Università Ca' Foscari Venezia
Graduate School

in cotutela con

Leuphana University Lüneburg
Faculty of Sustainability

# Specific Molecular Markers in Lake Sediment Cores for Biomass Burning Reconstruction during the Holocene

Dottorato di Ricerca
in Scienze Ambientali
27° Ciclo
Anno di Discussione 2015

**Tesi di Dottorato di:**
Torben Kirchgeorg
Matricola: 955945

**Settore Scientifico Disciplinare:** CHIM/1

**Coordinatore del Dottorato:**
Prof. Dr. Gabriele Capodaglio
Department of Environmental Sciences, Informatics and Statistics, Ca' Foscari University of Venice

**Tutors:**
Prof. Dr. Carlo Barbante
Department of Environmental Sciences, Informatics and Statistics, Ca' Foscari University of Venice and Institute for the Dynamics of Environmental Processes - National Research Council of Italy

Prof. Dr. Ralf Ebinghaus
Institute for Coastal Research, Helmholtz Centre Geesthacht and Faculty of Sustainability, Leuphana University of Lüneburg

# Abstract

Fire plays an important role in the earth system by influencing ecosystems and climate, but climate also influences fire. The system became more complex when humans started using fire as a tool. Understanding the interaction of humans, fire and climate is the major aim of paleofire research. Understanding changes in these three aspects in the past will help predications of future climate, fire and human interactions. The use of lake sediment cores as natural archives for reconstructing past fire activity by counting charcoal particles is well established. This present dissertation is dedicated to the evaluation and application of specific organic molecular markers for biomass burning: levoglucosan, mannosan and galactosan as proxies for reconstructing past fire activity in lake sediments thorough the entire Holocene. First, a new analytical method was developed using high performance anion exchange chromatography combined with mass spectrometry to separate and detect these three monosaccharide anhydrates in lake sediments. The suitability of this analytical method was proven by comparing the levoglucosan, mannosan and galactosan results in selected lake sediment samples from Lake Kirkpatrick, New Zealand and correlating the results with macroscopic charcoal. Furthermore, the method was successfully applied to a lake sediment core from Lake Petén Itzá, Guatemala to reconstruct regional Holocene fire history. The analyses of levoglucosan was combined with fecal sterols to reconstruct late Holocene human fire interactions at Lake Trasimeno, Italy, demonstrating low fire activity during the Roman period. This combination of studies proves that these molecular markers are valid fire proxies in sediments from multiple locations around the globe. Comparison of levoglucosan, mannosan and galactosan concentrations with macroscopic charcoal trends in Lake Kirkpatrick and Lake Petén Itzá, suggest that the molecular markers represent more regional fire history and low temperature fires in contrast with macroscopic charcoal which is a local fire proxy. In addition, vegetation changes (Lake Kirkpatrick and Lake Petén Itzá) and charcoal morphotypes (Lake Petén Itzá) were compared to the levoglucosan/mannosan and levoglucosan/(mannosan+galactosan) ratios suggesting that these ratios may be a suitable tool to track burned fuel. Biodegradation tests demonstrate the potential degradation of levoglucosan, mannosan and galactosan if dissolved in water, but findings in ancient sediment samples suggest that particle-bound levoglucosan, mannosan and galactosan can be buried in sediments over millennial time scales. Although these uncertainties still exist, the results of this research suggests that organic molecular markers are a suitable regional fire proxy and isomer ratios may help to understand changes in burned vegetation.

# Zusammenfassung

Waldbrände beeinflussen das Ökosystem und das Klima der Erde, allerdings beeinflusst auch das Klima die Entwicklung von Waldbränden. Dieses System wurde durch den Menschen komplexer, als diese Feuer als Werkzeug entdeckt haben. Die Erforschung der Wechselwirkung zwischen Menschen, Feuer und Klima ist eine der Hauptfragen der Paleofeuerforschung. Erkenntnisse über die Vergangenheit können helfen die zukünftigen Wechselwirkungen vorherzusagen. Die Analyse von Kohlepartikel im Sediment ist eine gängige Methode zur Untersuchung der vergangenen Feueraktivität. Sedimentbohrkerne aus Seen werden hierbei als natürliche Archive genutzt. Diese Dissertation beschäftigt sich mit der Anwendung und Bewertung der spezifischen organischen Proxys für Biomasseverbrennung: Levoglucosan, Mannosan und Galactosan, die nur bei der Verbrennung von Cellulose entstehen, und deren Anwendungsmöglich zur Untersuchung der Feueraktivität während des Holozäns. Dafür wurde zunächst eine Messemethode zur Analyse der drei Isomere mittels Hochleistungsflüssigkeitsanionaustauschchromatographie in Kombination mit Massenspektroskopie entwickelt. Die Methode wurde auf Sedimentproben von Lake Kirkpatrick (Neuseeland) angewendet und die Ergebnisse zeigen eine Korrelation mit den makroskopischen Kohlepartikeln in den Proben. Zusätzlich wurde ein Sedimentbohrkern vom Lago Petén Itzá (Guatemala) untersucht, um die dortige regionale Waldbrandgeschichte während des Holozäns zu rekonstruieren. Darüber hinaus wurde die Analyse von Levoglucosan mit der von fäkalen Sterolen kombiniert und diese Methode wurde auf einen Sedimentbohrkern vom Lago diâTrasimeno (Italien) angewendet. Dies ermöglichte die Untersuchung der Mensch-Feuer Interaktion im späten Holozän. Der Vergleich von Levoglucosan, Mannosan und Galactosan mit makroskopischen Kohlepartikeltrends am Lake Kirkpatrick und Lago Petén Itzá zeigen, dass diese Proxys tendenziell regionale Waldbrandgeschichte und Brände mit geringeren Temperaturen widerspiegeln, im Gegensatz zu makroskopischen Kohlepartikeln, als lokaler Proxy. Zusätzliche haben wir die Verhältnisse zwischen den Isomeren Levoglucosan/Mannosan und Levoglucosan/(Mannosan+Galactosan) mit dem Wechsel der Vegetation (Lake Kirkpatrick und Lago Petén Itzá) und mit den morphologischen Eigenschaften von den Kohlepartikeln (Lago Petén Itzá) verglichen. Die Ergebnisse zeigen, dass dies ein Ansatz sein könnte, die verbrannte Vegetation (Bäume oder Gras) zu bestimmen. Tests zum Bioabbau zeigen, dass ein Teil von Levoglucosan, Mannosan und Galactosan abgebaut wird, wenn diese im Wasser gelöst sind. Allerdings zeigen die Ergebnisse der Untersuchen von verschieden Sedimenten, dass zumindest partikel-gebundes Levoglucosan, Mannosan und Galactosan stabil genug sind, um im Sediment über lange Zeiträume abgelagert zu werden. Obwohl diese Unsicherheiten weiterhin bestehen, weisen die Ergebnisse dieser Untersuchungen darauf hin, dass diese organischen Verbindungen ein geeigneter Proxy zur Rekonstruktion der regionalen Waldbrandgeschichte sind und die relativen Verhältnisse der Isomere Hinweise auf die verbrannte Vegetation geben können..

# Sintesi

Il fuoco gioca un ruolo importante nel sistema terrestre influenzando gli ecosistemi e il clima, ma anche il clima influenza il fuoco. Il discorso diviene più complesso quando gli umani hanno iniziato ad usare quotidianamente il fuoco come strumento. Lo scopo principale della ricerca paleoincendiaria è lo studio delle interazioni fra l'uomo, il fuoco e il clima. Comprendere come questi tre aspetti siano cambiati nel passato aiuterà a prevedere le loro interazioni nel futuro. L'uso di carote di sedimento lacustre come archivi naturali per la ricostruzione della passata attività incendiaria attraverso il conteggio delle particelle di charcoal é un metodo ben consolidato. Il presente lavoro di tesi è dedicato alla valutazione e all'applicazione di indicatori molecolari organici per l'individuazione di eventi di biomass burning: levoglucosan, mannosan e galactosan sono usati come proxies per la ricostruzione della passata attività incendiaria in sedimenti di lago nel corso dell'Olocene. Inizialmente, ho sviluppato un nuovo metodo analitico basato su tecniche di cromatografia a scambio ionico ad alte prestazioni interfacciate alla spettrometria di massa per la separazione e quantificazione di questi tre monosaccaridi anidri in sedimenti lacustri. L'applicabilità di questo metodo analitico è stata dimostrata confrontando i risultati relativi alla presenza di levoglucosan, mannosan e galactosan in alcuni campioni di sedimento provenienti dal Lago Kirkpatrick (Nuova Zelanda) con quelli relativi alle analisi dei macroscopic charcoal. Successivamente ho applicato, con esito positivo, il suddetto metodo a sedimenti lacustri provenienti dal Lago Petén Itzá (Guatemala) per la ricostruzione dell'attività incendiaria su scala regionale durante l'Olocene. Abbiamo impiegato sia analisi di levoglucosan che di steroli fecali in carote provenienti dal Lago Transimeno (Italia) per ricostruire le interazioni fra il fuoco e la presenza umana durante l'Olocene, osservando bassa attività incendiaria durante il periodo Romano. Tale combinazione di tecniche dimostra che i marker molecolari sono validi fire proxies quando analizzati in campioni di sedimento in diverse località. Il confronto fra il trend descritto dalle concentrazioni di levoglucosan, mannosan e galactosan e quello relativo al macroscopic charcoal nel Lago Kirkpatrick e nel Lago Petén Itzá suggerisce che i marker molecolari descrivono la storia dell'attività del fuoco su scala regionale in relazione ad incendi a basse temperature, a differenza del macroscopic charcoal che risulta essere un proxy su scala geografica più locale. Sono, inoltre, stati confrontati i cambiamenti nella vegetazione (Lago Kirkpatrick e Lago Petén Itzá) e nel charcoal morphotypes (Lago Petén Itzá) con i rapporti fra levoglucosan/mannosan e levoglucosan/(mannosan+galactosan), suggerendo che tali rapporti potrebbero risultare utili strumenti per l'individuazione della biomassa combusta. I test di biodegradazione dimostrano una potenziale degradazione di levoglucosan, mannosan e galactosan quando si trovano dissolti in soluzione acquosa, ma la loro presenza in "'antichi"' campioni di sedimento suggeriscono la loro possibile conservazione per millenni se legati a particelle nel sedimento. Sebbene esistano ancora incertezze, i risultati di questa ricerca suggeriscono che i marker molecolari organici testati sono validi fire proxies su scala regionale e che il rapporto fra i loro isomeri può aiutare ad individuare cambiamenti nella vegetazione combusta

# Preface

The research leading to the results of this cumulative thesis was conducted between September 2011 and July 2015 mainly at the Department of Environmental Sciences, Informatics and Statistics at the Ca' Foscari University of Venice, Italy and the Institute of Sustainable and Environmental Chemistry, Leuphana University Lüneburg, Germany.

The thesis consists of an extended summary, which includes a general introduction to the objectives of this thesis, an overview of the developed methods, followed by a synthesis of the applied results and future outlook. The extended summary is followed by the cumulative dissertation consisting of four manuscripts, where two of them are already published in international peer-reviewed journals. The studies are focused on method development for the analyses of specific molecular markers for biomass burning in lake sediments, the application of the method to different lake sediments for reconstructing Holocene fire history and the potential biodegradation of the analysed compounds. The section includes reprints of the published and two unpublished manuscripts.

# Table of Contents

# List of Tables

# List of Figures

# Abbreviations

| | |
|---|---|
| $^{13}C_2$-CHL | Cholesterol-3,4-$^{13}C_2$ |
| $^{13}C_3$-CHL | $^{13}C$-labeled Cholesterol-25,26,27-$^{13}C_3$ |
| a.s.l. | above sea level |
| AD | anno domini |
| ASE | accelerate solvent extraction |
| BA | late Bronze Age |
| BC | before christ |
| BF | Braccio da Montone |
| BOD | biological oxygen demand |
| BP | Perusine War |
| BP | before present |
| BTSFA | N,O-Bis(trimethylsilyl)trifluoroacetamide |
| CAS number | chemical abstracts service number |
| CHAR | particles cm$^{-2}$ yr$^{-1}$ |
| CoP | coprostanol |
| DCM | methylene chloride |
| e-CoP | epi-coprostanol |
| e.g. | example given |
| ER | Etruscan-Roman Period |
| ESI | electrospray ionization |
| FD | Franks Domination |
| FD | Perugia Domination |
| FeSt | fecal sterols |
| GC-MS | gas chromatography-mass spectrometry |

| | |
|---|---|
| GL | Gothic and Longobard Domination |
| HPAEC | high performance anion-exchange chromatography |
| HPLC-MS/MS | high performance liquid chromatography-tandem mass spectrometry |
| IC | ion chromatograph |
| IC-MS | ion chromatography-mass spectrometry |
| L/(M+G) | levoglucosan/(mannosan + galactosan) |
| L/M | levoglucosan/mannosan |
| LOD | limit of detection |
| LOQ | limit of quantification |
| lvg | levoglucosan |
| m/z | mass to charge ratio |
| MA | monosaccharide anhydrates |
| MeOH | methanol |
| MS | mass spectrometry |
| MS/MS | tandem mass spectrometry |
| NaOH | sodium hydroxide |
| OECD | Organization for Economic Cooperation and Development |
| RSD | relative standard deviation |
| SIM | single ion monitoring |
| SPE | solid phase extraction |
| SRM | standard reference material |
| Stg | stigmastanol |
| TB | Battle of Trasimeno |
| ThOD | theoretical oxygen demand |
| TMCS | trimethylchlorosilane |
| TOC | total organic carbon |

# Acknowledgements

# A Extended Summary

# 1 Introduction

## 1.1 Fire and Fire History

Fire has played an important role in the Earth system since the first occurrence of terrestrial plants. Fire widely impacts the local ecosystem, including altering vegetation and soil composition (González-Pérez et al., 2004), but fire also affects global systems such as the carbon cycle (Crutzen, 2002; Bowman et al., 2009; Carcaillet et al., 2009). In addition, fire can influence the climate through emitting greenhouse gases and aerosols, and changing vegetation and surface properties (Randerson et al., 2006). Climate change also controls fire activity (e.g. weather conditions, biomass production) (Kloster et al., 2014). In addition to these natural factors that control fire on long and short time scales, humans complicated fire regimes once they routinely started using fire in daily life (Glikson, 2013). Humans can increase local to regional fire activity by using slash-and-burn techniques for forest clearance or through residential fire, but humans can also suppress fire activity through fire management techniques or through altering vegetation (Bowman et al., 2011). The early impact of fire on climate by agricultural activities was summarized in a hypotheses by Ruddiman (2003) which proposed that humans already influenced the climate as early as 5000 years ago.

The understanding of the fire history in the past is important to comprehend and predict interactions between fire, climate and humans in the future (Daniau et al., 2012). Remote sensing techniques have only been available for approximately the last 30 years (Lentile et al., 2006). To understand pre-industrial fire activity and changes of fire over longer time scales, it is necessary to investigate natural archives. Dendrochronological records from fossil trees can provide records up to 10,000 years (Nicolussi et al., 2009). Ice cores can be used to reconstruct past fire history over glacial-interglacial time scales and up to seasonal resolution by analyzing different non-specific proxies (Fischer et al., 2008; Savarino and Legrand, 1998). These ice core records of fire history include the innovative specific proxy for biomass burning (levoglucosan) that has been analyzed in ice cores from the Arctic and the Himalaya (Yao et al., 2013; Zennaro et al., 2014). Lake sediment cores are another major form of reconstructing fire history, mainly by analyzing charcoal as a fire proxy (Conedera et al., 2009; Mooney and Tinner, 2011; Whitlock and Larsen, 2001). Although charcoal is an established proxy for biomass burning, recent studies proposed the analyses of specific molecular markers in lake sediment as

an additional tool for reconstructing past fire activity (Elias et al., 2001; Kuo et al., 2008, 2011b). In contrast to ice cores, lakes are often located close to human settlements and macroscopic charcoal analyses provide a local fire signal. The synthesis of lake sediment data can provide information about the fire history over wide spatial and temporal scales (Marlon et al., 2013; Power et al., 2010). Sediments are multi-proxy archives where e.g. stable isotopes, calcium carbonate, and diatoms provide climate information (Birks and Birks, 2006; Leng and Marshall, 2004; Mueller et al., 2009), fecal sterols demonstrate the presence of humans (D'Anjou et al., 2012) and pollen assemblages provide information on changing vegetation (Davis, 1963; Huntley and Webb III, 1988). This information helps differentiate between fire, humans, climate, and vegetation (Iglesias and Whitlock, 2014).

## 1.2  Fire Proxies in Lake Sediment

### 1.2.1  Charcoal

The most common approach for paleofire reconstruction from lake sediments is the analysis of charcoal. Analyzing microscopic charcoal (< 100 μm) from pollen slides began in the 1940s (Iversen, 1941) and expanded with the introduction of macroscopic charcoal (> 100 μm) analyses in the 1980s (Clark, 1988; Clark and Hussey, 1996; Clark et al., 1998), Many of these studies are currently compiled in the Global Charcoal Database (Power et al., 2010). Charcoal originates from incomplete combustion processes and is generated in a temperature range between 300 °C and 600 °C. Charcoal is assumed to be resistant against degradation and persists in sediment records over millennia, making it suitable as a proxy for biomass burning. The charcoal size-classes are assumed to represent different spatial scales, where microscopic charcoal can originate from distance sources, and macroscopic charcoal is assumed to represent local fire (Clark, 1988; Clark et al., 1998). However, the type of fire, intensity, temperature and fuel conditions may influence the transport of charcoal particles (Tinner et al., 2006). In addition, charcoal can originate from primary and secondary sources. Primary charcoal is directly deposited to the water surface after a fire, whereas secondary charcoal can be deposited in the lake by streams, surface processes, and sediment mixing years after the fire took place (Conedera et al., 2009; Whitlock and Larsen, 2001).
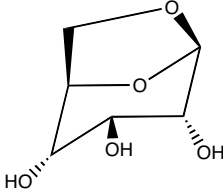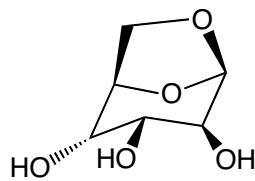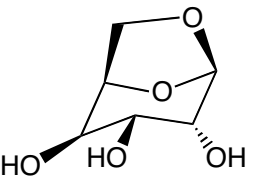
Microscopic charcoal identification is normally conducted on the same slides as for pollen analysis (Carcaillet et al., 2001). Macroscopic charcoal analyses lacks standardization, but the most convenient and most frequently applied method is sieving (Conedera et al., 2009; Mooney and Tinner, 2011; Whitlock and Larsen, 2001). For this method, samples (> 1 cm$^3$) are normally soaked in a deflocculant for days, followed by a bleaching step for dark organic matter. Samples are sieved, whereas the mesh size can differ (100-250 μm). Charcoal is

manually counted under a stereomicroscopic or through image analyses, other method using estimations of the volume or particle size (Ali et al., 2009). Recent studies also aim to distinguish between different morphotypes (e.g. grass, leaf or wood) to enhance the paleofire information obtained from charcoal analyses (Crawford and Belcher, 2014; Enache and Cumming, 2007; Jensen et al., 2007; Mustaphi and Pisaric, 2014; Umbanhowar Jr. and McGrath, 1998). This differentiation may help estimate the type of burnt fuel. Due varying sediment accumulation rates, charcoal concentrations are normally transformed into fluxes (particles $cm^{-2}$ $yr^{-1}$, often CHAR) using the cores chronological information (Mooney and Tinner, 2011). Recent approaches, e.g. the Global Charcoal Data Base, aim to homogenize charcoal raw data by standardizing the statistical treatments for a better comparison of the data between diverse sites (Blarquez et al., 2014; Power et al., 2010).

### 1.2.2 Monosaccharide Anhydrates

Recent studies and reviews propose using the monosaccharide anhydrates (MAs) levoglucosan, mannosan and galactosan (Table 1.1) as potential specific molecular markers for biomass burning in lake sediment cores (Conedera et al., 2009; Elias et al., 2001; Kuo et al., 2008, 2011a). These compounds, and in particular levoglucosan, are generated in high concentrations during cellulose and hemicellulose combustion (Fabbri et al., 2009; Simoneit, 2002) suggesting that they are a specific molecular marker for biomass burning.

**Table 1.1:** General information about levoglucosan, mannosan and galactosan.

| Name | Formula | CAS number | Chemical structure |
|------|---------|------------|--------------------|
| Levoglucosan (1,6-anhydro-$\beta$-D-glucopyranose) | $C_6H_{10}O_5$ | 498-07-7 |  |
| Smilecode: C1[C@@H]2[C@H]([C@@H]([C@H]([C@H](O1)O2)O)O)O | | | |
| Mannosan (1,6-anhydro-$\beta$-D-mannopyranose) | $C_6H_{10}O_5$ | 14168-65-1 |  |
| Smilecode: O[C@H]2[C@@H](O)[C@H](O)[C@@H]1O[C@H]2OC1 | | | |
| Galactosan (1,6-anhydro-$\beta$-D-galactopyranose) | $C_6H_{10}O_5$ | 644-76-8 |  |
| Smilecode: O[C@@H]2[C@H](O)[C@@H](O)[C@@H]1OC[C@H]2O1 | | | |

Initial studies suggested that levoglucosan, mannosan and galactosan are produced in the same temperature range as charcoal (above 350 °C; Simoneit, 2002), whereas later studies detected the emission maxima between 200 and 350 °C, depending on the isomer (Kuo et al., 2011b). MAs therefore cover the initial part of the combustion continuum followed by charcoal (> 300-600 °C) and soot (> 600 °C) (Kuo et al., 2008). This means that MAs may provide additional information regarding burning temperatures and low temperature fires compared to only charcoal analyses.

Although MAs do degrade in the atmosphere (Hennigan et al., 2010; Hoffmann et al., 2010), MAs are actively used as tracers for biomass burning in aerosols (Bae et al., 2012; Schkolnik and Rudich, 2006; Zangrando et al., 2013). Due to the relatively low volatility of MAs, they tend to be ab/adsorbed to primarily fine fraction (> 10 μm) aerosols in the atmosphere (Barbaro et al., 2015; Engling et al., 2009). Recent studies demonstrate the long-range atmospheric transport of MAs as determined from aerosols and snow pits in the Arctic (Kehrwald et al., 2012; Zangrando et al., 2013). This possibility of long-range transport means that MAs may also provide different spatial information in contrast to the primarily local macroscopic charcoal. In addition, the emission ratios between levoglucosan, mannosan and galactosan in smoke can differentiate between burned source types (hardwood, softwood, grass) (Fabbri et al., 2008 and references within). This approach is limited because recent studies suggested that temperature and fire conditions also influence these ratios (Engling et al., 2006; Kuo et al., 2011b). It is still unclear if the preservation of these ratios in lake sediments can provide information regarding vegetation changes, as the three isomers have not yet been analyzed in lake sediments.

The stability of MAs over decal to millennial time scales and the suitability as a specific molecular marker for biomass burning in ice cores was demonstrated by the reconstruction of biomass burning from Arctic and Tibetan Plateau ice cores (Yao et al., 2013; Zennaro et al., 2014). Using the same specific marker in ice cores and lake sediments may also be an additional tool for comparing and synchronizing these two different archives. However, the suitability of MAs as a biomass-burning proxy in lake sediments is actively discussed, because the stability of MAs in sediments is not well known. Only a single laboratory experiment determined an aquatic half-life of 3-4 days for dissolved levoglucosan (Norwood et al., 2013). In addition different bacteria are know to be capable of metabolizing levoglucosan (Kitamura et al., 1991; Yasui et al., 1991). However, several studies analyzed lake or marine sediments and detected levoglucosan in up to 20 kyr old sediments (Elias et al., 2001; Hopmans et al., 2013; Kuo et al., 2011a; Sikes et al., 2013) suggesting that levoglucosan entrapped in particles may be stable over long time scales (Elias et al., 2001).

Several analytical methods are published for the analyses of MAs in atmospheric aerosols. The first methods used gas chromatography-mass spectrometry (GC-MS) with derivatization with the capability to separate the three isomers (Medeiros and Simoneit, 2007; Schkolnik and Rudich, 2006). To avoid derivatization, liquid techniques using high performance liquid chromatography-tandem mass spectrometry (HPLC-MS/MS) using

C18 columns were developed to analyze levoglucosan in ice cores (Gambaro et al., 2008; Yao et al., 2013; Zennaro et al., 2014). This method is sensitive but cannot separate the three isomers. Recently, Hopmans et al. (2013) published a HPLC-MS/MS method for marine sediments which was able to separate all three isomers by applying hydrophilic interaction chromatography. Other recent studies use high performance anion-exchange chromatography (HPAEC) with amperometric detection and MS or MS/MS to analyze MAs in different matrixes (Engling et al., 2006; Piot et al., 2012; Saarnio et al., 2010). These methods can separate all three isomers, where such separation was not possible with the previous techniques. HPAEC with MS may be a promising analytical approach to analyze levoglucosan, mannosan and galactosan in lake sediment samples but this approach has not yet been applied to lake cores.

# 2 Research Objectives

The overall aim of this thesis is to increase our understanding of the suitability of the specific molecular markers levoglucosan, mannosan and galactosan as proxies for biomass burning in lake sediment cores for reconstructing past fire activity during the Holocene.

Suitable methods for analyzing all three MAs in lake sediment samples are rare. A fast and sensitive method is crucial for the analyses of levoglucosan, mannosan and galactosan. One objective of this thesis was to develop a sensitive method without needing derivatization, thereby creating a faster method than GC-MS techniques. Furthermore, the method has to separate the three isomers, where such separation is difficult with HPLC-MS/MS methods. High-performance anion-exchange chromatography demonstrated good separation of the three isomers in previous atmospheric aerosol studies (Saarnio et al., 2010) and this research served as the starting point for developing methods of MAs in lake sediments.

Previous studies analyzed levoglucosan in a small number of lake or marine sediment cores and correlations with macroscopic charcoal were observed in some of these studies assuming that levoglucosan can be used as a fire proxy in lake sediment samples (Elias et al., 2001; Kuo et al., 2011a). It is still unknown if mannosan and galactosan can independently serve as molecular markers of biomass burning. Here, macroscopic charcoal and MAs in different lake sediments were compared to increase our understanding of the relationship between macroscopic charcoal and MAs.

Reconstructing the fuel sources of past fires remains a major research challenge in fire science. Methods that analyze the three isomers (levoglucosan, mannosan and galactosan) allow calculating ratios of these isomers, which in turn may provide data on the past vegetation. One of the objectives of this study is to investigate if these isomeric ratios are preserved in lake sediment cores and if they can track past burned fuel.

Another major research question in fire science is if humans or climate were the major drivers for past fire activity. Here, a novel approach was included to try to disentangle these influences by augmenting levoglucosan analyses with fecal sterol concentrations in the same sediment samples. Fecal sterols are a marker of human presence in a local area. The method was applied to a Lake Trasimeno (Italy) sediment core to prove the suitability of this method.

This thesis analyzes lake sediment samples from three different regions: New Zealand, Guatemala and

Italy. Each individual study focuses on its specific region, where the synthesis of these studies increases our understanding of the suitability of MAs in lake sediment samples as fire markers. This overview is focused on this synthesis, whereas the fire history of the different regions is discussed in detail in each individual study of this cumulative thesis.

**Study I: Method for the determination of specific molecular markers of biomass burning in lake sediments.**

Development of an analytical method for the determination of levoglucosan, mannosan and galactosan using accelerated solvent extraction followed by high-performance anion-exchange chromatography-mass spectrometry and application of the method on a limited sample set from a sediment core from Lake Kirkpatrick, New Zealand.

**Study II. Combining charcoal sediment and molecular markers to infer a Holocene fire history in the Maya lowlands of Petén, Guatemala.**

The aim of the study was the first application of the HPAEC-MS method to a full Holocene record from a lake sediment core from Lake Petén Itzá, Guatemala. The reconstruction of the fire history of the Holocene was discussed in context of the occupation of the Mayas in this region. In addition, we analyzed macroscopic charcoal as a comparison between fire proxies. Pollen records from previous studies were used to prove the suitability of the MA ratios as proxy for vegetation changes.

**Study III. Late Holocene record of humans and fire at Lake Trasimeno (Italy): An organic molecular marker approach**

The aim of the third study was to use a single extraction method for the analyses of MAs and fecal sterols in the same sample as proxies for fire and human appearance, respectively. The study focuses on the discussion of the suitability of this method and was applied to sediment samples from Lake Trasimeno, Italy. The obtained results were used to investigate the interaction between human and fire in this region during the last 3100 years.

**Study IV. Biodegradation of levoglucosan, mannosan and galactosan: Implications for paleoclimate research**

In order to better understand the post-depositional behavior of MAs, preliminary biodegradation tests of dissolved levoglucosan, mannosan and galactosan were performed and the implications for paleoclimate research were discussed.

# 3 Experimentel Section

## 3.1 Samples

Samples analyzed in the three studies originate from different regions in the world (see Figure 3.1): Lake Kirkpatrick, New Zealand (Study I), Lake Petén Itzá, Guatemala (Study II) and Lake Trasimeno, Italy (Study III).
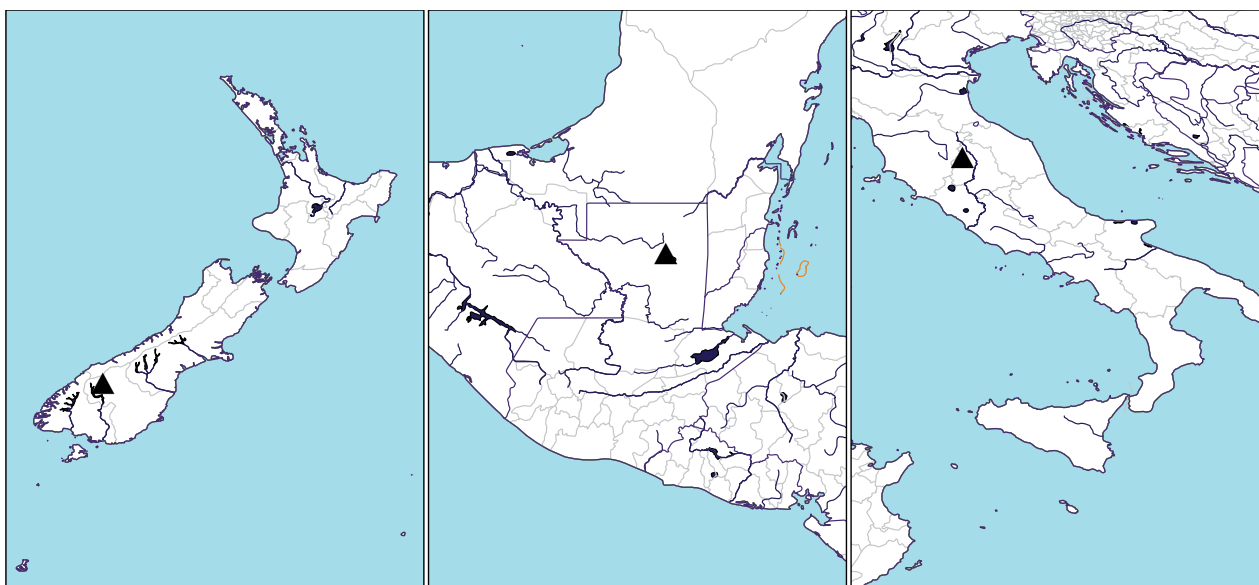


**Figure 3.1:** The three sample locations are marked with a triangle. From right to left: Lake Kirkpatrick (New Zealand), Lake Petén Itzá (Guatemala) and Lake Trasimeno (Italy). Maps made with Natural Earth, http://www.naturalearthdata.com.

**Lake Kirkpatrick, South Island, New Zealand (45°03'S, 168°57'E, 570 m a.s.l. (above sea level))**
The lake (ca. 3.5 ha) is characterized by a small catchment area ($< 10$ km$^2$) with a relatively closed basin with no known significant surface inflow. The macroscopic charcoal is assumed to originate from local aerosol ($< 1$-$3$ km$^2$). The core was drilled in 2009 by a Montana University research team and twelve samples from a depth of 75-129 cm and 119-129 cm were shipped to Venice in 2012. The age of the samples were between

1240-1311 AD (119-129 cm) and between 1479 and 1533 AD (75-88 cm) corresponding to a pre-Maori period with a low fire activity and high fire period after the arrival of the Maori, respectively. Details of the drilling site and charcoal and pollen measurements are given in McWethy et al. (2009).

**Lake Petén Itzá, Guatemala (17°00'N, 89°50'W; 110 m a.s.l.)**

The lake is one of the largest and deepest lakes in Central America (ca. 165 m). The lake has now significant outflow and receives water from surface runoff, rainfall, subsurface groundwater and a small input stream (Correa-Metrio et al., 2012). The current water level is mainly regulated by rainfall. The surrounding region was strongly influenced by the Maya, one of the major ancient Mesoamerican civilizations. The first Maya settlements and agricultural activity were dated around 3000 BP at Lake Petén Itzá. We analyzed 110 samples from the 550 cm log core (PI 5 VI 02 11B) drilled in Lake Petén Itzá in June 2002 (Hillesheim et al., 2005). 110 subsamples for MA, charcoal and total organic carbon (TOC) measurements were selected at the University of Florida, USA and shipped in 2012 to Venice. The sampling resolution was 5.5 cm from 18 to 300 cm depth, and 1 cm from 300 and 514 cm depth. The core covers the entire Holocene until ca. 11,000 cal yr BP. Details regarding the dating, drilling, and sampling site are available in Anselmetti et al. (2006) and Mueller et al. (2009).

**Lake Trasimeno, Umbria, Italy (43°08'N, 12°06'W; 260 m a.s.l.)**

Lake Trasimeno has been a natural crossroad throughout human history including the famous Trasimeno battle between the Romans and the army of Hannibal in 217 BC. The lake is one of the largest lakes in Italy with a diameter of 10 km and surface area of ca. 120 km$^2$. A very low maximum depth of 6 m characterizes the lake, where the lake only receives minor direct river inflows without any outflows. The low depth and minimal inflows leads to periodical changes in the water level. The 360 cm core was subsampled at 5 cm resolution for a total number of 72 samples. Preliminary age-depth model based on 4 dating points suggests that the first 235 cm covers approximately 3100 cal yr BP; followed by a section with a low sedimentation rate until 260 cm, going back until 8100 cal yr BP. The occurrence of dry periods is evident in the last part of the core and dated material reaches back to 23,000 yr BP.

## 3.2 Chemicals

Following chemicals were used in this thesis: 1,6-anhydro-$\beta$-D-glucopyranose (levoglucosan), 1,6-anhydro-$\beta$-D-mannopyranose (mannosan) and 1,6-anhydro-$\beta$-D-galactopyranose (galactosan) were purchased at Molekula SRL (Rimini, Italy). Mass labelled $^{13}C_6$-levoglucosan, 5$\beta$-cholestan-3$\beta$-ol (CoP), 5$\beta$-cholestan-3$\alpha$-ol (e-CoP), 5$\beta$-stigmastanol (Stg), $^{13}C$-labeled Cholesterol-25,26,27-$^{13}C_3$ ($^{13}C_3$-CHL) and Cholesterol-3,4-$^{13}C_2$ ($^{13}C_2$-

CHL) and derivatisation reagent N,O-Bis(trimethylsilyl)trifluoroacetamide (BSTFA) with 1 % trimethylchlorosi-
lane (TMCS) were purchased from Sigma Aldrich (Milan, Italy). Pesticide grade methylene chloride (DCM),
n-hexane, acetone and methanol (MeOH) (Romil Ltd., Cambridge, UK) were used. Anhydrous sodium sulphate
(Carlo Erba Reagenti S.p.A., Milano, Italy) was oven-dried at 150 °C for 24 h, washed with DCM, n-hexane
and then stored in this latter. Metallic copper (Carlo Erba Reagenti S.p.A.) was activated by rinsing three times
with hydrochloric acid and solvents (water, acetone and DCM) and stored in deaerated n-hexane. Diatomaceous
earth and Ottawa sand (Applied Separations Inc., Allentown, PA, USA) were used as inert materials for the
extractions. Ultra pure water was produced with a PURE Lab System (ELGA LabWater, Marlow, UK).

