Evidence for carbon cycling in a large freshwater lake in the Balkans over the last 0.5 million years using the isotopic composition of bulk organic matter

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Abstract

In the DEEP core from the Lake Ohrid ICDP drilling project, the carbon isotope composition of bulk organic matter ($\delta^{13}C_{TOC}$) over the last 516 ka shows a negative correlation with total organic carbon (TOC) and total inorganic carbon (TIC). This relationship is marked by periods of lower $\delta^{13}C_{TOC}$ values corresponding to higher TIC and TOC. Along with TOC/TN, the correlation between $\delta^{13}C_{TOC}$ and $\delta^{13}C_{TIC}$ suggests that most of the organic matter in the core is from aquatic primary production within the lake. The combination of TOC, TIC, and $\delta^{13}C_{TOC}$ is able to disentangle long-term glacial/interglacial cycles and, to a lesser extent, millennial scale climate variability. Over the longer term, $\delta^{13}C_{TOC}$ shows modest variability, indicating that the $\delta^{13}C$ of the dissolved inorganic carbon (DIC) pool is stabilised by the supply of karst spring water characterised by $\delta^{13}C_{DIC}$ influenced by the bedrock $\delta^{13}C$ value, and the long residence time of the lake water and well mixed upper water column promoting equilibration with atmospheric CO_2. However, comparison between arboreal pollen (AP%), TIC and TOC data indicates that the $\delta^{13}C_{TOC}$ signal is modulated by the leaching of soil CO_2 through runoff and spring discharge, changes in primary productivity, and recycling of organic matter within the lake, all affecting $\delta^{13}C_{DIC}$. Exceptionally low $\delta^{13}C_{TOC}$ during some interglacial periods (e.g. MIS7 and MIS9) possibly indicate rapid intensification of organic matter recycling and/or increasing stratification and enhanced methanogenesis, even if the latter process is not supported by the sedimentological data.

Keywords: Pleistocene, Paleolimnology, Europe, stable isotopes, organic matter, Lake Ohrid.

1. Introduction

A defining feature of the Quaternary Period is the quasi-periodic expansion (glacial) and contraction (interglacial) of Northern Hemisphere ice sheets (Lisiecki and Raymo, 2005). According to the astronomical theory of ice ages proposed by Milankovitch (1941), these glacial-interglacial cycles are driven by variations in the Earth's axial inclination and orbit around the sun that affect the seasonal and latitudinal distribution of incoming solar radiation. Although the general characteristics of climate during glacial and interglacial phases are global in extent, detailed regional investigations show that they have different expressions in different archives, as a result of the complexity of the climate system and its regional components. Glacial to interglacial climatic changes produce different effects on individual components of the continental environment (e.g. soil, vegetation, hydrology, and fauna), which can be disentangled using multi-proxy comparisons between regional archives. Most of the information on long-term (multiple glacial cycles) climatic variability comes from marine records (e.g. Lisiecki and Raymo, 2005; Hodell et al., 2013), and ice cores (e.g. EPICA-community-members, 2004; North Greenland Ice Core Project Members, 2004). However, one of the limitations when understanding the complexity of the climate system and its impact on the terrestrial environment is the paucity of long and continuous archives on land (Past Interglacials Working Group of PAGES, 2016).

In recent years, very long and important lacustrine records have been obtained as part of the International Continental scientific Drilling Program (ICDP; e.g. Litt et al., 2014; Johnson et al., 2016; Wagner et al., 2017). Proxies from lake records, for example pollen and stable isotopes, can potentially provide regional paleoenvironmental and paleoclimatic information (e.g. Tzedakis et al., 1997; Sadori et al., 2008), and can be used to correlate change across regions (e.g. Milner et al., 2013; Zanchetta et al., 2016). Other proxies (e.g. organic matter, biogenic silica, chemical composition) provide more local information e.g. primary productivity or lake stratification (Hodell and Schelske, 1998; O'Beirne et al., 2017), and/or information on erosion of the catchment including input of nutrients and soil development (Mourier et al., 2010; Arnaud et al., 2016). Overall, different proxies recovered and measured in lake archives can provide crucial information on the different responses of the terrestrial environment to climate forcing over glacial-interglacial timescales (e.g. Wilson et al., 2015). Here we focus on the carbon isotope composition of bulk organic matter ($\delta^{13}C_{TOC}$), which can help in understanding past lake evolution in relation to catchment vegetation and soil development, together with productivity and recycling processes, complementing inferences obtained from other proxies such as pollen and other biological indicators (e.g. Meyer, 1994; Meyers and Lallier-Vergès, 1999; Leng et al., 2010, 2013; Whittington et al., 2015).

We measured $\delta^{13}C_{TOC}$ in the "DEEP" core retrieved from Lake Ohrid under the umbrella of the ICDP project: Scientific Collaboration on Past Speciation Conditions in Lake Ohrid (SCOPSCO) (Wagner et al., 2014ab, 2017). Here, we compare our $\delta^{13}C_{TOC}$ data with other proxy data obtained from the same sediment core over the last 516 ka (Wagner et al., 2017). Previous studies on Lake Ohrid have shown that the sediments contain components (e.g. biogenic silica, organic matter and carbonate content) that are highly sensitive to environmental changes (e.g. Wagner et al., 2008, 2009, 2010; Vogel et al., 2010; Leng et al., 2013; Lacey et al., 2014, 2016; Francke et al., 2016; Sadori et al., 2016), and these have revealed how the lake has evolved through more than half a million years of climate change (Wagner et al., 2017).

2. General setting

Lake Ohrid is located on the southern Balkan Peninsula between Albania and Macedonia at an altitude of 693 m a.s.l. (Fig. 1). The lake developed in a tectonic depression (graben) formed during the latter phases of the Alpine Orogeny in the Pliocene (Aliaj et al., 2001; Lindhorst et al., 2015). The lake is ca. 30 km long and 15 km wide, with a maximum water depth of 293 m and a total volume of 50.7 km^3 (Lindhorst et al., 2015). The hydrological catchment of Lake Ohrid comprises an area of 2393 km^2 incorporating Lake Prespa (848 m a.s.l.) and its catchment (Popovska and Bonacci, 2007) as the two lakes are connected via a karst aquifer passing through the Galicica and Suva Gora mountain ranges. Karst springs depleted in nutrients and minerogenic matreial represent about 55% of hydrologic input to Ohrid (this may have been higher in the past, see Lacey and Jones, this volume). Up to 50% of the karst waters are estimated to have originated from Lake Prespa (Anovski et al., 1992; Matzinger et al., 2007). Direct precipitation on the lake, together with river and surface runoff account for the remaining 45% of the hydrologic input. The surface outflow through the river Crn Drim (60%) and evaporation (40%) represent the main hydrologic outputs (Matzinger et al., 2006a). According to Matzinger et al. (2006a) the estimated theoretical hydraulic water residence time is ca. 70 years (Matzinger et al., 2006a), which should be reduced to 45 years if evaporation is considered (Wagner et al., 2017). However, Wagner et al. (2017) suggested that the real residence time could be much longer (up to 3 to 4 times).

Presently, Lake Ohrid is oligotrophic and oligomictic, although the littoral zones of the lake exhibit a slightly higher trophic state (Wagner et al., 2017). Whilst the upper c. 150 m of the lake water mix every year, the lake is stratified by salinity below 150 m, where mixing occurs on a sub-decadal cycle (Matzinger et al. 2006b). However, the oligotrophic conditions allow bottom-water oxygen concentrations of above 4 mg L^{-1} even during years without complete overturn (Matzinger et al., 2006b).

Due to the sheltered position of the lake in a relatively deep basin surrounded by high mountain ranges and due to the proximity of the Adriatic Sea, the climate of the Lake Ohrid watershed shows both Mediterranean and continental characteristics (Watzin et al., 2002; Panagiotopoulos et al., 2013). The average annual air temperature for the period between 1961 and 1990 was +11.1°C, with a maximum temperature of +31.5°C and a minimum temperature of –5.7°C. The average annual precipitation for the Ohrid catchment amounts to approximately 900 mm (Popovska and Bonacci, 2007), and the prevailing wind directions follow the N–S axis of the Ohrid valley.

