportions of vascular vs. non-vascular (mostly charophyte) litter input. The effect of perturbations is

apparent in the extended presence of char, particularly abundant in fire-prone areas. OM quantity and quality do not seem to depend on hydrology (although seasonal flooding is associated with less organic wetland soils) or soil characteristics. Dominant vegetation and fire are the main drivers of OM content and composition. Structural carbohydrate, protein and lipid (> 60% of total organic fraction) dominate. Widespread anaerobic conditions and the recent character of the sediments could explain the preservation of different fractions of the original detritus composition (due to different vegetation and presence of microbes).

## 1. Introduction

In spite of their reduced surface, wetlands represent important and active C reservoirs within the global carbon cycle (Gorham 1991; Mitsch and Gosselink 1993; Bridgham et al., 2006). Most wetlands accumulate organic matter (OM), because primary production exceeds slow mineralization rates, caused by permanent or temporary anaerobiosis associated with the waterlogged conditions (Nieder and Bembli, 2008). Wetlands represent ca. 6% (Mitsch, 1994) of the Earth's surface. However, it is difficult to give an exact proportion, considering the discrepancies in the definition of wetlands and the changing nature of such ecosystems. As a whole, wetlands contain up to  $450 \times 10^{12}$  kg C (Jokic, 2003), which represents about a quarter of the global soil organic carbon (OC) inventory. Their desiccation has given rise to concern, by way of a significant positive feedback in global warming (Davidson and Janssens, 2006; Fissore et al., 2009).

Notwithstanding its role in the global carbon cycle, wetland OM has been comparatively less studied than OM accumulated in other ecosystems (terrestrial soil,

lakes, rivers, oceans...), although some systems such as peatlands and estuaries, that are wetlands in a broad sense, have been studied extensively. Elemental and functional group analysis of OM from freshwater wetlands, as well as research on OM stabilization and humus formation, is rare (Visser, 1983; Jokic et al., 2003; Hernández and Mitsch, 2007; Alam et al., 2003). Studies of salt marshes (Filip and Alberts, 1994; Santin et al., 2008; Mendonça et al., 2004), coastal and marine sediments (El Sayed et al., 1996; Calace et al., 2006), estuarine sediments (Sardessai and Wahidullah, 1997; Krull et al., 2009) and lakes (Calace et al., 1999; review by Meyers and Ishiwatari 1993; He et al., 2008; Gołębiewska et al., 1996) are more frequent.

It has been stated that the process of soil OM (SOM) production from living organism litter is to a large extent microbially mediated and tends to generate —from diverse original detritus inputs— metabolic products and derived macromolecules of a more similar composition than the original detritus composition (Plante, 2008). This could explain the relative overall similarity in SOM bulk composition around the world (see also Mahieu, 1999), an outstanding example being the narrowing of the C/N ratio in the initial transformation of SOM (Meyers and Ishiwatari, 1993). Chemical and thermal (fire- induced) processes, on the other hand, could play a decisive role in the transformation of those metabolic products and partially degraded macromolecules, to produce humic-type substances and black carbon (Almendros, 2008).

Nelson and Baldock (2005) compared the composition of vegetation samples, and the SOM directly derived from them, using the molecular mixing model (MMM; Nelson and Baldock, 2005; Baldock et al., 2004). In that study, of three adjacent Canadian soils, where only the vegetation differed (maple tree, alfalfa and corn), litter and SOM composition were different, with a drastic decrease in carbohydrate content from the former to the latter, and a concomitant increase in protein and lignin content.

The “final” SOM compositions of the three soils were much more alike than the original litter compositions. Apparently, soil conditions had a bigger impact on the quality of SOM than the initial litter composition, which is in line with the above statements. Unfortunately, no comparable report is available for wetland OM, although for a lagoonsediment, Krull (2009) found differences which could be related to either planktonic or vascular plant litter. In comparison with soil, sedimentary OM may allow better preservation of the compositional differences in the original litter. It is clear that wetland soil carbon dynamics may differ considerably from soil carbon dynamics. Particularly, OM composition and transformation in semi-arid wetlands with drastic changes in hydroperiod and vegetation are poorly known.

Therefore, the aims of the present work were to: (i) characterise the main features of wetland SOM in a Mediterranean semiarid wetland and (ii) explore the origin and transformation of the OM via its elemental and functional composition, taking advantage of a molecular mixing model applied to  $^{13}\text{C}$  NMR spectra and the elemental analysis of wetland samples. It was expected that a more precise knowledge of the molecular composition of SOM would allow formulation of sound conclusions on its origin and transformation, as well as quantification of its main molecular components, including char. The latter may be an important long term carbon sink, since it represents carbon transferred from the fast biosphere cycle to the much slower geological cycle (Preston and Schmidt, 2006; Forbes et al., 2006). We also assumed that such a basic knowledge about SOM could be of value in understanding the different mechanisms of carbon accumulation and the fate of wetland OC with respect to climate change.

## 2. Materials and methods

### 2.1. Study site

The Tablas de Daimiel is a freshwater wetland in Central Spain on the River Guadiana watershed (39° 08' N, 3° 43' W; Fig. 1). Historically, it formed through three processes: (i) upwelling of groundwater from the Spanish 4.04 aquifer, (ii) flooding of two rivers (the northeastern Gigüela river and the eastern Guadiana river) and (iii) the slowing of discharge by way of the dams of 14 watermills, which changed the pristine riverine conditions to more lacustrine conditions.

Since the 1970s, the Tablas of Daimiel has been characterized by heavily disturbed hydrological processes. Desiccation, channeling, irrigation with groundwater and natural, prolonged dry periods were the main culprits. Anthropogenic changes in the second half of the 20th Century resulted in large-scale ecosystem shifts. These included drainage of 130 km<sup>2</sup>, which disrupted surface flooding in the 1960s and early 1970s, and reduced the flooded surface from ca. 150 km<sup>2</sup> (in 1940) to less than one seventh of this (ca. 19.3 km<sup>2</sup>). Since 1973, the study site has been a National Park (Parque Nacional de las Tablas de Daimiel, TDNP hereafter) and in 1982 it was included in the RAMSAR convention list of wetlands of international importance ([www.ramsar.org](http://www.ramsar.org)). In 1986, the main inflow at the eastern part of TDNP, the Guadiana River, disappeared permanently as a result of aquifer overexploitation resulting from intensive agricultural irrigation practices in the catchment.

Physiognomically, the Tablas is a mixture of water tables (“tablas”, or seasonal shallow ponds <1 m depth), that can be colonized by submerged plants, belonging mainly to the *Chara* genus (a division of green algae, common in continental waters (Cirujano et al., 2008), and helophyte patches. In spite of the relatively high species richness of the emergent vegetation (up to 21 species; Cirujano, 1996), two

taxa (*Cladium mariscus* (L.) Pohl, sawgrass; *Phragmites australis* (Cav.) Trin. ex Steudel, common reed) have dominated (>90% of the overall helophyte cover) the wetland for many years, being much more important in biomass and cover than charophytes (common name for green algae of the *Chara* genus). Phytoplankton comprises the greatest part of the algal biomass. In recent years (2006 to the beginning of 2010), the wetland landscape has been dominated by terrestrial plants (*Rumex* sp., *Conyza* spp., *Chenopodium* sp., *Cochlearia* sp.), as a consequence of prolonged drying out.

The historical vegetation (before the 1960s) was sawgrass (with marginal reed stands) and a rich aquatic flora, with two different zones (the “Tablas” and the “Cañas”), associated with the influence of Gigüela and Guadiana rivers, respectively (Fig. 1). The Gigüela River, with seasonal saline water, was the water source in the NE sector of the Park, creating the “tablas”, with large charophyte formations. Aquatic vegetation in the Guadiana influence zone (from Guadiana entrance to the W–SW, including springs), with permanent, deeper (max. depth 4–5 m) fresh water, was more diverse, with *Nymphaea*, *Nuphar*, *Ceratophyllum*, *Utricularia*, *Myriophyllum* and *Potamogeton*, besides charophytes (Álvarez-Cobelas and Cirujano, 1996).

The TDNP includes wide areas of peat (> 12–18% of TOC, Mitsch and Gosselink, 1993), associated particularly with the las Cañas area wetland soils. In the 1980s, when Tablas dried out, some wildfires affected a wide area of the peat deposits.

## 2.2. Sampling and analysis

In March 2007, a total of 20 wetland soil samples, intended to be representative of the entire local variation in the wetland environment, were taken from the Tablas area (Fig. 1). Points 17 and 17' were a few m apart from each other, and were collected to

ascertain the spatial heterogeneity of wetland soil. At each point only the upper 20–30 cm were collected with a spade. The entire Tablas surface, except ca. 20 ha, was dry at the time of sampling.

Samples were air-dried, ground and sieved to pass 2 mm. An aliquot of each sample was decarbonated with 6M HCl, followed by dialysis. An additional aliquot (ca. 10 g) was further ground to 1mm and dried (105 °C, 12 h) prior to analysis. Total C, H and N contents were determined with a Perkin-Elmer 2400 Series 2 CNHS/O elemental analyzer. Two sub-samples were used for each analysis and further sub-samples were analyzed if the results did not fit with the estimated error interval. Total P was determined using the method of Bray and Kurtz (1945).

OC content was determined by subtracting the inorganic C content (determined with a Bernard calcimeter) from the total C content. The OM fraction was estimated as the loss in ignition of decarbonated samples heated (6 h) at 550 °C.

Solid state  $^{13}\text{C}$  NMR spectra were obtained with a Bruker DSX 200 (4.7 T) operating at 50.29 MHz for  $^{13}\text{C}$ . Magic angle spinning (MAS) was performed at 6.84 kHz with zirconium dioxide rotors (diam. 7 mm) in a commercial double bearing probe. Spinning side band intensities were rather small, so were neglected. The recycle delay was 300 ms. Cross polarization (CP) contact time was 1 ms and a ramped  $^1\text{H}$  pulse was used to account for Hartmann-Hahn mismatches. Depending on the sample, the number of accumulated scans was between 3000 and 20000 and the line broadening ranged between 25 and 75 Hz. The chemical shifts were calibrated to tetramethylsilane (0 ppm) using glycine as external reference. For spectral interpretation the following ranges and assignments were considered: 0–45 ppm= alkyl (13= methyl, 21= acetate, 30= polymethylene), 46–110 ppm= *O*-alkyl (56= methoxyl/ $\alpha$ -amino, 73= glucopyranosyde-derived, 103= anomeric C in carbohydrate, 105= quaternary aromatic carbons in

tannins); 110–160 ppm= aromatic/unsaturated [ca. 135= unsubstituted, ca. 145 heterosubstituted: guaiacyl (G) lignins/dihydroxys of tannins; ca. 153: ether-linked (syringyl (S) lignins)/tannins]; 160–200 ppm= carbonyl (172= carboxyl/amide, 198= ketone/aldehyde: Wilson, 1987; Wilson et al., 1988; Preston, 1992; Preston et al., 1997; Huang et al., 1998).

Areas in nine spectral regions were measured with ImageJ software (<http://rsb.info.nih.gov/ij/>) and normalized to 100. Statistical calculations were carried out with the Statistica software (Stat-ITCF 1995).