## 3.3  Sample Preparation and Extraction

For all analyses wet lake sediment samples were freeze-dried, milled and homogenized. Two different extraction
methods were used, depending on the range of proxies analyzed.

a)  Study I and II: Levoglucosan, mannosan and galactosan were extracted from an aliquot of 200 mg of freeze-
dried sediment using accelerate solvent extraction (ASE) (PSEone, Applied Separations, Hamilton, USA)
with methanol (2 cycles of 5 min each, 100 °C at 100 bar). Prior to the extraction, samples were spiked with
100 µL of the internal standard ($^{13}C_6$-levoglucosan; concentration: 1 µg mL$^{-1}$ in methanol). The extract was
filtered (0.2 µm, PTFE, Phenomenx, USA) and evaporated under nitrogen to dryness (Turbovap, Biotage,
Uppsala, Sweden) and finally dissolved in 500 µL ultra pure water (ELGA Pure Lab, UK), shortly sonicated
and centrifuged if solid residues were visible. Sample was transferred to measurement vials and stored at
4 °C until analysis.

b)  Study III: Extraction was modified due to additional analyses of fecal sterols (FeSt; 5$\beta$-cholestan-3$\beta$-
ol (CoP), 5$\beta$-cholestan-3$\alpha$-ol (e-CoP), 5$\beta$-stigmastanol (Stg). Due to the low expected concentrations;
extracted sediment volume was increased to up to 5 g. The six FeST and three MAs were extracted with
a dichlormethane:methanol (9:1; v:v) mixture (ASE 200, Dionex, 1500 psi, 2 cycles of 10 min). Copper
was added to remove sulfur prior to the extraction and $^{13}C_6$-levoglucosan and $^{13}C$-labeled Cholesterol-
25,26,27-$^{13}C_3$ were added as internal standards. Extracts were pre-concentrated to ca. 2 mL with a vacuum
evaporator (EZ-2 vacuum evaporator, Genevac Ltd, Ipswich, UK) for 40 min at 26 °C. Samples were further
pre-concentrated to a volume of ca. 500 µL (Turbovap) prior to sample clean-up and separation into two
fractions (FeST and MAs) using solid phase extraction cartridges (Discovery SPE DSC-SI Silica Tube 12 mL,
2 g, Supelco, US). Cartridges were cleaned and conditioned with ca. 30 mL DCM. Samples were loaded onto
the cartridge and the FeSt fraction was eluted with 80 mL DCM, while the MA fraction was eluated with

20 mL methanol. The MA fraction was treated as previously described. The FeSt fraction was evaporated to dryness and the residues were dissolved in an internal standard solution (Cholesterol-3,4–$^{13}C_2$). Samples were derivatizated by adding 100 µL of N,O-Bis(trimethylsilyl)trifluoroacetamide with 1% trimethylchlorosilane and kept at a temperature of 70 °C for one hour prior to the analyses by GC-MS.

## 3.4 Biodegradation Test

In order to estimate the biodegradation of dissolved levoglucosan, mannosan and galactosan, we performed a manometric respirometry test according to the OECD 301F/1992 at the Leuphana University Lüneburg, Germany in 2015. In brief, a known concentration of the MAs corresponding to theoretical oxygen demand (ThOD) of 30 mg L$^{-1}$ was stirred in an inoculated mineral medium in a closed flask at a constant temperature of 20 °C for 28 days. The inoculum was taken from wastewater treatment effluent. Biodegradation was tested in parallel and in addition an abiotic sterile control and a toxicity control was performed for each compound. During the 28 days the pressure changes due to the consumption of oxygen are automatically registered by the OxiTop system (WTW GmbH, Weilheim, Germany). The final biodegradation corresponds to the ratio between the biological oxygen demand (BOD), and the ThOD. All details are given in the guidelines of the OECD for testing chemicals (OECD, 1992).

## 3.5 Charcoal Analyses

The analyses of macroscopic charcoal (Study II) were performed at the University of Bern, Switzerland in 2014. A wet subsample of 1 cm$^3$ of sediment was first soaked for several hours in a deflocculating solution (5 % sodium hexametaphosphate), followed by a treatment with hydrogen peroxide (10 %) for approx. 12 hours to bleach the organic content, and finally sieved with a 100 µm mesh. A stereomicroscope with 40x magnification was used to count macroscopic charcoal particles. Different charcoal morphotypes were distinguished as described in Colombaroli et al. (2014) to differentiate between grass and woody vegetation. Grass cuticles usually occur in flat sheets, with their epidermal cells arranged in parallel rows, and with stomata between the rows (Jensen et al., 2007). Grass-based charcoal mainly has a length to width ratio $\geq$ 4:1. Woody material presents a thicker structure than the grass cuticle and the length to width ratio is normally $\leq$ 4:1 (Umbanhowar Jr. and McGrath, 1998). Leaf fragments can be separated from other charred material due to the presence of leaf veins, which are characterized by a divergence of branches from a node (Jensen et al., 2007). All details can be found in Beffa (2012). Once the different vegetation types are identified, charcoal counts (particles cm$^{-3}$) are converted into fluxes (CHAR; particles cm$^{-2}$ yr$^{-1}$) based on the deposition rates obtained from the age-depth

model.

## 3.6 Instrumental Analyses

### 3.6.1 Monosaccharide Anhydrates

Levoglucosan, mannosan and galactosan were separated with an ion chromatograph (IC; Dionex ICS 5000, Thermo Scientific, Waltham, US). For Study I the system was equipped with a CarboPac™ PA1 column and a CarboPac™ P10 column (Thermo Scientific, each 2 mm x 250 mm). In addition, we used a CarboPac™ PA 10 guard column (2 x 50 mm) and an AminoTrap™ column (2 x 50 mm) to trap amino acids. An injection volume of 25 µL was used. The sodium hydroxide gradient was: 20 mM (0-15 min; seperation), 100 mM (15-40 min; column cleaning), 20 mM (40-60 min; equilibration). The flow was 0.250 mL min$^{-1}$. Sodium hydroxide was removed by a suppressor (ASRS 500, 2 mm, Thermo Scientific) before entering the MS source. For the protection of the MS during the cleaning step, the flow was switched to waste after a total run time of 15 min. Ammonia in methanol (7‰) was added post-column with an external pump (flow: 0.02 mL min$^{-1}$) to improve the ionization of the aqueous eluent.

For the analyses of the samples from Lake Petén Itzá and Lake Trasimeno (Studies II and III) Thermo Scientific provided a CarboPac™ MA1 column (Thermo Scientific, 2 x 250 mm), which has a higher separation capacity compared to the previous used columns. For protecting the column we used an AminoTrap™ column (Thermo Scientific, 2 x 50 mm). We slightly modified the eluent gradient to obtain a better separation. A gradient of NaOH: 20 mM (0-20 min; seperation), 100 mM (20-40 min; cleaning), 20 mM (40-60 min; equilibration) was used. In addition, the injection volume was increased from 25 µL to 50 µL.

The three MAs were detected with a single quadrupole mass spectrometer (MSQ Plus™, Thermo Scientific) after the separation on the IC. The MS was equipped with an electrospray ionization (ESI) source used in negative ionization mode. Source temperature was 350 °C, and needle voltage was -3.5 kV and cone voltage -50 V. Levoglucosan, mannosan and galactosan were analyzed using single ion monitoring (SIM) with the mass to charge ratio (m/z) 161 and the internal standard $^{13}C_6$ levoglucosan m/z 167.

Samples were quantified using the response factor of levoglucosan, mannosan and galactosan with the mass-labeled internal standard ($^{13}C_6$-levoglucosan). The advantage of using internal standards is the ability to correct potential analyte loss during the extraction process and handling. A Chromeleon 6.8 Chromatography data system (Thermo Scientific) was used for data acquisition and elaboration. For a better comparison with the charcoal data and under consideration of different sedimentation rates, MA concentrations (ng g$^{-1}$ dry weight) were converted into fluxes (ng cm$^{-2}$ yr$^{-1}$) based on the deposition rates obtained from the age-depth model.

### 3.6.2 Fecal Sterols

Details of the GC-MS analyses are given in the Study III and in Battistel et al., 2015. Briefly, analyses were performed with a gas chromatograph (6890 N GC System; Agilent Technologies, Santa Clara, CA, USA) coupled to a quadrupole mass spectrometer (5973, Agilent Technologies, Santa Clara, CA, USA). The silylated steroids were separated on a HP-5MS column (60 m length, 0.25 mm inside diameter, and 0.25 µm film thickness, Agilent Technologies, Santa Clara, CA, USA). Quantification was performed by analysing in SIM using target and qualifier ions for each compound. The response factor between the target analytes and the internal standards was used for quantification.

# 4 Results and Discussion

## 4.1 Method Development

### 4.1.1 Extraction and Clean-up

In studies I and II we used an ASE extraction with only methanol as a solvent, following the suggestions in Hopmans et al. (2013). The advantage of only using methanol is to reduce the matrix, compared when using dichloromethane:methanol mixtures. By using methanol, it was only necessary to filter the samples prior to their injection into the IC-MS. Certificated reference materials for MAs in lake sediments are not available, thus we based our extraction efficiency based on spiked blanks. The relative recovery of MAs from spiked blank samples (n = 5), was 99.8% ($\pm$ 1.3%) (levoglucosan), 97.7% ($\pm$ 6.4%) (mannosan) and 94.1% ($\pm$ 7.6%) (galactosan). The lower relative recovery for mannosan and galactosan may be due to the missing corresponding internal standard and were corrected by the internal standard $^{13}C_6$-levoglucosan. The extraction method was modified for the additional analyses of more proxies (Study III), because sediment sample materials were very limited. A single extraction method was preferred for the analyses of MAs and FeSt in the samples to both reduce sample volume and extraction time. A dichloromethane:methanol mixture (9:1, v:v) was used to extract the more non-polar FeSt together with MAs and a SPE clean-up was necessary to separate the samples into two fractions for analyses with GC-MS (FeSt) and HPAEC-MS (MAs). Detailed information for the FeSt is given in Battistel et al. (2015).

### 4.1.2 Chromatographic Separation

One problem in the analyses of levoglucosan, mannosan and galactosan is the separation of the three isomers. HPAEC can separate the three isomers well when using CarboPac™ columns (Piot et al., 2012; Saarnio et al., 2010). However, even these columns have problems to achieve a baseline separation of levoglucosan and mannosan. As described in the method section, a combination of two columns (CarboPac™ PA 1 and CarboPac™ PA 10) was used and we achieved a baseline separation of all three isomers, with retention times of 4.8 min, 6.4 min and 9.1 min for levoglucosan, mannosan and galactosan, respectively (Figure 4.1). Thermo

Scientific later provided us with a CarboPac™ MA1 column that was not yet available on the market. With this CarboPac™ MA1 column we were able to increase the separation to 6.5, 10.2, and 17.7 min for levoglucosan, mannosan, and galactosan, respectively, which help to reduce potential interferences in complex sediment matrixes. Unfortunately the multi proxy extraction method and clean-up method applied in Study III influenced the separation and only levoglucosan data can be reported. For mannosan and galactosan retention time shifts and peak enlargement was observed, leading to the impossibility of quantifying mannosan and galactosan. The source of this problem remains unknown and requires future investigations.
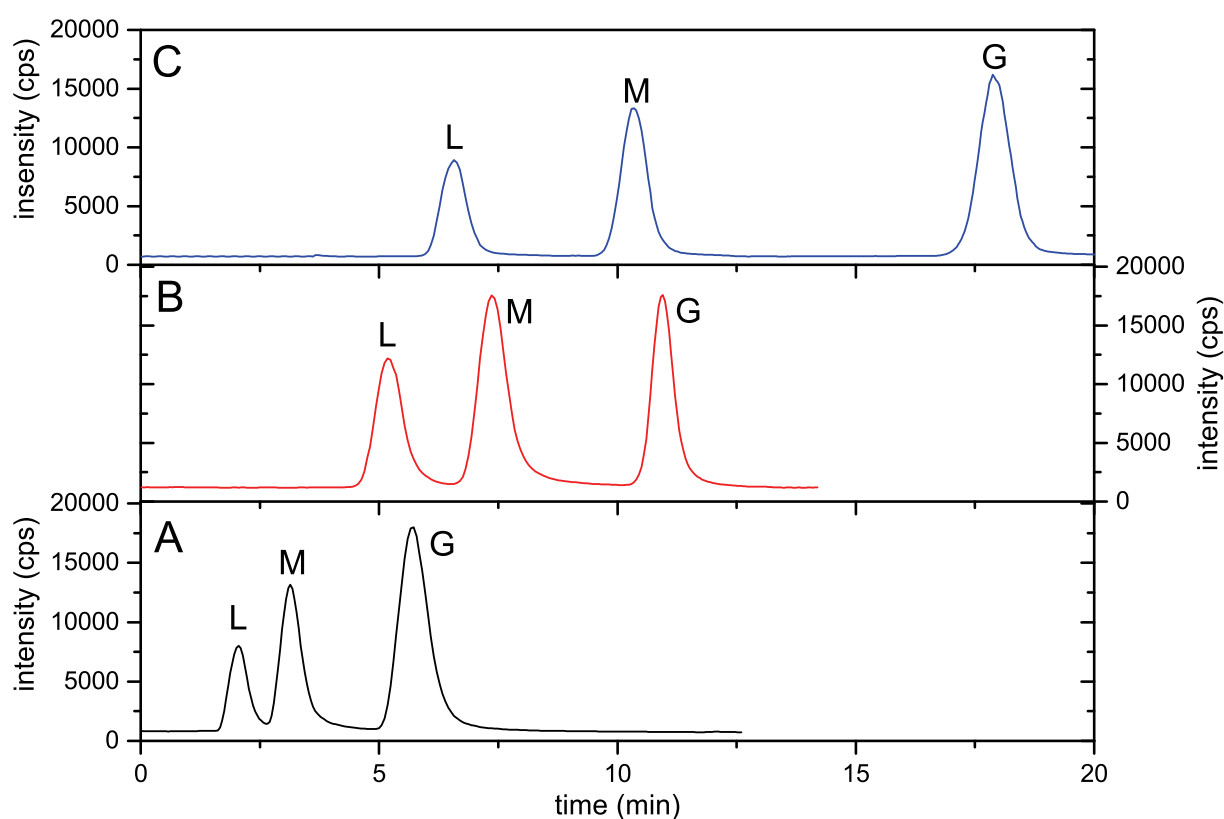


**Figure 4.1:** HPAEC-MS chromatogram (SIM m/z 161) of levoglucosan (L), mannosan (M) and galactosan (G) of a 100 ng mL$^{-1}$ standard solution with different columns: A: CarboPac™ PA10; B: CarboPac™ PA1 + CarboPac™ PA10; C: CarboPac™ MA1.

## 4.1.3  Analytical Performance

Instrumental precision was tested by a 10-fold analysis of low (25 ng mL$^{-1}$) and high (300 ng mL$^{-1}$) concentration standards of levoglucosan, mannosan and galactosan, resulting in a relative standard deviation (RSD) of 3.8 % and 6.6 %, 3.2 % and 2.3 %, and 3.7 % and 2.8 %, respectively. The linearity for levoglucosan, mannosan and galactosan was proven by a 8 point calibration from 5-500 ng mL$^{-1}$ and with a r$^2$ = 0.99 for all

three isomers. We calculated the instrumental limits of detection (LOD) and limits of quantification (LOQ) corresponding to signal-to-noise of 3 (LOD) and 10 (LOQ), affording LOD and LOQ of 0.9-1.8 ppb and 3.2-5.8 ppb, respectively. The LOD and LOQ using the CarboPac™ MA1 column were 0.4-1.3 ng mL$^{-1}$ and 1.4-4.4 ng mL$^{-1}$ as a result of the increase of injection volume. This detection limits in the low ng mL$^{-1}$ range in the two different column set-ups are similar to previously published HPAEC-MS methods (Saarnio et al., 2010). Standard reference materials (SRM) for lake sediments is not available for levoglucosan, mannosan and galactosan, so we used the urban dust standard SRM 1649b, which was also used to validate previous methods (Kuo et al., 2008; Louchouarn et al., 2009). We obtained concentrations of 168 ppm ($\pm4.5$), 15.7 ppm ($\pm0.7$) and 5.0 ppm ($\pm0.2$) for the three isomers, which agreed with the previously published concentrations of 160.5 ppm ($\pm5.0$), 16.7 ppm ($\pm0.7$) and 4.8 ppm ($\pm0.2$) (Louchouarn et al., 2009). In addition we performed several parallel extractions of different samples with a good reproducibility (results are given in Study I and III). The estimated overall method uncertainty is 4.7 %, 9.9 % and 8.7 % for levoglucosan, mannosan and galactosan, respectively. The increased uncertainty of mannosan and galactosan compared to levoglucosan may be due to the lack of internal standards for mannosan and galactosan. For study III, we modified the extraction (DCM:MeOH instead of only MeOH) and added a clean-up step (SPE clean-up for separating FeSt fraction). A higher uncertainty was observed in Study III based on the results from parallel extractions and spiked blank samples, where spiked blanks demonstrated a good reproducibility with a standard deviation of 6 % and 2 % (n=5) for concentrations 100 ng mL$^{-1}$ and 300 ng mL$^{-1}$, while extraction of samples in triplicates resulting in 6-34 %. This difference may be due to losses during the clean-up and the increased number of pre-concentrations steps.

### 4.1.4 Blank contribution

Blank concentrations in all our studies are between 2-15 ng mL$^{-1}$, which is a limiting factor to obtaining better detection limits. Levoglucosan, mannosan and galactosan are ubiquitous in the air, and their atmospheric concentrations especially increase with home heating with wood stoves in the winter. In order to account for these local atmospheric aerosols, blanks were analyzed every day and were finally subtracted from all reported MA concentrations. The elevated blank contribution and the low MA concentrations detected in the samples from Lake Petén Itzá both suggested the necessity of increasing the amount of extracted sediment in the later studies.

## 4.2 Paleofire Reconstructions

We applied the successfully-developed method to samples from three different lakes, where we compared the MA concentrations with different proxy data for reconstructing past fire activity. Lake Petén Itzá represents a full Holocene fire record; Lake Trasimeno focuses on the late Holocene fire history, whereas Lake Kirkpatrick is only a limited dataset for method validation. Macroscopic charcoal data evaluates the suitability of MAs as a fire proxy at both Lake Kirkpatrick and Lake Petén Itzá. At Lake Trasimeno we introduced fecal sterols in the analytical method and evaluate the advantages of this approach. Here, the discussion is focused on the evaluation of MAs as a fire proxy while the full discussion of the local fire histories are present in their corresponding publications.

### 4.2.1 MAs and Charcoal

Macroscopic charcoal is a proxy for local fire histories and previous studies compare charcoal with MA concentrations in lake sediments (Elias et al., 2001; Kuo et al., 2011a). However, correlations between the two proxies were not observed in all of these studies (Sikes et al., 2013). To further evaluate this relationship macroscopic charcoal was compared to MA concentrations from Lake Kirkpatrick and Lake Petén Itzá samples.
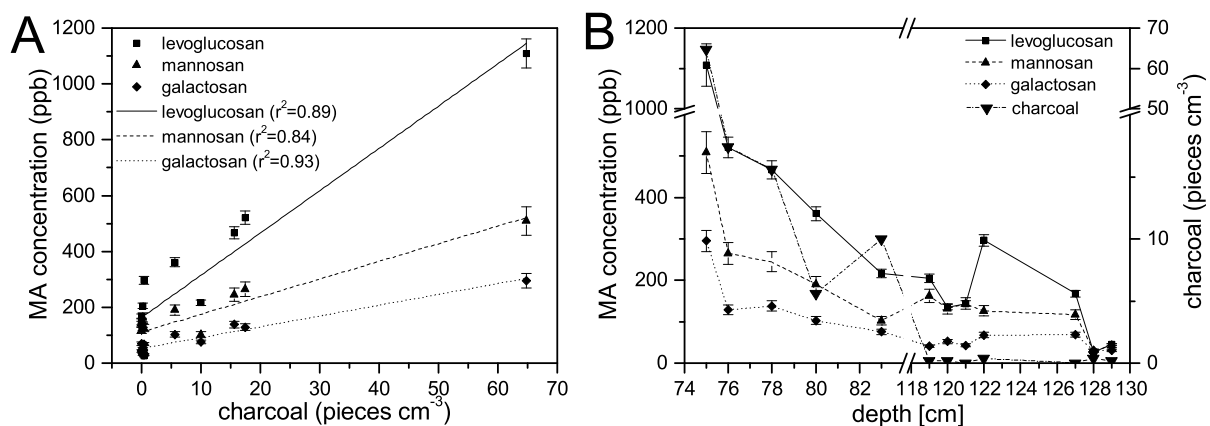


**Figure 4.2:** A: MA concentrations (dry wt. concentration in ppb) related to macroscopic (> 125 μm) charcoal concentration (pieces cm$^{-3}$). Lines represent linear fits of the data. Error bars represent the estimated method uncertainty. B: MA concentration (dry wt. concentration in ppb) and charcoal concentration (pieces cm$^{-3}$) related to sample depth (cm). Note the axis breaks. Reprint from Study I.

Lake Kirkpatrick samples were selected due to their known fire history that encompasses the initial burning period with the arrival and dispersal of the Maori in New Zealand. Low charcoal concentrations and thus low fire activity were observed in the samples originating from the period before the Maori arrival (1240-1311 AD) and higher charcoal concentrations in the samples after the arrival (1479-1533 AD) (McWethy et al., 2009). The

analyses of MAs in these samples demonstrate similar results (Figure 4.2). MAs correlate with macroscopic charcoal, with a $r^2$ of 0.89, 0.84 and 0.93 for levoglucosan, mannosan and galactosan (Figure 4.2). However, the data set is small and we selectively chose samples with high and low charcoal.
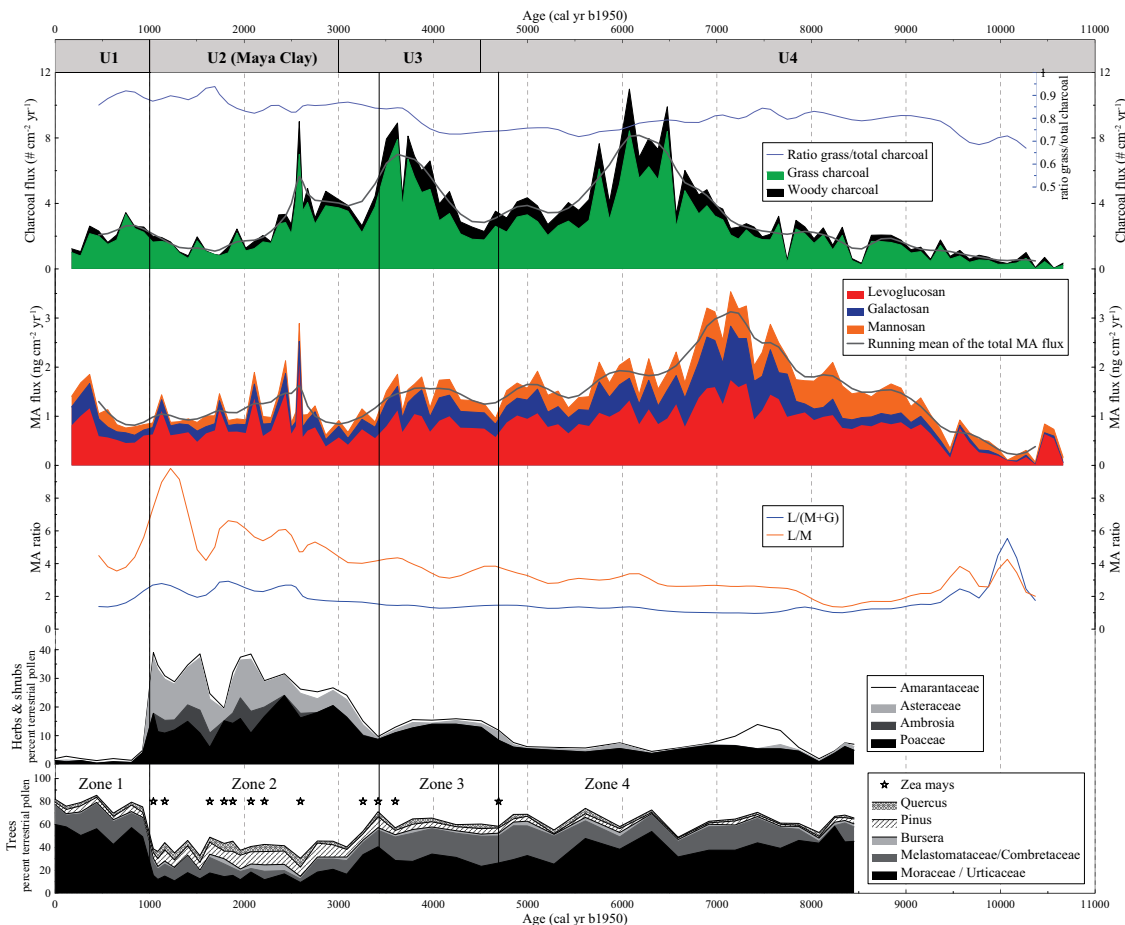


**Figure 4.3:** Top: Grass charcoal (green) and woody charcoal (black), total charcoal corresponds to the upper envelope of the woody charcoal line. The ratio of grass to total charcoal is indicated as a blue line. The grey line indicates a 7-point Gaussian running mean of the total charcoal flux. Middle: Fluxes of MAs (red: levoglucosan, blue: galactosan, orange: mannosan). The grey line indicates a 7-point Gaussian running mean of the total MA flux. The ratio of levoglucosan/(mannosan + galactosan) (L/(M+G)) is shown as solid orange line, and the levoglucosan/mannosan (L/M), ratio is shown as solid blue line. Bottom: Pollen records from Lake Puerto Arturo (Wahl et al., 2006) are subdivided into grass and shrub pollen types (upper panel) and in arboreal pollen types (bottom panel). In the arboreal pollen panel, filled colours correspond to local trees, whereas hatched areas indicate disturbances in arboreal types. Stars indicate core sections with Zea mays Pollen Zones as defined by Wahl et al. (2006) are indicated by black vertical lines, see Study II for details. On top the lithologic and lithostratigraphic units described in detail by Hillesheim et al. (2005) and Mueller et al. (2009) are indicated (U1-U4), U2 corresponds to the low organic content Maya clay layer. All records are plotted linearly versus age. Reprint from Study II.

We successfully reconstructed a regional fire history for the entire Holocene at Lake Petén Itzá, Guatemala and results are shown in Figure 4.3. The MA and charcoal fluxes demonstrating three main fire periods:

9500-6000 cal yr BP, 3700 cal yr BP and 2700 cal yr BP. These fire periods agree with previous reconstructions of Mesoamerican fire history (Curtis et al., 1998; Rosenmeier et al., 2002). Climate conditions were assumed as the driver for the first period between 9500-6000 cal yr BP (e.g. insolation maximum during this period), while the last maximum is attributed to anthropogenic activity. The rapid change between burned vegetation types at the 3700 cal yr BP fire maximum may be attributed to human activity. The charcoal and MAs behave similarly in the record from Lake Petén Itzá. The mannosan and galactosan flux only correlates in the middle section of the core (7000-2000 cal yr BP) with $r^2$ of 0.53 and 0.46 (p < 0.01), where this correlation is thus weaker than that observed in Lake Kirkpatrick. The major difference between macroscopic charcoal and MAs at Lake Petén Itzá is the earlier increase in MA concentrations (around 7000 cal yr BP) compared with macroscopic charcoal (around 6000 cal yr BP). This earlier increase at Lake Petén Itzá and the occurrence of MAs in samples without any macroscopic charcoal at Lake Kirkpatrick may be explained with the differences between MAs and macroscopic charcoal in a) transport behavior b) burning conditions and c) post depositional effects.

a) Transport behavior: Macroscopic charcoal is a local fire proxy (Whitlock and Larsen, 2001) and e.g. at Lake Kirkpatrick a catchment of 1-3 $km^2$ was assumed (McWethy et al., 2009). This charcoal catchment size is mainly related to the particle size of macroscopic charcoal (> 100 μm) which interferes with or prohibits long distance transport. In contrast, MAs are mainly distributed in the fine fraction (> 10 μm) of aerosols (Barbaro et al., 2015; Engling et al., 2009). Several findings in Arctic and Antarctic aerosols demonstrate that MAs are able to undergo long range atmospheric transport (Barbaro et al., 2015; Zangrando et al., 2013). Levoglucosan data from the NEEM ice core (Greenland) represents supra-regional to semi-hemispheric fire history (Zennaro et al., 2014). These different transport distances may explain the presence of MAs at Lake Kirkpatrick in samples without any macroscopic charcoal. The idea that regional or supra-regional fire history is recorded in the sediment at Lake Kirkpatrick is demonstrated by the occurrence of microscopic charcoal in the samples without macroscopic charcoal (McWethy, personal communication). Microscopic charcoal has a larger catchment area than macroscopic charcoal and can represent regional fire history (Carcaillet et al., 2001; Whitlock and Bartlein, 2003). At Lake Petén Itzá the MA record peaks almost 1000 years earlier than the macroscopic charcoal in the fire period between 6500-9000 cal yr. BP. This earlier increase at Lake Petén Itzá may be linked to biomass burning across much of Mesoamerica or southern North America, especially as the literature demonstrates that fire activity was not evenly distributed in the early Holocene in this region (Power et al., 2008, 2012). This earlier increase is also evident from other charcoal records from Mesoamerica, where early Holocene fire activity increased at the same time as the MAs at Lake Petén Itzá (Lozano-García et al., 2005, 2013; Piperno, 2006). This correspondence between regional microcharcoal and MAs means that observed differences between macrocharcoal and MAs at Lake Kirkpatrick and Lake Petén

Itzá may result from different catchments, where macroscopic charcoal represents local fire history, while MAs represent a more regional to supra-regional signal.

b) Burning conditions: Previous studies demonstrate that levoglucosan generation peaks at 250 °C and is not present in char above 350 °C (Kuo et al., 2008, 2011a). Macroscopic charcoal is generated at temperatures between 350-500 °C, which may explain the differences in both records. At Lake Petén Itzá low temperature fires and smouldering fires may prevail during the early Holocene and are not captured in the macroscopic charcoal records. This difference in fire types corroborates with the relatively open stands in the highlands where low intensity fire are favored.
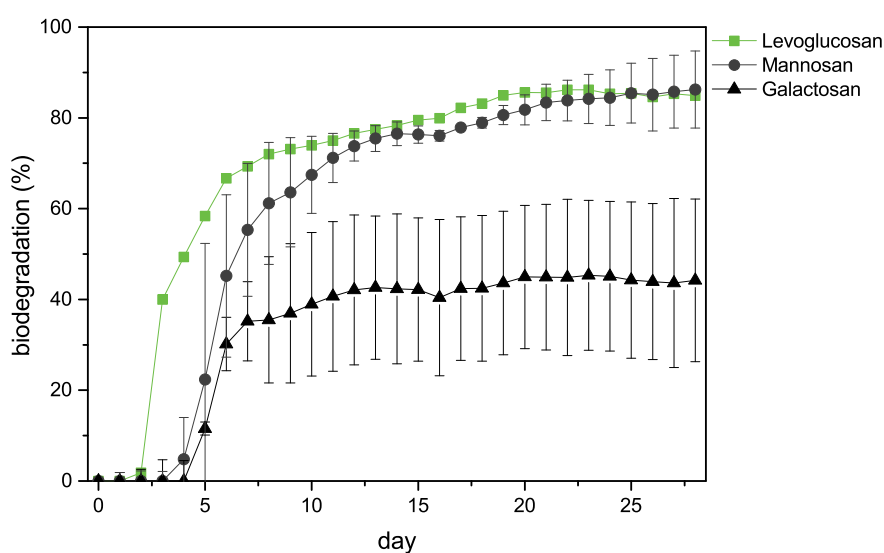


**Figure 4.4:** Results of the biodegradation test (%) of dissolved levoglucosan, mannosan and galactosan. Error bars corresponding to the standard deviation between the replicates of each compound.

c) Post-depositional behavior: The differing behavior of the MAs and charcoal may be related to different sedimentation and/or post-depositional processes, such as the redistribution of these materials by pore-water, potential biodegradation (Xie et al., 2006) or degradation processes depending on different sediment types and conditions. However, these post-depositional processes have not yet been tested. Biodegradability tests (Study IV) demonstrates that dissolved levoglucosan, mannosan and galactosan can degrade (Figure 4.4), corroborating previous findings by Norwood et al. (2013) for dissolved levoglucosan. This degradation requires specific microorganisms which catalyze the metabolism and are usable by the levoglucosan kinase (Xie et al., 2006; Yasui et al., 1991). Nevertheless, MAs are mainly deposited to the lake surface being attached to particles. This particle attachment may protect MAs from degradation in lake water and lake sediments, due to the reduced bioavailability. The literature assumes that this particle-bound deposition

enables the sedimentation of levoglucosan in lake sediment cores (Elias et al., 2001). This deposition style may explain the presence of MAs in ancient lake sediments samples in previous studies (Elias et al., 2001; Hopmans et al., 2013; Kuo et al., 2011b; Sikes et al., 2013). The results form Lake Petén Itzá and Lake Trasimeno demonstrate the occurrence of MAs over the entire core. All of these post-depositional processes may influence the MA concentrations over the entire core, but such declining trends were not observed at Lake Petén Itzá and Lake Trasimeno. In summary, MAs appear to be a suitable tool for reconstructing past fire history and may provide additional information about types of fires or spatial distribution in comparison of only macroscopic charcoal data. However, several aspects regarding post-depositional effects and catchments need to be further evaluated.

### 4.2.2 MAs and Fecal Sterols

In Study III, we applied a multi-proxy extraction method to analyze both levoglucosan and FeSt in the same samples. The suitability of these FeSt in paleoclimate records was already demonstrated by D'Anjou et al. (2012). Here the sum of CoP + e-Cop was used as a proxy of the local appearance of humans along the lakeshore and Stg as a proxy for livestock. The complex chronology of the lake sediment core allows only a detailed discussion of fluxes of levoglucosan, CoP + e-Cop and Stg fluxes in the upper 235 cm of the core (see Figure 4.5), corresponding to approximately the last 3100 cal yr BP. From 235 cm until the end of the core at 360 cm, sedimentation rates are low resulting in poor temporal resolution for this part of the core.

At Lake Trasimeno levoglucosan does not correlate with any of the FeSt ($r^2 < 0.4$), suggesting that regional fire activity may not be primarily influenced by the local occupation of the lake. In contrast CoP, Stg and e-CoP ($r^2 \geq 0.7$, p = 0.01) are significantly correlated suggesting that livestock occurrence at Lake Trasimeno coincides with the appearance of humans at the lake. Archeological excavations demonstrate that the lake was already occupied during the late Bronze Age ~3000 cal yr BP (Angelini et al., 2014), but archeological evidence of human occupation for the period between 3000 and 2500 cal yr BP are missing (Gambini, 1995). The highest fire activity occurs during this period, but FeSt did not significantly increase. Regional lake levels were low in the beginning of this period demonstrating drier climate conditions. Followed by increasing lake levels starting at around 2750 and reaching a maximum around 2500 (Magny et al., 2007, 2012; Vannière et al., 2008). Lake Trasimeno is mainly receiving water from precipitation, thus lake level increase may a result of colder temperatures or increase of precipitation. Although archeological evidence is missing at the lakeshore, humans might have influenced fire, thus the difference between the FeSt record and levoglucosan may be due to the following reasons: population density was too low for a significant measurable impact of FeSt in lake sediments, but slash-and-burn techniques for clearing land may produce a measurable signal of levoglucosan.

