A taxon-specific analysis of chironomid $\delta^{13}C$ to assess carbon cycling of Blakemere Moss during the early Holocene

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I applied my mind to study and to explore by wisdom all that is done under the heavens. What a heavy burden God has laid on mankind! I have seen all the things that are done under the sun; all of them are meaningless, a chasing after the wind.

What is crooked cannot be straightened; what is lacking cannot be counted.

I said to myself, “Look, I have increased in wisdom more than anyone who has ruled over Jerusalem before me; I have experienced much of wisdom and knowledge.” Then I applied myself to the understanding of wisdom, and also of madness and folly, but I learned that this, too, is a chasing after the wind.

Ecclesiastes 1 13-17

ABSTRACT

Chironomid remains of the *Chironomus* genus and Chironomini tribe were extracted and studied for carbon isotopic content from a lake sediment sequence from Blakemere Moss during the early Holocene (10,743 to 10,482 Cal BP). Evidence derived from bulk geochemical proxies and chironomid assemblage was used to elucidate the factors that influenced the chironomid isotopic content. The spatial heterogeneity of carbon processing was revealed by vastly different carbon isotopic signatures ($\Delta\delta^{13}C_{HC}$; $\Delta\delta^{13}C_{\textit{Chironomus}\ -\ \text{Chironomini}}$ = –0.7 to 7.0‰). A climatic amelioration beginning the sequence, indicated by a rapid increase in chironomid-inferred temperatures, was mirrored by a rapid fall in *Chironomus* $\delta^{13}C_{HC}$ and $\delta^{13}C_{OM}$ with the former exhibiting the greater ^{13}C depletion. This indicates that in our shallow paleolake, an increase in methane-derived carbon to *Chironomus* is likely linked to a strengthened summer thermal stratification. Due to the hydrologically closed nature of Blakemere Moss, a small shift in precipitation/evaporation balance is thought to have induced an ecosystem state shift from algal to macrophyte dominance and increased faunal diversity, indicated by an increase in C/N and $\delta^{15}N$ reduction. Despite a concurrent shift in the dominating functional feeding group from collectors to shredders, Chironomini $\delta^{13}C_{HC}$ continued to represent the $\delta^{13}C$ of aquatic organic matter, indicating that bulked samples of littoral dwelling Chironomini can be used to faithfully reconstruct the $\delta^{13}C$ of aquatic organic matter through time. This supports the use of littoral-dwelling Chironomini for tracing temporal variations of aquatic organic matter, and for quantifying methane-derived carbon uptake via ingestion of methane-oxidising bacteria in isotope mixing models.

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Thank you to my family for their commitment to my well-being and success. I especially thank my grandparents who have supported me beyond belief, I am truly very lucky to have you in my life. Grandma, I think you deserve an honorary taxi driving licence by this point! Also, this thesis would be filled with many grammatical errors if it were it not for the diligent proofreading and editing of my grandfather Philip Weston.

Finally, I thank my Lord and Saviour Jesus Christ, without whom, this thesis would have gone uncompleted.

DECLARATION

This thesis has not been submitted in support of an application for another degree at this or any other university. It is the result of my own work and includes nothing that is the outcome of work done in collaboration except where specifically indicated. Many of the ideas in this thesis were the product of discussion with my supervisor Peter Wynn and co-supervisor Philip Barker. The word count for this thesis is 18,727 and does not exceed the maximum word count.

The radiocarbon dates used to create the age–depth model are from Beta Analytic. They have not been previously published and are the property of the University of Manchester Geography Laboratories, funded by Manchester Geographical Society.

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CHAPTER I

INTRODUCTION

In recent years, the role of the terrestrial carbon cycle has been viewed as increasingly important in predicting Earth's future climate (Cole et al., 2007; Prairie, 2008; Schimel et al., 2015), and in particular, the response of lacustrine systems to climatic and anthropogenic forcing (McGowan et al., 2016; Hillman et al., 2018). Most lakes are considered to be a net source of carbon to the atmosphere, emitting CO_2 and CH_4, yet simultaneously sequester a considerable amount of carbon in their sediments (Dean and Gorham, 1998; Tranvik et al., 2009; Cole, 2013). A major challenge therefore exists in trying to quantify relative rates of carbon emission and sequestration in lakes in the context of previous environmental change (McGowan et al., 2016; Praetzel et al., 2020). The transition into the Holocene (11,700 Cal BP) was the most recent period of significant environmental change and was characterised by significant deglaciation and meltwater flux in the northly latitudes (Heinrich, 1988; Clark et al., 2002). This had a strong influence on the Atlantic meridional overturning circulation (AMOC) and consequently the UK temperature and precipitation (Rahmstorf, 2007; Lang et al., 2010; Brooks and Langdon, 2014). The reconstruction of lake carbon cycling during this period may therefore offer insights into the response of lakes to future climate warming (Randsalu-Wendrup et al., 2014).

Past changes in lake carbon cycling can be reconstructed using proxies such as chironomid stable isotope signatures to infer carbon flux in the basal resources of aquatic food webs (van Hardenbroek et al., 2009; Rawcliffe et al., 2010). In particular, the role of the benthos for carbon flux in the food web has been recognised (Vadeboncoeur et al., 2002; Wagner et al., 2012; Kivilä et al., 2019), as has sedimentary organic matter (Nogaro et al., 2009; Ferland et al., 2014). Studies have demonstrated time- and space-sensitive consumption of both autochthonous and allochthonous resources (Belle et al., 2017a, 2018; Kivilä et al., 2019), as well as feeding on ^{13}C-depleted methane-oxidising bacteria (van Hardenbroek et al., 2018, and references therein).

The uptake of methane-derived carbon (MDC) in chironomids has recently been linked to lake stratification strength and frequency (Grey, 2016). This is driven either by factors relating to anthropogenic eutrophication (Frossard et al., 2015; Belle et al., 2016;

Schilder et al., 2017) or by climate (Wooller et al., 2012; van Hardenbroek et al., 2013; Belle et al., 2017b, 2018). However, quantifying the MDC contribution has proven problematic, particularly in shallow lake systems which generally produce less methane, thus leading to chironomid $\delta^{13}C$ isotope values in the 'zone of contention' (–30 to –40‰; see Section 2.7.4.3) (Deines and Grey, 2006; Grey, 2016). Furthermore, drivers of spatial variability within lake basins and how interspecific chironomid isotope measurements can elucidate these differences remain largely understudied (van Hardenbroek et al., 2018). Similarly, more studies in a range of climatic and environmental contexts are required to understand better the relationship between climate and methane production, particularly for shallow productive lakes like the one in the present study (Belle et al., 2017a).

Isotope mixing models have also proven useful in quantifying the uptake of methane-derived carbon by comparing estimated values for algae and methane oxidising-bacteria MOB with $\delta^{13}C_{HC}$ values, however, this method is prone to uncertainties due to the spatial (and temporal) variation in $\delta^{13}C$ values of methane and algae between lakes (van Hardenbroek et al., 2018, and references therein). The present study therefore attempts to use bulked littoral-dwelling Chironomini $\delta^{13}C_{HC}$ values to demonstrate that an account for these variations and thus recommend their use in future studies to inform isotope mixing models.

CHAPTER II

LITERATURE REVIEW

2.1 The Lacustrine Record

Understanding the global carbon cycle is critical in providing future scenarios of climate change (Intergovernmental Panel on Climate Change [IPCC], 2021). The climate system can be studied through the development and application of proxies which provide long-term paleoclimate records of environmental change; these include lake cores, speleothems, tree rings, sand dunes, marine cores, and ice cores (Lowe and Walker, 2014). Lake sediments provide a chronologically structured record of lake history by collecting and preserving valuable information in their sediment (Smol and Cumming, 2000). Sediment sources include types from within (autochthonous) and outside the lake (allochthonous). Organic matter accumulation in lakes can be autochthonous (phytoplankton), allochthonous (terrestrial vegetation), or from the marginal macrophyte community. Climate constitutes an overriding influence on sediment flux and transformation because it is intimately and complexly linked to the physical, chemical, and biological processes that occur in the lake (Cohen, 2003). These linkages must therefore be understood to realise the full potential of lake sediments as paleoclimate archives (Battarbee, 2000).

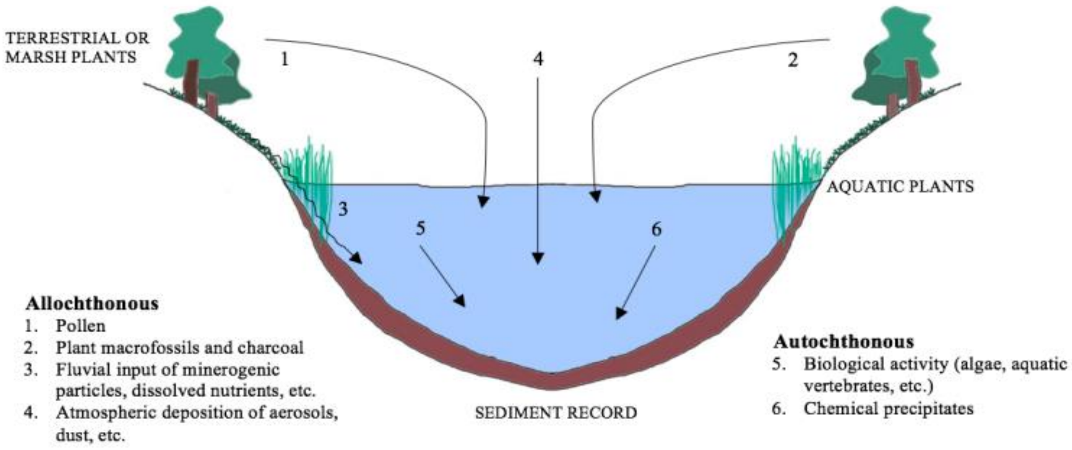


Figure 2.1: Schematic of allochthonous and autochthonous material that may be used to derive proxy data in a lake environment: *Source*: Swallow (2019).

Lake sediment records contain a wealth of multi-proxy information. Fossil remains of past biota such as invertebrates (e.g., Chironomidae) have proven particularly useful for reconstructing the environmental, climatic, and lake conditions of the past (Porinchu and Macdonald, 2003; Lowe and Walker, 2014). The geochemical analysis (elemental and stable isotopes, e.g., δD, $\delta^{13}C$, $\delta^{15}N$, and $\delta^{18}O$) of lake sediment can permit inferences into the hydrological nature of lake systems and the reconstruction of the long-term dynamics of terrestrial and aquatic ecosystems (Leng et al., 2005). The combination of these approaches (i.e., the stable carbon isotopic analysis of invertebrate remains) has yielded valuable information on carbon processing in aquatic food webs and the processes which govern the availability of various carbon sources, including methane (Heiri et al., 2012; Grey, 2016; van Hardenbroek et al., 2018).

Underpinning all paleolimnological reconstructions is the need for accurate and precise chronologies as they provide the timescales for events and for which the occurrence of patterns and processes can be assessed (Birks et al., 2012; Bradley, 2015). This is of particular importance for high-frequency records, which may reveal short-term changes in climate or ontological environmental regime shifts (Perga et al., 2015). Radiocarbon (^{14}C) dating techniques are well suited for late Quaternary sediments (up to ~50k years), as the half-life of C (5730 years) enables sub-centennial shifts to be identified (Poluianov et al., 2016).

2.2 The Lacustrine Carbon Cycle

2.2.1 The importance of the lacustrine carbon cycle

Concern over climate change and the role of greenhouse gases (GHGs) has led to efforts to understand the global carbon cycle better (IPCC, 2013). The relationship between sinks and sources and the processes that govern carbon transport and transformation has been of particular focus (Falkowski, 2000; Liu et al., 2013). As a result, inland waters are now considered a site of globally significant carbon processing and a net GHG source rather than a passive site of transportation (Cole et al., 2007; Battin et al., 2009). Some of the carbon is buried in freshwater bodies (0.15 Pg C yr^{-1}) (Mendonça et al., 2017); however, a significant proportion returns to the atmosphere via outgassing, with estimates in the region of 1.2–1.4 Pg C yr^{-1} for inland waters (Tranvik et al., 2009; Raymond et al., 2013). Freshwater areas are the largest natural contributor of methane to the atmosphere (26%)

(IPCC, 2021); however, they also account for the greatest uncertainty (Pham-Duc et al., 2017).

2.2.2 The lacustrine carbon cycle defined

Carbon in gaseous, dissolved, and solid forms may be sequestered or transferred through a lake system through complex and interlinked pathways. Both biotic and abiotic processes transform carbon received from the catchment or atmosphere. These processes are influenced by environmental and climatic factors, including nutrient availability, water temperature, and precipitation, leading to complex interactions (Hillman et al., 2018). An important distinction is made between the epilimnion and hypolimnion as the transformation of carbon varies significantly between the two regions owing to differences in the availability of light and oxygen.

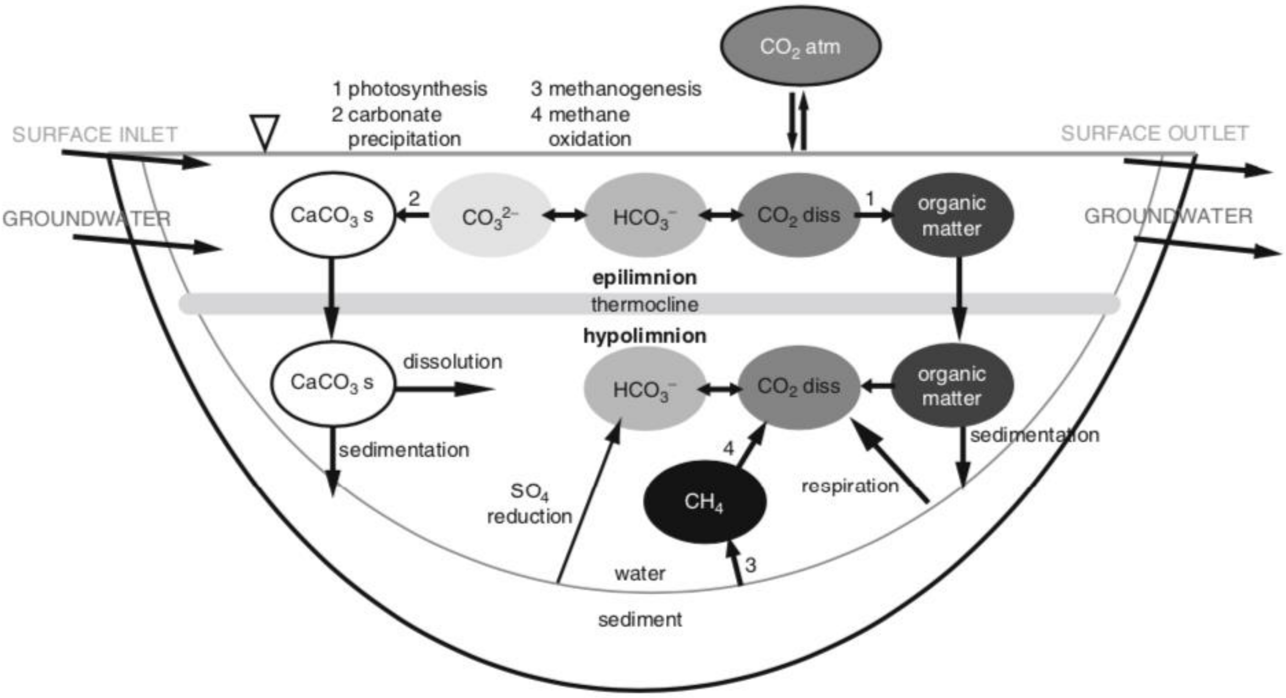


Figure 2.2: Schematic diagram of the major carbon cycle processes in lakes. DOC is excluded from the diagram for simplicity as it is a source for many microbial and abiogenic processes. *Source*: Mybro (2012).

2.2.2.1 Dissolved inorganic carbon (DIC)

The dominant input of carbon is atmospheric CO_2, which may dissolve in lake water in a variety of forms depending on the lake pH. Collectively termed dissolved inorganic carbon (DIC), these forms include carbon dioxide (CO_2), carbonic acid (H_2CO_3) and bicarbonate (HCO_3^-). Lakes also receive DIC through terrestrial soil/respiration processes and from weathering reactions (Fry, 2006; Campeau et al., 2017). Under most conditions, CO_2 rapidly dissolves to form carbonic acid (Walter and Matt, 2017). Once dissolved, CO_2 may leave this part of the cycle by being assimilated into organic matter via photosynthesis, via diffusion into the atmosphere, or via ground/surface water flow (Soumis et al., 2004; Tremblay et al., 2005; Prairie and Cole, 2009). Dissolved CO_2 may also be precipitated as $CaCO_3$ biologically or chemically (e.g., foraminifera or water pH >8.0, respectively) (Lehmann et al., 2004).

In non-acidic lakes, H^+ ions in the carbonic acid dissociate to form bicarbonate. This is known as the bicarbonate equilibrium and is dictated by the pH of the lake system (availability of H^+ ions). The sum of dissolved inorganic compounds within the lake is referred to as TDIC (total dissolved inorganic carbon) and it has important implications for the availability of CO_2 for photosynthesis (Mybro, 2012) (see Section 2.3.2.2).

$$CO_2 + H_2O \leftrightharpoons H_2CO_3 \leftrightharpoons H^+ + HCO_3^- \leftrightharpoons 2H^+ + CO_3^{2-}$$

carbon dioxide ⇋ carbonic acid ⇋ bicarbonate ⇋ carbonate

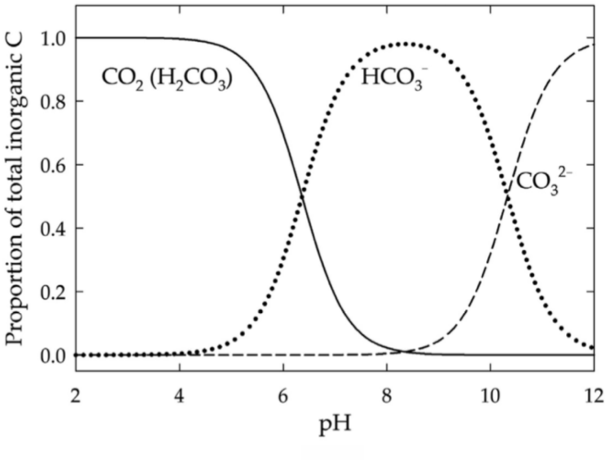


Figure 2.3: Relative concentrations of inorganic compounds involved in the bicarbonate equilibrium as a function of pH. *Source*: Walter and Matt (2017).

2.2.2.2 Organic carbon

In limnology, organic carbon (OC) is most broadly classified as either dissolved (DOC) or particulate organic carbon (POC). The largest fraction (~90%) is DOC (<45 μm) and comprises a diversity of organic compounds that vary in their chemical characteristics and, consequently, in their functionality in lakes (Fellman et al., 2010). The dissolved pool of organic carbon can be divided into two major classes – humic and non-humic. Humic compounds are produced from the decay of plant material (aquatic or terrestrial), they have high molecular weights, and they lend a brownish colour to water, which attenuates photosynthesis (Karlsson et al., 2009) (see Section 2.2.3.1). The non-humic fraction includes sugars and other carbohydrates, amino acids, urea, proteins, pigments, and lipids, which are more easily metabolised by aquatic microbes (Walter and Matt, 2017). Heterotrophic (CO_2-producing) microbial processes break down non-humic substances to yield humic compounds as a degradation by-product (Stumm and Morgan, 1981). Particulate organic carbon (POC) (>45 μm) forms a smaller fraction of total organic carbon (TOC) and is produced via the breakdown of plant biomass (Wetzel, 2001).

2.2.2.3 Photosynthesis

Photosynthesis involves the assimilation of dissolved inorganic carbon into organic matter. For most lakes, phytoplankton are the dominant organism producing primary organic matter through photosynthesis (Meyers, 1997). The rate of this production is highly dependent on the relationship between non-living mineral materials and living algal organic matter and the availability of light (Walters and Matt, 2017). Phosphorus and nitrogen are also important limiting nutrients (see Section 2.4.1).

The stoichiometry of carbon fixation by aquatic photoautotrophs generally follows the empirical reaction:

$$106CO_2 + 106H_2O + 16NH_3 + H_3PO_4 \leftrightarrows (CH_2O)_{106}(NH_3)_{16}(H_3PO_4) + 106O_2$$

in accordance with the Redfield ratio (C:N:P = 106:16:1), which refers to the average elemental composition of aquatic biomass. The reverse reaction (i.e., to the left) corresponds to aerobic respiration (see the next section).

Phytoplankton and macrophytes are primary producers (photoautotrophs) and are at the base of the food web (basal resource; lowest trophic level). Heterotrophic bacteria and zooplankton feed on primary producers (primary consumers) and thus belong to the second trophic level. Fish and other consumers of primary producers are of higher trophic levels; aquatic communities at all trophic levels are therefore affected by changes in terrestrial and aquatic organic carbon inputs (Pace et al., 2004). Terrestrial plants and emergent macrophytes use atmospheric CO_2 as their main carbon source, whereas lacustrine algae and submerged macrophytes and phytoplankton mainly utilise dissolved CO_2. The shift to HCO_3^- utilisation is caused by high water temperatures, changes in pH or salinity, or enhanced productivity caused by an increase in nutrients (Meyers and Ishiwatari, 1993).

2.2.3 Transformation of carbon under oxic conditions

Dissolved inorganic carbon can be transformed to POC or DOC via photosynthesis, respiration, and degradation (Herczeg, 1987). Compared with allochthonous carbon, the DOC produced by autochthonous production (i.e., phytoplankton and macrophytes) is often colourless and is usually rapidly broken down by bacteria (Nalewajko and Marin, 1969; Gergel et al., 1999). The concentration of DOC in lakes is controlled by (1) the physical properties of the catchment (Hanson et al., 2011); (2) morphometric characteristics of the lake and regional climate (Sobek et al., 2007); (3) autochthonous production of DOC inside the lake (see figure 2.4), and (4) biotic and abiotic mineralisation of DOC (Benoy et al., 2007) (see Section 2.2.3.1).

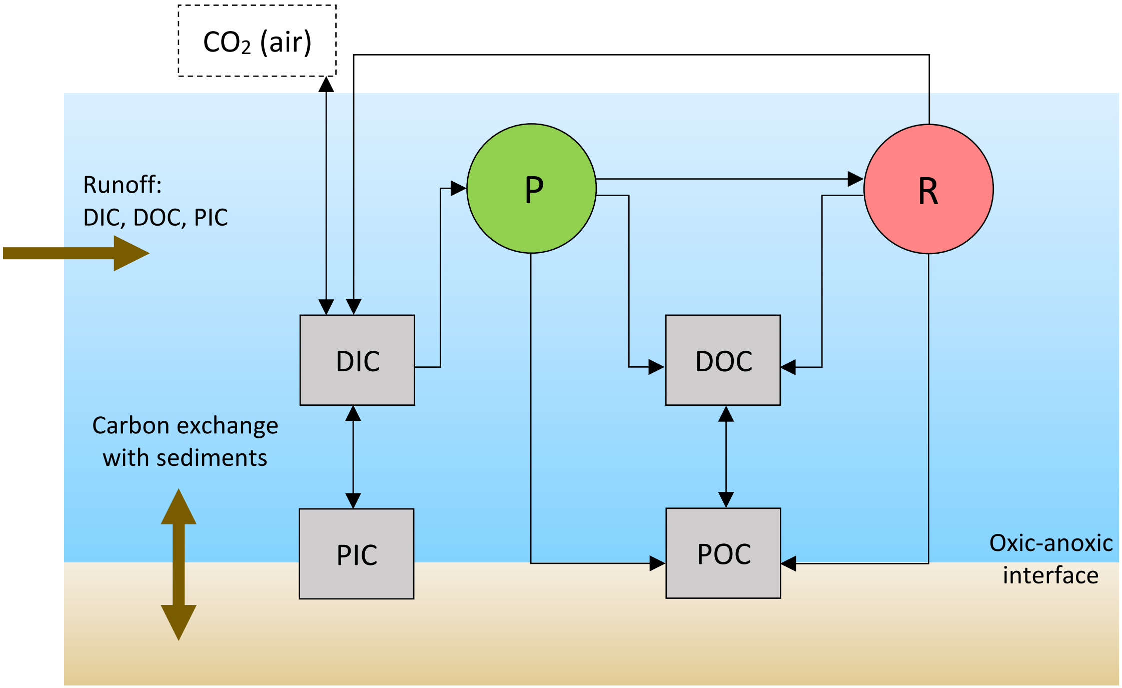


Figure 2.4: Forms and transformations of aquatic inoranic and organic carbon. Arrows indicate direction of carbon transfer. P and R are photosynthesis and respiration, respectively. *Modified from*: Reitsema et al. (2018).