### *2.3 . Assessment of composition of wetland soil OM*

A tentative determination of the composition using a molecular mixing model (MMM) allows characterization of different depositional environments and OM origins. It has been applied to a series of terrestrial (Baldock et al., 2004; Kaal, 2008), sedimentary (Baldock and Nelson, 2005; Dickens, 2006) and estuarine (Krull, 2009) samples, as well as to OM and peat (Kaal, 2007). In addition, Hockaday (2009) and Masiello (2008) used the MMM to determine the carbon oxidation state and oxidation ratio of plant litter and soils and sediments.

A detailed description of the MMM is given by Nelson and Baldock (2005) and Baldock et al. (2004). Briefly, the  $^{13}\text{C}$  NMR spectrum is expressed as a linear combination of the spectra of six basic components, associated with five classes of common biomacromolecules (carbohydrate, protein, lignin, lipid and char) and an additional carbonyl component. To each component, a  $^{13}\text{C}$  NMR signal intensity is associated with seven chemical shift regions (Table 1).

Signal intensities for the six components in the seven spectral regions were taken from Baldock et al. (2004) for soil OM. These authors also supplied the molecular



elemental ratios (normalized against C) H/C, N/C and O/C for the six organic components.

Mathematically, if A, B, C, D, E, and F are the fractions of the six components (carbohydrate, protein, lignin, lipid, char and carbonyl), i.e. the unknowns, and  $\alpha_i$   $\beta_i$   $\gamma_i$   $\delta_i$   $\varepsilon_i$   $\zeta_i$   $\eta_i$  the signal intensity in each spectral region  $\alpha$ ,  $\beta$ , etc, associated with each component  $i$  ( $i = a, b, \dots, f$ ), i.e. the equation coefficients, the experimental spectral areas in a sample ( $\alpha_{\text{SAMPLE}}, \beta_{\text{SAMPLE}}, \dots, \eta_{\text{SAMPLE}}$ ) can be expressed as:

$$\alpha_a A + \alpha_b B + \alpha_c C + \alpha_d D + \alpha_e E + \alpha_f F = \alpha_{\text{SAMPLE}}$$

$$\beta_a A + \beta_b B + \beta_c C + \beta_d D + \beta_e E + \beta_f F = \beta_{\text{SAMPLE}}$$

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$$\eta_a A + \eta_b B + \eta_c C + \eta_d D + \eta_e E + \eta_f F = \eta_{\text{SAMPLE}}$$

This is a system of equations with seven equations and six unknowns. The system was solved for each sample using the Generalized Reduced Gradient nonlinear optimization code in the Solver add-in of Microsoft Excel, with two restrictions:

$$A + B + C + D + E + F = 100 \text{ and } A, B, C, D, E, F \geq 0$$

The method calculates the sample composition that minimizes the sum of the squares of the difference between the predicted and experimental spectral areas. In this way, the composition for a sample can be calculated (Table 6; Fig. 4).

### 3. Results

#### 3.1. General

The wetland soils are rich in organic C, N and P (Table 3a); whereas the average TOC content of Spanish soils is 7.59 kg C m<sup>-2</sup> (to 1 m depth; Rodríguez-Murillo, 2001), the

upper 20 cm in Tablas contain ca. 20 kg C m<sup>-2</sup> (assuming a bulk density of 1 x 10<sup>3</sup> kg m<sup>-3</sup>). TOC varies greatly spatially and with no clear pattern. The soils are also rich in carbonate; samples 1, 3, 5, 6, 12, 13—all from the upper Tablas—and 17 and 17b, from the lower end of TDNP, are essentially a mixture of carbonate (>60%) and OM (up to 30%), with only ca. 10% of other minerals (ash). Such “carbonated mud” shows a typical white or light grey colour, a conspicuous feature of dried out wetland soils of upper Tablas, formed mainly by charophyte remains. Soil associated with present or past sawgrass stands (the historically dominant helophyte) is typically darker (less carbonate and/or more OM). The remainder of the samples contain variable amounts (20–80%) of minerals other than carbonate. The composition is variable across the wetland and the amount of TOC does not show a clear pattern, although it tends to increase in the Guadiana-influenced zone.

We have explored the relationships of wetland SOM and soil environmental conditions. Mean % C is higher in areas with permanent (11.5 ± 9.9) and irregular flooding (10.4 ± 6.2) than in areas with seasonal flooding (5.76 ± 2.03), but differences between flooding regimes are not statistically significant. Other environmental factors must drive soil C content. Similarly, % C is not strongly related to any of soil parameters in Table 3; we only found significant (P < 0.05) relationships with pH (negative relationship, R<sup>2</sup> 0.27, P 0.018) and %Mg (positive relationship, R<sup>2</sup> 0.39, P 0.0032). Mg is an important element in plants, either qualitatively (present in chlorophyll) and quantitatively (Mg content in plants is of the order of that found in our soils) and it is not surprising that its amount is related to the amount of OM. Soil pH varies little across the wetland and its influence might be obscured by other factors, but such a weak relationship with %C precludes any conclusions.

Several spectral ranges were defined in  $^{13}\text{C}$  NMR spectra. Table 4 shows the relative contribution of nine spectral regions, as well as the total intensity in the aliphatic (alkyl, methoxyl and carbohydrate carbon) and aromatic chemical shift regions. A visual inspection of sample spectra (Fig. 2) allows classification of the samples into four groups:

Group I: High intensity from 110 to 165 ppm with a distinct signal between 140 and 165 ppm (O-aryl C) indicating a typical lignin pattern. It includes samples 7, 10, 11, 17b and 18, with low carbonate content.

Group II: Intermediate aromaticity. Includes samples 8, 9, 14, 15 and 16, with low carbonate content.

Group III: Highly alkyl samples 2, 4, 13, 17 and 19, with high carbonate content.

Group IV: High aliphaticity samples 1, 3, 5, 6 and 12, with high carbonate content.

A series of correlations between functional group content as determined via  $^{13}\text{C}$  NMR and the amount of organic and inorganic C were calculated (Table 5). Since no significant relationship ( $P < 0.05$ ) between the proportion of OC and organic functional group content was found, we assume that the amount of OC is not directly related to OM quality or composition. However, there exist significant relationships between the aromatic content and the aliphatic content (aromaticity and aliphaticity in Table 4) and inorganic carbon.

### 3.2. Comparison with global data.

To compare our spectral areas with those in a global database of soil OM composition (Mahieu et al., 1999), we considered four different chemical shift regions in the NMR spectra, corresponding to different organic functionalities:

0–45 ppm: alkyl region, including methyl and methylene groups in aliphatic structures (alkanes, fatty acids and waxes),

46–110 ppm: *O*-alkyl region, including  $\alpha$ -amines, methoxyl groups from lignin, and the six different carbon types in carbohydrates, as well as quaternary aromatic carbons in tannins,

111–165 ppm: aromatic and unsaturated C region, including unsubstituted and substituted aromatic carbons,

165–200 ppm: carbonyl region, including carboxyl groups in aliphatic and aromatic acids, carbonyl groups in amides, and carbonyl in ketones, aldehydes and quinones.

We also distinguish a total aliphatic region, consisting of the two first regions, alkyl and *O*-alkyl.

In Fig. 3, the relative areas of the four defined spectral regions are represented for the 20 samples. The order of relative intensity is *O*-alkyl > alkyl > aromatic > carbonyl, comparable to that described by Mahieu et al. (1999). However, our samples have more alkyl carbon and (to a lesser extent) more aromatic and carbonyl carbon, and less *O*-alkyl carbon, which could be at first sight a consequence of their aquatic nature (Aiken et al., 1985).

### 3.3. Sample OM composition as given by the MMM

Analysis of  $^{13}\text{C}$  NMR data using the MMM makes possible the characterization of the wetland soil OM as a mixture of six constituents (Table 6). In order to assess the performance of the MMM method, two error analyses of the prediction of spectral areas of functional groups in  $^{13}\text{C}$ - NMR spectra were carried out.

First, errors in predicted spectral areas were evaluated for each spectral zone in each sample (Table 7). The sum of spectral area errors for each sample gives an indication of the accuracy of the application of MMM to that sample. The mean average error (0.93) is better than NMR spectrometer accuracy (Baldock and Smernik, 2002) for the whole samples and the spectral zones (ca. 3.9% of total signal intensity). The biggest error is in the  $O_2$ -alkyl region (95–110 ppm). Second, for each sample, a lineal regression method was applied to test the agreement between the experimental and the calculated spectrum —Software IRENE (<http://www.isci.it/tools>) (Fila et al., 2003). The calculated intensity in each spectral region,  $C$ , is regressed against the experimental spectral area,  $E$ :

$$C = b + mE$$

If  $b = 0$  and  $m = 1$  simultaneously, then the coincidence between calculated and experimental would be perfect. Taking into account measurement and assignment errors and an incomplete accounting of each molecular component in the MMM, we would expect some deviation from total agreement. The analysis calculates the correlation coefficient,  $b$ , and  $m$ , and tests the hypothesis that  $b$  is not different from 0 and  $m$  is not different from 1 simultaneously, giving the degree of significance of the hypothesis,  $P$  (Table 8). As shown there, the correlation  $C$  vs.  $E$  is very good, although simultaneous occurrence of  $b = 0$  and  $m=1$  has a degree of significance variable, and usually lower than 0.95. Calculated areas tend to be slightly overestimated with MMM, as  $b$  is generally  $>0$ .

We concluded that the  $^{13}\text{C}$  NMR spectra of our samples can be simulated by way of a mixture of six “generic” organic components, within the typical experimental errors of the spectra. The advantage of the approach over the direct determination of the content of functional groups via intensity distribution among the respective chemical

shift regions in the  $^{13}\text{C}$  spectra is that, in addition to identification and determination of compound classes such as char or proteins, it provides the possibility of establishing the origin of soil OM from different precursor macromolecular entities. Difficulties in detecting non-protonated aromatic carbons, such as present in char, by way of CPMAS spectra are well known, but an efficient cross polarization must exist for char derived from vegetation fires under our analytical conditions (Knicker et al., 2005).

Principal component analysis (PCA) using the six compositional variables for the 20 samples, confirmed the initial visual classification of the samples into four classes (Fig. 5). Four clusters occur in different regions of the PCA plot: Group I is to a large extent defined by lignin amount and char amount, II is made up of samples characterized by a balanced char and carbohydrate-protein content, III has high lipid content and IV contains dominant carbohydrate-protein moieties. A discriminant analysis with forward selection of variables, using the compositions as variables and the groups as classification factor, separates the four groups by way of the variables carbohydrate, lipid and protein (Fig. 6).

The distribution of the different OM components is variable spatially across the TDNP, reflecting the variation in detrital plant input, in transformation environment and perturbation factors, especially fire. Samples with predominantly aliphatic nature (carbohydrate, lipid and protein) are characteristic of the Tablas sector (group IV and part of III), whereas predominantly aromatic samples (groups I and II) are typical of the Las Cañas sector (Fig.1). Group III (high lipid component) is present in both sectors. The four groups of samples are ordered according to their aromaticity as: I > II > III > IV (Fig. 4). Aromaticity is explained mainly by the sum of lignin and char content ( $R^2=0.94$ ), since ca. 95% of the aromaticity is due to the presence of lignin and char.

We analyzed the molecular composition as deduced from the  $^{13}\text{C}$  NMR spectra by examining the correlation coefficients between component (carbohydrate, protein, lignin, lipids, carbonyl and char) content and other variables such as C% (organic and inorganic), N%, C/N ratio, clay% and P. This was carried out using the 20 samples simultaneously (Table 9) and for each group of samples separately.