The lake is considered to be the oldest in continuous existence in Europe. Preliminary analyses from DEEP core sediments indicate that the age of Lake Ohrid is between 1.3 and 1.9 Ma (Baumgarten et al., 2015; Lindhorst et al., 2015; Wagner et al., 2014ab, 2017). The extended limnological history, hydrological conditions, and the presence of >300 endemic species make Lake Ohrid a hotspot of biodiversity and a site of global significance (Albrecht and Wilke, 2008; Föller et al., 2016).

3. Material and methods

A total of 1526 m of sediment cores were recovered from six parallel core holes at Lake Ohrid's DEEP site (5045-1) in 2013 using the Deep Lake Drilling System (DLDS) of DOSECC (Wagner et al., 2014ab; 2017). The main coring site for the drilling project was chosen after systematic hydroacoustic surveys, which were carried out between 2004 and 2009. The DEEP site (N 41°02'57'', E 020°42'54"; Fig. 1) is located in the central basin of Lake Ohrid in a basement depression with an estimated maximum sediment fill of 680 m and 243 m water depth (Fig. 1; Lindhorst et al., 2015; Wagner et al., 2017).

Here, we use the samples from the DEEP site composite profile, which cover the last 516 kyr (based on the age model proposed by Francke et al., 2016). This age model is based on 11 tephra layers (Leicher et al., 2016) and was refined by tuning the bio-geochemical proxy response to local summer insolation. For the interval corresponding to Marine Isotope Stage (MIS) 5, additional tuning points were obtained by comparison with regionally well-dated continental archives (Zanchetta et al., 2016).

Detailed core descriptions and methodologies for the chronology, geochemical measurements and pollen analyses can be found in Francke et al. (2016), Lacey et al. (2016), Leicher et al. (2016), and Sadori et al. (2016), and are not therefore discussed here. Pollen data have been updated by new counts (Sadori et al., 2018) for some intervals. $\delta^{13}C_{TOC}$ data from the DEEP sediments is presented here for the first time. 315 samples were analysed at 64 cm intervals, corresponding to an average temporal resolution of ca. 1600 years. $\Delta^{13}C_{TOC}$ data are compared to total organic carbon (TOC), total inorganic carbon (TIC), TOC/total nitrogen (TOC/TN, given as the atomic ratio), TOC/total sulphur (TOC/TS), and biogenic silica (BSi) published in Francke et al. (2016), $\delta^{13}C$ of TIC ($\delta^{13}C_{TIC}$) published in Lacey et al. (2016), and arboreal pollen percentage (AP%) published in Sadori et al. (2016). In the DEEP core, $\delta^{13}C_{TOC}$ from the Late Glacial-Holocene was not considered in detail because of the nearby high-resolution study from the LINI site (Fig. 1; Lacey et al., 2015). Data from the LINI site are included here to complete the DEEP core $\delta^{13}C_{TOC}$ record for this interval.

Surface soil samples from 14 different locations around Lake Ohrid (Fig. 1), were collected to cover a range in altitudes and vegetation types (Table 1). The soil samples were dried at 40°C, after which root and plant remains were discarded by hand-picking and sieving to <2 mm. Samples were then ground by hand before being immersed in 10% HCl to remove TIC, rinsed several times with deionized water to near-neutral pH, and oven dried at 40°C for 24 h. The $\delta^{13}C_{TOC}$ data were obtained at the IGG-CNR of Pisa by producing CO_2 via combustion using a Carlo Erba 1108 elemental analyser, interfaced to a Finnigan DeltaPlusXL via the Finnigan MAT Conflo II interface. Results were calibrated against the Vienna Pee Dee Belemnite (VPDB) scale using international standards (ANU-sucrose and polyethylene foil from NIST) and a within-run standard (graphite) in order to correct for drift. Average analytical reproducibility for these samples was ±0.10 ‰.

Total Carbon (TC) and TN contents of soils were measured using a Carlo Erba 1108 elemental analyser. TOC was quantified from the difference between TC and TIC, which was measured with a De Astis calcimeter (reproducibility obtained on pure grade calcite and samples was < ±5%). These measurements were also used to calculate the TOC/TN ratio.

We correlate our data to the Marine Isotope Stages (MISs) but acknowledge there are different responses within marine and continental proxies (e.g. Sánchez-Goñi et al., 1999; Shackleton et al., 2003). We use the correlation proposed by Francke et al. (2016) between the DEEP record and MISs defined on the stacked benthic isotope curve of Lisieki and Raymo (2005). When we use “warmer periods” or “warm stages” we refer to the entire duration of periods typically associated with odd-numbered MISs, conversely “cold periods” is used for glacial periods usually correlated with even-numbered MISs. The definition of “interglacial” is complex (e.g. Past Interglacials Working Group of PAGES, 2016 and references therein) and it is important to note that the term “warm stage” (e.g. MIS 5, 7) is not synonymous with the term “interglacial”, which usually corresponds to a much shorter interval of time (e.g. Railsback et al., 2015; Past Interglacials Working Group of PAGES, 2016).

We use the term “transition” instead of “termination” for the passage between glacial and interglacial periods, as suggested by Kukla et al. (2002), as the definition of “termination” is defined by the benthic marine isotope records (e.g. Broecker and van Donk, 1970). Govin et al. (2015) suggested to use the term "Penultimate deglaciation" to refer to the climatic transition occurring between full MIS 6 glacial and MIS 5e interglacial conditions, where the terms "transition" and "deglaciation" could be used interchangeably and also for older "transitions" before MIS 6.

4. Results

The TOC content of the soil samples ranges from 1.4 to 12.3 % (mean 5.5 %) and N contents from 0.1 to 1.2 % (mean 0.4 %). The TOC/TN ratio of the topsoil samples has an average of 16.8 ±4.7, ranging from 11.7 to 27.2 (Table 1, Fig. 2). $\delta^{13}C_{TOC}$ values range from –22.8 ‰ to –25.5 ‰. These values are similar to topsoils dominated by C3 vegetation in the Mediterranean region (e.g., Hartman and Danin, 2010; Prendergast et al., 2017).

Figure 3 shows $\delta^{13}C_{TOC}$ data from the core alongside TOC, TIC, TOC/TS, BSi and TOC/TN. Through the core $\delta^{13}C_{TOC}$ range from –26‰ to –34 ‰. In general, sediments from colder stages (MIS 12, 10, 8, 6, 4, 2) are characterized by higher $\delta^{13}C_{TOC}$ values, ranging from –25.7 to –29.4 ‰, with an average of –27.1 ± 1.0 ‰. These intervals contain lower TIC, TOC and BSi, and show very low TOC/TN (<6) and TOC/TS (<20) (Francke et al., 2016). They also correspond to silty clayey, massive and mottled hemipelagic sediment (lithotype 3 of Francke et al. (2016)). The $\delta^{13}C_{TOC}$ average value calculated for the warmer stages (–28. 7 ± 1.4 ‰) is slightly lower than the average value for the glacial periods (–27.1 ± 1.0 ‰) and warmer stages generally show more millennial-scale variability compared to glacial phases. In particular, MIS 9 and 7 have more variable $\delta^{13}C_{TOC}$ from –31.9 to –27.0 ‰ and from –32.6 to –26.5 ‰, respectively. In contrast, MIS 13, MIS 5 and the Holocene have higher mean $\delta^{13}C_{TOC}$ in comparison with the others warmer periods (–28.1±1.0, –28.1±0.9, and –27.2±0.9 ‰, respectively). Warmer stages also have higher TIC, TOC, and BSi contents, and higher TOC/TN and TOC/TS (Francke et al., 2016). These sediments correspond to hemipelagic massive or mottled calcareous-silty-clay or slightly calcareous-silty-clay (lithotype 1 and 2 of Franke et al., 2016).

