Evaluating the ‘conservative’ behavior of stable isotopic ratios ($\delta^{13}\text{C}$, $\delta^{15}\text{N}$, and $\delta^{18}\text{O}$) in humic acids and their reliability as paleoenvironmental proxies along a peat sequence

C. Zaccone^{a,*}, G. Casiello^b, F. Longobardi^b, L. Bragazza^{c,d,e}, A. Sacco^b, T.M. Miano^f

^a Department of Agro-Environmental Sciences, Chemistry and Plant Protection, University of Foggia, via Napoli 25, 71122 Foggia, Italy

^b Department of Chemistry, University of Bari, via Orabona 4, 70126 Bari, Italy

^c WSL Swiss Federal Institute for Forest, Snow and Landscape Research, Site Lausanne, Case postale 96, station 2, CH-1015 Lausanne, Switzerland

^d Ecole Polytechnique Fédérale de Lausanne (EPFL), School of Architecture, Civil and Environmental Engineering (ENAC), Laboratory of Ecological Systems (ECOS), Batiment GR, Station 2, CH-1015 Lausanne, Switzerland

^e Department of Biology and Evolution, University of Ferrara, Corso Ercole I d'Este 32, 44100 Ferrara, Italy

^f Department of Biology and Chemistry of Agro-Forestry and Environment, University of Bari, via Amendola 165/A, 70126 Bari, Italy

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ABSTRACT

Although several studies have used bogs in order to reconstruct paleoclimatic conditions and the historical trends of pollutants, scientific literature is still rather controversial about the role of ombrotrophic bogs as reliable record of past environmental changes. Consequently, understanding whether all vegetational and climatic “information” are effectively preserved in peat deposits during humification becomes an essential aspect to be tested before using bogs as natural archives.

The present work focuses on stable isotopic ratios, i.e., $^{13}\text{C}/^{12}\text{C}$ ($\delta^{13}\text{C}$), $^{15}\text{N}/^{14}\text{N}$ ($\delta^{15}\text{N}$) and $^{18}\text{O}/^{16}\text{O}$ ($\delta^{18}\text{O}$), and is based on the assumption that, if bogs are consistent archives of environmental changes, these types of “information” should be recorded also into humic acids (HA), i.e. the fraction of peat more recalcitrant and refractory to degradation.

Thus, an 81-cm long peat core, covering the last 2000 years, was collected from the Etang de la Gruère bog (Jura Mountains, Switzerland), cut into 3 cm slices, and HA were isolated from each age dated layer. Stable isotopic ratios ($\delta^{13}\text{C}$, $\delta^{15}\text{N}$ and $\delta^{18}\text{O}$) were then determined in bulk peat and corresponding HA samples.

An increase in the humification degree and a decrease of the C/N ratio were observed along the profile. The $\delta^{13}\text{C}$ of both peat and HA showed a significantly similar trend with depth ($p=0.0001$), and the same significant correlation was observed for the $\delta^{15}\text{N}$ ($p<0.0001$). Also the ratio between $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ closely resembled the C/N ratio observed in the peat and in HA ($p<0.0001$), thus underlining that the trend of these isotopic ratios is preserved along the studied bog profile. Consequently, our data seem to support both the role of HA as recalcitrant, stable molecules with a long-term residence time, and the potential of ombrotrophic bogs to be used as “archives” of vegetational changes occurring (at least) in the last 2000 years. Although a certain relationship ($p<0.05$) between peat and corresponding HA was found also for $\delta^{18}\text{O}$, our data did not allow the solving of the issue of its reliability as paleoenvironmental proxy.

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1. Introduction

Peat bogs are unique archives because their genesis is directly linked to the atmospheric conditions occurring during peat formation. Peatlands are widely distributed across the globe under temperate-cold climatic conditions and account for about 5% of the land area of the Earth (Kivinen and Pakarinen, 1981). Peat accumulation is the result of an imbalance between the annual rates of primary productivity and decomposition, so that, on the long term, a net

surplus of partially decomposed litter accumulates as peat (Kuhry and Vitt, 1996; Bragazza et al., 2009). Ombrotrophic bogs, in particular, are domed peatlands which receive water and nutrients only by atmospheric deposition (Damman, 1978; Clymo, 1983). Such deposits have been often used as a record of historical deposition of pollutants caused by human activity (e.g., Pfeiffer-Madsen, 1981; Shotyk et al., 1996, 1998; Martínez-Cortizas et al., 1999; Zaccone et al., 2007a, 2009a) and could be a useful tool in palaeoclimatic and paleovegetation reconstructions (e.g., Aaby, 1976; van Geel, 1978; Moore et al., 1991; Tarek et al., 2004).

Several approaches have been used for palaeoclimatic and paleovegetational reconstruction from peat deposits, including: i) plant macrofossil analysis (e.g., Barber et al., 1994; Hughes et al.,

* Corresponding author.

E-mail address: c.zaccone@unifg.it (C. Zaccone).

URL: <http://www.claudiozaccane.net> (C. Zaccane).