2.2.3.1 Allochthonous organic matter and DOC processing

Allochthonous OM represents one of the most important lateral exchanges of carbon between land and aquatic ecosystems (Duarte and Prairie, 2005). Upon entry, it fuels the metabolism of heterotrophic microorganisms in the water column (i.e., bacterioplankton), which results in the production of CO_2 (Tranvik, 1988). However, owing to its dark colour, it has an attenuating effect on photosynthesis (Karlsson et al., 2009). Therefore, most lakes tend towards net heterotrophy as respiration exceeds primary production, leading to net CO_2 production within the water column (Del Giorgio and Peters, 1994; Jansson et al., 2000).

The diffusion of CO_2 across the water–air interface should lead to equilibrium between lake and atmosphere; however, the rate of allochthonous carbon input exceeds that of CO_2 evasion, leading to CO_2 supersaturation (Cole et al., 1994). Two main pathways of carbon input have been identified: (1) allochthonous OM may be mineralised bacterially or photochemically once it has entered the lake (Sobek et al., 2003, 2005; McCallister and del Giorgio, 2008; Roehm et al., 2009); and (2) carbon may be respired terrestrially and then enter the lake system as dissolved CO_2 via surface/groundwater flow

(Carignan et al., 2000; Dubois et al., 2009; Stets et al., 2009). The relative importance of these pathways depends on interlinked processes, e.g., catchment productivity (Wetzel, 1990, 1992), soil respiration rates (Öquist et al., 2009; Wallin et al., 2013), and colour of allochthonous DOC (Karlsson et al., 2009).

2.2.3.2 Relative significance of autochthonous and allochthonous inputs

Del Giorgio and Cole (1998) showed that bacterial heterotrophic productivity tends to be higher on autochthonous DOC. This probably occurs since allochthonous DOC tends to be more recalcitrant and resistant to degradation (Benoy et al., 2007). However, for lakes which are small relative to their catchment and have low productivity rates, the primary source of DIC is through the oxidation of terrestrial DOC (Cole et al., 2002; Sobek et al., 2003). Kritzberg et al. (2004) showed this preference within a single lake as bacteria switched to respiring allochthonous carbon when the availability of autochthonous carbon was diminished. Studies by Hessen (1992) and Hanson et al. (2004) suggest that terrestrial DOC may bypass the food web altogether and degas if the lake is humic and dystrophic.

The dominant mode of bacterial production in lakes depends on the lability of autochthonous DOC as it tends to be more labile and, consequently, has a higher biological availability than most allochthonous sources (Wetzel, 2001; del Giorgio and Davis, 2003). This leads to a more conservative carbon cycle in which carbon produced via algal production is readily assimilated into microbial communities or heterotrophic algae and transferred to higher trophic levels (Benoy et al., 2007). However, the high variability in the lability of terrestrial vegetation means that lakes cannot be assumed to metabolise solely autochthonous or allochthonous carbon, but rather a mixture (Jackrel et al., 2016).

2.2.4 Carbon exchange with sediments

Following transformation in the photic zone, carbon may leave the lacustrine carbon cycle in two ways once it has been transported downwards to the sediment–water interface as particulate organic matter. Here, it is processed and degraded (mineralisation) to CO_2 or CH_4 by microbial activity and may subsequently evade into the atmosphere (Prairie and Cole, 2009; Sobek et al., 2009). Alternatively, carbon may be buried through sequestration in lake sediments (Ferland et al., 2014); the burial efficiency of organic carbon is mostly dependent on factors that affect and regulate mineralisation, including temperature (Gudasz et al., 2010; Chmiel et al., 2016) and oxygen exposure (Sobek et al., 2009). During carbon (and organic matter) sequestration, diagenetic alterations relating to burial efficiency (Gudasz et al., 2012) can obscure paleoenvironmental interpretations of various proxies derived from lake sediments (reviewed in the relevant following sections) (Meyers and Ishiwatari, 1993).

2.2.4.1 Oxidation of carbon via inorganic compounds

Under anoxic conditions at the sediment–water interface, carbon in organic matter may be oxidised to produce CO_2 via inorganic compounds such as nitrate, sulfate, and iron. This generally occurs at a slower rate than aerobic respiration, leading to slower rates of CO_2 production (Zehnder and Svensson, 1986). The efficiency of these oxidations is determined by the oxidation state of the compound. For example, as O_2 is the most oxidised compound, organisms can extract the greatest amount of energy through aerobic respiration. Then, organisms turn to other compounds in order of redox potential, which is defined by the relative availability of electrons for reducing or oxidising chemical reactions (Walter and Matt, 2017).

The community of organisms involved in this process is ‘syntrophic’, meaning that no one species can complete the decomposition process. This leads to competition for energy-yielding inorganic compounds and gives rise to selection based on conversion efficiency (i.e., organisms capable of oxidising the more efficient compounds will outcompete others). Thus, for example, methanogenesis is unlikely to occur if there are sufficient alternative inorganic compounds available (see table 2.1).

Process	**Substrates**		**Final products**		**$-\Delta G°$ (kJ mol^{-1} e, pH = 7)**
	e⁻ donor	e⁻ acceptor			
Aerobic respiration	OM	O_2	CO_2	H_2O	125
Denitrification	OM	NO_3^-	CO_2	N_2	112
Manganese reduction	OM	Mn(IV)	CO_2	Mn(III), Mn(II)	95
Iron reduction	OM	Fe(III)	CO_2	Fe(II)	24
Fermentation	OM	OM	CO_2	LMWOM, H_2	5–60
Sulfate reduction	LMWOM	SO_4^{2-}	CO_2	S^{2-}	18
Methanogenesis	LMWOM, H_2	LMWOM, CO_2	CO_2	CH_4	14–28

Table 2.1: Redox reactions used in the microbial degradation of organic matter in order of decreasing efficiency (most efficient at the top). The principle of these metabolic processes is that organisms gain energy by transferring electrons from an electron donor to an electron acceptor. *Modified from*: Bastviken (2009).

2.2.4.2 Methanogenesis and methane oxidation

Methane (CH_4) is an end product of the decomposition of organic matter (OM) in freshwater environments when other substrates (see table 2.1) are limited (Kiene, 1991; Conrad, 2005). It is produced (methanogenesis) by anaerobic archaea which generate methane via the disproportionation of acetate (CH_3COOH) or by utilising CO_2 as the terminal electron acceptor (Berg et al., 2010; Su, 2020). During decomposition, fermenting bacteria and archaea convert complex organic matter molecules to smaller substrates referred to as low molecular weight organic matter (LMWOM) and energy (Walter and Matt, 2017). LMWOM is available for methanogenic archaea to produce methane in deep, anoxic waters or in anoxic lake sediment (Bartlett and Harriss, 1993; Rudd and Taylor, 1980) via two pathways:

Autotrophic methanogenesis:

$$CO_2 + 4H_2 \rightarrow CH_4 + 2H_2O$$

Acetoclastic methanogenesis (acetate disproportionation):

$$CH_3COOH \rightarrow CH_4 + CO_2$$

CH_4 is both produced and consumed (oxidised) in aquatic environments. This results in local within-system carbon cycling, enabling carbon transfer between anoxic and oxic processes or habitats (Bastviken, 2009). Once produced, methane diffuses back into the water column across the oxic–anoxic boundary, where it may return to the atmosphere via diffusion or ebullition or transport via vascular plants (Bastviken et al., 2008; Kankaala et al., 2007; Walter et al., 2007). Alternatively, it may be oxidised to CO_2 by methanotrophic bacteria, which are subsequently consumed by benthic invertebrates such as Chironomidae (Striegl and Michmerhuizen, 1998; Steinmann et al., 2008). Owing to the isotopically distinct carbon signature of these bacteria (Whiticar, 1999), food web investigations are an effective means to trace or reconstruct CH_4 flux in lakes (e.g., Kelly et al., 2004; Banning et al., 2005).

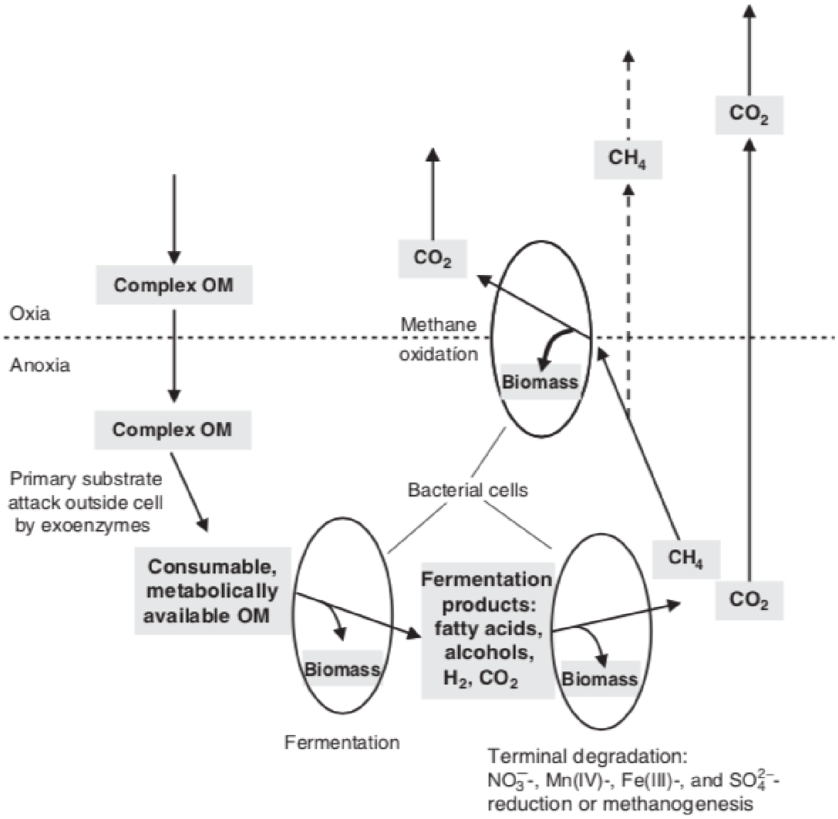


Figure 2.5: Illustration of anoxic degradation of organic matter and of CH_4 oxidation in aquatic sediment. Production of CH_4 represents one of several possible anoxic degradation processes. *Source*: Bastviken (2009).

Methanotrophs gain the most energy via aerobic oxidation by using CH_4 as an energy and carbon source and O_2 as an electron acceptor (see Section 2.2.4.1). Therefore, aerobic oxidation of methane is thought to be the dominant mode of oxidation in lake environments as it is more commonly observed where there is a constant supply of O_2 (Murase et al., 2003; Samad and Bertilsson, 2017), particularly at shallower depths (Jedrysek, 2005; Gunthel et al., 2019). Furthermore, indirect evidence from Su (2020) suggests that some aerobic methanotrophs may catalyse anaerobic methanotrophs.

Anaerobic oxidation of methane (AOM) has been mostly studied in marine environments and is generally coupled to sulfate reduction as the dominant methane-consuming process in anoxic marine environments (Reeburgh, 2007; Knittel and Boetius, 2009; Egger et al., 2018). However, understanding of AOM in freshwater environments is still rudimentary. While AOM has been demonstrated in freshwater systems (Sivan et al., 2011; Norði et al., 2013), metabolic pathways are unresolved, and the consortia of organisms involved are not fully identified or understood (Valentine and Reeburgh, 2000; Su, 2020).

The amount of methane produced is determined by the availability and degradability of organic matter, the presence of electron acceptors, and the levels of H_2 and acetate as substrates for methanogenesis (Conrad, 1999; Fenchel et al., 2012). The strength and duration of hypoxic conditions are also important in allowing the build-up of dissolved CH_4 in the hypolimnion (Borrel, 2011; Clayer, 2016). Studies that have quantified past methane output are limited (Walter et al., 2007); however, in the past decade, comparisons of the carbon isotopic content of invertebrate remains with sedimentary organic matter have been able to reconstruct the relative contribution of methane-oxidising bacteria into food webs as a proxy for methane availability (Grey, 2016; van Hardenbroek et al., 2018). Recently, studies have indicated the importance of temperature (van Hardenbroek et al., 2014; Belle et al., 2017b) and productivity (Schilder et al., 2017) as drivers of increased CH_4 production and accumulation in the hypolimnion.

2.3 $\delta^{13}C$ in Lake Sediments

2.3.1 Defining bulk $\delta^{13}C$

Stable carbon isotope analysis of bulk carbon from lake sediments provides a useful paleoenvironmental proxy in many cases. In lake sediments, organic matter (OM) supports a $\delta^{13}C$ signal ($\delta^{13}C_{OM}$) which is mainly driven by the source of OM, including terrestrial plants, aquatic macrophytes, phytoplankton, and bacteria, which makes their interpretation complex (Ficken et al., 2000; Street-Perrott et al., 2004). For terrestrial plants and floating and emergent aquatic macrophytes, the $\delta^{13}C$ will reflect the relative input from C_3 plants ($\delta^{13}C$ = –22‰ to –33‰ V-PDB) and C_4 plants ($\delta^{13}C$ = –9‰ to –16‰ V-PDB) (Bender, 1971; Deines, 1980; O'Leary, 1981). Algal biomass $\delta^{13}C$ commonly ranges between –30 and –25‰ V-PDB (Boutton, 1991; Meyers and Lallier-Vergès, 1999).

The distinct $\delta^{13}C$ of lake sediment sources means that the $\delta^{13}C_{OM}$ signal may reflect changes in the relative contributions of sources in the terrestrial and aquatic carbon cycles (Leng and Marshall, 2004) (e.g., Schelske and Hodell, 1991) shown by figure 2.6. These data may be further corroborated by combining other proxies such as C/N, TOC, and magnetic susceptibility (Leng et al., 2005).

Diagenetic alterations of ^{13}C in sediments tend to be minor (Schelske and Hodell, 1995), although microbial activity can select for light C isotopes, increasing the $\delta^{13}C$ value of the sequestered OM (Nadelhoffer and Fry, 1988). Furthermore, aquatic plants and bacteria contain high levels of labile compounds and decompose more rapidly than vascular plants (Wetzel, 2001; del Giorgio and Davis, 2003), and therefore become less significant to the bulk organic C/N and $\delta^{13}C$. However, relative differences in $\delta^{13}C$ values are maintained throughout the burial process, enabling relative inputs to be understood through time (Meyers and Ishiwatari, 1993; Boschker et al., 1995).

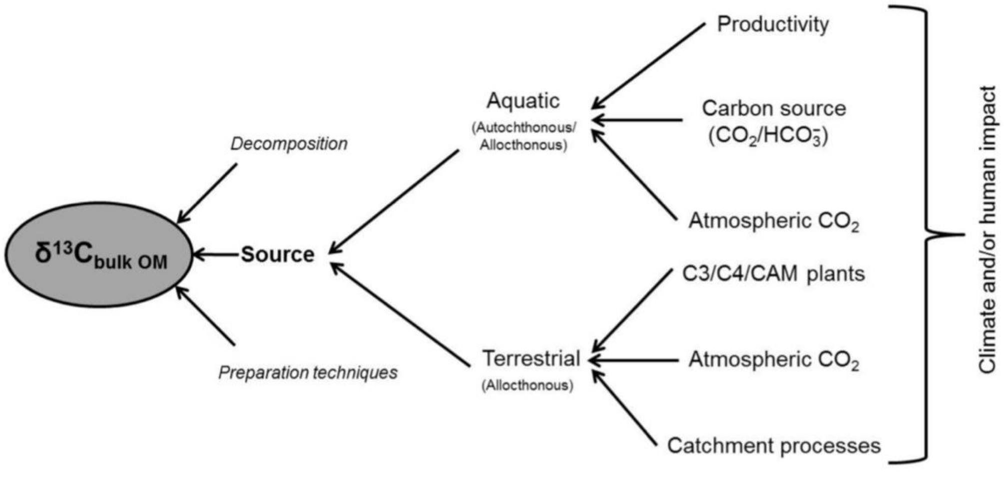


Figure 2.6: Controls on the carbon isotope composition of organic matter in lacustrine sediments. *Source*: Leng and Lewis (2015).

2.3.2 Factors influencing $\delta^{13}C_{OM}$

One of the key drivers of $\delta^{13}C_{OM}$ is the photosynthetic pathway of the catchment vegetation and emergent macrophytes due to their varying isotopic fractionation of atmospheric CO_2 (Meyers and Teranes, 2001; Leng et al., 2005); indeed, all photosynthetic organisms preferentially take up ^{12}C for greater efficiency (O'Leary, 1988). For example, if allochthonous organic matter inputs dominate the lake sediment composition, $\delta^{13}C_{OM}$ will mainly reflect the photosynthetic pathway of the vegetation comprising it (Leng and Marshall, 2004). In contrast, however, the $\delta^{13}C$ of submergent aquatic plants and phytoplankton is influenced by the $\delta^{13}C$ of the TDIC pool ($\delta^{13}C_{TDIC}$) from which they fix their carbon rather than the degree of fractionation (Ariztegui et al., 1996; Meyers and Lallier-Verges, 1999). In this regard (if autochthonous production dominates), $\delta^{13}C_{OM}$ may be influenced by shifts in the $\delta^{13}C$ of the sources to the TDIC pool and the type of carbon available to aquatic plants (CO_2 vs. HCO_3^-) (Smith and Walker, 1980; Lucas, 1983; Leng and Marshall, 2004; Leng et al., 2005). On a global scale, the $\delta^{13}C$ values of all organic materials are affected by the changing amount and composition of atmospheric CO_2 and climate (Meyers and Teranes, 2001).

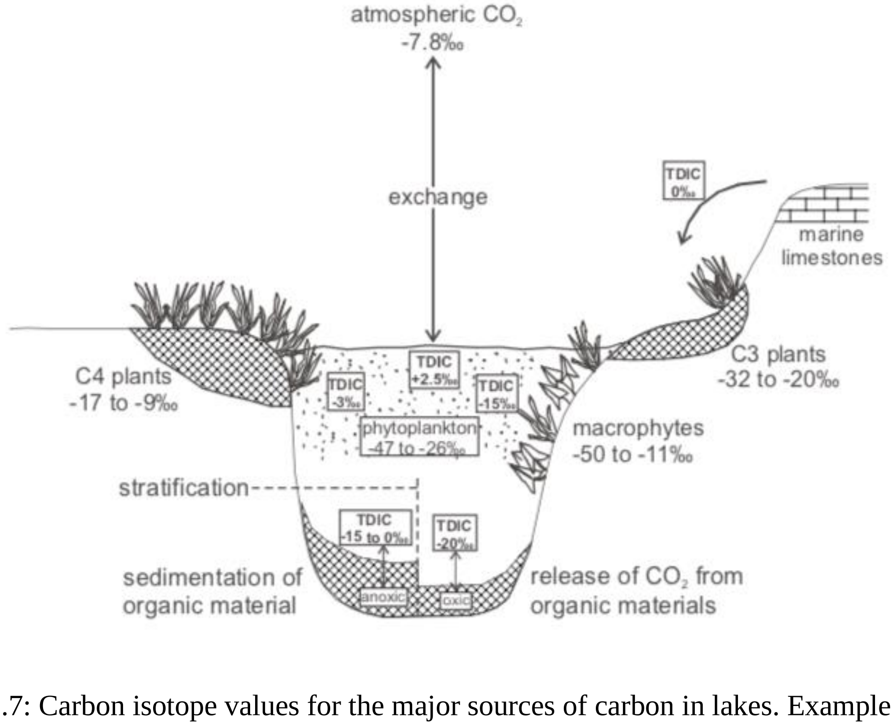


Figure 2.7: Carbon isotope values for the major sources of carbon in lakes. Examples of the range of the resulting $^{13}C_{TDIC}$ are included. The $^{13}C_{TDIC}$ is higher (around –3‰ as opposed to –15‰) when groundwater flows in an area dominated by C_4 plants due to reduced ^{13}C discrimination during photosynthesis. Lake water stratification may lead to a ^{13}C-enriched TDIC pool as anoxic bottom waters slow organic oxidation, which is a source of ^{12}C to the system. *Source*: Leng (2005).

2.3.2.1 Photosynthetic pathways

The $\delta^{13}C$ composition of terrestrial plants and emergent macrophytes is largely dependent upon their photosynthetic pathway (O'Leary, 1988). Because the photosynthetic pathway is largely dictated by environmental conditions (most notably particular temperature, aridity, and atmospheric CO_2 concentration) (Ehleringer et al., 1997; Boom et al., 2001), $\delta^{13}C_{OM}$ can be a valuable proxy for environmental conditions, particularly if it is dominated by catchment vegetation (Leng and Marshall, 2004).

There are three main photosynthetic pathways: C_3 (Calvin cycle), C_4 (Hatch–Slack), and CAM (Crassulacean acid metabolism) (Ehleringer et al., 1997), which discriminate against ^{13}C at varying levels. C_3 photosynthesis is the most common, especially in dicotyledonous plants such as trees and shrubs (Brown, 1999; Sage et al., 1999). They are adapted to cooler environments, are the least efficient in their water use (Huang et al., 2001), and produce organic matter that has $\delta^{13}C$ values between –35 and –20‰ V-PDB (Bender, 1971; O'Leary, 1981). Aquatic macrophytes that fix their carbon from the atmosphere tend to produce $\delta^{13}C$ values in line with C_3 plants (Leng et al., 2005). C_4 plants minimise water loss by closing their stomata more often; they therefore outcompete C_3 plants in areas with lower precipitation and in open areas with greater amounts of light and higher temperature and produce organic material with $\delta^{13}C$ values ranging from –10 to –14‰ V-PDB (Bender, 1971; O'Leary, 1981). CAM plants combine the C_3 and C_4 pathways and have intermediate $\delta^{13}C$ values in the range –10 to –20‰ V-PDB (O'Leary, 1988).

2.3.2.2 TDIC source

Within the water column, aquatic plants source their carbon for photosynthesis from the TDIC pool; therefore, changes in $\delta^{13}C_{TDIC}$ can have major effects on $\delta^{13}C_{OM}$, particularly if these sources of carbon dominate the total organic matter production for the lake system (Leng and Marshall, 2004). Aquatic plants can utilise both bicarbonate (HCO_3^-) and dissolved CO_2 (Benedict et al., 1980) for photosynthesis, as can phytoplankton (Reynolds, 2006). However, they will preferentially take up dissolved CO_2 (Miller and Coleman, 1980; Miller et al., 1990), which has lower $\delta^{13}C$ values (–7‰ if in equilibrium with atmospheric CO_2) than HCO_3^- (~0‰; Keeley and Sandquist, 1992). Furthermore, the degree of fractionation is greater when CO_2 is utilised (Smith and Walker, 1980;

Lucas, 1983), which can lead to their ^{13}C being depleted relative to that of the TDIC (Smith and Walker, 1980; Beardall et al., 1982).

HCO_3^- utilisation occurs when lakes become CO_2 limited, under conditions of high pH and temperature (Holmes et al., 1999), and is coupled to lower lake levels, perhaps due to lack of rainfall (Leng et al., 2005). Li and Ku (1997) suggested that for closed lakes which have long residence times, this mechanism could lead to reduced $\delta^{13}C_{OM}$ values as the TDIC exchange with isotopically heavier atmospheric carbon is pronounced. Conversely, during wet periods, the lake carbon pool is refreshed from a greater influx of soil-derived CO_2, which has lower $\delta^{13}C$, although this is dependent on the catchment vegetation photosynthetic pathway, see figure 2.7 (Leng et al., 2005).

2.3.2.3 Temporal shifts in $\delta^{13}C_{TDIC}$

$\delta^{13}C_{TDIC}$ is influenced by the relative contribution of its sources (which have varying $\delta^{13}C$), including carbonate rock weathering and the photosynthetic pathway of catchment vegetation which enters the lake through surface/ground water flow (see figure 2.7; Leng et al., 2005). For example, C_4-dominated catchment vegetation can lead to a TDIC relatively enriched with ^{12}C due to the reduced fractionation of their photosynthetic pathway. Of greater importance for lowland forested sites of closed hydrology is atmospheric CO_2 exchange ($\delta^{13}C$ = –7‰) and aquatic organic matter respiration/photosynthesis ($\delta^{13}C$ values outlined above) (Fry, 2006; Campeau et al., 2017).

