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# Advances in the determination of humification degree in peat since Achard (1786): applications in geochemical and paleoenvironmental studies

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**Abstract**

The humification process is one of the least understood and most intriguing aspects of humus chemistry and vital to the global carbon (C) cycle. Peatlands represent the largest terrestrial reservoirs of organic C and support a unique biodiversity, but are also natural archives of climate and environmental changes. In fact, cores from ombrotrophic peatlands are commonly used to reconstruct environmental impacts by human activities during the past decades, centuries and millennia. Understanding the extent to which bogs may serve as reliable archives is of paramount importance in order to ensure that chronological information about natural and human-induced environmental changes are effectively preserved in peat deposits rather than irretrievably affected by humification. Structural changes of the organic matter which constitutes peat are often evaluated by various indirect measures of the degree of humification. Numerous methods and proxies have been proposed and used during the last century, often providing contrasting or inconsistent results. Here we tested multiple physical, chemical, spectroscopic and thermal approaches using peat samples collected from nine bogs on four continents. Despite the different climatic conditions, botanical composition, depth and age of peat formation, we show that the H/C ratio is the simplest, most widely valid and cost-effective humification proxy and should find universal application to multi-proxy geochemical and paleoenvironmental studies. Moreover, we show that three-quarters of the organic C in the studied bogs is in a comparatively undecomposed state, and thus vulnerable to climate changes.

Keywords: C cycle; decomposition; organic matter decay; bogs; H/C

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## 1. Introduction

The humification process, whereby remains of plants and animals are transformed through biochemical and abiotic pathways into complex and heterogeneous mixtures of polydispersed materials known as humic substances, was defined by Stevenson (1982) as "*one of the least understood aspects of the humus chemistry and one of the most intriguing*". More than 230 years after the first extraction of organic matter (OM) carried out by Achard (1786) for the study of a peat soil, the nature, structure and fate of OM is today more controversial than ever, and often under debate (Tan, 2003; Schmidt et al., 2011; Schnitzer and Monreal, 2011; Lehmann and Kleber, 2015).

Understanding the evolution of OM residues into humic substances is of key importance in elucidating carbon (C) biogeochemistry and all ecosystem services provided by organic soils and wetlands, including C sequestration and climate change mitigation. These are particularly important in peatlands, as they represent the largest terrestrial global reservoirs of organic C, approaching one-third of the global soil C stock (Gorham, 1991; Jenkinson et al., 1991). Here, the accumulation of OM as peat is a function of the imbalance between net productivity and decay rate (Clymo, 1983), and is not uniform in peatlands even in the same climatic region or even within the same peatland. Generally speaking, although vegetation in peatlands may grow at different rates, depending for example on moisture and availability of nutrients, the net accumulation of organic C in these environments (~550 PgC in boreal peatlands alone; Yu et al., 2010) is mainly due to very slow decomposition processes, that are in turn driven mainly by temperature, oxygen availability and water saturation, litter quality and pH (Waksman, 1930; Clymo, 1991; Philben et al., 2015; Bragazza et al., 2016). Actually, while the paradigm of OM recalcitrance controlling long-term decomposition rates in soils has been roundly criticized during the last decades (Schmidt et al., 2011; Lehmann and Kleber, 2015), peatlands are an interesting exception to the principles that are being

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developed to explain long-term preservation of OM in mineral soils. In fact, peat is a complex material that consists of partly fossilized plants differing in botanical composition and degree of decomposition and humification, along with a minor component of amorphous and crystalline inorganic compounds (Fig. 1). Consequently, as in peat the mineral content is very low, the OM preservation in the peatland ecosystem, all environmental conditions being equal (mainly pH and oxygen availability), is conditioned by the inherent recalcitrance of organic inputs rather than to the physical and chemical stabilization by OM-mineral associations.

Ombrotrophic (*i.e.*, rain-fed) bogs are unique archives of past environmental conditions (Damman, 1978; Clymo, 1983). Since pioneering studies by Aiton (1805), and for at least two centuries, peat has been recognized as an excellent record of both natural and human-induced environmental changes. Although hundreds of studies have been carried out during the last decades using cores from bogs in order to reconstruct past climatic and vegetation changes (*e.g.*, van Geel, 1978; Barber et al., 1994; Tareq et al., 2004; Chambers et al., 2012), as well as the historical depositional trend of trace elements, dusts and organic pollutants from natural processes as well as anthropogenic activities (*e.g.*, Shotyk et al., 1998; Martínez-Cortizas et al., 1999; Björck and Clemmensen, 2004; Sapkota et al., 2007; Zaccone et al., 2009a; Shotyk et al., 2016). However, the scientific literature is still rather controversial about their reliability to record some forms of environmental change because of concerns about selective enrichments or losses of chemical constituents during OM decay.

Actually, problems related to the rate of peat formation and the relationship between growth rate and degree of humification have troubled scientists since the beginning of investigations on raised bogs at the start of the 19$^{th}$ century (Aaby and Tauber, 1975). In fact, variations of environmental signals (such as concentrations, enrichment factors and isotopic composition) within a given peat profile are not only the result of changing rates or sources of atmospheric deposition, but may also be consequences of differences in the rate of plant

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growth and peat accumulation, of changes in the botanical composition of peat-forming vegetation, as well as differences in peat decomposition, humification and mineralization over time (Zaccone et al., 2012, 2013). Consequently, understanding whether paleoenvironmental information is effectively preserved in peat deposits rather than irretrievably lost during humification becomes a fundamental aspect to be considered before using bogs as environmental archives. However, despite the universal importance of the problem, it has received remarkably little attention.

One of the main drawbacks in discussing and comparing changes in peat decomposition and humification addressed in many studies is that a small number of proxies or indices are compared, and often neglect the possibility that humification may evolve in different directions mainly depending on differences in peat-forming vegetation, climatic conditions, peat depth, trophic status, pH, and age. In short, complex relationships among all these factors result in multiple pathways in peat chemistry. In addition to the limited number of humification methods and/or proxies generally compared, studies are generally carried out among cores from the same geographical area or having similar features.

Here, we test and compare an array of physical, chemical, spectroscopic and thermal approaches to describe peat humification using samples collected from nine bogs on four continents. The sites selected for this study are characterized by extremely different climatic conditions, botanical composition, trophic status, depth and age of formation. Our main objective is to identify the simplest, most representative, robust and cost-effective humification proxy to be applied as a part of geochemical and paleoenvironmental studies. Furthermore, due to the distribution of our study sites, we provide some new insights into the humification process at global scale.

## 2. Determining the degree of humification in peat: an historical background

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The evolution of OM occurring during peat formation is difficult to quantify and has been generally assessed by indirect measurements of the degree of humification, *i.e.*, approximated using a measure of one or more of the physical or chemical characteristics that change with advanced decomposition. "*One of the main reasons for this situation probably lies in the fact that we have no very clear conception of the exact meaning of the degree of humification: we do not know what we try to determine*" (Kaila, 1956). For that reason, to date there is no universally accepted definition of humification (Table 1).

The first isolation and fractionation of humic materials was carried out by Achard (1786), who treated peat with potassium hydroxide, added acid to the dark solution and obtained an amorphous dark precipitate. He noted that more precipitate was obtained from the darker (more humified) layers rather than from the less decayed plant debris from the upper layers. However, a full understanding of the transformations of OM in soils was hindered because developments in microbiology lagged behind those of chemistry; the advances in microbiology focussed interest on the roles that microorganisms have in the transformations of organic debris in soils (Hayes and Swift, 2018). Several studies carried out in the second half of 19$^{th}$ century (*e.g.*, von Post, 1862; Ramann, 1888) clearly indicated that the genesis of humus was a biological process.

Several methods later became very popular, but some of them were highly dependent on the skill and experience of the investigator. The von Post scale (H1-H10), despite its simplicity, has proven to be effective for describing peat humification in the field, based on the colour and turbidity of squeezed porewaters and the structure of remaining solid material (von Post, 1922, 1924). In the lab, two methods became popular: the pyrophosphate test, based on the percentage of peat soluble in pyrophosphate (Kaila, 1956), and the morphologically-defined decomposition scale (fibric, mesic and sapric), based on the amount and size of fiber material (Farnham and Finney, 1965).

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Most of the studies about peat humification were carried out during the first half of the 20$^{th}$ century, especially in order to classify organic soils for agricultural management, but also to help characterize peat for fuel. Melin and Odén (1916) probably developed one of the first quantitative colorimetric approaches for the determination of the degree of peat humification, resulting in a relative value obtained by comparing the colour intensity of the alkali (NaOH) extract at boiling temperature of a sample with that of a standard preparation (*Acidus huminicum*, by Merck). This method was criticized by Springer (1940), who proposed the ratio, in %, between acetyl bromide insoluble C and total organic C or, in samples poor in ash, the ratio of insoluble organic material to the loss of ignition (LOI). Some of the oversimplifications of these methods were later overlooked by Kaila (1956) who used diluted sodium pyrophosphate (0.025 M) to extract, from 220 peat samples, in a semi-quantitative way, the dark-coloured products of decomposition; the colour intensity of filtered and diluted extracts was then measured using a colorimeter (with a monochromatic light filter having a transmission peak at 550 nm), resulting in the well-known pyrophosphate index.

In the 1920s-30s, Keppeler (1920, 1932) proposed as a rapid way for measuring the degree of decomposition the total amount of reducing sugars in the acid hydrolysate of the peat sample compared to that found for an undecomposed *Sphagnum* moss. Various methods were also mentioned by Waksman (1930, 1932, 1938), all of them based either on the amount of residual carbohydrate or accumulation of lignins and other C-rich compounds (*e.g.*, cellulose, hemicellulose, uronic acids) or on the change in colour. Bouyoucos (1934) developed a novel method for determining the degree of decomposition of plant debris (including *Sphagnum* and sedge moss and peat samples) by comparing samples as found with their fresh state. In detail, a volatilization index, obtained by dividing the percentage of loss at 310 °C (due to the easily decomposable substances) by the percentage of LOI at 550 °C

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(recalcitrant fraction), was calculated for both fresh and corresponding decomposed plant material.

In Finland, Salmi (1949) observed that the calorific value of peat was affected by peat type and ash content, and that it increased with humification, as determined according to the von Post method. According to Segeberg (1952), the increase in the volume weight of the ash-free dry matter (expressed as a percentage of the corresponding weight of the volume for the sample to be analysed) and the maximum water holding capacity provided estimations of the degree of humification of peat samples.

In the 1960s, Schnitzer and co-authors suggested that the concentration of oxygen-containing functional groups (Schnitzer and Desjardins, 1965, 1966), the peak height in differential thermogravimetric curves around 300 °C (Schnitzer and Hoffman, 1966; Stewart et al., 1966) and the proportion of C concentrations in humic (HA) *vs.* fulvic (FA) acids (Schnitzer, 1967) could all be used as diagnostic tools for estimating the humification degree in peat samples, as determined by solubility in diluted pyrophosphate solution. A few years later, several authors observed a strong correlation between bulk density of peat and the degree of decomposition, both expressed as the amount and size of peat fiber (Boelter, 1965, 1969), as well as the von Post degree of humification (Päivänen, 1969; Puustjärvi, 1970).