A strong correlation ( $r > 0.8$ ) was found between protein and lignin content (negative correlation; Table 9), which may correspond to the relative proportions of non-vascular, lignin-lacking plants (such as phytoplankton or charophytes, which have high carbohydrate and protein content) and vascular plants (macrophytes, helophytes or hydrophytes, and terrestrial plants, with lignin and comparatively less protein) in detrital OM. No significant relationships were found between OM constituents and the other variables (C%, H%, clay%, %Mg, pH and P), except the negative char-inorganic carbon ( $r -0.653$ ) relationship which could be fortuitous (fewer fires, i.e. less char in the more carbonate-rich Tablas zone) and weaker, hard to explain relationships [carbonyl-clay ( $r -0.552$ ,  $P 0.012$ ) and lipid-pH ( $r = 0.533$ ,  $P = 0.014$ )]. Significant relationships between the aromatic content and the aliphatic content and inorganic carbon (Table 5) could be due to the association of more aliphatic charophytes with carbonate.

As found in the analysis of spectral regions, the composition of wetland SOM (OM quality), bears no relationship with the proportion of OC (OM quantity). Another relatively strong correlation was found for char vs. carbohydrate ( $r -0.69$ ); this is expected, as carbohydrate is preferentially burned in fires and transformed to char (González-Pérez, 2004).

OM quality is not significantly different in the areas with permanent, irregular, or seasonal flooding, as the means of the six OM constituents assessed by the Fisher's least significant differences test show no significant ( $P < 0.05$ ) differences.

Differences between samples were assessed as differences in mean values of variables in the four groups (Table 10). OC content, N content, C/N and P content are not significantly different for the four groups. Inorganic carbon is significantly higher in IV than in I and II, III having an intermediate content. Groups III, I and IV are significantly different from the other three in carbohydrate and char (IV), lignin and protein (I), and lipid content (III). Group II shows an intermediate character, as it is not significantly different from the other three in any of the variables considered.

#### 4. Discussion

##### 4.1. General

The TDNP has very high productivity and sedimentation rate, being therefore an active carbon and nutrient sink. Sánchez Carrillo et al. (2000) found a mean accretion rate of 2.22 and 2.47 cm yr<sup>-1</sup> for 1997 and 1998. Domínguez Castro et al. (2006) reported an average sedimentation rate of 0.184 cm yr<sup>-1</sup> for the last 100 yr. A recent, unpublished dating of a sedimentary core gives a mean sedimentation rate of 0.31 cm yr<sup>-1</sup> for the last 60 yr (Álvarez-Cobelas, personal communication). From data of Sánchez Carrillo (2001), we estimate that an average of 1.43 kg m<sup>-2</sup> OC (3.14 kg of OM and 10.64 kg of mineral matter), and 0.79 kg m<sup>-2</sup> inorganic carbon (mainly CaCO<sub>3</sub>) were deposited yearly during 1997–1998. During that period, the carbonate fraction collected in sediment traps was 48%, the same as the mean carbonate content of our samples. The OC fraction was 10.4%, well in the range of C% (10.0 ± 7.8 %) measured for our samples (Table 3).

It is tempting, but very difficult, to try to relate the amount and characteristics of wetland SOM to the hydroperiod of each sample point, as soil humidity and inundation should be the chief determinants of SOM transformation (Mitsch and Gosselink, 1993).



The difficulty lies in the drastic changes in extent of flooding and, very likely, in flooding patterns brought about by mill dam disappearance and channel building within the last forty years (Álvarez-Cobelas and Cirujano 1996). An even more severe limitation occurs in trying to characterize the historical vegetation and its evolution at each particular point. However, we have taken advantage of the relatively stable flooding pattern in the sampling points until ca. 1973 and have roughly characterized the flooding regime of each sample (Table 3).

The highly variable environment in TDNP, in addition to the long history of anthropogenic change, results in difficulties in performing an analysis of detrital OM in terms of its origin and transformation. In addition, wetland water movement and sediment transport can mix the organic detritus from different areas. Nevertheless, some patterns are apparent, as discussed below.

An obvious observation is the widespread distribution of the six categories of OM in every sample; in addition, sample composition is variable in the wetland. More interesting is the presence of significant amounts of lignin and char all over the wetland, but in relatively more variable quantities (sum of lignin and char between 10% and 50%) vs. the other three main constituents (sum of carbohydrate, protein and lipid accounting for 50% to 90% of the SOM). OM in the upper sediment (20–30 cm) of Tablas is of recent origin (ca. 100–150 yr), given the high sedimentation rate. Bearing in mind that aging and humification of OM leads to a more similar material, the striking quality differences in the samples are in accord with the “recentness” of this OM. Prevalent anaerobic conditions in our samples could also help preserve the original differences in SOM.

OM quality does not show a significant relationship to historical flooding regime. Clearly, other environmental factors (such as vegetation and fire) must drive

OM composition besides hydrology. The lack of dependence of either the quantity as well as the quality of the OM on soil characteristics and known hydrology listed in Table 3 is surprising, but some important information on soil conditions (detailed degree of anaerobiosis) is lacking, so a more exact analysis is not possible. We are at present not able to explain the differences in OM content in the different zones of Las Tablas; different productivity in different areas, besides different degree of anaerobiosis may explain the differences in OM content. Anyway, the quality of the wetland SOM does not seem to keep any relationship with OM content. What is clear is that plant composition is the main control on OM composition, with an influx of allochthonous lignin and char, and fire in some samples.

Variability in the OM composition could then be interpreted as the result of accumulation (and preservation) of detrital material of different origin, although the influence of different sediment age cannot be ruled out. The samples of Group I accumulated lignin, those of group III and IV lipids and carbohydrates, respectively, with a statistically significant difference compared to the remainder of the groups in each case. Group II has not a statistically different content of any component. Groups I and IV are clearly extremes (and so appear in PCA and discriminant analyses; Figs. 6 and 7) and every component (with the exception of lipid) has an average significantly different when comparing I and IV. Geographically, group IV belongs to the northern and central Tablas, dominated by charophyte vegetation, which could explain the high protein and carbohydrate contents. Group I belongs to the Cañas sector, with a more mixed vegetation and an important vascular macrophyte contribution (high lignin content).

Perturbations are frequent and widespread in the Cañas zone (Álvarez-Cobelas and Cirujano, 1996), particularly fires, which can explain the high char content; the

autochthonous char content could then be considered a signal of partially terrestrial conditions. Lower char content in group IV is coherent with the historically less fire-perturbed Tablas area. Char and lignin could also be of allochthonous origin: it has been estimated that 35–55% of the OM accumulated in 1997–1998 in the Tablas was allochthonous in nature (Sánchez-Carrillo et al., 2000) and a sizeable proportion of the external OM should be either lignin or char or both. Either way, differences in lignin content could be attributed basically to differences in initial detritus composition (more lignin = more vascular plant residue), and differences in char content to differences in fire frequency and intensity (more frequent fires in the Cañas zone). Groups II and III are of intermediate character. This is particularly true for group II (Cañas area), which has a high char content, but intermediate lignin content and high protein content. Char content indicates fire, i.e. a more terrestrial environment than IV, and lignin content is mainly a signal of vascular plant origin of detritus, which is intermediate between groups I and IV. Group III is characterized by high lipid content. This could be caused by OM of microbial origin, preserved under aquatic conditions. Samples of group III are located either in the Tablas or in the Cañas zones.

Differences in sample constituents could also be influenced by the different depositional environments. Such differences in sediment physical conditions (e.g. degree of hydromorphism), would favour differential preservation of components; this could be investigated by comparing humic acid and whole sample compositions.

#### *4.2. Mechanisms of carbon accumulation*

Significant OC accumulation in soils is related to the formation of recalcitrant SOM compounds. Depending on the initial OM and the degradation conditions,

different components can be preserved. Perturbations can also offer paths for formation or preservation of long lived organic compounds (Almendros, 2008). Due to the diversity of original OM sources and transformation conditions, wetlands are suitable, albeit difficult, sites for studying OC accumulation pathways and mechanisms.

The TDNP wetland has been an active carbon sink, via high vegetation productivity and fast sedimentation. Besides anaerobic preservation of dead OM caused by flooding, fast sedimentation could also play a role in OM protection. Areas of high productivity (masiega stands) are associated with carbon-rich, peaty sediments. In any case, carbon accumulation is widespread, and the OC content bears no relationship with OM quality. This is noteworthy, as higher carbon content is expected to be related to the presence of more recalcitrant components (char, lignin, some lipids). Preservation of (in principle) more labile components such as carbohydrates and proteins, which has been attributed to charophyte vegetation and, perhaps, younger sediments (Group IV samples and, to a lesser extent, Group II -protein and carbohydrate- and Group III -protein-), seems to be an efficient mechanism of carbon sequestration in the Tablas. Thus, for the Group I and II samples, char formation is a relevant mechanism for carbon accumulation, and for Group IV, lipid preservation is important. However, the presence of structural carbohydrate, protein and lipid is dominant in TDNP SOM (>60% OM in every group). This confirms that the preservation of such constituents is a general feature of TDNP wetland soils.

It is noteworthy (Sánchez-Carrillo et al 2001) that more than a third of the carbon deposited in TDNP is inorganic (carbonate). Part could be of biogenic origin, because historically dominant charophyte vegetation under free water precipitates up to 70% of its dry weight as carbonate in a lagoon complex (Lagunas de Ruidera; unpublished data).

Fire is an important factor in biospheric carbon fluxes globally and particularly in Spain (Rodríguez-Murillo, 1994). Widespread fires are probably a new phenomenon in Tablas de Daimiel wetland, associated with extended desiccation and cultivation, have left in spite of this a considerable amount of char. Char and other pyrogenic C forms are C sinks in the long term, even if their production by fire implies a CO<sub>2</sub> release, as they are (relatively) recalcitrant materials, and the CO<sub>2</sub> released will eventually be fixed by the new growing vegetation (Forbes et al., 2006). At present, we have not tried to evaluate the effects of fire and char formation on C storage in the wetland. If fires were mere surface fires, affecting only the vegetation (and accepting that there is no long term degradation of that vegetation caused by fire influx), we could consider fire as a C sink process in Las Tablas. But the fires have also affected a big area of peat in Las Tablas, surely destroying a long term, recalcitrant carbon store (Rein 2010), and the final balance could well represent a carbon source, even in the middle-long term.

## 5. Conclusions

Elemental analysis and <sup>13</sup>C NMR spectroscopic analysis of wetland soil samples from the Mediterranean semi-arid wetland, Tablas de Daimiel, allow the molecular characterization of SOM using the MMM in terms of carbohydrate, protein, lignin, lipid, char and carbonyl fractions. Correlation of fractions of these six OM components, and comparison of sample composition, make possible the identification of the main sources of OM in the wetland (via protein/lignin contents and correlations), as well as the effects attributed to fire apparent in the char content.

The TDNP is an active carbon and nutrient sink. Carbon content is not significantly different between the groups of samples, roughly associated with the two main TDNP environments, but peat deposits are more abundant in the Cañas area. On the other hand, there is no relationship between C quantity and OM composition (C quality), and C content is not related to other environmental or site characteristics (P or clay content). Other local factors, such as microtopography, perturbations or mineral composition could be responsible for the observed differences in organic carbon content.