2000); ii) pollen records (e.g., van der Knaap et al., 2000); iii) testate amoebae analysis (e.g., Charman et al., 2000); iv) determination of humification indexes (C/N, dry density, absorbance measurements of alkaline peat extracts, von Post scale) (e.g., von Post, 1924; Blackford and Chambers, 1993; Kuhry and Vitt, 1996; Roos-Barraclough et al., 2004); v) determination of bulk and compound-specific signature of stable isotopes [$^{13}\text{C}/^{12}\text{C}$ ($\delta^{13}\text{C}$), $^2\text{H}/^1\text{H}$ ($\delta^2\text{H}$), $^{15}\text{N}/^{14}\text{N}$ ($\delta^{15}\text{N}$), and $^{18}\text{O}/^{16}\text{O}$ ($\delta^{18}\text{O}$)] in peat and plant remnants (e.g., White et al., 1994; Aucour et al., 1999; Moschen et al., 2009; Bingham et al., 2010; Loisel et al., 2010; Yamamoto et al., 2010). In particular, for what concerns the use of isotopic signatures, it is generally assumed that:

- Carbon isotope variations in continental settings primarily reflect isotopic variations in land plants over time (e.g., Nordt et al., 1994; Fredlund and Tieszen, 1997). Peat-forming vegetation includes C_3 and C_4 plants that can be clearly distinguished by their $\delta^{13}\text{C}$ values, which are the function of the photosynthetic pathway used to fix CO_2 (Smith and Epstein, 1971). In detail, the C_3 plants (i.e., all trees, most shrubs and herbs, and many grasses) have $\delta^{13}\text{C}$ values (ca. -27‰ , average value) that are much lower than those of atmospheric CO_2 (-7.7‰ , average value), while the C_4 ones (e.g., dry/warm climate grasses and some sedges and herbs), as a result of their more efficient CO_2 fixation, show $\delta^{13}\text{C}$ values more similar to those of the atmosphere (ca. -13‰ , average value) (Deines, 1980; O'Leary, 1988). Furthermore, Brader et al. (2010) demonstrated that $\delta^{13}\text{C}$ values of bulk organic matter (OM) and C_{23} n -alkane from *Sphagnum* moss are species-dependent. Finally, relationships between plant C isotopic ratios and a variety of environmental factors have been proposed (Ménot and Burns, 2001 and refs. therein).
- Processes by which nitrogen is lost from soils lead to ^{15}N enrichment of soil nitrogen relative to atmospheric N_2 ; as a result, N_2 -fixing plants generally have slightly lower $\delta^{15}\text{N}$ values than non-fixing ones (Nadelhoffer and Fry, 1994). Meyers (1997) applied $\delta^{15}\text{N}$ to differentiate between OM residues derived from aquatic ($\delta^{15}\text{N}$ values around 10‰ or higher) and terrestrial plants (ca. 0‰), while Bragazza et al. (2005) utilized $\delta^{15}\text{N}$ signature of *Sphagnum* plants to identify atmospheric N sources.
- As the $\delta^{18}\text{O}$ of meteoric water is correlated with temperature, oxygen isotopes of plants have been used to reconstruct ancient climatic conditions including surface temperature and relative humidity (DeNiro and Epstein, 1979; Burk and Stuiver, 1981).

Despite several studies that have been carried out using the stable isotopes, very little is known about the influence of decay/humification processes on isotopic signature in peat and, to the best of our knowledge, no studies on stable isotopic composition of humic material from undisturbed profiles in relation to paleoenvironmental changes have been published. Indeed, if ombrotrophic bogs are reliable archives of climatic and vegetational changes, we must be sure that historical information on environmental changes, as mirrored in isotopic signature, are effectively preserved in peat deposits over time. Consequently, to evaluate such a central issue, it is important to clarify what occurs during humification processes in this type of ecosystem.

In detail, during the decay and transformation of plant and microbial remains, fresh C is metabolized by microbes. The OM fraction that does not degrade completely to CO_2 forms humic substances (HS); plant lignin and its transformation products, as well as polysaccharides, melanin, cutin, proteins, lipids, nucleic acids, fine char particles, are important components taking part in this process (Stevenson, 1994; Tan, 2003). Several authors (e.g., Wershaw, 1993; Piccolo, 2001; Sutton and Sposito, 2005) now describe HS as aggregates of relatively small biopolymers (200–3000 Da) in various stages of degradation, where hydrogen bonding and weak dispersive forces hold the molecular components together in supramolecular structures. On the other hand, the more traditional and renowned theories describe HS as recalcitrant and refractory high molecular weight heteropolymers with a backbone

of single or polynuclear aromatic rings, joined by various ether, ester, and aliphatic linkages with numerous terminal hydroxyl and carboxyl groups (e.g., Schulten and Schnitzer, 1993; Stevenson, 1994; Schulten and Leinweber, 2000).

Regardless of the structural model considered, humic acids (HA), representing the terminal or semi-terminal step of humification processes, provide a long-term sink for C in soils and sediments (often defined as a “passive” C pool) and show mean residence times up to few millennia (Jenkinson and Rayner, 1977; Stevenson, 1994, 1999; Tan, 2003; Torn et al., 2009). Consequently, if ombrotrophic bogs are reliable archives, climatic and vegetational changes should be recorded also into the HA fraction.

To this aim, our study assesses the isotopic signature of ^{13}C , ^{18}O and ^{15}N in bulk peat (“vertical” changes) and in the corresponding HA fraction (variations occurring within each sample) along an undisturbed chronosequence back to about 2100 years before the present and determines if these isotopic signatures are preserved during the humification process.