Using the method proposed by Bahnson (1968) (modified from Overbeck, 1947), which consisted of a colorimetric determination carried out using UV absorbance on an alkaline extract of peat, Aaby and Tauber (1975) measured differences in the degree of humification based on the colour intensity of extracts. Following this study, several other approaches, involving diluted alkaline extraction followed by UV-Vis absorption, were developed, and several proxies proposed (*i.e.*, molar extinction coefficients and specific UV absorbance values at several wavelengths) (*e.g.*, Chen et al., 1977; Chin et al., 1994; Weishaar et al.,

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2003; Chambers et al., 2011). A similar approach has been also used to characterize the dissolved OM in the porewater, *i.e.*, in the liquid phase of peat.

The free radical (commonly or predominantly substituted semiquinones) content of alkaline extracts of peat was suggested by Schnitzer and Lévesque (1979) as a humification proxy; in fact, signal height in Electron Spin Resonance (ESR) spectra was found to be positively correlated with the degree of humification, as measured by solubility of the peat in dilute sodium pyrophosphate solution. In the same year, comparing various means of measuring the degree of decomposition of virgin peat materials, Lévesque and Mathur (1979) included the rate of biodegradation, expressed as the concentration of C lost through aerobic soil respiration over time, as a novel index.

One of the common proxies used to describe the humification pattern in contemporary studies of peat profiles is the C/N ratio (*e.g.*, Malmer and Holm, 1984; Kuhry and Vitt, 1996; Zaccone et al., 2007a; Biester et al., 2014; Schellekens et al., 2015): this approach is based on the observation that, during OM decay, an enrichment of N relative to C generally occurs, thus resulting in a decrease in the C/N ratio (Waksman, 1938; Stevenson, 1982). Other atomic ratios (*i.e.*, H/C and O/C) have also been adopted (*e.g.*, Outridge and Sanei, 2010; Zaccone et al., 2011a; Biester et al., 2014; Magnan et al., 2018), and data frequently reported in the form of a van Krevelen (1950) diagram.

Several other spectroscopic approaches, as well as improvements and revisions of the previous ones, have been proposed and used during the last five decades to find potential descriptors of the humification process in peat, including Fourier Transform Infrared (FT-IR), molecular fluorescence and Nuclear Magnetic Resonance (NMR) spectroscopy, Gas Chromatography (GC) and GC-Mass Spectrometry (GC-MS), and Isotope Ratio Mass Spectrometry (IRMS) (*e.g.*, Preston et al., 1987; Lehtonen and Ketola, 1993; Cocozza et al., 2003; Zaccone et al., 2008, 2011b; Biester et al., 2014; Philben et al., 2015; Schellekens et al.,

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2015), just to mention some of them. A brief (and not exhaustive) summary of proxies used to investigate peat humification is reported in Table 2.

## 3. Peat cores selection and determination of humification proxies

Nine bogs from four continents (America, *n* = 3; Asia, *n* = 1; Europe, *n* = 4; Oceania, *n* = 1) were included in this study (Fig. 2). These bogs were selected because of their great diversity in respect to climatic conditions (from polar to tropical), botanical composition (predominantly either mosses, grasses, shrubs or trees), extent of peat accumulation (from less than 1 m to >14 m) and age of formation (from a few decades to thousands of years). From each core, four to five representative peat samples were randomly collected at various depths (total *n* = 40; Table 3), and each of them was tested using a broad array of physical, chemical, spectroscopic and thermal proxies of humification (Table 4).

### *3.1. Study sites*

1) Bracebridge Inlet (*BI*) is a typical Arctic peatland in the Polar Bear Pass National Wildlife Area (Bathurst Island, Nanavut, Canada). The coring site was located 5 km from the ocean, with an elevation of 130 m a.s.l. The surface layer of the peat deposit was fractured into large blocks along gullies, probably resulting from the melting of this layer. Here, a 90-cm peat core (code: BI2b) was collected in July 2000: the first 14 cm as blocks of non-frozen peat, whereas the other 76 cm as cores of frozen peat (Givelet et al., 2004). Samples were relatively uniform and well decomposed, and consisted of 70-80% small debris and 10-30% sedges roots (only two moss stems, probably *Tomenthypnum nitens*, were found); the whole core represented *ca.* 6,000 years of peat accumulation (Givelet et al., 2004). More information about peat stratigraphy on Bathurst Island are reported elsewhere (Blake et al., 1974). The samples included in this study are BI2b #32, #36, #47 and #52 (Table 3).

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2) Sifton (*SIF*) is an acidic *Sphagnum* bog located in Ontario, Canada. The bog (*ca.* 28 ha of surface) is formed in a large depression, left behind as a glacial block melted about 13,000 years ago (Givelet et al., 2003). The peatland today consists of a small pond surrounded by a floating mat of *Sphagnum* moss, flanked by a marginal hardwood swamp (Givelet et al., 2003). The central portion of the bog contains as much as 10 m of peat accumulation (Warner and Charman, 1994). The thickness of peat accumulation at the sampling site was about 6 m; here, a short peat core (code: SIF) was collected in July 2000 (Givelet et al., 2003). The samples included in this study, namely SIF #02, #16, #32, #47 and #62, were selected at random from a 68 cm deep core (Table 3).

3) Harberton (*HB*) is a *S. magellanicum* bog east of Estancia Harberton along the eastern Beagle Channel, Tierra del Fuego, Argentina (Markgraf and Huber, 2010). Here, a 1024-cm-long peat core (code: HB) was collected in 1987; it was composed of *Drepanoaladus* peat from the gravel base to 800 cm, overlain by sedge peat with few *Drepanocladus* moss fragments and abundant fungal remains, transitioning at 730 cm to *S. magellanicum* peat (Markgraf and Huber, 2010). More detailed information on this peatland and on the chemical features of peat with depth can be found in Schellekens and Buurman (2011) and Schellekens et al. (2015). The samples included in this study are HB #5-6, #225-250, #475-500 and #625-650 (Table 3).

4) Fleck's Loch (*FL*) is a blanket bog located on the Island of Foula (3 km wide and 5 km long), Shetland Islands, Scotland. The bog is *ca.* 500 m long and up to 275 m wide; the perimeter of the bog is extensively eroded, with some bare peat banks, deep gulleys, and small streams which frequently arise out from and disappear into the thick blanket peat (Shotyk, 1997). A peat core (code: 6b) was collected in September 1991 from the domed area, where the surface was 2 to 3 m higher than the edges of the bog (Shotyk, 1997). The stratigraphy of the top 100 cm of the profile was dominated by *Sphagnum-Eriophorum* peat

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that, basing on the von Post scale, was poorly decomposed (H3) within the first 38 cm, and well decomposed (H7) below (Shotyk, 1997). The samples included in this study are 6b #03, #27, #61 and #85 (Table 3).

5) Loch Laxford (*LL*) is a small blanket bog located in Scotland, approximately 15 km from the open ocean. Here, a peat core (code: 7c) was collected in October 1991 (Shotyk, 1997). The stratigraphy of the top 100 cm of the profile was dominated by *Sphagnum-Eriophorum* peat, varying in degree of decomposition on the von Post scale (0-25 cm, H3; 25-39 cm, H5; 39-65 cm, H4; 65-100 cm, H6) (Shotyk, 1997). The samples included in this study are 7c #01, #26, #54 and #82 (Table 3).

6) Etang de la Gruère (*EGR*) is a bog located in the Jura Mountains of Switzerland, and represents the longest continuous record of ombrotrophic peat in the northern hemisphere. Here, 6.5 m of peat has accumulated during the past *ca.* 15,000 years on a small island of siliceous sediment (Shotyk et al., 1998). In the 17$^{th}$ century, an artificial dam was built to operate a mill, and drainage ditches were dug into some areas of the bog to improve soil fertility and provide a new grazing area; no drainage or peat cutting was carried out on the peninsula where peat cores used in several previous studies were taken (Roos-Barraclough et al., 2004). In fact, EGR is one of the most well studied bogs, with several cores investigated in the last 25 years; detailed information about OM features and changes in peat humification with depth are reported elsewhere (Cocozza et al., 2003; Roos-Barraclough et al., 2004; Zaccone et al., 2007a, 2008, 2011b). According to Steinmann and Shotyk (1997), the stratigraphy of the top 130 cm at EGR bog, with the degree of decomposition on the 10-point scale of von Post given in brackets, is: *Sphagnum*-dominated peat with *Eriophorum* down to 25 cm (H2); *Sphagnum*-dominated peat with few *Carex* and *Eriophorum* remains from 25 to 50 cm (H4); *Sphagnum-Eriophorum* peat from 50 to 60 cm (H4); *Sphagnum-Eriophorum* peat from 60 to 130 cm (H5). A peat core (code: 2T) was collected in June 2005 from the central,

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domed area, where the surface peat layers are clearly elevated up to 4 m beyond the edge of the bog (Zaccone et al., 2007b). The samples included in this study, namely 2T #04, #34, #69 and #90 (Table 3), were selected at random from the first 100 cm, spanning >2,000 yr of peat accumulation (Zaccone et al., 2007b).

7) Posta Fibreno (*PF*) is a small, relic free-floating island located in central Italy, having an ombrotrophic core of *S. palustre* (Casella et al., 2010). This site can be classified as a transitional mire with a secondary ombrotrophic local dominance induced by buoyancy, where the *Sphagnum* development probably witnesses the moment of hydrogeological isolation of the inner-core of the free-floating island, *i.e.*, when precipitations become the only source of water and nutrients (Zaccone et al., 2017). With 4 m of peat accumulated in less than 700 yr, PF shows the thickest accumulation of peat reported for this type of ecosystem in the scientific literature, and an average growth rate 10-fold higher than many other peatland ecosystems (Zaccone et al., 2017). Moreover, this sub-Mediterranean site shows some microbial taxa characterized by an unusual concurrent species convergence with far Nordic or circumpolar environments (Concheri et al., 2017). Here, a peat core (code: PF2) was collected in July 2012 from the centre of the island (Zaccone et al., 2017). Five samples from PF, namely PF2 #01, #27, #62, #97 and B1#09, were included in this study. These samples, consisting of well preserved (almost undecomposed) *Sphagnum* material, were selected at random from the top 120 cm of depth that represent less than 60 years of peat accumulation (Zaccone et al., 2017). $^{210}$Pb age dates of core PF peat samples are summarized in Table 3.

8) The peat deposit of Palangka Raya is located in the Central Kalimantan (*KAL*), Borneo, Indonesia. The peatland is strongly domed, with >12 m of peat accumulation in the centre; here, the dominant forest type is a low pole forest (Shepherd et al., 1997; Page et al., 1999). A peat core (code: SA6.5) was collected between August-September 1995. The samples included in this study, namely SA6.5 #01, #17, #28, #40 and #58, were selected at

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random from the 10 m deep profile. According to Weiss et al. (2002), the first 80 cm consisted of poorly to moderately decomposed organic material, mainly peaty humus with some wood; the sections from 80 to 840 cm consisted of well-decomposed to very well-decomposed peat with wood and herbaceous material, whereas the sediment-peat interface occurred at 840-860 cm (SA6.5 #52; 22,120±320 $^{14}$C yr BP). Radiocarbon age dates of core SA6.5 peat samples are summarized in Table 3.