During periods of enhanced lake productivity, or in lakes with relatively high autochthonous production, the TDIC pool may become enriched in $\delta^{13}C$ over time (Leng et al., 2005) due to the ^{12}C in the TDIC pool being assimilated into organic matter (O'Leary 1988). This mechanism is reversed when organic matter is oxidised at the lake bed, releasing ^{12}C back into the carbon pool (McKenzie, 1985). The addition of allochthonous organic matter can therefore be a source of ^{12}C to the TDIC by increasing the rate of organic matter oxidation. Lake water stratification (and accordingly anoxia) directly influences this mechanism by slowing organic matter oxidation as microbes involved in degradation must find alternative electron donors which are more reduced and consequently less efficient (Zehnder and Svensson, 1986; Bastviken, 2009). This can lead to a more ^{13}C-depleted TDIC, as shown by figure 2.7 (Leng et al., 2005).

2.4 The Lacustrine Nitrogen Cycle

Nitrogen (N) is an essential nutrient to produce life-dependent biomolecules such as amino acids, proteins, and nucleic acids. As such, along with phosphorus, they determine the extent of productivity available to higher trophic levels as they are limiting nutrients for photosynthesis (see Section 2.2.2.3) (Smith and Schindler, 2009; Moss, 2012). All stages of the global nitrogen cycle (nitrogen fixation, nitrification, nitrate assimilation, ammonification, and denitrification) occur in, or are directly connected to, the lacustrine system (Bernhard, 2010; Stein and Klotz, 2016).

The largest reservoir of N is triple-bonded N_2 gas (78% of the atmosphere). Despite its continual availability for aquatic systems (Ferber et al., 2004), it is inaccessible to most organisms for use as a nutrient for growth as it must first be fixed by diazotrophic microorganisms into reactive nitrogen (defined as N bonded to C, O, or H, as in organic N) (Vitousek et al., 1997). Therefore, the availability of reactive N may limit primary production as it is dependent on the rate of microbial fixation (Lebauer and Treseder, 2008; Scott and McCarthy, 2010).

2.4.1 The lacustrine nitrogen budget

Sources of nitrogen entering lake systems include (a) precipitation falling directly onto the lake surface, (b) inputs from surface and groundwater drainage, and (c) direct N_2 fixation in both the water and the sediments by cyanobacteria (Ferber et al., 2004; Brönmark and Hansson, 2005). Nitrogen inputs into the biosphere are highly influenced by human activity (Galloway et al., 1995; Smil, 1997; Boyer et al., 2002), such as energy/fertiliser production, which contributes ~150–210 Tg N yr^{-1} (Wetzel, 2001; Fowler et al., 2013). In comparison, natural fixation by diazotrophs yields only 90–130 Tg N yr^{-1} (Wetzel, 2001). Nitrogen may be removed from the lacustrine nitrogen cycle via (a) surface and groundwater outflow, (b) sedimentation of inorganic and organic nitrogen-containing compounds (Findlay et al., 1994), and (c) denitrification with subsequent return of N_2 to the atmosphere (Scott et al., 2008). The rate of nitrogen flux in lacustrine systems is highly dependent on the rate of nitrogen fixation and is therefore influenced the by the interactions between the N fixer and other organisms or its environment, which include a variety of biotic and abiotic controls such as temperature, pH, and competition for recourses (Vitousek et al., 2002).

2.4.2 Nitrogen transformation in lake systems

Inorganic nitrogen occurs in several oxidation states, which leads to complex cycling of nitrogen through the various inorganic and organic reservoirs. In aquatic environments, ammonium (NH_4^+), nitrate (NO_3^-), and nitrite (NO_2^-) are important components of the dissolved inorganic nitrogen (DIN) reservoir, as they source primary production. Traditionally, N transformation has been divided into three biological processes – N_2 fixation, nitrification, and denitrification. The products generated from these processes can be transported into another reservoir, assimilated by autochthonous production, resuspended, or deposited on the lake floor (Syn, 2018; Uuemaa et al., 2018). These processes occur with various intensities depending on the characteristics of the surface water; for example, in dynamic environments, no deposition as is the case in rivers, which is in contrast to relatively stagnant water bodies (Saunders and Kalff, 2001).

The role of stratification is also an important environmental control of nitrogen transformation as the nutrient transfer between the sediment and photic zone is important for supplying photoautotrophic biomass (Wetzel, 2001). In deep lakes, the nutrients released from the sediment tend to accumulate in the hypolimnion, particularly during summer when the temperature (and consequently stratification) is greatest (Jankowski et al., 2006; MacIntyre et al., 2006). In contrast, the shallow lakes (namely polymictic lakes; where the influence of wind is incidentally greater) tend to have well-mixed conditions that create an immediate interaction between the sediment and the photic zone, thus delivering nutrients to primary producers during the whole growth season (Nixdorf and Deneke, 1995). Furthermore, the relatively small water volume relative to the size of the lake bottom in shallow lakes means that a given rate of phosphorus release, for example, will lead to a more rapid increase in the concentrations of the water phase than in deep lakes with their greater water volumes (Sondegaard, 2007).

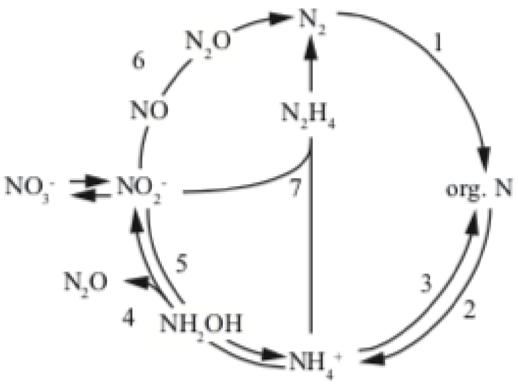


Figure 2.8: Representation of the microbial nitrogen cycle. (1) N_2 fixation, (2) mineralisation, (3) assimilation, (4) nitrification, (5) dissimilatory nitrate reduction to ammonium (DNRA), (6) denitrification, and (7) anaerobic ammonium oxidation (ammox). *Source*: Wenk (2014).

2.4.2.1 Biological processes

Nitrogen fixation is the process by which atmospheric nitrogen enters the biological cycle via assimilation into organic matter (pathway 1 in figure 2.8). Due to the triple bond of N_2, it is an energetically demanding process which can only be accomplished by diazotrophs either as free-living organisms or symbiotic partnerships between species (Howard and Rees, 1996). In lakes with severe nitrogen limitation, cyanobacteria (blue–green algae) can fix nitrogen, leading ultimately to phosphorus limitation (Schindler et al., 2008; Darcy et al., 2018).

Under anoxic conditions, mineralisation results from the degradation of organically bound N to NH_3 or NH_4^+ (pathway 2). The ammonia produced may then be nitrified to nitrate (ammonia oxidation) in which three cohorts of microorganisms gain energy (most using carbon dioxide as carbon source; Bock and Wagner, 2006): (1) ammonia oxidisers that oxidise ammonia to nitrite (nitritation) (Clark and Schmidt, 1967); (2) nitrite oxidisers (nitrite to nitrate); and (3) complete ammonia oxidisers that oxidise ammonia to nitrate, skipping the nitrite stage (comammox) (Van Kessel et al., 2015). This processes is dependent on environmental factors: substrate and oxygen concentration, temperature, and pH (Daufresne and Loreau, 2001). Nitrification was previously thought to strictly aerobic (Kowalchuk and Stephen, 2001); however, methane-oxidising bacteria and archaea have recently been shown to oxidise ammonia (Burgin and Hamilton, 2007).

Conversely, nitrogen may also be assimilated into organic matter via ammonia (NH_4^+) and nitrate (NO_3^-) assimilation (pathway 3). In highly productive lakes, the exploitation of these reservoirs leads to dissolved NH_4^+ and NO_3^- limitation, in which case nitrite (NO_2^-) provides an additional dissolved N source (Talbot, 2002). Nitrite may alternatively be reduced microbially to N_2 via denitrification (intermediate products are shown in figure 2.7; Knowles, 1982). N_2 may then be released into the atmosphere and is therefore the main process of nitrogen elimination from lacustrine environments (Zumft, 1997). Most bacteria involved can perform the multiple steps of denitrification, which is dependent on nitrate concentrations, availability of labile organic matter, temperature, and oxygen conditions (Risgaard-Petersen et al., 2006).

2.5 C/N in Lake Sediments

The ratio of percentage organic carbon to percentage total nitrogen (C/N) found in lake sediment is a useful indicator of the relative proportions of autochthonous and allochthonous sources of lacustrine organic matter (Talbot and Lærdal, 2000). Compared with aquatic plants, terrestrial plants have more carbon-rich molecules (e.g., cellulose) and fewer nitrogen-rich molecules (e.g., protein), thus leading to distinct differences in their C/N ratio (Talbot and Johannessen, 1992; Meyers and Teranes, 2001). For example, ratios of <10 indicate lacustrine algae, whereas values between 10 and 20 indicate submergent and floating aquatic macrophytes or a mixed source, and values >20 indicate emergent macrophytes and terrestrial plants (Holmes et al., 1997, 1999; Meyers and Lallier-Verges, 1999).

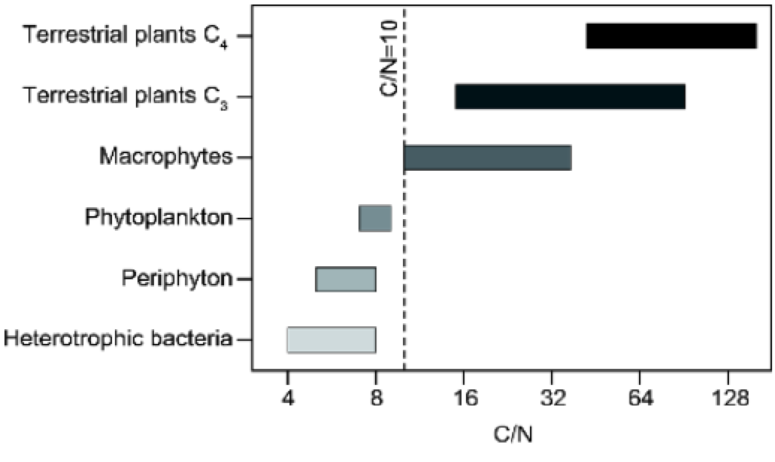


Figure 2.9: C/N ranges for various biological sources to lake sediment. *Source*: Liu et al. (2019).

By analysing the C/N ratio of lake sediment, it is possible to draw conclusions about the OM source (Silliman et al., 1996). At times of high lake productivity when the OM is dominated by phytoplankton and macrophytes (i.e., autochthonous production), C/N ratios will be much lower than during periods dominated by allochthonous OM. A mix of allochthonous and autochthonous OM is common in lakes, which produces intermediate C/N ratios (~15); however, shifts over time can be used to deduce their relative contribution and is aided by $\delta^{13}C_{OM}$, which is also dependent on different $\delta^{13}C$ values of end members (Leng et al., 2005; see Section 2.3.2).

The C/N ratio of OM may be altered during deposition and burial (Thornton and McManus, 1994) and should therefore be considered for palaeoecological investigations. For example, the C/N ratio of algae can increase during diagenesis due to selective degradation of nitrogen-rich proteins, whereas the C/N ratio of woody materials tends to decrease over time as carbon-rich sugars are more quickly broken down (Meyers et al., 1984; Meyers and Teranes, 2001). Also, owing to the higher lability of autochthonous carbon, it is disproportionately accumulated in sediments, which is reflected in the C/N ratio (Wetzel, 2001; Gudasz et al., 2012). Under most circumstances, however, diagenetic alterations remain consistently below the microbially active top section of the lake sediment, and thus can be considered negligible for most paleolimnological reconstructions (which do not cross this boundary) (Meyers, 1994; Meyers and Teranes, 2001).

2.6 $\delta^{15}N$ in Lake Sediments

The nitrogen cycle in freshwater environments involves a wide range of biological and physical processes that cause isotopic fractionation, which leads to highly variable $\delta^{15}N$ signatures (Talbot, 2002; Finlay and Kendall, 2007). This is due to the isotopically distinct source pools of dissolved inorganic nitrogen [ammonium (NH_4^+), nitrate (NO_3^-), and nitrite (NO_2^-)] (Talbot and Johannessen, 1992; Kendall, 1998; Wolfe et al., 1999) and the large isotopic fractionations during nitrogen transformation which are highly sensitive to local environmental conditions (Talbot and Lædal, 2000; Guiry, 2019). As such, nitrogen isotopes are more difficult to interpret than carbon isotopes and consequently are less commonly used (Leng et al., 2005). However, they can provide valuable information on the paleoenvironment such as determining sources of OM

(Meyers and Ishiwatari, 1993; Brenner et al., 2006) and indicating trophic position (Brenner et al., 1999; Post, 2002), particularly when combined with other geochemical data (Talbot, 2002). Diagenetic alterations to $\delta^{15}N$ of organic matter during sedimentation are considered negligible (Meyers and Ishiwatari, 1993; Teranes and Bernasconi, 2000; Ogawa et al., 2001).

The $\delta^{15}N$ values of organic matter found in lake sediments are influenced by the $\delta^{15}N$ of the source from which it derives its nitrogen, but also generally decreases with increasing trophic state, perhaps reflective of the greater relative contribution of ^{15}N-depleted, nitrogen-fixing cyanobacteria to the sediment organic matter pool at higher trophic states (Brenner et al., 1999). Nitrogen fixation from atmospheric N_2 (^{15}N = 0‰ by definition) causes only limited nitrogen isotope fractionation (–2 to +2‰) (Casciotti, 2009, and references therein). Variations in the $\delta^{15}N$ of terrestrial plants are therefore controlled by both extrinsic (nitrogen sources) and intrinsic (e.g., N-fixing or non-N-fixing plants, variable discrimination during assimilation) factors (Evans, 2001; Robinson, 2001). Most terrestrial plants utilising atmospheric N_2 will have a $\delta^{15}N$ value of approximately 1‰ (Meyers and Ishiwatari, 1993), but it can be as high as +10‰ (Talbot, 2002). Cyanobacteria produce organic matter in the range –3‰ to +1‰ (Fogel and Cifuentes, 1993).

2.6.1 Within-lake processes

Isotope fractionation is based on the fact that different isotopes have distinct masses and therefore, in most microbially-mediated reactions, molecules containing the lighter ^{14}N react more quickly than those containing ^{15}N. Consequently, aquatic nitrogen pools (e.g. NO_3^-) become enriched in ^{15}N over time. The rate of nitrogen transformation is therefore an important control on the organic matter which derives dissolved aqueous nitrogen. These pools also exhibit significant diurnal and seasonal variation (generally –6 to +6‰) (Cornell et al., 1995; Russell et al., 1998), which, combined with the effect of climate (Heaton, 1987; Handley et al., 1999), is reflected in the large range for aquatic organic matter (Talbot, 2002; Chappuis et al., 2017). This makes correlations with ^{13}C or C/N difficult as ^{15}N values are less well constrained (Leng et al., 2005).

The main nitrogen sources in aquatic systems are atmospheric deposition, mineralisation of organic matter, and animal waste. The $\delta^{15}N$ signature of these sources is variable and depends on factors such as basin land use, the presence of N-fixing plants in the watershed, the presence of reducing conditions, and local processes (e.g., assimilation, nitrification, denitrification, and mineralisation; Finlay and Kendall, 2007). Nitrification and denitrification influence the $\delta^{15}N$ of NO_3^- in opposing ways, with the former causing ^{15}N enrichment and vice versa (Finlay and Kendall, 2007). Aquatic plants use inorganic nitrogen forms, mainly NH_4^+ and NO_3^- (Barko et al., 1991; Peipoch et al., 2014) and organic nitrogen (e.g., amino acids). Autochthonous sources of OM, which utilise dissolved nitrate as the nitrogen source, will have a $\delta^{15}N$ value of approximately 8‰ (Meyers and Ishiwatari, 1993).

2.7 Chironomids in Lake Sediments

2.7.1 Overview

Chironomids (Insecta: Diptera) are a cosmopolitan group of flies (non-biting midges), which as larvae are often the most abundant bottom-dwelling macroinvertebrates in aquatic ecosystems (Cranston, 1995; Holmes, 2014). They have a particular suitability for use in paleolimnological-based reconstructions for a number of reasons: (1) they are extremely abundant in sediments, thus allowing high temporal resolution analyses (Birks, 1998); (2) they inhabit a wide range of biotopes owing to their ability to tolerate extremes of pH, salinity, oxygen concentration, temperature, and productivity (Armitage, 1995; Brooks, 2006); (3) the ecological optima of individual species are narrow, especially temperature, making them precise indicators (Verbruggen et al., 2011; Holmes, 2014); (4) the response of chironomids to any change in conditions is local and fast owing to their short life cycle (<1–3 years) (Tokeshi, 1986); (5) their chitinous head capsules are well preserved in lake sediment (Miller, 1991; Verbruggen et al., 2009), enabling species-level identifications (Brooks et al., 2007); and (6) the ^{13}C value of their head capsules reflects that of their diet, permitting the reconstruction of within-lake carbon cycling (Wang et al., 2009; Frossard et al., 2013a; van Hardenbroek, 2009; Verbruggen et al., 2009).

2.7.2 Biology and ecology

Chironomids experience complete metamorphism, through four life stages, e.g., larvae, pupa, and imago (Porinchu and MacDonald, 2003; Walker, 2013). Their eggs are laid in a hygrophilous jelly matrix dropped into the water or attached at one end to a substrate such as macrophytes (Chang, 2015). Once the eggs have hatched, they live as larvae in the benthos, undergoing ecdysis for four instars of progressively larger size (Oliver, 1971; Holmes, 2014). The timing and availability window of adult emergence from pupae to imago vary between species and with climate (Self, 2010). Adults (imago) typically live for a few days and mate briefly in swarms. The duration of this cycle ranges between multiple times per year to every 3 years (Tokeshi, 1986).

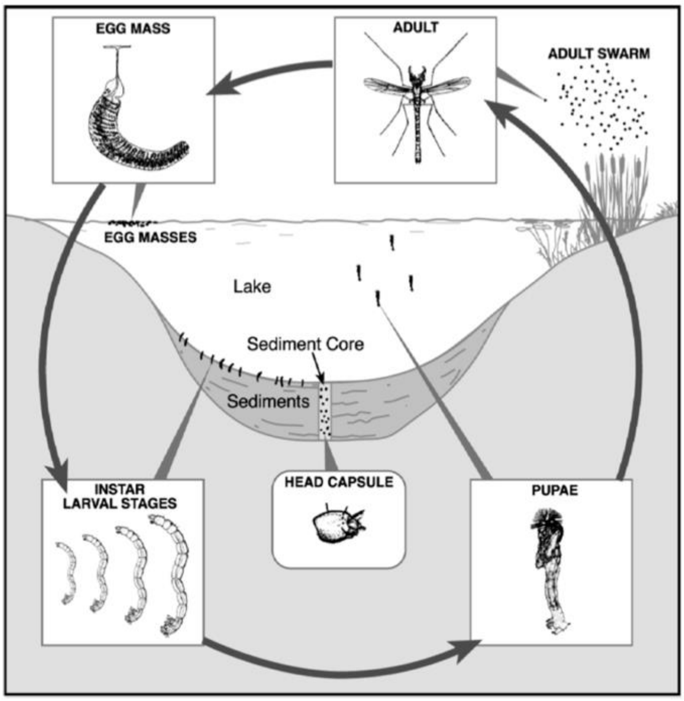


Figure 2.10: Life cycle of aquatic Chironomidae. Ecdysis between instars leads to the sedimentation of chitinous head capsules (subfossil Chironomidae). *Source*: Porinchu and Macdonald (2003).

2.7.2.1 Feeding mechanisms

As larvae, chironomids feed on a wide variety of food sources, including (a) algae and macrophytes, (b) detritus and associated microorganisms (e.g., methane-oxidising bacteria), (c) woody debris, and (d) other invertebrates (including other Chironomidae) (Berg, 1995). They therefore constitute an important energy link to higher trophic levels as they are an important food source for invertebrates, fish, birds, and even amphibian consumers (Berg, 1995).

Different chironomid species exhibit distinct functional traits related to their biological and physiological properties (Usseglio-Polatera et al., 2000; Serra et al., 2016), which are indicative of environmental changes (response traits) or driving such changes in the ecosystems (effect traits) (Nock et al., 2016). For example, distinctions of the mentum and ventromental plates can be made based on feeding types as they exhibit a range of modes including collector-gatherers, collector-filterers, scrapers, shredders, engulfers, and piercers (Antczak-Orlewsk et al., 2021). However, the plasticity inherent to species' feeding behaviour is problematic for categorisation and for identifying the driving factors behind adaptations in feeding habits (Berg, 1995; Serra et al., 2017). Functional feeding groups (FFGs) help overcome this issue by combining the feeding mode with preferred type of nutrition (Mandaville, 2002; Merritt et al., 2008), thus enabling species compositions to be assessed with regard to environmental changes and benthic resource utilisation (Kivila, 2019, 2020).

Algae are known to be a major food resource for chironomids (Ali et al., 2002); depending on feeding mode, chironomids feed on benthic, epiphytic, suspended, and/or benthic macro-algae (Berg, 1995). Filter-feeding chironomids ingest suspended algae; collector-gatherers feed on deposited algae and detritus; scrapers remove or feed on algae from the surfaces of plants and other detrital objects; and shredders mainly feed on coarse particulate organic matter including submerged wood, leaf litter, and living macrophytes on macro-algae (Berg, 1995). *Chironomus* can also ingest methane-oxidising bacteria due to their tube building into the anoxic sediment (see Section 2.7.4.1).

2.7.2.2 Food webs and basal resources

Tracing the origin of organic matter that sustains food webs is an ongoing challenge in aquatic ecology (Tanentzap et al., 2017). Food webs are highly complex due to the spatial variability of their components: nutrients, detritus, and organisms (Polis et al., 1997). The base of the food web (i.e. basal resources) is the combined primary production (photoautotrophs) in a system and is where primary consumers such as chironomids acquire their nutrients (Post, 2002, Karlsson et al., 2012). Despite the high abundance of terrestrial organic matter in most lacustrine water columns, aquatic organic matter often is preferentially assimilated by aquatic consumers due to its higher nutritive value and lability (Lau et al., 2014; Guo et al., 2016). Novel paleolimnological approaches which measure the carbon isotopic content of invertebrates have provided insights into long-term resource partitioning in aquatic consumers and have shown that invertebrates select for aquatic resources (Belle et al., 2017a, 2019; Rantala et al., 2015).

2.7.3 Indicators of paleoenvironment and paleoclimate

In descending order of importance, influencing factors on the species composition of chironomids in any given lake are temperature (Walker et al., 1991b), trophic status (Brooks et al., 2001; Brodersen and Andersen, 2002), oxygen conditions (Verneaux and Aleya, 1998), nature and amount of organic matter in the sediment (Brinkhurst, 1974; Verneaux and Aleya, 1998), and type of substrate (McGarrigle, 1980; Palomaki, 1989). Under unique circumstances, assemblages may be driven by salinity (Charles and Smol, 1994; Walker, 1995; Vershuren et al., 2004) and pH (Brodersen and Quinlan, 2006; Velle et al., 2010). The mechanism by which these factors influence chironomid assemblages is highly complex and subject to ongoing debate (McKeown et al., 2019; Stivrins et al., 2021).

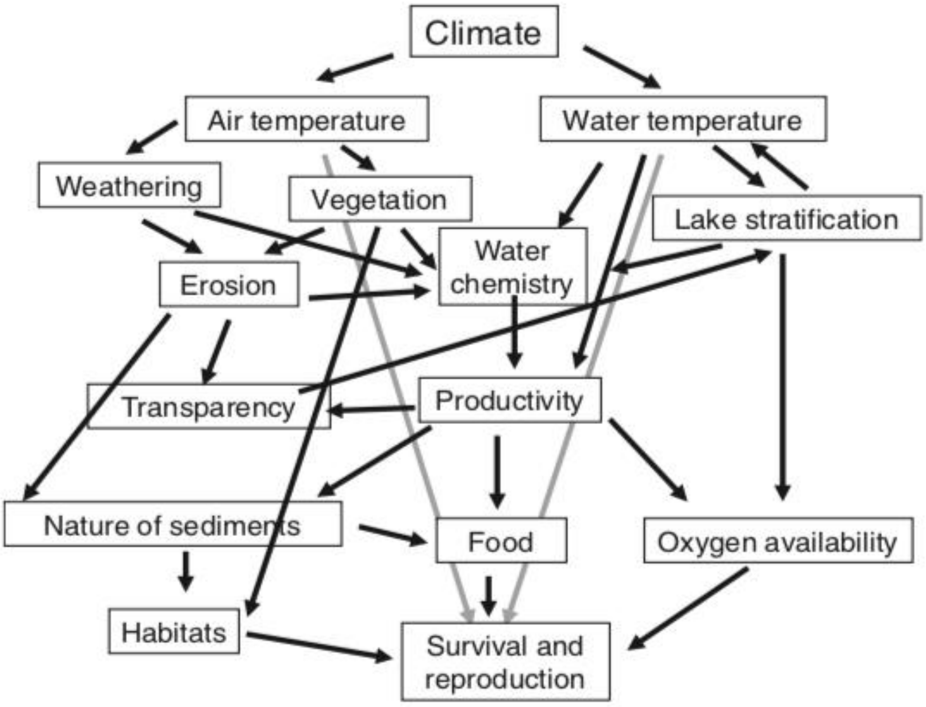


Figure 2.11: Direct (black) and indirect (grey) effects of temperature on chironomid assemblage, and the relationships between variables. Indirect temperature effects (so-called confounding variables) are mediated through changes in the catchment and chemical/physical lake properties. *Source*: Eggermont and Heiri (2012).