2. Material and methods

2.1. Sampling site features

The study site, called Etang de la Gruère (EGr), is a well-preserved ombrotrophic bog in the Jura Mountains of Switzerland ($47^\circ 14' \text{N}$, $7^\circ 02' \text{E}$). The bog is developed at an elevation of 1005 masl on a small island of siliceous sediment. The climate is moist continental, with an annual average temperature of 5.5°C and average precipitation exceeding 1300 mm yr^{-1} (Steinmann and Shotyk, 1997). As the bog is characterized by high acidity ($\text{pH} \sim 4$), the present vegetation (i.e., small shrubs or trees, sedges, and mosses) is quite sparse and dominated by *Sphagnum* species such as *S. cuspidatum*, *S. fuscum*, *S. magellanicum*, *S. nemoreum*, *S. papillosum*, *S. recurvum*, *S. rubellum* and *S. tenellum* (Feldmeyer-Christe, 1990).

The EGr bog shows a maximum peat accumulation of approximately 6.5 m (Joray, 1942), so representing more than 14.5 ka of peat formation (Shotyk et al., 1998). Previous paleovegetation studies based on tree pollen and plant microfossil stratigraphy have evidenced a superficial layer at the sampling site composed of *Sphagnum*-dominated peat with *Eriophorum* down to 25 cm; *Sphagnum* peat, with few *Eriophorum* and *Carex*, from 25 to 50 cm; *Sphagnum*–*Eriophorum* peat from 50 to 250 cm; *Sphagnum*-dominated peat with few *Eriophorum* from 250 to 420 cm, and *Carex* peat with *Eriophorum* from 420 to 650 cm of depth (Joray, 1942).

2.2. Sample collection

A peat profile (core 2 h, $10 \times 10 \times 81 \text{ cm}$), covering the last ca. 2000 years (Zaccone et al., 2008b), was collected in the central domed area of the EGr bog using a Wardenaar profile sampler (Wardenaar, 1987), wrapped in plastic foil, and kept frozen at -18°C until analysis. The core was sliced frozen into 3 cm sections; the edges were systematically discarded being considered as potentially contaminated during the sampling. The first slice ($+3$ – 0 cm) corresponds to the living *Sphagnum* material present on the bog surface. Chemical, physical, and spectroscopic features of both the studied profile (core 2 h) and its corresponding HA fraction have been reported elsewhere (Zaccone et al., 2007b; 2008a), and some of them briefly summarized in Table 1.

Three additional deep samples from a parallel core (core 2p), dated to ca. 9300–11,500 ^{14}C years BP (Shotyk et al., 1998), were also considered in this study in order to test the preservation of isotopic signatures on a millennial scale.

2.3. Humic acids isolation and humification degree indices

Humic acids were extracted from all samples of the 2 h core and from 3 deep peat samples of the 2p core, according to a modified procedure of

Table 1

Average elemental composition of peat and HA samples along the EGr 2 h profile. Corresponding sample depths (in cm) are reported in brackets.

	Ash (mg g ⁻¹)	C (mg g ⁻¹)	N (mg g ⁻¹)	O (mg g ⁻¹)	C/N
<i>Peat</i>					
Maximum value	68.3 (–34.5)	577.7 (–73.5)	25.3 (–28.5)	452.0 (–1.5)	96.8 (–10.5)
Minimum value	4.1 (–13.5)	481.8 (–1.5)	5.9 (–10.5)	328.3 (–73.5)	24.6 (–34.5)
Mean ± St.dev. (n = 26)	24.8 ± 15.9	525.8 ± 29.0	15.4 ± 6.0	394.3 ± 35.8	47.2 ± 20.5
<i>HA</i>					
Maximum value	32.5 (–7.5)	597.6 (–61.5)	34.5 (–34.5)	368.0 (–10.5)	43.8 (–13.5)
Minimum value	11.9 (–31.5)	559.5 (–10.5)	15.3 (–13.5)	318.2 (LL ^a)	19.6 (–34.5)
Mean ± st.dev. (n = 26)	21.1 ± 0.3	580.1 ± 10.6	23.8 ± 4.8	338.2 ± 13.5	29.6 ± 6.0

^a LL, living layer.

the International Humic Substances Society (Swift, 1996). Briefly, peat samples were treated with a 0.5 M NaOH extracting solution at room temperature for about 12 h. After stirring, centrifugation, and filtration through Whatman GF/C filter, the alkaline extract was acidified with HCl to pH 1.5, and allowed to precipitate for 24 h. The sediment was separated from the supernatant by centrifugation, washed with a solution of Milli-Q water + HCl (pH = 3), manually shaken, and centrifuged. Milli-Q water was added to the sediment and stirring was carried out for 3 h. Finally, the HA fraction was transferred to a dialysis membrane (Spectra/Por membrane, MWCO 6–8000) and dialyzed against Milli-Q water, successively replaced at frequent intervals (6–7 h) in order to remove Cl⁻ until the EC of discharged water decreased below 10⁻² dS m⁻¹. Following these treatments, the HA were freeze-dried.

For the preparation of all solutions, high purity water (18.2 MΩ cm) from a Milli-Q Element system (Millipore, Molsheim, France), and high purity grade reagents were used.