9) Kopouatai (*KOP*) is an essentially unmodified restiad bog of *ca.* 10,000 ha, situated in the Hauraki Lowlands on North Island, New Zealand. The raised nature of the bog is evident: 1500 m from its edge the bog surface is 4 m above surrounding ground level (Shearer, 1997). Peat in the deposit reaches maximum thicknesses of about 8 m in the north and 12 m in the south, resulting in an age at base of peat ranging from 6,000-13,000 yr BP (Newnham et al., 1995; Shearer, 1997). According to Shearer (1997), peat character displays a vertical trend, with peat being less degraded up the core, *i.e.* the younger the peat the less degraded it was. A peat core (code: KOP 1500) was collected in 1995. The samples included in this study are KOP #03, #19, #37, #84 and #115 (Table 3). The area where this core was collected developed more than 6,000 BP after marine transgression covered the majority of Kopouatai bog (Newham et al., 1995).

*3.2. Determination of humification proxies*

Bulk density was determined by dividing the dry weight (obtained at 105 °C overnight) by the volume of each peat slice. The ash content, expressed as a percentage of the original dry weight, was determined for each peat sample by combustion in a muffle furnace at 550 °C for 12 h.

Dried peat samples were ground using a Retsch agate ball mill (PM 400, Haan, Germany) with 250 mL jars containing three 30 mm balls at 300 rpm for four x 2.5 minutes.

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Total C, H, N and S concentrations in peat samples were determined in triplicate, using dry combustion with an elemental analyzer (Fisons EA1108, Milan, Italy). The instrument was calibrated using both BBOT [2,5-Bis-(5-tert-butylbenzoxazol-2-yl)-thiophen] and Phenanthrene Enriched standard (Fison/Carlo Erba Italia s.p.a.). Total organic C was determined by difference between total C and inorganic C (TOC = TC–IC), the latter determined, using the same elemental analyzer, on peat samples pre-dried at 420 °C for 12 h; in this case, urea was used as standard. Oxygen content was calculated by difference: O% = 100 - (C + H + N + S)%. All samples were analyzed in triplicate, and obtained data corrected for ash and moisture content. Ratios between elements (*i.e.*, C/N, H/C and O/C) were determined as atomic ratios, considering as C concentration those of the TOC.

For the determination of total extractable C (TEC), humic acid C ($C_{HA}$), and fulvic acid C ($C_{FA}$), 40 mL of a solution 0.1 M $Na_4P_2O_7$ and 0.1 M NaOH were added to 150 mg of ground peat sample and the mixture shaken for 24 h at room temperature. The supernatant solution (TEC fraction) was then separated by centrifugation and filtration through Whatman GF/C filter, and analysed for organic C content using a Shimadzu TOC-VCSH. Twenty-five mL of the supernatant were acidified to pH 1.5 with 95–98% $H_2SO_4$ to precipitate the HA fraction. The acidic supernatant (FA + non-humic fraction) was separated from the HA fraction by centrifugation and analysed for the organic C content with a Shimadzu TOC-VCSH. Humic acid C ($C_{HA}$) content was calculated by subtracting $C_{FA}$ from TEC content.

Solid-state Cross-Polarization Magic Angle Spinning $^{13}C$ Nuclear Magnetic Resonance ($^{13}C$ CPMAS NMR) spectra were obtained using a Bruker AVANCE II 400 MHz spectrometer operating at a MAS frequency of 5,000 Hz, a recycle delay of 1 s, and a contact time of 1 ms. The free induction decay signal was digitized and multiplied by an exponential function corresponding to 80 Hz line broadening in the final transformed spectrum. The spectra were then baseline corrected, calibrated relative to adamantine, and integrated into the

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following chemical shift regions: 0–45 ppm, unsubstituted alkyl C; 45–65 ppm, N-substituted alkyl/methoxyl C; 65-95 ppm, O-substituted alkyl C; 95–110 ppm, anomeric C; 110–160 ppm, aromatic/phenolic C; 160–220 ppm, carboxyl, amide and carbonyl C (Simpson and Simpson, 2009).

Isotopic ratios $^{13}C/^{12}C$ and $^{15}N/^{14}N$ were determined on dried peat samples by Continuous Flow-Isotope Ratio Mass Spectrometry (CF-IRMS, Delta Plus, ThermoFisher) coupled with an elemental analyser (CHNS-O mod. EA 1110 ThermoFisher). The Elemental Analyser was interfaced with the IRMS through the Conflo III (Thermo) dosing the samples and the reference gases ($N_2$ and $CO_2$). The pyrolyser was equipped with a PorapackQS (GC column). Isotopic values were expressed in δ(‰) relative to V-PDB (Vienna-Pee Dee Belemnite) for carbon ($\delta^{13}C$), and AIR (Atmospheric nitrogen) for nitrogen ($\delta^{15}N$), according to the formula:

$$\delta\ (‰) = [(R_{sample}/R_{standard})\text{-}1] \times 1000$$

where $R_{sample}$ and $R_{standard}$ are the $^{13}C/^{12}C$ or $^{15}N/^{14}N$ ratios of sample and standard, respectively. All analyses were carried out in triplicate.

Ultraviolet–visible (UV-Vis) and fluorescence spectroscopic analyses were conducted on solutions of 10 mg $L^{-1}$ of dried and milled peat at a pH of 7 and an ionic strength of 0.1 M. Stocks solutions were prepared by dissolving 10 mg of dried peat in 70 mL of deionized distilled water and 10 mL of 1 M KOH. The mixture was stirred for 30 min under $N_2$ gas, and then 10 mL of 1 M HCl was added. Subsequently, the pH was adjusted to a value of 7 by addition of 0.1 M KOH and the volume was finally brought to 100 mL with deionized distilled water. Stocks solutions were 1:10 diluted with 1 M KCl at a pH of 7 for analysis. UV-Vis analysis was conducted using a Shimadzu UV-1800 spectrophotometer. The absorbance and the corresponding specific absorptivity values were measured at several wavelengths. The molar absorptivity (or molar extinction coefficient) was recorded at 280 nm

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($\varepsilon_{280}$) (L mole $OC^{-1}$ $cm^{-1}$), *i.e.*, in the region where the $\pi \rightarrow \pi^*$ electron transitions occur for a number of aromatic substances (Chin et al., 1994), and at 540 nm ($\varepsilon_{540}$) (Chambers et al., 2011). Specific UV absorbance at 254 nm ($SUVA_{254}$) (L g $OC^{-1}$ $cm^{-1}$) was calculated by normalizing the absorbance at this wavelength by the concentration in organic C of the corresponding sample (Weishaar et al., 2003). Finally, the $E_4/E_6$ ratio was calculated as the ratio of absorbance at 465 and 665 nm (Chen et al., 1977). Fluorescence spectra were recorded using a Perkin-Elmer LS55 luminescence spectrometer equipped with FL WinLab software (version 4.00.03). Emission and excitation slit widths were set at 10 nm, and a scan speed of 500 nm $min^{-1}$ was selected for the emission monochromator. The wavelength emission range was from 250 to 600 nm, while the excitation wavelength was increased sequentially from 250 to 600 nm by 5-nm steps. A fluorescence-based humification index (HIX) was calculated from the fluorescence emission spectrum obtained at an excitation wavelength of 254 nm by dividing the area from 435 to 480 nm by the area from 300 to 345 nm (Zsolnay, 2003) after correcting for both primary and secondary inner filter effects (Ohno, 2002).

Simultaneous thermogravimetric (TG) and differential thermal (DTA) analyses were performed using the TG-DTA92B instrument managed by a SETSOFT2000 software (Setaram, France) on all samples to record continuous weight losses during sample heating and the corresponding energy changes as endothermic or exothermic reactions. The beginning and the end of mass loss for each reaction was calculated by DTG (the first derivative of TG curve, which is the rate at which the mass of decomposing samples changes with respect to its changing temperature). DTG curve allows converting the TG trace into well defined peaks. Two high purity standards indium and aluminum (both 99.99%) were used to calibrate TG-DTA for both temperature (°C) and enthalpy (J $g^{-1}$). A heating rate of 10 °C $min^{-1}$ from 30 to

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800 °C under dynamic air flow (8 L $h^{-1}$) was used (Montecchio et al., 2006). All analyses were carried out in triplicate.

Statistical correlations were performed using the Statistica Version 7 software (StatSoft Inc., 2004), considering a significance level of $p<0.05$.

## 4. Variability of humification proxies

The variability of each humification proxy selected for this study is summarized in Table 5.

### *4.1. Physical proxies*

Physical parameters, such as dry bulk density and ash content, are easy to determine and could represent simple and useful humification proxies. Density is generally assumed to increase during humification and this impacts the hydraulic conductivity (Boelter, 1965); in fact, as decomposition proceeds, the volume of the organic residues decreases due to physical degradation and biochemical reactions, resulting in the reduction of noncapillary pore spaces and greater bulk density (Boelter, 1969). Density has been commonly reported to increase with depth, because of the weight of the overlying peat column. Here, density ranges from 0.024 (at PF, living layer) to 0.258 g $cm^{-3}$ (at FL) (Table 5), with greater values of this parameter sometimes found in the central part of the profile rather than at the bottom (Supplementary Fig. S1); in general, no significant linear correlation was found between peat density and depth (Table 6).

During decomposition and humification, a loss of mass occurs through OM mineralization, resulting in a relative increase of the ash content. For this reason, the ash content has been proposed as a possible humification proxy (Leifeld et al., 2011). However, in peat cores it is difficult to distinguish between changing rates of dust deposition over time *vs.* increases in ash content due to OM mineralization (Zaccone et al., 2012, 2013). For example,

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many bogs worldwide show episodic increases in volcanic ash shards and aeolian dusts (*e.g.*, Weiss et al., 2002; Björck and Clemmensen, 2004; Shotyk et al., 2005; Sapkota et al., 2007), thus limiting the possibility to use the bulk mineral matter content as an index of humification. In fact, Steinmann and Shotyk (1997), analyzing both the ash and the acid-insoluble ash (AIA) fraction along a peat core at EGR, demonstrated that most of inorganic solids (*i.e.*, 23-86%, mean 49%) consisted of atmospheric soil dusts derived from the weathering of crustal rocks; similar values (*i.e.*, 28-84%, mean 55%) were found by Zaccone et al. (2013) analyzing another core from the same bog. The observed positive and significant correlation ($p<0.05$) between ash content and depth (Table 6) is due to the transition, generally occurring in deeper layers, between ombrotrophic (*i.e.*, rain-fed) and minerotrophic (*i.e.*, groundwater-fed) conditions during terrestrialization (*i.e.*, basin-filling). This phenomenon is particularly evident in deeper layers at HB, KAL and KOP (Supplementary Fig. S1).