2.7.3.1 Transfer functions

Temperature is reported to be the single most important explanatory variable for chironomid assemblages (Walker et al., 1991b; Eggermont and Heiri, 2012). Owing to their narrow ecological optima, assemblages can be used for numerical inference models (so-called transfer functions) to quantitatively reconstruct past lake temperature (Birks, 1995, 1998). Such reconstructions involve two discrete steps: (1) modelling the relationship between modern chironomid distributions and modern lake temperatures using a transfer function or inference model (e.g., Brooks and Birks, 2000; Heiri et al., 2011; Chang, 2015) and (2) applying the model to the paleo assemblage to derive a reconstruction of past climate/environmental change (e.g., Walker et al., 1991a).

Several assumptions necessarily follow these steps but are rarely met (Telford et al., 2004; Juggins, 2013). The most problematic of these is that variables other than temperature are considered negligible (Birks et al., 2010), which is an issue particularly for Holocene records when temperature fluctuations were relatively small compared with the influence of confounding factors (Velle et al., 2010). To account for this, interpretations should include good ecological data on taxa and sites should be carefully selected, e.g., buffered against pH changes (Brooks et al., 2012a; Velle et al., 2012).

2.7.3.2 Relationship between climate, catchment vegetation, and chironomid assemblages

Many studies have demonstrated that lake sediment organic matter content and dissolved organic matter in lake water can be strong and statistically significant explanatory variables for the distribution of chironomid assemblages in lakes (Eggermont and Heiri, 2012, and references therein). As climate is a major determinant of vegetation in the watershed, it may influence the input of allochthonous organic matter and hence food quality and quantity, lake pH, lake transparency, and dissolved organic carbon (DOC) in the water column (Schindler, 1997). As the food resource availability in lakes is strongly linked to vegetation composition/cover (Heino, 2008), traits related to resource exploitation in chironomid communities are therefore well constrained by catchment characteristics such as vegetation type (Engels et al., 2020; Stivrins et al., 2021). Belle and Goedkoop (2021) demonstrated this effect across an elevation gradient showing that for warm lakes with forested catchments, the incidence of sediment-feeding (and larger) taxa was greater compared with cold environments and above the tree line.

2.7.4 $\delta^{13}C$ in chironomids

Carbon stable isotope analysis of the anterior part of the larvae exoskeleton (the head capsule, HC; $\delta^{13}C_{HC}$) is used to reconstruct temporal changes in carbon resources incorporated into the chironomid biomass (van Hardenbroek et al., 2013; Frossard et al., 2014; Belle et al., 2017b). Chironomid larvae are ideal organisms to study within-lake carbon cycle variability as (1) they show a high divsity in feeding modes and link the pelagic and benthic food webs in different ways (Serra et al., 2016, 2017; Belle and Goedkoop, 2021), (2) their carbon isotopic composition ($\delta^{13}C_{HC}$) reflects that of their diet (DeNiro and Epstein, 1978; Akerblom and Goedkoop, 2003) and is chemically preserved in lake sediments (Verbruggen et al., 2009; van Hardenbroek et al., 2009), and (3) $\delta^{13}C_{HC}$ can be used to reconstruct the dietary source and transfer pathway of carbon due to variable source $\delta^{13}C$ signatures (Wooller, 2012; Frossard et al., 2014; van Hardenbroek, 2013, 2014; Belle et al., 2016, 2017b), for example, ^{13}C-depleted MOB (Bunn and Boon, 1993; Kiyashko et al., 2001; Grey et al., 2004; Kelly et al., 2004).

The carbon isotopic signature of insects is determined by their diet (DeNiro and Epstein, 1978; Vander Zanden and Rasmussen, 1999). The $\delta^{13}C$ of chironomid tissue therefore largely reflects the isotopic values of the food ingested by the larvae and will give insight into the carbon cycling within lake food webs (Akerblom and Goedkoop, 2003). A number of studies in a controlled laboratory environment have confirmed this with minor offsets (–0.8 to +1.2‰) between $\delta^{13}C$ values of the food and chironomid biomass (Goedkoop et al., 2006; Wang et al., 2009), particularly for fourth instar specimens (Heiri et al., 2012; Frossard et al., 2013a). Adding to these data, Belle et al. (2020) showed that temperature did not significantly impact carbon and nitrogen isotopic fractionation values, thus reducing the confounding effects of climate change on food availability.

2.7.4.1 Feeding ecology and $\delta^{13}C_{HC}$

Members of the *Chironomus* genus have haemoglobin (Brodersen and Andersen, 2008) and can therefore tolerate the low oxygen conditions in which methane-derived carbon is available (Saether, 1979; Deines et al., 2007a). As a typical filter-feeder, *Chironomus plumosus* builds U-shaped dwelling tubes in the loose sediment; here they can transport oxygen and food into the tube by undulating their body (Johnson, 1987; Brooks et al., 2007). Additionally, *C. anthracinus*-type is a typical deposit-feeder that inhabits vertical I-shaped tubes in the mud; it sweeps the sediment surface surrounding its tube, and food is focused down to the interface between oxidised and reduced sediment (Johnson, 1985; Grey and Deines, 2005).

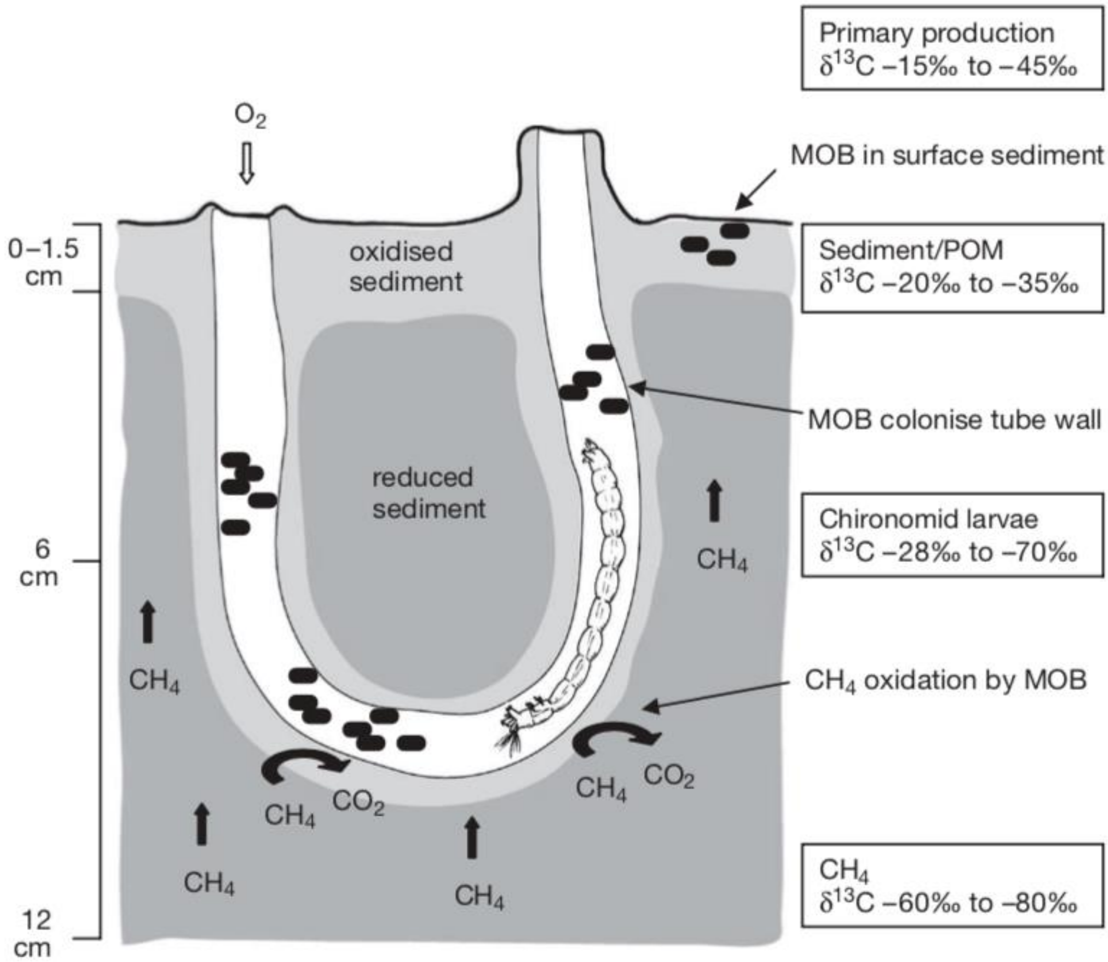


Figure 2.12: Cross-section through a *Chironomus plumosus* tube. The chironomid is thought to maintain an interface of oxic–anoxic conditions by pumping water through, which creates a microhabitat for MOB. Typical δ^{13}C values are shown by the boxes with arrows indicating direction of carbon transfer. *Source*: Deines et al. (2007a).

Hershey et al. (2006) hypothesised that the tube-dwelling nature of some chironomids may allow increased utilisation of methanotrophs which are ^{12}C enriched

($\delta^{13}C$ <–50‰ (Whiticar, 1999; Grey et al., 2004). Indeed, larvae assimilating settling phytoplankton should exhibit relatively ^{13}C-enriched signatures, reflecting the use of atmospheric or dissolved carbon dioxide and bicarbonate (see Section 2.7.2.1) (Fry, 2006). Using a two-source isotope-mixing model, Eller et al. (2005) estimated the contribution of methane-derived carbon to the diet of *C. plumosus*-type was between 16 and 30%, with the more significant contribution occurring in the dimictic lake. Eller et al. (2007) suggested that the oxic/anoxic interface of the chironomid larval tube may provide a constant source of MOB for *Chironomus* larvae but did not find direct evidence for MOB consumption. Jones et al. (2008) measured the carbon isotopic content of chironomids from modern lakes and found exceptionally low ^{13}C values (as low as –72.3‰), confirming the assimilation of MOB, perhaps constituting as much as 70% of their total biomass during periods of prolonged oxygen depletion.

2.7.4.2 Indicators of methanogenesis

Laboratory experiments with ^{13}C-labelled methane first provided direct evidence that chironomids can incorporate methane-derived carbon into their head capsules (e.g., Deines et al., 2007b; van Hardenbroek, 2009). Site-specific studies provided the first direct evidence of MOB assimilation in chironomids in modern lake settings. Deines and Grey (2006) measured the chironomid carbon isotopic content of *Chironomus plumosus* and *C. anthracinus*-type across a depth gradient in Esthwaite Water and demonstrated a significant relationship with methane flux (increasing with depth) and $\delta^{13}C_{HC}$ values. CH_4 flux has also been correlated with $\delta^{13}C_{HC}$ by van Hardenbroek et al. (2012), who sampled seven Swedish lakes and found a significant relationship between the $\delta^{13}C$ of Chironomini and both CH_4 release at the lake surface and sediment. This implies that the study of fossil head capsules should provide robust indicators of past methane production in lakes.

Studies that have quantified past methane output are limited (Walter et al., 2007); however, in the past decade, comparisons between the carbon isotopic content of fossil invertebrate remains with sedimentary organic matter have allowed the reconstruction of the relative contribution of methane-oxidising bacteria to food webs as a proxy for methane availability (Grey, 2016 and references therein). Isotope mixing models have also proven useful in quantifying the uptake of methane-derived carbon by comparing estimated values for algae and MOB with $\delta^{13}C_{HC}$, although this approach may be prone to uncertainty due to variations in $\delta^{13}C$ values of methane and algal material between lakes (and through time) (van Hardenbroek et al., 2018 and references therein).

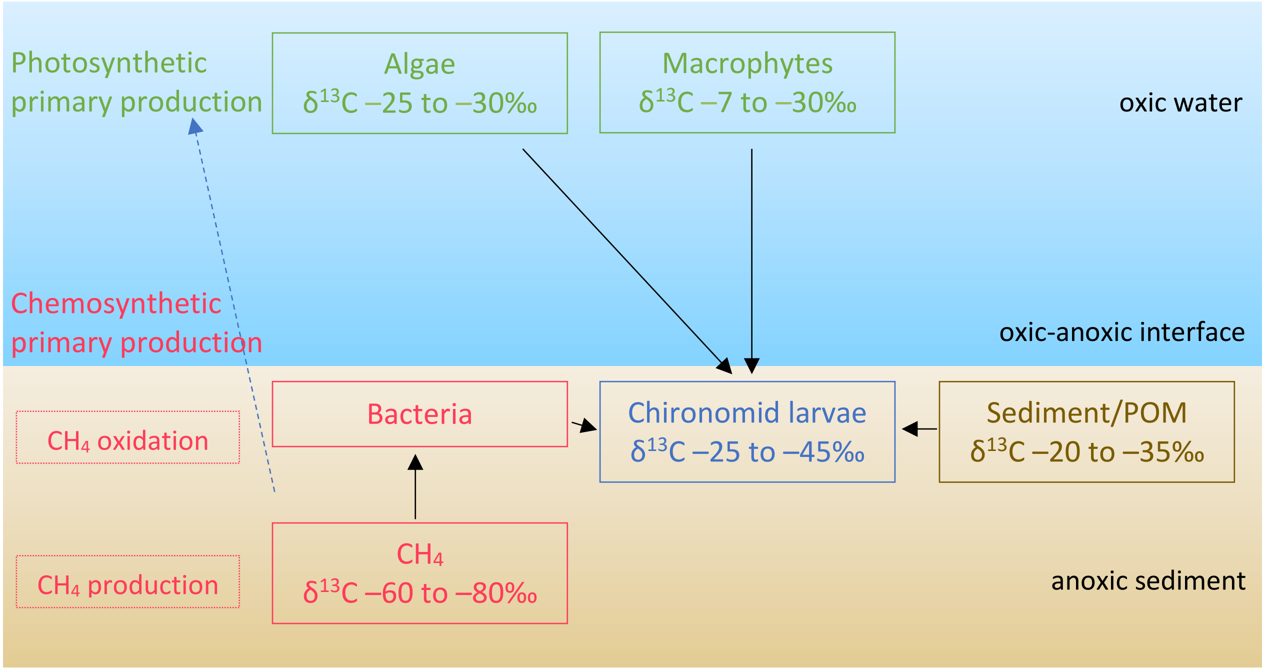


Figure 2.13: Scheme of trophic interactions in the lake ecosystem with common associated $\delta^{13}C$ signatures. Arrows indicate the direction of carbon transfer. References for photosynthetic primary production $\delta^{13}C$ values are in Appendix E. Blue dashed arrow signifies methane oxidation into the aqueous CO_2 (TDIC) pool which can indirectly cause low $\delta^{13}C_{HC}$ values by depleting cause $\delta^{13}C$ of phytoplankton. *Modified from*: Deines (2006).

Space–time studies which measure invertebrate $\delta^{13}C_{HC}$ assume that changes in consumer isotope values reflect temporal changes in the baseline isotope compositions (i.e., food) (Boecklen et al., 2011; van Hardenbroek, 2018). In theory, therefore, the $\delta^{13}C$ values of chironomid remains should follow that of sedimentary organic matter, which has been demonstrated (e.g., van Hardenbroek et al., 2014; Belle et al., 2017a; Anas et al., 2019). However, the carbon isotopic content of food sources varies spatially (see figure 2.13) and chironomids can consume multiple food sources, sometimes switching depending on food source availability (Belle et al., 2017a; Serra et al., 2017; Leite-Rossi et al., 2019). Indeed, Borderelle et al. (2008) observed lower $\delta^{13}C$ values in profundal Chironomini larvae compared with those from the littoral in a modern lake setting. This difference became larger with increasing hypolimnetic anoxia, suggesting the importance of depth and oxygen conditions for methane-derived carbon availability and subsequent incorporation.

Warm temperatures can induce oxygen depletion in the profundal zone of lakes by strengthening the thermal stratification (Jankowski et al., 2006), and organic matter is degraded by methanogenic archaea under anoxic conditions (Borrel et al., 2011). Thus, the importance of temperature has been recognised as a driver of increased CH_4 production, indicated by the $\delta^{13}C$ of invertebrate remains (van Hardenbroek et al., 2013, 2014; Belle et al., 2017b). Moreover, Belle et al. (2018) observed a statistically significant relationship between the $\delta^{13}C_{HC}$ of *Chironomus anthracinus*-type and reconstructed air temperature. Productivity, coupled to temperature (van Hardenbroek et al., 2014; Belle et al., 2017b) and nutrient availability, (Frossard et al., 2015; Schilder et al., 2017), has also been demonstrated as an important driver of chironomid $\delta^{13}C$ values by increasing the strength and duration of hypoxic conditions which induce CH_4 build-up, although studies on the role of nutrient conditions which predate the Anthropocene are limited (Kivila et al., 2019).

Methane production can vary spatially in lakes mainly due to OM quality and depth, particularly in small lakes which may experience accelerated carbon cycling under climate shifts (see the next section) (Praetzel et al., 2020). Studies of chironomid larvae from contemporary lakes have shown this effect, leading to the suggestion that larvae from deeper sites, which are more susceptible to prolonged anoxia, exhibited greater intraspecific variability, and were significantly ^{13}C depleted (Grey et al., 2004; Deines and Grey, 2006). Frossard et al. (2015) demonstrated this effect through time by comparing cores from a range of depths; chironomid $\delta^{13}C$ values became progressively lower with depth, but with the onset of eutrophication spread to the shallower cores, indicating a spatial shift in the anoxic hypolimnion to shallower depths.

2.7.4.3 Other sources of ^{13}C-depleted $\delta^{13}C_{HC}$ values

In many cases, the $\delta^{13}C_{HC}$ values found are only a few ‰ more depleted than POM and bulk sediment. Although many studies make strong arguments for the uptake of methane as the cause of the difference, other methods of generating depleted isotopic values cannot be ruled out. Grey (2016) refers to this as the ‘zone of contention’ in which chironomid $\delta^{13}C$ values ranging from –30 to –40‰ can be theoretically explained due to the incorporation of other chemosynthetic bacteria involved in OM degradation (see table 2.1) or photoautotrophic biomass with low $\delta^{13}C$ due to influences of the TDIC (Kohzu et al., 2004). This is a pertinent issue for such studies of shallow lakes, which usually produce less methane due to more regular mixing, and thus constitutes a smaller proportion of their diet (Nixdorf and Deneke, 1995; Deines and Grey 2006).

The $\delta^{13}C$ of aquatic primary producers are dependent on the $\delta^{13}C_{TDIC}$ values, which are variable. High rates of respiration and microbial decomposition compared with photosynthesis can generate greater amounts of ^{13}C-depleted CO_2 (opposed to preferential ^{12}C uptake via photosynthesis), leading to $\delta^{13}C_{TDIC}$ values as low as –20‰ (Lennon et al., 2006; Kapl et al., 2010; Hecky and Hesslein, 1995). Such a scenario may occur during periods of high DOC concentrations in which photosynthesis may be attenuated and the carbon supply for degradation is high (Rau, 1978; Karlsson et al., 2009). It stands to reason, therefore, that algal $\delta^{13}C$ values can be as low as –35‰ (e.g., Grey et al., 2000), or even lower if the degree of fractionation by phytoplankton is large (up to 15‰; see figure 2.7) (O'Leary, 1988; Bade et al., 2006). Van Hardenbroek et al. (2013) noted that such a process would be expected to affect the $\delta^{13}C_{OM}$ and $\delta^{13}C_{HC}$ values of all benthic invertebrates and can therefore be used to confirm the uptake of methane-derived carbon. Furthermore, turbulence and thermally induced mixing regimes can influence the delivery of chemosynthetically produced CO_2 to primary producers and, in cases where transfer between the benthic and pelagic environments is high, the discrimination of the heavier ^{13}C isotope is increased, which is reflected by lower $\delta^{13}C_{OM}$ values (Belle et al., 2021).

2.8 Research Aims and Questions

The aim of the project was to assess past environmental change and within-lake variability of carbon cycling during the early Holocene. Blakemere Moss has already yielded pollen and chironomid stratigraphical data during the Late-glacial and early Holocene. Approximately 1000 years after the Holocene transition, a very high abundance of littoral-dwelling chironomid taxa was observed (Weston, 2020). The aim was to study these taxa for carbon isotopic content, along with profundal-dwelling *Chironomus*, to test whether their combined use could reveal lake carbon cycling spatial heterogeneity and improve understanding of the relationship between local environmental conditions and methane flux for our shallow lake in a forested catchment setting.

This will be achieved by examining bulk sediment geochemical indicators (C/N, $\delta^{13}C_{OM}$, and $\delta^{15}N$) and chironomid carbon isotopic content ($\delta^{13}C_{HC}$) and taxonomy to produce a temperature reconstruction. The taxon-specific analysis of chironomid carbon isotopic content of both will be used to reconstruct the benthic food web carbon flux for the profundal and littoral and also to assess the extent to which a *Chironomus* diet selects for aquatic organic matter. The drivers of $\delta^{13}C_{HC}$ will also be assessed regarding climate, lake conditions (e.g., productivity, anoxia), food availability, and taxon-specific feeding habits. A high temporal resolution will be used to elucidate sub-centennial drivers of $\delta^{13}C_{HC}$, bulk geochemical proxies, and local environmental change (Perga et al., 2015).

In order to address the knowledge gaps highlighted in the preceding sections, the following questions were asked and will be used to guide the discussion:

- Can bulk geochemical indictors (C/N, $\delta^{15}N$, $\delta^{13}C_{OM}$) and a chironomid-inferred temperature reconstruction (CI-TR) be used to identify drivers of environmental change and interpret chironomid $\delta^{13}C_{HC}$?
- Can *Chironomus* $\delta^{13}C_{HC}$ be used to reconstruct methanogenesis for a shallow lake?
- Is littoral Chironomini $\delta^{13}C$ representative of the carbon isotopic composition of aquatic organic matter?
- Does $\delta^{13}C_{HC}$ correlate with chironomid-inferred temperatures and can differences between *Chironomus* and (littoral) Chironomini be explained using bulk geochemical data?
- Can $\delta^{13}C$ of littoral Chironomini be used to aid interpretations of *Chironomus* diet by revealing the carbon isotopic composition of aquatic organic matter?

CHAPTER III

STUDY AREA

Blakemere Moss is situated in the Delamere Forest in the Mersey basin, Cheshire (52°54′1″N 2°52′1″W), at 73 m asl. Now a peat bog, it is 1000 m long and varies in width (200–500 m). Stratigraphic evidence suggests that during the early Holocene, the maximum depth was ~6–7 m; however, the middle 300 m of the lake was 1–3 m deep (Fairhurst, 1988) (see figure 3.2). The most recent glaciation created the Mouldsworth Gap and the outwash plain on which Blakemere Moss was created (Leah, 1997). The catchment geology of glacial sand and gravel suggests that major paleo stream in-/outputs are unlikely, nor does visible modern evidence exist. The high density of local kettle hole lakes combined with Blakemere's relatively large area means that the catchment to lake area ratio is low (<5).