Preservation of carbohydrates, lipids and protein is more apparent in Groups IV (carbohydrate), II, III and IV (protein), and III (lipid), but is widespread in Tablas. The variability in the quality of the OM could then be interpreted as a result of accumulation (and preservation) of detrital material of different origins, although the influence of different sediment age cannot be ruled out. High lipid content in III could be attributed to a more microbial source of the biomass. The high lignin contribution to Group I is best explained by a predominant vascular origin of the OM. Terrestrial input should be greater for Groups II and III, which may also have experienced more fires. Lignin and char are not confined to zones with vascular vegetation and burned zones, as OM, including allochthonous OM, moves around the wetland,

Inorganic carbon (carbonate) also accumulates in Tablas de Daimiel. It is associated with less aromatic OM. This could be due to preferential char accumulation, as a consequence of repeated fires, in the less carbonated areas, and more OM of vascular origin (i.e. more aromatic) and fewer charophytes (which are usually associated with highly carbonated sediments) in these areas.

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## **References**

- Aiken, G.R., McKnight, D.M., Wershaw, R.L., MacCarthy, P. (Eds.), 1985. Humic Substances in Soil, Sediment, and Water. J. Wiley and Sons, New York.
- Alam, A.K.M.R., Hossain, A.B.M.E., Hoque, S., 2003. Carbon content of humus in some freshwater wetland soil in Bangladesh. Bangladesh Journal of Botany 32, 5-9.

–Almendros, G., Knicker, H., González-Vila, F., 2003. Rearrangement of carbon and nitrogen forms in peat after progressive thermal oxidation as determined by solid-state  $^{13}\text{C}$ - and  $^{15}\text{N}$ -NMR spectroscopy. *Organic Geochemistry* 34, 1559–1568.

–Almendros, G., 2008. Humic substances. In: Chesworth, W. (Ed.), *Encyclopedia of Soil Science*. Springer, pp. 315-323.

–Álvarez-Cobelas, M., Cirujano, S. (Eds.), 1996. *Las Tablas de Daimiel, Ecología acuática y Sociedad*. Spanish Ministry of Environment, Madrid.

–Baldock, J.A., Masiello, C.A., Gélinas, Y., Hedges, J.I., 2004. Cycling and composition of organic matter in terrestrial and marine ecosystems. *Marine Chemistry* 92, 39–64.

–Baldock, J.A., Smernik, R.J., 2002. Chemical composition and bioavailability of thermally altered *Pinus resinosa* (Red pine) wood. *Organic Geochemistry* 33, 1093-1109.

–Bray, R.H., Kurtz, L.T., 1945. Determination of total organic and available forms of phosphorus in soils. *Soil Science* 59, 39-45.

–Bridgham, S.D., Megonigal, J.P., Keller, J.K., Bliss, N.B., Trettin, C., 2006. The carbon balance of North American Wetlands. *Wetlands* 26, 889-916.

–Calace, N., Cardellicchio, N., Petronio, B.N., Pietrantonio, M., Pietroletti, M., 2006.



Sedimentary humic substances in the northern Adriatic sea (Mediterranean sea). *Marine Environmental Research* 61, 40–58.

–Calace, N., Capolei, M., Lucchese, M., Petronio, B.M., 1999. The structural composition of humic compounds as indicator of organic carbon sources. *Talanta* 49, 277–284.

–Cirujano, S., 1996. Bentos vegetal. Flora y vegetación superior. In: Alvarez-Cobelas, M., Cirujano, S. (Eds.), *Las Tablas de Daimiel: Ecología Acuática y Sociedad*. Spanish Ministry of Environment, Madrid, p 129.

–Cirujano, S., Cambra, J., Sánchez Castillo, P.M., Meco, A., Flor Arnau, N., 2008. In: Cirujano, S. (Ed.), *Flora Ibérica. Algas Continentales. Carófitos (Characeae)*. Real Jardín Botánico-CSIC, Madrid.

–Cirujano, S., Velayos, M., Castilla, F., Gil, M., 1992. *Criterios Botánicos para la Valoración de las Lagunas y Humedales Españoles*. ICONA, Madrid.

–Davidson, E.A., Janssens, I.A., 2006. Temperature sensitivity of soil carbon decomposition and feedbacks to climate change. *Nature* 440, 165–173.

–Dickens, A.F., Baldock, B.A., Smernik, R.J., Wakeham, S.G., Arnarson, T.S., Gélina, Y., Hedges, J.I., 2006. Solid-state  $^{13}\text{C}$  NMR analysis of size and density fractions of marine sediments: Insight into organic carbon sources and preservation mechanisms. *Geochimica et Cosmochimica Acta* 70, 666–686.

–Domínguez-Castro, F., Santisteban, J.I., Mediavilla, R., Dean, W.E., López-Pamo, E., Gil-García, M.J., Ruiz-Zapata, M.B., 2006. Environmental and geochemical record of human-induced changes in C storage during the last millennium in a temperate wetland(Las Tablas de Daimiel National Park, central Spain). *Tellus B* 58, 573–585.

El-Sayed, M.A., Wafica, M., Naga, A., Ali I. Beltagy, A.I., Halim, Y., 1996. Sedimentary humic substances isolated from a coastal lagoon of the Nile delta: physical and chemical characteristics. *Estuarine, Coastal and Shelf Science* 43, 205-215.

Fila, G., Bellocchi, G., Acutis, M., Donatelli, M., 2003. IRENE: a software to evaluate model performance. *European Journal of Agronomy* 18, 369-372.

–Filip, Z., Alberts, J.J., 1994. Microbial utilization resulting in early diagenesis of salt-marsh humic acids. *Science of the Total Environment* 144, 121–135.

–Fissore, C., Giardina, C., Randall P., Kolka, K., Trettin, C., 2009. Soil organic carbon quality in forested mineral wetlands at different mean annual temperature. *Soil Biology & Biochemistry* 41, 458–466.

–Forbes, M.S., Raison, R.J., Skjemstad, J.O., 2006. Formation, transformation and transport of black carbon (charcoal) in terrestrial and aquatic ecosystems. *Science of the Total Environment* 370, 190–206.

–Gołębiewska, D., Mielnik, L., Gonet, S., Fründ, R., Lüdemann, H.D., 1996. Characteristics of humic acids in bottom sediments of lobelia lakes. *Environment International* 22, 571-578.

Gołębiewska, D., 1989. The quantitative analysis of solution- and CPMAS <sup>13</sup>C NMR spectra of humic material. *Science of the Total Environment* 81/82, 157-168.

–González-Pérez, J.A., González-Vila, F.J., Almendros, G., Knicker, H., 2004. The effect of fire on soil organic matter—a review. *Environment International* 30, 855–870.

–Gorham, E. 1991. Northern peatlands: role in the carbon cycle and probable responses to climatic warming. *Ecological Applications* 1, 182–195.

–Haumaier, L., Zech, W., 1995. Black carbon—possible source of highly aromatic components of soil humic acids. *Organic Geochemistry* 23, 191-196.

–He, M., Shi, Y., Lin, C., 2008. Characterization of humic acids extracted from the sediments of the various rivers and lakes in China. *Journal of Environmental Science* 20, 1294–1299.

–Hernández, M.E., Mitsch, W.J., 2007. Denitrification potential and organic matter as affected by vegetation community, wetland age, and plant introduction in created wetlands. *Journal of Environmental Quality* 36, 333–342.

- Hockaday, W.C., Masiello, C.A., Randerson, J.T., Smernik, R.J., Baldock, J.A., Chadwick, O.A., Harden, J.W., 2009. Measurement of soil carbon oxidation state and oxidative ratio by  $^{13}\text{C}$  nuclear magnetic resonance. *Journal of Geophysical Research* 114, G02014.
- Huang R.D., Feather M.S.,1988.  $^{13}\text{C}$  NMR study of some Maillard reaction products arising from D-glucose-DL-alanine interactions. *Journal of Agriculture and Food Chemistry* 36, 673-676.
- ITCF. Institut Technique des Céréales et des Fourrages. 1997. Manuel d'Utilisation. Atelier, Paris.
- Jokic, A., Cutler, J.N., Ponomarenko, E., Van Der Kamp, G., Anderson, D.W., 2003. Organic carbon and sulphur compounds in wetland soils: Insights on structure and transformation processes using K-edge XANES and NMR spectroscopy. *Geochimica et Cosmochimica Acta* 67, 2585–2597.
- Kaal, J., Brodowski, S., Baldock, J.A., Nierop, K.G.J., Martínez-Cortizas, A., 2008. Characterisation of aged black carbon using pyrolysis-GC/MS, thermally assisted hydrolysis and methylation (THM), direct and cross-polarisation  $^{13}\text{C}$  nuclear magnetic resonance (DP/CP NMR) and the benzene polycarboxylic acid (BPCA) method. *Organic Geochemistry* 39, 1415–1426.
- Kaal, J., Baldock, J.A., Buurman, P., Nierop, K.G.J., Pontevedra-Pombal, X., Martínez-Cortizas, A., 2007. Evaluating pyrolysis-GC/MS and  $^{13}\text{C}$  CPMAS NMR

in conjunction with a molecular mixing model of the Penido Vello peat deposit, NW Spain. *Organic Geochemistry* 38, 1097–1111.

Knicker, H., Totsche, K.-U., Almendros, G., González-Vila, F.J., 2005. Condensation degree of burnt peat and plant residues and the reliability of solid-state VACP MAS  $^{13}\text{C}$  NMR spectra obtained from pyrogenic humic material. *Organic Geochemistry* 36, 1359-1377.

–Kögel-Knabner, I., 2000. Analytical approaches for characterizing soil OM. *Organic Geochemistry* 31, 609-625.

–Kögel-Knabner, I., 2002. The macromolecular organic composition of plant and microbial residues as inputs to soil OM. *Soil Biology and Biochemistry* 34, 139-162.

– Krull, E., Haynes, D., Lamontagne, S., Gell, P., McKirdy, D., Hancock, G., McGowan, J., Smernik, R., 2009. Changes in the chemistry of sedimentary OM within the Coorong over space and time. *Biogeochemistry* 92, 9–25.

–Masiello, C.A., Gallagher, M.E., Randerson, J.T., Deco, R.M., Chadwick, O.A., 2008. Evaluating two experimental approaches for measuring ecosystem carbon oxidation state and oxidative ratio. *Journal of Geophysical Research* 113, G03010.

–Mahieu N., Powlson D.S., Randall E.W., 1999. Statistical analysis of published C-13CPMAS NMR spectra of soil OM. *Soil Science Society of America Journal* 63, 307-319.

–Mendonça, A., Duarte, A.C., Santos, E.B.H., 2004. Spectroscopic properties of sedimentary humic acids from a salt marsh (Ria de Aveiro, Portugal): comparison of sediments colonized by *Halimione portulacoides* (L.) Aellen and non-vegetated sediments. *Biogeochemistry* 69, 159–174.

–Meyers, P.A., Ishiwatari, R., 1993. Lacustrine organic geochemistry and overview of indicators of OM sources and diagenesis in lake sediments. *Organic Geochemistry* 20, 867-900.

–Mitsch, W. J., Gosselink, J.G., 1993. *Wetlands*. Van Nostrand Reinhold. New York.

–Mitsch, W. J., Mitsch, R.H., Turner, R.E., 1994. Wetlands of the old and new world: ecology and mangement. In: Mitsch , W.J. (Ed.), *Global Wetlands: Old World and New*. Elsevier, Amsterdam, pp. 3-56.

–Nelson, P.N., Baldock, J.A., 2005. Estimating the molecular composition of a diverse range of natural organic materials from solid state  $^{13}\text{C}$  NMR and elemental analyses. *Biogeochemistry* 72, 1–34.