The $\delta^{13}C_{TOC}$ data is negatively correlated with TOC, TOC/TN, and TIC (Table 2, Fig. 2). When considering the intervals characterised by high TIC contents, which correspond mostly to warmer stages, and where calcite is thought to be the main mineral almost exclusively of endogenic origin (Francke et al., 2016; Lacey et al., 2014, 2016), $\delta^{13}C_{TOC}$ and $\delta^{13}C_{TIC}$ show a positive correlation (Fig. 4). Colder periods are often marked by very low or no TIC (Francke et al., 2016), and sometimes the occurrence of small amounts of diagenetic siderite (Lacey et al., 2016).

5. Discussion

5.1 Origin of organic matter in the lake

Organic matter within lake sediments can have different origins in response to changes in aquatic primary production, input from catchment vegetation, and the amount of particulate and dissolved material transferred to the lake. In addition, organic matter can also be subject to differential degradation and dilution effects resulting from different abundances of inorganic compounds (Meyers and Terranes, 2001). Hence, the $\delta^{13}C_{TOC}$ of the organic matter in lake sediment may reflect a variety of environmental factors (e.g. Meyer and Ishiwatari, 1994).

Biomarkers in the more littoral cores from Lake Ohrid (Holtvoeth et al., 2016, 2017), show the presence of organic matter that originated from the terrestrial environment (specifically, pollen, litter and soils). However, most of the DEEP record shows TOC/TN ratios <10, suggesting that the majority of organic matter is most likely aquatic in origin (e.g. Meyer and Ishiwatari, 1994). Only intervals within some of the warm stages show values >10 (in particular the interglacials MIS 5e and MIS11c, Fig. 3). TOC/TN > 10 could suggest an increased contribution of terrestrial organic matter. An increase in terrestrial organic matter input during the warmer phases could be the result of increased runoff and vegetation cover, as indicated by the pollen record (Sadori et al., 2016, Fig. 3). However, Francke et al. (2016) consider a high contribution of allochthonous organic matter to the DEEP site to be unlikely and suggested that higher TOC/TN could be the result of early diagenetic selective loss of N (Cohen, 2003). This concurs with the results of Rock-Eval pyrolysis analyses from the nearby LINI site Holocene record, which is thought to contain very low amounts of terrestrial material (Lacey et al., 2015). Proxies for clastic input (e.g. the amount of K, Fig. 3) substantially decrease during warmer phases (Francke et al., 2016), which might imply overall less allogeneic supply to the central part of the lake including eroded terrestrial organic matter. This is also in agreement with modern top soil organic matter, which has both higher TOC/TN and higher $\delta^{13}C_{TOC}$ values than the DEEP core sediments (Fig. 2). However, it should also be considered that the modern $\delta^{13}C$ values of terrestrial organic matter are not necessarily representative of past conditions. The $\delta^{13}C$ of terrestrial organic matter changes with variations in atmospheric CO_2 concentration and climatic conditions (O'Leary, 1981; Ehleringer et al., 1997; Kohn, 2010; Diefendorf et al., 2010) as well as due to the recent decrease in the $\delta^{13}C$ of atmospheric CO_2 caused by anthropogenic greenhouse gas emissions (e.g. Keeling, 1979). Cold periods generally have lower atmospheric CO_2 concentrations (Jouzel et al., 2007), where colder and drier conditions lead to higher $\delta^{13}C$ of terrestrial plants (Kohn, 2010; Diefendorf et al., 2010).

If most of the organic matter from the DEEP core sediment is from aquatic primary producers, changes in $\delta^{13}C_{TOC}$ should largely depend on changes in the $\delta^{13}C$ of the lacustrine dissolved inorganic carbon pool ($\delta^{13}C_{DIC}$) (e.g. Whittington et al., 1996; Zanchetta et al., 2007a). As shown in Figure 4, $\delta^{13}C_{TOC}$ and $\delta^{13}C_{TIC}$ of the DEEP core sediment show a strong positive correlation (r = 0.60) during warmer intervals that contain primarily endogenic calcite, further evidence for the prevalent autochthonous nature of the organic matter.

5.2 $\delta^{13}C_{TOC}$ changes at the glacial-interglacial scale

It is interesting to note that the average value of $\delta^{13}C_{TIC}$ and $\delta^{13}C_{TOC}$ are +0.42±0.62 ‰ and –27.96±1.45 ‰, respectively. In particular, $\delta^{13}C_{TIC}$ indicates that the $\delta^{13}C_{DIC}$ has been continuously high and reasonably stable over the past 516 ka. This can be related to the significant and relatively constant contribution of waters from karst springs sourced from marine carbonate bedrock which have an average $\delta^{13}C$ of +1.1‰ (Leng et al., 2010). It will also be supported by the long residence time of the lake water and a well-mixed upper water column which promote equilibration with atmospheric CO_2 (Leng and Marshall, 2004). However, fluctuations beyond this "baseline" over glacial to interglacial cycles need to be evaluated.

As discussed above in Section 5.1, if most of the organic matter originated within the lake, the differences in $\delta^{13}C_{TOC}$ between colder and warmer periods is mostly dependent on $\delta^{13}C_{DIC}$. During glacial periods, restricted formation and/or poor preservation of endogenic calcite in the DEEP core prevents the measurement of $\delta^{13}C_{TIC}$, but in this absence, $\delta^{13}C_{TOC}$ should be a good first-order proxy for long-term changes in lake $\delta^{13}C_{DIC}$. $\delta^{13}C_{TOC}$ is negatively correlated with TIC and TOC (Table 2), which are good indicators of primary productivity in the lake (Francke et al., 2016), and negatively correlated with Arboreal Pollen percentage (AP%, Fig. 5), which is a good indicator of wet/dry and cold/warm conditions (Sadori et al., 2016). This suggests that $\delta^{13}C_{TOC}$ (and $\delta^{13}C_{DIC}$) is dependent on glacial to interglacial conditions, forced fundamentally by climatic factors. Decreasing $\delta^{13}C_{DIC}$ values are often derived from CO_2

sourced from the oxidation of organic matter, where two main processes can control $\delta^{13}C_{DIC}$. One is the external input of oxidized organic matter from either increasing the rate of productivity and/or quantity of CO_2 leaching from catchment soils (Diefendorf et al., 2008; Lézine et al., 2010; Lacey et al., 2015). The amount of soil CO_2 derived by root and microbial respirations is largely influenced by local vegetation typology and climate, with higher CO_2 production is related to wetter and warmer conditions (e.g. Raich and Schlesinger, 1992). $\delta^{13}C_{DIC}$ in rivers and springs is also mediated by the weathering of different lithologies, biological activity, and mineral precipitation (e.g. Yang et al., 1996; Karim and Veizer, 2000; Kanduč et al., 2007), which can change over seasonal to longer- (i.e. glacial to interglacial) time scales. Despite these complications, it is reasonable to expect that the amount of soil-derived CO_2 will increase during periods of wetter and warmer climatic conditions. Higher temperatures and precipitation during these intervals would have supported the expansion of forests in the catchment leading to greater soil productivity and CO_2 formation in soils, as well as increasing the subsequent leaching of this CO_2 by rivers, groundwaters and springs to the lacustrine system. Conversely, soil-derived CO_2 will decrease during periods of colder and drier conditions where vegetation is less developed, soil biological activity is reduced and drier conditions would have reduced CO_2 leaching (e.g. Zanchetta et al., 2007a; Lézine et al., 2010; Regattieri et al., 2015). A significant correlation between AP% and $\delta^{13}C_{TOC}$ suggests that soil CO_2 delivered to the lacustrine system is an important mechanism to drive $\delta^{13}C_{DIC}$, considering the significant contribution of karst springs to the lake's water budget.