*4.2. Chemical proxies*

Atomic ratios are probably the humification proxy more commonly used in paleoenvironmental and geochemical studies than any other parameter (*e.g.*, Malmer and Holm, 1984; Kuhry and Vitt, 1996; Zaccone et al., 2007a; Outridge and Sanei, 2010; Zaccone et al., 2011a; Biester et al., 2014). Generally speaking, it is assumed that the increase in peat humification is associated with a decrease in C/N, H/C and O/C ratios in response to a relative enrichment of total N relative to organic C, as well as a residual enrichment of more recalcitrant aromatic and aliphatic compounds (Zolcinski, 1928; Stevenson, 1982). Here, the H/C and the O/C ratios show a similar trend throughout each profile and a variability on the order of 2 times (0.91-1.88 and 0.44-0.96, respectively), whereas a different pattern and a greater variability (20-110) was observed for the C/N ratio (Table 5 and Supplementary Fig. S2).

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Total extractable C (TEC) represents the organic C fraction soluble in alkaline solution, *i.e.*, a mixture of humic (HA) and fulvic (FA) acids, but also containing a small quantity of some other, non-humic substances (non-HS) (Sequi et al., 1986). The TEC, in this study, ranges between 43 and 282 g $kg^{-1}$ (Table 5), meaning that the humified organic C fraction accounts for 4 to 28% of bulk peat. These values increase (9.9-45.6%) when TEC is normalized to total organic C (TOC), illustrating how the C content in the humified fraction may represent anywhere from 10% (at PF) to 46% (at KOP) of TOC (Supplementary Fig. S3). Moreover, according to most of the conventional theories, humification may evolve in different directions depending on environmental conditions and OM sources, thus resulting in different proportions of C stored in HA ($C_{HA}$) rather than in FA ($C_{FA}$) (Stevenson, 1982). Pioneering works carried out by Schnitzer (1967) on twenty organic soil samples suggested that the more the OM was humified, the more FA it contained, and that the principal reaction mechanism governing humification was the oxidative degradation of HA to FA. Consequently, ratios like $C_{HA}$/TEC, $C_{HA}$/TOC and $C_{HA}/C_{FA}$ may provide useful indications about the degree of humification of peat as well as about the dominance of fragmentation *vs.* aggregation and/or polymerization processes among humic molecules. Here, the $C_{HA}$ represents, on average, more than 60% of the TEC, with greatest values ranging from 75 to 95% at KAL; at the same time, the $C_{HA}$ accounts on average for only 14±6% of the TOC, with values ranging from <6% (at PF) to 43% (at KAL) (Table 5 and Supplementary Fig. S4). With only a few exceptions, HA is the humus fraction storing most of the TEC (on average, twice that of FA+non-HS); values of $C_{HA}/C_{FA}$ ratio range between 0.7 at SIF and 17.8 at KAL (Table 5), probably mirroring the different climatic conditions that characterize our study sites and that drive the humification process.

*4.3. Spectroscopic proxies*

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Solid-state $^{13}C$ CPMAS NMR spectroscopy has been utilised to characterise changes in chemical composition of C found in a wide range of natural organic residues including peat. In particular, it was observed that, with increasing extent of peat decomposition, the proportion of aromatic and alkyl C increase, while that of O- and N-substituted alkyl C decreased (Baldock et al., 1997). As a consequence, A/O-A (unsubstituted alkyl-C / O substituted alkyl-C), A/O,N-A (unsubstituted alkyl-C / O, N substituted alkyl-C) and Ar/O,N-A ($sp^2$-hybridized C / O, N substituted alkyl-C) ratios generally increase during humification. Here, these three proxies behave similarly and are significantly ($p$<0.001) correlated with each other (Table 6), identifying common zones along the profiles characterized by more humified peat (Supplementary Fig. S5). Generally speaking, A/O-A, A/O,N-A and Ar/O,N-A ratios of more humified peat differ by one unit of magnitude compared to undecomposed peat material (*e.g.*, from 0.16 to 1.79 in the case of A/O-A; Table 5). Smaller variations of these ratios were observed at PF, while larger differences were found at KAL and KOP (Supplementary Fig. S5).

The use of C and N stable isotopes fail to provide unambiguous measures of peat humification (Biester et al., 2014). For example, degradation of OM generally produces a $^{13}C$-enrichment in residual material because microorganisms responsible will preferentially metabolize the $^{12}C$-rich fraction, thus resulting in an increase of the $\delta^{13}C$ yielding less negative values (*e.g.*, Balesdent et al., 1987, 1993). At the same time, however, the C isotope composition of plant detritus gradually changes during decomposition and humification processes as polysaccharides (*e.g.*, cellulose) are preferentially destroyed, leaving behind a material that is relatively enriched in lignin-derived C and depleted in $^{13}C$ (*e.g.*, Benner et al., 1987; Zaccone et al., 2011b). Thus, decay processes in peatlands can act to both increase and decrease $\delta^{13}C$ simultaneously. Values of $\delta^{13}C$ in the bogs studied here range between -30.2 to -23.2‰ ($C_3$ plants show a $\delta^{13}C$ of *ca.* -27‰), without revealing a common trend; the same

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occurs for $\delta^{15}N$, whose values range from -6.8 to 1.7‰ (Table 5 and Supplementary Fig. S6). The stable isotope ratios of C and N do not show a significantly correlated signature (Table 6).

Following elemental analysis and the determination of atomic ratios (C/N, O/C and H/C), the utilization of an aqueous alkaline extract examined using either UV-Vis or molecular fluorescence spectroscopy is probably the most common approach to estimate, in a simple and inexpensive way, the humification degree of peat samples (Table 2). Generally speaking, and with the exception of the $E_4/E_6$ ratio, all the spectroscopic proxies investigated in this study were in agreement with one another (Supplementary Figs. S7 and S8), showing a range of variation, between minimum and maximum values, on the order of 10-50 times (Table 5).

*4.4. Thermal proxies*

Thermal analysis was used since the 1950s in order to investigate the characteristics of peat and coal as fuels. Analysing peat and plant samples, Lévesque and Dinel (1978) found three main regions of weight loss in DTG curves: a first region (275-325 °C), associated with a variety of readily oxidizable compounds such as sugars and cellulosic materials, a second (360-460 °C) region associated with humic substances, and a third region (500-560 °C) due to non-hydrolyzable residues (Schnitzer and Hoffman, 1966). Consequently, these soil scientists found a general occurrence of exothermic peaks due to decomposition and combustion reactions of organic compounds having different thermal stability and this, in turn, reflected the degree of humification.

Here, the first peak ($Exo_1$), generally associated with less humified compounds, occurred in all samples between 316 and 357 °C and represented between 4% (at KAL) and 67% (at PF) of the sample; the second peak ($Exo_2$), generally associated with OM having a greater humification degree, occurred between 362 and 530 °C and ranged between 9 and 59%

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of the sample (both at KAL) (Table 5). The $Exo_2/Exo_1$ ratios ranged from 0.15 at PF to 2.17 at KAL (Table 5; Supplementary Fig. S9).

## 5. Comparing humification proxies

The extremely high variability of peat humification degree from our sample collection is evident in Table 5, where, for most of the proxies included in this study, there is up to one order of magnitude difference between the minimum and the maximum value recorded.

### *5.1. Humification degree vs. botanical composition of peat*

Contrary to mineral soils, the OM recalcitrance, intrinsic of each peat-forming plant tissue, may have a major role in peatlands, affecting both humification process and its measurement. For example, long ago Overbeck (1947) demonstrated that the light absorbance values on NaOH extracts from fresh bog species were different, while Garcia et al. (1993) reported different extraction yields and HA chemical properties for *Carex* vs. *Sphagnum* peats. Coulson and Butterfield (1978) shown that rates of decomposition were correlated to the chemistry of peat-forming plants, while Johnson and Damman (1991) suggested different decay rates even among *Sphagnum* species. In contrast, Lévesque and Dinel (1978), analyzing both bulk peats and peat-forming plants by thermal methods, concluded that thermal curves of fresh plant materials were not related to those of partially decomposed remains, *i.e.*, that specific features of TG curves cannot be tied back to dominant plant species in peat.

Although our sites were characterized by peat showing a different botanical composition, most of selected proxies included in this study are significantly correlated with each other and show common patterns, thus suggesting that peat-forming vegetation either did not affect them, or (most likely) affected these proxies in the same way (Supplementary Table S1) thus making them comparable.

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*5.2. Humification degree vs. peat depth*

On the opposite, selected proxies rarely correlated with either depth or age (Table 6 and Supplementary Table S1; Supplementary Figs. 1-9). It means that peat humification does not consistently increase with depth, and this finding is in agreement with several surveys on peat stratigraphy reporting poorly decomposed peat underlying well decomposed layers (*e.g.*, Shotyk et al., 1990; Williams and Yavitt, 2003; Roos-Barraclough et al., 2004; Zaccone et al., 2007a; Payne and Blackford, 2008; Magnan et al., 2018; Supplementary Fig. S10). Within a given climatic periodic, peat humification might increase with depth but, because a peat profile may reflect accumulation of OM through various climate periods, humification does not simply increase with depth. In fact, the environmental conditions governing peat accumulation vary during peatland development, and the resulting changes in peat-forming vegetation may have a strong impact on decomposition rates of plant debris. Therefore, the occurrence of relatively undecomposed deep peat layers is probably the result of environmental conditions in the past that were more conducive to rapid peat accumulation (*i.e.*, slower decomposition rates) (Williams and Yavitt, 2003), also when a profile is characterized by botanically similar peat. In summary, while there may often be an overall trend toward more humified peat with depth, the data presented here show that peat humification does not always and unconditionally increase with depth and age.

*5.3. Relationships among proxies of peat humification degree*

We note further that there is a general lack of correlation between the C/N ratio and most of the other humification proxies (Table 6). While the C/N ratio is often used, the pioneering study by Kivinen (1933) already showed long ago that the C/N ratio and the humification index (determined by the von Post method) failed to agree. Ash content also is not correlated with

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most of the humification proxies, suggesting that, in the ombrotrophic peatlands included in this study, variations in ash content are mainly the result of changes in the rate of supply of dust particles rather than due to differences in the degree of OM mineralization. As a consequence, bulk density values, which depend upon the ash contents, do not always reflect changes in OM humification.

The signatures of stable isotopes ($\delta^{13}C$, $\delta^{15}N$) were not correlated with most of the humification proxies (Table 6), confirming how the contrasting indications obtained by their evolution render them unsuitable universal measure of humification. On the opposite, a general agreement is found among remaining spectroscopic (UV-Vis, molecular fluorescence, NMR) indicators of OM humification, with the only exception being the $E_4/E_6$ ratio (Table 6); in fact, this index was proposed by Chen et al. (1977) to obtain information on humic material rather than on bulk (peat) OM. At the same time, being some of them (*i.e.*, $SUVA_{254}$, $\varepsilon_{280}$, $\varepsilon_{540}$, HIX) determined on peat extracts (generally in alkali) or on peat liquid phase (*i.e.*, porewaters), they may represent artifacts or be not in agreement with the information recovered from the corresponding solid phase, respectively. However, despite there is some evidence that alkali extraction procedure may have an influence (*e.g.*, Stevenson, 1982; Caseldine et al., 2000; Yeloff and Mauquoy, 2006; Zaccone et al., 2007d), these spectroscopic proxies generally follow the same trend obtained using chemical and thermal methods, thus making them still attractive for researchers.