–Nieder, R., Benbi, D.K., 2008. *Carbon and Nitrogen in the Terrestrial Environment*. Springer Science + Business Media B.V.

- Plante, A.F., Magrini-Bair, K., Vigil, M., Paul, E.A., 2008. Pyrolysis-molecular beam mass spectrometry to characterize soil OM composition in chemically isolated fractions from differing land uses. *Biogeochemistry* 10.1007/s10533-008-9218-3.
- Preston, C.M., Schmidt, M.W.I., 2006. Black (pyrogenic) carbon in boreal forests: a synthesis of current knowledge and uncertainties. *Biogeosciences Discussions* 3, 211–71.
- Preston, C.M., J.A. Trofymow, J.A., B.G. Sayer, B.G., Niu, J., 1997.  $^{13}\text{C}$  nuclear magnetic resonance spectroscopy with cross-polarization and magic-angle spinning investigation of the proximate analysis fractions used to assess litter quality in decomposition studies. *Canadian Journal of Botany* 75, 1601-1613.
- Preston, C.M., 1992. The application of NMR to OM inputs and processes in forest ecosystems of the Pacific Northwest. *Science of the Total Environment* 3, 107-120.
- Rein, G., 2010. Overview of Subsurface Peat Fires and the 2009-2010 Fire in Las Tablas de Daimiel National Park. *Proceedings of the XV Meeting of the IHSS*, Vol.1:295–302. Puerto de la Cruz, Tenerife, Spain.
- Rodríguez-Murillo, J.C., 1994. The carbon budget of the Spanish forests. *Biogeochemistry* 25, 197-217.

–Rodríguez-Murillo, J.C., 2001. Organic carbon content under different types of landuse and soil in peninsular Spain. *Biology and Fertility of Soils* 33, 53-61.

–Sánchez-Carrillo, S., 2001. Hidrología y sedimentación actual de Las Tablas de Daimiel. PhD Thesis, Universidad Autónoma de Madrid, Madrid.

–Sánchez Carrillo, S., Álvarez Cobelas, M., Angeler, D.G., Vizcayno Muñoz, C., 2000. Tasas de acreción y características de los sedimentos actuales en el parque nacional Las Tablas de Daimiel (Ciudad Real). *Estudios Geológicos* 56, 239-250.

–Santín, C., González-Pérez, M., Otero, X.L., Vidal-Torrado, P., Macías, F., Álvarez, M.Á., 2008. Characterization of humic substances in salt marsh soils under sea rush (*Juncus maritimus*). *Estuarine, Coastal and Shelf Science* 79, 541-548.

–Sardesai, S., Wahidullah, S., 1998. Structural characteristics of marine sedimentary humic acids by CP/MAS  $^{13}\text{C}$  NMR spectroscopy. *Oceanologica Acta* 21, 543-550.

Stat-ITCF, 1995. *STATISTICA for Windows* [Computer program manual]. Tulsa, OK: StatSoft, Inc., 2300 East 14th Street, Tulsa, OK, 74104-4442, (918) 749-1119, fax: (918) 749-2217, e-mail: info@statsoft.com, WEB: <http://www.statsoft.com>.

–Stevenson, F. J., 1982. *Humus Chemistry*. Wiley, New York.



–Visser, S.A., 1983. Comparative study on the elementary composition of fulvic and humic acids of aquatic origin and from soils and microbial substrates. *Water Research* 17, 1393-1396.

–Wilson, M. A., 1987. *NMR Techniques and Applications in Geochemistry and Soil Chemistry*. Pergamon Press, Oxford, UK.

–Wilson, M.A., Hatcher, P.G. 1988. Detection of tannins in modern and fossil barks and in plant residues by high-resolution solid-state  $^{13}\text{C}$  nuclear magnetic resonance. *Organic Geochemistry* 12, 539, 546.

## TABLES

**Table 1**

Definition of spectral regions in  $^{13}\text{C}$  NMR spectra.

Spectral region	Chemical shift
“Carbonyl”	210–165 ppm
<i>O</i> -aromatic	165–145 ppm
Aromatic	145–110 ppm
$\text{O}_2$ -alkyl	110–95 ppm
<i>O</i> -alkyl	95–60 ppm
N-alkyl-methoxy	60–45 ppm
Alkyl	45–0 ppm

**Table 2**

Distribution of  $^{13}\text{C}$  NMR signal intensity for each model component (% total signal intensity for the component).<sup>a</sup>

Chemical shift region (ppm)	Carbohydrate	Protein	Lignin	Lipid	Carbonyl	Char
0–45	0.0	35.4	10.5	75.6	0.0	1.3
45–60	0.0	22.6	13.8	4.5	0.0	1.2
60–95	83.3	3.5	12.5	9.0	0.0	1.3
95–110	16.7	0.0	8.6	0.0	0.0	6.3
110–145	0.0	8.9	30.6	3.6	0.0	64.9

145–165	0.0	1.3	19.5	0.7	0.0	17.5
165–215	0.0	28.3	4.6	6.6	100.0	7.7
Molar elemental ratio (normalized vs. C)						
C	1.00	1.00	1.00	1.00	1.00	1.00
N	0.00	0.27	0.00	0.00	0.00	0.00
H	1.67	1.10	1.24	1.94	1.00	0.45
O	0.83	0.16	0.43	0.24	2.00	0.41

<sup>a</sup>From Baldock et al. (2004).

**Table 3**

General analytical characteristics of whole samples from Tablas de Daimiel wetland.

**a**

Sample	Organic carbon		P (mg kg <sup>-1</sup> )	Inorg. C		Ash (%)	OM (%)	Carbonate (%)
	(g kg <sup>-1</sup> )	N (g kg <sup>-1</sup> )		(g/100g)	C/N			
1	149	13.5	67.6	7.63	11.1	7.20	29.3	63.5
2	78	5.7	7.4	4.34	13.6	41.9	22.0	36.2
3	49	4.8	74.1	9.07	10.1	12.9	11.6	75.5
4	39	3.4	41.4	3.44	11.4	51.5	19.8	28.7
5	105	9.3	61.0	8.53	11.3	11.1	17.9	71.1
6	58	5.5	89.4	10.0	10.5	7.97	8.50	83.5
7	53	3.8	100	0.10	14.2			
8	86	7.6	12.2	4.21	11.2	41.5	23.4	35.1

9	365	22.6	56.7	0.25	16.1	25.7	72.3	2.05
10	83	7.5	52.3	3.68	11.1	50.8	18.6	30.7
11	148	10.6	78.5	1.59	13.9	56.2	30.5	13.3
12	92	8.5	14.0	7.37	10.9	12.5	26.2	61.3
13	88	9.8	19.6	8.33	8.9	13.0	17.7	69.4
14	133	12.0	17.4	6.22	11.0	21.8	26.5	51.8
15	200	18.4	19.6	3.21	10.8	34.1	39.1	26.8
16	19	1.1	30.5	4.66	16.5	56.5	4.70	38.8
17	132	10.4	15.3	8.60	12.7	3.63	24.8	71.7
17b	14	0.9	17.4	10.5	15.0	10.1	2.87	87.0
18	37	4.1	32.7	0.73	9.0	83.2	10.8	6.07
19	73	3.9	105	5.67	18.9			
Mean $\pm$ S.D.	100 $\pm$ 78	8.4 $\pm$ 5.4	52 $\pm$ 34	5.4 $\pm$ 3.3				

Table 3<sup>a</sup>

b

Sample	K (mg/100g)	Ca (mg/100g)	Na (mg/100g)	Mg (mg/100g)	pH	Sand %	Clay %	Flooding regime
1	21.3	756	3.8	28.5	7.13	60	10	I
2	9.3	2170	26.2	106	7.26	25	11	I
3	8.6	603	4	27.9	7.36	29	15	S
4	9	2180	28.5	153	7.71	15	6	S
5	6.4	1040	3.2	34.6	7.28	42	11	I
6	16.1	715	3.8	17.7	7.21	42	11	S
7	36	2000	15.9	66.5	7.08	23	3	I
8	40.7	2040	59.1	184	7.47	36	7	S
9	19	2480	47.1	238	7.04	55	12	P
10	22.8	825	17.7	73.2	7.26	57	9	P

11	36.1	994	28.9	126	7.01	49	19	P
12	13.3	1340	2.6	39.5	7.4	29	9	P
13	8.4	1220	61.5	106	7.62	16	12	P
14	13.3	891	39.1	127	7.34	28	10	P
15	32.1	1580	23.4	133	7.28	41	10	I
16	12.8	649	2.3	17.7	7.3	50	6	P
17	12	637	36.7	106	7.37	28	16	P
17b	8.1	541	11.9	54.1	7.6	24	14	P
18	40.4	1140	14.5	75.8	7.42	40	4	I
19	15.4	788	48.5	113	7.5	31	11	P

<sup>a</sup> Flooding regime before 1973 (approximate onset of drastic hydrological change in the sampled areas; Cirujano and Álvarez-Cobelas, private communication): I, irregular flooding; S, seasonal flooding; P, permanent flooding.

**Table 4**

Integration values of NMR areas of samples.<sup>a</sup>

Spectral Range (ppm)											
	0–45	46–58	59–65	66–94	95–110	111–146	147–167	168–188	189–200		
	<i>H</i>										
Sample	Alkyl	Methox	C6	O-alk	C1	arom	<i>N</i> -arom	Carboxyl	Carbonyl	Aromaticity	Aliphaticity
1	26.9	8.89	6.07	26.8	6.30	11.3	3.47	9.24	0.95	14.8	75.0
2	37.7	9.36	3.90	11.8	3.08	15.9	4.92	11.8	1.56	20.8	65.8
3	30.7	9.77	5.46	21.8	6.21	11.6	3.43	9.95	1.15	15.0	73.9

4	34.7	7.57	3.81	13.6	3.42	16.5	4.71	13.9	1.75	21.2	63.1
5	34.5	9.71	5.30	21.1	5.66	9.85	3.25	9.78	0.80	13.1	76.3
6	31.2	9.26	5.65	23.8	6.10	8.05	2.44	12.3	1.17	10.5	76.0
7	25.4	8.32	3.74	15.2	5.17	19.5	7.44	13.3	1.99	26.9	57.8
8	29.0	8.95	4.31	16.0	5.26	19.7	5.99	9.55	1.24	25.7	63.6
9	25.7	9.38	4.72	18.0	6.14	17.3	6.35	10.7	1.64	23.7	64.0
10	25.9	8.86	4.77	15.8	5.45	19.7	7.53	10.3	1.83	27.2	60.7
11	21.4	8.67	4.03	12.6	4.18	27.3	10.3	9.33	2.17	37.6	50.9
12	32.9	8.10	4.09	20.8	4.57	11.7	3.54	12.9	1.35	15.2	70.5
13	32.2	10.1	4.53	16.7	4.99	15.3	4.66	10.5	1.13	20.0	68.4
14	26.1	9.43	4.63	17.1	5.39	18.5	6.11	11.2	1.53	24.6	62.7
15	29.0	9.06	4.53	15.6	4.76	18.2	5.90	11.4	1.56	24.1	62.9
16	29.8	8.98	4.50	15.5	5.29	16.9	6.19	11.4	1.40	23.1	64.0
17	37.2	9.41	3.78	13.4	3.86	15.1	4.62	10.9	1.62	19.7	67.7
17b	25.0	8.42	4.21	14.8	5.53	19.8	8.39	12.2	1.70	28.2	58.0
18	28.0	7.77	3.45	11.3	4.40	21.7	9.36	12.4	1.72	31.1	54.8
19	33.4	8.63	3.81	14.0	4.09	16.1	5.86	12.3	1.72	22.0	64.0

<sup>a</sup> “Alkyl”, alkyl +  $\alpha$ -amino; “Methoxyl”, methoxyl +  $\alpha$ -amino; “C6”, “C1”, C6 and C1 carbons in glucopyranoside structures; “O-alkyl”, C2, C3, C4 and C5 carbons in glucopyranoside structures; “H-aromatic”, aromatic C unsubstituted; “N-aromatic”, aromatic C heterosubstituted; “Carboxyl”, carboxyl + amide; “Carbonyl”, ketone and aldehyde. “Aliphaticity”, area between 0 and 110 ppm. “Aromaticity”, area between 111 and 167 ppm.