In addition, different transport mechanisms and catchment sizes also influence the concentrations of each type of proxy.
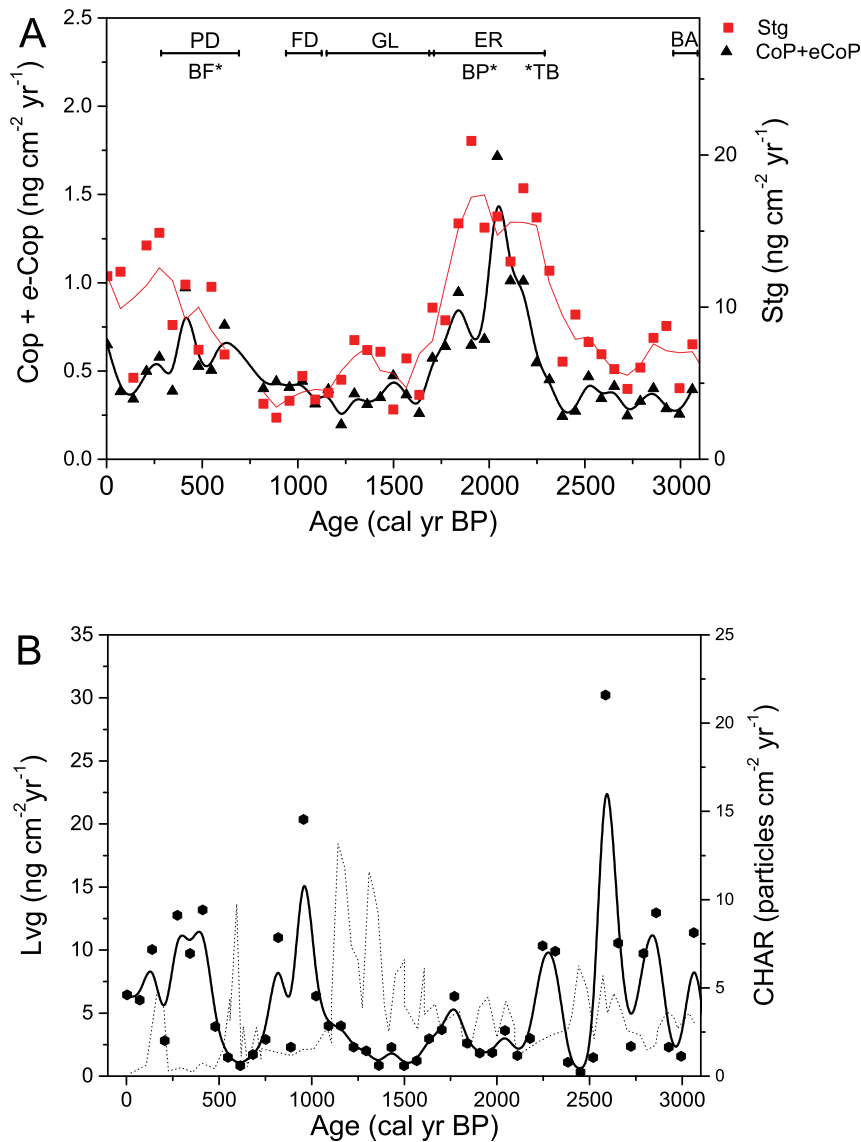


**Figure 4.5:** A: Cop+e-Cop (black triangles; black-line: 3-point moving average) and Stg fluxes (red squares; red-line: 3-point moving average). Historical periods are marked as followed: Late Bronze Age settlements (BA), Etruscan-Roman Period (ER), Gothic and Longobard Domination (GL), Franks Domination (FD); Perugia Domination (PD). Important events at Lake Trasimeno are marked with an asterisk: Battle of Trasimeno (TB), Perusine War (BP) and Braccio da Montone (BF). B: Levoglucosan (Lvg) fluxes (black dots; black line: 3-point moving average) at Lake Trasimeno. Dotted-line: Lake dell'Accesa macroscopic charcoal record (Vannière et al., 2008).

The Bronze Age settlements were on the opposite shores from the sampling location where the periodic high discharge of human feces into the lake coupled the low water depth led to sedimentation close to the source. In

general, run-off and direct discharge into the water body are the major sources of FeSt, where these markers thus represent a local signal.

The most prominent feature in the Lake Trasimeno record is a high flux of FeSt between 1750 cal yr BP and 2250 cal yr BP. This time period encompasses the Etruscan-Roman period, where Lake Trasimeno was an important military and economic center. During this period, two major battles took place at Lake Trasimeno: the famous battle of Trasimeno at 217 BC and the Perusine War around 40-42 BC. The military camp with approximately 40,000 soldiers was located at the northern shore close to the sampling location, and the input of human fecal matter may have significantly increased during this period creating the high fluxes of Cop + e-Cop.

Interestingly, during this period when the FeSt fluxes are at their peak in Lake Trasimeno sediments, fire activity is lower than in previous and later periods. This difference in human habitation and fire activity markers may be a result of changes in the agricultural practices and silviculture of the Romans where they may have actively suppressed the natural fire activity. Such fire suppression includes removing dead biomass from the forests (Tinner et al., 1998), or else open-land vegetation already existed and land clearance by fire was no longer necessary. The fact that the Roman period does not always linked to an increase in fire activity is also evident in different charcoal records: e.g. Lake dell'Accesa (Colombaroli et al., 2007; Vannière et al., 2008). Between 750-1650 cal yr BP, CoP + e-CoP fluxes decrease as a result of a general depopulation during this period (Di Pasquale et al., 2014). The Lake Trasimeno fire peaks at ca. 1000 cal yr BP; these occurs slightly earlier (1100-1400 cal yr BP) at Lake dell'Accesa and Lake Massaccioli (Colombaroli et al., 2007; Vannière et al., 2008). During this peak fire activity, high Lake Trasimeno levels flooded agricultural land, necessitating opening new land for breeding and cultivation. A final increase of the levoglucosan flux around 500 cal yr BP until 300 cal yr may correspond to the medieval increase of the population on local and regional scale, as evident from ancient maps (Gambini et al. 1995 and references within).

Summarizing, these first results from Lake Trasimeno suggest the suitability of the multi-extraction method for analyzing FeSt and levoglucosan. As previously mentioned, mannosan and galactosan were not quantified in this study. The results suggest that the additional analyses of FeSt can provide information about human impacts and livestock on the aquatic system. It has to be considered that the catchment areas of these compounds are quite local, where in contrast levoglucosan represents a more regional fire proxy. The combination of MAs and FeSt and the possibility of using different analytical techniques provide a wide range of additional organic molecular markers to interpretations of lake sediments.

## 4.3 MA Isomer Ratios

Levoglucosan, mannosan and galactosan emissions during biomass burning depend on vegetation type, burning conditions and temperature (Alves et al., 2010; Fabbri et al., 2009; Kuo et al., 2011b). The change in the relative abundance of the different isomers and the ratios between levoglucosan/mannosan (L/M) and levoglucosan/(mannosan + galactosan) (L/(M+G)) may track changes in past burned fuel. One major problem is that these ratios obtained from burning experiments overlap for the three main fuel types: softwood, hardwood and grass which therefore may limit conclusions about the burned fuel (Engling et al., 2006; Fabbri et al., 2009; Kuo et al., 2011a; Oros and Simoneit, 2001a,b; Oros et al., 2006). Engling et al. (2006) detected a ratio of 108 for Savannah Grass and 27 for hardwood (Oak); and Oros and Simoneit (2001a,b) reported L/M ratios between 2-33 (average 14) for different types of grasses and 0.2-8.2 (average 4.9) for different types of hardwood. Ratios depending on the fire temperature were reported by Kuo et al. (2011a) with cordgrass L/M ratio of 33 and honey mesquite (hardwood) ratio of 4.4 at the levoglucosan maximum production temperatures (250 °C). Grasses in all of these studies have higher L/M and L/(M+G) ratios compared to hardwood and we therefore assumed that this tendency to higher ratios may indicate more burned grass. At Lake Kirkpatrick and Lake Petén Itzá these ratios were compared to the local vegetation history inferred from pollen data.

**Table 4.1:** Lake Kirkpatrick ratios of levoglucosan/mannosan (L/M) and levoglucosan/(mannosan + galactosan) L/(M + G).

| depth (cm) | L/M | L/(M + G) |
|---|---|---|
| 75 | 2.2 | 1.4 |
| 76 | 2.0 | 1.3 |
| 78 | 1.9 | 1.2 |
| 80 | 1.9 | 1.1 |
| 83 | 2.1 | 1.2 |
| 119 | 1.3 | 1.0 |
| 120 | 1.0 | 0.7 |
| 121 | 1.0 | 0.8 |
| 122 | 2.4 | 1.5 |
| 127 | 1.4 | 0.9 |
| 128 | 0.9 | 0.4 |
| 129 | 1.1 | 0.6 |

Lake Kirkpartrick has L/M and L/(M+G) ratios of 1.3 ±0.5 and 0.9 ±0.3 in the deeper samples (Table 4.1) where the primary vegetation was mainly hardwood with beech (*Nothofagus spp.*) and podocarp forests (McWethy et al., 2009). The ratios increase to 2.0 ±0.1 and 1.3 ±0.1 where a shift to more grassy vegetation was observed with bracken (*Pteridium*), native grasses (*Poaceae*) and shrubs (*Coriaria* and *Coprosma spp.*). This shift was assumed as a result of the deforestation in this region (McWethy et al., 2009). This change in

pollen types corresponds with the assumption that higher ratios represent grass-dominated fuel.

The changes in the isomer ratios at Lake Petén Itzá are depicted in third panel of Figure 4.3. In addition the pollen records based on the data from Wahl et al. (2006) are shown in panel 4 and 5. The period of stable MA ratios (1.2 (L/M) and 0.9 (L/(G+M))) corresponds to a stable vegetation which began around 8000 cal yr BP, although fire activity was changing during this period. During the mid-Holocene (~7-4.5 kyr BP) climate conditions (temperature and moisture) were relatively stable in Central America. The vegetation in Petén slowly shifted to more open, savannah-like type (Islebe et al., 1996; Leyden, 2002; Wahl et al., 2006) which may explain the slight increase in the MA ratios (L/M: from 2.7 to 3.2, L/(M+G): from 1.0 to 1.4) during this period. Finally, the rapid shift in vegetation from a tree-dominated landscape to primarily herbs and shrubs occurring at circa 3000 cal yr BP coincides with increased L/M and L/(M+G) ratios (L/M up to 5.2, L/(M+G) up to 1.7) and may result from enhanced burning of grass-like vegetation. This increase of the relative abundance of burned grass is also evident in charcoal morphotype analyses, with an increase in the amount of grass charcoal in the total charcoal during the same period (Figure 4.3 Panel 1).

Post-depositional effects on these MA ratios is still unknown and a different biodegradation behaviour was observed for galactosan, which may limit application of L/(M+G) ratios; but results of the galactosan biodegradation test were out of the validation range and have to be confirmed in future studies. Nevertheless, the results from Lake Kirkpatrick and Lake Petén Itzá suggest that these ratios may be a promising tool to reconstruct general changes in the primary burned vegetation (grass vs. wood), thereby increasing the value of using these molecular markers in paleofire reconstructions.

## 4.4 Advantages and Limitations

The advantages of analyzing levoglucosan, mannosan and galactosan is that MAs can provide additional information of past fire history and improve our understanding of fire dynamics. MAs in combination with macroscopic charcoal records can provide fire histories on different spatial scales. The catchments of MAs may be regional to supra regional, but this idea needs to be further evaluated by e.g. comparison with microcharcoal and pollen data. MAs may also provide information on low temperature fires, which may be not covered by macroscopic charcoal records, because higher temperatures are necessary for charcoal generation. Another advantage of using MAs is the fact that their is ratios successfully track past burned fuel types and may be used in future studies to reconstruct general changes in fuel.

Although the results demonstrate the suitability of MAs as a proxy of biomass burning, there are still limiting factors which need to be addressed in future investigations. The post-depositional behavior of MAs is still unknown and the biodegradation test demonstrated that, at a minimum, dissolved MAs can degrade. The

stability and depositional behavior of MAs attached to particles needs to be evaluated. It is still unclear if different sediment types or lake water conditions can influence MA concentrations.

HPAEC-MS with ASE extraction is a suitable and sensitive method to detect the three isomers levoglucosan, mannosan and galactosan in lake sediment samples. However, sample preparation and extraction is quite time consuming, and the method was initially limited to the analyses of only the three MAs. In order to distinguish if biomass burning is driven by human or changing climate conditions, the MAs were included in a multi-proxy extraction method, which includes the analyses fecal sterols from humans and grassing mammals (CoP + e-Cop and Stg). Separation in different fraction allows the application of different analytical techniques (e.g. GC-MS and HPAEC-MS). Although the extraction method did not allow the quantification of mannosan and galactosan, the analyses of the Lake Trasimeno core demonstrate the suitability of the method. The advantage of this multi-extraction method is that a wide range of proxies may be included (e.g. other combustion markers such as polycyclic aromatic hydrocarbons). The combination of several organic molecular markers in one extraction method will allow reducing the time of sample preparation while increasing the information obtained from any single sample.

# 5 Conclusions and Outlook

We successfully developed a HPAEC-MS method to analyze levoglucosan, mannosan and galactosan in lake sediment samples and applied this method on three different sets of lake sediment samples. The work includes the first Holocene fire histories using these novel methods. Results from Lake Petén Itzá and Lake Trasimeno demonstrate the suitability of MAs a specific molecular marker for biomass burning in lake sediments. In combination with macroscopic charcoal, MAs analyses have the potential to reconstruct fire history on different spatial scales and may indicate the influence of low temperature fires. We also successfully reconstructed changes in burned fuel types by calculating MA ratios, which may help estimate changes in burned fuel. The results demonstrate promising correlations between pollen assemblages and MAs, thereby adding an additional value to the analyses of MAs in paleofire science. Finally, we included MAs in a multi-proxy extraction method, where analyzing multiple proxies from a single extraction helps both reduce sample preparation time and increase the number of proxies analyzed from single sediment samples. The analyses of specific molecular markers and the analyses of additional proxies such as fecal sterols may help to discriminate if fire is human or climate driven. Initial levoglucosan and fecal sterol results from Lake Trasimeno are promising, but this combination of proxies has to be further evaluated in future studies.

Several uncertainties still exist, including the stability of the compounds, post-depositional behavior, and more detailed catchment estimates and future research has to reduce these uncertainties. Nevertheless, the results from this research demonstrates that the organic molecular markers levoglucosan, mannosan and galactosan have the potential to be an additional tool in paleofire reconstruction, by providing fire information on different scales and changing fuel types. The inclusion of levoglucosan in a multi-proxy extraction method makes it possible to analyze a wide range of proxies and may improve the interpretation of drivers and sources of biomass burning.

# 6 References

Ali, A. A., Higuera, P. E., Bergeron, Y., Carcaillet, C., 2009. Comparing fire-history interpretations based on area, number and estimated volume of macroscopic charcoal in lake sediments. Quaternary Research 72 (3), 462–468.

Alves, C. A., Gonçalves, C., Evtyugina, M., Pio, C. A., Mirante, F., Puxbaum, H., 2010. Particulate organic compounds emitted from experimental wildland fires in a Mediterranean ecosystem. Atmospheric Environment 44 (23), 2750–2759.

Angelini, P., De Angelis, M. C., Guerzoni, R. P., Gigante, D., Rubini, A., Properzi, A., Venanzoni, R., 2014. Wood identification of pile dwellings from the Bronze Age San Savino site (Lake Trasimeno, central Italy). Plant Biosystems 148 (February), 713–722.

Anselmetti, F., Ariztegui, D., Hodell, D., Hillesheim, M., Brenner, M., Gilli, A., McKenzie, J., Mueller, A., 2006. Late Quaternary climate-induced lake level variations in Lake Petén Itzá, Guatemala, inferred from seismic stratigraphic analysis. Palaeogeography, Palaeoclimatology, Palaeoecology 230 (1-2), 52–69.

Bae, M.-S., Lee, J. Y., Kim, Y.-P., Oak, M.-H., Shin, J.-S., Lee, K.-Y., Lee, H., Lee, S. Y., Kim, Y.-J., 2012. Analytical methods of levoglucosan, a tracer for cellulose in biomass Burning, by four different techniques. Asian Journal of Atmospheric Environment 6 (March), 53–66.

Barbaro, E., Kirchgeorg, T., Zangrando, R., Vecchiato, M., Piazza, R., Barbante, C., 2015. Sugars in Antarctic Aerosol. Atmospheric Environment, under review.

Beffa, G., 2012. Analysis of sediment charcoal morphotypes from Gorgo Basso (Sicily). Bachelor thesis, Universtity of Bern.

Birks, H. H., Birks, H. J. B., 2006. Multi-proxy studies in palaeolimnology. Vegetation History and Archaeobotany 15 (4), 235–251.

Blarquez, O., Vannière, B., Marlon, J. R., Daniau, A.-L., Power, M. J., Brewer, S., Bartlein, P. J., 2014. Computers & Geosciences paleofire : An R package to analyse sedimentary charcoal records from the Global Charcoal Database to reconstruct past biomass burning. Computers and Geosciences 72, 255–261.

Bowman, D. M. J. S., Balch, J., Artaxo, P., Bond, W. J., Cochrane, M. a., D'Antonio, C. M., Defries, R., Johnston, F. H., Keeley, J. E., Krawchuk, M. a., Kull, C. a., Mack, M., Moritz, M. a., Pyne, S., Roos, C. I., Scott, A. C., Sodhi, N. S., Swetnam, T. W., 2011. The human dimension of fire regimes on Earth. Journal of Biogeography 38 (12), 2223–2236.

Bowman, D. M. J. S., Balch, J. K., Artaxo, P., Bond, W. J., Carlson, J. M., Cochrane, M. a., D'Antonio, C. M., Defries, R. S., Doyle, J. C., Harrison, S. P., Johnston, F. H., Keeley, J. E., Krawchuk, M. a., Kull, C. a., Marston, J. B., Moritz, M. a., Prentice, I. C., Roos, C. I., Scott, A. C., Swetnam, T. W., van der Werf, G. R., Pyne, S. J., 2009. Fire in the Earth system. Science 324 (5926), 481–484.

Carcaillet, C., Ali, A. A., Blarquez, O., Genries, A., Mourier, B., Bremond, L., 2009. Spatial variability of fire history in subalpine forests: From natural to cultural regimes. Ecoscience 16 (1), 1–12.

Carcaillet, C., Bouvier, M., Fréchette, B., Larouche, A., Richard, P., 2001. Comparison of pollen-slide and sieving methods in lacustrine charcoal analyses for local and regional fire history. The Holocene.

Clark, J., Lynch, J., Stocks, B., Goldammer, J., 1998. Relationships between charcoal particles in air and sediments in west-central Siberia. The Holocene 8 (1), 19–29.

Clark, J. S., 1988. Particle motion and the theory of charcoal analysis: Source area, transport, deposition, and sampling. Quaternary Research 30 (1), 67–80.

Clark, J. S., Hussey, T. C., 1996. Estimating the mass flux of charcoal from sedimentary records: effects of particle size, morphology, and orientation. The Holocene 6 (2), 129–144.

Colombaroli, D., Marchetto, A., Tinner, W., 2007. Long-term interactions between Mediterranean climate, vegetation and fire regime at Lago di Massaciuccoli (Tuscany, Italy). Journal of Ecology 95 (4), 755–770.

Colombaroli, D., Ssemmanda, I., Gelorini, V., Verschuren, D., 2014. Contrasting long-term records of biomass burning in wet and dry savannas of equatorial East Africa. Global Change Biology 20 (9), 2903–14.

Conedera, M., Tinner, W., Neff, C., Meurer, M., Dickens, A. F., Krebs, P., 2009. Reconstructing past fire regimes: methods, applications, and relevance to fire management and conservation. Quaternary Science Reviews 28 (5-6), 555–576.

Correa-Metrio, A., Bush, M. B., Cabrera, K. R., Sully, S., Brenner, M., Hodell, D. a., Escobar, J., Guilderson, T., 2012. Rapid climate change and no-analog vegetation in lowland Central America during the last 86,000 years. Quaternary Science Reviews 38, 63–75.

Crawford, A. J., Belcher, C. M., 2014. Charcoal morphometry for paleoecological analysis: The effects of fuel type and transportation on morphological parameters. Applications in Plant Sciences 2 (8), 1–10.

Crutzen, P. J., 2002. Geology of mankind. Nature 415 (6867), 23.

Curtis, J. H., Brenner, M., Hodell, D. A., Balser, R. A., Islebe, G. A., Hooghiemstra, H., 1998. A multi-proxy study of Holocene environmental change in the Maya Lowlands of Peten, Guatemala. Journal of Paleolimnology 19 (2), 139–159.

Daniau, A. L., Bartlein, P. J., Harrison, S. P., Prentice, I. C., Brewer, S., Friedlingstein, P., Harrison-Prentice, T. I., Inoue, J., Izumi, K., Marlon, J. R., Mooney, S., Power, M. J., Stevenson, J., Tinner, W., Andrič, M., Atanassova, J., Behling, H., Black, M., Blarquez, O., Brown, K. J., Carcaillet, C., Colhoun, E. a., Colombaroli, D., Davis, B. a. S., D'Costa, D., Dodson, J., Dupont, L., Eshetu, Z., Gavin, D. G., Genries, A., Haberle, S., Hallett, D. J., Hope, G., Horn, S. P., Kassa, T. G., Katamura, F., Kennedy, L. M., Kershaw, P., Krivonogov, S., Long, C., Magri, D., Marinova, E., McKenzie, G. M., Moreno, P. I., Moss, P., Neumann, F. H., Norstrm, E., Paitre, C., Rius, D., Roberts, N., Robinson, G. S., Sasaki, N., Scott, L., Takahara, H., Terwilliger, V., Thevenon, F., Turner, R., Valsecchi, V. G., Vannière, B., Walsh, M., Williams, N., Zhang, Y., 2012. Predictability of biomass burning in response to climate changes. Global Biogeochemical Cycles 26 (4), 1–12.

D'Anjou, R. M., Bradley, R. S., Balascio, N. L., Finkelstein, D. B., 2012. Climate impacts on human settlement and agricultural activities in northern Norway revealed through sediment biogeochemistry. PNAS 109 (50), 20332–7.

Davis, M. B., 1963. On the theory of pollen analysis. American Journal of Science 261 (10), 897–912.

Di Pasquale, G., Buonincontri, M. P., Allevato, E., Saracino, A., 2014. Human-derived landscape changes on the northern Etruria coast (western Italy) between Roman times and the late Middle Ages. The Holocene 24 (11), 1491–1502.

Elias, V. O., Simoneit, B. R. T., Cordeiro, R. C., Turcq, B., 2001. Evaluating levoglucosan as an indicator of biomass burning in Carajás, Amazonia: A comparison to the charcoal record. Geochimica et Cosmochimica Acta 65 (2), 267–272.

Enache, M. D., Cumming, B. F., 2007. Charcoal morphotypes in lake sediments from British Columbia (Canada): An assessment of their utility for the reconstruction of past fire and precipitation. Journal of Paleolimnology 38 (3), 347–363.

Engling, G., Carrico, C. M., Kreidenweis, S. M., Collett, J. L., Day, D. E., Malm, W. C., Lincoln, E., Min Hao, W., Iinuma, Y., Herrmann, H., 2006. Determination of levoglucosan in biomass combustion aerosol by high-performance anion-exchange chromatography with pulsed amperometric detection. Atmospheric Environment 40, 299–311.

Engling, G., Lee, J. J., Tsai, Y.-W., Lung, S.-C. C., Chou, C. C.-K., Chan, C.-Y., 2009. Size-Resolved Anhydrosugar Composition in Smoke Aerosol from Controlled Field Burning of Rice Straw. Aerosol Science and Technology 43 (7), 662–672.

Fabbri, D., Torri, C., Simoneit, B. R. T., Marynowski, L., Rushdi, A. I., FabiaÅska, M. J., 2009. Levoglucosan and other cellulose and lignin markers in emissions from burning of Miocene lignites. Atmospheric Environment 43 (14), 2286–2295.

Fischer, H., Behrens, M., Bock, M., Richter, U., Schmitt, J., Loulergue, L., Chappellaz, J., Spahni, R., Blunier, T., Leuenberger, M., Stocker, T. F., 2008. Changing boreal methane sources and constant biomass burning during the last termination. Nature 452 (7189), 864–867.

Gambaro, A., Zangrando, R., Gabrielli, P., Barbante, C., Cescon, P., 2008. Direct determination of levoglucosan at the picogram per milliliter level in antarctic ice by high-performance liquid chromatography/electrospray ionization triple quadrupole mass spectrometry. Analytical Chemistry 80 (5), 1649–1655.

Gambini, E., 1995. Le oscillazioni di Livello del Lago Trasimeno. Museo della Pesca, Grafiche Piemme S.n.c., Perugia.

Glikson, A., 2013. Fire and human evolution: The deep-time blueprints of the Anthropocene. Anthropocene 3 (2013), 89–92.

González-Pérez, J. A., González-Vila, F. J., Almendros, G., Knicker, H., 2004. The effect of fire on soil organic matter–a review. Environment International 30 (6), 855–870.

Hennigan, C. J., Sullivan, A. P., Collett, J. L., Robinson, A. L., 2010. Levoglucosan stability in biomass burning particles exposed to hydroxyl radicals. Geophysical Research Letters 37 (9), L09806.

Hillesheim, M. B., Hodell, D. A., Leyden, B. W., Brenner, M., Curtis, J. H., Anselmetti, F. S., Ariztegui, D., Buck, D. G., Guilderson, T. P., Rosenmeier, M. F., Schnurrenberger, D. W., 2005. Climate change in lowland central America during the late deglacial and early Holocene. Journal of Quaternary Science 20 (4), 363–376.

Hoffmann, D., Tilgner, A., Iinuma, Y., Herrmann, H., 2010. Atmospheric stability of levoglucosan: A detailed laboratory and modeling study. Environmental Science and Technology 44 (2), 694–699.

Hopmans, E. C., dos Santos, R. A. L., Mets, A., Sinninghe Damsté, J. S., Schouten, S., 2013. A novel method for the rapid analysis of levoglucosan in soils and sediments. Organic Geochemistry 58, 86–88.

Huntley, B., Webb III, T., 1988. Vegetation history, 1st Edition. Kluwer Academic Publishers.

Iglesias, V., Whitlock, C., 2014. Fire responses to postglacial climate change and human impact in northern Patagonia (41-43°S). Proceedings of the National Academy of Sciences 111 (51), 5545–5554.

Islebe, G. A., Hooghiemstra, H., Brenner, M., Curtis, J. H., Hodell, D. A., 1996. A Holocene vegetation history from lowland Guatemala. The Holocene 6 (3), 265–271.

Jensen, K., Lynch, E. a., Calcote, R., Hotchkiss, S. C., 2007. Interpretation of charcoal morphotypes in sediments from Ferry Lake, Wisconsin, USA: Do different plant fuel sources produce distinctive charcoal morphotypes? The Holocene 17 (7), 907–915.

Kehrwald, N., Zangrando, R., Gabrielli, P., Jaffrezo, J. L., Boutron, C., Barbante, C., Gambaro, A., 2012. Levoglucosan as a specific marker of fire events in Greenland snow. Tellus, Series B: Chemical and Physical Meteorology 64 (1), 1–9.

Kitamura, Y., Abe, Y., Yasui, T., 1991. Metabolism of Levoglucosan (1,6-Anhydro-beta-D-glucopyranose) in Microorganisms. Agriculture Biological Chemistry 55 (2), 515–521.

Kloster, S., Brücher, T., Brovkin, V., Wilkenskjeld, S., 2014. Controls on fire activity over the Holocene. Climate of the Past Discussions 10 (6), 4257–4275.

Kuo, L. J., Herbert, B. E., Louchouarn, P., 2008. Can levoglucosan be used to characterize and quantify char/charcoal black carbon in environmental media? Organic Geochemistry 39 (10), 1466–1478.

Kuo, L. J., Louchouarn, P., Herbert, B. E., 2011a. Influence of combustion conditions on yields of solvent-extractable anhydrosugars and lignin phenols in chars: Implications for characterizations of biomass combustion residues. Chemosphere 85 (5), 797–805.

Kuo, L. J., Louchouarn, P., Herbert, B. E., Brandenberger, J. M., Wade, T. L., Crecelius, E., 2011b. Combustion-derived substances in deep basins of Puget Sound: Historical inputs from fossil fuel and biomass combustion. Environmental Pollution 159 (4), 983–990.

Leng, M. J., Marshall, J. D., 2004. Palaeoclimate interpretation of stable isotope data from lake sediment archives. Quaternary Science Reviews 23 (7-8), 811–831.

Lentile, L. B., Holden, Z. A., Smith, A. M. S., Falkowski, M. J., Hudak, A. T., Morgan, P., Lewis, S. A., Gessler, P. E., Benson, N. C., 2006. Remote sensing techniques to assess active fire characteristics and post-fire effects. International Journal of Wildland Fire 15 (3), 319–345.

Leyden, B. W., 2002. Pollen evidence for climatic variability and cultural disturbance in the Maya lowlands. Ancient Mesoamerica 13 (1), 85–101.

Louchouarn, P., Kuo, L. J., Wade, T. L., Schantz, M., 2009. Determination of levoglucosan and its isomers in size fractions of aerosol standard reference materials. Atmospheric Environment 43 (35), 5630–5636.

Lozano-García, S., Sosa-Nájera, S., Sugiura, Y., Caballero, M., 2005. 23,000 yr of vegetation history of the Upper Lerma, a tropical high-altitude basin in Central Mexico. Quaternary Research 64 (1), 70–82.

Lozano-García, S., Torres-Rodríguez, E., Ortega, B., Vázquez, G., Caballero, M., 2013. Ecosystem responses to climate and disturbances in western central Mexico during the late Pleistocene and Holocene. Palaeogeography, Palaeoclimatology, Palaeoecology 370, 184–195.

Magny, M., de Beaulieu, J. L., Drescher-Schneider, R., Vannière, B., Walter-Simonnet, A. V., Miras, Y., Millet, L., Bossuet, G., Peyron, O., Brugiapaglia, E., Leroux, A., 2007. Holocene climate changes in the central Mediterranean as recorded by lake-level fluctuations at Lake Accesa (Tuscany, Italy). Quaternary Science Reviews 26 (13-14), 1736–1758.

Magny, M., Peyron, O., Sadori, L., Ortu, E., Zanchetta, G., Vannière, B., Tinner, W., 2012. Contrasting patterns of precipitation seasonality during the Holocene in the south- and north-central Mediterranean. Journal of Quaternary Science 27, 290–296.

Marlon, J. R., Bartlein, P. J., Daniau, A. L., Harrison, S. P., Maezumi, S. Y., Power, M. J., Tinner, W., Vanniére, B., 2013. Global biomass burning: A synthesis and review of Holocene paleofire records and their controls. Quaternary Science Reviews 65, 5–25.

McWethy, D. B., Whitlock, C., Wilmshurst, J. M., McGlone, M. S., Li, X., 2009. Rapid deforestation of South Island, New Zealand, by early Polynesian fires. The Holocene 19 (6), 883–897.

Medeiros, P. M., Simoneit, B. R. T., 2007. Analysis of sugars in environmental samples by gas chromatography-mass spectrometry. Journal of Chromatography A 1141 (2), 271–278.

Mooney, S., Tinner, W., 2011. The analysis of charcoal in peat and organic sediments. Mires and Peat 7 (9), 1–18.

Mueller, A. D., Islebe, G. A., Hillesheim, M. B., Grzesik, D. A., Anselmetti, F. S., Ariztegui, D., Brenner, M., Curtis, J. H., Hodell, D. A., Venz, K. A., 2009. Climate drying and associated forest decline in the lowlands of northern Guatemala during the late Holocene. Quaternary Research 71 (2), 133–141.

Mustaphi, C. J. C., Pisaric, M. F. J., 2014. A classification for macroscopic charcoal morphologies found in Holocene lacustrine sediments. Progress in Physical Geography 38 (6), 734–754.

Nicolussi, K., Kaufmann, M., Melvin, T. M., van der Plicht, J., Schiessling, P., Thurner, A., 2009. A 9111 year long conifer tree-ring chronology for the European Alps: a base for environmental and climatic investigations. The Holocene 19 (6), 909–920.

Norwood, M. J., Louchouarn, P., Kuo, L. J., Harvey, O. R., 2013. Characterization and biodegradation of water-soluble biomarkers and organic carbon extracted from low temperature chars. Organic Geochemistry 56 (2013), 111–119.

OECD, 1992. Guidelines for the Testing of Chemicals - Test No. 301: Ready Biodegradability.

Oros, D. R., Abas, M. R. B., Omar, N. Y. M. J., Rahman, N. A., Simoneit, B. R. T., 2006. Identification and emission factors of molecular tracers in organic aerosols from biomass burning: Part 3. Grasses. Applied Geochemistry 21 (6), 919–940.

Oros, D. R., Simoneit, B. R. T., 2001a. Identification and emission factors of molecular tracers in organic aerosols from biomass burning Part 1. Temperate climate conifers. Applied Geochemistry 16 (13), 1513–1544.

Oros, D. R., Simoneit, B. R. T., 2001b. Identification and emission factors of molecular tracers in organic aerosols from biomass burning Part 2 . Deciduous trees. Applied Geochemistry 16 (13), 1545–1565.

Piot, C., Jaffrezo, J.-L., Cozic, J., Pissot, N., El Haddad, I., Marchand, N., Besombes, J.-L., 2012. Quantification of levoglucosan and its isomers by high performance liquid chromatography-electrospray ionization tandem mass spectrometry and its applications to atmospheric and soil samples. Atmospheric Measurement Techniques 5 (1), 141–148.

Piperno, D. R., 2006. Quarternary environmental histroy and agricultural impact on vegetation in Central America. Annals of the Missouri Botanical Garden 93 (2), 274–296.

Power, M. J., Marlon, J., Ortiz, N., Bartlein, P. J., Harrison, S. P., Mayle, F. E., Ballouche, A., Bradshaw, R. H. W., Carcaillet, C., Cordova, C., Mooney, S., Moreno, P. I., Prentice, I. C., Thonicke, K., Tinner, W., Whitlock, C., Zhang, Y., Zhao, Y., Ali, a. a., Anderson, R. S., Beer, R., Behling, H., Briles, C., Brown, K. J., Brunelle, A., Bush, M., Camill, P., Chu, G. Q., Clark, J., Colombaroli, D., Connor, S., Daniau, A. L., Daniels, M., Dodson, J., Doughty, E., Edwards, M. E., Finsinger, W., Foster, D., Frechette, J., Gaillard, M. J., Gavin, D. G., Gobet, E., Haberle, S., Hallett, D. J., Higuera, P., Hope, G., Horn, S., Inoue, J., Kaltenrieder, P., Kennedy, L., Kong, Z. C., Larsen, C., Long, C. J., Lynch, J., Lynch, E. a., McGlone, M., Meeks, S., Mensing, S., Meyer, G., Minckley, T., Mohr, J., Nelson, D. M., New, J., Newnham, R., Noti, R., Oswald, W., Pierce, J., Richard, P. J. H., Rowe, C., Sanchez Goñi, M. F., Shuman, B. N., Takahara, H., Toney, J., Turney, C., Urrego-Sanchez, D. H., Umbanhowar, C., Vandergoes, M., Vanniere, B., Vescovi, E., Walsh, M., Wang, X., Williams, N., Wilmshurst, J., Zhang, J. H., 2008. Changes in fire regimes since the last glacial maximum: An assessment based on a global synthesis and analysis of charcoal data. Climate Dynamics 30 (7-8), 887–907.

Power, M. J., Marlon, J. R., Bartlein, P. J., Harrison, S. P., 2010. Fire history and the global charcoal database: A new tool for hypothesis testing and data exploration. Palaeogeography, Palaeoclimatology, Palaeoecology 291 (1-2), 52–59.

Power, M. J., Mayle, F. E., Bartlein, P. J., Marlon, J. R., Anderson, R. S., Behling, H., Brown, K. J., Carcaillet, C., Colombaroli, D., Gavin, D. G., Hallett, D. J., Horn, S. P., Kennedy, L. M., Lane, C. S., Long, C. J., Moreno, P. I., Paitre, C., Robinson, G., Taylor, Z., Walsh, M. K., 2012. Climatic control of the biomass-burning decline in the Americas after AD 1500. The Holocene August 14, 1–11.

Randerson, J. T., Liu, H., Flanner, M. G., Chambers, S. D., Jin, Y., Hess, P. G., Pfister, G., Mack, M. C., Treseder, K. K., Welp, L. R., Chapin, F. S., Harden, J. W., Goulden, M. L., Lyons, E., Neff, J. C., Schuur, E. A. G., Zender, C. S., 2006. The impact of boreal forest fire on climate warming. Science 314 (5802), 1130–1132.

Rosenmeier, M. F., Hodell, D. A., Brenner, M., Curtis, J. H., Guilderson, T. P., 2002. A 4000-Year Lacustrine Record of Environmental Change in the Southern Maya Lowlands, Petén, Guatemala. Quaternary Research 57 (2), 183–190.

Ruddiman, W., 2003. The anthropogenic greenhouse era began thousands of years ago. Climatic Change 61, 261–293.

Saarnio, K., Teinilä, K., Aurela, M., Timonen, H., Hillamo, R., 2010. High-performance anion-exchange chromatography-mass spectrometry method for determination of levoglucosan, mannosan, and galactosan in atmospheric fine particulate matter. Analytical and Bioanalytical Chemistry 398 (5), 2253–2264.

Savarino, J., Legrand, M., 1998. High northern latitude forest fires and vegetation emissions over the last millennium inferred from the chemistry of a central Greenland ice core. Journal of Geophysical Research 103 (D7), 8267.

Schkolnik, G., Rudich, Y., 2006. Detection and quantification of levoglucosan in atmospheric aerosols: a review. Analytical and bioanalytical chemistry 385 (1), 26–33.