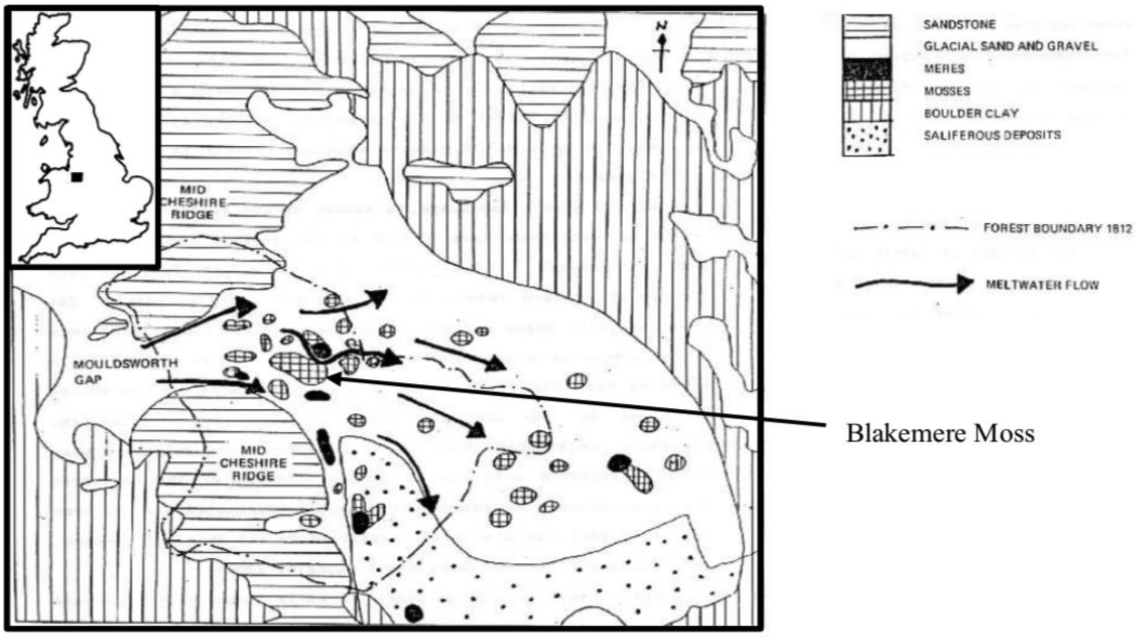


Figure 3.1: Geological map of the area surrounding Blakemere Moss. *Modified from*: Fairhurst (1988).

The absence of elevated local topography removes the possibility of transient cirque glaciation in the area. Atmospheric and meltwater effects of glaciers greatly affect water temperature and therefore chironomid paleo assemblages (e.g., Brooks et al., 2012b).

Although figure 3.1 illustrates meltwater flow from the West, it is highly likely that the ice sheet had retreated before Blakemere Moss became a site of sedimentation (i.e., when the dead ice had melted) (Wilson and Lord, 2014; Ballantyne and Small, 2018). The transition from silt to *Sphagnum* of 4 m depth (at the core location on figure 3.2) suggests that the paleolake infilled around 7000 Cal BP.

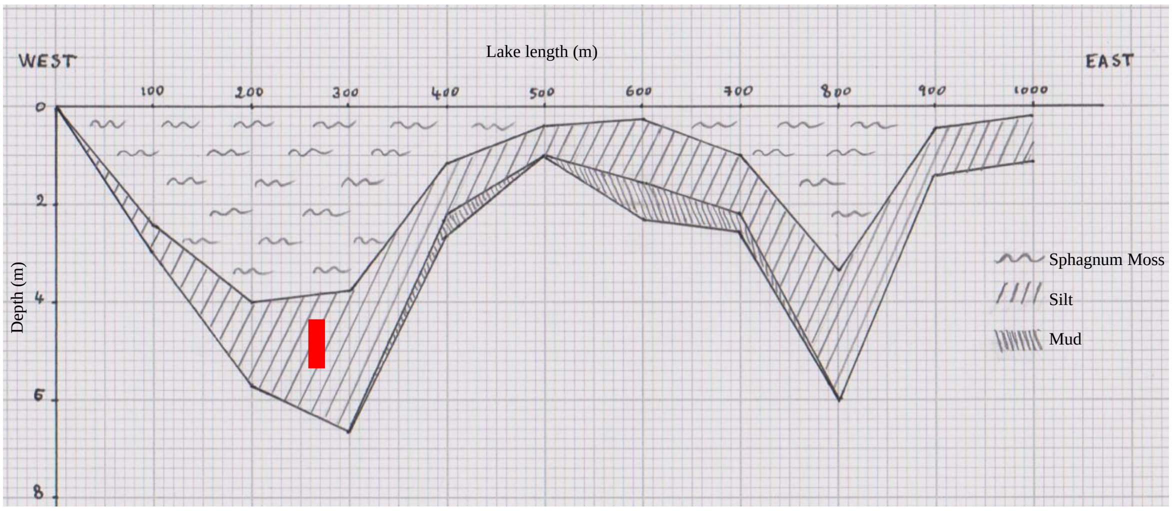


Figure 3.2: Profile and stratigraphy of Blakemere Moss. Approximate core location is indicated in by the red rectangle. *Redrawn from*: Fairhurst (1988).

Unusually, Blakemere Moss is comprised of two kettle hole depressions connected by a shallow plateau leading to a disproportionately large littoral zone. Weston (2020) suggested that the bathymetry may have engendered a littoral-dominated chironomid assemblage, thus inflating chironomid-inferred temperatures for the early Holocene (18 °C MST). The site therefore represents a unique opportunity to test the assumptions of lake ontogeny and biogeochemical cycling during the early Holocene.

CHAPTER IV

METHODOLOGY

4.1 The Core

In 2017, a Late Glacial–Early Holocene sequence (BKM 08/17) was recovered from the western paleo-profundal zone of Blakemere Moss using a Russian-type corer. It has been documented that profundal sediments represent an integration of paleo chironomid assemblages from the entire lake (Tarrats et al., 2018), especially in smaller lakes (Walker and Mathewes, 1987; Heiri, 2004). Four groups of three contiguous 5 mm samples separated by 5 mm intervals were extracted by staff at The University of Manchester.

4.2 Chronology

An age–depth model (figure 4.1) was constructed with seven radiocarbon dates (table 4.1) using the BACON package in RStudio (Blaaw and Christen, 2013). Median age from a 1σ range was calculated using Bayesian statistical methods (Reimer and Reimer, 2007) and data from Reimer et al. (2020) (see Appendix A). The 1σ range in the age-depth model (figure 4.1) ranged from 1653 to 1681 years (see Appendix A). Interpretations of the data with decadal and centennial scale variations are therefore made with these large errors in mind wherever ages are reported.

Depth (cm)	Radiocarbon age (Cal BP)	2σ Calibration	Median (Cal b2k)
86.5	3210 ± 30	3422–3518	3470
143.5	3660 ± 30	3948–4137	4043
177.5	3860 ± 30	4276–4460	8736
579.5	8990 ± 50	10166–10293	10230
666.5	11090 ± 80	12882–13176	13029
683.5	11880 ± 30	13657–13769	13713
692.5	12090 ± 40	13860–14007	13934

Table 4.1: ^{14}C radiocarbon dating-inferred ages.

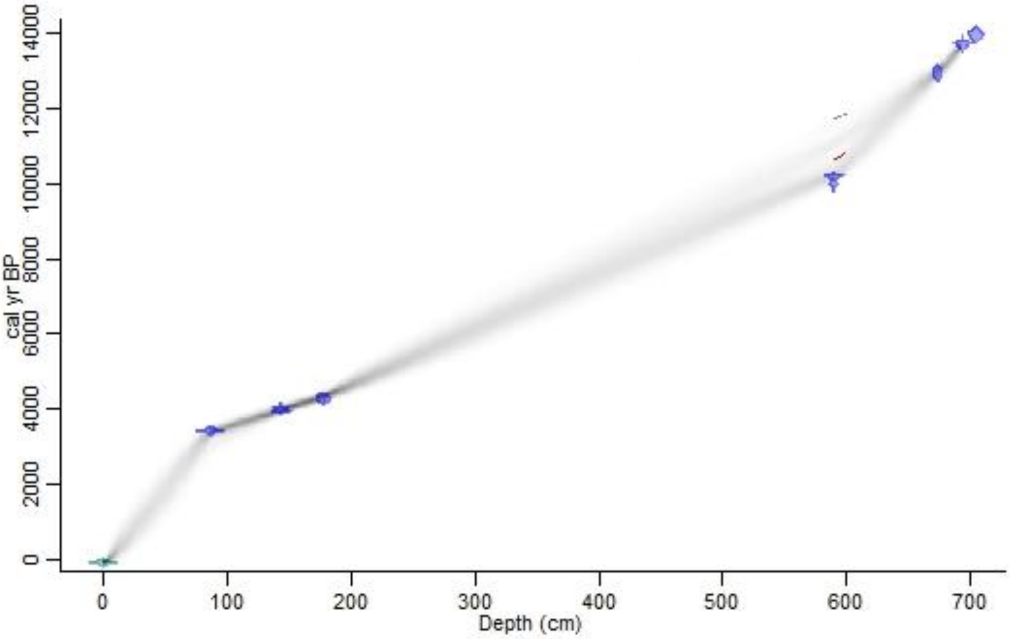


Figure 4.1: Age–depth model derived from radiocarbon-inferred dates 14,000 Cal BP–present. Inferred dates are shown by the red line.

4.3 Bulk Sediment Geochemistry

4.3.1 Pretreatments

Aliquots weighing 0.5 g were extracted, dried at 50 °C overnight and homogenised with a pestle and mortar. Subsamples of 0.25 g were taken from the aliquot to perform carbon and nitrogen stable isotope analyses separately due to the influence of HCl treatment on $\delta^{15}N$ values (Larson et al., 2008). The subsample for $\delta^{13}C$ analysis was acidified using 40 ml of 20% (v/v) HCl to remove excess carbonates. The pH was measured using litmus paper to ensure that the HCl was not neutralised via carbonate reaction. Excess acid was then drained. All subsamples were rinsed with MilliQ water three times using a centrifuge at 3000 rpm. Samples for $\delta^{13}C$ analysis were then measured again for pH to ensure that all acid had been neutralised. After being dried at 50 °C overnight, all subsamples were transferred to tin capsules, weighed, and crimped.

4.3.2 $\delta^{13}C$, $\delta^{15}N$, and C/N measurements

Initially, a 'junk' run was carried out to ascertain %C and %N for random samples across the sequence. From these data, an appropriate weight to achieve a consistent peak height of 10 nA was calculated. The analyses were performed following standard procedures using an elemental analyser interfaced to an Isoprime 100 IRMS. The lake sediment reference material (–27.2‰ V-PDB) used was a secondary standard of known relation to international standards: IAEACH6 (–10.449‰ V-PDB), IAEA600 (–27.771‰ V-PDB; 1‰ air), and USGS41 (47.57‰ air).

Replicate sample measurements (n = 6) on the calibrated lake sediment standard gave an analytical error of ±0.07‰ (1 SD) for stable carbon isotopes. Results are expressed in delta notation with Vienna Pee Dee Belemnite as the standard: $\delta^{13}C$ (‰) = $(R_{\text{sample}}/R_{\text{standard}} - 1)/1000$, where $R = {}^{13}C/{}^{12}C$. For stable nitrogen isotope analysis, replicate sample measurements (n = 12) on the calibrated lake sediment standard gave an analytical error of ±0.09‰ (1 SD). Results are expressed in delta notation with air (N_2) as the standard: $\delta^{15}N$ (‰) = $(R_{\text{sample}}/R_{\text{standard}} - 1)/1000$, where $R = {}^{15}N/{}^{14}N$. C/N was obtained from %C and %N data from samples for $\delta^{15}N$ measurements.

4.4 Chironomids

4.4.1 Pretreatments

The remainder of the samples were deflocculated using 10% KOH solution for 2 h at room temperature and sieved at >150 and >90 μm intervals. The sediment was then exposed to 2.5% (v/v) HCl for 20 min to remove excess carbonates. These pretreatments have been shown to have no significant effect on the head capsule stable carbon isotope ratios (van Hardenbroek et al., 2009). Samples were subsequently rinsed with MilliQ water using a >90 μm sieve.

4.4.2 Extraction and identification

A dissecting microscope (10–60×), forceps, and an adapted Bogorov counting tray were used to extract chironomid head capsules (HCs) from the sediment matrix and stored in MilliQ water. Only HCs from the >150 μm interval were extracted to save time; furthermore, the abundance of 3rd and 4th instar Chironomini HCs was extremely low for the 90 μm interval (van Hardenbroek et al., 2010). *Chironomus* and littoral-dwelling species of the 3rd and 4th instar (Frossard et al., 2013a) from the Chironomini tribe were identified to the highest possible taxonomic resolution and separated into subsamples with reference to Brooks et al. (2007).

Chironomus specimens were identified only to genus level owing to the lack of mandibles and sufficient zoom to identify variable length of the fourth lateral tooth of the mentum (Hofmann, 1971). Littoral species [*Endochironomus, Polypedilum nubeculosum* (hereafter *Polypedilum*), *Glyptotendipes, Cladopelma lateralis, Microtendipes pedellus* (hereafter *Microtendipes*), and *Phaenospectra flavipes* (hereafter *Phaenospectra*)] were selected based on being associated with aquatic macrophytes or observed as inhabiting the littoral zone (Pinder and Reiss, 1983; Brodin, 1986; Walker and Macdonald, 1995; Olander et al., 1997; Klink and Moller-Pillot, 2003; Brooks et al., 2007; Merritt et al., 2008). Subsamples were transferred into tin capsules using forceps and dried at 40 °C overnight, subsequently crimped, and stored in a desiccator. When the HC numbers were too low, adjacent HC samples were pooled together. The new sample age corresponded to the mean age of the pooled samples.

4.4.3 δ^{13}C measurements ($\delta^{13}C_{HC}$)

Chironomid samples were analysed on an elemental analyser interfaced to an Isoprime 100 IRMS. The chitin reference material used was a secondary standard of known relation to the international standards used for bulk carbon isotope analysis. A blank subtraction was carried out on the calibrated data as the blank peak exceeded 20% of the sample signal at times.

Estimating the number of fossils necessary to meet these minimum mass requirements is complicated by the different sizes and weights of larval head capsules of different chironomid species and instars (Heiri et al., 2012). Van Hardenbroek et al. (2009) showed that 20 μg was sufficient to generate a signal of 1.0 V (the minimum voltage considered to produce reliable δ^{13}C analyses with EA-IRMS equipment). Between 5 to 10 head capsules were obtained from the sediment matrix to meet these mass requirements (Van Hardenbroek et al. 2009). The minimum sample mass for δ^{13}C analysis of chironomid remains depends on the type and quality of the equipment used. With our sample mass of ~10 μg, replicate sample measurements ($n = 16$) on the internal standard gave an analytical error of ±0.31‰ (1 SD) when applied to blank-subtracted values. Results are expressed in delta notation with Vienna Pee Dee Belemnite as the standard: $\delta^{13}C\ (‰) = (R_{sample}/R_{standard} - 1)/1000$, where $R = {}^{13}C/{}^{12}C$.

4.5 Temperature Inference

Mean July air temperature estimates were derived from square root transformed chironomid percentage data using the 154 lake Norwegian chironomid-based temperature calibration dataset (Brooks and Birks, 2001). Four lakes were removed due to glacial meltwater interference with the temperature signal. The lakes used span mean July air temperatures of 3.5–16 °C, latitudes from 80 to 58°N, altitudes from 0 to 1600 m, and 142 chironomid taxa.

Using the Rioja package in R, a two-component weighted averaging partial least-squares (WA-PLS) regression model was applied to the palaeodata (Juggins, 2009). This was cross-validated by leave-one-out jack-knifing, which has a root mean square error of prediction ($\mathrm{RMSEP}_{\mathrm{jack}}$) of 1.06 °C, a coefficient of determination (r^2_{jack}) of 0.91, and a maximum bias of 2.37 °C. Sample-specific errors of the inferred temperatures were estimated by 1000 bootstrap cycles (Telford and Birks, 2011).

Taxa below 5% abundance were excluded to improve reliability (Velle et al., 2012). Quinlan and Smol (2001) have established that 50 head capsules are sufficient for obtaining reliable temperature estimates. On the other hand, Heiri and Lotter (2001), indicated that the number of head capsules required for a reliable analysis is case dependent, and in instances where abundances are low, fewer head capsules are needed. Only the Chironomini measured for carbon isotopic content were used for the temperature reconstruction owing to time limitations. This introduced bias to higher temperatures (Eggermont and Heiri, 2012) and, combined with low head counts (20–30), increased uncertainties in (see Appendix D). However, extraneous noise was minimised as the total number of chironomids picked for each sample was high (50–100+).

The results from the chironomid-inferred temperature reconstruction (CI-TR) are comparable and broadly synchronous to the results from Weston (2020) (see Appendix G). Furthermore, species shifts were consistent with temperature changes for both reconstructions, this places confidence in using the present CI-TR to interpret the bulk lake sediment indicators and chironomid isotopic results despite high uncertainties (see Appendix D).

CHAPTER V

RESULTS

5.1 Bulk Sediment Geochemistry and Inferred Temperature

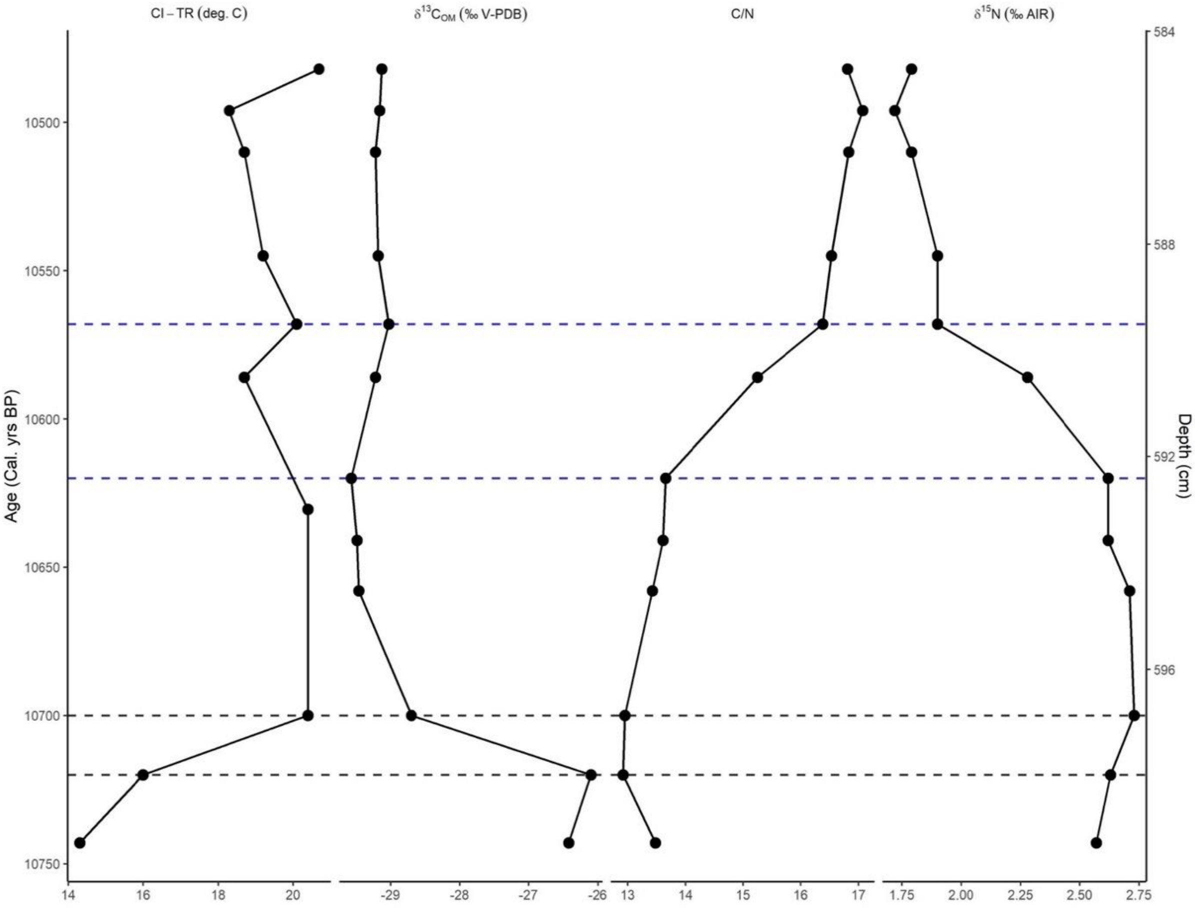


Figure 5.1: Bulk lake sediment indicators ($\delta^{13}C_{OM}$, $\delta^{15}N$, and C/N) and chironomid-inferred mean summer temperatures (sample-specific errors are shown in Appendix D). Key shifts are indicated by the dotted lines at 10,720, 10,700 (black) 10,641, and 10,568 Cal BP (blue).

The Holocene transition is characterised for $\delta^{13}C_{OM}$, C/N, and $\delta^{15}N$ for organic matter and chironomid-inferred temperatures in figure 5.1. At the beginning of the sequence, a rapid shift in carbon isotopic composition from –26.10 to –28.70‰ V-PDB in a period of 20 years (10,720 and 10,700 Cal BP) is indicated by the black dotted lines. In the same period, chironomid-inferred temperature rise significantly from 16 to 20 °C. Thereafter, both $\delta^{13}C_{OM}$ and inferred temperatures remain stable for the remainder of the study period, although inferred temperature does fall by 2 °C for two brief periods before returning to 20 °C at the end of the sequence. The variability of $\delta^{13}C_{OM}$ and CI-TR appears to be independent of C/N and $\delta^{15}N$.

Approximately 100 years after the rapid shift in $\delta^{13}C_{OM}$ and CI-TR, both C/N and $\delta^{15}N$ changed synchronously, with most of the variation occurring between 10,620 and 10,545 Cal BP; indeed, the two indicators were very highly correlated ($R^2 = 0.98$). During this period, C/N and $\delta^{15}N$ shifted from 13.66 to 16.38 and from 2.62 to 1.90‰ (air), respectively. Outside this period, C/N and $\delta^{15}N$ did not change significantly.

5.2 Chironomid $\delta^{13}C_{HC}$

5.2.1 Difference between $\delta^{13}C_{OM}$ and littoral Chironomini ($\delta^{13}C_{HC}$)

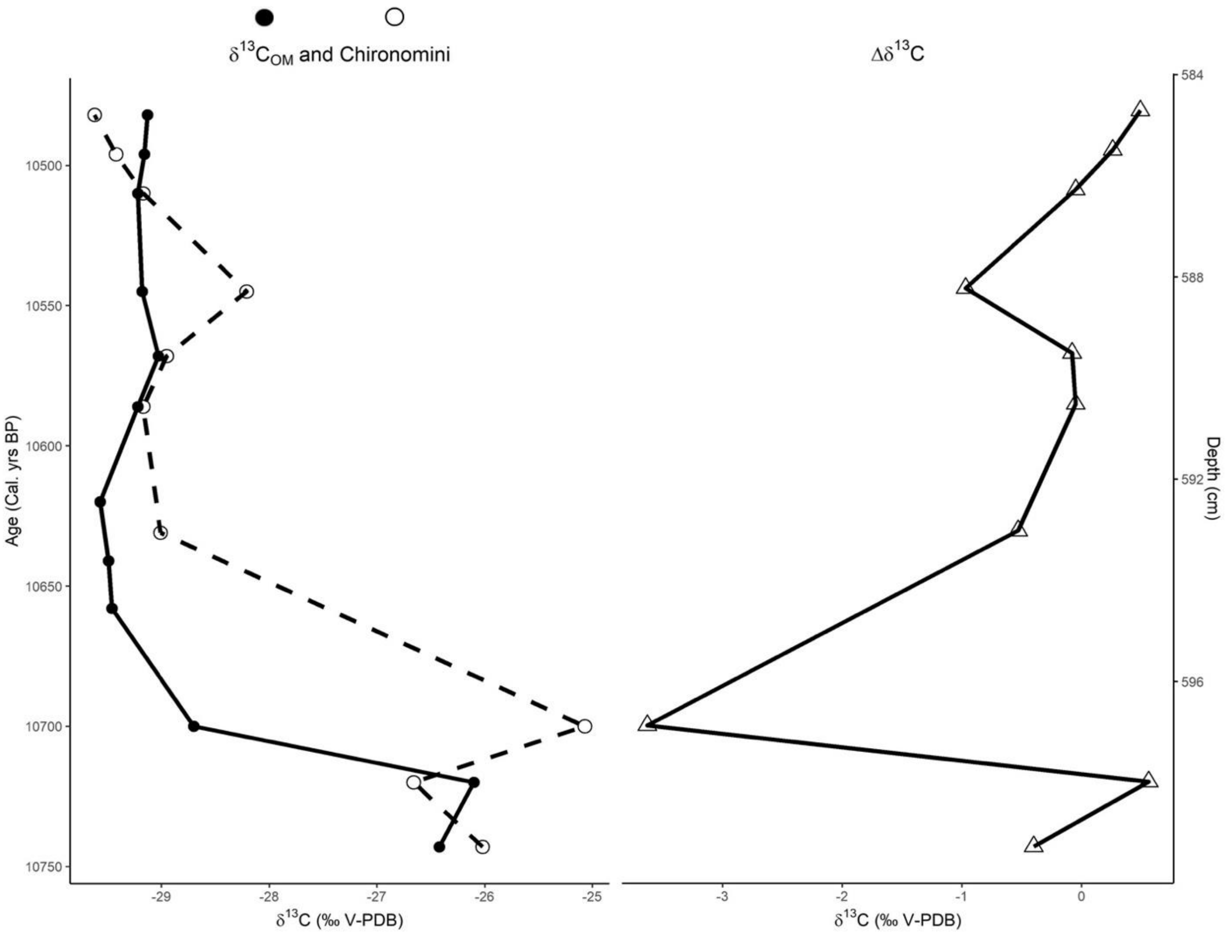


Figure 5.2: Trends in carbon isotopic composition of organic matter ($\delta^{13}C_{OM}$; ‰), carbon isotopic composition of Chironomini remains ($\delta^{13}C_{HC}$; ‰), and difference between carbon isotopic composition of organic matter and Chironomini remains ($\Delta\delta^{13}C = \delta^{13}C_{OM} - \delta^{13}C_{HC}$).