Although similar criticisms could be raised when TEC, $C_{HA}$ and $C_{FA}$ are extracted, these proxies allowed to quantify the C content in humified peat. Based on our survey of selected peat samples from a diverse array of bogs, we estimate that, on average, only 22±6% of TOC is stored in humic molecules (*i.e.*, extractable in alkali), and that C stored in the HA fraction represents only the 14±6% of the total C in peat (Table 5). In short, three-quarters of the organic C is in a comparatively undecomposed state, the result of current hydrological

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circumstances and climatic conditions. However, as climate changes and surface and groundwater hydrology along with it, there is a vast store of OM which is vulnerable to decomposition and humification, and this may have important consequences for the global C cycle (*e.g.*, Hodgkins et al., 2014). Of the peatlands studied here, the frozen peat from Bathurst Island, in the high Arctic of Canada, is probably the most vulnerable to climate change.

A simplified scheme summarizing the trend of the most reliable proxies of humification degree, as resulted from the present study, is reported in Fig. 3.

*5.4. Comparison with high resolution records from other studies*

Matching peat cores is extremely complex and often dangerous because of differential growth rates, variable compression during coring, micro-topographic differences, etc., even if they are collected from the same bog and during the same year, and especially when they differ in resolution or detailed age dating are lacking (Zaccone et al., 2012).

Although only few samples for each site were included in this study, a general agreement was found with detailed percentage light transmission (at 550 nm) and C/H profiles reported by Roos-Barraclough et al. (2004) [core 2G; 1991] and Zaccone et al. (2007a) [core 2H; 1991], respectively, for the EGR bog (samples included in this study were selected from a third core [2T] collected in 2005), with detailed C/H ratios reported by Zaccone et al. (2017) for the PF floating mire, and detailed C/N ratios reported by Schellekens et al. (2015) for the HB bog. However, measurements of the humification degree are perfectly comparable exclusively when different proxies are determined on the same peat slice.

## 6. Implications for (paleo)environmental reconstructions and geochemical studies

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Although the degree of humification is a very important property of peat, probably none of the methods proposed during the last two centuries for assessing it is completely satisfactory, with each having both benefits and drawbacks. The choice is much more complex when disturbing factors (*e.g.*, fire events, permafrost aggradation) affect the OM chemistry (*e.g.*, Zaccone et al., 2014; Magnan et al., 2018), as all proxies could be irretrievably compromised. At the same time, there is a need for a simple means of measuring humification, especially in geochemical and paleoenvironmental studies, or when highly degraded samples are collected from peatlands where plant macrofossils and testate amoebae are not well preserved. Moreover, in contrast to visible peat stratigraphy, which may suggest abrupt changes, humification degree shows generally a gradual pattern, although ecological knowledge and botanical changes may strongly help interpreting chemical proxies (*e.g.*, Schellekens et al., 2015; Magnan et al., 2018; van Bellen et al., 2018).

Additionally, detailed paleoenvironmental reconstructions may be carried out using peat profiles that could be 10 m or more deep. If the peat cores are cut into 1 cm slices for high resolution reconstructions, many hundreds of samples need to be analysed for a broad range of parameters. Given that peat corers typically collect samples which are only 5-10 cm in diameter, that peat *in situ* consists up to 95% of water, and that the dry bulk density of this material may be as low as 0.02 g $cm^{-3}$, the amount of sample available per analysis is extremely limited. Moreover, state-of-the-art, detailed reconstructions involve a broad range of physical, chemical and biological analyses, commonly including some combination of radiometric age dating ($^{14}C$ and $^{210}Pb$ for recent peat layers), pollen and plant macrofossils (including charcoal particles), testate amoebae, elemental composition, ash content (including volcanic ash), major and trace elements, persistent organic pollutants, stable and radiogenic isotopes; ideally, all of these measurements are undertaken using the same slice of peat (*e.g.*, Givelet et al., 2004). In consequence, there is generally insufficient material for all of the analyses of interest, especially

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when Hiller (Fries and Hafsten, 1965) or Belarus (Jowsey, 1966) corer are used, and each analytical procedure is constrained by the amount of sample available for study. A simulation of sample availability for peat slice collected using a Belarus *vs.* a Wardenaar (Wardenaar, 1987) corer is reported in Supplementary Table S2.

As a result, the index or proxy used to summarize a complex phenomenon such peat humification needs not only to be simple and representative of the process, but also cost-effective and consume the smallest possible amount of material at the same time. The H/C ratio seems to meet all these requirements, and is a good compromise among quantity of sample required (generally 3-8 mg per replicate), duration of the analysis (a few minutes per sample) and cost (10-15 USD per sample) (Fig. 4). Examples of detailed H/C ratio profiles for EGR and PF are reported in Zaccone et al. (2007a) and Zaccone et al. (2017), respectively. Moreover, elemental analysis (CHNS-O) does not require any sample dissolution (*i.e.*, thus avoiding any risk of possible artifact creation) and also allows data to be organized in the form of the well-known van Krevelen plot (Fig. 5).

While the thermal parameter $Exo_2/Exo_1$ could also be used as a reliable surrogate to describe humification throughout peat profiles, a larger quantity of sample (15-20 mg per replicate) and longer time of analysis (more than 70 min per sample, at 10 °C $min^{-1}$) are required to have reliable data. Finally, also UV-Vis and fluorescence based proxies are still attractive for researchers, being their determination cost-effective and requiring small quantity of sample; however, the possible influence of the alkali extraction procedure needs to be considered.

It is clear that more powerful analytical techniques (*e.g.*, NMR) may provide detailed, structural information, but they generally need hundreds of mg of sample (not always available), are quite expensive (200-300 USD per sample) and are time-consuming (hours per analysis).

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## 7. Fundamental studies on peat humification

Significant advances in studies of peat chemistry can be obtained, for example, using Electrospray Ionization Fourier Transform Ion Cyclotron Resonance Mass Spectrometry (ESI-FTICR-MS), allowing to assign accurate molecular weights and formulas to thousands of individual components without the need for prior separation (Hartman et al., 2015), as well as by X-ray Absorption Near-Edge Structure (XANES) spectroscopy, allowing the determination of C compound classes or ratios of C compound classes (*e.g.*, phenols:ketones and carboxylates:ketones) (Gillespie et al., 2014). Also (Pyrolysis-)GC-MS provides, besides molecular biomarkers allowing the reconstruction of vegetation changes occurring in the past (Pancost et al., 2002), semi-quantitative data about the relative abundance of easily degradable versus more recalcitrant compounds, as resulting during burial and diagenesis of plant matter in bogs (Lehtonen and Ketola, 1990, 1993; Schellekens and Buurman, 2011).

While these (or others) sophisticated tools and approaches help us to much better understand the humification process, they have limited availability (*e.g.*, FTICR-MS), are far too time-consuming and expensive, and provide a huge quantity of data to be managed; that makes them difficult to be used as a routine measure of peat humification as part of a paleoenvironmental reconstruction or a geochemical study. Consequently, they could be extremely useful when detailed or fundamental studies of the humification pattern need to be carried out on individual samples rather than on complete, deep peat profiles.

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**Author contributions**

C.Z. designed and granted funding for this study, provided samples from two peat cores (EGR, PF), interpreted the results and wrote the manuscript. C.P. led fluorescence, $^{13}C$ CPMAS NMR and UV-Vis analysis. C.C. led CF-IRMS and TG-DTA analysis. C.Z. and T.M.M. led physical and elemental analysis. W.S. provided samples from seven peat cores (BI, SIF, HB, FL, LL, KAL, KOP) and edited the manuscript. All authors discussed the results and contributed to the final version of the paper.

**Supplementary Information**

The authors declare no competing financial interests. Supplementary information accompanies this paper. Correspondence and requests for materials should be addressed to C.Z.

**Figure 1.** Examples of peat heterogeneity, showing the diversity of peat-forming plants even in the same slice (*i.e.*, same depth) (photos by C.Z.).

**Figure 2.** Location of the bogs selected for this study. Numbers in brackets indicate the number of peat samples collected from each core. **1)** Bracebridge Inlet, Bathurst Island, Canada (BI; *n* = 4); **2)** Sifton, Ontario, Canada (SIF; *n* = 5); **3)** Harberton, Tierra del Fuego, Argentina (HB; *n* = 4); **4)** Fleck's Loch, Island of Foula, Shetland Islands, Scotland (FL; *n* =

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4); **5)** Loch Laxford, Scotland (LL; *n* = 4); **6)** Etang de la Gruère, Switzerland (EGR; *n* = 4); **7)** Posta Fibreno, Italy (PF; *n* = 5); **8)** Setya Alam, Kalimantan (Borneo), Indonesia (KAL; *n* = 5); **9)** Kopouatai, North Island, New Zealand (KOP; *n* = 5).

**Figure 3.** Simplified scheme summarizing the trend of the most reliable humification proxies (according to results obtained in the present study).

**Figure 4.** 3D scatterplot showing the relationship among quantity of sample, cost and time required per analysis. Data are obtained averaging values obtained from 5 laboratories, and consider also sample preparation (if needed).

**Figure 5.** Van Krevelen diagrams. Relationships between O/C *vs.* H/C atomic ratios for the studied peat samples (a). In panel (b), the peat age, when available (Table 3), is provided as *z*-axis. When age is provided as third dimension (b), it becomes clear that variations in the humification degree cannot be attributed simply, or exclusively, to differences in the age of formation and, consequently, in peat depth. Corresponding bi-dimensional plots are reported in Supplementary Fig. S11.

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**Table 1.** Some of the most commonly used definitions of humification (and/or humic substances). A more comprehensive review can be found in Hayes and Swift (2018).