**Table 5**

Pearson linear correlation coefficients  $r$  for functional C content and organic and inorganic C content <sup>a</sup>

	Alkyl C	O-alkyl C	Aliphatic C	Aromatic C	Carbonyl C	C <sub>org</sub>	C/N
C <sub>org</sub>	-0.173	0.138	0.008	0.005	-0.255		0.136
C <sub>inorg</sub>	0.386	0.439	0.661**	-0.650**	-0.226	-0.326	-0.221
**: $P < 0.01$							

<sup>a</sup> Meanings of structural unit names are as in Table 4.

**Table 6**

Calculated composition of samples (%).

Sample	Carbohydrate	Protein	Lignin	Lipid	Carbonyl	Char
1	34.48	28.23	7.77	21.07	0.00	8.45
2	12.28	29.02	7.37	34.99	1.29	15.05
3	27.23	30.64	10.11	24.68	0.00	7.34
4	15.25	23.47	3.47	34.10	5.14	18.57
5	25.40	28.04	12.03	30.76	0.00	3.77
6	30.20	31.51	7.01	25.53	2.35	3.39
7	16.17	18.98	20.64	21.53	6.21	16.47
8	18.69	25.79	11.17	24.34	0.00	20.01
9	21.26	25.74	18.51	19.15	1.92	13.41
10	17.95	21.17	21.09	21.09	2.47	16.24
11	12.95	24.03	19.57	13.95	0.47	29.03
12	24.41	26.20	4.69	30.43	3.80	10.46
13	19.41	30.51	11.12	26.46	0.00	12.50
14	20.63	29.44	12.42	18.71	1.19	17.61
15	18.43	27.82	10.74	23.47	1.67	17.87
16	17.43	23.21	17.52	25.86	2.75	13.24
17	14.27	29.06	8.24	34.29	0.62	13.52



17b	15.26	14.90	28.13	21.96	5.81	13.93
18	9.21	9.36	30.08	28.10	6.88	16.37
19	14.48	21.96	14.97	31.60	4.04	12.93

**Table 7**

Errors in predicted spectral area, relative to measured area (errors are expressed in absolute values; total measured area normalized to 100).

Sample	0–45	45–60	60–95	95–110	110–145	145–165	165–215	Sum
1	0.06	0.39	0.22	0.66	0.20	0.04	0.20	1.78
2	0.02	0.03	0.15	0.55	0.04	0.23	0.04	1.06
3	0.00	0.25	0.07	0.33	0.09	0.40	0.23	1.38
4	0.02	0.03	0.17	0.59	0.04	0.24	0.04	1.14
5	0.02	0.28	0.02	0.14	0.11	0.33	0.23	1.13
6	0.01	0.02	0.07	0.24	0.02	0.11	0.02	0.50
7	0.01	0.01	0.09	0.34	0.02	0.13	0.02	0.63
8	0.02	0.24	0.04	0.08	0.11	0.20	0.18	0.86
9	0.01	0.02	0.05	0.15	0.02	0.07	0.02	0.34
10	0.01	0.02	0.10	0.38	0.02	0.15	0.02	0.71
11	0.06	0.09	0.41	1.46	0.11	0.61	0.10	2.84
12	0.02	0.03	0.16	0.57	0.04	0.24	0.04	1.11
13	0.02	0.29	0.02	0.01	0.12	0.28	0.22	0.96
14	0.00	0.01	0.06	0.23	0.01	0.09	0.01	0.42
15	0.01	0.02	0.10	0.37	0.02	0.15	0.02	0.69
16	0.01	0.01	0.02	0.04	0.01	0.03	0.01	0.12
17	0.00	0.00	0.02	0.08	0.00	0.03	0.00	0.13
17b	0.01	0.01	0.08	0.31	0.02	0.12	0.02	0.57

18	0.03	0.04	0.21	0.75	0.05	0.31	0.05	1.44
19	0.01	0.02	0.12	0.43	0.03	0.17	0.03	0.81

**Table 8**Agreement between experimental and calculated spectra.<sup>a</sup>

Sample	r	Intercept_RMA	Slope_RMA	P(F_RMA) intercept = 0 & slope = 1
M1	0.9996	0.1665	0.9886	0.7722
M2	0.9998	0.0857	0.9944	0.8850
M3	0.9997	0.0013	1.0002	0.9995
M4	0.9997	0.1055	0.9930	0.8673
M5	0.9998	0.0292	0.9982	0.9820
M6	1.0000	-0.0320	1.0024	0.8990
M7	0.9998	0.1203	0.9921	0.7435
M8	0.9999	0.0893	0.9943	0.8154
M9	1.0000	-0.0399	1.0032	0.7735
M10	0.9969	-0.1195	0.9088	0.9717
M11	0.9959	0.5802	0.9602	0.7249
M12	0.9997	0.0956	0.9936	0.8730
M13	0.9998	0.0677	0.9956	0.9133
M14	0.9999	0.0731	0.9954	0.7741
M15	0.9998	0.0986	0.9936	0.8066
M16	1.0000	-0.0048	1.0008	0.6823
M17	1.0000	0.0163	0.9992	0.8489
M17b	0.9998	0.1193	0.9922	0.7246
M18	0.9992	0.2264	0.9848	0.7736
M19	0.9998	0.0884	0.9942	0.8486

Avg. 0.9993 0.1308 0.9913 0.8213

<sup>a</sup> Intercept, slope and degree of significance  $P$  calculated with reduced major axis (RMA) method.

**Table 9**

Pearson linear correlations between composition and other variables in the 20 samples.<sup>a</sup>

	Carbohydr	Protein	Lignin	Char	% Org C
Protein	0.587				
	$P=0.0066$				
Lignin	-0.482	-0.822			
	$P=0.032$	$P<0.0001$			
Lipid			0.451		
			$P=0.046$		
Carbonyl	-0.459	-0.666	0.501		
	$P=0.042$	$P<0.0001$	$P=0.024$		
Char	-0.690				
	$P=0.0008$				
% Org C					
%N					0.953
					$P<0.0001$
% Inorg C	0.519			-0.653	
	$P=0.019$			$P=0.0076$	

<sup>a</sup> Only significant correlations ( $P<0.05$ ) are given. C/N, clay and P are not shown, as they do not have any significant correlation with any variable.

**Table 10**

Differences between sample groups (Fisher's least significant differences test or Kruskal-Wallis test).<sup>a</sup>

Group	Organic C	Inorganic C	N	P	C/N	Carbohydrate	Protein	Lignin	Lipid	Carbonyl	Char
I	X	X	X	X	X	X	X	X	X	X	X
II	X	X	X	X	X	X	X	X	X	X	X
III	X	XX	X	X	X	X	X	XX	X	XX	X
IV	X	X	X	X	X	X	X	X	X	X	X

<sup>a</sup> Crosses under each variable mean significant ( $P < 0.05$ ) difference between groups.

Crosses form a group of means without statistically significant differences.

## FIGURE CAPTIONS

**Fig. 1.** Location of sampling points in Las Tablas de Daimiel Natural Park. Dashed lines indicate the area of influence area of the Gigüela and Guadiana rivers, respectively.

**Fig. 2.**  $^{13}\text{C}$  NMR spectra of samples.

**Fig. 3.** Comparison of Daimiel and global data of organic functionalities in soil OM. Bars represent one standard deviation.

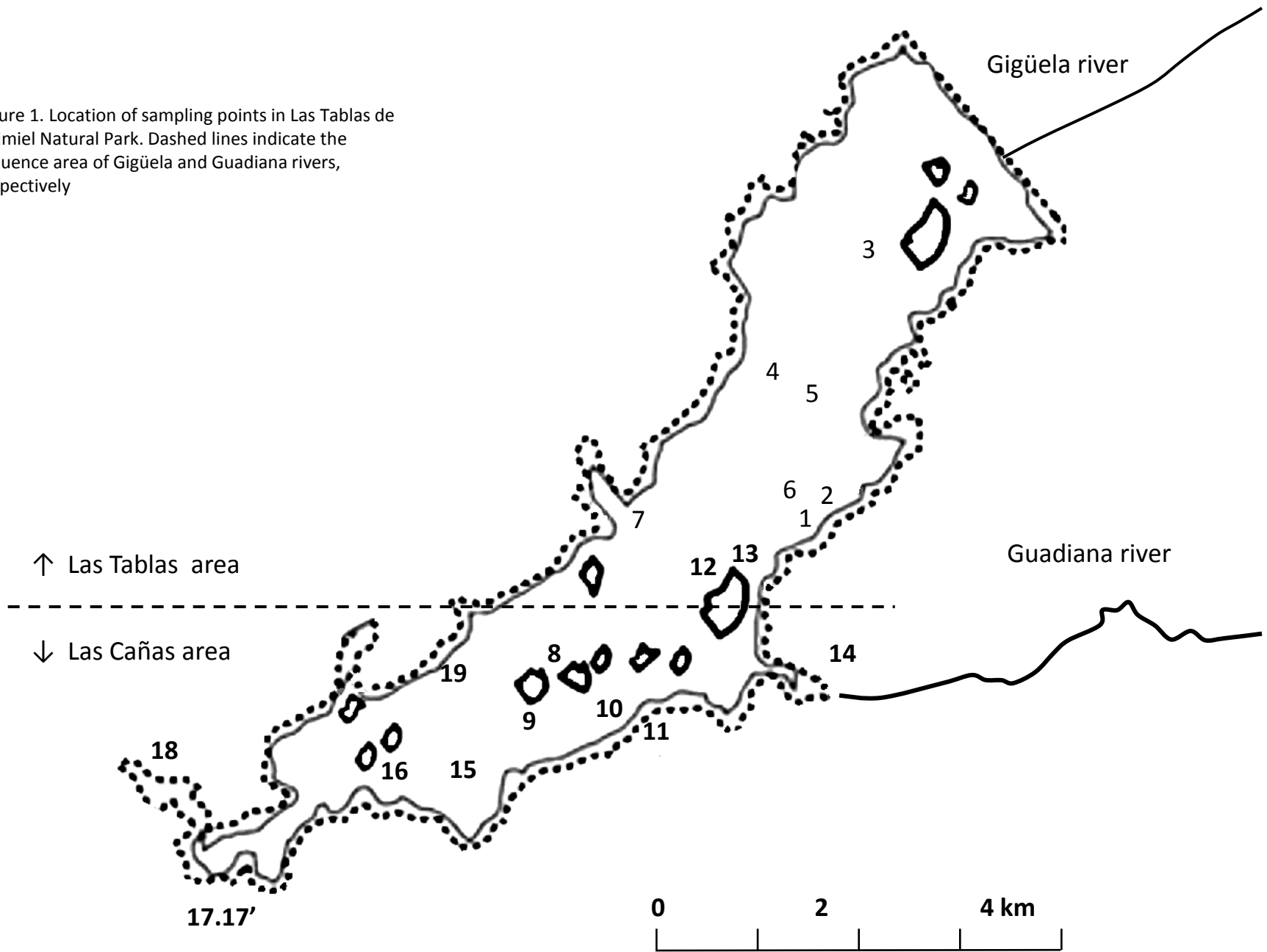
**Fig. 4** Sample composition, as calculated with MMM. Samples are ordered by aromaticity, from bottom (lower aromaticity) to top (higher aromaticity).