Sikes, E. L., Medeiros, P. M., Augustinus, P., Wilmshurst, J. M., Freeman, K. R., 2013. Seasonal variations in aridity and temperature characterize changing climate during the last deglaciation in New Zealand. Quaternary Science Reviews 74, 245–256.

Simoneit, B., 2002. Biomass burning–a review of organic tracers for smoke from incomplete combustion. Applied Geochemistry 17, 129–162.

Tinner, W., Ammann, B., Conedera, M., Gaggeler, H., Sagesser, B., Gedye, S., Jones, R., 1998. Pollen and charcoal in lake sediments compared with historically documented forest fires in southern Switzerland since AD 1920. The Holocene 8 (1), 31–42.

Tinner, W., Hofstetter, S., Zeugin, F., Conedera, M., Wohlgemuth, T., Zimmermann, L., Zweifel, R., 2006. Long-distance transport of macroscopic charcoal by an intensive crown fire in the Swiss Alps-implications for fire history reconstruction. The Holocene 16 (2), 287–292.

Umbanhowar Jr., C., McGrath, M., 1998. Experimental production and analysis of microscopic charcoal from wood, leaves and grasses. The Holocene 8 (3), 341–346.

Vannière, B., Colombaroli, D., Chapron, E., Leroux, A., Tinner, W., Magny, M., 2008. Climate versus human-driven fire regimes in Mediterranean landscapes: the Holocene record of Lago dell'Accesa (Tuscany, Italy). Quaternary Science Reviews 27 (11-12), 1181–1196.

Wahl, D., Byrne, R., Schreiner, T., Hansen, R., 2006. Holocene vegetation change in the northern Peten and its implications for Maya prehistory. Quaternary Research 65 (3), 380–389.

Whitlock, C., Bartlein, P. J., 2003. Holocene fire activity as a record of past environmental change. Developments in Quaternary Science 1 (1986), 479–490.

Whitlock, C., Larsen, C., 2001. Charcoal as a fire proxy. In: Tracking environmental change using lake sediments-Vol 3 Terrestrial algal and siliceous indicators. Vol. 3. pp. 75–97.

Xie, H., Zhuang, X., Bai, Z., Qi, H., Zhang, H., 2006. Isolation of levoglucosan-assimilating microorganisms from soil and an investigation of their levoglucosan kinases. World Journal of Microbiology and Biotechnology 22 (9), 887–892.

Yao, P., Schwab, V. F., Roth, V. N., Xu, B., Yao, T., Gleixner, G., 2013. Levoglucosan concentrations in ice-core samples from the Tibetan Plateau determined by reverse-phase high-performance liquid chromatography-mass spectrometry. Journal of Glaciology 59 (216), 599–612.

Yasui, T., Kitamura, Y., Nakahara, K., Abe, Y., 1991. Short Communication Metabolism of Levoglucosan. Journal of Agricultural Chemistry 55 (7), 1927–1929.

Zangrando, R., Barbaro, E., Zennaro, P., Rossi, S., Kehrwald, N. M., Gabrieli, J., Barbante, C., Gambaro, A., 2013. Molecular markers of biomass burning in Arctic aerosols. Environmental Science and Technology 47 (15), 8565–8574.

Zennaro, P., Kehrwald, N., Mcconnell, J. R., Schüpbach, S., Maselli, O., 2014. Fire in ice: two millennia of Northern Hemisphere fire history from the Greenland NEEM ice core. Climate of the Past 10, 809–857.

# B Cumulative Thesis

# 1 Study I: Method for the determination of specific molecular markers of biomass burning in lake sediments

T. Kirchgeorg[a,b], S. Schüpbach[a,c], N. Kehrwald[a] D. B. McWethy[d] and C. Barbante[a,e,f]

[a]Department of Environmental Sciences, Informatics and Statistics, University Ca' Foscari, Venice, 30123, Italy

[b]Institute of Sustainable and Environmental Chemistry, Leuphana University of Lüneburg, Lüneburg, 21335, Germany

[c]Climate and Environmental Physics, Physics Institute, and Oeschger Centre for Climate Change Research, University of Bern, Bern, 3012, Switzerland

[d]Department of Earth Sciences, Montana State University, Bozeman, MT 59717, United States

[D]Institute for the Dynamics of Environmental Processes-CNR, Venice, 30123 Italy

ELSEVIER

CrossMark

# Method for the determination of specific molecular markers of biomass burning in lake sediments

T. Kirchgeorg [a,b,*], S. Schüpbach [a,c], N. Kehrwald [a], D.B. McWethy [d], C. Barbante [a,e,f]

[a] Department of Environmental Sciences, Informatics and Statistics, University Ca'Foscari, Venice 30123, Italy
[b] Institute of Sustainable and Environmental Chemistry, Leuphana University of Lüneburg, Lüneburg 21335, Germany
[c] Climate and Environmental Physics, Physics Institute, and Oeschger Centre for Climate Change Research, University of Bern, Bern 3012, Switzerland
[d] Department of Earth Sciences, Montana State University, Bozeman, MT 59717, USA
[e] Institute for the Dynamics of Environmental Processes-CNR, Venice 30123, Italy
[f] Centro Linceo B. Segre, Accademia Nazionale dei Lincei, Rome 00165, Italy

## ARTICLE INFO

## ABSTRACT

Fire has an influence on regional to global atmospheric chemistry and climate. Molecular markers of biomass burning archived in lake sediments are becoming increasingly important in paleoenvironmental reconstruction and may help determine the interaction between climate and fire activity. Here, we present a high performance anion exchange chromatography–mass spectrometry method to allow separation and analysis of levoglucosan, mannosan and galactosan in lake sediments, with implications for reconstructing past biomass burning events. Determining mannosan and galactosan in Lake Kirkpatrick, New Zealand (45.03°S, 168.57°E) sediment cores and comparing these isomers with the more abundant biomass burning markers levoglucosan and charcoal represents a significant advancement in our ability to analyze past fire activity. Levoglucosan, mannosan and galactosan concentrations correlated significantly with macroscopic charcoal concentration. Levoglucosan/mannosan and levoglucosan/ (mannosan + galactosan) ratios may help determine not only when fires occurred, but also if changes in the primary burned vegetation occurred.

## 1. Introduction

Human activity has influenced fire regimes by changing fire ignition rate, the fuel, land use and land cover for millennia (Bowman et al., 2009). Data from natural archives, including sediment and ice cores, can help understand past fire activity over longer temporal and spatial scales. Charcoal data from lake sediment cores represent a well known proxy for biomass burning and are used to reconstruct past fire activity on a local scale to a regional scale (e.g. Whitlock and Larsen, 2001). Molecular markers of biomass burning archived in lake sediments are increasingly important in paleoenvironmental reconstruction and may help determine the interaction between climate and fire activity.

One group of biomass burning markers is the monosaccharide anhydrides (MAs) levoglucosan (1,6-anhydro-$\beta$-D-glucopyranose), mannosan (1,6-anhydro-$\beta$-D-mannopyrose) and galactosan (1,6-anhydro-$\beta$-D-galactopyranose), which are specific indicators of fire

activity. In contrast to polycyclic aromatic hydrocarbons, MAs are only generated by biomass burning at temperatures > 300 °C (Simoneit, 2002) and are present in combustion residues from biomass containing cellulose and hemicellulose. Due to their relatively low volatility they tend to be ab-/adsorbed to aerosols in the atmosphere.

Several studies have used MAs as specific markers for biomass burning in atmospheric aerosols (Simoneit and Elias, 2000; Jordan et al., 2006). While a proportion of levoglucosan can be degraded in the atmosphere, these compounds are still able to undergo long range atmospheric transport due to their stability and the significant emissions of levoglucosan during biomass burning (Fraser and Lakshmanan, 2000; Hoffmann et al., 2010). Snow pit and aerosol studies from remote Arctic regions demonstrate the applicability of levoglucosan as a fire activity tracer up to thousands of km from potential sources (Kehrwald et al., 2012; Zangrando et al., 2013). Zennaro et al. (2014) demonstrated the stability and suitability of levoglucosan as a proxy for biomass burning by analyzing a Greenland ice core covering the last two millennia.

In contrast to ice core and aerosol studies, the stability of MAs in lake sediments is not well known. Only in one laboratory

---

* Corresponding author at: Department of Environmental Sciences, Informatics and Statistics, University Ca'Foscari, Venice 30123, Italy. Tel.: +39 041 2348545.
*E-mail address:* kirchgeorg@unive.it (T. Kirchgeorg).

experiment was an aquatic half-life of 3–4 days for dissolved levoglucosan determined (Norwood et al., 2013). The capability of MAs to remain in lake and marine sediments for timescales as along as 20,000 yr (Elias et al., 2001; Kuo et al., 2011a; Hopmans et al., 2013) suggests that, at a minimum, the levoglucosan entrapped in particles deposited on the lake surface should be stable in the aquatic phase and incorporated into sediments (Elias et al., 2001).

The literature demonstrates methods for determining MAs in different matrixes using gas chromatography–mass spectrometry (GC–MS) based on derivatization (Schkolnik and Rudich, 2006; Medeiros and Simoneit, 2007), high performance anion exchange chromatography (HPAEC) with amperometric detection, mass spectrometry (MS) or MS/MS (Engling et al., 2006; Saarnio et al., 2010; Piot et al., 2012) and high performance liquid chromatography tandem mass spectrometry (HPLC–MS/MS; Gambaro et al., 2008; Hopmans et al., 2013). Only a few of these methods have been applied to sediments (Elias et al., 2001; Kuo et al., 2011a; Hopmans et al., 2013) and to the best of our knowledge only levoglucosan data have been published from sediment cores.

The aim of this study was to develop a method for analyzing all three MAs in lake sediment cores based on the existing HPAEC methods used for other matrices (Saarnio et al., 2010; Piot et al., 2012). The method has the advantage of not requiring derivatization, which is necessary for GC–MS. The selectivity and sensitivity of the HPAEC–MS method are better than for amperometric methods and HPAEC results in better separation of the three isomers than HPLC (Gambaro et al., 2008; Hopmans et al., 2013). A major benefit of analyzing all three isomers is the possibility of calculating the emission ratios of levoglucosan/mannosan and levoglucosan/(mannosan + galactosan) that might help determine changes in burned vegetation. Laboratory burning experiments and smoke analysis demonstrated characteristic emission ratios of the three isomers depending on the type of vegetation burned (Fabbri et al., 2009). For method validation we applied the new method to 12 selected samples from a Lake Kirkpatrick (New Zealand) sediment core and compared our MA results with the known charcoal concentration in the samples.

## 2. Material and method

### 2.1. Material

Levoglucosan was from Sigma Aldrich (St. Louis, USA), and mannosan and galactosan (> 99%) were from Molecula (Shaftesbury, UK). $^{13}C_6$-levoglucosan (> 98%) was from Cambridge Isotope Laboratories Inc. (Andover, MA, US). MeOH (Ultrapure) was from Romil LTD (Cambridge, UK) and $NH_4OH$ (Fluka, 25%) from Sigma Aldrich. Ultra-pure water was produced by PURELAB Pulse and PURELAB Flex (ELGA LabWater, Marlow, UK). He and $N_2$ of purity 5.0 was from SIAD (Bergamo, Italy).

### 2.2. Samples

The test samples ($n$ = 12) with known macroscopic charcoal concentration (> 125 μm) originated from a sediment core drilled by the Montana State University research team at Lake Kirkpatrick (South Island, New Zealand; 45.03°S, 168.57°E, 570 m above sea level) in 2009. This small lake (ca. 3.5 ha) is characterized by a small catchment area (< 10 km$^2$) with a relatively closed basin with no significant surface inflow. The counted macroscopic charcoal was assumed to originate from local aerosols (< 1–3 km$^2$), eolian deposition from airborne particles and surface erosion. The samples were from a depth of 75–129 cm. Those from 119–129 cm originated from 1240–1311 A.D. and those between 75 and 88 cm from 1479–1533 A.D. Charcoal concentration in the samples was between 0 and 64.8 pieces/cm$^3$ (Table 1). Relative to the overall charcoal variation at Lake Kirkpatrick, samples between 5.6 and 64.8 pieces/cm$^3$ were indicated as samples with high charcoal values, significantly influenced by local biomass burning. Samples with charcoal concentration between 0 and 0.4 pieces/cm$^3$ were assumed to be low charcoal samples. Detailed information regarding the drilling site and charcoal measurements are available from McWethy et al. (2009). Samples were shipped from Montana State University to Venice in 2013.

### 2.3. Sample preparation and extraction

Wet samples were freeze-dried, milled and homogenized. Prior to extraction the samples were spiked with 100 μl of internal standard (1 ppm in MeOH) containing $^{13}C$ labeled levoglucosan. The freeze-dried sediment (ca. 0.2 g) was extracted using pressurized solvent extraction (PSE; PSEone, Applied Separations, Hamilton, USA) with MeOH (2 cycles of 5 min each, 100 °C at 100 bar). The sample was filtered (0.2 μm, PTFE), evaporated under a stream of $N_2$ to dryness (Turbovap, Biotage, Uppsala, Sweden), dissolved in 0.5 ml ultra-pure water and sonicated to avoid any adsorption to the walls of the evaporation glass. Finally, the sample was centrifuged (5 min, 14,000 rpm) and transferred to the measurement vials. A blank was extracted with each batch of 5 samples.

### 2.4. Instrumental analysis and quantification

Separation of levoglucosan, mannosan and galactosan was performed with an ion chromatography (IC) instrument (Dionex ICS 5000, Thermo Scientific, Waltham, US) equipped with a CarboPac PA1™ column and a CarboPac P10™ column (Thermo Scientific, each 2 mm × 250 mm). In addition, we used a CarboPac PA 10™ guard column (2 × 50 mm) and an AminoTrap column (2 × 50 mm) to trap amino acids. The three MAs were detected with a single quadrupole mass spectrometer (MSQ Plus™, Thermo Scientific) after IC separation. The injection volume was 25 μl.

NaOH was produced as a carrier solvent by an eluent generator (Dionex ICS 5000 EG, Thermo Scientific). The gradient was: 20 mM (0–15 min), 100 mM (15–40 min; column cleaning), 20 mM (40–60 min; equilibration). The flow was 0.250 ml/min. The NaOH was removed by a suppressor (ASRS 300, 2 mm, Thermo Scientific) before entering the MS source. For the protection of the MS instrument during the cleaning step, the flow was switched to waste after a total run time of 15 min. MeOH/$NH_4OH$ was added post-column (0.02 ml/min) to improve ionization of the aqueous eluent. Ultrapure water for eluent generation and the MeOH mixture for the post-column infusion were kept under a He atmosphere to avoid external contamination and pressure fluctuation.

The MS instrument was equipped with an electrospray ionization (ESI) source used in negative ionization mode. Due to the post-column injection of MeOH/$NH_4OH$, we slightly modified the MS parameters from those described by Saarnio et al. (2010), to improve performance. The source temperature was reduced to 350 °C, the needle voltage was −3.5 kV and the cone voltage −50 V. Levoglucosan, mannosan and galactosan were analyzed using $m/z$ 161, where $m/z$ 101 and 113 were used as qualifiers when sufficient concentrations were present. The $^{13}C$ labeled internal standard was determined using $m/z$ 167.

Samples were quantified using the response factor of levoglucosan, mannosan and galactosan vs. the $^{13}C$ labeled internal standard. The advantage of using an internal standard is the ability to correct potential analyte loss during extraction and handling. A Chromeleon 6.8 Chromatography data system (Thermo Scientific) was used for data acquisition and elaboration.

**Table 1**
Levoglucosan, mannosan, and galactosan concentrations (ppb dry wt. sediment), macroscopic charcoal counts (> 125 μm pieces/cm$^3$) and the ratios of levoglucosan/mannosan ($L/M$) and levoglucosan/(mannosan + galactosan) [$L/(M + G)$] in lake sediment samples vs. depth (cm).

| Depth (cm) | Levoglucosan (ppb) | Mannosan (ppb) | Galactosan (ppb) | Charcoal (pieces/cm$^3$) | $L/M$ | $L/(M + G)$ |
|---|---|---|---|---|---|---|
| 75 | 1109 | 509 | 295 | 64.8 | 2.2 | 1.4 |
| 76 | 521 | 265 | 129 | 17.4 | 2.0 | 1.3 |
| 78 | 467 | 245 | 138 | 15.6 | 1.9 | 1.2 |
| 80 | 361 | 190 | 103 | 5.6 | 1.9 | 1.2 |
| 83 | 216 | 102 | 76 | 10.0 | 2.1 | 1.2 |
| 119 | 205 | 163 | 41 | 0.2 | 1.3 | 1.0 |
| 120 | 135 | 131 | 52 | 0.2 | 1.0 | 0.7 |
| 121 | 144 | 144 | 43 | 0.0 | 1.0 | 0.8 |
| 122 | 297 | 126 | 68 | 0.4 | 2.4 | 1.5 |
| 127 | 168 | 117 | 69 | 0.0 | 1.4 | 0.9 |
| 128 | 27 | 30 | 31 | 0.4 | 0.9 | 0.4 |
| 129 | 45 | 42 | 31 | 0.2 | 1.1 | 0.6 |

## 3. Results and discussion

### 3.1. Sample extraction

Sonic bath, shaking and PSE techniques have been discussed as potential extraction methods for MAs from filters, soil or sediments (Saarnio et al., 2010; Piot et al., 2012; Hopmans et al., 2013). We applied PSE using MeOH (cf. Hopmans et al., 2013). Multiple extractions led to only a low MA concentration in the second extract (< limit of quantification; LOQ) and third (< limit of detection; LOD) extract. We therefore only used one PSE extraction with 2 extraction cycles as described above in order to keep the volume of MeOH as small as possible. The samples were evaporated to dryness and dissolved in water prior to injection.

### 3.2. Chromatographic separation

The suitability of CarboPac™ columns for the separation of levoglucosan, mannosan and galactosan has been discussed recently (Saarnio et al., 2010; Piot et al., 2012). Initially we used a CarboPac™ PA 10 column with an AminoTrap column and achieved a similar separation to Saarnio et al. (2010). However, due to the complex matrix of the samples some co-eluting compounds interfered with MA separation. Changing NaOH concentration in the eluent did not improve the separation from the matrix. The retention time of levoglucosan was not significantly affected by changing NaOH concentration between 0.1 mM and 20 mM. However, the introduction of a second column (CarboPac™ PA 1) in series with the CarboPac™ PA 10 column improved the separation substantially (Fig. 1D).

Retention time was 4.8 min, 6.4 min and 9.1 min for levoglucosan, mannosan and galactosan, respectively. We achieved baseline separation of levoglucosan and mannosan when both columns were used in series, thereby resolving the separation problem in the method of Saarnio et al. (2010) and increased the separation of the isomers (Fig. 1A) compared with Piot et al. (2012). In addition, we were able to separate levoglucosan from the complex background matrix (Fig. 1B–D), where Fig. 1D demonstrates the improved separation when using both columns vs. the CarboPac PA 10™ column only.

To further reduce matrix influence, we tested smaller injection volumes of 10 μl and 25 μl, compared with published ones of 50 μl for HPAEC–MS (Saarnio et al., 2010) or 449 μl for HPAEC–MS/MS (Piot et al., 2012). Both resulted in reduced matrix effects, so we used 25 μl due to the potentially low concentration of the analytes in lake sediment samples.

Due to the use of two columns, a long cleaning and reconditioning step was required after each sample injection, leading to a 60 min run time for a single analysis. The cleaning was necessary to remove all residual sugars in order to avoid carryover effects, as MAs can be generated in the ESI source > 300 °C if other sugars are present (Saarnio et al., 2010).

### 3.3. Analytical performance

We examined linearity via a 7 point response factor calibration with concentrations of levoglucosan, mannosan and galactosan from 25 to 500 ppb, with $r^2$ 0.99 for all three MAs. The LOD and LOQ were calculated for the standard solution from signal/noise 3 (LOD) and 10 (LOQ), affording LOD and LOQ of 0.9–1.8 ppb and 3.2–5.8 ppb, respectively. These values are in the same range as in other studies (Saarnio et al., 2010), although we reduced the injection volume and introduced a second column. Using a 0.2 g sediment aliquot, which is less than the amount of sediment or soil analyzed using GC–MS (Elias et al., 2001) or HPAEC–MS/MS (Piot et al., 2012), corresponded to a LOD between 2.3 and 4.5 ppb and a LOQ between 8 and 14.5 ppb.

Instrumental precision was tested by a ten fold analysis of low (25 ppb) and high (300 ppb) concentration standards of levoglucosan, mannosan and galactosan, resulting in a relative standard deviation (RSD) of 3.8% and 6.6%, 3.2% and 2.3%, and 3.7% and 2.8% for the low and high concentration standard of levoglucosan, mannosan and galactosan, respectively.

Standard reference materials for MAs in lake sediments were not available, so we tested the method with the NIST reference material for urban dust SRM 1649b. MAs in this urban dust standard have been analyzed (Louchouarn et al., 2009), with reported concentrations (standard deviation in brackets) of 160.5 (± 5.0), 16.7 (± 0.7) and 4.8 (± 0.2) ppm for levoglucosan, mannosan and galactosan, respectively. Due to these high concentrations we only used a small aliquot (0.01 mg) and had to dilute the sample after extraction. We obtained concentrations of 168 (± 4.5), 15.7 (± 0.7) and 5.0 (± 0.2) ppm. Although levoglucosan and galactosan concentrations were slightly higher and mannosan concentration lower than the values in the literature, our results agree with the published values. The method accuracy based on the relative recovery of the MAs from spiked blank samples ($n$ = 5), was 99.8% (± 1.3%) (levoglucosan), 97.7% (± 6.4%) (mannosan) and 94.1% (± 7.6%) (galactosan). The estimated overall method uncertainty was 4.7%, 9.9% and 8.7% for levoglucosan, mannosan and galactosan, respectively. The increased uncertainty for mannosan and galactosan compared with levoglucosan may be due to the lack of internal standards for mannosan and galactosan.

We also performed parallel extractions of sediment samples, resulting in concentrations of 348 and 333 ppb, 163 and 145 ppb and 70 and 80 ppb for levoglucosan, and mannosan and galactosan, respectively. In addition, we performed a second parallel extraction of sediment samples from 76 cm and 119 cm, respectively.
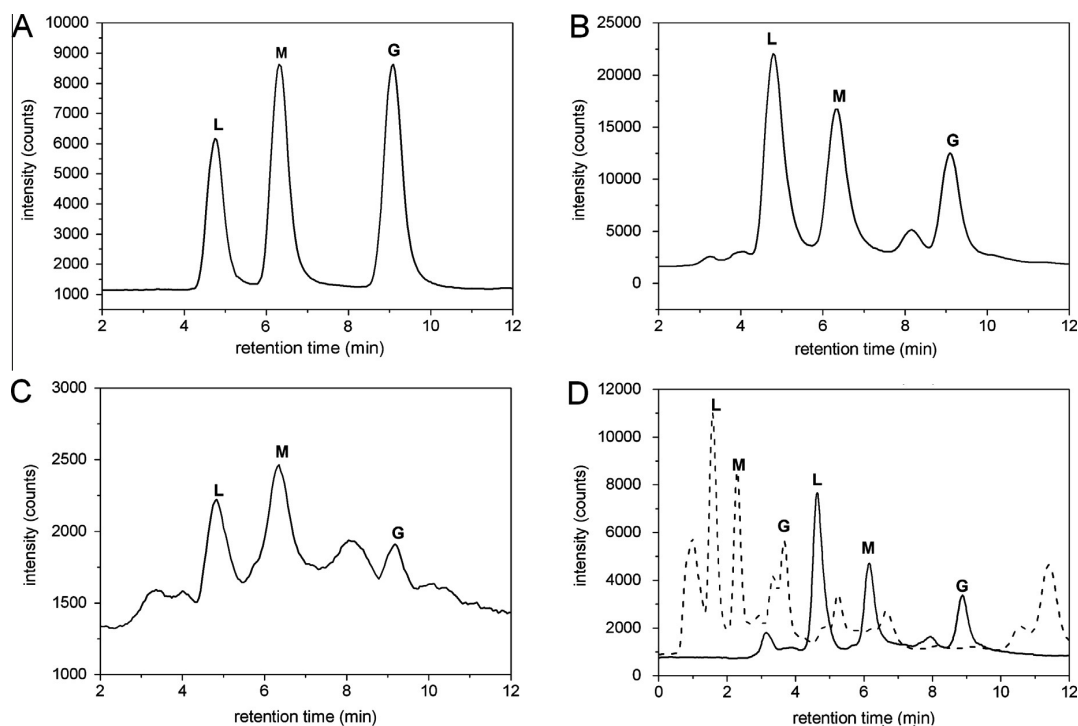
**Fig. 1.** (A) chromatogram (SIM) of a standard mixture (all 100 ppb) of levoglucosan (*L*), mannosan (*M*) and galactosan (*G*). The retention times are 4.8, 6.4 and 9.1 min, respectively. (B) chromatogram (SIM) of the three compounds in a New Zealand lake sediment sample with high charcoal concentration. (C) chromatogram (SIM) of the three compounds in a New Zealand lake sediment sample with low charcoal concentration. (D) comparison of the separation using only one column (dashed line) and the current method using two columns applied to a real sample. Note the different dimensions of the *y*-axis.

We extracted the second set of samples 3 weeks after the first set. The concentrations of levoglucosan, mannosan and galactosan were 521 and 512 ppb, 265 and 256 ppb and 129 and 117 ppb for the 76 cm sample, and 205 and 199 ppb, 163 and 157 ppb, and 41 and 44 ppb for the 119 cm sample, respectively. All parallel extractions demonstrated good method reproducibility and the variation was in the range of the estimated method uncertainty.

We measured blanks ($n = 3$) by treating them as real samples and also analyzed blanks within the different batches of five samples. The blank concentrations for these replicates ($n = 3$) were $10 \pm 6$, $2 \pm 4$ and $2 \pm 3$ ppb for levoglucosan, mannosan and galactosan, respectively The average blank concentrations during real sample analyses were between $15 \pm 1$ ppb for levoglucosan, $8 \pm 7$ ppb for mannosan and $4 \pm 6$ ppb for galactosan, thereby slightly higher. We corrected all sample concentrations using the blank values for the corresponding batch.

### 3.4. Sediment sample concentration

The method was tested and validated by analyzing lake sediment samples and comparing the results with the charcoal concentration of the samples. The samples were treated in random order to avoid any trend in the data that could possibly be influenced by the extraction or measurement procedures. In addition, we analyzed three blanks subjected to the same treatment as the samples.

The concentrations of levoglucosan, mannosan and galactosan in samples with charcoal counts between 5.6 and 64.8 pieces/cm$^3$ were 216–1109, 102–509 and 76–295 ppb, respectively. The concentrations of the three MAs in the samples with low charcoal counts (0–0.4 pieces/cm$^3$) were 27–297, 42–144 and 31–69 ppb.

An overview of the concentrations is given in Table 1 and plotted in Fig. 2B. All concentrations were blank corrected.

Reports of levoglucosan concentration in lake sediments are rare, so we used the limited published data as an initial estimate of the Lake Kirpatrick MAs concentration range even if the studies covered different time periods and sampling regions. Two marine cores from the main basin of Puget Sound (USA), had a comparable concentration to the present study, with values of 60–782 ppb (1700–2000 A.D.; Kuo et al., 2011a). Levoglucosan concentration from the present study was lower than values from a New Zealand marine sediment core record (9 ppm and 1045 ppm) covering a period from 9116 to 26,066 yr B.P. (i.e. before 1950 A.D.; Sikes et al., 2013) and lake sediment cores from the Southern Serra dos Carajás, Brazil, covering a period from 100–7000 yr B.P. (0.1–41.4 ppm; Elias et al., 2001). All previous studies used GC–MS. To the best of our knowledge, this is the first study reporting mannosan and galactosan concentrations in a sediment core.

### 3.5. Comparison of MAs with charcoal

Charcoal in sediment is a biomass burning marker and laboratory studies have demonstrated the occurrence of levoglucosan in low temperature charcoal (Kuo et al., 2008). Therefore a comparison between levoglucosan, mannosan and galactosan with associated charcoal in the same core is essential. The literature demonstrates a relationship between charcoal and levoglucosan in sediments (Elias et al., 2001; Kuo et al., 2011a). Our Lake Kirkpatrick levoglucosan data correlated strongly ($r^2$ 0.89, $p < 0.01$) with charcoal counts in samples from the same depth (Fig. 2A). Mannosan and galactosan concentrations displayed a similar trend ($r^2$ 0.84 and 0.93, $p < 0.01$) to the levoglucosan and charcoal concentrations (Fig. 2A). The high
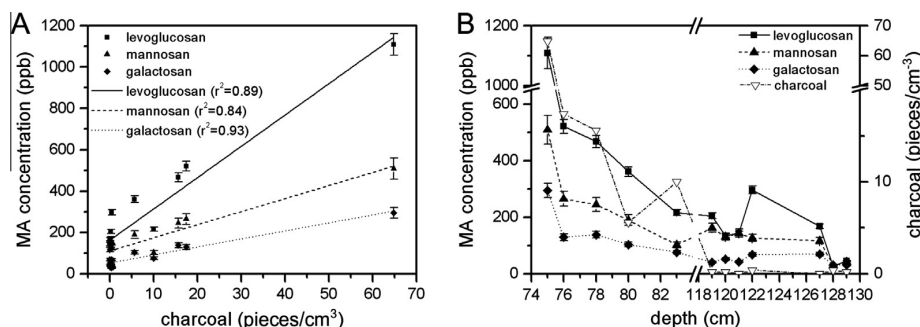
**Fig. 2.** (A) MA concentrations (dry wt. concentration in ppb) related to macroscopic (> 125 μm) charcoal concentration (pieces/cm³). Lines represent linear fits of the data. Error bars represent the estimated method uncertainty (B) MA concentration (dry wt. concentration in ppb) and charcoal concentration (pieces/cm³) related to sample depth (cm). Note the axis breaks.

value of the sample from 75 cm influenced the correlation and the exclusion of this sample led to a weaker correlation ($r^2$ 0.65, 0.47 and 0.76), demonstrating that MAs in lake sediments are not only imported by macroscopic charcoal. This relationship may explain why MAs were also detected in samples without any macroscopic charcoal (> 125 μm).

Microscopic (< 50 and > 50 μm) charcoal was analyzed and detected in the core including samples from 75, 80 and 121 cm (McWethy et al., personal communication). Previous studies demonstrated that levoglucosan, mannosan and galactosan were detected in the aerosol size fractions PM 2.5 and PM 10 in the atmosphere, and burning experiments demonstrated levoglucosan concentration maxima in the coarser fractions (> PM 10) and the ultrafine fraction (< 0.49 μm; Jordan et al., 2006; Engling et al., 2009). These aerosols may be deposited in lake sediments and these fine particle fractions were not included in the charcoal analysis. Thus, analysis of MAs in lake sediments is an additional tool for reconstructing biomass burning. The present data suggest that the MAs in sediment reflect not only local the impact of biomass burning, but also record regional fire activity, since fine aerosol fractions survive long distance transport.

*3.6. Comparison with MA emission ratios*

Pollen analysis of the core demonstrate a change in the primary vegetation from beech (*Nothofagus* spp.) and podocarp forests in the samples from 119–129 cm to beech (*Nothofagus* spp.) and podocarp forests with increasing amounts of bracken (*Pteridium*), native grasses (*Poaceae)* and shrubs (*Coriaria* and *Coprosma* spp.) in the samples from 75–83 cm (McWethy et al., personal communication). The correlation of levoglucosan, mannosan and galactosan with each other ($p < 0.01$; Spearman rank correlation) indicates a similar transport and deposition behavior of all three isomers. If we further assume that all three isomers are equally stable in the atmosphere and in lake sediments, levoglucosan/ mannosan and levoglucosan/(mannosan + galactosan) ratios may be a tool for determining changes in vegetation at the source regions of the aerosol particles. The average ratios of levoglucosan/ mannosan and levoglucosan/(mannosan + galactosan) in the samples from 119–129 cm were 1.3 ± 0.5 and 0.9 ± 0.3 and for the samples from 75–83 cm 2.0 ± 0.1 and 1.3 ± 0.1 (see Table 1). The different ratios may reflect the change in the primary vegetation, corresponding with the Lake Kirkpatrick pollen data (McWethy et al., personal communication).

However, the differences are not significant and the greater standard deviation for the deeper samples (119–129 cm) is due to the ratio for the 122 cm sample, which is more similar to the ratios of the upper samples. The 122 cm sample had the highest

levoglucosan concentration in the 119–129 cm section and may be impacted by relatively distant sources as discussed above.

In addition, the ratios are in the range of published (Fabbri et al., 2009 and references within) emission ratios of (levoglucosan/ mannosan and levoglucosan/(mannosan + galactosan) for different types of vegetation, such as softwood (0.6–13.8, 0.4–6.1), hardwood (3.3–22, 1.5–17.6) and grass (2.0–33.3, 1.7–9.5). The wide range in ratios for the same type of vegetation might be a result of different combustion conditions, which yield different emission ratios (Kuo et al., 2011b). This influence on the emission of MAs may further limit the use of the ratios to track back to specific vegetation.

If the MA ratios for lake sediment cores can be used to reconstruct specific burned vegetation and not only demonstrate general changes, burning experiments with the primary local vegetation would be necessary and should be compared with MAs in sediment cores over longer timescales. In the present study we analyzed only selected samples to validate the analytical method and, thus, the small dataset limits a deeper evaluation of the suitability of these ratios. However, the results are promising, with evidence that a change in primary vegetation may influence MA ratios in sediment cores.

## 4. Conclusions

The molecular markers levoglucosan, mannosan and galactosan in sediment samples were successfully separated and determined using high performance anion-exchange chromatography–mass spectrometry. All three markers correlated with charcoal, a local marker for biomass burning. However, the MA records may also have been influenced by more distant sources than charcoal. To the best of our knowledge the data are the first record of the three MAs from lake sediment samples. Therefore, some uncertainty exists, which may bias the new records, including multiple burned vegetation types, different combustion conditions, distance to potential sources and stability of the compounds in water and sediment. Future work should examine whether post-depositional effects such as degradation or redistribution change the ratios to the point that they no longer represent specific source emission ratios. The analysis of molecular markers in sediment cores is a fairly new but developing field with the possibility of addressing fundamental assumptions and unknowns in fire science.

*Associate Editor—***B. van Dongen**

## References

Bowman, D.M.J.S., Balch, J.K., Artaxo, P., Bond, W.J., Carlson, J.M., Cochrane, M.A., D'Antonio, C.M., Defries, R.S., Doyle, J.C., Harrison, S.P., Johnston, F.H., Keeley, J.E., Krawchuk, M.A., Kull, C.A., Marston, J.B., Moritz, M.A., Prentice, I.C., Roos, C.I., Scott, A.C., Swetnam, T.W., van der Werf, G.R., Pyne, S.J., 2009. Fire in the earth system. Science 324, 481–484.

Elias, V.O., Simoneit, B.R.T., Cordeiro, R.C., Turcq, B., 2001. Evaluating levoglucosan as an indicator of biomass burning in Carajás, Amazônia: a comparison to the charcoal record. Geochimica et Cosmochimica Acta 65, 267–272.

Engling, G., Carrico, C.M., Kreidenweis, S.M., Collett Jr., J.L., Day, D.E., Malm, W.C., Lincoln, E., Min Hao, W., Iinuma, Y., Herrmann, H., 2006. Determination of levoglucosan in biomass combustion aerosol by high-performance anion-exchange chromatography with pulsed amperometric detection. Atmospheric Environment 40, 299–311.

Engling, G., Lee, J.J., Tsai, Y.-W., Lung, S.-C.C., Chou, C.C.-K., Chan, C.-Y., 2009. Size-resolved anhydrosugar composition in smoke aerosol from controlled field burning of rice straw. Aerosol Science and Technology 43, 662–672.

Fabbri, D., Torri, C., Simoneit, B.R.T., Marynowski, L., Rushdi, A.I., Fabiańska, M.J., 2009. Levoglucosan and other cellulose and lignin markers in emissions from burning of Miocene lignites. Atmospheric Environment 43, 2286–2295.

Fraser, M.P., Lakshmanan, K., 2000. Using levoglucosan as a molecular marker for the long-range transport of biomass combustion aerosols. Environmental Science and Technology 34, 4560–4564.

Gambaro, A., Zangrando, R., Gabrielli, P., Barbante, C., Cescon, P., 2008. Direct determination of levoglucosan at the picogram per milliliter level in Antarctic ice by high-performance liquid chromatography/electrospray ionization triple quadrupole mass spectrometry. Analytical Chemistry 80, 1649–1655.