$\delta^{13}C_{HC}$ broadly trends with $\delta^{13}C_{OM}$ as both values fall from –26 to –29‰ (±0.5‰) over the entire sequence. However, no significant correlation was observed, largely due to the high $\Delta\delta^{13}C$ value of –3.87‰ at 10,700 Cal BP; consequently, this was also the highest $\delta^{13}C_{HC}$ value in the sequence (25.07‰). The removal of this sample leads to a statistically significant correlation between $\delta^{13}C_{OM}$ and $\delta^{13}C_{HC}$ ($R^2 = 0.86$). A second $\Delta\delta^{13}C$ peak of –0.97‰ occurred at 10,545 Cal BP but quickly returned to the $\delta^{13}C_{OM}$ range. Following this, the littoral $\delta^{13}C_{HC}$ trended downwards, with positive $\Delta\delta^{13}C$ values for the last two samples.

5.2.2 Difference between $\delta^{13}C_{OM}$ and profundal *Chironomus* ($\delta^{13}C_{HC}$)

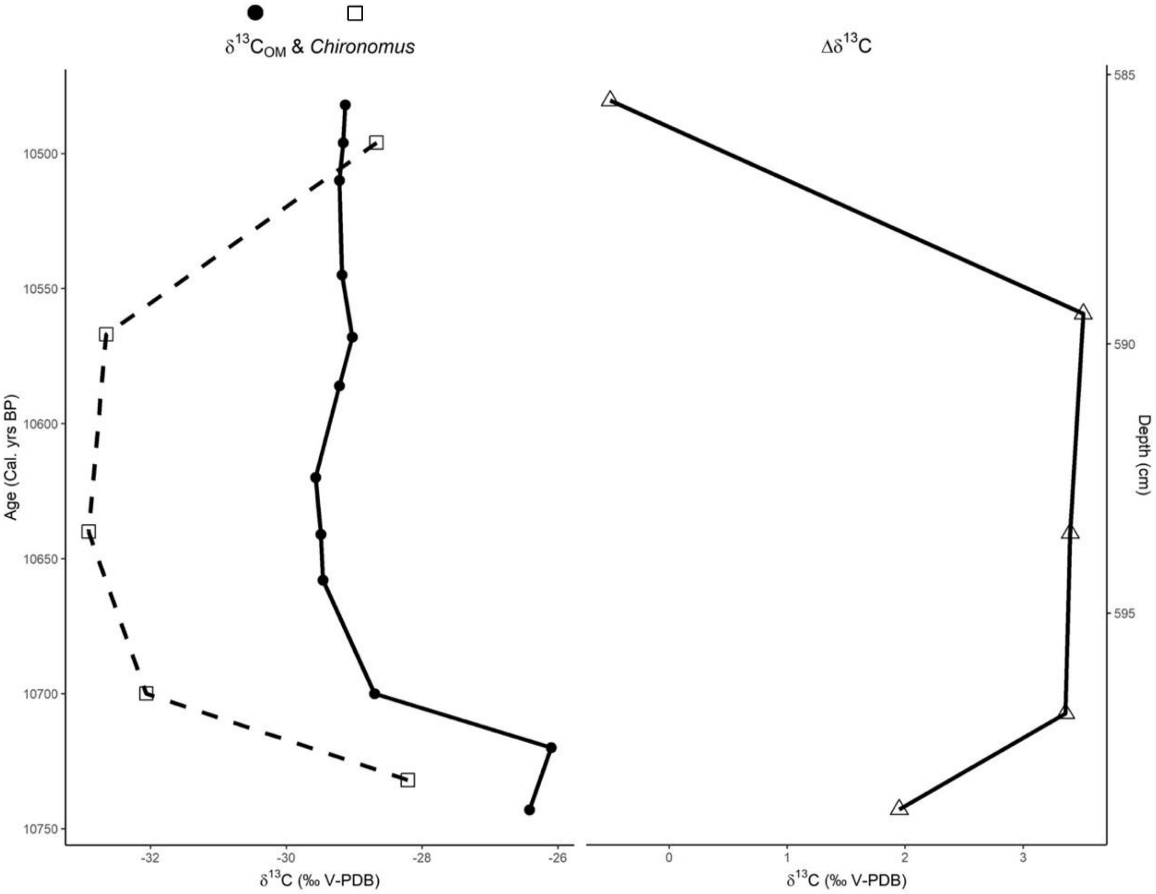


Figure 5.3: Trends in carbon isotopic composition of organic matter ($\delta^{13}C_{OM}$; ‰), carbon isotopic composition of *Chironomus* remains ($\delta^{13}C_{HC}$; ‰) and difference between carbon isotopic composition of organic matter and *Chironomus* remains ($\Delta\delta^{13}C = \delta^{13}C_{OM} - \delta^{13}C_{HC}$).

Chironomus $\delta^{13}C_{HC}$ exhibits ^{13}C depletion compared with $\delta^{13}C_{OM}$ for all samples except the youngest at 10,496 Cal BP. ^{13}C depletion relative to $\delta^{13}C_{OM}$ increased at the beginning of the sequence, indicated by a $\Delta\delta^{13}C$ shift from 1.95 to 3.36‰ between 10,732 and 10,700 Cal BP. Following this, the most depleted $\delta^{13}C_{HC}$ (-32.91‰) value was recorded at 10,640 Cal BP and remained consistent until the final sample at 10,486 Cal BP, at which point *Chironomus* became isotopically enriched in ^{13}C compared with $\delta^{13}C_{OM}$ for the first time in the sequence ($\Delta\delta^{13}C$ = –0.74‰). *Chironomus* did not correlate to $\delta^{13}C_{OM}$ however, the removal of the youngest sample (10,496 Cal BP) results in a highly significant correlation ($R^2 = 0.99$).

5.3 Chironomid Taxa and Key Proxies (CI-TR, C/N, $\delta^{13}C_{OM}$, and Chironomini $\delta^{13}C_{HC}$)

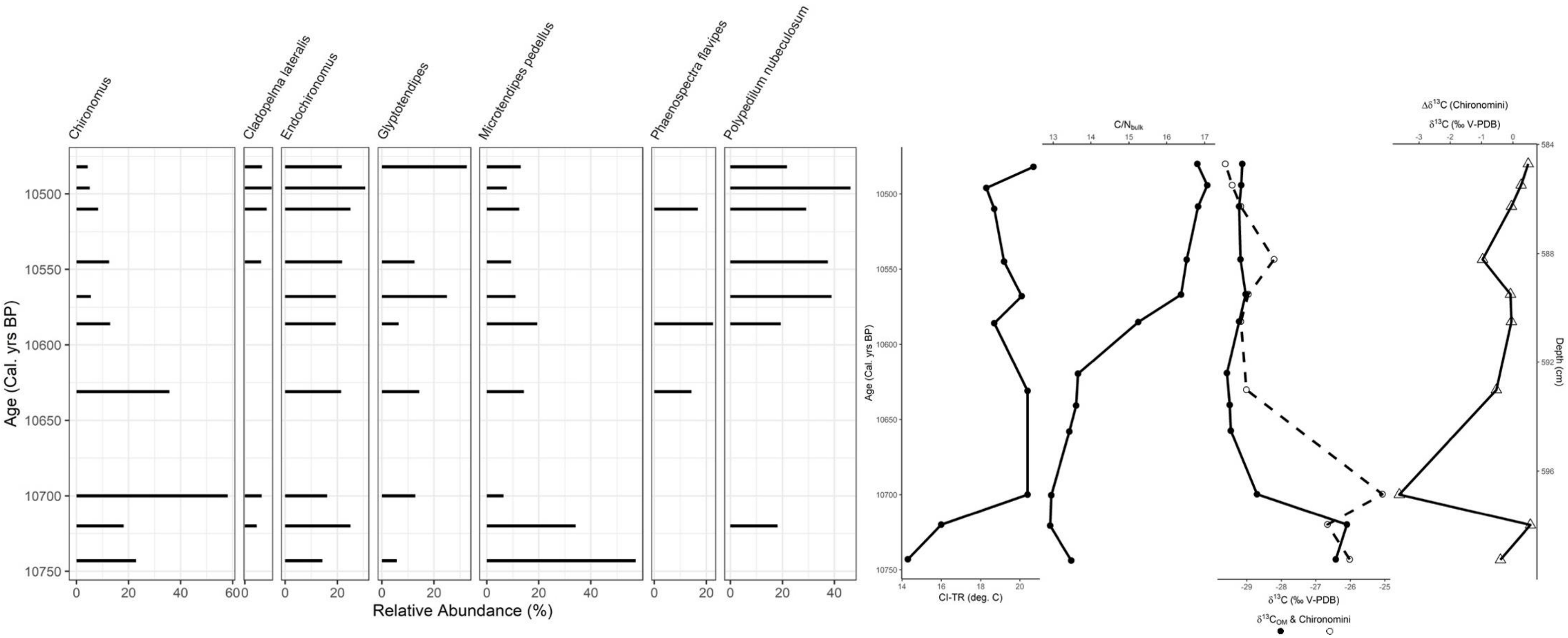


Figure 5.4: Stratigraphic diagram of chironomid taxa, Chironomid-inferred temperature reconstruction (mean summer temperature, °C), C/N, and carbon isotopic content of littoral Chironomini compared with $\delta^{13}C_{OM.}$ $\Delta\delta^{13}C$ (Chironomini) = $\delta^{13}C_{OM} - \delta^{13}C_{HC}$.

Littoral-dwelling chironomid taxa *Endochironomus*, *Glyptotendipes*, and *Microtendipes pedellus* are present throughout the sequence with the former exhibiting the greatest stability. *Microtendipes pedellus* was particularly dominant in the first two samples (10,743 and 10,720 Cal BP) in which the highest $\delta^{13}C_{OM}$ values (~26‰ V-PDB) and lowest inferred temperatures (15 ± 1 °C) were recorded. This was followed by the collapse of the littoral chironomid community (*Microtendipes pedellus* in particular) as profundal species *Chironomus* dominated the assemblage (~60% abundance); this also coincides with a peak in Chironomini $\Delta\delta^{13}C$ of –3.63‰. As an early coloniser, the dominance of *Chironomus* dominance is indicative of significant environmental change (Brooks et al., 1997). Similar species shifts were also observed by Weston (2020), placing confidence in the use of these species in the temperature reconstruction, despite high uncertainties (see appendixes D and G).

The rise in C/N from 13.66 to 16.38 (10,631 – 10,586 Cal BP) coincides with the re-emergence of *Polypedilum nubeculosum* along with new species *Phaenospectra flavipes* and the decline of *Chironomus*. Thereafter, *Chironomus* remains at its lowest relative abundance (<15%) and *Polypedilum nubeculosum* dominates (>20%) for the remainder of the sequence. This coincided with a rise in *Chironomini* $\delta^{13}C_{HC}$ values above that of $\delta^{13}C_{OM}$ ($\Delta\delta^{13}C$ = -0.5‰) Following the shift in C/N, Chironomini $\delta^{13}C_{HC}$ is isotopically lighter than $\delta^{13}C_{OM}$ ($\Delta\delta^{13}C$ = 1.0‰) for one sample at 10,546 Cal BP before returning to the $\delta^{13}C$ range of OM by 10,510 Cal BP. Species diversity was greatest following the shift in C/N and lowest beforehand.

CHAPTER VI

DISCUSSION

Interpreting the carbon isotopic content of chironomids requires an understanding of source signatures, chironomid feeding behaviour (species specific), lake biogeochemical cycling, and the overriding influence of climate (Serra et al., 2017; van Hardenbroek et al., 2018; Kivilä et al., 2020). Here, evidence derived from bulk geochemical proxies and chironomid assemblage is used to aid interpretations of the carbon isotopic content of both littoral and profundal chironomid species. These data demonstrate that lake carbon cycling spatial heterogeneity can be revealed through the combined measurements of *Chironomus* and Chironomini $\delta^{13}C_{HC}$. The viability of Chironomini $\delta^{13}C_{HC}$ to reveal the ^{13}C of aquatic organic matter is also used to assess the contributions of carbon sources to *Chironomus* diet.

6.1 Climatic and Environmental Change Inferred from Bulk Organic Matter and Chironomid-Inferred Temperature Reconstruction

6.1.1 C/N indicators of organic matter characteristics

Low C/N values throughout the sequence (values of 13–17) suggest that the lake sediments examined were dominated by autochthonous production (Talbot and Lærdal, 2000). This is likely due to the hydrologically closed nature of the lake, the relatively small catchment size compared with the lake size, and relatively large littoral zone (see figure 3.2) (Meyers and Ishiwatari, 1993). Figures 6.1 and 6.3 indicate that sedimentary organic matter transitions away from a pool containing a greater relative proportion of algal biomass at 10.7 kya to one containing relatively more macrophyte-dominated material and/or C3 vegetation towards 10.5 kya. However, the absolute contribution of macrophyte versus a mix of algal and catchment vegetation sources is not distinguishable from the isotope space plots alone due to samples plotting in overlapping areas. This variation in C/N and $\delta^{15}N$ was independent of $\delta^{13}C_{OM}$ and CI-TR, indicating an ontological rather than climatically driven process, perhaps in response to the climatic shift observed at 10.7 kya (see figure 5.1).

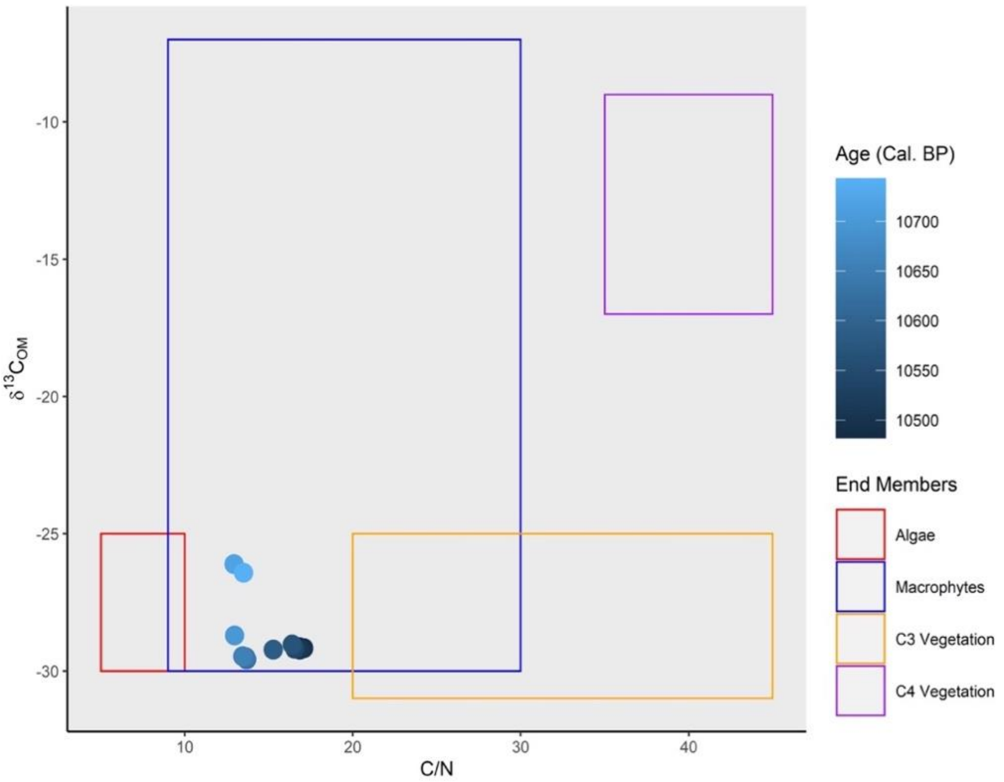


Figure 6.1: Isotope space plot of C/N against $\delta^{13}C_{OM}$. References for endmember values are outlined in appendix E.

6.1.2 Climatic controls on the isotopic signature of bulk organic matter at 10.7 kya.

At the beginning of the sequence (10.7 kya), both $\delta^{13}C_{OM}$ and CI-TR shifted rapidly, with most of the variation occurring in a 20-year period (10,720 and 10,700 Cal BP). This variation was independent of C/N change and is therefore likely not driven by a shift in the relative contributions of autochthonous and allochthonous production (Leng and Marshall, 2004). The shift in $\delta^{13}C_{OM}$ is therefore likely explained by climatic control which regulates $\delta^{13}C_{TDIC}$ supplying inorganic carbon to autochthonous photosynthetic production. An increase in temperature alone could cause ^{13}C depletion of the TDIC pool by (1) increasing productivity and carbon recycling (indicated as respiration in figure 6.2), which is known to occur in lakes with high DOC concentrations and could be the case due to the lowland forested setting of Blakemere Moss (Lennon et al., 2006; Karlsson, 2007), and/or (2) enhancing stratification duration or strength and subsequent methane oxidation (Jankowski et al, 2006; Finlay and Kendal, 2008; Borrel et al., 2011). The latter mechanism is supported by the dominance of *Chironomus* in the assemblage as, unlike most other taxa, it is tolerant of low oxygen conditions (Saether, 1979; Brodersen and Andersen, 2008).

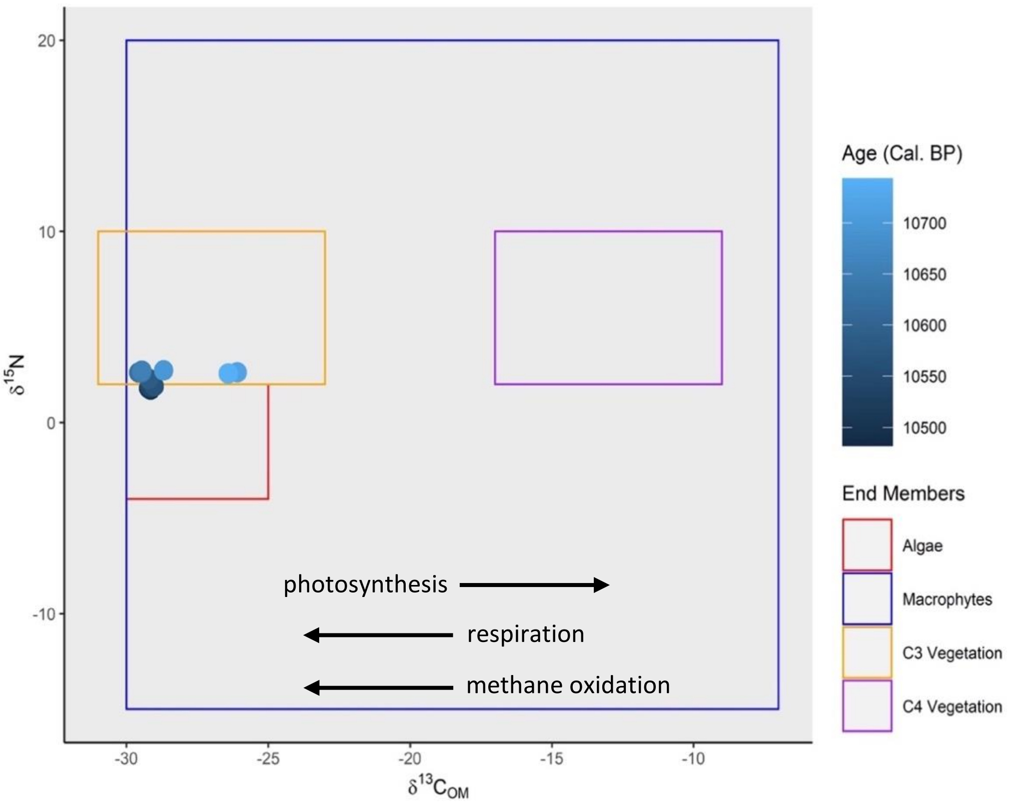


Figure 6.2: Isotope space plot of $\delta^{13}C_{OM}$ against $\delta^{15}N$. Relevant biogeochemical processes that control $\delta^{13}C_{TDIC}$ or $\delta^{15}N_{NO_3^-}$, the $\delta^{13}C$ or $\delta^{15}N$ of the aquatic plants are shown by the arrows, which indicate the effect of an increased amount of the specified process (Finlay and Kendall, 2007). Algal $\delta^{15}N$ values above 2‰ occur due to processes that affect the isotopic composition of the dissolved inorganic nitrogen pool, whereas values below 2‰ are for N sources from the atmosphere (Talbot, 2002). References for values are outlined in appendix E.

The proximity of Blakemere Moss to the coast (~10 km) means that maritime climate influences were highly important; this effect was demonstrated by Lang et al. (2010), who reconstructed late glacial–early Holocene summer air temperatures for five lakes in the Lake District, UK, with varying distances from the coast. Following extensive early Holocene warming, the associated meltwater effects in the northerly latitudes are known to impact AMOC, and likely dominated over atmospheric effects in Northern Europe (Rahmstorf, 2007). Brooks and Langdon (2014) showed this effect with chironomid-based records of temperature change in North-West Europe and between 11 and 10.5 kya the UK climate appears to have been warmer due to an increased maritime influence, perhaps as a result of the retreat of the Fenoscandinavian ice sheet and/or reduced meltwater effects (Rahmstorf, 2007). Regional climatic forcing for the 10.7 kya event is also suggested by the Greenland Isotope record (Seierstad, 2014; see appendix F).

6.1.3 C/N and $\delta^{15}N$ change indicative of reduced lake levels and macrophyte dominance?

In a 75-year period, (10,620 to 10,545 Cal BP), both C/N and $\delta^{15}N$ shifted from 13.66 to 16.38‰ and from 2.62 to 1.90‰ (air), respectively. This was independent of $\delta^{13}C_{OM}$ and inferred temperature, although relatively high uncertainties mean that the usefulness of the CI-TR is limited following the 10,700 Cal BP. Commensurate with this shift in chemical composition of the bulk organic matter is a change in the chironomid species assemblage resembling a higher availability of leaf litter for chironomids as the dominant functional feeding group shifts from collectors (*Chironomus*, *Microtendipes*) to shredders (*Polypedilum*, *Glyptotendipes*) (Berg, 1995; Leite-Rossi et al., 2019). This corroborates the indication in figures 6.1 and 6.3 that a shift from algal- to macrophyte-dominated production occurred during this period (Brooks et al., 2007; Merritt et al., 2008). Indeed, the stability of Chironomini $\delta^{13}C_{HC}$ and $\delta^{13}C_{OM}$ supports the notion that autochthonous production continued to comprise sedimentary organic matter rather than an increased terrestrial vegetation input driving C/N increase.