A second source of ^{13}C-depleted DIC is the internal recycling of organic matter in the water-column and bottom sediments. Oxidation of organic matter produces CO_2 with $\delta^{13}C$ values close to those of the original organic matter, which is ^{12}C-enriched during photosynthesis (Deines, 1980). Therefore, well-ventilated lakes may recycle organic matter rapidly and efficiently, producing lower $\delta^{13}C_{DIC}$ and subsequently lower $\delta^{13}C_{TOC}$, owing to successive steps of oxidation and photosynthesis. Although partial stratification of the water column takes place (by temperature above 150 m depth and by salinity below 150 m) due to holomictic conditions, bottom waters are never depleted in oxygen due to the lake being oligotrophic. Therefore, organic matter recycling is probably efficient in the upper part of the water column in both glacials and interglacials. Moreover, enhanced primary productivity during warmer periods, as indicated by higher TIC and TOC, would have increased the amount of ^{12}C-enriched CO_2 from the oxidation of organic matter available to be recycled by photosynthetic organisms, thereby driving lower $\delta^{13}C_{TOC}$. In addition, very low $\delta^{13}C_{TOC}$ of less than –32‰ occur at ca. 220 ka (MIS7c), 243 ka (MIS7e), 288 ka (MIS9a) and 313 ka (MIS9c), which may suggest periods of enhanced organic matter recycling and/or methane oxidation. Methane oxidation produces strongly ^{13}C-depleted CO_2 (e.g. Whiticar et al., 1986), and methanogenesis could have been important during periods of higher TOC accumulation promoting anoxic conditions and stratification. However, sedimentological observations suggest complete anoxia at the bottom of the lake has not taken placed (in agreement with present day oxygen concentration; Matzinger 2006a), possibly suggesting that this mechanism is a less likely the driver of very low $\delta^{13}C_{TOC}$.

Overall, lower $\delta^{13}C_{TOC}$ values during warmer periods at DEEP site could be related to the release of a higher amount of soil CO_2, associated with an increase in soil respiration and/or to an increased flux of dissolved soil CO_2 to the lake (both related to warmer and wetter conditions). This would be consistently accompanied by higher primary productivity in the lake (as suggested by higher TIC, TOC and BSi, Francke et al. (2016), Fig. 3) and increased organic matter recycling, which in turn drives lower $\delta^{13}C_{DIC}$, and provides a mechanism to explain the lowest $\delta^{13}C_{TOC}$ values. The increased storage of ^{13}C-depleted organic matter in the lake sediment (i.e. higher TOC) during warmer periods does not affect the total isotope budget of the DIC and drive higher $\delta^{13}C_{DIC}$, suggesting that the DIC pool is fully replenished by external soil-derived CO_2, recycled, and atmospheric CO_2.

On the contrary, higher $\delta^{13}C_{TOC}$ values during glacial periods may be related to reduced soil CO_2 production in forest soils. Colder conditions promote the development of ecosystems charaterised by more open and grass vegetation (Sadori et al., 2016), which have soils characterised by lower CO_2 soil respiration rates (Brook et al., 1983; Raich and Schlesinger, 1992). Furthermore, drier conditions typically associated with glacial phases can also reduce soil CO_2 production (Harper et al., 2005). Within these drier conditions, there would also be a change between C3 and C4 vegetation (Boretto et al., 2017) with the latter having significantly higher $\delta^{13}C$ values (Deines, 1980). For instance, this could be due to the increase of grass and sedge, which include species that utilise the C4 photosynthetic pathway (in particular *Amaranthaceae*; e.g. Ehleringer et al., 1997). This is also supported by a strong decrease in AP% and increases in herbs (non arboreal pollen, NAP) during glacial times, as evident in the pollen record of the DEEP core (Fig. 3, Sadori et al., 2016). A reduction in external soil CO_2-production and ^{12}C DIC-replenishment then increases the importance of equilibration with the atmospheric CO_2 in producing higher $\delta^{13}C_{DIC}$ values (Leng and

Marshall, 2004). All these processes can account for higher $\delta^{13}C_{TOC}$ during glacials. In addition, there is an internal mechanism for increasing $\delta^{13}C_{DIC}$ related to enhanced aquatic primary productivity, which leads to a ^{13}C-enrichment in the DIC due to preferential uptake of ^{12}C by phytoplankton during photosynthesis (Hollander and McKenzie, 1991; Meyers, 1994; Bade et al., 2004). If photosynthetic activity is associated with a higher rate of organic matter burial and a reduced rate of soil CO_2 replenishment to the lake, strong ^{13}C-enrichment of $\delta^{13}C_{DIC}$ may also occur (Zanchetta et al., 2007a). Although higher $\delta^{13}C_{DIC}$ could be related to enhanced aquatic primary productivity (e.g. Hollander and McKenzie, 1991; Meyers, 1994; Bade et al., 2004) glacial intervals have low TOC, TIC and BSi accumulation (Francke et al., 2016; Figure 3), suggesting overall low productivity.

Overall, periods of particularly low $\delta^{13}C_{TOC}$ can be related to the "right" combination of temperature, and the amount and seasonal distribution of precipitation allowing greater soil CO_2 production along with environmental conditions that promote CO_2 leaching and delivery to the lake at times of increased aquatic primary production. This combination does not necessarily correspond uniquely to interglacials characterised by higher temperature. For instance, periods of drought during the main algal bloom, which usually occurs between late spring to early autumn (Lacey et al., 2016), may reduce the soil CO_2 flux into the lake alongside additional increased evaporation favouring CO_2-outgassing and fractionation (Talbot, 1990) and progressive ^{13}C-enrichment due to within-lake photosynthesis. This may be the case during MIS 5e, which does not have a particularly prominent minima in the $\delta^{13}C_{TOC}$ record (Fig. 3, 6) and for which regional data suggest a climate regime characterised by high seasonality with an extended period of summer drought (Milner et al., 2012; Sinopoli et al., 2018). Conversely, for MIS 9a, which has a prominent $\delta^{13}C_{TOC}$ minima, regional high-resolution (Fig. 3, 6) pollen data suggest wetter and cooler conditions throughout the year (Fletcher et al., 2013), whereas MIS 9e, again with a prominent minima in $\delta^{13}C_{TOC}$ (Fig. 3, 6), also appears to be marked by very wet conditions from speleothem data from the Ohrid catchment (Regattieri et al., 2018).

5.3 Glacial to interglacial transitions

Looking in detail at the correlation between $\delta^{13}C_{TOC}$ and the other lake proxies, some differences emerge during glacial-interglacial transitions (Fig. 6). Although part of these differences may be amplified by the variable resolution of the proxies, and by the fact that TIC and TOC are probably affected by dissolution/degradation during glacial periods, the overall differences appear consistent. When changes in $\delta^{13}C_{TOC}$ are in phase with other proxy records this may suggest a complacent behaviour of the different parts of the system previously described. In case of glacial/interglacial transitions where $\delta^{13}C_{TOC}$ lags AP % by several thousand years, it may indicate a late response of $\delta^{13}C_{TOC}$ to the warming and wetting of the climate. For instance, an abrupt lowering of $\delta^{13}C_{TOC}$ occurs at ca. 430 ka, during the MIS 12-MIS 11 transition. This is in phase with increased AP %, however TIC and TOC start to increase ca. 2000-5000 years later. The transition between MIS 10 and MIS 9 appears less sharp than MIS 12-MIS 11, and the precise timing of the transition is hard to define, even if the highest rate of $\delta^{13}C_{TOC}$ lowering seems to lag AP % by ca. 2000 years (taking into account record resolution). The transition between MIS 8 and MIS 7e shows a rapid decrease of $\delta^{13}C_{TOC}$ values at ca. 245 ka, lagging behind the increase of AP % by ca. 1500 years (at the limit of our records resolution), with TIC and TOC in phase with the change observed in $\delta^{13}C_{TOC}$. The transition between MIS 7d and MIS 7c is complex, but the decrease in $\delta^{13}C_{TOC}$ and the increase in AP %, TIC and TOC appear closely in phase. During the MIS 6-MIS 5e transition, AP % shows a sharp increase at ca. 130 ka, while $\delta^{13}C_{TOC}$ starts to decrease around 3000 years later, but both reach “interglacial” values at ca. 127 ka.