Hoffmann, D., Tilgner, A., Iinuma, Y., Herrmann, H., 2010. Atmospheric stability of levoglucosan: a detailed laboratory and modeling study. Environmental Science and Technology 44, 694–699.

Hopmans, E.C., Santos, R.L.Dos., Mets, A., Sinninghe Damsté, J.S., Schouten, S., 2013. A novel method for the rapid analysis of levoglucosan in soils and sediments. Organic Geochemistry 58, 86–88.

Jordan, T.B., Seen, A.J., Jacobsen, G.E., 2006. Levoglucosan as an atmospheric tracer for woodsmoke. Atmospheric Environment 40, 5316–5321.

Kehrwald, N., Zangrando, R., Gabrielli, P., Barbante, C., Gambaro, A., 2012. Levoglucosan as a specific marker of fire events in Greenland snow. Tellus B 64, 1–9.

Kuo, L.-J., Herbert, B.E., Louchouarn, P., 2008. Can levoglucosan be used to characterize and quantify char/charcoal black carbon in environmental media? Organic Geochemistry 39, 1466–1478.

Kuo, L.-J., Louchouarn, P., Herbert, B.E., Brandenberger, J.M., Wade, T.L., Crecelius, E., 2011a. Combustion-derived substances in deep basins of Puget Sound: historical inputs from fossil fuel and biomass combustion. Environmental Pollution 159, 983–990.

Kuo, L.-J., Louchouarn, P., Herbert, B.E., 2011b. Influence of combustion conditions on yields of solvent-extractable anhydrosugars and lignin phenols in chars: implications for characterization of biomass combustion residues. Chemosphere 85, 797–805.

Louchouarn, P., Kuo, L.-J., Wade, T.L., Schantz, M., 2009. Determination of levoglucosan and its isomers in size fractions of aerosol standard reference materials. Atmospheric Environment 43, 5630–5636.

McWethy, D.B., Whitlock, C., Wilmshurst, J.M., McGlone, M.S., Li, X., 2009. Rapid deforestation of South Island, New Zealand, by early Polynesian fires. The Holocene 19, 883–897.

Medeiros, P.M., Simoneit, B.R.T., 2007. Analysis of sugars in environmental samples by gas chromatography-mass spectrometry. Journal of Chromatography A 1141, 271–278.

Norwood, M.J., Louchouarn, P., Kuo, L.-J., Harvey, O.R., 2013. Characterization and biodegradation of water-soluble biomarkers and organic carbon extracted from low temperature chars. Organic Geochemistry 56, 111–119.

Piot, C., Jaffrezo, J.-L., Cozic, J., Pissot, N., El Haddad, I., Marchand, N., Besombes, J.-L., 2012. Quantification of levoglucosan and its isomers by High Performance Liquid Chromatography – Electrospray Ionization tandem Mass Spectrometry and its applications to atmospheric and soil samples. Atmospheric Measurement Techniques 5, 141–148.

Saarnio, K., Teinilä, K., Aurela, M., Timonen, H., Hillamo, R., 2010. High-performance anion-exchange chromatography-mass spectrometry method for determination of levoglucosan, mannosan, and galactosan in atmospheric fine particulate matter. Analytical and Bioanalytical Chemistry 398, 2253–2264.

Schkolnik, G., Rudich, Y., 2006. Detection and quantification of levoglucosan in atmospheric aerosols: a review. Analytical and Bioanalytical Chemistry 385, 26–33.

Sikes, E.L., Medeiros, P.M., Augustinus, P., Wilmshurst, J.M., Freeman, K.R., 2013. Seasonal variations in aridity and temperature characterize changing climate during the last deglaciation in New Zealand. Quaternary Science Reviews 74, 245–256.

Simoneit, B.R.T., 2002. Biomass burning—a review of organic tracers for smoke from incomplete combustion. Applied Geochemistry 17, 129–162.

Simoneit, B.R.T., Elias, V.O., 2000. Organic tracers from biomass burning in atmospheric particulate matter over the ocean. Marine Chemistry 69, 301–312.

Whitlock, C., Larsen, C., 2001. Charcoal as a fire proxy. In: Smol, J.P., Birks, H.J., Last, W.M. (Eds.), Tracking Environmental Change Using Lake Sediments: Terrestrial Algal and Siliceous Indicators, vol. 3. Kluwer Academic Publishers, Dordrecht, pp. 75–97.

Zangrando, R., Barbaro, E., Zennaro, P., Rossi, S., Kehrwald, N.M., Gabrieli, J., Barbante, C., Gambaro, A., 2013. Molecular markers of biomass burning in arctic aerosols. Environmental Science and Technology 47, 8565–8574.

Zennaro, P., Kehrwald, N., McConnel, J.R., Schüpbach, S., Maselli, O., Marlon, J., Vallelonga, P., Leuenberger, D., Zangrando, R., Spolaor, A., Borrotti, M., Barbaro, E., Gambaro, A., Barbante, C., 2014. Fire in ice: two millennia of Northern Hemisphere fire history from the Greenland NEEM ice core. Climate of the Past Discussion 10, 809–857.

# 2 Study II: Combining charcoal sediment and molecular markers to infer a Holocene fire history in the Maya lowlands of Petén, Guatemala

S. Schüpbach[a, b, c], T. Kirchgeorg[a,d], D. Colombaroli[c,e], G. Beffa[c,e], M. Radaelli[f], N. Kehrwald[a] and C. Barbante[a,f,g]

[a]Department of Environmental Sciences, Informatics and Statistics, University Ca' Foscari, Venice, 30123, Italy

[b]Climate and Environmental Physics, Physics Institute, University of Bern, Bern, 3012, Switzerland

[c]Oeschger Centre for Climate Change Research, University of Bern, Bern, 3012, Switzerland

[d]Institute of Sustainable and Environmental Chemistry, Leuphana University of Lüneburg, Lüneburg, 21335, Germany

[e]Institute of Plant Sciences, University of Bern, Bern, 3013, Switzerland

[f]Institute for the Dynamics of Environmental Processes-CNR, Venice, 30123 Italy

[g]Centro Linceo B. Segre, Accademia Nazionale dei Lincei, Rome, 00165, Italy

CrossMark

# Combining charcoal sediment and molecular markers to infer a Holocene fire history in the Maya Lowlands of Petén, Guatemala

Simon Schüpbach [a, b, c, *], Torben Kirchgeorg [a, d], Daniele Colombaroli [c, e], Giorgia Beffa [c, e], Marta Radaelli [f], Natalie M. Kehrwald [a], Carlo Barbante [a, f, g]

[a] Department of Environmental Sciences, Informatics and Statistics, University Ca'Foscari, Venice 30123, Italy
[b] Climate and Environmental Physics, Physics Institute, University of Bern, Bern 3012, Switzerland
[c] Oeschger Centre for Climate Change Research, University of Bern, Bern 3012, Switzerland
[d] Institute of Sustainable and Environmental Chemistry, Leuphana University of Lüneburg, Lüneburg 21335, Germany
[e] Institute of Plant Sciences, University of Bern, Bern 3013, Switzerland
[f] Institute for the Dynamics of Environmental Processes-CNR, Venice 30123, Italy
[g] Centro Linceo B. Segre, Accademia Nazionale dei Lincei, Rome 00165, Italy

## ARTICLE INFO

## ABSTRACT

Vegetation changes in the Maya Lowlands during the Holocene are a result of changing climate conditions, solely anthropogenic activities, or interactions of both factors. As a consequence, it is difficult to assess how tropical ecosystems will cope with projected changes in precipitation and land-use intensification over the next decades. We investigated the role of fire during the Holocene by combining macroscopic charcoal and the molecular fire proxies levoglucosan, mannosan and galactosan. Combining these two different fire proxies allows a more robust understanding of the complex history of fire regimes at different spatial scales during the Holocene. In order to infer changes in past biomass burning, we analysed a lake sediment core from Lake Petén Itzá, Guatemala, and compared our results with millennial-scale vegetation and climate change available in the area. We detected three periods of high fire activity during the Holocene: 9500—6000 cal yr BP, 3700 cal yr BP and 2700 cal yr BP. We attribute the first maximum mostly to climate conditions and the last maximum to human activities. The rapid change between burned vegetation types at the 3700 cal yr BP fire maximum may result from human activity.

## 1. Introduction

Fire in the earth system plays an important ecological role by influencing biogeochemical and carbon cycles, atmospheric chemistry, aerosols, land-surface properties and human activities on a local to a global scale (Carcaillet et al., 2002; Bowman et al., 2009). Climate conditions such as moisture levels and temperature control fire activity through biomass accumulation and the likelihood of its spread. In turn, biomass burning influences climate at a more global scale by the emission of aerosols and greenhouse gases, like carbon dioxide and methane. Humans used fire to clear landscapes at least since the introduction of agriculture, thus

influencing the long-term fire variability. Scientists actively debate if such early anthropogenic activities impacted the climate and, if so, when this influence began (Ruddiman, 2003). The potential of fire to affect the climate system is demonstrated by recent carbon dioxide emissions from fire, which account up to 50% of the total emitted carbon dioxide (Bowman et al., 2009).

Evidence of early human agriculture activity in Mesoamerica as early as 7000 BP is based on *Zea mays* pollen in sediment cores from northwest Mexico (Pohl et al., 2007). However, other studies demonstrate that the initiation of maize cultivation varied between different Mesoamerican regions (Pope et al., 2001). The Maya Lowlands, consisting of the Yucatan Peninsula (Mexico) and parts of Guatemala, are known as the center of the rise and fall of the Maya culture. The history of the Maya began ca 4000 BP, and they became the largest and highly developed culture in Mesoamerica until declining rapidly around 900 BP. The literature refers to this

population decline as the "*Classic Maya collapse*" which may be due to several severe droughts (e.g. Haug et al., 2003; Kennett et al., 2012).

Pollen records and lake sediment patterns from the Maya Lowlands show a rapid vegetation change (e.g. forest openings, increase in pioneer tree species) and increase of soil erosion during the Holocene and particularly when the Maya were the prominent culture (e.g. Islebe et al., 1996; Leyden, 2002; Anselmetti et al., 2007). The soil erosion resulted in an inorganic sediment layer, the "*Maya Clay*" observed in several sediment cores in the Maya Lowlands (e.g. Curtis et al., 1998; Anselmetti et al., 2007; Mueller et al., 2009). Researchers still discuss if the major driving forces behind this vegetation change and the rapid soil erosion were changing climate conditions or early Maya deforestation. The role of fire, anthropogenic or natural, on this system during the Holocene, was only partly discussed in these studies.

The aim of this study is to reconstruct Holocene fire activity at Lake Petén Itzá and to compare this biomass burning record with previously published multi-proxy studies in Lake Petén Itzá sediment cores and other regional sampling sites for a better understanding of the interaction between climate, humans and fire. In order to reach this aim, we use a novel multi-proxy approach which combines lake sediment macroscopic charcoal (a proxy for biomass burning at local-scale, see (Whitlock and Larsen, 2001)) and concentrations of the monosaccharide anhydrates (MAs) levoglucosan (1,6-anhydro-$\beta$-D-glucopyranose), mannosan (1,6-anhydro-$\beta$-D-mannopyranose) and galactosan (1,6-anhydro-$\beta$-D-galactopyranose) as specific molecular markers for biomass burning in lake sediments (Elias et al., 2001; Conedera et al., 2009; Kuo et al., 2011b; Kirchgeorg et al., 2014). Comparing the two biomass burning proxies may help increase our understanding about advantages and limitations of molecular markers as proxies for past fire reconstruction.

## 2. Study area

Lake Petén Itzá (17°00′N, 89°50′W, 110 m above sea level) is located in the Department of Petén in the northern part of Guatemala (Fig. 1). It is the deepest lake in the Maya Lowlands of Central America with a maximal depth of approximately 165 m. Previous studies demonstrated that the lake did not desiccate during long dry periods and had a continuous sedimentation (Mueller et al., 2009). Lake Petén Itzá receives water from surface runoff, rainfall, subsurface groundwater, and a small input stream, without any significant outflows (Correa-Metrio et al., 2012). Due to the fact that Lake Petén Itzá receives most of its water by rainfall, the water level strongly depends on rainfall and evaporation. The annual mean temperatures in this region are on average 25 °C with a high variability of precipitation between 900 and 2500 mm yr$^{-1}$, which occurs mainly between June and October, followed by a dry winter. The climate is quite sensitive to the migration of the Intertropical Convergence Zone (ITCZ), which controls the regional rainfall (Deevey et al., 1980; Hillesheim et al., 2005; Hodell et al., 2008).

Prior to the Maya the vegetation at Lake Petén Itzá was characterized by closed-forest taxa including *Moraceae* and *Urticaceae*. During the occupation of the Maya, the pollen records show a shift to more open vegetation, including *Amaranthaceae*, *Ambrosia*, *Compositae*, *Cyperaceae*, as well as *Poaceae* and other disturbance-adapted taxa, which are assumed to be a result of the deforestation (Curtis et al., 1998; Leyden, 2002; Rosenmeier et al., 2002). These changes were observed at several other sites in the Maya Lowlands (Leyden, 2002). The forest recovered to pre-Maya taxa after the *Classic Maya Collapse*.

The Maya settlements and the beginning of the agricultural activity in Petén was dated around 3000 BP, corroborated by the first findings of *Zea mays* pollen and the beginning of the stratigraphic section of *Maya Clay* in the cores from lakes in Petén (e.g. Wahl et al., 2006; Anselmetti et al., 2007; Mueller et al., 2009). The *Classic Maya Collapse* diminished the Maya population in Petén but historical documents and archaeological studies demonstrate that Lake Petén Itzá was still surrounded by several Maya settlements after the *Classic Maya Collapse* and migration towards the lake may have occurred until the arrival of Spanish conquerors (Rice et al. 1998).

## 3. Materials and methods

### 3.1. Samples

The 110 lake sediment samples originate from the core PI 5 VI 02 11B collected in Lake Petén Itzá in June 2002 (Hillesheim et al., 2005). The 11B core is one out of six (11A–11F) piston cores along seismic line 11, recovered using a Kullenberg type piston corer triggered by a mud–water interface corer (Hillesheim et al., 2005; Anselmetti et al., 2006). The 550 cm long core 11B was chosen because it covers the entire Holocene, it is well dated, and various proxy data are available from this or parallel cores from Lake Petén Itzá and from surrounding lakes (e.g. Rosenmeier et al., 2002; Hillesheim et al., 2005; Anselmetti et al., 2006; Mueller et al., 2009; Correa-Metrio et al., 2012). The samples of approximately 5 g wet weight were subsampled from the original core at the University of Florida, USA, and shipped to the University of Venice, Italy in 2012. The subsamples were taken from a depth of 18 to 514 cm. One sample covers a depth of 2 cm. Distance between two samples is 5.5 cm (i.e. 7.5 cm sampling resolution) from 18 to 300 cm depth, and 1 cm (3 cm sampling resolution) from 300 to 514 cm depth. In Venice, samples were divided into two parts: 1 cm$^3$ of wet sample was used for the macroscopic charcoal analyses, while the remaining sample was freeze-dried and homogenized for molecular biomass burning marker and total organic content (TOC) analyses. As sampling was not contiguous our fire history reflects low frequency changes in biomass burning over the millennial time scale.

### 3.2. Analytical methods

The monosaccharide anhydrates levoglucosan (1,6-anhydro-$\beta$-D-glucopyranose), mannosan (1,6-anhydro-$\beta$-D-mannopyranose) and galactosan (1,6-anhydro-$\beta$-D-galactopyranose) were analysed using an aliquot of 200 mg of freeze-dried and homogenized sediment. The compounds were extracted by pressurized solvent extraction (PSEone, Applied Separations, Hamilton, USA) with methanol (Ultrapure, Romil LTD, Cambridge, UK), filtered (0.2 μm, PTFE), evaporated to dryness (Turbovap, Biotage, Uppsala, Sweden), and dissolved in ultra-pure water (ELGA LabWater, Marlow, UK). MAs were separated and quantified by high performance anion exchange chromatography–mass spectrometry (Dionex ICS 5000 – MSQ Plus™, Thermo Scientific, Waltham, US) with the internal standard method using mass labelled $^{13}C_6$-levoglucosan. We improved the chromatographic separation of the previously published method (Kirchgeorg et al., 2014) by using a CarboPac™ MA1 column (Thermo Scientific, 2 × 250 mm) and an AminoTrap column (Thermo Scientific, 2 × 50 mm). Chromeleon 6.8 Chromatography data system (Thermo Scientific) was applied for data acquisition and elaboration. All modifications of the original method are given in the Supporting information.

The total organic carbon was analysed by an aliquot of 100 mg of each freeze dried sample, following the EPA procedure (EPA
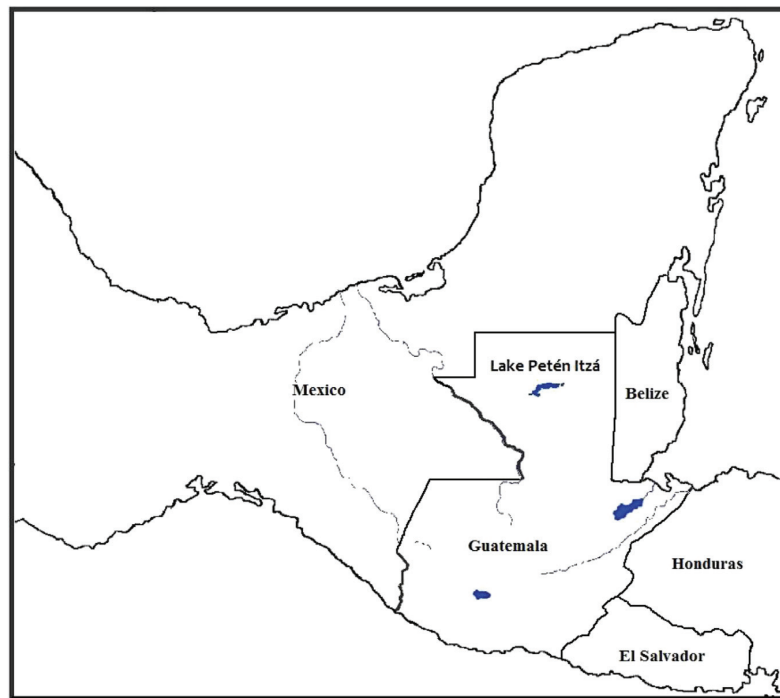
**Fig. 1.** Map of Mesoamerica, showing the study site at Lake Petén Itzá, Guatemala.

Method 9060). Inorganic carbon was removed prior to analysis by adding hydrochloric acid (2.5N) to the sample and then incubating it for 30 min at 120 °C. Excessive water was removed by oven-drying the sample at 105 °C overnight. Finally the dried sample was analysed with the TOC elemental analyser (TOC 5050, Shimadzu), by combustion at elevated temperatures using air as carrier gas. The $CO_2$ produced was measured by Fourier transform infrared spectroscopy.

Macroscopic charcoal analyses were performed at the University of Bern, Switzerland. For the analyses, 1 $cm^3$ of wet sample was first soaked for several hours in a deflocculating solution (5% sodium hexametaphosphate), treated with hydrogen peroxide (10%) for approx. 12 h to remove the organic content, and sieved over a 100 μm mesh. Macroscopic charcoal particles were estimated under a stereomicroscope at 40× magnification. We distinguished different types of charcoal morphotypes as in Colombaroli et al. (2014). Grass cuticles usually occur in flat sheets, and epidermal cells are arranged in parallel rows, with stomata within the rows (Jensen et al., 2007), and mostly have a length to width ratio $\geq$ 4:1 (Umbanhowar and Mcgrath, 1998). Woody material presents a thicker structure than the grass cuticle and the length to width ratio is normally lower (<4:1; Umbanhowar and Mcgrath, 1998). Leaf fragments can be separated from other charred material due to the presence of leaf veins, which are characterized by a divergence of the branches from a node (Jensen et al., 2007). Further details can be found in Beffa (2012) and Colombaroli et al. (2014).

### 3.3. Age-depth model

We calculated the depositional time (yrs $cm^{-1}$) of each level, using CLAM Version 2.2 (Blaauw, 2010), applying a spline interpolation (1000 iterations) between neighbouring levels and calibrating with the northern hemisphere terrestrial calibration curve

from Reimer et al. (2013). Radiocarbon data (a total of 12 ^{14}C dates) from this and a parallel core were published by Mueller et al. (2009) and Hillesheim et al. (2005). Ten out of these 12 ^{14}C dates had been determined on a parallel core (11A), and only two on the 11B core. The core 11A covers about the same age and is about equally long as 11B, and it has been drilled 500 m north of 11B during the same drilling campaign in June 2002. However, from 11A the top 50 cm are missing (corresponding to about 600 years), whereas for 11B only the top 18 cm (approximately 200 years) are missing, see (Hillesheim et al., 2005; Anselmetti et al., 2006) for a detailed description of the various cores drilled along seismic line 11 in Lake Petén Itzá. The dates determined on the 11A core were projected onto the 11B core by matching visual stratigraphy by Mueller et al. (2009). One of the two dates determined on the 11B core was based on a charcoal sample (at 365 cm depth, 6735 cal yr BP), which we have discarded for our age model calculations because charcoal samples often show a bias towards older ages. All other samples were either wood pieces (ten) or leaves (one).

Different smoothing factors for the upper and the lower core sections have been chosen to better constrain rapid change in accumulation rates in the upper section, due to erosion (Maya Clay layer, e.g. Anselmetti et al., 2007; Mueller et al., 2009). We used 0.2 as a spline interpolation smoothing factor (Blaauw, 2010) for the upper section (up to and including the Maya Clay layer, i.e. approx. 3100 cal yr BP), and a 0.4 smoothing factor below the Maya Clay layer. In the lower core section we applied a slightly stronger smoothing in order to smooth out additional uncertainties introduced by the projection of the 11A ^{14}C dates onto the 11B core. Alternative age models including all ^{14}C dates and applying different smoothing factors illustrating the effect on the flux records are included in the Supplementary information.

The resulting depth-age model is shown in Fig. 2. We applied the depth-age model to calculate the fluxes of charcoal
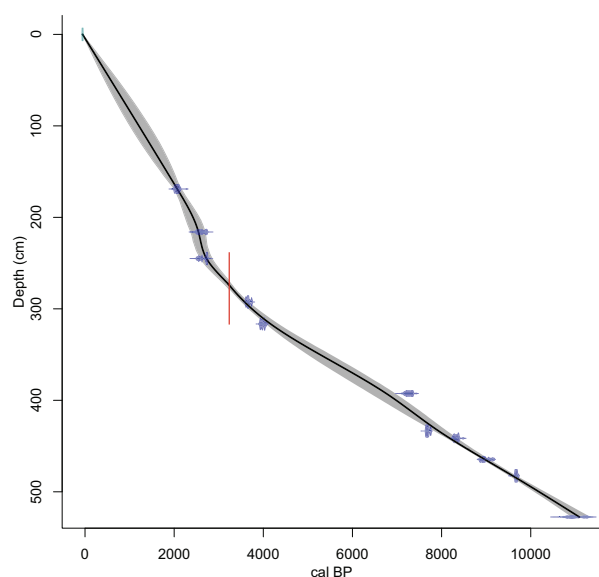
**Fig. 2.** Depth-age model based on eleven radiocarbon determinations and produced using CLAM 2.2 (Blaauw, 2010). The shift in the smoothing factor from 0.2 to 0.4 is indicated by the vertical red line. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

(pieces $cm^{-2}$ $yr^{-1}$) and of levoglucosan, mannosan and galactosan (ng $cm^{-2}$ $yr^{-1}$).

## 4. Results

In this section we present the results of the charcoal and MA analyses performed on the PI 5 VI 02 11B core. Additionally, the total organic carbon (TOC) profile of the core is shown in Fig. 3. The TOC in the entire core is between 1 and 10 % which is in agreement with the values in the Petén Itzá sediment cores which were discussed by Mueller et al. (2009). It also shows the typical Maya clay layer (approx. 3000—1000 cal yr BP) with low TOC content observed in many Petén lake cores (e.g. Islebe et al., 1996; Curtis et al., 1998; Rosenmeier et al., 2002; Anselmetti et al., 2006). This inorganic clay layer is generally attributed to soil erosion resulting from ancient Maya forest clearance (Anselmetti et al., 2007; Mueller et al., 2009).

### 4.1. Charcoal

Macroscopic charcoal was detected in all investigated samples. Different morphotypes were distinguished when counting the charcoal particles (see "Materials and Methods"). Charcoal particles were mainly grasses (79% of total charcoal pieces), the second most important morphotype was wood-like charcoal (20%), and finally leaves, needles and others materials accounted for less than 1% of the total number of charcoal pieces. Total charcoal and the two main morphotypes of wood and grass concentrations are shown in Fig. 3, with concentrations between 0—176 pieces $cm^{-3}$ and 1—582 pieces $cm^{-3}$, respectively.

In general, trends of charcoal fluxes (Fig. 4) mirror original concentrations, with the two main peaks at 370 cm (6100 cal yr BP) and 305 cm (3700 cal yr BP). However, the calculated charcoal fluxes are complemented by a third and less prominent peak at 225 cm (2600 cal yr BP), which is not visible in charcoal concentration records and is a result of the different deposition rates

during this period. In the bottom section of the core the charcoal flux is very low (<2 pieces $cm^{-3}$ $yr^{-1}$), demonstrating a slightly increasing trend with time. The trend of total charcoal flux of the bottom section is in good agreement with the charcoal data of the same core in the age interval 11k—5k cal yr BP presented by Hillesheim et al. (2005). Over the entire core the highest charcoal fluxes were found between 210 and 400 cm (2500—7000 cal yr BP). All charcoal types show a similar phasing during the first peak around 370 cm. Interestingly, during the second maximum around 305 cm the different charcoal types show a different pattern: They start to increase simultaneously at the onset of the increase of total charcoal around 305 cm, but the percentage of woody charcoal suddenly drops, while grass-like charcoal concentrations still increase before slowly decreasing after reaching its peak value (see Figs. 3 and 4). Above the second maximum, and especially above 250 cm, total charcoal influx is dominated by grass-like charcoal. In general, the two charcoal morphotypes follow a similar trend over the entire depth range. However, in the top 250 cm charcoal particles are almost exclusively grass-like particles (>90%), whereas from 300 cm downwards wood-like charcoal is relatively more abundant (approx. 75% grass vs. 25% wood).

### 4.2. Monosaccharide anhydrides

As in the charcoal record, the MA concentrations are generally low in the upper part of the core, as well as in the bottommost part (Fig. 3), and at an elevated level in the middle part from 300 to 490 cm (3700—9900 cal yr BP). In contrast to charcoal the MA concentrations show no clear maximum during this period, apart from galactosan around 400 cm (7000 cal yr BP). It is evident that levoglucosan and mannosan start to increase at 10k—9.5k cal yr BP and, thus, well before charcoal which begins to increase at 7000 cal yr BP and galactosan which begins to increase at 8000 cal yr BP. The highest values of levoglucosan concentrations are reached at 400 cm, after which it slowly decreases until dropping at 305 cm (3700 cal yr BP) along with TOC and (mainly wood-like) charcoal. In the upper half of the core, where low charcoal and MA concentrations prevail, slightly elevated levoglucosan concentrations can be observed between 200 and 80 cm depth (2400—960 cal yr BP). These elevated levoglucosan concentrations are even more evident in the flux record in Fig. 4. While the charcoal flux is constantly low after the 2600 cal yr BP peak, levoglucosan remains at an elevated level, although with a downward trend. Levoglucosan flux reaches its local minimum around 900 cal yr BP, after which it increases again along with mannosan and galactosan.

Generally, the three distinct maxima in the charcoal flux record are mirrored in the levoglucosan flux record, although the first levoglucosan maximum occurs approximately one thousand years before the charcoal maximum. Around 6000 cal yr BP, where sediment charcoal peaks for the first time, a local maximum in the levoglucosan flux is also evident. This levoglucosan peak height is in good agreement with the height of the charcoal peak relative to the two other peaks of charcoal and levoglucosan.

The flux records of the two isomers mannosan and galactosan significantly correlate with levoglucosan ($R^2$ = 0.53 and 0.46, respectively, p-values of $9.2 \cdot 10^{-10}$ and $2.8 \cdot 10^{-8}$, respectively, with N = 52 data points) during the middle section (7000—2000 cal yr BP), whereas in the bottom and the top sections the isomers trends differ from one another. While levoglucosan shows a constant increase from 10k—8k cal yr BP, galactosan shows only a slow increase at the beginning and then abruptly increases by a factor of 4 within about five centuries after ~8000 cal yr BP. Mannosan on the other hand increases faster in the oldest core section, but peaks at 8300 cal yr BP. Between 2000 and 800 cal yr BP the three isomers
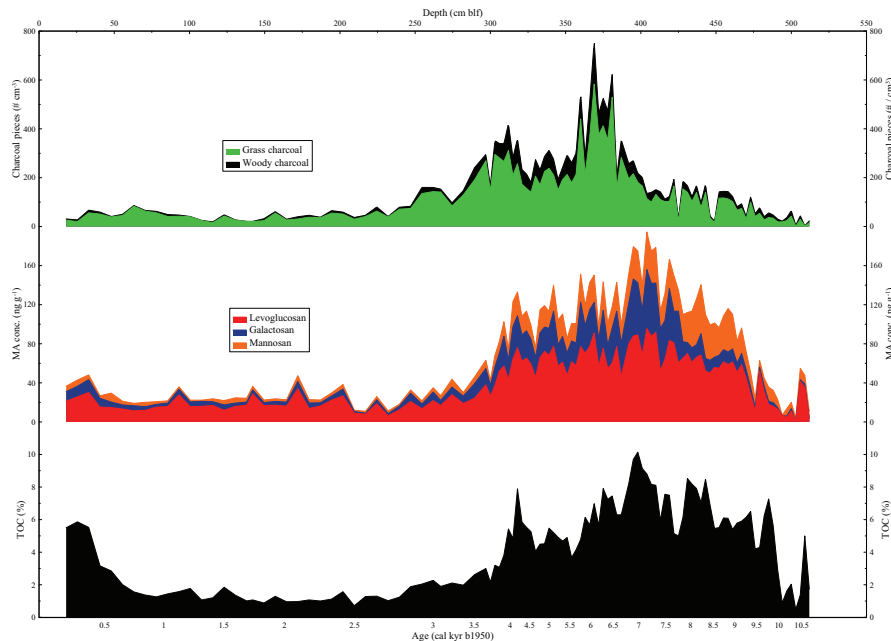
**Fig. 3.** Top: Concentrations of charcoal particles (black: woody charcoal, green: grass-like charcoal). Middle: Concentrations of MAs (red: levoglucosan, blue: galactosan, orange: mannosan), Bottom: Total Organic Content in the 11B core. All species are shown linearly versus depth (top axis), at the bottom the corresponding age is indicated. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

appear to be completely uncorrelated, however, after 800 BP they all increase together, even though mannosan is not increasing as fast as the other two. The concentrations of the three isomers are influenced by the type of burned vegetation and the burning conditions (mainly temperature) (Fabbri et al., 2009 and references therein; Alves et al., 2010 and references therein; Kuo et al., 2011a). The abundance of the isomers is discussed in more detail in Sect. 5.

## 5. Discussion

The fire proxy records from Lake Petén Itzá represent fire history in lowland Guatemala during the entire Holocene. Here, we compare the fire history results with Holocene natural and human induced vegetation changes.

Before the transition into the Holocene, climate in Central American lowlands was probably drier and 4—8 °C cooler than during the early Holocene (e.g. Brenner et al., 2002; Hodell et al., 2008). At the transition into the Holocene the climate shifted to warmer and wetter conditions (Hodell et al., 2008), and the period from the early to the mid-Holocene (10—4.5 ka BP) was dominated by relatively stable warm and wet climate conditions (Hillesheim et al., 2005; Mueller et al., 2009). Pollen studies from Petén Department indicate that the vegetation shifted towards tropical forest from ~12.5 ka BP onwards, and that it was established by about 10 ka BP (e.g. Leyden et al., 1994; Hillesheim et al., 2005). Also based on pollen studies Islebe et al. (1996) found that a semi-evergreen and closed-forest (*Urticaceae* and *Moraceae*) was already established by ~8600 yr BP surrounding Lake Petén Itzá.

Fire activity was low during this transition of vegetation to tropical forest. The charcoal record demonstrates low values with a slightly increasing trend, whereas levoglucosan starts at low values at the very beginning of the Holocene but shows a substantially stronger increase during the early Holocene. The increasing fire

activity may be explained by advancing forestation and subsequent higher fuel abundance.

Different behaviour at different burning conditions might be a reason for differences between MA and charcoal concentrations in the sediment. It is known that levoglucosan generation peaks at 250 °C and is not present in char above 350 °C, and galactosan and mannosan generation is peaking at 200 °C, depending on the burned vegetation type (Kuo et al., 2008, 2011a). On the other hand, charcoal is assumed to be generated at temperatures between 250 °C—500 °C. The earlier increase of the molecular markers might therefore be a result of low temperature and smouldering fires prevailing in the region during the early Holocene, which is captured less efficiently by the sediment charcoal proxy. This would be in line with more opened stands in the highlands of Guatemala because it was typically drier there during that period. In open stands, low intensity fires are prevailing and would thus explain an earlier increase of the MAs compared to sedimentary charcoal.

The different catchment areas of MAs compared to charcoal may also explain the observed differences among the two proxies. Macroscopic charcoal is usually transported only a few hundred meters to a few kilometres and is thus mostly a local fire proxy (Whitlock and Larsen, 2001), whereas levoglucosan can be transported as aerosol hundreds of kilometres and is therefore a regional to hemispheric fire proxy which integrates supra-regional fires (Zennaro et al., 2014). Therefore, the earlier increase of the MAs compared to charcoal might also be attributed to an increased fire activity linked to the forestation over the entire region of Mesoamerica or southern North America and not only in the lowlands near Lake Petén Itzá. Regional charcoal syntheses demonstrate that early-Holocene fire activity was not homogenously distributed over Central and North America and depended instead on regional climate conditions and occupation history (Power et al., 2012; Marlon et al., 2013). At Lake Ixtacola, Mexico, charcoal starts to increase at approx. 9500 cal yr BP (Piperno et al., 2007), whereas in
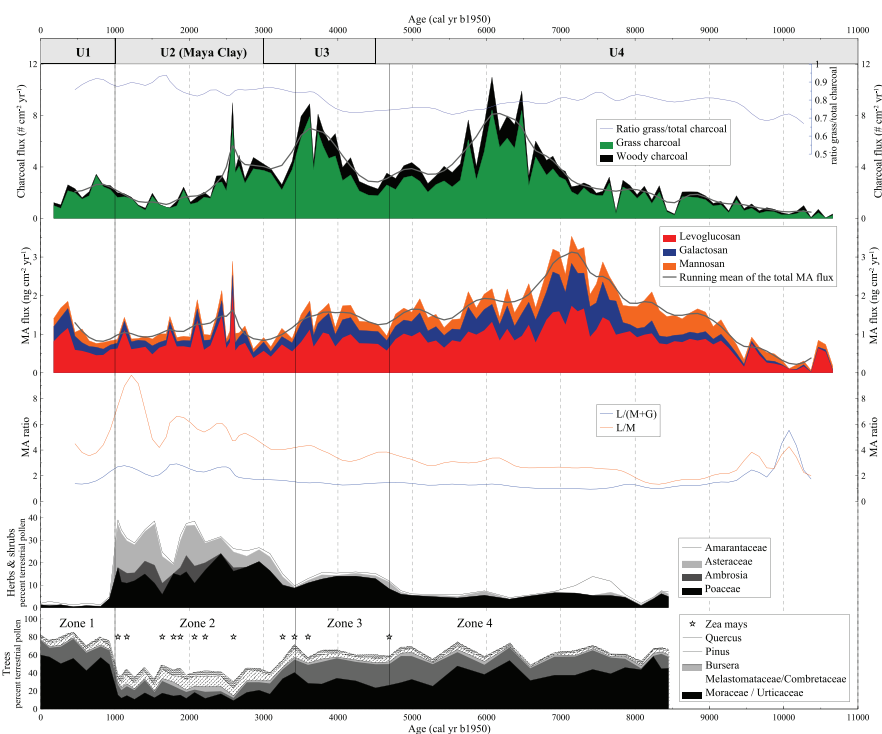
**Fig. 4.** Top: Grass charcoal (green) and woody charcoal (black), total charcoal corresponds to the upper envelope of the woody charcoal line. The ratio of grass to total charcoal is indicated as a blue line on a separate axis. The grey line indicates a 7-point Gaussian running mean of the total charcoal flux. Middle: Fluxes of MAs (red: levoglucosan, blue: galactosan, orange: mannosan). The grey line indicates a 7-point Gaussian running mean of the total MA flux. The ratio of levoglucosan/(mannosan + galactosan) (L/(M + G)) is shown as solid orange line, and the levoglucosan/mannosan (L/M) ratio is shown as solid blue line. Both ratios are indicated as 7-point Gaussian running means of the raw signal. Bottom: Pollen records from Lake Puerto Arturo (Wahl et al., 2006) are subdivided into grass and shrub pollen types (upper panel) and in arboreal pollen types (bottom panel). In the arboreal pollen panel, filled colours correspond to local trees, whereas hatched areas indicate disturbance arboreal types. Stars indicate core sections where *Zea mays* has been encountered. Pollen Zones as defined by Wahl et al. (2006) are indicated by black vertical lines, see text for details. On top the lithologic and lithostratigraphic units described in detail by Hillesheim et al. (2005) and Mueller et al. (2009) are indicated (U1: organic rich silt; U2: low organic content Maya Clay layer; U3: Deep water carbonate; U4: Organic-rich clay). All records are plotted linearly versus age. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

a core from the Upper Lerma, Mexico, charcoal maxima were detected between 7000 and 8500 cal yr BP (Lozano-García et al., 2005), and finally, Lozano-García et al. (2013) found charcoal peaks around 9200 and 8000 cal yr BP in western central Mexico.