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|---|---|
| Kononova (1966) | "…a complex two-stage process in which organic residues of plant and animal origin undergo profound transformations involving: 1. The decomposition of the original components of tissues and their conversion by micro-organisms into simpler chemical compounds and partially to products of complete mineralization ($CO_2$, $NO_2$, $NO_3$, $NH_3$, $CH_4$, $H_2O$, etc.). 2. The synthesis of organic compounds with the formation of high molecular-weight humus substances of specific nature" |
| Hayes and Swift (1978) | "Transformed products, humus, that bear no morphological resemblances to their source materials. These are generally referred to as humified products. The humified products are further subdivided into (a) amorphous polymeric, brown-coloured humic substances (differentiated on the basis of their solubility characteristics into […]) and (b) compounds belonging to recognisable classes, such as carbohydrates, peptides, altered lignins, fats, waxes, cutins and cutans. These can be synthesised by microorganisms, or they can arise from alterations of similar materials in the original debris" |
| Stevenson (1982) | "…a series of high-molecular-weight, yellow to black substances formed by secondary synthesis reactions" |
| Aiken et al. (1985) | "…a category of naturally occurring, biogenic, heterogeneous organic substances that can be generally characterized as being yellow-to-black in color, of high molecular weight, and refractory" |
| Piccolo (2001) | "…supramolecular associations of self-assembling heterogeneous and relatively small molecules deriving from the degradation and decomposition of dead biological material" |
| Wershaw (2004) | "…a three step process of (1) degradation of components of plant tissue followed by (2) reassembly of the degradation products into NOM, and (3) the degradation of the NOM formed in step 2." |
| Schnitzer and Monreal (2011) | "…a portion of the total SOM that is extracted and solubilized with dilute alkali" |
| IHSS (2018) | "…complex and heterogeneous mixtures of polydispersed materials formed in soils, sediments, and natural waters by biochemical and chemical reactions during the decay and transformation of plant and microbial remains (a process called humification)" |

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**Table 2.** A short (not exhaustive) summary of main humification proxies used for peat.

| Method and/or proxy | References (examples) |
|---|---|
| *Physical and thermal approaches* | |
| Colour and turbidity of squeezed porewaters and structure of remaining solid material | Von Post, 1922, 1924 |
| Water holding capacity | Segeberg, 1952 |
| Bulk density | Boelter, 1965; Boelter, 1969; Päivänen, 1969; Silc and Stanek, 1977 |
| Fiber content and dimensions | Farnham and Finney, 1965; Lynn et al., 1974 |
| Loss of Ignition (LOI) | Bouyoucos, 1934; Leifeld et al., 2011 |
| Calorific value | Salmi, 1949; Makila, 1980; Keys, 1983 |
| Thermogravimetry | Schnitzer and Hoffman, 1966, 1967; Stewart et al., 1966; Lozbin and Smolyaninov, 1974; Lévesque and Dinel, 1978; Sheppard and Forgeron, 1987; Francioso et al., 2003 |
| *Chemical and spectroscopic approaches* | |
| Colorimetric approaches; UV-Vis absorbance measurements of alkaline peat extracts | Melin and Odén, 1916; Kaila, 1956; Bahnson, 1968; Aaby and Tauber, 1975; Blackford and Chambers, 1993; Caseldine et al., 2000; Roos-Barraclough et al. 2004; Yeloff and Mauquoy, 2006; Klavins et al., 2008; Payne and Blackford, 2008; Chambers et al., 2011 |
| Proportion of oxygen-containing functional groups | Schnitzer and Desjardins, 1965, 1966 |
| Extraction of humified C | Schnitzer, 1967; Ciavatta et al., 1996; Cavani et al., 2003 |
| ESR and EPR: abundance of free radicals | Schnitzer and Lévesque, 1979; Wikander and Nordén, 1988; Cocozza et al., 2003 |
| Isoelectric focusing | Ciavatta et al., 1996; Cavani et al., 2003 |
| Atomic ratios (C/N, O/C and H/C), determined using elementar analysis | Malmer and Holm, 1984; Meyer, 1994; Kuhry and Vitt, 1996; Zaccone et al., 2007a; Klavins et al., 2008; Zaccone et al., 2011a; Andersson et al., 2012; Biester et al., 2014; Schellekens et al., 2015; Magnan et al., 2018 |
| Rock Eval© Pyrolysis | Outridge and Sanei, 2010; Zaccone et al., 2011a; Delarue et al., 2013; Biester et al., 2014 |
| GC and (Pyr-)GC-MS; HPLC | Lehtonen and Ketola, 1990, 1993; González et al., 2003; Williams and Yavitt, 2003; Zaccone et al., 2008; Schellekens and Buurman, 2011; Biester et al., 2014; Philben et al., 2015; Schellekens et al., 2015 |

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| | |
|---|---|
| IRMS: signature of stable (mainly C, N) isotopes | Meyer, 1994; Zaccone et al., 2011b; Andersson et al., 2012; Biester et al., 2014; Krüger et al., 2015 |
| Solid State NMR | Preston et al., 1987; Krosshavn et al., 1992; Baldock et al., 1997; González et al., 2003 |
| Fluorescence properties of peat extracts, peat humic molecules and porewaters | Cocozza et al., 2003; Zaccone et al., 2007c, 2009b |
| FT-IR | Cocozza et al., 2003; González et al., 2003; Zaccone et al., 2007a, 2011a; Delarue et al., 2013; Biester et al., 2014 |
| *Biological approaches* | |
| Biodegradation | Lévesque and Mathur, 1979; Gillespie et al., 2014 |
| *Comparisons among (more than two) methods* | |
| | Kaila, 1956; Raitio and Huttunen, 1976; Stanek and Silc, 1977; Levesque and Mathur, 1979; Schnitzer and Lévesque, 1979; Tolonen, 1982; Klemetti and Keys, 1983; Preston et al., 1987; Blackford and Chambers, 1993; Francioso et al., 2003; González et al., 2003; Yeloff and Mauquoy, 2006; Klavins et al., 2008; Zaccone et al., 2011a,b; Hansson et al., 2013; Biester et al., 2014; Gillespie et al., 2014; Schellekens et al., 2015 |

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**Table 3.** Details of the cores and samples included in this study.

| Sample code | Depth | Age | | References |
|---|---|---|---|---|
| | (cm) | ($^{14}$C yr BP) | (cal. yr BP; *AD/BC*) | |
| | | | | |
| **1) Bracebridge Inlet, Canada (BI) [**core code: BI2b; collected in 2000**]** | | | | |
| BI2b #32 | 31-32 | 4,585±60 | *3386-3093* | Givelet et al., 2004 |
| BI2b #36 | 35-36 | | *3760 BC* † | |
| BI2b #47 | 46-47 | 5,305±60 | *4255-3985 BC* | |
| BI2b #52 | 51-52 | | *4000 BC* † | |
| **2) Sifton, Ontario, Canada (SIF) [**core code: SIF; collected in 2000**]** | | | | |
| SIF #02 | 0-1.1 | -785±45 (bomb pulse) | *AD 1999-2000* | Givelet et al., 2003 |
| SIF #16 | 14.9-15.9 | -1,460±55 (bomb pulse) | *AD 1958-1959* | |
| SIF #32 | 31.9-32.9 | | *AD 1925±10* | |
| SIF #47 | 47.8-48.9 | | *AD 1888±25* | |
| SIF #62 | 63.7-64.8 | | *AD 1812±125* | |
| **3) Harberton, Argentina (HB) [**core code: HB; collected in 1987**]** | | | | |
| HB #5-6 | 5-6 | | | Markgraf and Huber, 2010 |
| HB #225-250 | 225-250 | | | |
| HB #475-500 | 475-500 | | | |
| HB #625-650 | 625-650 | A volcanic ash at 678 cm depth is most likely the Hudson H1 ash (6850±160 $^{14}$C BP). | | |
| **4) Fleck's Loch, Scotland (FL) [**core code: 6b; collected in 1991**]** | | | | |
| 6b #03 | 2-3 | | | Shotyk, 1997 |
| 6b #27 | 26-27 | | | |
| 6b #61 | 60-61 | | | |
| 6b #85 | 84-85 | | | |
| **5) Loch Laxford, Scotland (LL) [**core code: 7c; collected in 1991**]** | | | | |
| 7c #01 | 0-1 | | *AD 1991* | Shotyk, 1997 |
| 7c #26 | 25-26 | | | |
| 7c #54 | 53-54 | | | |
| 7c #82 | 81-82 | | | |
| **6) Etang de la Gruère, Switzerland (EGR) [**core code: 2T; collected in 2005**]** | | | | |
| 2T #04 | 2.1-3.1 | | *AD 2002±1* | Zaccone et al., 2007b |
| 2T #34 | 34.0-35.2 | | *AD 1845±20* | |
| 2T #69 | 77.5-78.9 | | | |

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|---|---|---|---|---|
| 2T #90 | 101.2-103.8 | For comparison, sample 2T #91 (103.8-110.0 cm of depth) showed a radiocarbon age of 2,110±30 $^{14}$C yr BP | | |
| **7) Posta Fibreno, Italy (PF) [**core code: PF2; collected in 2012**]** | | | | |
| PF2 #01 | 2.9-3.9 | | *AD 2012±1* | Zaccone et al., 2017 |
| PF2 #27 | 24.6-25.4 | | *AD 1996±2* † | |
| PF2 #62 | 57.3-58.4 | | *AD 1976±3* † | |
| PF2 #97 | 87.6-88.4 | | *AD 1966±4* | |
| PF2 B1#09 | 110.6-112.5 | 130.9±0.3 pMC | *AD 1963±4* | |
| **8) Kalimantan, Indonesia (KAL) [**core code: SA6.5; collected in 1995**]** | | | | |
| SA6.5 #01 | 0-3 | | | Weiss et al., 2002 |
| SA6.5 #17 | 170-190 | 3,960±80 | 4,417 | |
| SA6.5 #28 | 390-410 | 8,280±100 | 9,279-9,395 | |
| SA6.5 #40 | 610-630 | 9,060±100 | 10,177-10,358 | |
| SA6.5 #58 | 960-980 | 20,350±130 | | |
| **9) Kopouatai, New Zealand (KOP) [**core code: KOP 1500; collected in 1995**]** | | | | |
| KOP #03 | 0-3 | | | Shearer, 1997 |
| KOP #19 | 48-51 | | | |
| KOP #37 | 135-140 | For comparison, sample KOP #46 (180-185 cm of depth) seems to correspond to 700 BP (Kaharoa eruption) | | |
| KOP #84 | 380-390 | | | |
| KOP #115 | 690-700 | For comparison, sample KOP #109 (630-640 cm of depth) seems to correspond to 6,150 BP (Tuhua eruption) | | |

† Interpolated ages (samples not directly measured)

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**Table 4.** Humification proxies considered in this study.

| Type of index | Technique | Humification proxies |
|---|---|---|
| Physical | | Depth, density |
| | Combustion | Ash content |
| | LB-γ spectrometry ($^{210}$Pb), AMS ($^{14}$C) | Age |
| Chemical | Flash combustion (Elemental Analysis) | Atomic ratios (C/N, H/C, O/C) |
| | Extraction and determination of humified C fractions | TEC, TEC/TOC, $C_{HA}$/TOC, $C_{HA}$/TEC, $C_{HA}/C_{FA}$ * |
| Spectroscopic | $^{13}$C CPMAS NMR | A/O-A, A/O,N-A, Ar/O,N-A ratios ‡ |
| | UV-Vis | $E_4/E_6$, $SUVA_{254}$, $\varepsilon_{280}$, $\varepsilon_{540}$ † |
| | Molecular fluorescence | HIX ‡ |
| | CF-IRMS | Signature of stable isotopes ($\delta^{13}$C, $\delta^{15}$N) |
| Thermal | TG-DTA | $Exo_1$, $Exo_2$, $Exo_2/Exo_1$ § |

* **TEC:** *total extractable organic C;* **TOC:** *total organic carbon;* **$C_{HA}$, $C_{FA}$:** *organic C in the humic and fulvic fraction, respectively*