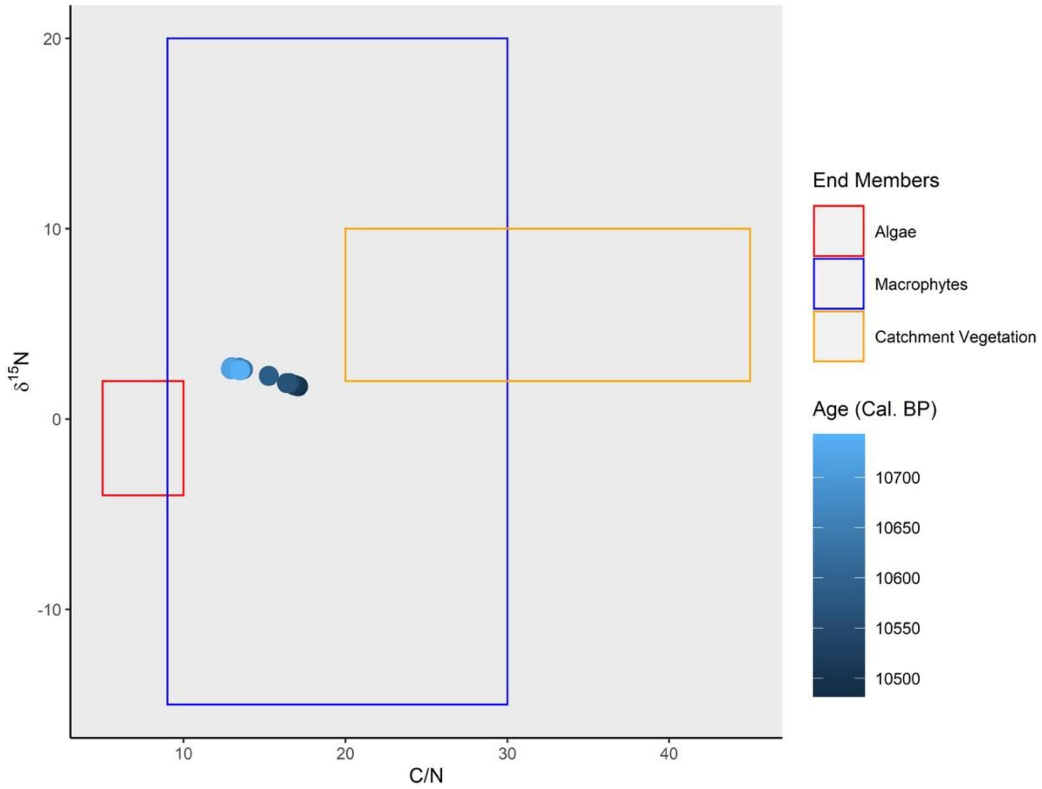


Figure 6.3: Isotope space plot of C/N against $\delta^{15}N$. References for values are outlined in appendix E.

Both %C and %N decreased throughout the sequence and the rate of decrease was greatest during the 10.6 kya event in which the rate of %N loss exceeded the rate of %C loss (hence rising C/N ratio). The fall in $\delta^{15}N$ and relative nitrogen abundance (C/N) indicates relatively reduced uptake of N into organic matter compared with the size of the aqueous N pool. Because the uptake of nitrogen via assimilation fractionates towards the lighter ^{14}N isotope (leaving the heaver ^{15}N isotope in the pool of aqueous nitrogen), a reduction in N assimilation relative to the size of the N pool will yield organic matter that is ^{14}N enriched, which was observed in our sequence. This may be explained by a shift to macrophyte rather than algal production (indicated by figures 6.1 and 6.3), although a larger shift in $\delta^{13}C_{OM}$ would be expected due to their ability to utilise atmospheric N unless the macrophytes were submergent. However, the process by which this occurs is not clear although it may be related to differing response of macrophytes to nutrient delivery related to thermal stratification (Belle et al., 2021).

Diagenetic alterations are also known to influence the $\delta^{15}N$ of sedimentary organic matter (Robinson et al., 2012), and studies have demonstrated a progressive depletion of $\delta^{15}N$ through time under anoxic conditions (Lehmann et al., 2002; Gälman et al., 2009), thought to be driven by an increase in the relative abundance of isotopically depleted dissolved ammonium (NH_4^+). This mechanism for explaining falling $\delta^{15}N$ in our sequence was rejected, however, due to the magnitude of the $\delta^{15}N$ change and to the strong correlation between $\delta^{15}N$ and C/N ($R^2 = 0.98$). Alternatively, McLaughlan (2013) suggested that the global climatic amelioration following the last glacial maximum may lead to reduced sedimentary $\delta^{15}N$ values by increased N_2 fixation in terrestrial ecosystems. The terrestrial organic matter which is relatively isotopically depleted may then be accumulated in the lake, thus influencing sedimentary $\delta^{15}N$; however, this occurs on centennial to millennial timescales, which is much greater than the 75-year shift observed in our sequence.

A common feature of shallow lakes (defined as a mean depth <3 m) is their tendency to exist in one of two divergent ecological states across a range of nutrient conditions (Blindow et al., 1993; Scheffer et al., 1993). Scheffer (2001) highlights that a reduction in lake level may lead to a rapid shift from a state of phytoplankton with turbid water, sparse or no macrophytes, and a reduced faunal diversity to domination by macrophytes accompanied by clear water and a diverse faunal assemblage. Indeed, species diversity of the Chironomini examined here increased with C/N (see figure 5.4).

Furthermore, Weston (2020) identified a much larger range of chironomid species (64 unique taxa) and found that species diversity was significantly higher for samples after 10,623 Cal BP compared with the 2000 years prior (see appendix G). Due to the hydrologically closed nature of Blakemere Moss, a small change in precipitation/evaporation would result in large lake-level variations, perhaps as a legacy effect of the temperature increase via enhanced evaporation, or due to a fall in precipitation related to the role to the AMOC (see the previous section; Battarbee, 2000; Marshall et al., 2007).

6.2 Carbon Isotopic Content of Chironomids

Taxon-specific analysis of chironomid remains revealed vastly different carbon isotopic signatures ($\Delta\delta^{13}C_{\text{Chironomus – Chironomini}}$ = –0.7 to 7.0‰). Previous studies which have measured the carbon isotopic content of bulked Chironomini have found $\delta^{13}C$ values which are closely related to $\delta^{13}C_{OM}$, which is in line with the findings of this study (e.g. Wooller et al., 2012; van Hardenbroek, 2014; Belle et al., 2017a). Chironomini which feed on photosynthetically derived carbon are expected to exhibit less ^{13}C depletion (relative to $\delta^{13}C_{OM}$) compared with *Chironomus* (or other Chironomini such as *Sergentia coracina*-type and *Stictochironomus*), which build tubes on the lake bottom where ^{13}C-depleted methane-oxidising bacteria (MOB) are most available (Whiticar, 1999; Moller Pillot, 2009; Deines et al., 2007a). In the discussion below, we assess the extent to which our data reflect these expectations of carbon isotope shifts dependent on growth conditions and ambient lake conditions.

The 10.7 kya shift in $\delta^{13}C_{OM}$ is thought to be driven by a fall in $\delta^{13}C_{TDIC}$ caused either through enhanced carbon recycling or stratification and subsequent methane oxidation effects (Lennon et al., 2006; Karlsson, 2007; Finlay and Kendal, 2007). The mechanism by which this occurred should in theory be elucidated by the chironomid carbon data. However, two key samples in our data reduce the certainty with which conclusions may be drawn due to low head counts. The samples were Chironomini at 10,700 Cal BP and *Chironomus* at 10,496 Cal BP (discussed in Section 6.2.2.3) and had low peak heights of 2.7 and 3.19 nA, respectively. The way in which low head counts impacted the validity of the data is outlined in the limitations section (Section 6.3.2).

The Chironomini sample at 10,700 Cal BP appears to exhibit a lagged response to the $\delta^{13}C_{OM}$ shift. This seems to suggest that the 13C depletion observed in *Chironomus* must be due to the incorporation of methane-derived carbon, and not due to influences of the TDIC on photoautotrophic biomass. However, the next Chironomini sample (70 years later) was similar to that of $\delta^{13}C_{OM}$ ($\Delta\delta^{13}C$ = -0.52‰) and continued for the remainder of the sequence (see figure 6.4). Therefore, if the sample at 10,700 Cal BP is excluded due to low head counts, the shift in $\delta^{13}C_{TDIC}$ may be a plausible explanation for the Chironomus and $\delta^{13}C_{OM}$.

6.2.1 Is littoral Chironomini $\delta^{13}C_{HC}$ representative of $\delta^{13}C$ of aquatic organic matter?

Littoral Chironomini $\delta^{13}C_{HC}$ remained within 1‰ for the entire sequence except for the sample at 10,700 Cal BP which succeeded the rapid shift in $\delta^{13}C_{OM}$ and CI-TR. The removal of this sample leads to a statistically significant correlation between $\delta^{13}C_{OM}$ and $\delta^{13}C_{HC}$ ($R^2 = 0.86$) (see Section 6.3.2 for sample validity). This suggests that for the most part, Chironomini fed on the aquatic matter comprising $\delta^{13}C_{OM}$ which was likely phytoplankton (algae) followed by macrophytes after 10,620 Cal BP as C/N increased (see Section 6.1.3). While no correlation was observed between Chironomini $\delta^{13}C_{HC}$ and C/N, the contiguousness of Chironomini $\delta^{13}C_{HC}$ and $\delta^{13}C_{OM}$ during the shift in C/N suggests that the Chironomini continued to represent the $\delta^{13}C$ of aquatic organic matter which maintained a stable $\delta^{13}C$ owing to the continued uptake of carbon from the TDIC pool whose $\delta^{13}C$ also remained stable.

The relative stability of Chironomini during the shift in C/N is somewhat surprising given that the dominant functional feeding group (FFG) shifts from collectors (*Chironomus*, *Microtendipes*) to shredders (*Polypedilum*, *Glyptotendipes*) (Berg, 1995; Leite-Rossi et al., 2019). Due to the relatively omnivorous character of chironomids, the availability of specific food items may not be a major controller of the presence and abundance of the different FFGs (Berg, 1995; Serra et al., 2017). Instead, habitat-related factors, such as water depth or DOC and nutrient concentrations, may have driven the guide occurrence of FFGs (Luoto and Ojala, 2014; Kivilä et al., 2019). This suggests that the use of bulked Chironomini from the littoral zone may be used to faithfully reconstruct aquatic organic matter during shifts in the both the nature of aquatic organic matter and functional feeding group, although further research will be required to improve the impact of site-specific (e.g. allogenic versus autogenic inputs, closed versus open hydrology) and species-specific factors.

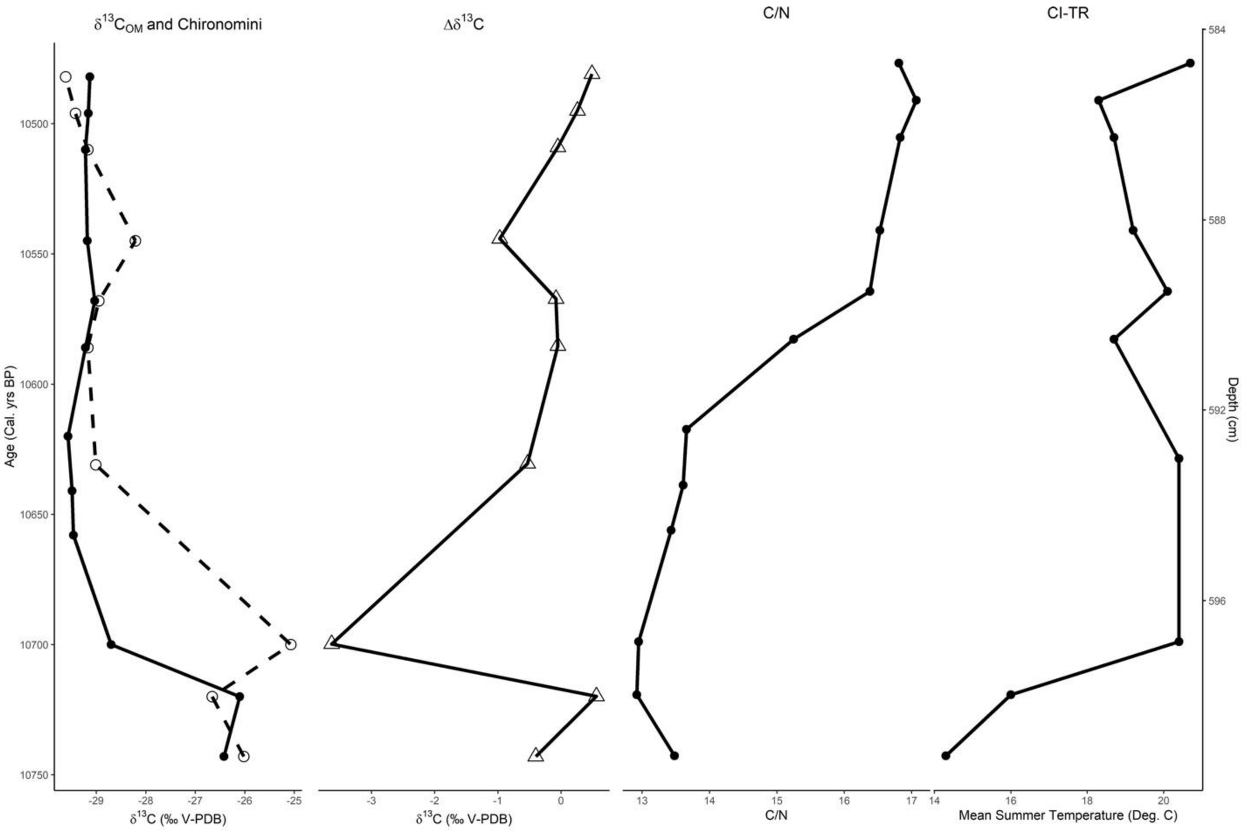


Figure 6.4: Trends in carbon isotopic composition of organic matter ($\delta^{13}C_{OM}$; ‰; closed circles); carbon isotopic composition of Chironomini remains ($\delta^{13}C_{HC}$; ‰; open circles); difference between carbon isotopic composition of organic matter and Chironomini remains ($\Delta\delta^{13}C = \delta^{13}C_{OM} - \delta^{13}C_{HC}$); C/N; and chironomid-inferred temperature.

6.2.1.1 10.7 kya event

Following the 10.7 kya event, Chironomini $\delta^{13}C_{HC}$ appears to lag behind $\delta^{13}C_{OM}$. Chironomini $\delta^{13}C_{HC}$ was ^{13}C enriched compared with $\delta^{13}C_{OM}$ ($\Delta\delta^{13}C_{HC}$ = –3.9‰), indicating the incorporation of a relatively ^{13}C-enriched carbon source. Due to the short life cycle of chironomids (1–3 years; Tokeshi, 1986), a lag between chironomid feeding and sedimentation is unlikely. Depositional or taphonomic processes are also likely not a driving factor as the species assemblage shifted significantly and concurrently with $\delta^{13}C_{OM}$. Instead, a low species abundance could have systematically impacted the result (see Section 6.3.2) or, indeed, the dietary source of Chironomini was different to that of organic matter comprising the lake sediment, perhaps due to species-specific feeding habits or a difference in the temporal integration of food sources.

Evidence of invertebrate $\delta^{13}C$ from cores at a range of depths from Frossard et al. (2015) indicated that the anoxic hypolimnion may migrate to shallower depths over decadal timescales. While this mechanism had not been observed to affect invertebrates for a shallow lake, it could explain the apparent lag in Chironomini $\delta^{13}C_{HC}$ values as the onset of hypoxia in the benthos could inhibit the delivery of chemosynthetically produced ^{13}C-depleted CO_2 to primary producers in the photic zone (Belle et al., 2021). In this case, sedimentary $\delta^{13}C_{OM}$ may have consisted of organic matter largely formed in the pelagic zone, and Chironomini inhabiting the littoral. Alternatively, the turbidity of the lake (see Section 6.1.3) may have led to the lag in a similar mechanism by increasing benthic CO_2 recycling with the onset of higher temperatures with a relatively smaller increase in photosynthesis (Lennon et al., 2006).

This mechanism may also be expected to cause relatively higher Chironomini $\delta^{13}C_{HC}$ values if the larvae fed on photoautotrophic biomass predominantly during the summer months in which the distribution of the ^{13}C-depleted CO_2 originating from benthic degradation processes can be especially restricted to the hypolimnion due to enhanced thermal stratification (van Breugel et al., 2005). *Glyptotendipes* and *Cladopelma lateralis*-type had higher relative abundances at 10,700 Cal BP compared with other samples in the sequence (31% and 15%, respectively) and could have species-specific characteristics that lead them to feed/live predominantly during the summer months, although such autecological knowledge does not exist. Alternatively, these species could have selectively fed on a ^{12}C-enriched food source as *Glyptotendipes* is an opportunistic omnivore and *Cladopelma lateralis*-type is known to graze on diatoms and detritus which may have been ^{12}C enriched compared with $\delta^{13}C_{OM}$ (Moog, 2002; Moller Pillot, 2009; Serra et al., 2017). In this sense, the Chironomini value of –25.1‰ could be reflective of species-specific food preferences although a correspondence analysis would be required to confirm this.

6.2.2 The difference between *Chironomus* $\delta^{13}C_{HC}$ and Chironomini $\delta^{13}C_{HC}$

6.2.2.1 ^{13}C-depleted Chironomus due to MOB incorporation?

Profundal dwelling *Chironomus* exhibited ^{13}C-depleted values (–28.2 to –32.9‰) compared with littoral $\delta^{13}C_{HC}$ and $\delta^{13}C_{OM}$, implying that *Chironomus* incorporated an isotopically lighter food source than the averaged sedimentary organic matter or via a small contribution of methane-derived carbon to their diet. This is in accordance with other studies that measured chironomid ^{13}C remains and $\delta^{13}C_{OM}$ (van Hardenbroek et al., 2013, 2014; Frossard, 2015; Belle et al., 2014, 2017b). *Chironomus* ^{13}C depletion relative to $\delta^{13}C_{OM}$ increased with temperature at the beginning of the sequence ($\Delta\delta^{13}C$ increased from 1.9 to 3.4‰ between 10,732 and 10,700 Cal BP; see figure 5.3), suggesting that a strengthened thermal stratification induced oxygen depletion in the profundal zone (Jankowski et al., 2006) with subsequent methanogenesis (Borrel et al., 2011). This mechanism has been previously proposed by Wooller et al. (2012) and Belle et al. (2017b). Unlike Belle et al., (2017b), however, a correlation was not observed between reconstructed air temperature and *Chironomus* $\delta^{13}C_{HC}$, largely due to the youngest Chironomus $\delta^{13}C_{HC}$ value of –28.67‰ at 10,496 Cal BP (see Section 6.3.2 for sample validity). Excluding this sample results in a highly statistically significant negative correlation ($R^2 = 0.92$) and thus suggests the incorporation of methane-derived carbon during the warmest period in the sequence.

Our *Chironomus* $\delta^{13}C_{HC}$ values are comparable to *Chironomus anthracinus*-type $\delta^{13}C$ values found in a shallow boreal lake, Lake Tollari, Estonia (–25 to –40‰) (Belle et al., 2017b), and are in the expected region for limited MOB incorporation given the relatively shallow depth (Grey et al., 2004; Deines and Grey, 2006). The shallow depth of Blakemere Moss means that the methane production and therefore the availability of methane-oxidising bacteria for *Chironomus* was low. Grey and Deines (2006) demonstrated a correlation between *Chironomus* $\delta^{13}C_{HC}$ values and depth (and increasing methane production). Our *Chironomus* $\delta^{13}C_{HC}$ values of ~–32‰ are therefore in the upper range of possibility for a small proportion of methane-derived carbon in *Chironomus* diet. This is also corroborated by the dominance of *Chironomus* in the assemblage, as unlike most other taxa it is tolerant of low oxygen conditions (Saether, 1979; Brodersen and Andersen, 2008).

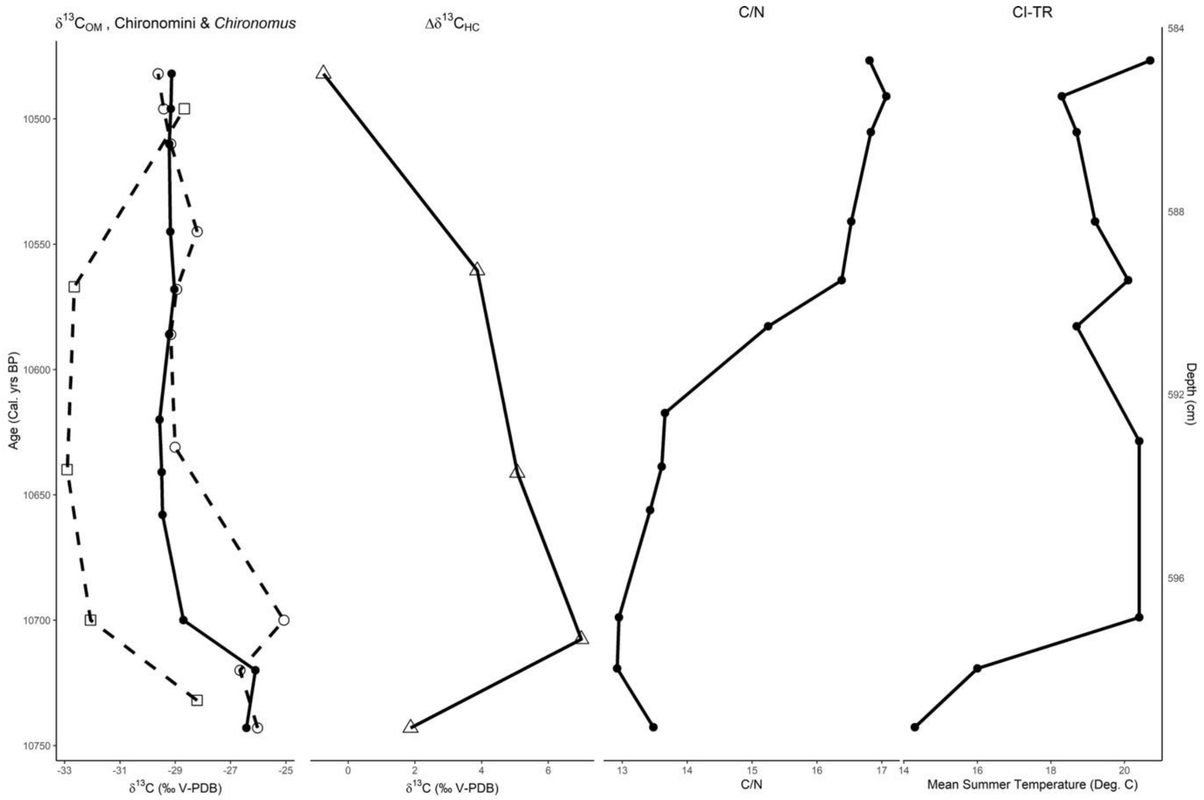


Figure 6.5: Trends in the carbon isotopic composition of organic matter ($\delta^{13}C_{OM}$; ‰; closed circles); carbon isotopic composition of *Chironomus* (open boxes) and Chironomini remains ($\delta^{13}C_{HC}$; ‰; open circles); the difference between carbon isotopic composition of *Chironomus* and Chironomini remains ($\Delta\delta^{13}C_{HC} = \delta^{13}C_{chironomus} - \delta^{13}C_{chironomini}$); C/N; and chironomid-inferred temperature.

6.2.2.2 *Other sources of isotopic depletion of Chironomus*

Chironomus $\delta^{13}C_{HC}$ values above –40‰ may also be isotopically lighter than organic matter due to the incorporation of other chemosynthetic bacteria or phytoplankton with low $\delta^{13}C$ (Kohzu et al., 2004; Grey, 2016). Indeed, van Hardenbroek et al. (2014) hypothesised that their ^{13}C-depleted daphnia could have resulted from the incorporation of algae which utilised ^{13}C-depleted heterotrophically respired carbon (Rau, 1978). In Section 6.1.2 it was suggested that $\delta^{13}C_{OM}$ values fell perhaps due to increased carbon recycling related to higher temperatures (under high DOC conditions) (Lennon et al., 2006). Therefore, owing to variable phytoplankton fractionation of CO_2 values, their $\delta^{13}C$ could have been low enough to induce the observed *Chironomus* $\delta^{13}C_{HC}$ values (Bade et al., 2006; Grey, 2016). This process would be expected to affect the carbon isotopic composition of all invertebrates remains, which was observed with the exception of one Chironomini sample at 10,700 Cal BP, and due to its questionable validity conclusions could be drawn either way (van Hardenbroek et al., 2013).

Alternatively, lower *Chironomus* $\delta^{13}C_{HC}$ values compared with those obtained from Chironomini could be related to a difference in the temporal integration of food sources. As mentioned in Section 6.2.1.1, Chironomini remains may have been fuelled by a higher proportion of phototrophic biomass grown in the epilimnion during summer. *Chironomus* have been observed to switch from a detrital-dominated diet in February to a more algal-biased diet, associated with the spring bloom, by Johnson (1987), and in this case could be fuelled by a higher proportion of respired carbon provided to the epilimnion after the spring turnover.

6.2.2.3 Dietary overlap between Chironomini and Chironomus

At the end of the sequence, *Chironomus* $\delta^{13}C_{HC}$ rose to values in the region of $\delta^{13}C_{OM}$ ($\Delta\delta^{13}C$ = –0.5‰), indicating that the larvae switched from an isotopically light food source to one that largely reflects that of sedimentary organic matter. If thermal stratification, and the subsequent influence on anoxia and CH_4 production, initially caused *Chironomus* ^{13}C depletion, then it is reasonable to assume that increased lake-water overturning reduced the MOB availability to *Chironomus*. Moreover, this occurred independently of Chironomini $\delta^{13}C_{HC}$ and $\delta^{13}C_{OM}$ values, ruling out the possibility of $\delta^{13}C_{TDIC}$ influences. The reduced availability of methane-derived carbon may be caused by a weaker stratification caused by lower temperatures; however, because uncertainty exceeded variability, this cannot be used to infer a substantial fall in temperature.