An additional reason for different behaviour of the MAs and charcoal may be different sedimentation and/or post-depositional processes, like the redistribution by pore-water, the potential biodegradation (Xie et al., 2006) or degradation processes depending on different sediment types (e.g. low organic content vs. organic rich sediment), which, however, have not been studied so far. Recent laboratory experiments observed degradation of dissolved levoglucosan in water (Norwood et al., 2013), but previous studies assumed that MAs attached to particles are stable enough for sedimentation and stored over long time scales in lake sediments (Elias et al., 2001). These post-depositional processes would influence the MA concentrations over the entire core; however, such trends in the MA concentrations were not observed in the core studied here.

The relative abundance of the different isomers and in particular the ratios between levoglucosan/mannosan and levoglucosan/(mannosan + galactosan) may be due to the type of vegetation burning and the burning conditions, mainly temperature of the fire (Fabbri et al., 2009 and references therein; Alves et al., 2010 and references therein; Kuo et al., 2011a). Oros et al. (2006) and Oros and Simoneit (2001a, b) reported MA ratios between 2—33 (average 14) for different types of grasses and 0.2—8.2 (average 4.9)

for different types of hardwood; Engling et al. (2006) reported ratios of L/M = 108 and L/(M + G) = 54 for savannah grass, and L/M = 28 and L/(M + G) = 14 for hardwood (Oak), and Kuo et al. (2011a) reported ratios depending on the fire temperature. At the temperature of the levoglucosan production maximum (250 °C) cordgrass shows ratios of 36 for L/M and 13 for L/(M + G), respectively, and honey mesquite (hardwood) shows ratios of 4.4 for L/M and 1.3 for L/(M + G), respectively. Comparing all studies mentioned above, the reported ratios are overlapping, which limits conclusions about prevailing vegetation based on these ratios. Nevertheless, in all these studies grass shows higher MA ratios compared to hardwood. Considering this tendency, higher ratios in the present study may be an indication for an increased amount of grass being burned relative to wood, if not influenced by temperature. The MA ratios in our samples (Fig. 4) are highly variable in the early Holocene (10.5k—9k cal yr BP; L/M 0.4—11; L/(M + G) 0.5—15.5) and decrease until ~8200 cal yr BP, where they reach values of 1.2 (L/M) and 0.9 (L/(G + M)), respectively. The high variability might be due to the changing wet and dry cycles during this period as described by Hillesheim et al. (2005). However, in this section MA concentrations are close to or even at the detection limits of our analytical setup, therefore this part of the record should be interpreted carefully due to potential contamination and biases of the low concentration data. The ratios stabilise from 8000 cal yr BP onwards, when the vegetation has changed to more stable forest vegetation (Hillesheim et al., 2005).

The timing and sequence of Holocene fire history presented here correspond well with sediment charcoal from Lake Petén Itzá presented by Correa-Metrio et al. (2012) (entire Holocene), and Hillesheim et al. (2005) (10.5k–5k cal yr BP). The first maximum in the charcoal record and its corresponding levoglucosan peak around 6000 cal yr BP indicate the first local Holocene fire maximum detected in the Petén Itzá core. Correa-Metrio et al. (2012) proposed a period of high fire activity in Mesoamerica between 10.5k–5k cal yr BP and attribute the increasing fire activity to rising temperatures at the onset of the Holocene. In addition to higher temperatures Correa-Metrio et al. (2012) also link the fire activity to more pronounced seasonality related to the insolation maximum during this period which may have increased fuel availability and intensified convective storms facilitating ignition at the beginning of the wet season. Based on the absence of anthropogenic pollen indicators suggesting the presence of humans at Petén during that period (i.e. no *Zea mays*) (e.g. Leyden, 2002; Wahl et al., 2006), we attribute the change in the fire regime in the early to mid-Holocene, including the fire maximum around 6 ky BP, mainly to climate. The slow decrease of grass-like charcoal contribution to total charcoal abundance during this fire maximum is consistent with a gradual shift towards tropical high forest recorded in various pollen records (e.g. Islebe et al., 1996; Leyden, 2002; Mueller et al., 2009). Grass-like charcoal decrease does not seem to be affected by the extensive fire activity prevailing at that time. This decrease is also present in the pollen records (Islebe et al., 1996; Leyden, 2002; Wahl et al., 2006; Mueller et al., 2009).

During the relatively stable (in sense of temperature and moisture) mid-Holocene (~7–4.5 ka BP), the vegetation in Petén slowly shifted to more open, savannah-like type (e.g. Islebe et al., 1996; Leyden, 2002; Wahl et al., 2006), which may explain the slight increase of the MA ratios (L/M: from 2.7 to 3.2, L/(M + G): from 1.0 to 1.4) during this period. During the mid-Holocene, fire activity remains at a low level after the initial charcoal and levoglucosan peak, until abruptly increasing before 4000 cal yr BP. At the beginning of this increase in fire activity the abundance of grass-like charcoal is relatively low (70–80 % of total charcoal) (Fig. 4, top panel). In contrast to the first fire maximum (6500–5500 cal yr BP) where the contribution of grass-like charcoal to total charcoal counts decreases gradually, the grass-like charcoal percentage increases abruptly from below 75% to above 85% within less than 300 years simultaneously with this second distinct fire maximum around 3700 cal yr BP.

The rapid increase in grass-like charcoal at 3700 cal yr BP coincides with the fire maximum and a relatively fast shift from mostly tree towards herb and shrub pollen as observed by Islebe et al. (1996) and Mueller et al. (2009). This increase in herbaceous pollen types is also visible in the pollen record of Lake Puerto Arturo in northern Petén about 70 km away from Lake Petén Itzá (Wahl et al., 2006) shown in Fig. 4. The four zones in the pollen diagram defined by Wahl et al. (2006) correspond to periods of high arboreal pollen abundance (zone 4, older than 4700 cal yr BP), the start of a significant increase in herbaceous pollen types (zone 3, 4700–3400 cal yr BP), amplification of the shift towards more herbaceous and less arboreal pollen types and presence of anthropogenic indicators (*Z. mays* and *Ambrosia*) (zone 2, 3400–1000 cal yr BP), and an abrupt change from herbaceous and disturbance taxa back to arboreal pollen types (zone 1, after 1000 cal yr BP). The above mentioned abrupt shift in the charcoal types corresponds to the change in the pollen profile from arboreal towards more herbaceous pollen types in the transition from zone 3 into zone 2. Considering the age uncertainties of the two sediment cores (approx. 500 yr of two sigma age range at 3200 cal yr BP) in the Lake Puerto Arturo sediment (Wahl et al., 2006) and 100 yr at 3500 cal yr BP in the Petén Itzá 11B core, the abrupt shifts

in the pollen records and in the fire proxy records are likely to happen simultaneously. Also the distinct decrease in *Brosimum* (Mueller et al., 2009) (not shown) fits temporally to the rapid change in the vegetation seen in the other records. This shift in the pollen records and the increase of grass-like charcoal coincide also with an increase of the MA ratios in this period (L/M up to 5.2, L/(M + G) up to 1.7) and might be a result of enhanced burning of grass-like vegetation. Local climate became drier in the period 4.5–3 ka BP (Mueller et al., 2009), which may have exacerbated the effect of human impact on vegetation. However, the shift towards drier climate and was a gradual process persistent during ~1.5 ka, whereas the changes in the pollen records and the distinct increase in grass-like charcoal took place within a few hundred years.

Initial evidence of agricultural activities in the Maya lowlands are dated to 4000–5000 BP, based on pollen studies where the increase of disturbance taxa (e.g. *Asteraceae* and *Ambrosia*) and of secondary forest taxa is attributed to forest clearance or is based on the presence of *Z. mays* which is an indicator for agricultural cultivation (e.g. Pope et al., 2001; Leyden, 2002; Wahl et al., 2006). Soil erosion histories point to a similar timing for the first appearance of human perturbation in the ecological system due to vegetation transformation (Anselmetti et al., 2007). The presence of a Maya Clay layer is a strong evidence for the presence of Maya settlements in the watershed of an investigated lake. In different lakes from the Petén region, such layers are present from about 3500 to 800 yr BP (Rosenmeier et al., 2002; Anselmetti et al., 2006; Mueller et al., 2009). The fact that the appearance of first human agricultural activities in the Maya lowlands is older than 4 ka BP indicates that the high fire activity at 3.7 ka BP may be attributed to humans rather than only climate. The presence of Maya clay layers in Petén lakes from ~3.5 ka BP onwards along with declining arboreal pollen abundance indicates that a considerable change in the vegetation occurred during that time. The coincident high fire activity provides a strong inference that fire clearance by early Maya populations may have started at this time. In general it is easier for humans to initiate fires in a drier environment, where the accompanying shift to a drier climate could have also favoured human-induced fires in this period. However, the first *Z. mays* pollen in Lake Petén Itzá or Lake Salpetén were found only after 3 ka BP (Leyden, 2002; Mueller et al., 2009). Thus, if the high fire activity in the area of Lake Petén Itzá around 3.7 ka BP is attributed to fire clearance by the Maya, this clearance would have happened considerably before the Maya started agricultural cultivation in this area. However, in Lake Puerto Arturo a single *Z. mays* grain was found at 4600 cal yr BP (Wahl et al., 2006), thus considerably earlier than in Lake Petén Itzá (Fig. 4, bottom panel). Wahl et al. (2006) discussed the early appearance of *Z. mays* at lake Puerto Arturo in the context of other early *Z. mays* appearances from coastal Veracruz around 7100 cal yr BP (Pope et al., 2001) and from nearby Belize as early as 5500 cal yr BP (Pohl et al., 1996). Another explanation for the different timing of the first appearance of *Z. mays* mentioned by Wahl et al. (2006) are the different sizes of the investigated lakes. Lake Puerto Arturo is small compared to Lake Petén Itzá, therefore the large size of the Petén lakes may have reduced the visibility of the weak *Z. mays* signal at 4600 cal yr BP. In summary, we conclude that the high fire activity in the Maya lowlands around 4000–3500 cal yr BP cannot be attributed clearly to either climate or human activities based on the data presented in this study and is likely to be influenced by both climate and humans.

The last of the three fire maxima at 2.7 ka BP observed in our proxy records lies well within the Maya Clay layer and corresponds with the maximum in the soil erosion rate (Anselmetti et al., 2007). Permanent Maya settlements and agriculture were established in the entire Maya lowlands at the mid-Preclassic period (about

2900–2400 yr BP). Even though the Maya population increased significantly only from the late-Preclassic onwards (2400–1750 yr BP), the local fire activity remains low after 2400 yr BP. This low fire activity can be explained by the low abundance of trees, seen e.g. in the low wood charcoal percentage as well as from the pollen assemblages from the Petén region (Fig. 4). Wahl et al. (2006) denoted the period from 3400 to 1000 cal yr BP as Zone 2 which is characterised by very low abundance of arboreal pollen and dominated by herbaceous and shrub pollen types and the presence of agricultural indicators (Z. mays and Ambrosia) (Fig. 4). In Zone 2 we also detected the lowest abundances of wood charcoal, i.e. total charcoal is dominated by grass like pieces (>90% in 2000–1000 cal yr BP), which is in line with the pollen records. Such grassland vegetation negates the need for extensive fire clearance. However, the regional fire signal (levoglucosan, see Fig. 4) remains at an elevated level with a steadily decreasing trend, which might be caused by a further expansion of the Maya settlements at the borders of the Maya territories. These additional fires would only be revealed in the levoglucosan and not in the charcoal record due to the different catchment areas of the two fire proxies. In addition, MA ratios demonstrate an elevated level (L/M is between 3 and 10, L/(M + G) is between 1.5 and 3.5) and high variability during this period. This variability may be an indication of a disturbance of the natural fire activity by the Maya by influencing e.g. type of burned biomass or fire conditions and intensity, which results in different MA ratios (Engling et al., 2006; Kuo et al., 2011a), because the period before the assumed arrival of the Maya at Lake Petén Itzá was characterized by quite stable (levoglucosan/ (mannosan + galactosan)) or only slowly increasing (levoglucosan/ mannosan) MA ratios, respectively. Based on the evidence as described in this paragraph, we attribute the elevated fire activity at 2.7 cal ka BP to fire clearance by the Maya.

After the Classic Maya collapse (~900 yr BP) the fire activity remains at a low level, probably accompanied by a natural forestation and, thus, build-up of fuel. As a consequence the wood charcoal percentage increases after 800 yr BP (Fig 4). After ~600 yr BP the fire activity starts to increase again, this is evident especially in the levoglucosan record. Also the MA ratios recover after the Maya collapse and they decrease towards values as before 4000 cal yr BP, clearly reproducing the rapid transition from mainly herbaceous to primarily arboreal pollen abundance as observed in the pollen records.

## 6. Conclusions

We provide a detailed reconstruction of biomass burning trends from the southern Maya Lowlands from a Lake Petén Itzá sediment core using two different fire proxies: macroscopic charcoal and the monosaccharide anhydrates levoglucosan, mannosan and galactosan. The combination of different fire proxies offers new insights into the fire history during the Holocene at different spatial scales across the Maya lowlands.

The Holocene fire record demonstrates a first period of high fire activity between 9000 and 6000 cal yr BP, which is attributed to climate conditions and corroborates corresponding results from previous studies. The fire maximum at 2700 cal yr BP can be linked to the agricultural activity of the Maya at Lake Petén Itzá. The fire maximum around 3700 cal yr BP might already be influenced by early anthropogenic agricultural activities in the region, based on the fast decrease in the relative abundance of wooden charcoal during the fire maximum. However, we cannot exclude that this fire maximum and the accompanying shift in the vegetation type were a result of the dry climate conditions facilitating ignition. To further constrain the causes for the high fire activity around 3700 cal yr BP

further investigations including several lake cores from this area with highly resolved data are needed.

An advantage of analysing MAs together with charcoal in lake sediments is the potential discrimination of different catchment areas of the proxies: While macroscopic charcoal represents a local fire signal, the MA record is influenced by regional to supra-regional fires, thus overlapping the spatial scale covered by microscopic charcoal (Whitlock and Larsen, 2001; Colombaroli et al., 2008; Conedera et al., 2009). Furthermore, higher concentrations in MAs might indicate that low temperature fires (<350 °C) where prevailing, while charcoal production is more efficient at temperatures between 250° and 500 °C. Analysing all three isomers enables the calculation of L/M and L/(M + G) ratios, which may help to estimate changes in types of burned vegetation. However, such conclusions are limited to the identification of general vegetation changes (wood vs. grass) and cannot identify specific vegetation compositions due to their temperature dependence and due to the large ranges of these ratios reported in the literature. Nevertheless, the promising correlation between the Petén Itzá MA ratios, pollen assemblages, and plant macrofossils demonstrates the added value of these molecular markers in paleo fire reconstructions.

## Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.quascirev.2015.03.004.

## References

Alves, C.A., Gonçalves, C., Evtyugina, M., Pio, C.A., Mirante, F., Puxbaum, H., 2010. Particulate organic compounds emitted from experimental wildland fires in a Mediterranean ecosystem. Atmos. Environ. 44, 2750–2759.

Anselmetti, F.S., Ariztegui, D., Hodell, D.A., Hillesheim, M.B., Brenner, M., Gilli, A., McKenzie, J.A., Mueller, A.D., 2006. Late Quaternary climate-induced lake level variations in Lake Petén Itzá, Guatemala, inferred from seismic stratigraphic analysis. Palaeogeogr. Palaeoclimatol. Palaeoecol. 230, 52–69.

Anselmetti, F.S., Hodell, D.A., Ariztegui, D., Brenner, M., Rosenmeier, M.F., 2007. Quantification of soil erosion rates related to ancient Maya deforestation. Geology 35, 915–918.

Beffa, G., 2012. Analysis of Sediment Charcoal Morphotypes from Gorgo Basso (Sicily). Institute of Plant Sciences. University of Bern, pp. 1–13 (Bachelor thesis).

Blaauw, M., 2010. Methods and code for 'classical' age-modelling of radiocarbon sequences. Quat. Geochronol. 5, 512–518.

Bowman, D.M.J.S., Balch, J.K., Artaxo, P., Bond, W.J., Carlson, J.M., Cochrane, M.A., D'Antonio, C.M., DeFries, R.S., Doyle, J.C., Harrison, S.P., Johnston, F.H., Keeley, J.E., Krawchuk, M.A., Kull, C.A., Marston, J.B., Moritz, M.A., Prentice, I.C., Roos, C.I., Scott, A.C., Swetnam, T.W., van der Werf, G.R., Pyne, S.J., 2009. Fire in the earth system. Science 324, 481–484.

Brenner, M., Rosenmeier, M.F., Hodell, D.A., Curtis, J.H., 2002. Paleolimnology of the Maya Lowlands – long-term perspectives on interactions among climate, environment, and humans. Anc. Mesoam. 13, 141–157.

Carcaillet, C., Almquist, H., Asnong, H., Bradshaw, R.H.W., Carrión, J.S., Gaillard, M.J., Gajewski, K., Haas, J.N., Haberle, S.G., Hadorn, P., Müller, S.D., Richard, P.J.H., Richoz, I., Rösch, M., Sánchez Goñi, M.F., von Stedingk, H., Stevenson, A.C., Talon, B., Tardy, C., Tinner, W., Tryterud, E., Wick, L., Willis, K.J., 2002. Holocene

biomass burning and global dynamics of the carbon cycle. Chemosphere 49, 845—863.

Colombaroli, D., Ssemmanda, I., Gelorini, V., Verschuren, D., 2014. Contrasting long-term records of biomass burning in wet and dry savannas of equatorial East Africa. Glob. Change Biol. 20, 2903—2914.

Colombaroli, D., Vannière, B., Emmanuel, C., Magny, M., Tinner, W., 2008. Fire-vegetation interactions during the Mesolithic-Neolithic transition at Lago del-l'Accesa, Tuscany, Italy. Holocene 18, 679—692.

Conedera, M., Tinner, W., Neff, C., Meurer, M., Dickens, A.F., Krebs, P., 2009. Reconstructing past fire regimes: methods, applications, and relevance to fire management and conservation. Quat. Sci. Rev. 28, 555—576.

Correa-Metrio, A., Bush, M.B., Cabrera, K.R., Sully, S., Brenner, M., Hodell, D.A., Escobar, J., Guilderson, T., 2012. Rapid climate change and no-analog vegetation in lowland Central America during the last 86,000 years. Quat. Sci. Rev. 38, 63—75.

Curtis, J.H., Brenner, M., Hodell, D.A., Balser, R.A., Islebe, G.A., Hooghiemstra, H., 1998. A multi-proxy study of Holocene environmental change in the Maya Lowlands of Peten, Guatemala. J. Paleolimnol. 19, 139—159.

Deevey, E.S., Brenner, M., Flannery, M.S., Yezdani, G.H., 1980. Lakes Yaxha and Sacnab, Petén, Guatemala: limnology and hydrology. Arch. Hydrobiol. 57, 419—460.

Elias, V.O., Simoneit, B.R.T., Cordeiro, R.C., Turcq, B., 2001. Evaluating levoglucosan as an indicator of biomass burning in Carajás, amazônia: a comparison to the charcoal record. Geochim. Cosmochim. Acta 65, 267—272.

Engling, G., Carrico, C.M., Kreidenweis, S.M., Collett Jr., J.L., Day, D.E., Malm, W.C., Lincoln, E., Min Hao, W., Iinuma, Y., Herrmann, H., 2006. Determination of levoglucosan in biomass combustion aerosol by high-performance anion-exchange chromatography with pulsed amperometric detection. Atmos. Environ. 40 (Suppl. 2), 299—311.

Fabbri, D., Torri, C., Simoneit, B.R.T., Marynowski, L., Rushdi, A.I., Fabiańska, M.J., 2009. Levoglucosan and other cellulose and lignin markers in emissions from burning of Miocene lignites. Atmos. Environ. 43, 2286—2295.

Haug, G.H., Günther, D., Peterson, L.C., Sigman, D.M., Hughen, K.A., Aeschlimann, B., 2003. Climate and the collapse of Maya civilization. Science 299, 1731—1735.

Hillesheim, M.B., Hodell, D.A., Leyden, B.W., Brenner, M., Curtis, J.H., Anselmetti, F.S., Ariztegui, D., Buck, D.G., Guilderson, T.P., Rosenmeier, M.F., Schnurrenberger, D.W., 2005. Climate change in lowland Central America during the late deglacial and early Holocene. J. Quat. Sci. 20, 363—376.

Hodell, D.A., Anselmetti, F.S., Ariztegui, D., Brenner, M., Curtis, J.H., Gilli, A., Grzesik, D.A., Guilderson, T.J., Müller, A.D., Bush, M.B., Correa-Metrio, A., Escobar, J., Kutterolf, S., 2008. An 85-ka record of climate change in lowland Central America. Quat. Sci. Rev. 27, 1152—1165.

Islebe, G.A., Hooghiemstra, H., Brenner, M., Curtis, J.H., Hodell, D.A., 1996. A Holocene vegetation history from lowland Guatemala. Holocene 6, 265—271.

Jensen, K., Lynch, E.A., Calcote, R., Hotchkiss, S.C., 2007. Interpretation of charcoal morphotypes in sediments from Ferry Lake, Wisconsin, USA: do different plant fuel sources produce distinctive charcoal morphotypes? Holocene 17, 907—915.

Kennett, D.J., Breitenbach, S.F.M., Aquino, V.V., Asmerom, Y., Awe, J., Baldini, J.U.L., Bartlein, P., Culleton, B.J., Ebert, C., Jazwa, C., Macri, M.J., Marwan, N., Polyak, V., Prufer, K.M., Ridley, H.E., Sodemann, H., Winterhalder, B., Haug, G.H., 2012. Development and disintegration of Maya political systems in response to climate change. Science 338, 788—791.

Kirchgeorg, T., Schüpbach, S., Kehrwald, N., McWethy, D.B., Barbante, C., 2014. Method for the determination of specific molecular markers of biomass burning in lake sediments. Org. Geochem. 71, 1—6.

Kuo, L.-J., Herbert, B.E., Louchouarn, P., 2008. Can levoglucosan be used to characterize and quantify char/charcoal black carbon in environmental media? Org. Geochem. 39, 1466—1478.

Kuo, L.-J., Louchouarn, P., Herbert, B.E., 2011a. Influence of combustion conditions on yields of solvent-extractable anhydrosugars and lignin phenols in chars: Implications for characterizations of biomass combustion residues. Chemosphere 85, 797—805.

Kuo, L.-J., Louchouarn, P., Herbert, B.E., Brandenberger, J.M., Wade, T.L., Crecelius, E., 2011b. Combustion-derived substances in deep basins of Puget Sound: historical inputs from fossil fuel and biomass combustion. Environ. Pollut. 159, 983—990.

Leyden, B.W., 2002. Pollen evidence for climatic variability and culturaldisturbance in the Maya Lowlands. Anc. Mesoam. 13, 85—101.

Leyden, B.W., Brenner, M., Hodell, D.A., Curtis, J.H., 1994. Orbital and internal forcing of climate on the Yucatan Peninsula for the past ca. 36 ka. Palaeogeogr. Palaeoclimatol. Palaeoecol. 109, 193—210.

Lozano-García, S., Sosa-Nájera, S., Sugiura, Y., Caballero, M., 2005. 23,000 yr of vegetation history of the Upper Lerma, a tropical high-altitude basin in Central Mexico. Quat. Res. 64, 70—82.

Lozano-García, S., Torres-Rodríguez, E., Ortega, B., Vázquez, G., Caballero, M., 2013. Ecosystem responses to climate and disturbances in western central Mexico during the late Pleistocene and Holocene. Palaeogeogr. Palaeoclimatol. Palaeoecol. 370, 184—195.

Marlon, J.R., Bartlein, P.J., Daniau, A.-L., Harrison, S.P., Maezumi, S.Y., Power, M.J., Tinner, W., Vannière, B., 2013. Global biomass burning: a synthesis and review of Holocene paleofire records and their controls. Quat. Sci. Rev. 65, 5—25.

Mueller, A.D., Islebe, G.A., Hillesheim, M.B., Grzesik, D.A., Anselmetti, F.S., Ariztegui, D., Brenner, M., Curtis, J.H., Hodell, D.A., Venz, K.A., 2009. Climate drying and associated forest decline in the lowlands of northern Guatemala during the late Holocene. Quat. Res. 71, 133—141.

Norwood, M.J., Louchouarn, P., Kuo, L.-J., Harvey, O.R., 2013. Characterization and biodegradation of water-soluble biomarkers and organic carbon extracted from low temperature chars. Org. Geochem. 56, 111—119.

Oros, D.R., Abas, M.R.b., Omar, N.Y.M.J., Rahman, N.A., Simoneit, B.R.T., 2006. Identification and emission factors of molecular tracers in organic aerosols from biomass burning: part 3. Grasses. Appl. Geochem. 21, 919—940.

Oros, D.R., Simoneit, B.R.T., 2001a. Identification and emission factors of molecular tracers in organic aerosols from biomass burning Part 1. Temperate climate conifers. Appl. Geochem. 16, 1513—1544.

Oros, D.R., Simoneit, B.R.T., 2001b. Identification and emission factors of molecular tracers in organic aerosols from biomass burning Part 2. Deciduous trees. Appl. Geochem. 16, 1545—1565.

Piperno, D.R., Moreno, J.E., Iriarte, J., Holst, I., Lachniet, M., Jones, J.G., Ranere, A.J., Castanzo, R., 2007. Late Pleistocene and Holocene environmental history of the Iguala Valley, Central Balsas watershed of Mexico. Proc. Natl. Acad. Sci. 104, 11874—11881.

Pohl, M.D., Pope, K.O., Jones, J.G., Jacob, J.S., Piperno, D.R., deFrance, S.D., Lentz, D.L., Gifford, J.A., Danforth, M.E., Josserand, J.K., 1996. Early agriculture in the Maya lowlands. Lat. Am. Antiq. 7, 355—372.

Pohl, M.E.D., Piperno, D.R., Pope, K.O., Jones, J.G., 2007. Microfossil evidence for pre-Columbian maize dispersals in the neotropics from San Andrés, Tabasco, Mexico. Proc. Natl. Acad. Sci. U. S. A. 104, 6870—6875.

Pope, K.O., Pohl, M.E.D., Jones, J.G., Lentz, D.L., Nagy, C.v., Vega, F.J., Quitmyer, I.R., 2001. Origin and environmental setting of ancient agriculture in the lowlands of Mesoamerica. Science 292, 1370—1373.

Power, M., Mayle, F., Bartlein, P., Marlon, J., Anderson, R., Behling, H., Brown, K., Carcaillet, C., Colombaroli, D., Gavin, D., Hallett, D., Horn, S., Kennedy, L., Lane, C., Long, C., Moreno, P., Paitre, C., Robinson, G., Taylor, Z., Walsh, M., 2012. Climatic control of the biomass-burning decline in the Americas after ad 1500. Holocene 23, 3—13.

Reimer, P.J., Bard, E., Bayliss, A., Beck, J.W., Blackwell, P.G., Bronk Ramsey, C., Buck, C.E., Edwards, R.L., Friedrich, M., Grootes, P.M., Guilderson, T.P., Haflidason, H., Hajdas, I., Hatté, C., Heaton, T.J., Hoffmann, D.L., Hogg, A.G., Hughen, K.A., Kaiser, K.F., Kromer, B., Manning, S.W., Niu, M., Reimer, R.W., Richards, D.A., Scott, E.M., Southon, J.R., Turney, C.S.M., van der Plicht, J., 2013. IntCal13 and Marine13 radiocarbon age calibration curves, 0—50,000 years cal BP. Radiocarbon 55, 1869—1887.

Rice, D.S., Rice, P.M., Pugh, T., 1998. Settlement Continuity and Change in the Central Peten Lakes Region: The Case of Zacpeten. In: Ruiz, A.C., et al. (Eds.), Anatomia de una Civilizacion: Aproximaciones Interdisciplinarias a la Cultura Maya. Sociedad Española de Estudios Mayas, Madrid, Spain, ISBN 978-84-923545-0-4, pp. 207—252.

Rosenmeier, M.F., Hodell, D.A., Brenner, M., Curtis, J.H., Guilderson, T.P., 2002. A 4000-year lacustrine record of environmental change in the southern Maya lowlands, Petén, Guatemala. Quat. Res. 57, 183—190.

Ruddiman, W.F., 2003. The anthropogenic greenhouse era began thousands of years ago. Clim. Change 61, 261—293.

Umbanhowar, C.E., Mcgrath, M.J., 1998. Experimental production and analysis of microscopic charcoal from wood, leaves and grasses. Holocene 8, 341—346.

Wahl, D., Byrne, R., Schreiner, T., Hansen, R., 2006. Holocene vegetation change in the northern Peten and its implications for Maya prehistory. Quat. Res. 65, 380—389.

Whitlock, C., Larsen, C., 2001. Charcoal as a fire proxy. In: Smol, J.P., Birks, H.J.B., Last, W.M. (Eds.), Tracking Environmental Change Using Lake Sediments. Kluwer Academic Publishers, Dordrecht, The Netherlands, pp. 1—23.

Xie, H., Zhuang, X., Bai, Z., Qi, H., Zhang, H., 2006. Isolation of levoglucosan-assimilating microorganisms from soil and an investigation of their levoglucosan kinases. World J. Microbiol. Biotechnol. 22, 887—892.

Zennaro, P., Kehrwald, N., McConnell, J.R., Schüpbach, S., Maselli, O.J., Marlon, J., Vallelonga, P., Leuenberger, D., Zangrando, R., Spolaor, A., Borrotti, M., Barbaro, E., Gambaro, A., Barbante, C., 2014. Fire in ice: two millennia of boreal forest fire history from the Greenland NEEM ice core. Clim. Past 10, 1905—1924.

## 2.1  Supplementary Information of the article: Combining charcoal sediment and molecular markers to infer a Holocene fire history in the Maya lowlands of Petén, Guatemala

**Supplementary Information of the article:**

**Combining charcoal sediment and molecular markers to infer a Holocene**

**fire history in the Maya lowlands of Petén, Guatemala**

Simon Schüpbach[a, b, c], Torben Kirchgeorg[a,d], Daniele Colombaroli[c,e], Giorgia Beffa[c,e], Marta Radaelli[a], Natalie Kehrwald[a], Carlo Barbante[a,f,g]


[a] Department of Environmental Sciences, Informatics and Statistics, University Ca'Foscari, Venice, 30123, Italy

[b] Climate and Environmental Physics, Physics Institute, University of Bern, Bern, 3012, Switzerland

[c] Oeschger Centre for Climate Change Research, University of Bern, Bern, 3012, Switzerland

[d] Institute of Sustainable and Environmental Chemistry, Leuphana University of Lüneburg, Lüneburg, 21335, Germany

[e] Institute of Plant Sciences, University of Bern, Bern, 3013, Switzerland

[f] Institute for the Dynamics of Environmental Processes-CNR, Venice, 30123 Italy

[g] Centro Linceo B. Segre, Accademia Nazionale dei Lincei, Rome, 00165, Italy

## 1      Analytical method

For a better separation of the three isomers, following parameters of the method by Kirchgeorg et al. (2014) have been adjusted. A different column (MA1, Thermo Scientific, 2 x 250 mm) with a slightly modified eluent gradient was used for a better separation. The MA1 column shows enhanced separation of the three isomers, with retention times of 6.5, 10.2, and 17.7 min for levoglucosan, mannosan, and galactosan, respectively. A gradient of NaOH was used (20 mM (0-20 min), 100 mM (20-40 min), 20 mM (40 – 60 min). In addition, the injection volume was increased from 25 uL to 50 uL. Selected Ion Monitoring (SIM) mode was used in the MS instrument to improve detection limits of levoglucosan, mannosan and galactosan (all m/z 161) and the internal standard (m/z 167).

The detection limits for levoglucosan, mannosan and galactosan were 1.3, 0.4, and 0.4 ng mL$^{-1}$ and method quantification limits were 4.4, 1.2, and 1.4 ng mL$^{-1}$, respectively, calculated based on a signal to noise ratio of 3 and 10 in the sediment samples, respectively.

## 2      Alternative age models and their effect on the flux records

In this section we demonstrate the results of alternative age model simulations. Alternatives to the one model used are: 1) Same C14 dates, different smoothing factor, and 2) Including all C14 dates, i.e. also the charcoal dating point which was discarded in the age model used in the main text. For calculating the age model the same CLAM Version 2.2 (Blaauw, 2010) was used, again with 1000 iterations, and calibrated with the northern hemisphere terrestrial calibration curve from Reimer et al. (2013).

An additional source of uncertainties in the depth-age relationship of core 11B is the projection of ages dated on a parallel core (11A) onto the 11B core. Especially in the lower part of the core this might introduce artefacts in the age model, which is illustrated in the following by different age models including only selected age markers.