As discussed previously, pollen analyses on the sediments of the DEEP site sequence revealed that high tree pollen percentages are broadly associated with warmer and wetter climate conditions on the Balkan Peninsula (Sadori et al., 2016). Changes in AP% is explained mainly by altitudinal upward migrations of the tree line in response to climate warming and increased rainfall. This implies that forests can expand rapidly at lower altitudes (also in terms of forest density) but they can take additional time to expand to higher altitudes in areas that experienced strong soil erosion linked to glacial conditions. Indeed, although still poorly studied, the Ohrid catchment appears to have been partially covered by glaciers in the past (Ribolini et al., 2011, 2018; Gromig et al., 2018), and the severity and extent of these glaciations probably had an effect on soil and vegetation recovery in the mountainous areas surrounding the lake, which have an important role in karst recharge. This may indicate that, at the catchment scale, soil development and CO_2 delivery to the lacustrine system is somewhat delayed compared to the regional climatic shift toward warmer/wetter conditions during glacial-interglacial transitions, as indicated by pollen (Holtvoeth et al., 2017), due to the slower soil recovery at higher altitude. Based on differences in the timing of response of oxygen and carbon isotopes, it has already been suggested that in some mountain areas of the Mediterranean soil development and soil-CO_2 delivery to karst aquifers is delayed compared to climate amelioration at glacial-interglacial transitions (Zanchetta et al., 2007b; Regattieri et al., 2014; Bajo et al., 2017). More specifically, the progressive development of soils after the sudden increase in precipitation has also been inferred from a speleothem record from the Ohrid catchment following the glacial maximum of MIS 8 (Regattieri et al., 2018). Thus, a delayed recovery of soil, meaning a delayed supply of soil CO_2 to aquifers, appears likely for some glacial/interglacial transitions at Ohrid, especially considering that the lake is largely fed by karst springs with an important recharge component sourced from high altitude. The degree of delay probably depends on a variety of factors, for example the intensity of the previous glacial period (e.g. presence of glaciers), which may affect the rock substratum and its alteration, influencing soil and vegetation development during the subsequent interglacial or warm period. Some of these aspects could be clarified through higher resolution records of single glacial to interglacial transitions and using soil biomarkers, as recently reported for a littoral core of lake Ohrid, which has showed complex patterns of soil and vegetation development and erosion during la last transition and the MIS5 (Holtvoeth et al., 2017).

6. Conclusions

Sediments stored in Lake Ohrid provide a continuous archive of long-term climate change and catchment landscape evolution. The comparison of $\delta^{13}C_{TOC}$ with other proxies (TIC, TOC, TOC/TN, TOC/TS, K, $\delta^{13}C_{TIC}$ and AP %) indicates that most of the organic matter in the sediments originates from aquatic primary production. On longer time scales, $\delta^{13}C_{TOC}$ shows relatively modest variability indicating that the $\delta^{13}C_{DIC}$ signal is probably stabilised by the long residence time of the lake and well mixed upper water column, promoting equilibration with atmospheric CO_2, and karst recharge from spring waters with a high component of dissolved carbonate bedrock. At the orbital scale, $\delta^{13}C_{TOC}$ variability is related to changes in the quantity and supply of soil CO_2, as well as the amount of primary productivity and recycling of organic matter, providing a complex interplay between catchment-scale terrestrial and lacustrine evolution. Although warmer periods are generally characterised by a change to lower $\delta^{13}C_{TOC}$, there is not a clear correlation between $\delta^{13}C_{TOC}$ values and periods traditionally associated with peak interglacial conditions (e.g. MIS 5e, MIS 11). This may depend on several factors, for example the amount and seasonal distribution of precipitation, which may regulate the seasonal amount of soil CO_2 produced and the rate of CO_2 leaching toward the lake, and/or different rates of mixing of the upper water column, which influence organic matter recycling efficiency.

Based on the different phase relationships between $\delta^{13}C_{TOC}$ and AP% for some glacial to interglacial transitions, a time lag may be present for soil recovery following stronger glacial periods compared to regional climate trends toward wetter and warmer conditions. This delay is probably related to the slow process of soil formation on bedrock (Holtvoeth et al., 2017) after local glaciation in the mountains surrounding Lake Ohrid, as shown for other mountainous areas in the Mediterranean and specifically supported by speleothem data for the end of the MIS 8 glacial maximum in the Ohrid catchment (Regattieri et al., 2018).

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Figure Captions

Figure 1. - a) Location Map of Lake Ohrid and soil sample positions; b) Detail of the Ohrid catchment.

Figure 2 - A) TOC/TN vs TOC % for the organic matter in the soil samples and lacustrine organic matter; B) TOC/TN vs $\delta^{13}C_{TOC}$ for the organic matter in the soil samples and lacustrine organic matter; C) TOC vs $\delta^{13}C_{TOC}$ for the organic matter in the soil samples and lacustrine organic matter; D) $\delta^{13}C_{TOC}$ vs TIC for the organic matter in the soil samples and lacustrine organic matter. TOC/TN and TOC for lacustrine sediments from Francke et al., 2016.

Figure 3 - Proxy records discussed in the text. From the bottom to the top: A) $\delta^{13}C_{TOC}$ of lacustrine sediments (this works), blue color data from the LINI core (Lacey et al., 2015); B) Total Organic Carbon % (TOC); C) Total Inorganic Carbon % (TIC); D) TOC/TN; E) Potassium (K) XRF core scanner data; F) Biogenic Silica (BSi %); G) Arboreal Pollen record (AP%) and Non Arboreal Pollen (NAP%); H) LR4 $\delta^{18}O$ stack (Lisiecki, L. E., Raymo, M. E., 2005). B, C, D, E and F from Francke et al., 2016, and G from Sadori et al. (2016).

Figure 4 - $\delta^{13}C_{TOC}$ *vs* $\delta^{13}C_{TIC}$. $\delta^{13}C_{TOC}$ and $\delta^{13}C_{TIC}$ show high correlation suggesting they are mostly controlled by $\delta^{13}C_{DIC}$. $\delta^{13}C_{TIC}$ data after Lacey et al. (2016).

Figure 5 - $\delta^{13}C_{TOC}$ vs AP% (AP% minus Pinus spp. after Sadori et al., 2016).