The HA extraction yield (EY_{HA}) has been calculated for each sample as follows:

$$EY_{HA} = \frac{W_{HA}}{W_{peat}} \cdot 100$$

where W_{HA} is the dry weight of extracted HA (in g), while W_{peat} is the dry weight of original peat material (in g).

Consequently, a peat humification index (HI_p) was proposed and determined as follows:

$$HI_p = \frac{[C_{HA}] \cdot EY_{HA}}{[C_{peat}]}$$

where $[C_{HA}]$ is the C concentration in HA (in mg kg⁻¹), while $[C_{peat}]$ is the C concentration in the bulk peat material (in mg kg⁻¹).

In order to validate the proposed HI_p, it was compared with other indices often used to estimate the humification degree, i.e., the C/N ratio and the E₄/E₆ ratio, the latter one calculated as the ratio of absorbance at 465 nm and 665 nm (Chen et al., 1977). The UV–vis absorption spectra of peat samples were recorded with a Perkin Elmer spectrophotometer, model Lambda 15.

2.4. Elemental composition

Total C and N content in peat and corresponding HA samples from the 2p core were determined in triplicate, using dry combustion with an elemental analyzer (Fisons EA1108, Milan, Italy). The instrument was calibrated by BBOT [2,5-Bis-(5-tert.-butyl-benzoxazol-2-yl)-thiophen] standard (ThermoQuest Italia s.p.a.). The obtained data were corrected for moisture and ash content.

2.5. Determination of stable isotopic ratios

Isotopic ratios of ¹³C/¹²C, ¹⁵N/¹⁴N, and ¹⁸O/¹⁶O were determined on peat and corresponding HA samples with an Isotopic Ratio Mass Spectrometer (IRMS, Finnigan Delta V Advantage, Thermo Fisher Scientific) coupled with an Elemental Analyser (FlashEA 1112 HT, Thermo Fisher Scientific). The Elemental Analyser was interfaced with the IRMS through the ConFlo III (Thermo) dosing the samples and reference gasses. This system enables the determination δ¹³C and δ¹⁵N of each sample in one run. The δ¹⁸O ratio was determined using a pyrolysis mode.

The Elemental Analyser and the Pyrolyser were both equipped with a PorapackQS (GC column). The isotopic values were expressed in δ(‰) vs. V-PDB (Vienna-Pee Dee Belemnite) for carbon, AIR (Atmospheric nitrogen) for nitrogen, and V-SMOW (Vienna-Standard Mean Ocean Water) for oxygen, according to the following formula:

$$\left[\frac{(R_{\text{sample}} - R_{\text{standard}})}{R_{\text{standard}}} \right] \times 1000$$

where R represents the ratio between the heavy and light isotopes.

Finally, for each run at least one in-house standard was analysed to check the accuracy of the analysis. The precision of measurements was usually ± 0.2‰ for δ¹³C and ± 0.3‰ for δ¹⁵N and δ¹⁸O, respectively.

2.6. Statistical analyses

Statistical correlation and analysis of variance (ANOVA) were performed using the Statgraphics Plus Version 5.1 software. Significant differences were calculated on the basis of the Tukey's test, considering a significance level of $p < 0.05$.

3. Results and discussion

3.1. Peat humification degree with depth

Humification index values range between 7.8% (at 0–3 cm) and 37.1% (at 54–57 cm) and, as expected, they generally increase with depth in the profile, reaching values between 22.5 and 37.1% (26.9 ± 3.8%, mean ± st.dev.) below 21 cm of depth vs. 7.8–20.8% (13.9 ± 4.1%, mean ± st.dev.) in the upper, less decomposed layer.

Thus, the HI_p values suggest that an increase in humification occurs with depth. This finding is consistent with the C/N ratio trend observed in peat along the profile ($R = -0.748$, $p < 0.0001$, Fig. 1), as the gradual transformation of plant raw material into humus leads to a decreasing of the C/N due to, for example, CO₂ production/release, C incorporation into microbial tissues, and chemical fixation of NH₃ and/or amines by lignin-like substances (Stevenson, 1994; Kuhry and Vitt, 1996). This aspect is particularly evident comparing the C/N ratio in peat against those in the more recalcitrant HA fraction (Fig. 2). Moreover, the HI_p trend is also significantly correlated with the E₄/E₆

Fig. 1. Relationship of humification index (HI_p) with C/N ratio and E_4/E_6 determined in peat.

ratio ($R = -0.576$, $p < 0.01$, Fig. 1), because the latter decreases with increasing molecular weight and condensation of aromatic constituents, i.e., with the humification degree (Chen et al., 1977; Stevenson, 1994).

Further details about the chemical and spectroscopic features of peat and corresponding HA from the studied bog are reported elsewhere (Zaccone et al., 2007b, 2008a).