The original age markers were originally published and described in detail by (Hillesheim et al., 2005) and (Mueller et al., 2009).
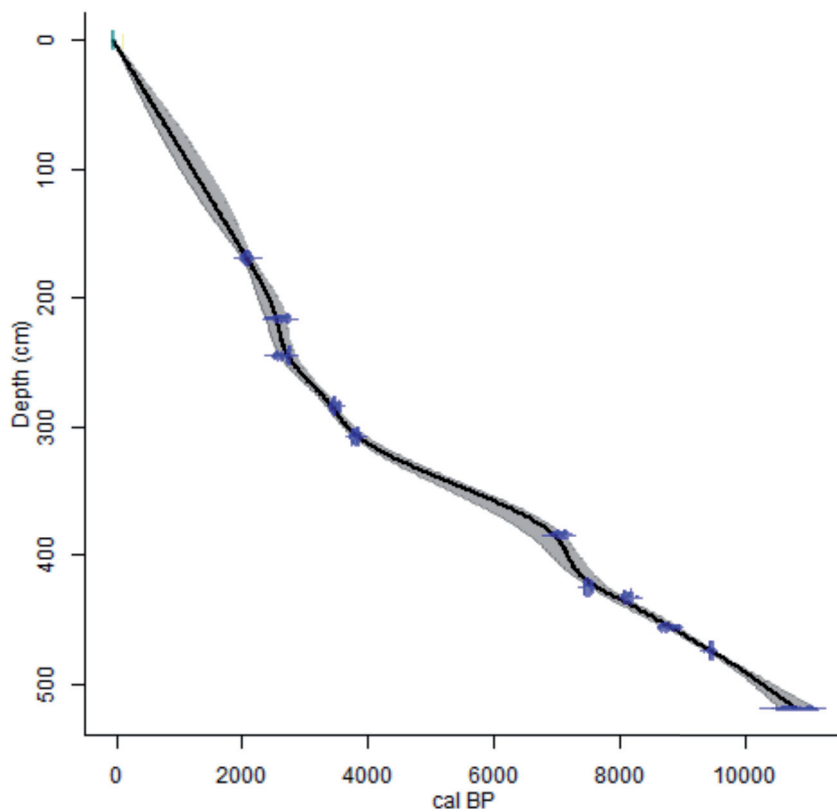


**Figure S1** The same C14 dating points as in the main text are included in this age model. However, here we applied a smoothing factor of 0.2 on the entire core. Thus, the top 280 cm of the core are unchanged, but the deeper part now shows a less straightened depth-age relation. Especially around 400 cm depth, the age model shows an excursion to reach the C14 date at 385 cm depth.
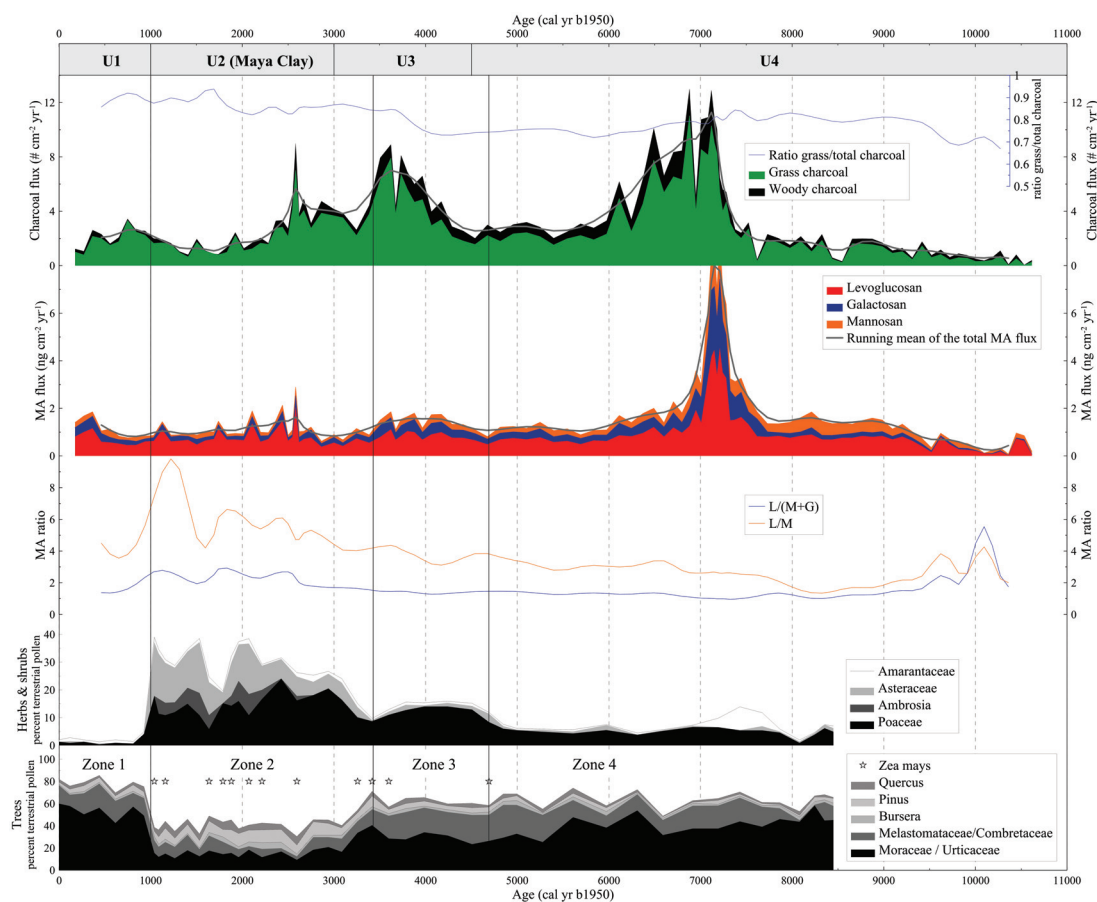
**Figure S2** The fluxes shown in Fig. 4 of the main text are now shown applying the age model shown in Fig. S1. It is evident that an artefact is introduced by this age model around 7 kyr BP. The charcoal and MA peaks are well in phase here, which is not surprising since the flux is entirely controlled by the deposition rate and no longer by the concentrations of the analysed proxies in the samples.
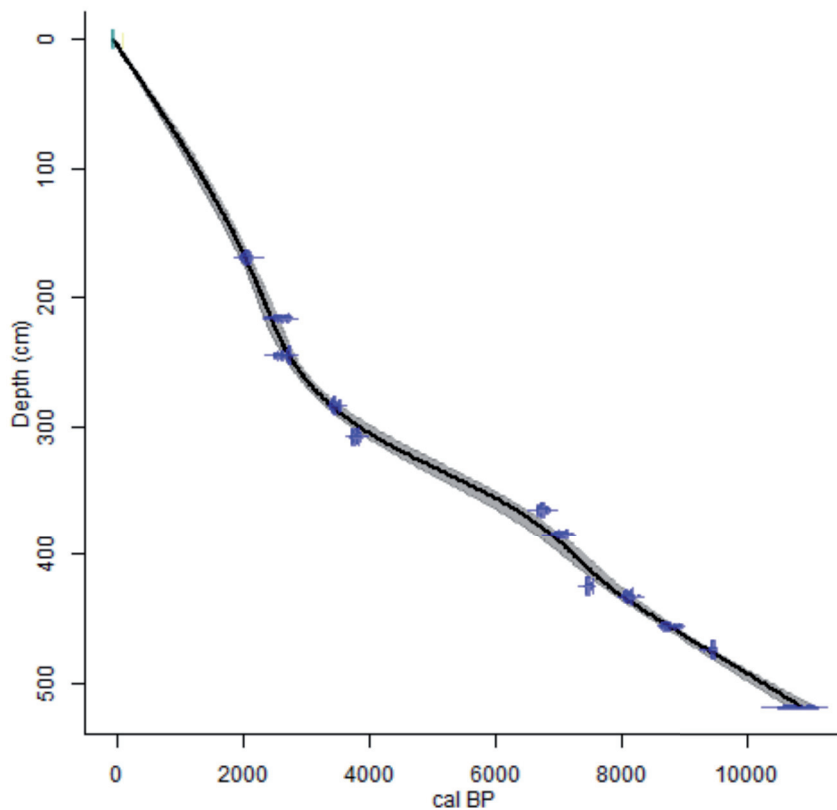
**Figure S3** Here we also introduced the discarded charcoal C14 dating point in the age model. The spline is applied with a smoothing factor of 0.4 on the entire core. Again, the main difference appears around 400 cm depth where the age model shows an excursion, now even with the stronger smoothing factor of 0.4 to reach the C14 dates at 384 cm and 365 cm depth, respectively.
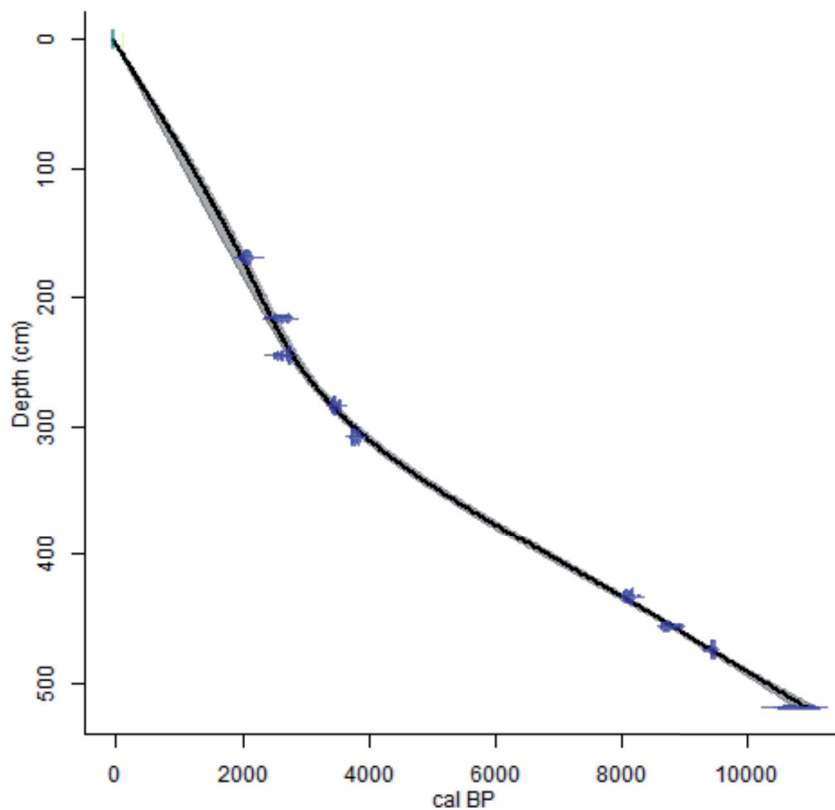
**Figure S4** Here we discarded the three points 365, 384, and 425 cm depth, which are all three at the edge of the age model with the spline applying a smooth factor of 0.4. Howerver, as there is no objective reason to discard the points at 384 and 425 cm depth (the point at 365 cm depth is dated on charcoal, and thus already discarded in the main text), they are both kept in the age model applied in the manuscript. Nevertheless, the age model used in the main text is very similar in the deeper part to the one shown here, since one of the points not shown here is overestimating the age (at 384 cm depth) and the other underestimating it (at 425 cm depth), and, thus, their effect on the age model is averaged out partly.

## References of the Supporting Information

Blaauw, M., 2010. Methods and code for 'classical' age-modelling of radiocarbon sequences. Quaternary Geochronology 5, 512-518.

Hillesheim, M.B., Hodell, D.A., Leyden, B.W., Brenner, M., Curtis, J.H., Anselmetti, F.S., Ariztegui, D., Buck, D.G., Guilderson, T.P., Rosenmeier, M.F., Schnurrenberger, D.W., 2005. Climate change in lowland Central America during the late deglacial and early Holocene. Journal of Quaternary Science 20, 363-376.

Mueller, A.D., Islebe, G.A., Hillesheim, M.B., Grzesik, D.A., Anselmetti, F.S., Ariztegui, D., Brenner, M., Curtis, J.H., Hodell, D.A., Venz, K.A., 2009. Climate drying and associated forest decline in the lowlands of northern Guatemala during the late Holocene. Quaternary Research 71, 133-141.

Reimer, P.J., Bard, E., Bayliss, A., Beck, J.W., Blackwell, P.G., Bronk Ramsey, C., Buck, C.E., Edwards, R.L., Friedrich, M., Grootes, P.M., Guilderson, T.P., Haflidason, H., Hajdas, I., Hatté, C., Heaton, T.J., Hoffmann, D.L., Hogg, A.G., Hughen, K.A., Kaiser, K.F., Kromer, B., Manning, S.W., Niu, M., Reimer, R.W., Richards, D.A., Scott, E.M., Southon, J.R., Turney, C.S.M., van der Plicht, J., 2013. IntCal13 and Marine13 radiocarbon age calibration curves, 0-50'000 years cal BP. Radiocarbon 55, 1869-1887.

# 3 Study III: Late Holocene record of humans and fire at Lake Trasimeno (Italy): An organic molecular marker approach

**- unpublished manuscript -**

## Abstract

The late Holocene history of human impact and regional fire activity in central Italy was investigated by the analysis of specific organic molecular makers in a sediment core collected from Lake Trasimeno, Italy. Fire history was reconstructed using levoglucosan as a tracer for biomass burning resulting in fire peaks at 2600-3000 cal yr BP, 2250 cal yr BP, 1750 cal yr BP, 1000-750 cal yr BP and 450-0 cal yr BP. Although levoglucosan does not always positively correlate with fecal sterols as a proxy for human presence near the lake, regional fire activity may relate to human activity. The most prominent human impact on the lake system, as evident from peak fecal sterol concentrations, occurred during the Roman occupation, including the period of the battle of Trasimeno (217 BC) and Perusine War (40-42 BC). However, fire activity was relatively low during the Roman occupation, which may be due to the decrease in fuel availability from previous forest clearance, or from fire suppression due to Roman land management. The study demonstrates that organic molecular markers provide valuable information about interactions between humans and fire.

## Introduction

Disentangling the interactions between fire, humans and climate remains a challenging question in paleofire research. Reconstructing fire history from lake sediment cores is well established by charcoal analyses (Conedera et al., 2009; Whitlock and Larsen, 2001). Recent approaches analyzed monosaccharide anhydrates and particularly levoglucosan (Lvg) as a molecular proxy for biomass burning in lake sediments (Elias et al., 2001; Kirchgeorg et al., 2014; Kuo et al., 2011; Schüpbach et al., 2015). Levoglucosan is generated in high concentrations only during cellulose combustion and is thus a specific proxy for biomass burning in contrast with other pyrolysis markers (e.g. polycyclic aromatic hydrocarbons and black carbon) which can also originate from other combustion sources (Simoneit, 2002). The stability of levoglucosan is still discussed where at least a portion of dissolved levoglucosan in the water phase degrades in laboratory experiments (Norwood et al., 2013). However, many studies report that levoglucosan was detected in sediments up to 20 kyr before present and suggest that at a minimum sediments archive particular-bound levoglucosan over long time scales (Elias et al., 2001; Kirchgeorg et al., 2014; Kuo et al., 2011; Schüpbach et al., 2015). Human activity and the potential influence on the fire system are difficult to measure in lake sediment cores over paleoclimatic time scales. Past studies primarily use indirect proxies such as soil erosion, changes in vegetation, and pollen of cultivated plants (Anselmetti et al., 2007; Drescher-Schneider et al., 2007; Ramrath et al., 2000). Recent research examines fecal sterols (FeSt) as a molecular proxy for human and livestock presence in a lake catchment to reconstruct population history on a Norwegian island (D'Anjou et al., 2012). These FeSt include $5\beta$-cholestan-3-ol (coprostanol) and $5\beta$-stigmastanol (Stg), where coprostanol (CoP) is comprises approximately 60 % of the sterols in human feces, whereas grazing animals mainly produce stigmastanol (Bull et al., 2002). Fecal sterols can undergo microbial degradation, where e.g. coprostanol degrades to $5\beta$-cholestan-$3\alpha$-ol (epi-coprostanol). Although the literature demonstrates additional degradation processes (Bull et al., 2002), FeSt remain sufficiently stable after burial in lake sediments. As a result the sum of coprostanol and epi-coprostanol (e-CoP) successfully records human fecal variations over time scales of thousands of years (D'Anjou et al., 2012). Combining FeSt and Lvg analyses may therefore provide information about both fire activity and the presence of humans near lake records. This study investigates if FeSt and levoglucosan in lake sediment cores can reconstruct human occupation and regional fire activity. Lake Trasimeno is an ideal test site for these types of analyses as archeological and historical records demonstrate the regional presence of humans for millennia. The shores of Lake Trasimeno incorporate late Bronze Age settlements (Angelini et al., 2014) and was the site of the famous Battle of Lake Trasimeno during the Second Punic War in 217 BC. Here, we use a multi-proxy extraction method to analyze levoglucosan and FeSt in Lake Trasimeno, Italy sediment core to investigate the interactions between human and fire in this region during the late Holocene. We applied

accelerated solvent extraction (ASE) followed by FeSt and levoglucosan determination using GC-MS (Battistel et al., 2015) and HPAEC-MS Kirchgeorg et al. (2014), respectively.

## Study Site

Lake Trasimeno is located in central Italy, in the region of Umbria (see Figure 1). The lake is the fourth largest lake in Italy with a diameter of 10 km and a surface area of ca. 120 km$^2$. Three islands (Maggiore, Minore and Polvese) are situated in the lake. Although it is one of the largest lakes in Italy, the maximum water depth is only 6 m. The lake receives the major water input from precipitation and only minor streams and only small artificial outflows are known. The low depth and minor inflow led to periodical changes in the lake water level, due to the precipitation-evaporation balance (Gaino et al., 2012). The floods and droughts caused by climate conditions resulting in early water management approaches by Etruscans and Romans to regulate the lake levels (Burzigotti et al., 2003) The current lake level is lower then during the late Holocene, which was assumed as a affect of changing climate (Gaino et al., 2012).
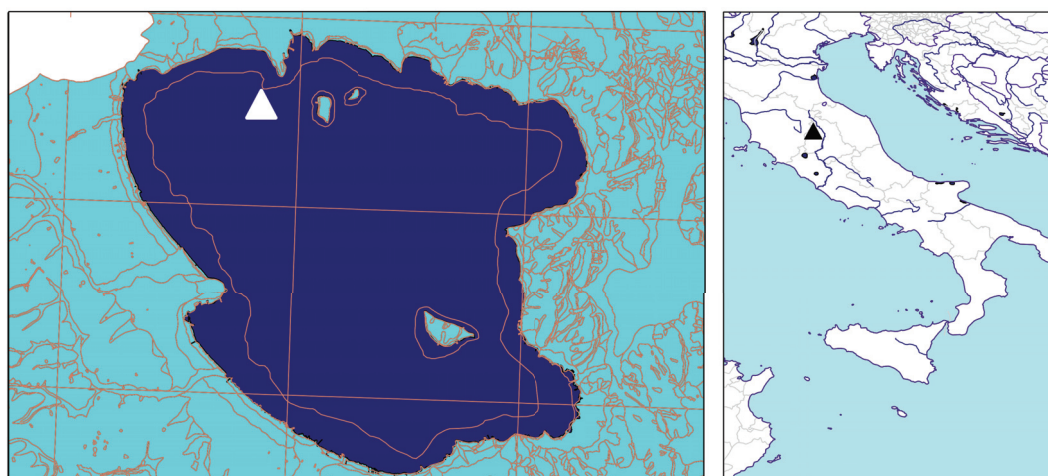


**Figure 1:** Map of Lake Trasimeno on the left, with drilling site marked with a white triangle. Right: Lake Trasimeno marked with a black triangle in central Italy.

## Materials and Methods

### Sampling and Sample Preparation

The 360 cm core was drilled in a water depth of 4 m with a piston corer near the northern shore of the lake close to Tuoro sul Trasimeno in 2013. The core was transported and stored at CNR-ISMAR (Bologna, Italy). We subsampled the core with a resolution of 5 cm resulting in a total of 72 samples (wet weight ranged from 4

to 9 g) for the entire core. Samples were transported to the University of Venice (Italy) and freeze-dried, milled and homogenized and stored at -20 °C until extraction. About 100 mg of each freeze-dried sample were used for the determination of total organic carbon (TOC).

## Age-Depth Model

The chronology is based on radiocarbon dating with 4 AMS ^{14}C dating points from shells and plant material from different core sections at 235, 260, 293 and 319 cm resulting in 2970 ($\pm$20), 8085 ($\pm$25), 20828 ($\pm$520) and 23280 ($\pm$80) years BP, respectively. The age-depth model was supported by grain-size and x-ray analyses of the core and stratigraphy features. We calculated the depositional time (yr cm$^{-1}$) of each level, using CLAM Version 2.2 (Blaauw, 2010), applying linear interpolation between neighbouring levels and calibrating with the northern hemisphere terrestrial calibration curve from Reimer (2013).

## Total Organic Carbon

TOC was analyzed following the EPA procedure (EPA Method 9060). Hydrochloric acid (2.5N) was used to remove inorganic carbon and samples were incubated for 30 minutes at 120 °C. The samples were oven-dried (105 °C) overnight to remove water. The dried samples were analyzed using a TOC elemental analyzer (TOC 5050, Shimadzu) by combusting the samples at elevated temperatures using oxygen as the carrier gas. Fourier transform infrared spectroscopy was used to measure the $CO_2$ produced during the combustion.

## Extraction of FeSt and Lvg

The extraction was performed with DCM:MeOH (9:1, v:v) mixture, using accelerated solvent extraction (ASE Dionex ASE 200, Thermo Fisher Scientific) at 1500 psi with two extraction cycles of 10 min. Prior to the extraction, samples were spiked with 100 µL of internal standards ($^{1}3C_3$-Cholesterol and $^{1}3C_6$-levoglucosan with concentrations of 1 µg mL$^{-1}$) and activated copper was added to the samples in order to remove sulfur interferences. The extracts (ca. 30 mL) were pre-concentrated in two steps: first under vacuum (40 min at 26 °C; EZ-2 vacuum evaporator, Genevac Ltd, Ipswich, UK) and then to a final volume of ca. 500 µL) under nitrogen stream (Turbovap, Biotage, Uppsala, Sweden). The clean-up and separation of the two fractions (FeSt and levoglucosan) was performed using solid phase extraction cartridges (Discovery SPE DSC-SI Silica Tube 12 mL, 2 g, Supelco, US). Cartridges were topped with anhydrous sodium sulphate for removing water traces eventually present in the samples, cleaned and conditioned with 40 mL DCM. The pre-concentrated sample was loaded onto the cartridge and the FeSt fraction was eluated with 80 mL DCM followed by 20 mL of MeOH for the Lvg fraction. FeST fraction was evaporated to dryness and the residues were dissolved in DCM. Prior to

the analyses by GC-MS, samples were derivatized by adding 100 µL of BSTFA with 1% TMCS and heated at 70 °C for one hour. The levoglucosan fraction was evaporated to dryness and residues were solved in 500 uL ultrapure water and centrifuged when solid residues were visible. The samples were stored at 4 °C prior the analyses with HPAEC-MS.

## Instrumental Analysis

The FeSt fraction was analyzed by GC-MS (GC: 6890 N GC System; MS: Agilent 5973; Agilent Technologies, Santa Clara, CA, USA). Separation was performed on a HP-5 MS column (60 m length, 0.25 mm inside diameter, and 0.25 µm film thickness, Agilent Technologies, Santa Clara, CA, USA). Helium was used as a carrier gas with a flow of 1 mL min$^{-1}$ where 2 µL of each sample was injected in split/splitless mode (splitless time 1.5 minutes). The temperature program was the following: 70 °C (held for 2 min) up to 210 °C with a rate of 20 °C min$^{-1}$ and then increased to 300 °C at 30 °C min$^{-1}$ and held for 8 min. An electron ionization source was used for mass detection. Single ion monitoring mode was performed for quantification, based on target ions and qualifiers. All instrumental details are reported in Battistel et al. (2015).

Levoglucsoan determination was carried out following the instrumental method proposed by Kirchgeorg et al. (2014) . Briefly, the levoglucosan fraction was analyzed with HPAEC-MS using an ion chromatographic system (Dionex, ICS 5000, Thermo Fisher Scientific) and a single quadrupole mass spectrometer (MSQplus, Thermo Fisher Scientific). MAs were separated on a CarboPac™ PA1 and PA10 column (both 2 x 250 mm, Thermo Fisher Scientific) and an AminoTrap pre-column (2 x 50 mm, Thermo Fisher Scientific) was used to protect the column. The MS operated with electrospray ionization in the negative mode. Lvg and the $^{13}C_6$-levoglucosan were detected in single ion monitoring mode (m/z 161 and 167, respectively).

## Reproducibility and Blanks

Extraction reproducibility was tested by extracting blanks spiked with known concentrations of levoglucosan and FeSt with two concentration levels (L1: 100 ng mL$^{-1}$ and L2: 300 ng mL$^{-1}$ for Lvg and L3: 10 ng mL$^{-1}$ for FeSt). In addition, three samples were extracted and analyzed in triplicate to prove the reproducibility of the results. Each of the replicates were extracted on different days in order to examine the reproducibility with changing lab conditions. Six extraction blanks and six blanks of the clean-up procedure were performed to evaluate blank concentrations of the different preparation steps. All reported concentrations in this study are blank corrected. The reproducibly is reported as the relative standard deviation of the extractions of spiked blanks with known concentration of FeSt and levoglucosan resulting in the following: CoP (L3: 7 %); e-CoP (L3: 8.1); Stg (L3: 31 %). The triplicate extraction of different samples results in the following uncertainties:

CoP (10-20 %), e-CoP (5-51 %), Stg (4-7 %). The mean FeST blank concentrations were (relative standard deviation in brackets): CoP: 0.4 ng mL$^{-1}$ ($\pm$0.6); e-CoP: 0.5 ng mL$^{-1}$ ($\pm$0.55); Stg 22 ng mL$^{-1}$ and for levoglucosan: 15 ng abs ($\pm$3). The standard deviation between the levoglucosan replicates of the extraction and analyses of spiked blanks are 6 % (L1) and 2 % (L2) for the different concentration levels and between 6-34 % for the triplicate extractions of sediment samples.

## Results and Discussion

### Chronology

The entire core covers approximately 27,000 yr (Figure 2). However, sediment type, x-ray and grain size analyses suggest that only the upper 235 cm (0-3100 cal yr BP) demonstrate a stable and continuous sedimentation. However, to confirm this stable sedimentation and thus reduce uncertainties in the age-depth model, further analyses are planned to verify the age-depth model of the last 235 cm. The section between 235 and 260 cm (3100 cal yr BP and 8100 cal yr BP) is characterized by a very high organic content, related to low lake level and a very low sedimentation rate. This results in a very low temporal resolution, which limits the discussion of these results. The section from 260-360 cm (8100-27,000 cal yr BP) of the core shows very low TOC values. Grain-sizes and visual analyses demonstrate sand layers in this section, suggesting that the sedimentation in this period was not continuous. Due to this discontinuity, the following discussion of TOC, levoglucosan and FeSt results focus on the section between 0 and 3100 cal yr BP due to the good temporal resolution between 0-235 cm, where each 1 cm samples correspond to ca. 12 years, and thus the 5 cm sampling resolution interval results in about 60 years.

### Total Organic Carbon and Lake Level

Total organic carbon is often influenced by lake level changes with lower TOC at higher lake levels and a higher TOC at low lake level (Meyers and Ishiwatari, 1993). TOC at Lake Trasimeno is ranging between 10-70 mg g$^{-1}$ between 3100 cal yr BP and today (Figure 3). Changes in the TOC may correspond to lake level changes during this period. Mediterranean lake level fluctuations due to changes in climate and precipitation are demonstrated in several paleoclimate studies from this region (Drescher-Schneider et al., 2007; Magny et al., 2007). Lake Trasimeno has no significant natural inflows or outflows, so lake level is strongly influenced by climate conditions, where these changes primarily encompass the precipitation-evaporation balance, or by changes of the catchment. In addition, historical documents give evidence of lake level changes at Lake Trasimeno, e.g. the loss of land for agriculture due to flooding. Humans were already aware of the flooding risk
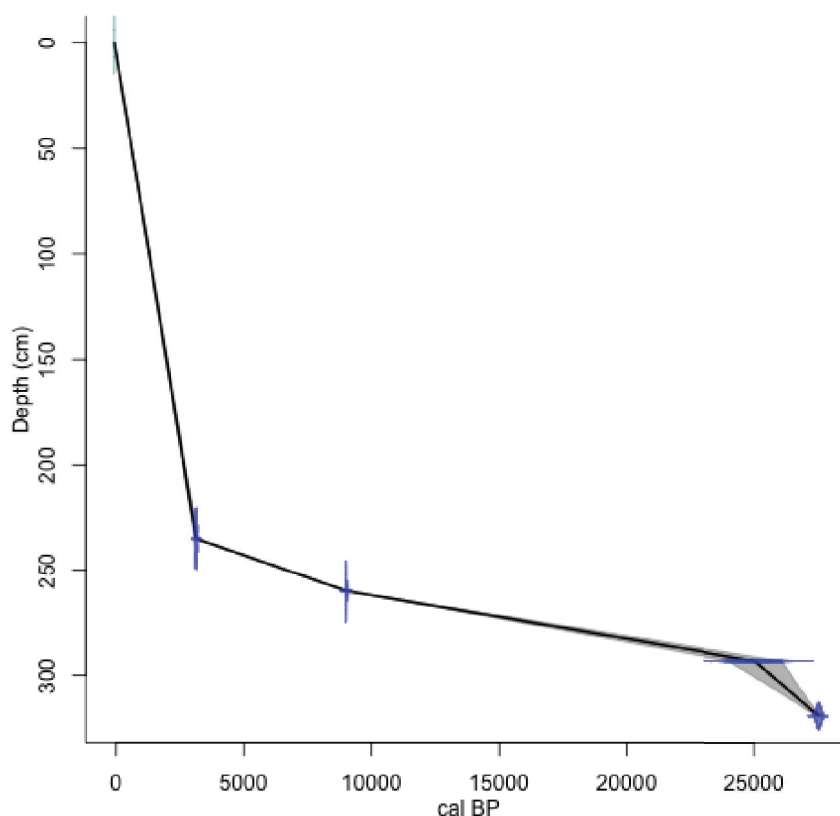
**Figure 2:** Age-depth model of the Lake Trasimeno core based on four radiocarbon determinations and produced using CLAM 2.2.

during Roman times and tried to regulate the lake level by constructing artificial outflows (Burzigotti et al., 2003). The relative changes of Lake Trasimeno TOC is similar to lake level changes at Lake dell'Accesa (Tuscany, Italy), which is approx. 100 km north west from Lake Trasimeno. The TOC is highest in the beginning of the period beginning at approximately 3000 cal yr BP, which may relate to the hot and dry period lasting until 3400 cal yr BP at Lake dell'Accesa (Colombaroli et al., 2007; Vannière et al., 2008). TOC decreases until 2500 cal yr BP as a result of more humid climate conditions, but increases again during the Roman Climatic Optimum which is similar to the lowering of Lake dell'Accesa during the same time period. In the following period, lake level at both lakes are increasing. Only the decrease of the lake level at Lake dell'Accesa at ca. 700 cal yr BP is not visible in the TOC at Lake Trasimeno. Gallorini (1992) reported an increase of the precipitation during this period. High lake levels are also the reason that peculiar fishing techniques were not longer applicable and disappeared between 200-400 cal yr BP (Cialini, 1985).

In recent years, the TOC is increasing and corresponds to the recent lake leves decrease at Lake dell'Accesa (Magny et al., 2007; Vannière et al., 2008). Although during this current period humans started to try to
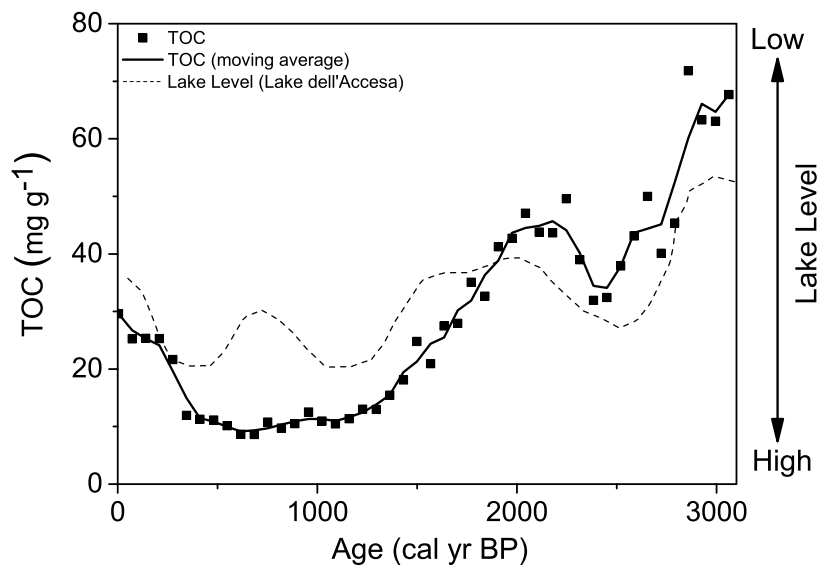
**Figure 3:** Total organic carbon (TOC) concentrations (mg g$^{-1}$), 3-point moving average of the TOC concentrations (black line) and relative lake level changes at Lake dell'Accesa, Italy (dashed-line) from Vannière et al. (2008) and Magny et al. (2007).

regulate the lake level, the main decrease at Lake Trasimeno is related to climate conditions, as this decrease was also observed at Lake dell'Accesa and other lakes (Magny et al., 2007; Vannière et al., 2008). The lake level decrease during the last century was also assumed as the reason for the decline of the sponge fauna at Lake Trasimeno (Gaino et al., 2012). In the present study, TOC values at Lake Trasimeno follow lake level changes at Lake dell'Accesa. These values can give a first rough estimate of relative changes of the lake level at Trasimeno, but TOC is also influenced by other parameters. A detailed discussion of lake level changes at Lake Trasimeno, based on multi-proxy and seismological analyses, will be discussed elsewhere and may resolve the gap between Lake Trasimeno and Lake dell'Accesa around 700 cal yr BP.

## Levoglucosan and Fire Activity

We used levoglucosan as a proxy for regional fire activity at Lake Trasimeno. Due to the compressed chronology below 235 cm, we mainly focus on the last 3100 years. From 3100 cal yr BP to 0 cal yr BP, we identified 5 fire activity periods with different intensities: 3000-2600 cal yr BP, 2250 cal yr BP, 1750 cal yr BP, 1000-750 cal yr BP and 450-0 cal yr BP.

Over the entire core, no significant correlation occurs between levoglucosan and FeSt (all $r^2 < 0.4$) and, thus, a positive direct and simple impact of humans to fire activity is still difficult to estimate. However, if regional to local fires are related to anthropogenic forest clearance, population density and thus discharge of FeSt to the

lake, these do not necessarily have to correlate with fire intensity. Furthermore, the different catchments and transport pathways of each marker influence the signals recorded in the sediments. Fecal sterols are washed directly into the lake from run-off, whereas levoglucosan can be atmospherically transported over long distances and deposited on the lake surface.
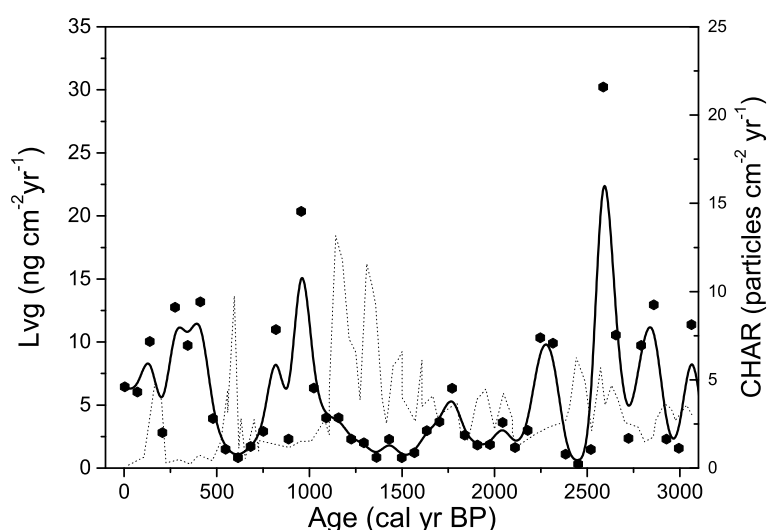


**Figure 4:** Levoglucosan fluxes (black dots; black line: 3-point moving average) at Lake Trasimeno. Dotted-line: Lake dell'Accesa macroscopic charcoal record from Vannière et al. (2008).

Levoglucosan flux and thus fire activity was highest between 2500-3000 cal yr BP, although this fire activity was highly variable. Late Bronze Age settlements were found along the shores of Lake Trasimeno around 3000 cal yr BP (Angelini et al., 2014), demonstrating the appearance of humans at the lake shore. Ecological and anthropological studies demonstrate that human strongly altered the landscape during this period (Valsecchi et al., 2006) and thus the high fire activity during this period may reflect anthropogenic activities. It is difficult to distinguish if this combination was also the case at Lake Trasimeno as the population density may be low during this period and the lower lake levels suggest dry climate conditions around 3000 cal yr BP. In addition archeological evidences for the period between 3000 and 2500 cal yr BP are missing (Gambini, 1995).During the Etruscan and early Roman period between 1750 and 2500 BP, when humans substantially impacted Lake Trasimeno, as identified from FeSt analyses, fire activity demonstrates more complex trends. Fire period is low at the beginning of the period and levoglucosan is peaking at ca. 2250 cal yr BP, followed second peak at 1750 cal yr BP where this second fire activity peak is lower than the previous peak (Figure 4). The region might have already been deforested by 2250 cal yr BP, thereby decreasing the amount of available fuel. The Bronze Age burning had already decimated forests in order to open the landscape for agriculture, and such

large scale burning was no longer possible or necessary. Low fire activity for this period was also reported from Lake dell'Accesa. This low biomass burning might result from Roman land management including reducing fire risk by removing dead biomass from the forests Tinner et al. (1998). The Roman period is followed by a low fire activity between 1600-1250 cal yr BP resulting either from cooler and more humid climate conditions in the regions and/or the depopulation of the region in the post Roman period as recorded in the low FeSt fluxes. Fire increases again from 1250 cal yr BP until reaching its maximum at ca. 1000 cal yr BP. This peak coincides with peaks of micro- and macroscopic charcoal at Lake dell'Accesa and Lake Massaccioli, to the north of Lake Trasimeno, where local fire maxima occur slightly earlier at 1200-1000 cal yr BP (Colombaroli et al., 2007; Vannière et al., 2008), where this difference may be related to uncertainties in the dating. These studies assume that agricultural activities and associated land clearance are the major reasons for the increased charcoal at these lakes, but also suggests, that warm and dry conditions during this period may also play a role. It is known that due to the lake level increase and the flooding of agricultural land the population around Lake Trasimeno had to open new landscapes for breeding and cultivations by using fire (Gallorini, 1992). This activity may explain the increase of levoglucosan in this record.The final period of high fire activity begins ∼500 cal yr BP, reaching a maximum at 300 cal yr BP before slightly decreasing until today. This period may correspond to a medieval population increase on local and regional scales as ancient maps already demonstrated several settlements around the lake (Burzigotti et al., 2003 and references within). Furthermore historical documents describe the annual burning of the cane thicket around the lake to maintain traditional fishing practices. Although it is not exactly clear when this practice started (it stopped in the 1980s) this annual burning may result in an additional input of levoglucosan to the lake surface, due to the fact that the cane thicket is located in the shallow lake boarder.