**Fig. 5.** PCA analysis of samples. Functional group contents are the 9 variables, and the 20 samples are the cases.

**Fig. 6.** Discriminant analysis of samples.

**Fig. 7.** Average content of OM components in the four groups of samples.

Figure 1. Location of sampling points in Las Tablas de Daimiel Natural Park. Dashed lines indicate the influence area of Gigüela and Guadiana rivers, respectively



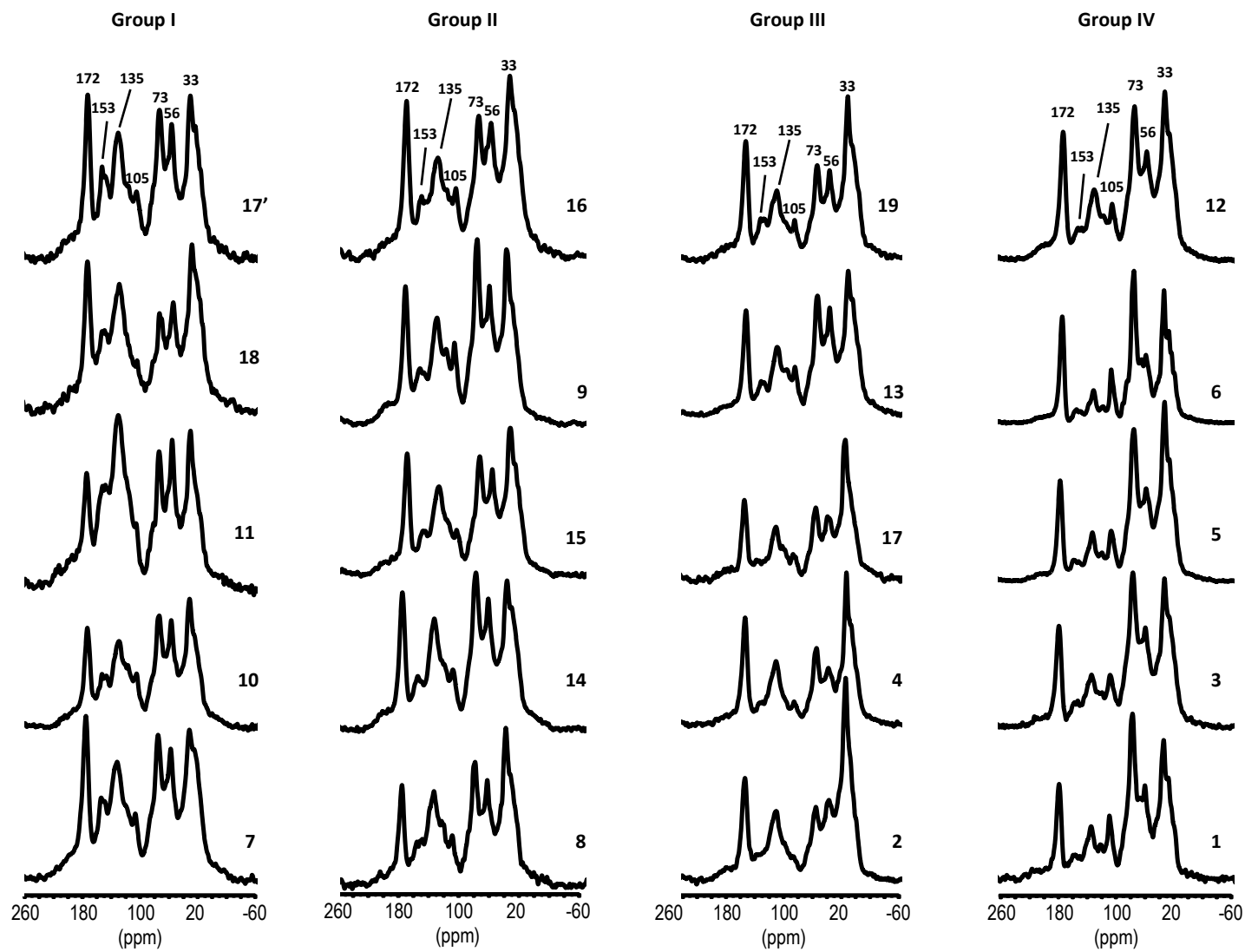


Figure 2.  $^{13}\text{C}$ -NMR spectra of samples

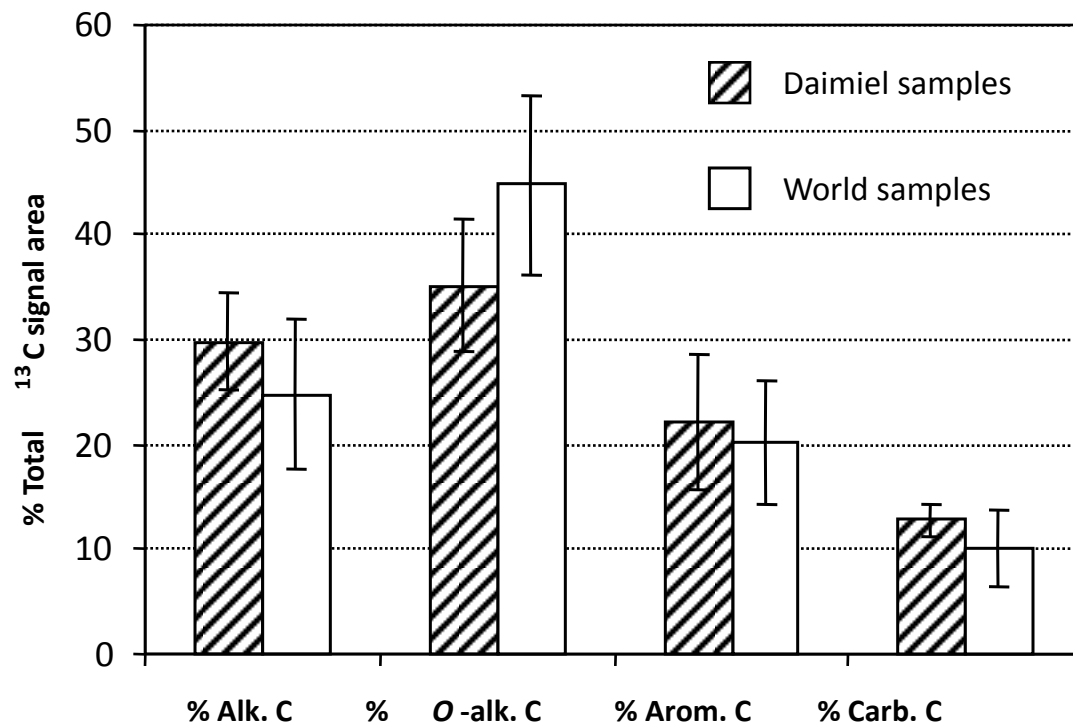


Figure 3. Comparison of Daimiel and global data of organic functionalities in soil organic matter. Bars represent one standard deviation



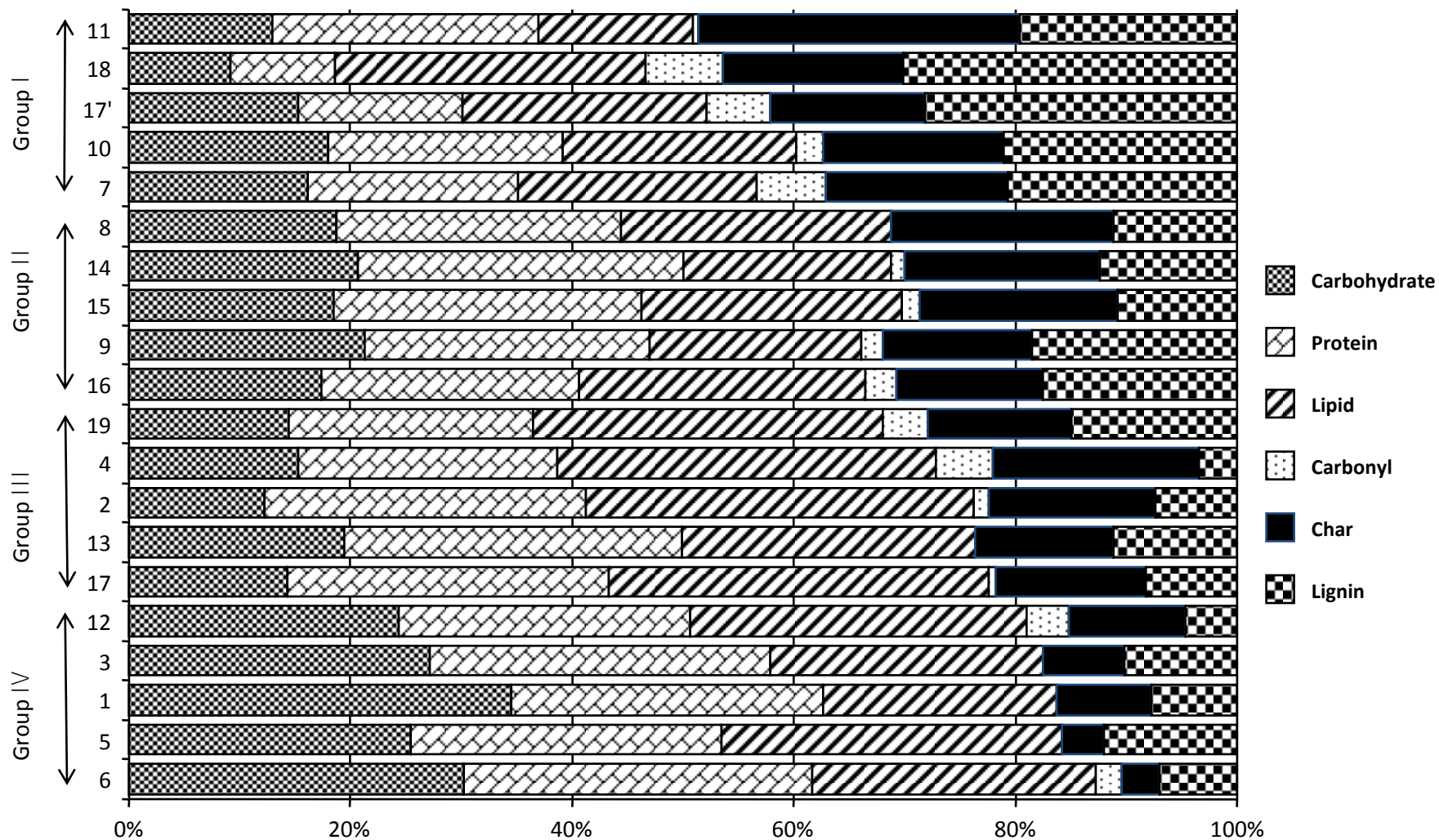


Figure 4 Sample compositions, as calculated with MMM. Samples are ordered by their aromaticity, from bottom (lower aromaticity) to top (higher aromaticity)