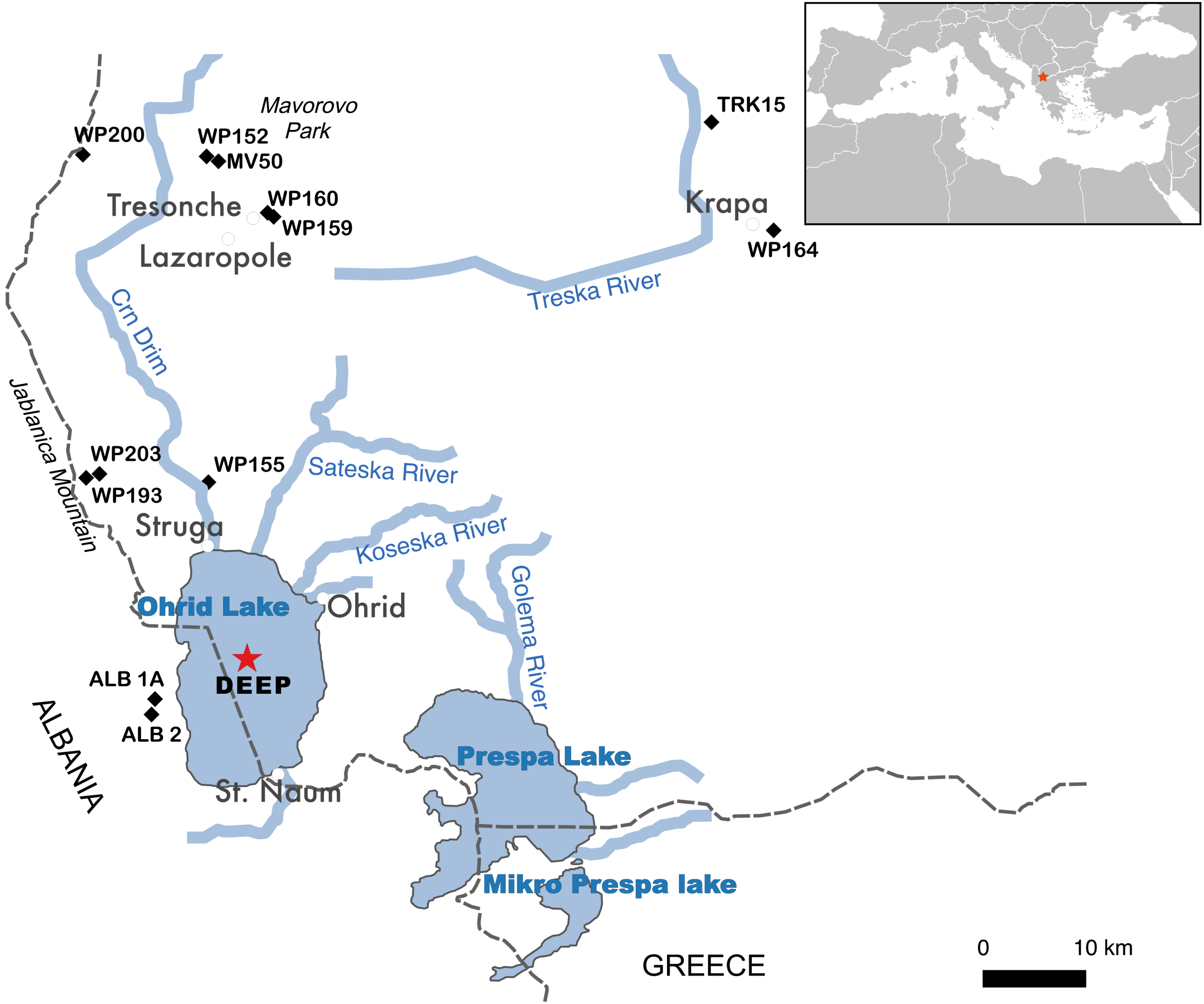
Figure 6 - Details of Ohrid proxies for several interglacial periods (from left bottom panel MIS5-MIS9-MIS11-MIS7); a) $\delta^{13}C_{TOC}$ (red, this study); b) TOC (yellow) and c) TIC, (grey) (Francke et al., 2016) ; d) Potssium (K) record (Francke et al., 2016; e) Arboreal pollen-pinus (from Sadori et al., 2016) (letters on MIS5 panel apply also for other interglacial).

Table 2. Soil samples location, ecosystem type and results of C-isotope composition, TIC, TOC, TN and TOC/TN molar ratio.

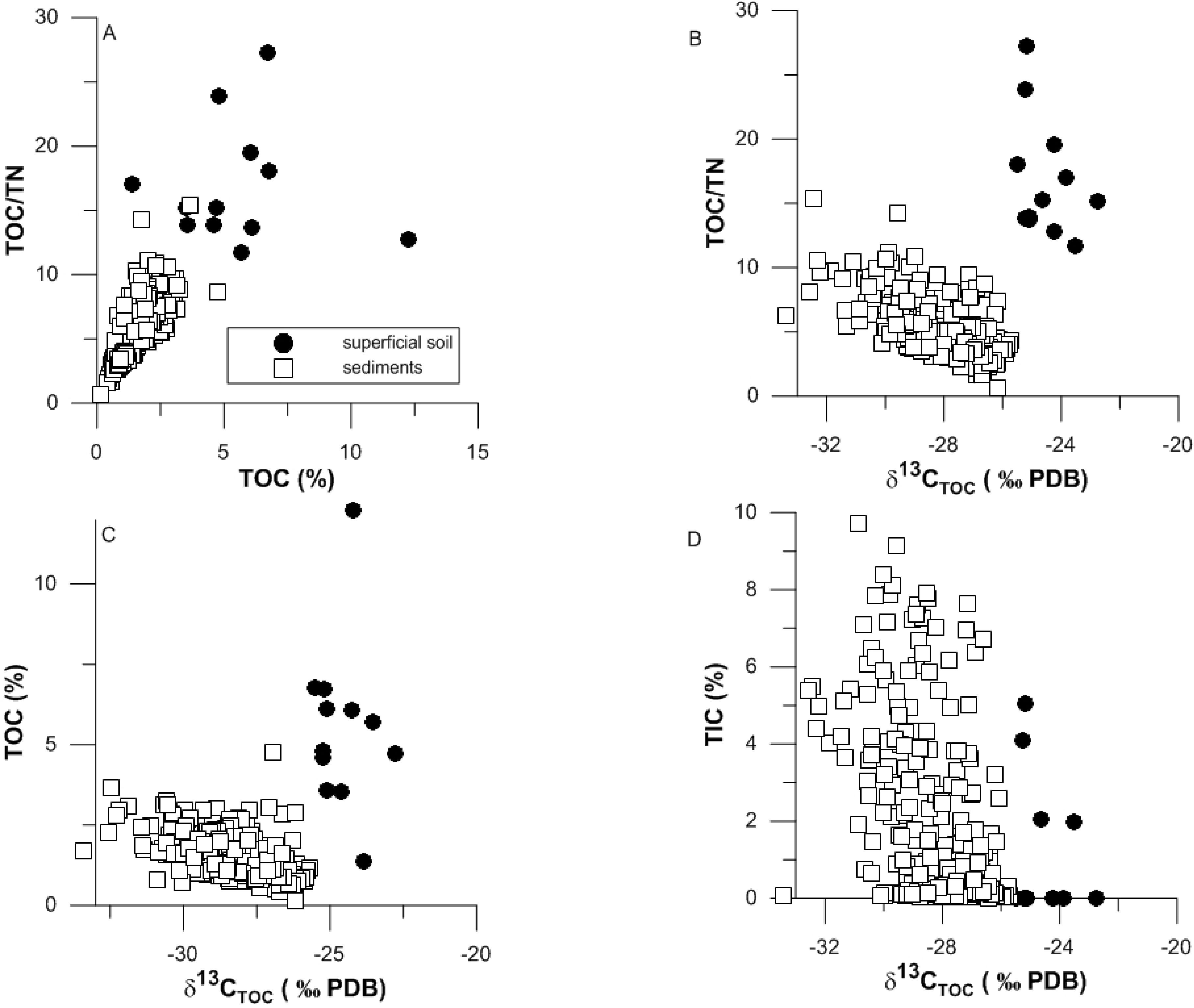
Sample	Coordinates	Location	Ecosystem type	$\delta^{13}C_{TOC}$ (‰ PDB)	TN %	TIC %	TOC %	TOC/ TN
WP152	41°37'56.16" N 20°40'5.40" E	soil on Mavrovo Mountain	grassland	-24.2	1.2	< 0.5	12.3	12.8
WP155	41°15'15.84" N 20°40'12.96" E	soil under unconsolidated glacial debris on Mavorovo Mountain	grassland	-23.5	0.6	2.0	5.7	11.7
MV50	41°37'35.9" N 20°40'55.1" E	soil on Mavrovo Mountain	grassland	-25.1	0.6	< 0.5	6.1	13.7
WP200	41°38'3.33" N 20°31'29.10" E	soil on Jablanica Mountain	grassland	-25.5	0.5	< 0.5	6.7	18.0
WP193	41°15'34.03" N 20°31'42.54" E	soil on Jablanica Mountain	grassland	-25.2	0.3	< 0.5	4.8	23.9
WP203	41°15'50.58" N 20°32'38.34" E	soil on Jablanica Mountain	forest	-24.3	0.4	< 0.5	6.1	19.5
TRK15	41°40'19.1" N 21°15'13.1" E	detritic soil on Treska river valley	trees and shrubs	-25.2	0.3	5.0	6.7	27.2
WP159	41°33'44.1" N 20°44'45.36" E	soil on Mountain near Tresonche(N-E Lazaropole)	forest	-25.3	0.4	4.1	4.6	13.9
WP160	41°34'1.02" N 20°44'20.10" E	soil on Mountain near Tresonche(N-E Lazaropole)	forest	-24.6	0.3	2.0	3.5	15.2
WP164	41°32'47.64" N 21°19'32.52" E	soil near Krapa	grassland	-22.8	0.4	< 0.5	4.7	15.2
ALB 1A	41°00'10.2" N 20°36'30.0" E	soil on Albanian Mountain ultramafic rocks (W-Ohrid)	grassland	-23.9	0.1	< 0.5	1.4	17.0
ALB 2	40°59'05.9" N 20°36'15.7" E	soil on Albanian Mountain ultramafic rocks (W-Ohrid)	grassland	-25.1	0.3	< 0.5	3.6	13.9

Table 1. Correlation coefficients (r; Pearson linear correlation coefficient) between variables measured on Lake Ohrid sediments (n=315). All the correlations are significant at the 0.05 level.