The low relative abundance of *Chironomus* at the end of the sequence (see figure 5.4) may also be an explanatory factor for their higher $\delta^{13}C_{HC}$ values by indicating the uptake of photosynthetic rather than chemosynthetic carbon. If the dominating mode of autochthonous production was macrophytes, the limited presence of Chironomus may be reflecting a change of available dietary source as the other species which have functional traits (e.g., mentum for food preference) which are better suited to that available food source (Serra et al., 2016; Antczak-Orlewsk et al., 2021). Moreover, *Chironomus* larvae have been known to inhabit both the littoral and the profundal and as such may have had access to isotopically lighter food sources than Chironomini ($\Delta\delta^{13}C_{HC}$ = –0.7) (Brooks et al., 2007).

Alternatively, Borderelle et al. (2008) suggested that the differences in the $\delta^{13}C$ values of macroinvertebrates between the littoral and deep zones ($\Delta\delta^{13}C_{HC}$) could be used to quantify the extent of heterotrophic processes within a lake system and we have already used this reasoning to argue for an alternative source of isotopic depletion of *Chironomus* relative to Chironomini $\delta^{13}C_{HC}$ and $\delta^{13}C_{OM}$. In this case, a high functional efficiency could lead to low values as differences in carbon sources (benthic algae, terrestrial organic matter) within littoral compared to the profundal likely play a higher role in driving $\Delta\delta^{13}C_{HC}$ in shallow lakes (Frossard et al., 2014).

6.3 Methodological Considerations

6.3.1 Reliability of chironomid-inferred temperature reconstruction

The chironomid-inferred temperature reconstruction (CI-TR) was performed using only chironomids from the Chironomini tribe. This introduced bias for our temperature reconstruction as they are larger and often found at the higher end of the temperature gradient (Eggermont and Heiri, 2012). Sample-specific errors, estimated by 1000 bootstrap cycles (Telford and Birks, 2011), were quite large for the present study (up to 1.5 °C), meaning that the variability in CI-TR for samples after 10,700 Cal BP never exceeded these uncertainties. The use of CI-TR to aid interpretations was therefore restricted to the temperature increase (14–20 °C) at the beginning of the sequence.

A previous reconstruction at Blakemere Moss using Chironomini, Tanytarsini and Orthocladiinae produced early Holocene mean summer temperatures of >18 °C, which exceeded the bounds of uncertainty for all other UK records (Weston, 2020, and references therein). This was likely due to the lowland forest setting of the site and large littoral zone which engendered a thermophilic chironomid population (Belle and Goedkoop, 2021). The use of the Norwegian calibration set (NCS) is not best suited for such (low-latitude) sites as the set does not include lakes with July air temperatures greater than 16 °C (Brooks and Birks, 2001). This leaves the temperature optima of highly thermophilic taxa (e.g., *Polypedilum nubeculosum* and *Phaenospectra flavipes*) ill-defined in the calibration set, which makes temperature estimations prone to the influence of secondary environmental gradients such as continentality (Self, 2010). Furthermore, only 14/157 lakes in the NCS exceed a mean July temperature of 15 °C, suggesting that there is a lack of data to allow a robust estimation of temperature from paleoassembalges of lakes in this latitude and elevation.

6.3.2 Number of HCs

The number of head capsules is an important issue for all studies which measure the carbon isotopic content of invertebrate remains (Van Hardenbroek et al., 2009). Owing to low chironomid abundances with respect to bulk organic matter, the study was limited by the number of head capsules because of time limitations for picking for the sequence. This limited the study in three key ways: (1) increasing the influence of intra-taxon variability (Davies, 2015; van Hardenbroek et al., 2018); (2) reducing the temporal resolution for both the Chironomini and *Chironomus* samples, and (3) reducing the strength of the signal on EA-IRMS, meaning that less confidence can be placed in the precision of the result (van Hardenbroek et al., 2009).

The influence of intra-taxon variability is more pronounced in samples of a limited number of HCs as *Chironomus* variability has shown to be as high as 3‰ by Davies (2015). However, owing to the shallow nature of the paleolake, intraspecific variability may be limited as larvae from deeper sites were found to exhibit greater intraspecific variability by Grey et al. (2004). Finally, reduced head counts also reduce the averaging out of seasonal variations of the $\delta^{13}C$ photoautotrophic biomass caused by summer stratification effects on dissolved inorganic carbon cycling, which can be reflected in the chironomid stable isotopic signatures due to their high growth or metabolic rates (see Section 6.2.2.2; Kankaala et al., 2010; van Hardenbroek et al., 2018, and references therein).

The low number of littoral HCs between 10,700 and 10,630 Cal BP reduced the temporal resolution as one sample had to be discarded and the subsequent two merged. This makes constraining the (timing of the) process of carbon cycling change at the time difficult. The timescale of the lagged response is not clear and also led to a lack of repeated results which could be used to support or deny the validity of the high Chironomini value at 10,700 Cal BP.

6.3.3 Level of identification of *Chironomus*

Chironomus could only be identified to the genus level, owing to the lack of mandibles likely lost during HCl rinsing. *C. plumosus* can exhibit more ^{13}C-depleted values compared with *C. anthracinus* due to the relatively higher reliance of the latter on (less ^{13}C-depleted) chemoautotrophic bacteria (Grey and Deines, 2005). Therefore, a shift in the balance of species could influence the *Chironomus* ^{13}C signal rather than indicating a shift in the isotopic baseline itself. A species shift is plausible given that previous work on Blakemere Moss has indicated the presence of both *C. plumosus* and *C. anthracinus* during the early Holocene (Weston, 2020).

This is most relevant for the beginning of the sequence, where an increase in the ^{13}C depletion of *Chironomus* relative to sedimentary organic matter, during a rise in inferred temperatures, was used as evidence for increased incorporation of methane-derived carbon (see Section 6.2.2.1). This increase is within the bounds possible to be explained by a shift from *C. anthracinus* to *C. plumosus* (Grey et al., 2004; Grey and Deines, 2005). Moreover, an increase in lake temperatures could drive this shift as the temperature optima of *C. plumosus* and *C. anthracinus* are 13.7 and 12.3 °C, respectively (Self, 2010).

CHAPTER VII

CONCLUSION

The present study aimed to measure the carbon isotopic content of *Chironomus* and littoral-dwelling Chironomini in order to reveal the carbon cycling spatial heterogeneity of Blakemere Moss during the early Holocene. The viability of littoral-dwelling Chironomini $\delta^{13}C_{HC}$ to reveal the $\delta^{13}C$ of aquatic organic matter was also tested in order to assess the varying contributions of isotopically distinct carbon sources to the *Chironomus* diet. The spatial heterogeneity of carbon processing was revealed by vastly different chironomid $\delta^{13}C_{HC}$ signatures which indicated spatial differences in the carbon isotopic composition of their food sources, including methane-derived carbon (MDC), which was shown to contribute to a greater proportion of *Chironomus* biomass when inferred temperatures increased. We therefore demonstrated that methane production can be reconstructed using *Chironomus* remains in shallow lakes such as Blakemere Moss and that it was positively correlated to temperature.

Aquatic organic matter was shown to be a constant food source for littoral-dwelling Chironomini (Belle et al., 2017a). It is therefore a useful proxy for estimating the carbon isotopic content of photoautotrophic biomass and hence may aid isotope mixing models to quantify the contribution of MDC though time to benthic invertebrates. This is particularly the case for studies of shallow lakes where ^{13}C depletion of MOB may be small (Deines and Grey, 2006), or when $\delta^{13}C_{OM}$ interpretations are obscured by mixed contributions from allochthonous and autochthonous sources. Furthermore, they continued to represent aquatic organic matter despite a shift in the likely dietary source from algae to leaf litter, indicated by a transition in functional feeding groups (coinciding with C/N increase) from collectors to shredders after 10.6 kya (Berg, 1995; Leite-Rossi et al., 2019). Moreover, the confluence of chironomid $\delta^{13}C_{HC}$ and $\delta^{13}C_{OM}$ indicated that the transition to macrophyte-dominated autochthonous production led to a more homogeneous food source for chironomids, or indeed that methane-derived carbon was no longer available due to climatic or lake conditions relating to stratification.

Future work at Blakemere Moss would benefit from a littoral core, particularly at 10.7 kya in order to ascertain whether the deviation of littoral Chironomini from $\delta^{13}C_{OM}$ was caused by temporal (i.e., seasonal) or spatial variations in the integration of food sources with associated isotopic offsets. Indeed, the influence of increasing temperature and thus rapid local environmental change may have destabilised the littoral Chironomini species assemblage, which in turn systematically impacted the validity of the result. Alternatively, the relative stability of *Endochironomus* makes it a promising prospect for species-specific $\delta^{13}C_{HC}$ analysis and could be compared with bulked littoral-dwelling Chironomini to assess the effectiveness of our method.

APPENDICES

Appendix A: Age–depth model calculations

Depth (cm)	Corrected depth (cm)	Minimum age (Cal BP (1σ range)	Maximum age (Cal BP (1σ range)	Median age (Cal BP)
583.75	590.9	10120	11801	10482
584.25	591.5	10137	11814	10496
584.75	592	10149	11819	10510
585.75	593.1	10175	11828	10545
586.25	593.7	10184	11848	10568
586.75	594.3	10193	11859	10586
587.75	595.4	10213	11890	10620
588.25	596.0	10231	11899	10641
588.75	596.5	10240	11904	10658
589.75	597.7	10261	11922	10700
590.25	598.2	10269	11925	10720
590.75	598.8	10279	11936	10743

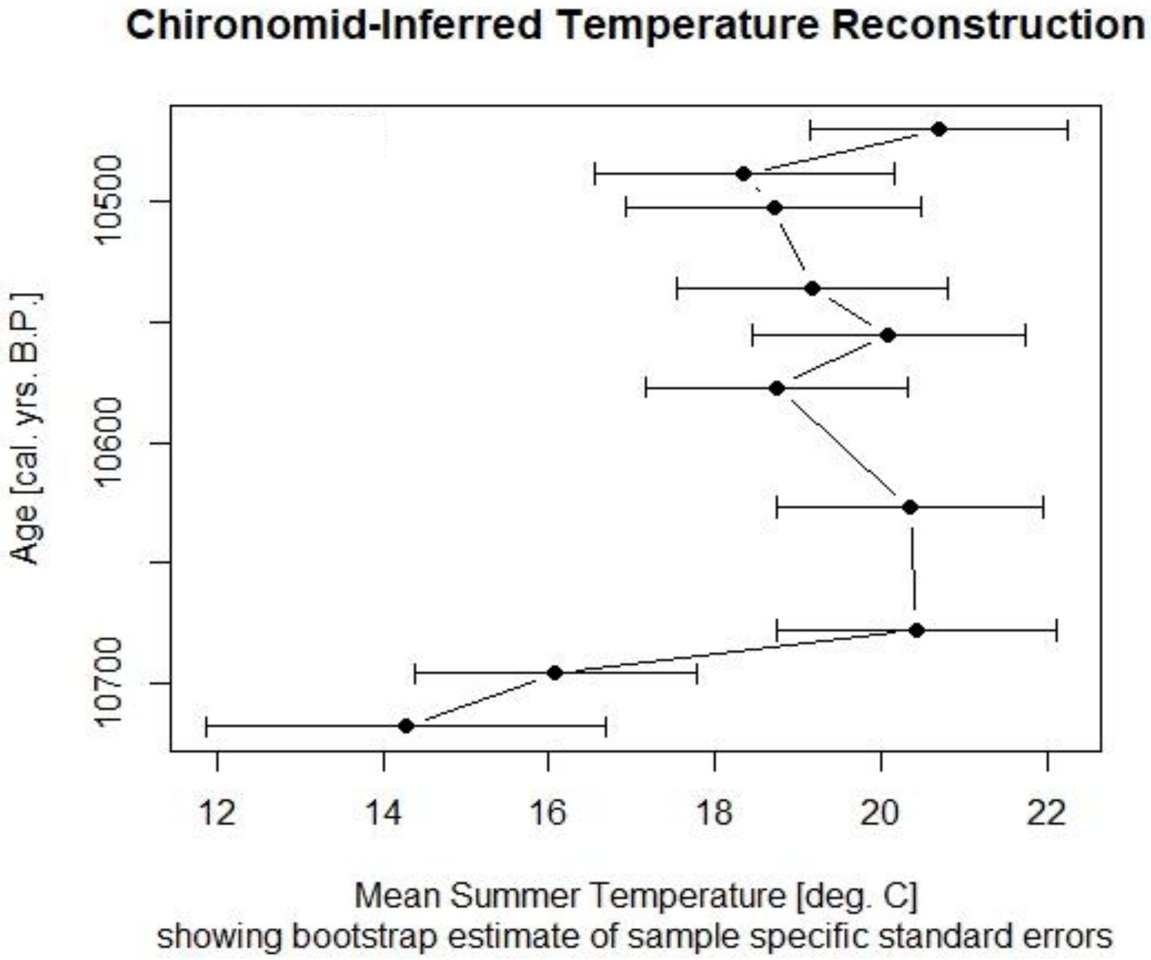
Appendix B: All collected data.

Age (Cal BP)	**Corrected depth (cm)**	**$\delta^{13}C_{OM}$ (‰ V-PDB)**	**$\delta^{15}N$ (‰ air)**	**C/N**	**$\delta^{13}C_{HC}$ (Chironomini) (‰ V-PDB)**	**$\delta^{13}C_{HC}$ (*Chironomus*) (‰ V-PDB)**	**Chironomid-inferred temperature (°C)**
10482	590.9	–29.13	1.79	16.81	–29.62	–28.67	20.7
10496	591.5	–29.16	1.72	17.07	–29.42		18.3
10510	592	–29.22	1.79	16.83	–29.17		18.7
10545	593.1	–29.18	1.90	16.53	–28.21	–32.65	19.2
10568	593.7	–29.03	1.90	16.38	–28.95		20.1
10586	594.3	–29.22	2.28	15.25	–29.17		18.7
10620	595.4	–29.57	2.62	13.66	–29.01	–32.91	20.4
10641	596.0	–29.49	2.62	13.61			
10658	596.5	–29.46	2.71	13.43	N/A		N/A
10700	597.7	–28.70	2.73	12.95	–25.07	–32.06	20.4
10720	598.2	–26.10	2.63	12.92	–26.66	–28.21	16.0
10743	598.8	–26.42	2.57	13.48	–26.02		14.3

Appendix: C: $\delta^{13}C_{OM}$, chironomid $\delta^{13}C_{HC}$, and the difference between the two: $\Delta\delta^{13}C = \delta^{13}C_{OM} - \delta^{13}C_{HC}$.

Age (Cal BP)	**Corrected depth (cm)**	**$\delta^{13}C_{OM}$ (‰ V-PDB)**	**$\delta^{13}C_{HC}$ (Chironomini) (‰ V-PDB)**	**$\Delta\delta^{13}C_{HC}$ (Chironomini) (‰ V-PDB)**	**$\delta^{13}C_{HC}$ (*Chironomus*) (‰ V-PDB)**	**$\Delta\delta^{13}C_{HC}$ (*Chironomus*) (‰ V-PDB)**	**$\Delta\delta^{13}C_{Chironomus\ -\ Chironomini}$ (‰ V-PDB)**
10482	590.9	–29.13	–29.62	0.49	–28.67	–0.5	–0.74
10496	591.5	–29.16	–29.42	0.26			
10510	592	–29.22	–29.17	–0.05			
10545	593.1	–29.18	–28.21	–0.97	–32.65	3.51	3.87
10568	593.7	–29.03	–28.95	–0.08			
10586	594.3	–29.22	–29.17	–0.05			
10620	595.4	–29.57	–29.01	–0.52	–32.91	3.4	5.07
10641	596.0	–29.49					
10658	596.5	–29.46	N/A	N/A			
10700	597.7	–28.70	–25.07	–3.63	–32.06	3.36	6.99
10720	598.2	–26.10	–26.66	–0.56	–28.21	1.95	1.87
10743	598.8	–26.42	–26.02	0.4			

Appendix D: Chironomid-inferred temperature reconstruction of Blakemere Moss with sample-specific errors obtained from 1000 bootstrap cycles shown by temperature uncertainty bars (Telford and Birks, 2011).



Appendix E: Typical values for end-members found in sedimentary organic matter. *Excluding Bryophytes.

End-members	**$\delta^{13}C_{OM}$ (‰ V–PDB)**	**C/N**	**$\delta^{15}N$ (‰ air)[†]**
Lacustrine algae[a]	–25 to –30[1]	5–10[2]	–4 to 2+[3]
Macrophytes*[b]	–7 to –30[1,2]	9–30[1]	–15 to 20[2]
C_3 plants[c]	–23 to –31[1]	20 to 45+[2]	2 to 10[3]
C_4 plants[d]	–9 to –17[1]	35 to 45+[2]	2 to 10[3]

[†] Higher $\delta^{15}N$ values are associated with higher trophic levels (Brenner et al., 1999; Post et al., 2002).

References:

[a] (1) Boutton (1991); Meyers and Lallier-Vergès (1999). (2) Meyers (2003). (3) Talbot (2002).

[b] (1) Keely and Sandquist (1992); Aichner et al. (2010). (2) Chappuis (2017).

[c] (1) Kohn (2010). (2) Meyers (2003). (3) Talbot (2002).

[d] (1) Deines (1980). (2) Meyers (2003). (3) Talbot (2002).

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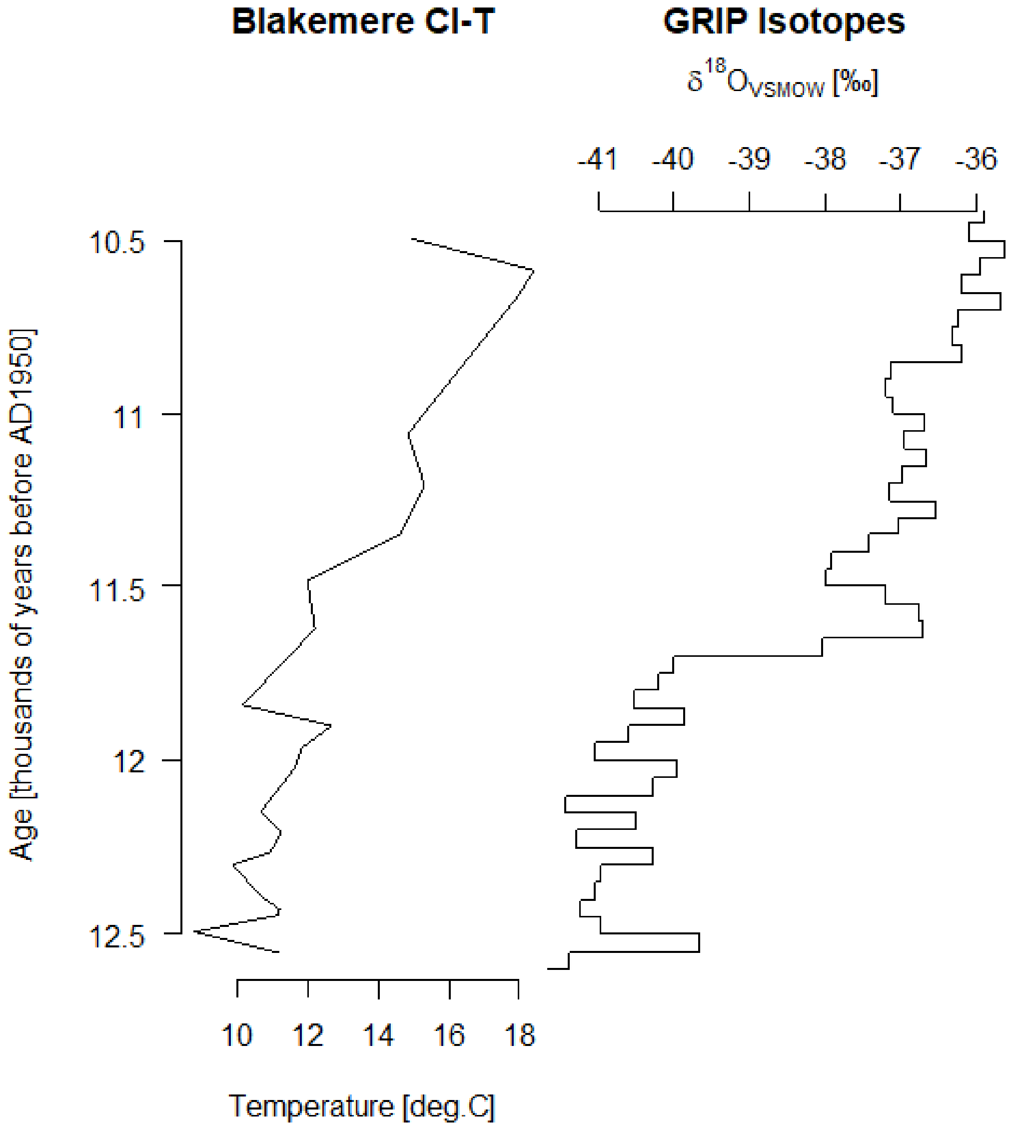
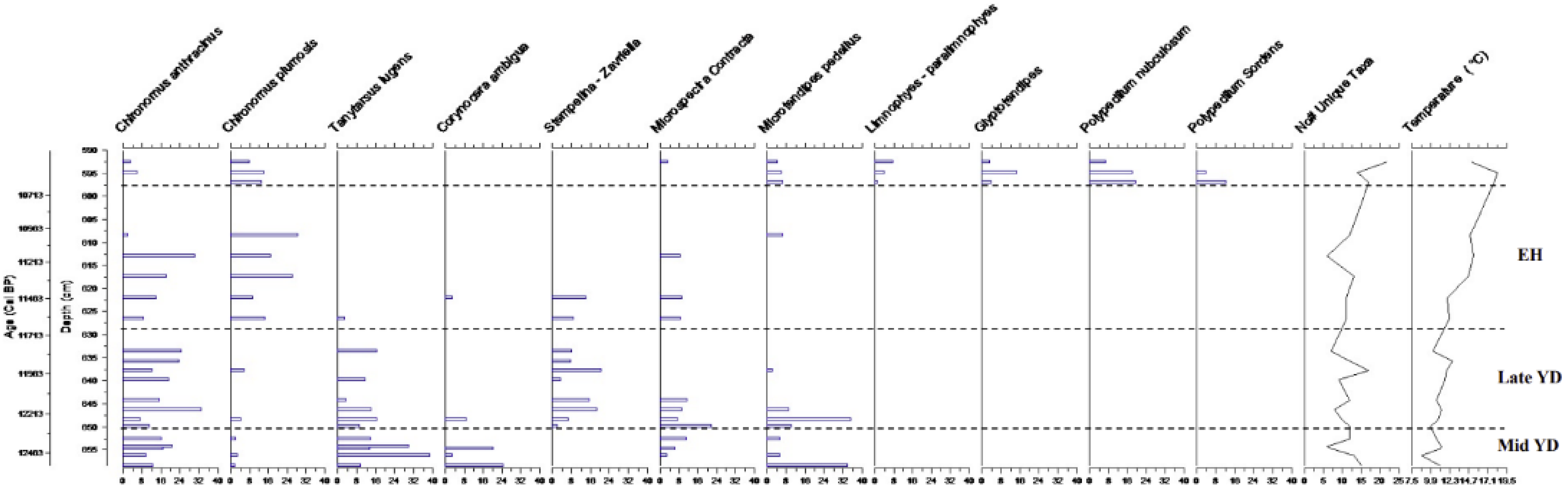
[a] (3) Talbot (2002): $\delta^{15}N > 2$ if DIN derived. $\delta^{15}N = –4$ to 2 if atmospheric derived.

[b] Macrophytes can exhibit C_3 ^{13}C if carbon source is atmospheric (Chappuis, 2017). This was corroborated by Aichner et al. (2010), who found that isotopic fractionation increases when more ^{13}C-enriched carbon sources (such as HCO_3^-) are assimilated. Values for Bryophytes (moss type) can be below –50‰ (Keely and Sandquist, 1992).

Appendix F: Correlation of chironomid-inferred mean July temperatures at Blakemere Moss correlated with the NGRIP oxygen isotope record using independent chronological controls. *Source*: Weston (2020).



Appendix G: Stratigraphic diagram of key taxa with species diversity and temperature at Blakemere Moss during the Holocene transition. *Source*: Weston (2020).



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