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

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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., $^{13}C/^{12}C$ ($\delta^{13}C$), $^{15}N/^{14}N$ ($\delta^{15}N$) and $^{18}O/^{16}O$ ($\delta^{18}O$), 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 ($\delta^{13}C$, $\delta^{15}N$ and $\delta^{18}O$) 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 $\delta^{13}C$ of both peat and HA showed a significantly similar trend with depth ($p = 0.0001$), and the same significant correlation was observed for the $\delta^{15}N$ ($p < 0.0001$). Also the ratio between $\delta^{13}C$ and $\delta^{15}N$ 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 $\delta^{18}O$, our data did not allow the solving of the issue of its reliability as paleoenvironmental proxy.

## 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., Pheiffer-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; Tareq 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.,

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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 [$^{13}C/^{12}C$ ($\delta^{13}C$), $^{2}H/^{1}H$ ($\delta^{2}H$), $^{15}N/^{14}N$ ($\delta^{15}N$), and $^{18}O/^{16}O$ ($\delta^{18}O$)] 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 $\delta^{13}C$ 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 $\delta^{13}C$ 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 $\delta^{13}C$ values more similar to those of the atmosphere (ca. −13‰, average value) (Deines, 1980; O'Leary, 1988). Furthermore, Brader et al. (2010) demonstrated that $\delta^{13}C$ 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 $\delta^{15}N$ values than non-fixing ones (Nadelhoffer and Fry, 1994). Meyers (1997) applied $\delta^{15}N$ to differentiate between OM residues derived from aquatic ($\delta^{15}N$ values around 10‰ or higher) and terrestrial plants (ca. 0‰), while Bragazza et al. (2005) utilized $\delta^{15}N$ signature of *Sphagnum* plants to identify atmospheric N sources.
- As the $\delta^{18}O$ 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°14′N, 7°02′ E). The bog is developed at an elevation of 1005 m asl 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 (pH ~ 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 × 10 × 81 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 addition 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