3.2. $\delta^{13}C$ and $\delta^{15}N$ signature

The signature of $\delta^{13}C$ in peat samples ranges between $-28.74 \pm 0.03\text{‰}$ (at 595 cm) and $-24.77 \pm 0.05\text{‰}$ (at 57–60 cm of depth) (Fig. 2). The average $\delta^{13}C$ (-26.2‰) and the range of variation (3.97‰) recorded along the EGr peat profile (spanning the period ca. 11,500 BP–1991 AD) seem to indicate both a dominant contribution of vascular C_3 plants besides *Sphagnum* (Fig. 2), and a possible change in the dominant *Sphagnum* specie (Brader et al., 2010). This finding is in agreement with the C/N ratio trend along the profile (Fig. 2) since, according to Meyers (1994), C/N ratio values >20 should indicate a prominent vascular plant source. In fact, $\delta^{13}C$ and C/N values feature mirror-trends, also underlining an upper part of the core characterized by both the lowest $\delta^{13}C$ values and the highest C/N ones (51–97) (Fig. 2). Also the ratios of syringyl to vanillyl (S/V) and cinnamyl to vanillyl (C/V) phenols reported in a previous work (Zaccone et al., 2008a) seem to indicate the presence of vascular plant sources that were mostly herbaceous angiosperms ($S/V > 0.5$ and $C/V \neq 0$) (Fig. 3). In HA, $\delta^{13}C$ ranges between $-29.01 \pm 0.07\text{‰}$ and $-25.42 \pm 0.05\text{‰}$, underlining a significant depletion of ^{13}C as compared with corresponding bulk peat samples (Table 2 and Fig. 2). The occurrence of lower $\delta^{13}C$ values in HA is

Fig. 2. Distribution of $\delta^{13}C$, $\delta^{15}N$ and C/N ratio between peat and corresponding HA samples along the EGr profile. Age dating is also showed. Data on C/N ratio (2 h core) are from Zaccone et al. (2007b).

Fig. 3. Plot of the syringyl to vanillyl ratio (S/V) vs. the cinnamyl to vanillyl one (C/V) of peat and HA samples from the EGr 2 h core. Ranges for gymnosperms (G), nonwoody gymnosperms (g), angiosperms (A), and nonwoody angiosperms (a) (according to Hedges and Mann, 1979) are also reported. Raw data are from Zaccone et al. (2008a).

probably due to the general depletion of ^{13}C in lignin material demonstrated by early studies (Park and Epstein, 1960; Smith and Epstein, 1971), as lignin and lignin-like substances, according to the most well-known humification theories, are the main precursors of humic molecules (see Stevenson, 1994). Although one might argue that the HA extraction procedure possibly alters the existing C isotopic distribution, it is unlikely. In one study, Prentice and Webb (2010) demonstrated that, in soils with relatively high C contents prior to extraction, $\delta^{13}\text{C}$ values of the humin fractions isolated were similar within them, as well as the $\delta^{13}\text{C}$ values of the humic acid fractions, regardless of extraction technique and extractant utilized (0.5 M NaOH or 0.1 M NaOH + 0.1 M $\text{Na}_4\text{P}_2\text{O}_7$). In another study, Spaccini et al. (2006), studying the isotopic $\delta^{13}\text{C}$ abundance of humic fractions from forested and cultivated soils in Ethiopia, reported similar values for humic and fulvic acids, and humin.

In contrast, $\delta^{15}\text{N}$ shows a significant enrichment of heavier ^{15}N in the HA fraction, with the only exception of the sample at 45–48 cm of depth (Fig. 2). $\delta^{15}\text{N}$ values range between $-4.99 \pm 0.21\text{‰}$ (in the living layer) and $0.46 \pm 0.10\text{‰}$ (at 555 cm of depth) in peat samples, and between $-4.15 \pm 0.05\text{‰}$ and $0.53 \pm 0.05\text{‰}$ in corresponding HA samples. Unlike $\delta^{13}\text{C}$, the $\delta^{15}\text{N}$ is not sensitive to the plant photosynthetic pathway, but is essentially a function of input and mineralization processes within the soil over time (Nadelhoffer and Fry, 1994). The significantly higher values of $\delta^{15}\text{N}$ recorded both in peat and HA samples between 525 and 595 cm of depth underline a change of the main peat-forming species (i.e., *Sphagnum*-dominated peat with *Eriophorum* vs. *Carex* peat with *Eriophorum*, see Joray, 1942), as well as a preferential loss of ^{14}N molecules through kinetic fractionation during biological transformation (Nadelhoffer and Fry, 1994). This isotopic enrichment, usually interpreted as reflecting a greater degree of decomposition (DeNiro and Hastorf, 1985; Nadelhoffer and Fry, 1994; Kendall, 1998), is supported by the decrease of C/N (Fig. 2) and E_4/E_6 ratios, and by the increase of HI_p values (Table 2) along the peat profile.

Thus, during decay/humification processes, a selective degradation of some macromolecules and/or a migration of low molecular weight substances, resulting in an isotopic ratios shift, might occur. Some authors (e.g., Benner et al., 1987; Wedin et al., 1995) state that, as different plant macromolecules (e.g. proteins, lipids, lignin, cellulose) have distinct $\delta^{13}\text{C}$ values, a differential loss of components during decomposition may lead to isotopic shifts, while Bird et al. (1994) hypothesized that even insoluble, mineral-occluded, organic C could be contaminated by “young” OM, thus suggesting that isotopic signatures in soils could be modified by dissolved organic matter (DOM) vertical migration. Furthermore, other authors (e.g., Meyers, 1997) suggest that the OM degradation might cause either depletion or enrichment of the ^{15}N due to the biosynthesis of inorganic N.