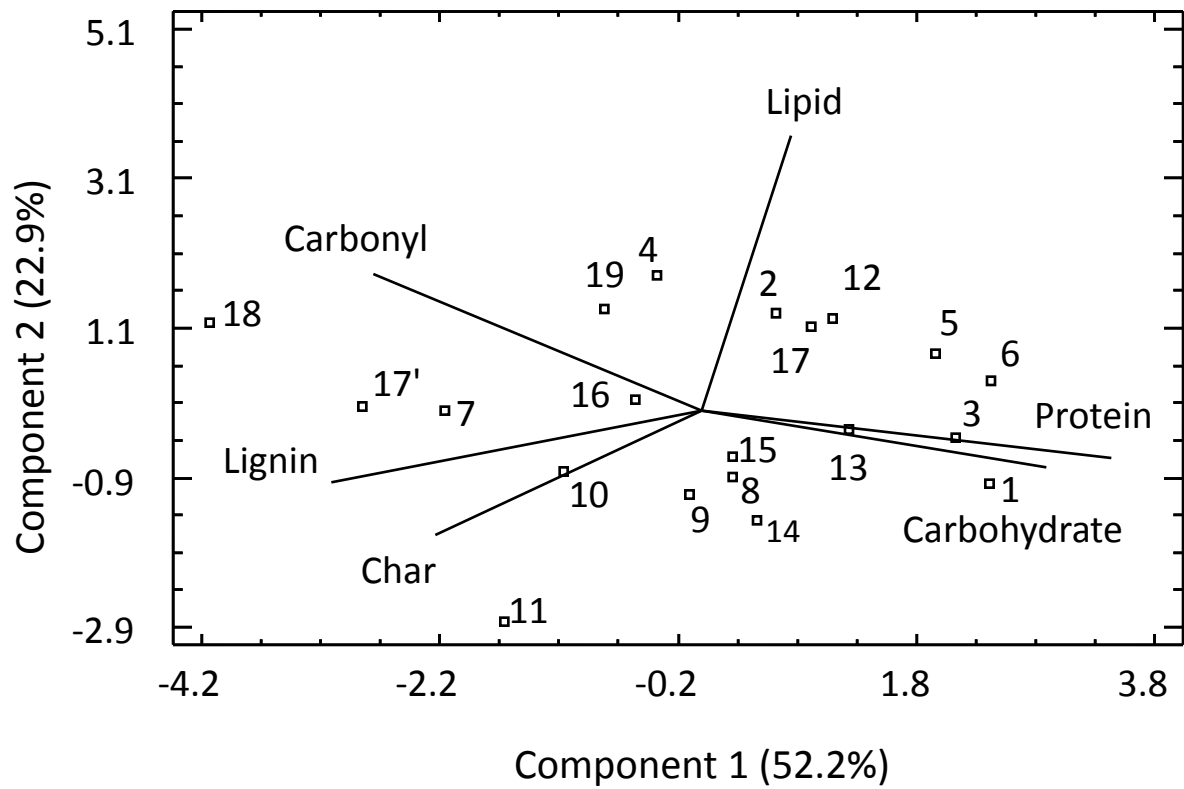


Figure 5. Principal component analysis of samples. Functional group contents are the 9 variables, and the 20 samples are the cases

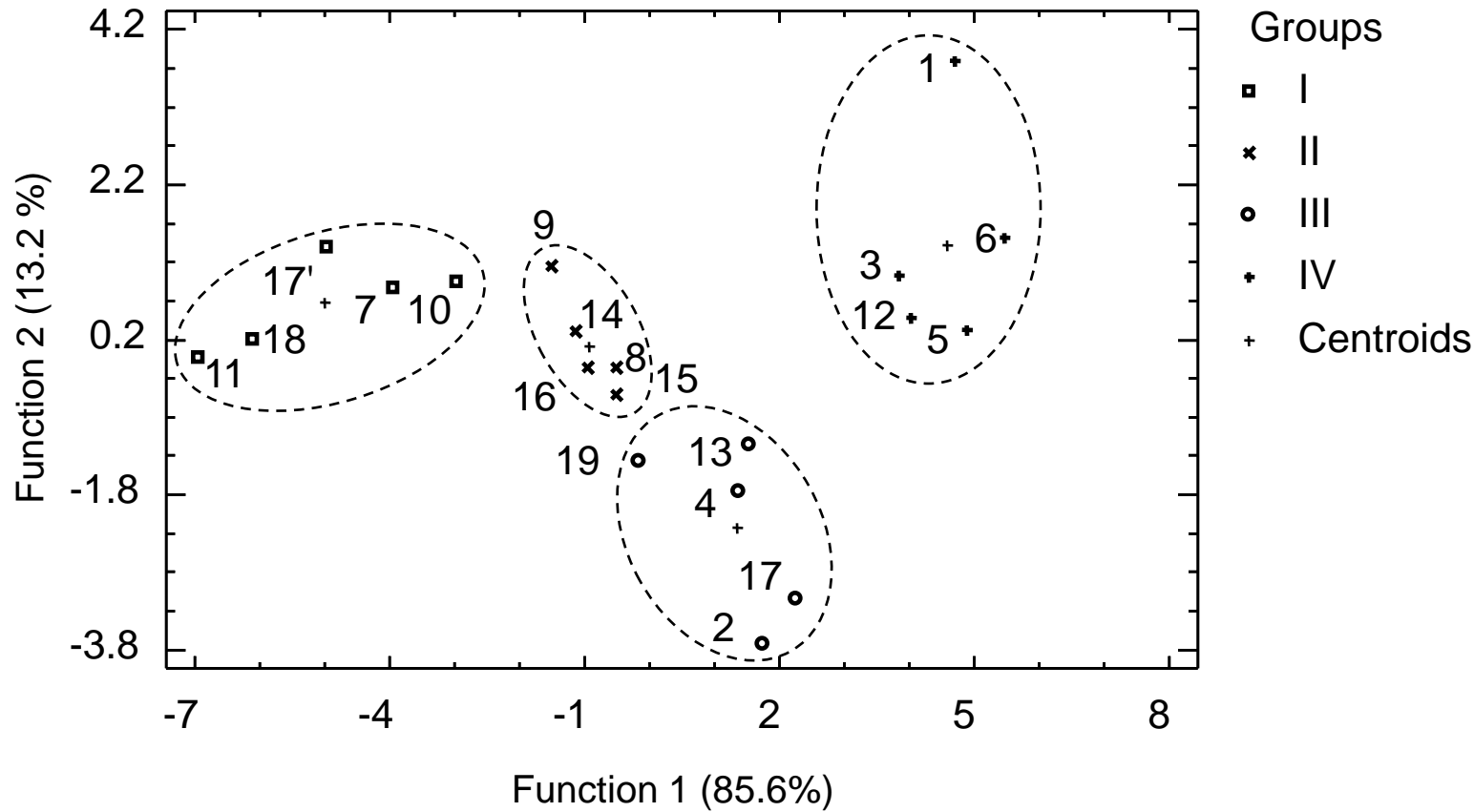


Figure 6. Discriminant analysis of samples

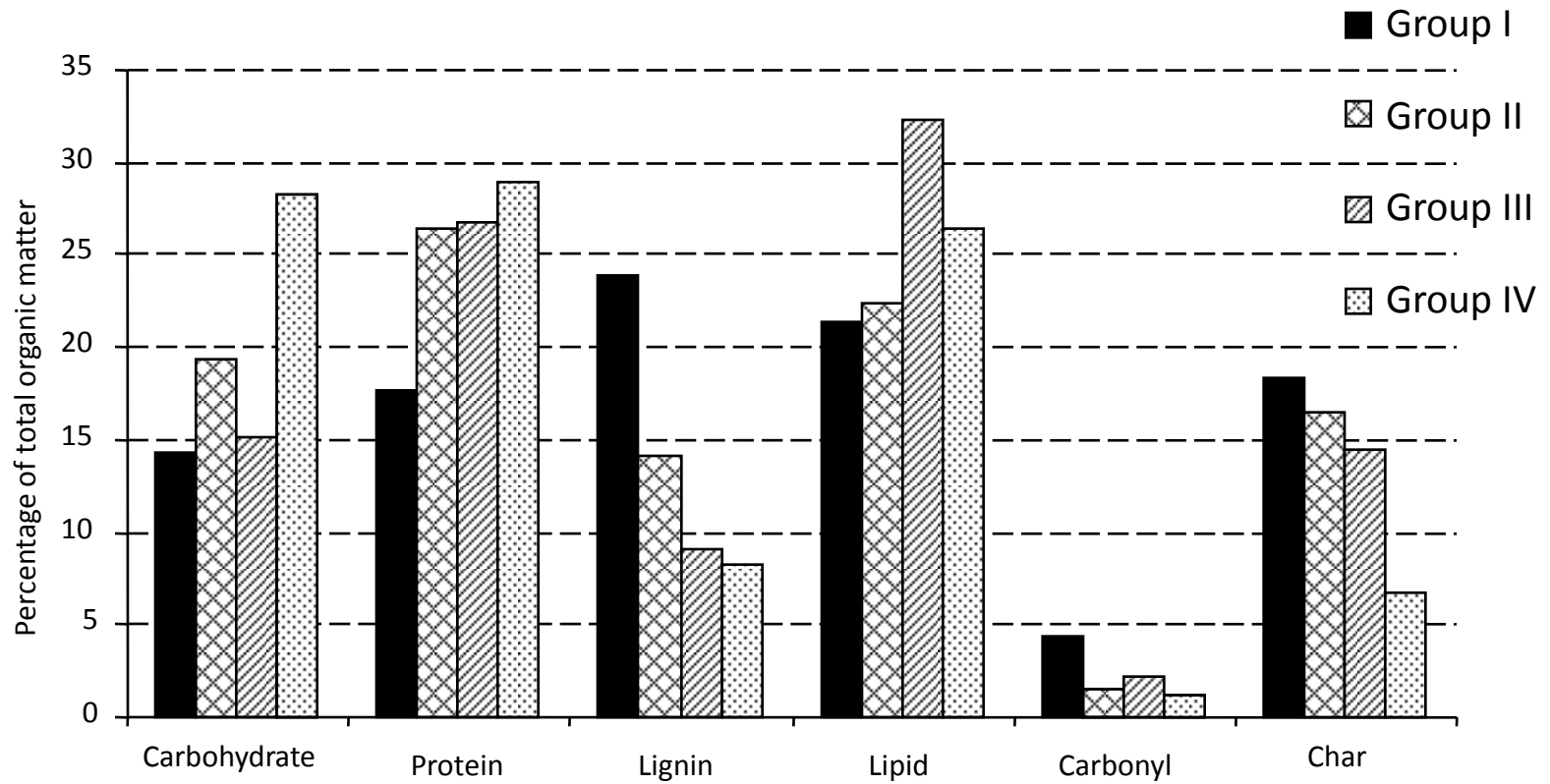


Figure 7. Average content of organic matter components in the four groups of samples

## Research highlights

- Characterization of wetland sedimentary organic matter using CPMAS  $^{13}\text{C}$  NMR spectroscopy.
- Origin and mechanisms of accumulation of organic matter in semiarid wetlands.
- Use of molecular mixing model to elucidate organic matter composition.