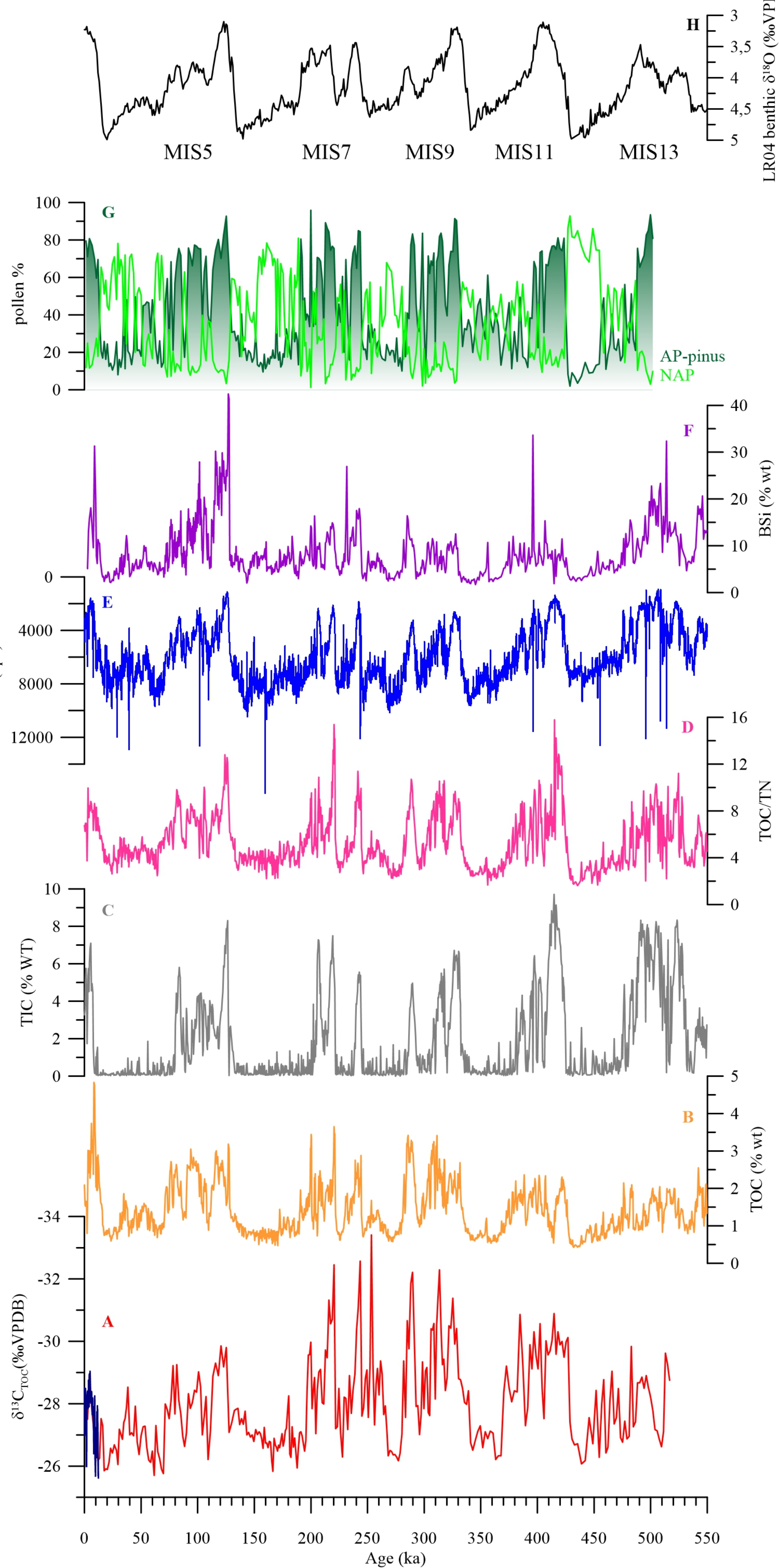
	$\delta^{13}C_{TOC}$	TIC	TOC	TOC/N
TOC/TS	- 0.51	0.40	0.82	0.72
TOC/N	- 0.65	0.73	0.79	
TOC	- 0.59	0.36		
TIC	-0.53			



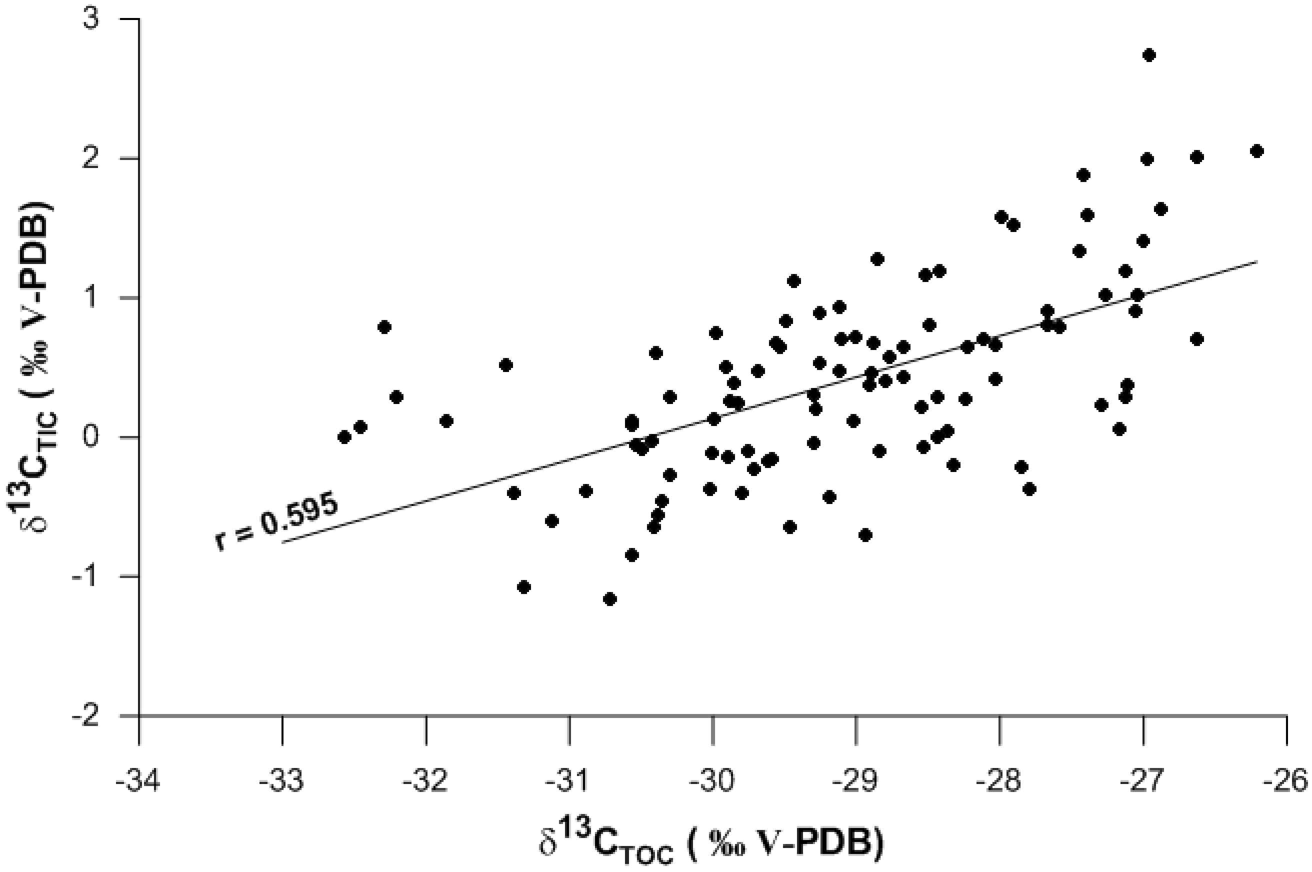
Mavorovo
Park
WP200
WP152
MV50
Tresonche
WP160
WP159
Lazaropole
TRK15
Krapa
WP164
Treska River
Crn Drim
Jablanica Mountain
WP203
WP193
WP155
Sateska River
Struga
Koseska River
Golema River
Ohrid Lake
Ohrid
DEEP
ALB 1A
ALB 2
ALBANIA
St. Naum
Prespa Lake
Mikro Prespa lake
GREECE
0
10 km