‡ **A/O-A:** *unsubstituted alkyl-C (0-45 ppm) / O substituted alkyl-C (65-110 ppm)*; **A/O,N-A:** *unsubstituted alkyl-C (0-45 ppm) / O, N substituted alkyl-C (45-110 ppm);* **Ar/O,N-A:** *$sp^2$-hybridized C (110-160 ppm) / O, N substituted alkyl-C (45-110 ppm)*

† **$E_4/E_6$:** *ratio of absorbance at 465 and 665 nm*; **$SUVA_{254}$:** *Specific UV Absorbance at 254 nm*; **$\varepsilon_{280,\ 540}$:** *Molar absorptivity (or molar extinction coefficient) at 280 and 540 nm, respectively*

‡ **HIX:** *ratio between the area in the upper quarter (435–480 nm) and the area in the lower quarter (300-345 nm) of the emission spectra*

§ **$Exo_1$, $Exo_2$, $Exo_2/Exo_1$:** *weight loss associated with the main exothermic peaks, and their ratio*

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**Table 5.** Minimum, maximum, average (±st.dev.) and median values recorded for each proxy.

| | Proxy | | Min | Max | Average | St.dev. | Median |
|---|---|---|---|---|---|---|---|
| Physical | Depth | (cm) | 0.5 | 970.0 | | | |
| | Age | (yr) | 1 | >20,000 | | | |
| | Density | (g $cm^{-3}$) | 0.024 | 0.258 | 0.085 | 0.055 | 0.068 |
| | Ash | (%) | 0.2 | 85.2 | 8.2 | 16.1 | 2.4 |
| Chemical | C/N | - | 20 | 110 | 55 | 23 | 53 |
| | H/C | - | 0.91 | 1.88 | 1.41 | 0.17 | 1.41 |
| | O/C | - | 0.44 | 0.96 | 0.66 | 0.12 | 0.63 |
| | TEC | (g $kg^{-1}$) | 43.0 | 281.7 | 116.3 | 40.1 | 114.9 |
| | TEC/TOC | (%) | 9.9 | 45.6 | 21.8 | 6.4 | 21.8 |
| | $C_{HA}$/TEC | (%) | 41.5 | 94.7 | 63.9 | 11.7 | 64.6 |
| | $C_{HA}$/TOC | (%) | 5.8 | 43.2 | 14.1 | 6.4 | 12.6 |
| | $C_{HA}/C_{FA}$ | - | 0.7 | 17.8 | 2.4 | 2.7 | 1.8 |
| Spectroscopic | A/O-A | - | 0.16 | 1.79 | 0.55 | 0.41 | 0.39 |
| | A/O,N-A | - | 0.14 | 1.26 | 0.42 | 0.28 | 0.31 |
| | Ar/O,N-A | - | 0.08 | 0.85 | 0.22 | 0.18 | 0.16 |
| | $\delta^{13}C$ | (‰) | -30.2 | -23.2 | -27.0 | 1.5 | -27.0 |
| | $\delta^{15}N$ | (‰) | -6.8 | 1.7 | -1.8 | 2.1 | -1.6 |
| | $\delta^{13}C/\delta^{15}N$ | - | -309 | 288 | 4 | 71 | 10 |
| | $E_4/E_6$ | - | 1.3 | 9.7 | 5.3 | 2.1 | 5.4 |
| | $SUVA_{254}$ | (L g $OC^{-1}$ $cm^{-1}$) | 4.2 | 42.0 | 12.0 | 6.8 | 10.6 |
| | $\varepsilon_{280}$ | (L mole $OC^{-1}$ $cm^{-1}$) | 37.7 | 428.5 | 125.3 | 70.9 | 110.8 |
| | $\varepsilon_{540}$ | (L mole $OC^{-1}$ $cm^{-1}$) | 0.8 | 32.3 | 6.9 | 5.6 | 5.7 |
| | HIX | - | 2.6 | 119.8 | 9.6 | 18.0 | 6.3 |
| Thermal | $Exo_1$ | (%) | 4.2 | 67.1 | 46.1 | 11.8 | 47.8 |
| | $Exo_2$ | (%) | 9.1 | 58.8 | 33.5 | 12.1 | 34.5 |
| | $Exo_2/Exo_1$ | - | 0.15 | 2.17 | 0.82 | 0.46 | 0.75 |

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**Table 6.** Statistical correlations and significance among the humification proxies under investigation. More details are reported in Supplementary Table S1.

| | **Physical parameters** | | | | **Chemical parameters** | | | | | | | | **Spectroscopic parameters** | | | | | | | | | | | **Thermal parameters** | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Depth | Age | Density | Ash | C/N | H/C | O/C | TEC | TEC/TOC | $C_{HA}$/TEC | $C_{HA}$/TOC | $C_{HA}$/$C_{FA}$ | A/O-A | A/O,N-A | Ar/O,N-A | $\delta^{13}C$ | $\delta^{15}N$ | $\delta^{13}C$/$\delta^{15}N$ | $E_4/E_6$ | $SUVA_{254}$ | $\varepsilon_{280}$ | $\varepsilon_{540}$ | HIX | $Exo_1$ | $Exo_2$ | $Exo_2/Exo_1$ |
| Depth | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Age | *** | | | | | | | | | | | | | | | | | | | | | | | | | |
| Density | NS | ** | | | | | | | | | | | | | | | | | | | | | | | | |
| Ash | * | NS | * | | | | | | | | | | | | | | | | | | | | | | | |
| C/N | NS | NS | * | ** | | | | | | | | | | | | | | | | | | | | | | |
| H/C | ** | NS | * | NS | NS | | | | | | | | | | | | | | | | | | | | | |
| O/C | * | ** | *** | NS | ** | *** | | | | | | | | | | | | | | | | | | | | |
| TEC | NS | NS | * | NS | NS | *** | *** | | | | | | | | | | | | | | | | | | | |
| TEC/TOC | NS | NS | NS | NS | NS | *** | *** | *** | | | | | | | | | | | | | | | | | | |
| $C_{HA}$/TEC | *** | * | ** | NS | NS | *** | *** | ** | * | | | | | | | | | | | | | | | | | |
| $C_{HA}$/TOC | NS | NS | ** | NS | NS | *** | *** | *** | *** | *** | | | | | | | | | | | | | | | | |
| $C_{HA}$/$C_{FA}$ | * | NS | NS | NS | NS | *** | *** | *** | *** | *** | *** | | | | | | | | | | | | | | | |
| A/O-A | *** | *** | *** | * | NS | *** | *** | * | NS | *** | ** | *** | | | | | | | | | | | | | | |
| A/O,N-A | *** | *** | *** | * | NS | *** | *** | * | NS | *** | *** | *** | *** | | | | | | | | | | | | | |
| Ar/O,N-A | *** | *** | ** | * | NS | *** | *** | * | NS | *** | ** | *** | *** | *** | | | | | | | | | | | | |
| $\delta^{13}C$ | NS | NS | NS | * | NS | * | NS | NS | NS | NS | NS | * | NS | NS | NS | | | | | | | | | | | |
| $\delta^{15}N$ | * | ** | * | ** | ** | NS | ** | NS | NS | NS | NS | NS | ** | ** | ** | NS | | | | | | | | | | |
| $\delta^{13}C$/$\delta^{15}N$ | NS | NS | NS | NS | * | ** | NS | * | * | * | * | *** | NS | NS | ** | NS | NS | | | | | | | | | |
| $E_4/E_6$ | NS | NS | NS | * | NS | *** | ** | *** | ** | * | ** | NS | NS | NS | NS | NS | NS | NS | | | | | | | | |
| $SUVA_{254}$ | * | NS | * | NS | NS | *** | *** | *** | *** | *** | *** | *** | *** | *** | *** | * | NS | ** | ** | | | | | | | |
| $\varepsilon_{280}$ | * | NS | NS | NS | NS | *** | *** | *** | *** | *** | *** | *** | *** | *** | *** | * | NS | ** | ** | *** | | | | | | |
| $\varepsilon_{540}$ | ** | * | * | NS | NS | *** | *** | *** | *** | *** | *** | *** | *** | *** | *** | NS | NS | ** | NS | *** | *** | | | | | |
| HIX | NS | NS | NS | NS | NS | *** | * | *** | *** | ** | *** | *** | ** | ** | *** | NS | NS | *** | NS | *** | *** | *** | | | | |
| $Exo_1$ | *** | *** | ** | *** | NS | *** | *** | NS | NS | *** | NS | * | *** | *** | *** | NS | ** | NS | NS | ** | ** | *** | NS | | | |
| $Exo_2$ | NS | NS | NS | ** | NS | ** | ** | *** | *** | *** | *** | ** | ** | ** | * | NS | NS | NS | *** | *** | ** | ** | * | NS | | |

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|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | * | | * | * | | | | | | | | | | | | | | | * | | | | | |
| $Exo_2/Exo_1$ | *** | *** | ** | NS | NS | *** | *** | * | NS | *** | ** | *** | *** | *** | *** | NS | * | NS | * | *** | *** | *** | ** | *** | *** | |
| | | | | | | | | | | | | | | | | | | | | | | | | | | |
| | Depth | Age | Density | Ash | C/N | H/C | O/C | TEC | TEC/TOC | $C_{HA}/TEC$ | $C_{HA}/TOC$ | $C_{HA}/C_{FA}$ | A/O-A | A/O,N-A | Ar/O,N-A | $\delta^{13}C$ | $\delta^{15}N$ | $\delta^{13}C/\delta^{15}N$ | $E_4/E_6$ | $SUVA_{254}$ | $\varepsilon_{280}$ | $\varepsilon_{540}$ | HIX | $Exo_1$ | $Exo_2$ | $Exo_2/Exo_1$ |