On the contrary, our data seem to underline a certain ‘conservative’ behavior of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ during peat humification. In detail, the net difference between $\delta^{13}\text{C}$ values recorded both in bulk peat and in corresponding HA samples ($\Delta^{13}\text{C}_{\text{p-HA}}$) ($0.65 \pm 0.56\text{‰}$, mean $\pm$ st.dev.), is quite low in the deepest part of the profile ($<0.3\text{‰}$ below 525 cm), while it reaches values of 1.5–1.7‰ in the first 10 cm of depth. Also the $\Delta^{15}\text{N}_{\text{p-HA}}$ ($0.53 \pm 0.48\text{‰}$, mean $\pm$ st.dev.) shows values ranging from 0.8 to 1.3‰ in the first 20 cm of depth, and $<0.5\text{‰}$ in the remaining part of the profile. Anyway, despite the differences in terms of absolute values observed between bulk peat and corresponding HA, $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ feature a significantly similar trend throughout the profile ($R^2 = 0.789$, $p < 0.0001$, and $R^2 = 0.926$, $p < 0.0001$, respectively) (Figs. 2 and 4). This finding is in agreement with the significant correlation between C/N

Table 2
Stable isotopic ratios in peat and corresponding HA samples throughout the EGr bog profile. Peat humification index (HI_p) values have been reported in table only for samples for which stable isotopic ratios have been determined.

Sample	Average depth (cm)	HI_p (%)	$\delta^{13}\text{C}$ (‰)			$\delta^{15}\text{N}$ (‰)			$\delta^{18}\text{O}$ (‰)		
			Peat [†]	HA	IEF ^{††}	Peat	HA	IEF	Peat	HA	IEF
2h01	1.5	14.0	-26.53 ± 0.01 ^d	-28.03 ± 0.05 ^c	---	-4.99 ± 0.21 ^a	-4.15 ± 0.05 ^b	+	23.38 ± 0.23 ^d	18.35 ± 0.03 ^d	---
2h04	−7.5	11.0	-25.60 ± 0.03 ^g	-27.32 ± 0.01 ^e	---	-4.61 ± 0.04 ^{a,b}	-3.33 ± 0.02 ^d	+	24.63 ± 0.37 ^{b,c}	19.27 ± 0.27 ^c	---
2h07	−16.5	20.8	-25.58 ± 0.06 ^g	-26.86 ± 0.02 ^f	---	-4.05 ± 0.20 ^c	-2.81 ± 0.09 ^e	+	24.16 ± 0.32 ^{b,c}	19.29 ± 0.22 ^c	---
2h08	−19.5	17.3	-25.82 ± 0.03 ^f	-26.94 ± 0.01 ^f	---	-2.36 ± 0.06 ^f	-1.06 ± 0.24 ^f	+	23.55 ± 0.13 ^b	20.09 ± 0.14 ^b	---
2h11	−28.5	24.0	-26.26 ± 0.05 ^e	-26.57 ± 0.09 ^g	---	-3.02 ± 0.00 ^e	-2.66 ± 0.11 ^e	+	22.57 ± 0.11 ^{b,c}	19.58 ± 0.06 ^{b,c}	---
2h14	−37.5	28.7	-25.80 ± 0.04 ^f	-26.17 ± 0.01 ^h	---	-4.90 ± 0.05 ^a	-4.72 ± 0.04 ^a	+	23.22 ± 0.10 ^a	21.23 ± 0.22 ^a	---
2h17	−46.5	28.2	-25.40 ± 0.04 ^h	-25.58 ± 0.03 ^{l,m}	---	-3.44 ± 0.09 ^d	-3.99 ± 0.04 ^{b,c}	+	23.12 ± 0.16 ^b	20.09 ± 0.09 ^b	---
2h21	−58.5	22.1	-24.77 ± 0.05 ⁱ	-25.42 ± 0.05 ^m	---	-4.13 ± 0.07 ^c	-3.80 ± 0.09 ^c	+	23.34 ± 0.32 ^{cd}	19.15 ± 0.29 ^c	---
2h22	−61.5	27.1	-25.14 ± 0.02 ⁱ	-25.70 ± 0.05 ^{l,i}	---	-4.33 ± 0.07 ^{b,c}	-4.10 ± 0.12 ^{b,c}	+	23.12 ± 0.23 ^c	19.18 ± 0.03 ^c	---
2h27	−76.5	22.5	-25.54 ± 0.00 ^g	-25.84 ± 0.01 ⁱ	---	-4.34 ± 0.18 ^{b,c}	-3.98 ± 0.00 ^{b,c}	+	22.83 ± 0.02 ^b	20.02 ± 0.10 ^b	---
2p43	−525.0	27.1	-27.38 ± 0.03 ^c	-27.54 ± 0.13 ^d	---	0.36 ± 0.08 ^g	0.39 ± 0.08 ^g	+	18.44 ± 0.30 ^e	16.30 ± 0.24 ^e	---
2p46	−555.0	27.8	-28.41 ± 0.02 ^b	-28.38 ± 0.03 ^b	---	0.46 ± 0.10 ^g	0.53 ± 0.05 ^g	+	18.70 ± 0.25 ^e	17.92 ± 0.19 ^f	---
2p50	−595.0	28.4	-28.74 ± 0.03 ^a	-29.01 ± 0.07 ^a	---	0.22 ± 0.06 ^g	0.30 ± 0.01 ^g	+	19.60 ± 0.32 ^f	19.21 ± 0.09 ^g	---

[†] Values (means $\pm$ st.dev.) within each column and followed by the same letter(s) are not significantly different at $p < 0.05$.