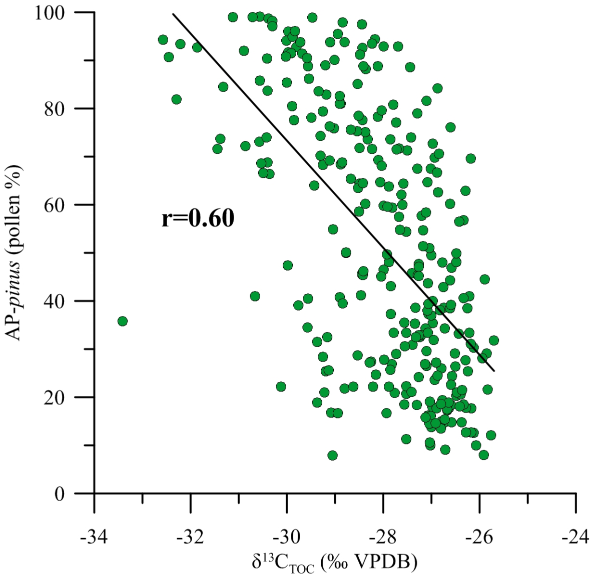
A
TOC/TN
TOC (%)
superficial soil
sediments
B
TOC/TN
$\delta^{13}C_{TOC}$ (‰ PDB)
C
TOC (%)
$\delta^{13}C_{TOC}$ (‰ PDB)
D
TIC (%)
$\delta^{13}C_{TOC}$ (‰ PDB)



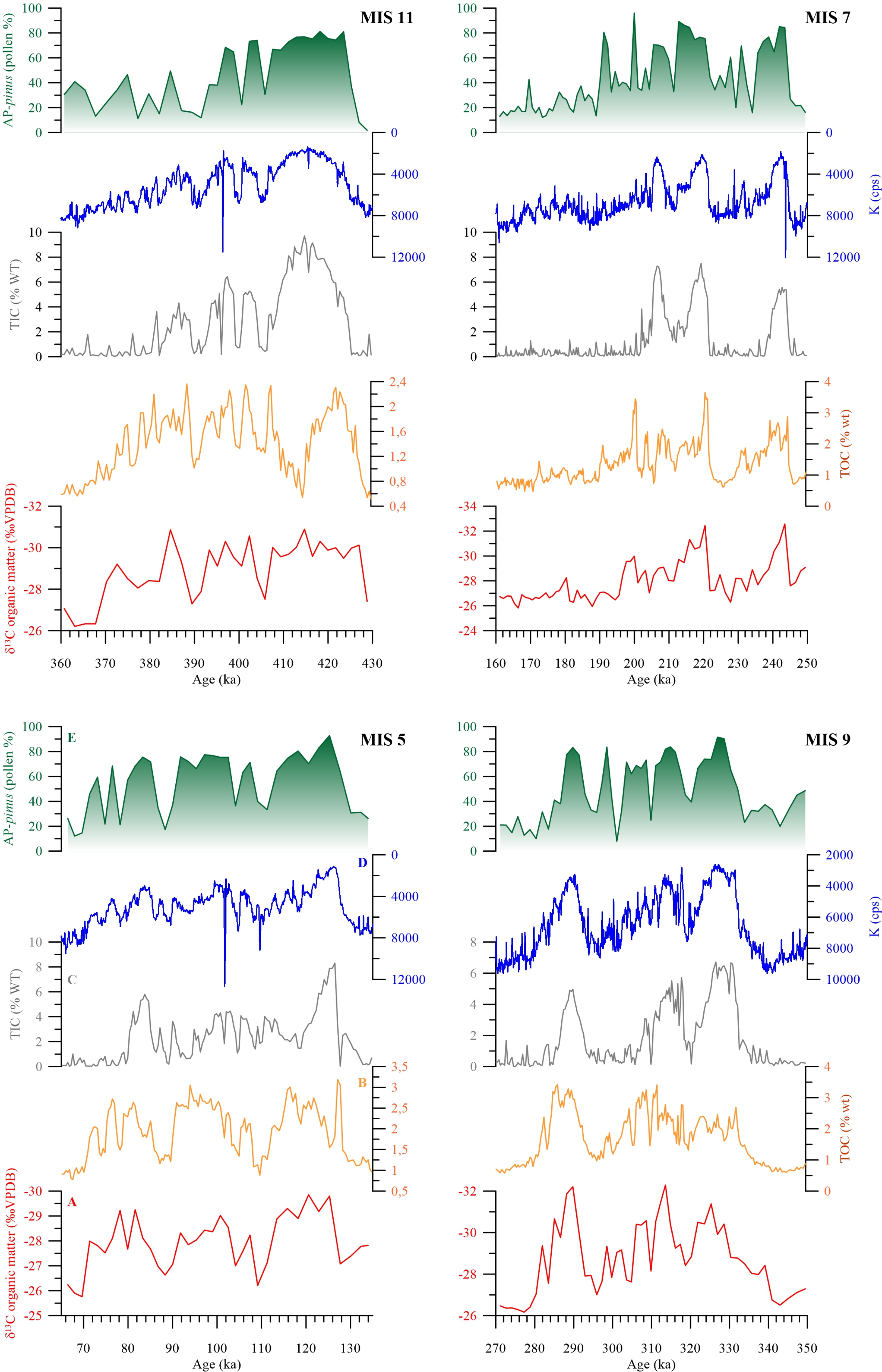
H
LR04 benthic δ18O (‰VPDB)
MIS5
MIS7
MIS9
MIS11
MIS13
G
pollen %
AP-pinus
NAP
F
BSi (% wt)
E
K (cps)
D
TOC/TN
C
TIC (% WT)
B
TOC (% wt)
A
δ13C TOC (‰VPDB)
Age (ka)



3
2
1
0
-1
-2
-34
-33
-32
-31
-30
-29
-28
-27
-26
r = 0.595
$\delta^{13}C_{TIC}$ (‰ V-PDB)
$\delta^{13}C_{TOC}$ (‰ V-PDB)



100
80
60
40
20
0
AP-*pinus* (pollen %)
r=0.60
-34
-32
-30
-28
-26
-24
δ13CTOC (‰ VPDB)


MIS 11
MIS 7
MIS 5
MIS 9
AP-pinus (pollen %)
K (cps)
TIC (% WT)
TOC (% wt)
δ13C organic matter (‰VPDB)
Age (ka)
A
B
C
D
E