***, **, *: *p<0.001, 0.01 and 0.05, respectively*; NS: *not significant*

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Figure 1

Figure 2

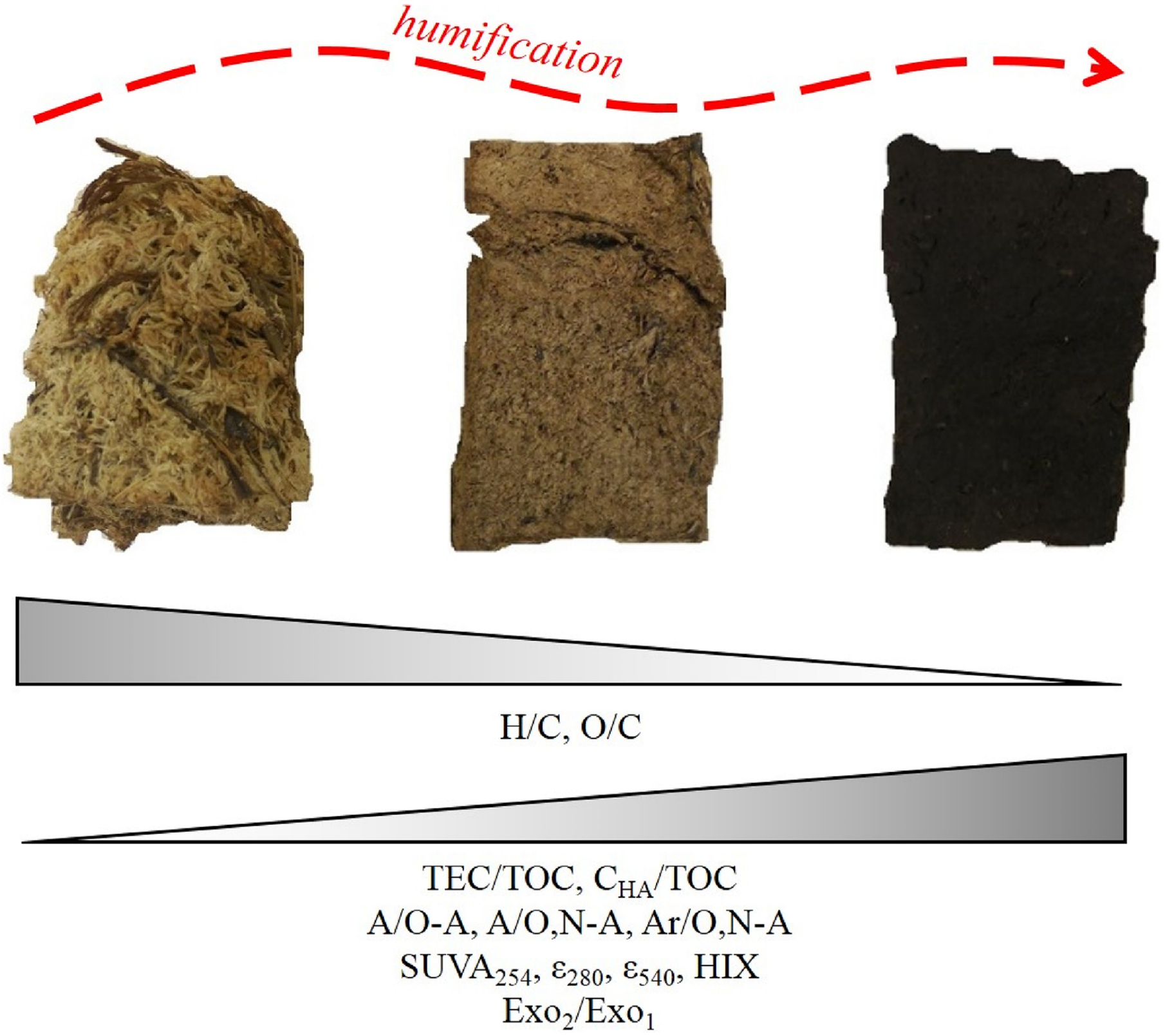
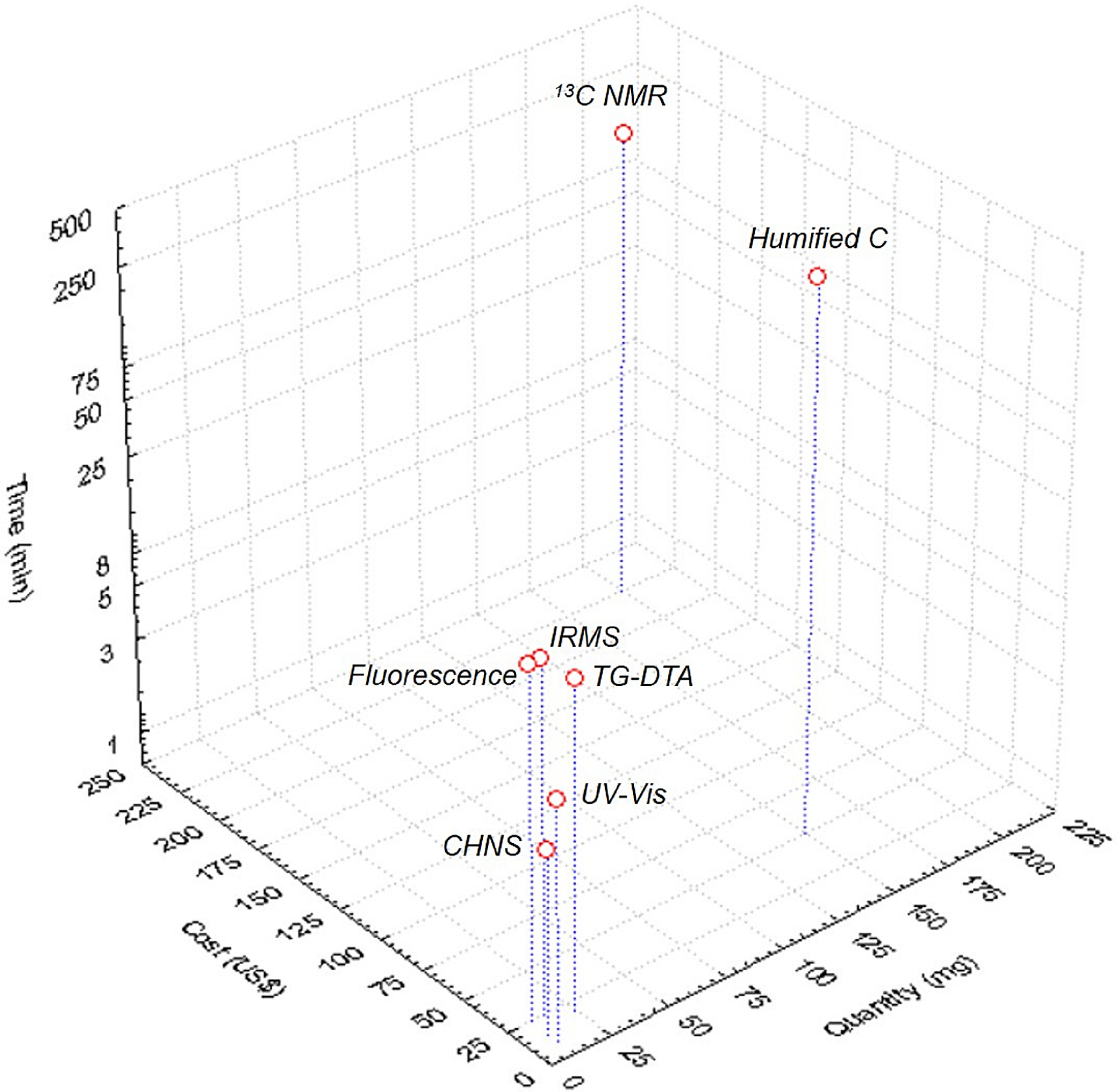

humification
H/C, O/C
TEC/TOC, $C_{HA}$/TOC
A/O-A, A/O,N-A, Ar/O,N-A
$SUVA_{254}$, $\varepsilon_{280}$, $\varepsilon_{540}$, HIX
$Exo_2/Exo_1$

Figure 3


$^{13}$C NMR
Humified C
IRMS
Fluorescence
TG-DTA
UV-Vis
CHNS
Time (min)
500
250
75
50
25
8
5
3
1
Cost (US$)
250
225
200
175
150
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25
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Quantity (mg)
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25
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Figure 4

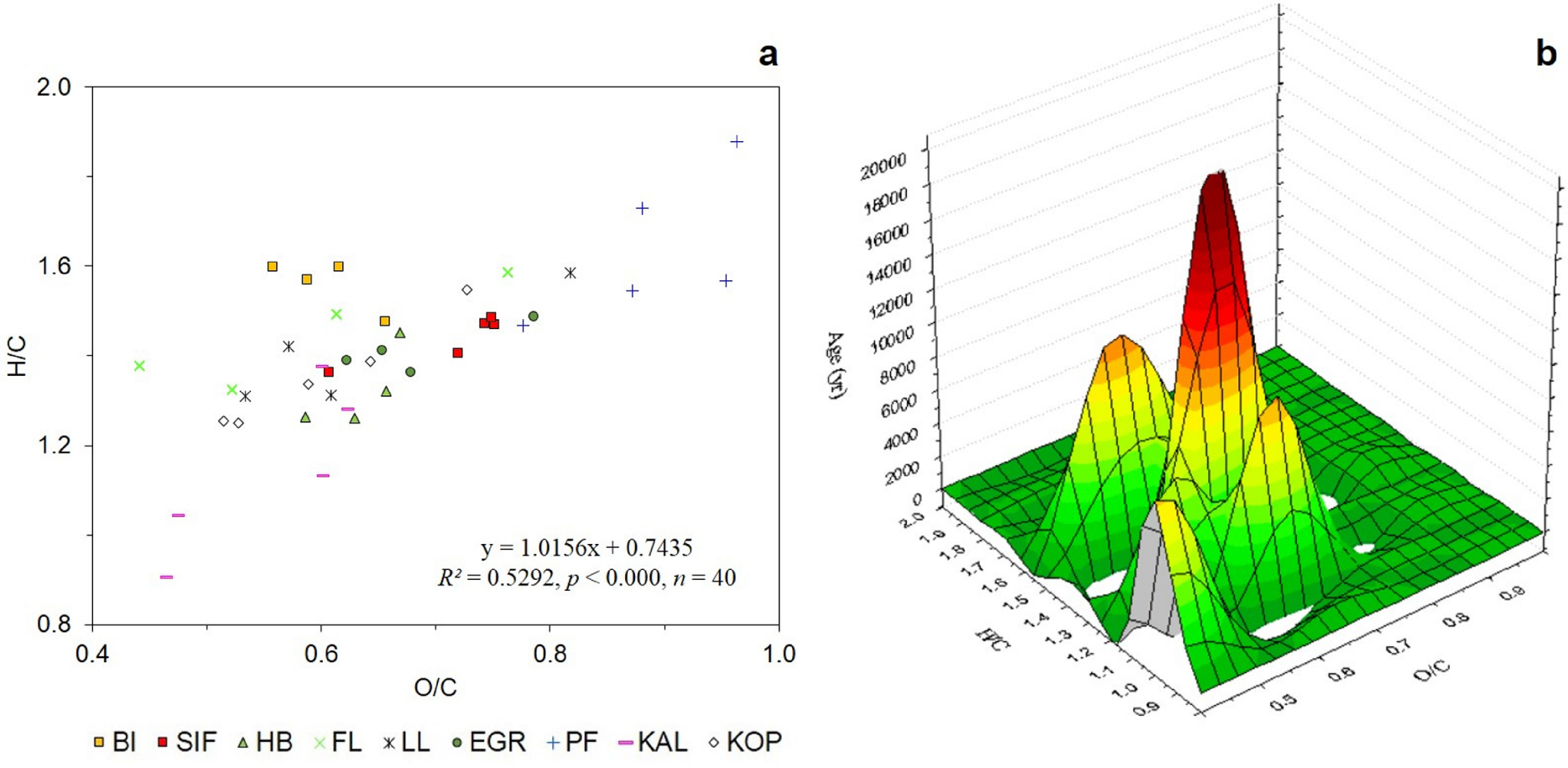
a
2.0
1.6
1.2
0.8
H/C
0.4
0.6
0.8
1.0
O/C
y = 1.0156x + 0.7435
R² = 0.5292, p < 0.000, n = 40
BI SIF HB FL LL EGR PF KAL KOP
b
Age (yr)
20000
18000
16000
14000
12000
10000
8000
6000
4000
2000
0
H/C
O/C

Figure 5

Figure 6