^{††} IEF, isotopic enrichment factor in HA vs. peat. “+” indicates an enrichment of the stable isotope under consideration occurring in HA vs. the corresponding peat samples; on the contrary, “−” underlines a depletion of the stable isotope under consideration occurring in HA vs. the corresponding peat samples. ns, *, **, ***: not significant or significant at $p < 0.05$, 0.01 or 0.001, respectively.

ratio values ($R^2 = 0.714$, $p < 0.0001$) recorded in both sample series, and especially with the relationship between $\delta^{13}\text{C}/\delta^{15}\text{N}$ ratios ($R^2 = 0.978$, $p < 0.0001$) (Fig. 4). Thus, C and N isotopic signatures do not seem to be compromised by the humification degree of peat (expressed by the HI_p , C/N ratio and E_4/E_6) (Table 3), which clearly increases along the profile. A similar lack of correlation was reported by Kaislahti Tillman et al. (2010), who compared $\delta^{13}\text{C}$ of cellulose with decay indices (C/N ratio and % Transmittance) in a *Sphagnum* peat sequence from the subarctic Canada.

In addition, $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ signatures seem to be unaffected also by possible DOM migration along the profile, in the sense that translocation processes of organic compounds from the peat solid phase to the aqueous phase could be assumed not to significantly contribute to their mobility throughout the profile. Actually, this finding is consistent with a previous study carried out on another core (2 T) from the same bog (Zaccone et al., 2009b) that hypothesized an OM stratification down the profile resulting in a rather limited vertical migration of DOC and any associated compounds (i.e., water movements predominantly horizontal rather than vertical).

3.3. $\delta^{18}\text{O}$ signature

In peat samples, $\delta^{18}\text{O}$ ranges from $18.44 \pm 0.30\text{‰}$ (at 525 cm) to $24.63 \pm 0.37\text{‰}$ (at 6–9 cm of depth), showing a significant variation around 30 cm of depth, probably due to the “Little Ice Age” event, ca. 1570–1730 AD (Bradley, 1993), as well as lower values in the deep part of the EGr profile (i.e., 2p46 and 2p50 samples), consistent with the last period of the “Younger Dryas” cold event (Shotyk et al., 2001). At the same time, some inconsistency has been observed, including the lowest $\delta^{18}\text{O}$ value ($18.44 \pm 0.30\text{‰}$) detected at 525 cm of depth, corresponding to ca. 9300 year BP, i.e., ca. 2000 years after the “Younger Dryas” event, as reported by Schwander et al. (2000) who described oxygen isotope records from Swiss lake sediments.

Although the $\delta^{18}\text{O}$ signature shows a certain correlation between peat and HA ($R^2 = 0.431$, $p < 0.05$) (Fig. 5), the oxygen isotopic composition does not seem to behave conservatively. In fact, among the stable isotopic ratios considered in this study, $\delta^{18}\text{O}$ is the only one whose absolute values show a significant correlation with both the HI_p ($R^2 = 0.349$, $p < 0.05$) and the C/N ratio ($R^2 = 0.520$, $p < 0.01$) (Fig. 5). In particular, the R values reported in Table 3 show a negative correlation between $\delta^{18}\text{O}$ and the HI_p , and a positive correlation between $\delta^{18}\text{O}$ and the C/N ratio, both underlining lower $\delta^{18}\text{O}$ in more humified peat samples. Furthermore, $\delta^{18}\text{O}$ shows the highest shifts,

Table 3

Correlation coefficient (R) and p -value for the humification proxies considered (HI_p , C/N ratio, E_4/E_6), compared with $\delta^{13}\text{C}$, $\delta^{15}\text{N}$, and $\delta^{18}\text{O}$ determined in peat.

	R	p	n
$\delta^{13}\text{C}$ vs. HI_p	−0.3234	0.2810, ns	13
$\delta^{15}\text{N}$ vs. HI_p	0.4655	0.1089, ns	13
$\delta^{18}\text{O}$ vs. HI_p	−0.5903	0.0337, *	13
$\delta^{13}\text{C}$ vs. C/N	0.4133	0.1604, ns	13
$\delta^{15}\text{N}$ vs. C/N	−0.5699	0.0420, *	13
$\delta^{18}\text{O}$ vs. C/N	0.7208	0.0054, **	13
$\delta^{13}\text{C}$ vs. E_4/E_6	0.2030	0.5060, ns	29
$\delta^{15}\text{N}$ vs. E_4/E_6	−0.4541	0.1190, ns	29
$\delta^{18}\text{O}$ vs. E_4/E_6	0.5192	0.0690, ns	29
HI_p vs. C/N	−0.7480	<0.0001, ***	29
HI_p vs. E_4/E_6	−0.5758	0.0011, **	29
C/N vs. E_4/E_6	0.5257	0.0034, **	29

ns Not significant.

* Significant at $p < 0.05$.

** Significant at $p < 0.01$.

*** Significant at $p < 0.001$.

with $\Delta^{18}\text{O}_{p\text{--}HA}$ ranging between 0.4 and 5.4‰ ($3.2 \pm 1.6\text{‰}$, mean $\pm$ st. dev.).

Compared to bulk peat, corresponding HA samples are depleted in ^{18}O , with $\delta^{18}\text{O}$ values ranging from $16.30 \pm 0.24\text{‰}$ to $21.23 \pm 0.22\text{‰}$ (Fig. 5 and Table 2). The different $\delta^{18}\text{O}$ trend between HA and bulk peat may reflect a ^{18}O depletion mainly due to a general decrease of the oxygen content in the HA fraction during the humification process (see O/C ratio values in HA vs. peat, Zaccone et al., 2007b), with a preferential loss of ^{18}O .

In addition, some influence of the procedure for HA isolation that possibly resulted in a $\delta^{18}\text{O}$ decrease and in a consequent ^{16}O enrichment should be also taken into account.

3.4. Implications for paleovegetational and paleoclimatic reconstructions

When peat undergoes decay/humification processes, some isotopic ratios shift might occur throughout bog profiles, thus altering the reliability of these records.

In order to validate whether isotopic ratios were affected by the humification degree, their absolute values were compared to several indices including the HI_p , the E_4/E_6 and the C/N ratio (Table 3). With the only exception of oxygen isotopes, the lack of correlation with the

Fig. 4. Correlation patterns of $\delta^{13}\text{C}$, $\delta^{15}\text{N}$, C/N ratio and $\delta^{13}\text{C}/\delta^{15}\text{N}$ ratios between peat and corresponding HA samples. Areas in gray highlight samples from the 2p core.

Fig. 5. Distribution and relationship of $\delta^{18}\text{O}$ between peat and corresponding HA samples (age dating is reported in italic). Relationships between $\delta^{18}\text{O}$ and the humification index (HI_p) and between $\delta^{18}\text{O}$ and the C/N ratio in peat are also showed. Areas in gray highlight samples from the 2p core.

humification proxies considered suggests that $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ trends might be preserved along the studied core.

Furthermore, the small differences in isotopic ratio values observed between bulk peat and its corresponding HA fraction raise two main issues.

First, our data clearly show that HA constitute the OM fraction responsible for the 'conservative' behavior of stable isotope signatures throughout the EGr peat profile. Thus, it is possible that molecules providing the stable isotopic signatures are either bound/associated to HA and protected from microbial degradation, producing recognizable, but refractory, biologically derived moieties (according to the new view about HS, see Sutton and Sposito, 2005 and ref. therein), or

assembled through condensation and polymerization in more recalcitrant and refractory high molecular weight heteropolymers (according to the traditional view, see Stevenson, 1994 and ref. therein). Consequently, regardless of the structural model considered, HA molecules could be utilized as proxies in paleoenvironmental studies in general, and in paleovegetational ones in particular, since the fractionation that may occur during decomposition seems to be quite small relative to the original fractionation during C fixation.

At the same time, Fig. 6 underlines that the extent of isotopic shifts between bulk peat and corresponding HA decreases with depth, i.e., with the increase of the HI_p . It is well known that, undergoing humification processes, peat first loses more labile OM, thus resulting in a more

Fig. 6. Relationship of isotopic net differences between bulk peat and corresponding HA samples [$\Delta^{13}\text{C}_{p\text{-HA}}$ (■), $\Delta^{15}\text{N}_{p\text{-HA}}$ (◇), $\Delta^{18}\text{O}_{p\text{-HA}}$ (▲)] and the humification index (HI_p).

refractory organic fraction richer in humic substances. Thus, as deep peat samples have a higher HA content if compared with the surficial ones ($65.6 \text{ mg}_{\text{HA}} \text{ g}_{\text{peat}}^{-1}$ at 0–3 cm of depth vs. $>250 \text{ mg}_{\text{HA}} \text{ g}_{\text{peat}}^{-1}$ below 40 cm), they show an isotopic signature that more closely will mirror that of HA. In any case, at least $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ isotopic trends seem to be preserved.

4. Conclusions

Data on isotopic signatures in bulk peat and corresponding HA along a Swiss undisturbed chronosequence of ca. 12,000 years suggest that ^{13}C and ^{15}N isotopic ratio trends (and, in a lower extent, also the ^{18}O) are well preserved in HA from peat.

In detail, results of the present study seem to support both the role of HA as recalcitrant, stable molecules with a long-term residence time in soils, and the potential of ombrotrophic bogs to be used as “archives” of climatic and vegetational changes occurring in the last 2000 years. This conclusion also has important implications for the global C and N cycles since their sequestration in bogs seem to be ensured by the recalcitrant HA fraction.

However, while the relative signatures of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ were largely unaffected by humification at this site, thus possibly resulting in reliable trends throughout the profile, some significant shifts in absolute isotope values as a result of humification were observed in the HA fraction when compared with corresponding bulk peat samples.

Although a certain relationship was found between peat and corresponding HA, our data do not resolve the issue of the reliability of $\delta^{18}\text{O}$ signature along peat profiles, as its trend seems to be affected by the humification degree of peat.

Thus, since climate and vegetation reconstructions rely on absolute isotope values as well as trends, data about stable isotopic ratios from ombrotrophic peat bogs should be interpreted cautiously in climate and vegetation reconstruction studies, and combined with other proxies to improve their interpretation.

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