## Human Impact and Livestock

The shores of Lake Trasimeno were already occupied in the late Bronze Age (around 3000 cal yr BP) as demonstrated by excavations of wooden samples from pile dwellings from the southern shore (San Savino) (Angelini et al., 2014). Radiocarbon dating demonstrates that the ages of these wooden samples were between 2900-3090 cal yr BP. Settlements and human impacts on lakes were also observed in the surrounding region, beginning as early as 3700 yr BP at Lago di Mezzano, based on sediment analyses and archeological findings (Ramrath et al., 2000; Sadori et al., 2004). Although these archeological findings demonstrate that humans lived near Lake Trasimeno, CoP + e-CoP fluxes are not significantly higher in this section, which may be due to the low population density during this period.
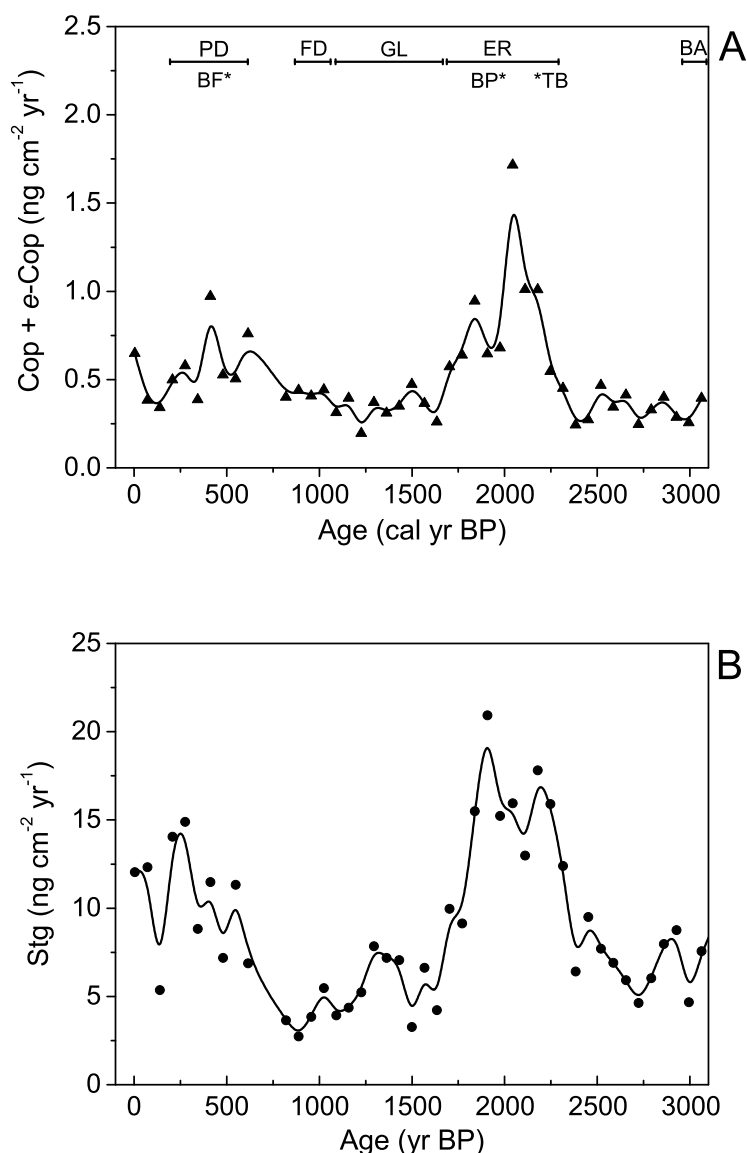
**Figure 5:** A: Cop+e-Cop fluxes (black triangles; black-line: 3-point moving average) as proxy for human appearances. Historical periods are marked as followed: Late Bronze Age settlements (BA), Etruscan-Roman Period (ER), Gothic and Longobard Domination (GL), Franks Domination (FD); Perugia Domination (FD). Important events at Lake Trasimeno are marked with an asterisk: Battle of Trasimeno (TB), Bellum Perusium (BP) and Braccio Fortebraccio da Montone (BF). B: STIG fluxes (black dots; black-line: 3-point moving average) as proxy for livestock appearances.

The pile dwelling excavation site was on the opposite side of the lake from our sampling location. Due to the size and shallow water of the lake, fecal material transported into the lake is deposited close to the source. However, the proxy for grass-eating animals, Stg shows a more significant peakt at 2800 cal yr BP, which may correspond to human occupation, because a significant correlation between CoP, Stg and e-CoP ($r^2 \geq 0.7$ p= 0.01) occurs over the entire core suggesting that the appearance of humans, as characterized by CoP and

e-CoP, may coincide with the occurrence of livestock (Stg) at Lake Trasimeno.



**Figure 6:** Correlation between Stg and Cop + e-Cop concentrations. Outlier (marked red) correspond to 90 BC.

Our results may also reflect the Roman occupation of the area. The period between 1750 cal yr BP and 2250 cal yr BP, with highest flux of CoP + e-CoP at 2000 cal yr BP corresponds to the Etruscan - Roman period. Historical documents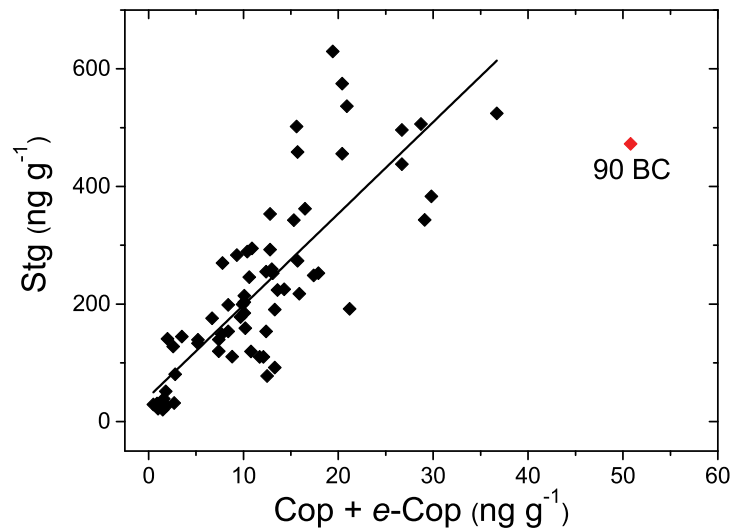 demonstrate that Lake Trasimeno was an important military and economic center for the Romans. In addition, the Battle of Trasimeno (217 BC) between the army of Hannibal and the Romans took place on the lakeshores close to the sampling location where approx. 50,000 soldiers were present along the northern part of lake and also during the erusine War, around 40-42 BC, Lake Trasimeno was an important passage of Roman troops. Although the military camp was only temporary, the impact on the aquatic system by human fecal matter may have significantly increased during this period and may correspond to the concentration peaks in the record. Interestingly, the correlation between CoP + e-CoP and Stg demonstrates an outlier during this period (Figure 6; 90 BC), which may correspond to one of theses event, as during this phase temporally a high number of humans were present compared to the livestock in the region. After this period Romans were still present in this region, as demonstrated by findings of Traiano and Constanzo Empire coins (98-306 AD; Ferugilo (1966)) and contemporaneous high CoP + e-Cop values compared to pre-roman times. CoP + e-CoP concentrations decrease and remain low level 750-1650 cal yr BP which may reflect rural depopulation after the end of the Roman Empire, as also observed in other regions of Italy (Di Pasquale et al., 2014). In addition, this period corresponds to the Gothic and Longobard Domination of the region around Lake Trasimeno and a general demographic decline during this period. The decrease is also evident in the Stg flux, thus also livestock around

Lake Trasimeno was reduced during this period.Historians actively debate if regional population increased from approx. 750 cal yr BP to the present (Campano, 1992; Gambini, 1995). However, our record suggests a strong increase of breeding activity in this region with increased Stg and CoP + e-CoP fluxes and also an increased input of FeSt. Fire also increased during this period, probably as a result of increasing population and thus additional landscapes were opened by fire. In addition new drainage works attempted, yet failed, to regulate the lake level. (Gambini, 1995). The most recent sample (60 yr BP) demonstrates the highest concentrations Stg and may correspond to modern agriculture. Interestingly, CoP+e CoP only slowly increases in this period, perhaps due to only small changes in the population density or to improves sewage-treatment conditions where human feces were not directly discharged into the lake.

## Conclusions

The results obtained from the analyses of the different organic molecular markers in the lake sediment core form Lake Trasimeno demonstrating a high impact of humans on the lake system during the Etruscan-Roman Period. During this period, two battles were fought at Lake Trasimeno, with the famous Battle of Trasimeno against Hannibal where almost 50,000 soldiers camped at the lakeshore, and perhaps significantly impacted the lake system. Fire intensity did not significantly increase during this period, suggesting either that the landscape was already successfully cleared in previous periods or Roman land management suppressed fire activity. Furthermore, breeding activities and human presence strongly correlate during this period.Fire reconstructions demonstrated different fire periods in the late Holocene record at 2600-3000 cal yr BP, 2250 cal yr BP, 1750 cal yr BP, 1000-750 cal yr BP and 450-0 cal yr BP. Although these fire periods may be strongly influenced by regional human activity, a direct human impact on these fire periods is difficult to estimate from the analyzed FeSt, due to the low variability and concentrations and different catchments. Concluding, the results of the analyses of organic molecular marker in a Lake Trasimeno sediment core demonstrates that organic molecular markers in lake sediments are a suitable tool to provide information about fire, human appearances and breeding activity in a lake catchment.

# References

Angelini, P., De Angelis, M. C., Guerzoni, R. P., Gigante, D., Rubini, A., Properzi, A., Venanzoni, R., 2014. Wood identification of pile dwellings from the Bronze Age San Savino site (Lake Trasimeno, central Italy). Plant Biosystems 148 (February), 713–722.

Anselmetti, F. S., Hodell, D. a., Ariztegui, D., Brenner, M., Rosenmeier, M. F., 2007. Quantification of soil erosion rates related to ancient Maya deforestation. Geology 35 (10), 915.

Battistel, D., Piazza, R., Marchiori, E., Argiriadis, E., Radaelli, M., Barbante, C., 2015. GC-MS method for the determination of caecal sterols in sediments and its application in anthropogenic impact reconstructions in river piave (Italy), in preperation.

Blaauw, M., 2010. Methods and code for 'classical' age-modelling of radiocarbon sequences. Quaternary Geochronology 5 (5), 512–518.

Bull, I. D., Lockheart, M. J., Elhmmali, M. M., Roberts, D. J., Evershed, R. P., 2002. The origin of faeces by means of biomarker detection. Environment International 27 (8), 647–654.

Burzigotti, R., Dragoni, W., Evangelisti, C., Gervasi, L., 2003. The role of Lake Trasimeno (central Italy) in the history of hydrology and water management. In: International Water History Association. Epypt.

Campano, G., 1992. Trasimeno felice. Trasimeni descriptio seu de felicitate Trasimeni, Testo e traduzione di C. Conti, introduzione e note E. Pianta. Folingo.

Cialini, G., 1985. Qualche dato sulla evoluzione della popolazione dellâIsola Maggiore dalla fine del XVI ad oggi. In: Centro di documentqazione del lago Trasimeno. Progetto generale e materiali di base. Regione dellâUmbria, Perugia., pp. 100–104.

Colombaroli, D., Marchetto, A., Tinner, W., 2007. Long-term interactions between Mediterranean climate, vegetation and fire regime at Lago di Massaciuccoli (Tuscany, Italy). Journal of Ecology 95 (4), 755–770.

Conedera, M., Tinner, W., Neff, C., Meurer, M., Dickens, A. F., Krebs, P., 2009. Reconstructing past fire regimes: methods, applications, and relevance to fire management and conservation. Quaternary Science Reviews 28 (5-6), 555–576.

D'Anjou, R. M., Bradley, R. S., Balascio, N. L., Finkelstein, D. B., 2012. Climate impacts on human settlement and agricultural activities in northern Norway revealed through sediment biogeochemistry. PNAS 109 (50), 20332–7.

Di Pasquale, G., Buonincontri, M. P., Allevato, E., Saracino, A., 2014. Human-derived landscape changes on the northern Etruria coast (western Italy) between Roman times and the late Middle Ages. The Holocene 24 (11), 1491–1502.

Drescher-Schneider, R., De Beaulieu, J. L., Magny, M., Walter-Simonnet, a. V., Bossuet, G., Millet, L., Brugiapaglia, E., Drescher, A., 2007. Vegetation history, climate and human impact over the last 15,000 years at Lago dell'Accesa (Tuscany, Central Italy). In: Vegetation History and Archaeobotany. Vol. 16. pp. 279–299.

Elias, V. O., Simoneit, B. R. T., Cordeiro, R. C., Turcq, B., 2001. Evaluating levoglucosan as an indicator of biomass burning in Carajás, Amazonia: A comparison to the charcoal record. Geochimica et Cosmochimica Acta 65 (2), 267–272.

Ferugilo, A., 1966. Rassegna degli scavi e delle scoperte. In: Storia Etrusca, Vol XXXIV (Serie II). Firenze, p. 305.

Gaino, E., Scoccia, F., Piersanti, S., Rebora, M., Bellucci, L. G., Ludovisi, A., 2012. Spicule records of Ephydatia fluviatilis as a proxy for hydrological and environmental changes in the shallow Lake Trasimeno (Umbria, Italy). Hydrobiologia 679 (1), 139–153.

Gallorini, S., 1992. Castiglion Fiorentino: Dalle origini etrusco-romane al 1384. Calosci, Cortona.

Gambini, E., 1995. Le oscillazioni di Livello del Lago Trasimeno. Museo della Pesca, Grafiche Piemme S.n.c., Perugia.

Kirchgeorg, T., Schüpbach, S., Kehrwald, N., McWethy, D. B., Barbante, C., 2014. Method for the determination of specific molecular markers of biomass burning in lake sediments. Organic Geochemistry 71, 1–6.

Kuo, L. J., Louchouarn, P., Herbert, B. E., Brandenberger, J. M., Wade, T. L., Crecelius, E., 2011. Combustion-derived substances in deep basins of Puget Sound: Historical inputs from fossil fuel and biomass combustion. Environmental Pollution 159 (4), 983–990.

Magny, M., de Beaulieu, J. L., Drescher-Schneider, R., Vannière, B., Walter-Simonnet, A. V., Miras, Y., Millet, L., Bossuet, G., Peyron, O., Brugiapaglia, E., Leroux, A., 2007. Holocene climate changes in the central Mediterranean as recorded by lake-level fluctuations at Lake Accesa (Tuscany, Italy). Quaternary Science Reviews 26 (13-14), 1736–1758.

Meyers, P. A., Ishiwatari, R., 1993. Lacustrine organic geochemistry-an overview of indicators of organic matter sources and diagenesis in lake sediments. Organic Geochemistry 20 (7), 867–900.

Norwood, M. J., Louchouarn, P., Kuo, L. J., Harvey, O. R., 2013. Characterization and biodegradation of water-soluble biomarkers and organic carbon extracted from low temperature chars. Organic Geochemistry 56 (2013), 111–119.

Ramrath, A., Negendank, J., Sadori, L., 2000. Sediments from Lago di Mezzano, central Italy: a record of Lateglacial/Holocene climatic variations and anthropogenic impact. The Holocene 10 (1), 87–95.

Reimer, P., 2013. IntCal13 and Marine13 Radiocarbon Age Calibration Curves 0-50,000 Years cal BP. Radiocarbon 55 (4), 1869–1887.

Sadori, L., Giraudi, C., Petitti, P., Ramrath, A., 2004. Human impact at Lago di Mezzano (central Italy) during the Bronze Age: A multidisciplinary approach. Quaternary International 113, 5–17.

Schüpbach, S., Kirchgeorg, T., Colombaroli, D., Beffa, G., Radaelli, M., Kehrwald, N. M., Barbante, C., 2015. Combining charcoal sediment and molecular markers to infer a Holocene fire history in the Maya Lowlands of Petén, Guatemala. Quaternary Science Reviews 115, 123–131.

Simoneit, B., 2002. Biomass burning–a review of organic tracers for smoke from incomplete combustion. Applied Geochemistry 17, 129–162.

Tinner, W., Ammann, B., Conedera, M., Gaggeler, H., Sagesser, B., Gedye, S., Jones, R., 1998. Pollen and charcoal in lake sediments compared with historically documented forest fires in southern Switzerland since AD 1920. The Holocene 8 (1), 31–42.

Valsecchi, V., Tinner, W., Finsinger, W., Ammann, B., 2006. Human impact during the Bronze Age on the vegetation at Lago Lucone (northern Italy). Vegetation History and Archaeobotany 15 (2), 99–113.

Vannière, B., Colombaroli, D., Chapron, E., Leroux, A., Tinner, W., Magny, M., 2008. Climate versus human-driven fire regimes in Mediterranean landscapes: the Holocene record of Lago dell'Accesa (Tuscany, Italy). Quaternary Science Reviews 27 (11-12), 1181–1196.

Whitlock, C., Larsen, C., 2001. Charcoal as a fire proxy. In: Tracking environmental change using lake sediments-Vol 3 Terrestrial algal and siliceous indicators. Vol. 3. pp. 75–97.

# 4 Study IV: Biodegradation of levoglucosan, mannosan and galactosan: Implications for paleoclimate research

**- unpublished manuscript -**

## Abstract

Levoglucosan, mannosan and galactosan are currently applied as a specific proxy for biomass burning in ice and lake sediments cores. Little is known about the stability of these compounds in these matrices and when exposed to environmental conditions. This study is the first evaluation of the stability of dissolved levoglucosan, mannosan and galactosan using a manometric respirometry test. The three monosaccharide anhydrates demonstrate a degradation of 85 % ($\pm$6.6 %), 86 % ($\pm$8.5 %) and 44 % ($\pm$17.9 %) after 28 days for levoglucosan, galactosan and mannosan, respectively. However, environmental conditions are more complex and may enhance the stability of these compounds under certain conditions, making them suitable as a proxy for biomass burning in natural archives.

## Introduction

Specific organic molecular markers for biomass burning, such as the monosaccharide anhydrates levoglucosan, mannosan and galactosan may be suitable proxies for reconstructing biomass burning in natural archives including lake sediments or ice cores. These compounds only originate from combustion of cellulose and hemicellulose and are emitted in high concentrations during biomass burning events (Simoneit and Elias, 2000). The stability of such organic molecular markers is an important aspect for their suitability as a proxy in natural archives, yet this aspect has currently only been partially investigated.

Previous studies examined the stability of levoglucosan in aerosols, due to their application as a tracer of biomass burning (Fraser and Lakshmanan, 2000). Hoffmann et al. (2010) tested the atmospheric stability of levoglucosan by conducting a modelling and laboratory study for the aqueous-phase oxidation with the atmospheric radicals $OH^-$, $NO_3$ and $SO_4^-$, demonstrating that reactions with $OH^-$ radicals are the main degradation process affecting levoglucosan in the atmosphere. Hennigan et al. (2010) exposed biomass burning particles to $OH^-$ radicals and estimated an atmospheric lifetime of levoglucosan of 0.7-2.2 days. A further study reported a heterogeneous oxidation lifetime for levoglucosan of 10.6 days (Kessler et al., 2010). Nevertheless, these studies suggest that monosaccharide anhydrates are suitable proxies for biomass burning due to the high emissions of levoglucosan, mannosan and galactosan. This conclusion corroborates with detection of levoglucosan, mannosan and galactosan in aerosols in remote regions like the Arctic and Antarctic, and demonstrate that monosaccharide anhydrates remain in the atmosphere even after long range atmospheric transport (Hu et al., 2013; Zangrando et al., 2013).

After the initial detection of levoglucosan in Arctic snow and their correlation with biomass burning events (Kehrwald et al., 2012), levoglucosan was used as a specific proxy for biomass burning in different ice cores (Yao et al., 2013; Zennaro et al., 2014), and researchers successfully reconstructed past fire histories with these results. Recently, levoglucosan, mannosan and galactosan were used as proxies for biomass burning in lake and marine sediments (Elias et al., 2001; Hopmans et al., 2013; Kirchgeorg et al., 2014; Kuo et al., 2008, 2011; Sikes et al., 2013; Schüpbach et al., 2015). Comparisons between levoglucosan and charcoal, a well-known proxy for biomass burning in lake sediment cores, suggest the suitability of levoglucosan as a proxy of biomass burning in lake sediments. The presence of levoglucosan in lake sediment samples demonstrates that levoglucosan may be sufficiently stable to be archived over thousands of years. Most studies assume at least that levoglucosan incorporated into particles are stable enough to be archived in lake sediments. However, detailed investigations of the stability of levoglucosan in lake sediments have not yet been performed. A recent laboratory experiment tested the stability of dissolved levoglucosan and estimated a lifetime of 7 days in water Norwood et al. (2013). Different studies demonstrate that levoglucosan can be metabolized by bacteria

(Kitamura et al., 1991; Nakahara et al., 1994; Xie et al., 2006) and 26 levoglucosan-assimilating microorganism have been isolated in Chinese soil samples (Xie et al., 2006). Studies have not yet examined the biodegradation of mannosan and galactosan.

In order to conduct the first evaluation of the stability of all three isomers in water, we compared the biodegradation of dissolved levoglucosan, mannosan and galactosan by applying a manometric respirometry test using the OECD 301F/1992 protocols to all three isomers and discuss the results in context of their suitability as proxies in paleoclimate research.

## Method

Manometric respirometry test following the OECD 301F/1992 protocols was performed. Levoglucosan (1,6-anhydro-$\beta$-D-glucsopyranose), mannosan (1,6-anhydro-$\beta$-D-mannopyranose) and galactosan (1,6-anhydro-$\beta$-D-galactopyranose) with a theoretical oxygen demand (ThOD) of 30 mg L$^{-1}$ was stirred in an inoculated mineral medium in a closed flask at a constant temperature of 20 °C for 28 days. The inoculum was taken from wastewater treatment effluent and filtered prior to the addition to the mineral medium. Biodegradation of the substances was tested with two replicates. In addition, an abiotic sterile control and a toxicity control were performed for each compound. In order to validate the tests, we performed both, one blank and a quality control in triplicate. In the beginning and in the end of the test, pH and total organic content was measured. During the 28 days of the degradation tests, the pressure changes due to the consumption of oxygen are automatically registered by the OxiTop Control A6/A12 system (WTW GmbH, Weilheim, Germany). The final biodegradation corresponds to the ratio between the BOD and the ThOD. All details are given in the guidelines of the OECD for testing chemicals (OECD, 1992).

## Results

After 28 days, a biodegradation for levoglucosan, mannosan and galactosan of 85 % ($\pm6.6$ %), 86 % ($\pm8.5$ %) and 44 % ($\pm17.9$ %) was detected, respectively (Figure 1). The results for galactosan were out of the validation range, because the difference between the two parallel samples was > 20 % with values of 57 % and 32 %. Abiotic degradation was not observed for any of the three compounds.

A time lag in degradation occurred at the beginning of the test for three isomers. Levoglucosan demonstrated low biodegradation for the first two days, followed by biodegradation of 40 % on the third day, while 75 % of the levoglucosan degraded by the eleventh day. We observed a similar time lag for mannosan, where the samples did not degrade in the first few days. Depending on the sample, mannosan biodegradation reached ca.

45 % on days five and seven and 75 % on days eleven and fifteen. Galactosan biodegraded more slowly than the other two isomers, reaching 30% of biodegradation in six to twelve days (depending on the sample).
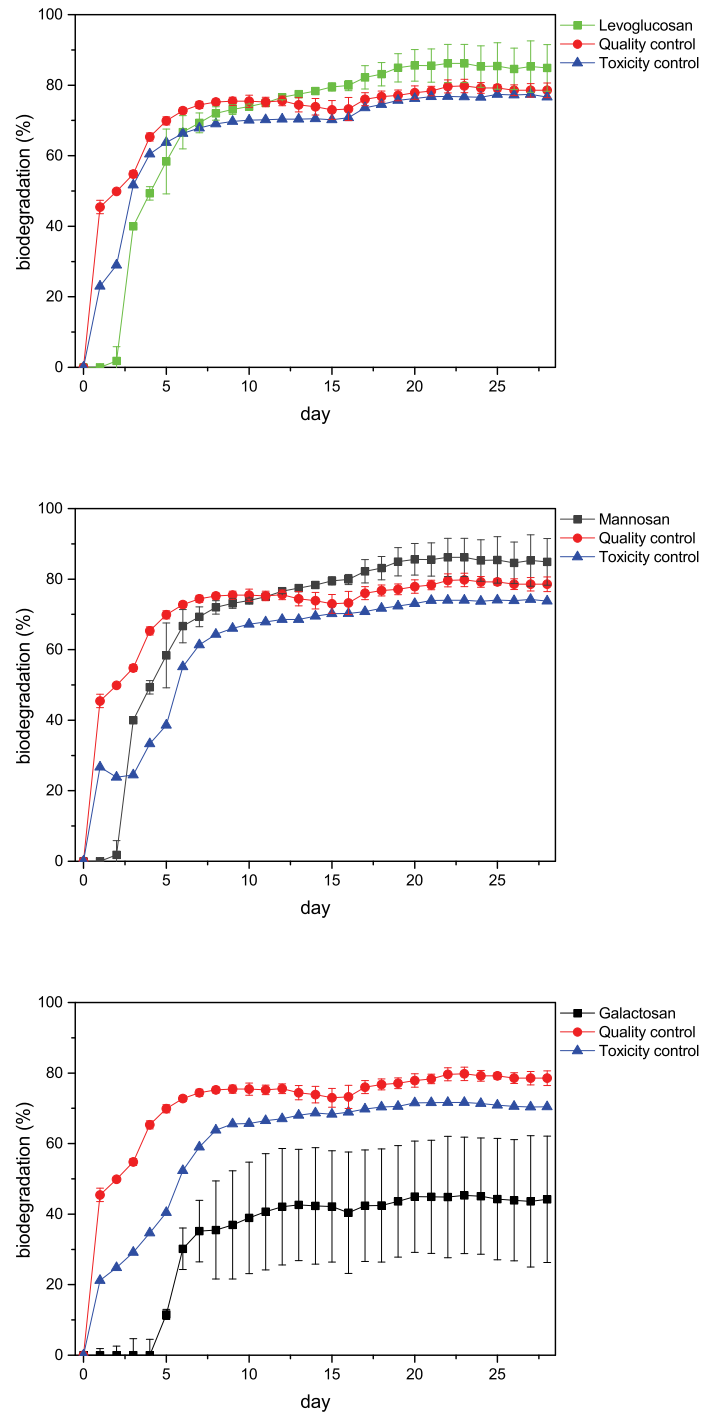


**Figure 1:** Results of the biodegradation test (%) of dissolved levoglucosan (top), mannosan (middle) and galactosan (bottom). Quality control (red) and toxicity control (blue) are plotted in each figure. Error bars represent the standard deviation between the replicates of each compound.

# Discussion

## Biodegradation

The degradation of 85 % of the initial levoglucosan in 28 days corroborates the findings of Norwood et al. (2013) who determined a similar degradation of dissolved levoglucosan extracted from cord grass and honey mesquite. Mannosan reacts similarly to levoglucosan where 86 % degrades after 28 days. Galactosan degradation substantially differs from both mannosan and levoglucosan where only 44 % degraded over the entire study period. However, the galactosan results were out of the validation range of the test and galactosan's lower biodegradation compared to levoglucosan and mannosan has to be confirmed in future studies.

A time lag between the beginning of the test and the start of biodegradation was observed levoglucosan, mannosan and galactosan. Norwood et al. (2013) also observes this lag for levoglucosan. The time lag was assumed as a result of the complex and slow assimilation by fungi or yeast, which need at least three steps until it will be reduced to glucose for consumption (Kitamura et al., 1991; Nakahara et al., 1994). Particular microorganisms with dehydrogenating enzymes have to be present for the initial step of metabolizing monosaccharide anhydrates (Kitamura et al., 1991; Nakahara et al., 1994; Xie et al., 2006).

## Implications for paleoclimate studies

The results demonstrate that levoglucosan, mannosan and galactosan degrade if they are dissolved in water and if bacteria that are capable of metabolizing these three compounds are present. However, monosaccharide anhydrates were detected in lake sediment samples up to 20,000 yrs old (Elias et al., 2001; Hopmans et al., 2013; Kirchgeorg et al., 2014; Kuo et al., 2008, 2011; Sikes et al., 2013; Schüpbach et al., 2015). The presence of these isomers in sediment samples from thousands of years ago may be explained by the transport behavior of monosaccharide anhydrates, which are generally emitted ad/absorbed in/onto aerosols into the atmosphere (Simoneit and Elias, 2000). If these particles are deposited on the lake surface, their bioavailability might be reduced thereby protecting at least the monosaccharide anhydrates incorporated in the particles from degradation. The presence of levoglucosan on particles in the ocean distant from potential sources suggests that levoglucosan may be transported over long distances in the water and thus also be buried in lake sediment cores, if attached onto or incorporated in particles (Kuo et al., 2011).

An increase in the stability of particle-bound substances was also observed for several micro organic pollutants (Warren et al., 2003). This sorption to particles in suspended matter or in sediments can decrease the bioavailability for microorganisms and thus increase their stability. The sorption behavior depends on both the physical-chemical properties of the compounds and the sediment as well as the particle composition and

structure. Monosaccharide anhydrates are highly water-soluble where dissolution into the surrounding water may occur only if these materials are adsorbed to the particle surface. Nevertheless, the interaction between water, suspended matter and sediments including micro bacterial activity is complex and several conditions can influence the stability of compounds in sediments after burial such as e.g. pore-water circulation, bioturbation, the presence of microorganisms or oxic-anoxic conditions (Warren et al., 2003).

Paleoclimate studies have to consider that at least part of the initially emitted monosaccharide anhydrates may degrade during transport (Hennigan et al., 2010; Hoffmann et al., 2010; Kessler et al., 2010), or may dissolve from the particle surfaces and degrade prior to the burial in lake sediments. Depending on the sediment conditions, levoglucosan, mannosan and galactosan may be stable, and the detection of these compounds confirms that they can be buried in lake sediments (Elias et al., 2001; Hopmans et al., 2013; Kirchgeorg et al., 2014; Kuo et al., 2008, 2011; Sikes et al., 2013; Schüpbach et al., 2015).

Recently, the ratios between the three isomers (levoglucosan/mannosan and levoglucosan/(mannosan + galactosan)) were used in sediment studies to differentiate if wood or grass was the major fuel as the type of burned fuel influences these ratios (Kirchgeorg et al., 2014; Schüpbach et al., 2015). In order to apply this this approach, it is necessary that the biodegradation behavior of each isomer is quite similar. The results of this study demonstrate that at least levoglucosan and mannosan biodegrade similarly, allowing the application of these ratios for levoglucosan and mannosan. Although galactosan is outside of the validation range, the biodegradation of galactosan may differ from that of levoglucosan and mannosan (Figure 1). This lower biodegradation rate may explain why the isomer composition in lake sediment differs from those detected on aerosol particles. In most aerosol studies, the contribution of galactosan to the total monosaccharide anhydrate concentration is quite low (e.g. average proportions of levoglucosan, mannosan and galactosan in aerosols from Finland are 85.8 ± 2.0 %, 9.7 ± 1.6 %, 4.5 ± 1.2 %, respectively (Saarnio et al., 2010) while lake sediment isomer proportions were 49.5 ± 9 %, 32.7 ± 6.2 % and 17.9 ± 6.8 % for the three isomers (Kirchgeorg et al., 2014). Nevertheless, it has to be considered that burning conditions and fuel type and thus the local setting also influence the isomer proportions (Kuo et al., 2011).

Levoglucosan was also successfully used as a proxy for biomass burning in ice cores (Yao et al., 2013; Zennaro et al., 2014) where the possibility that biodegradation may influence these records has not yet been investigated. Various studies demonstrate the presence of bacteria in ice cores (Christner et al., 2001; Sheridan et al., 2003; Zhang et al., 2008), but the low temperatures may reduce or even stop the bacterial activity. Bacteria concentrations may be significantly lower than in lake water and sediments and the specific bacteria with the levoglucosan kinase have to be present. However, this situation should only occur if climate conditions and temperatures are high enough to melt snow and enable bacterial activity and biodegradation. Zennaro (2013) assumed this potential degradation may have caused the absence of levoglucosan in melt layers from the basal

ice in the NEEM ice core. These basal samples originate from the warm Eemain period, where the Eemain surface snow may have melted after deposition.

## Conclusions

The result of the biodegradation test of the present study demonstrates that dissolved levoglucosan, mannosan and galactosan biodegrade with a loss of levoglucosan and mannosan of up to 85 % under the test conditions, but where only up to 57 % of the galactosan degrades. This difference in biodegradation between the three isomers may limit the application of isomer ratios in lake sediment samples. Only dissolved monosaccharide anhydrates were analyzed in this study, which limits the conclusions about the environmental behavior of these compounds and their deposition in lake sediments, due to their primarily particle-bound emission and complex interactions in both water and sediments. The fact that monosaccharide anhydrates are particle-bound may enhance their stability in the environment and may explain their presence in ancient sediments and their suitability as a paleoclimate proxy. Further investigations of the stability of particle-bound monosaccharide anhydrates and their distribution in natural sediments and the surrounding water are necessary to better understand the behavior of levoglucosan, mannosan and galactosan in the aqueous environment.

# References

Christner, B. C., Mosley-Thompson, E., Thompson, L. G., Reeve, J. N., 2001. Isolation of bacteria and 16S rDNAs from Lake Vostok accretion ice. Environmental Microbiology 3 (9), 570–577.

Elias, V. O., Simoneit, B. R. T., Cordeiro, R. C., Turcq, B., 2001. Evaluating levoglucosan as an indicator of biomass burning in Carajás, Amazonia: A comparison to the charcoal record. Geochimica et Cosmochimica Acta 65 (2), 267–272.

Hennigan, C. J., Sullivan, A. P., Collett, J. L., Robinson, A. L., 2010. Levoglucosan stability in biomass burning particles exposed to hydroxyl radicals. Geophysical Research Letters 37 (9), L09806.

Hoffmann, D., Tilgner, A., Iinuma, Y., Herrmann, H., 2010. Atmospheric stability of levoglucosan: A detailed laboratory and modeling study. Environmental Science and Technology 44 (2), 694–699.

Hopmans, E. C., dos Santos, R. A. L., Mets, A., Sinninghe Damsté, J. S., Schouten, S., 2013. A novel method for the rapid analysis of levoglucosan in soils and sediments. Organic Geochemistry 58, 86–88.

Hu, Q.-H., Xie, Z.-Q., Wang, X.-M., Kang, H., Zhang, P., 2013. Levoglucosan indicates high levels of biomass burning aerosols over oceans from the Arctic to Antarctic. Scientific Reports 3, 3119.

Kehrwald, N., Zangrando, R., Gabrielli, P., Jaffrezo, J. L., Boutron, C., Barbante, C., Gambaro, A., 2012. Levoglucosan as a specific marker of fire events in Greenland snow. Tellus, Series B: Chemical and Physical Meteorology 64 (1), 1–9.

Kessler, S. H., Smith, J. D., Che, D. L., Worsnop, D. R., Wilson, K. R., Kroll, J. H., 2010. Chemical sinks of organic aerosol: Kinetics and products of the heterogeneous oxidation of erythritol and levoglucosan. Environmental Science and Technology 44 (18), 7005–7010.

Kirchgeorg, T., Schüpbach, S., Kehrwald, N., McWethy, D. B., Barbante, C., 2014. Method for the determination of specific molecular markers of biomass burning in lake sediments. Organic Geochemistry 71, 1–6.

Kitamura, Y., Abe, Y., Yasui, T., 1991. Metabolism of Levoglucosan (1,6-Anhydro-beta-D-glucopyranose) in Microorganisms. Agriculture Biological Chemistry 55 (2), 515–521.

Kuo, L. J., Herbert, B. E., Louchouarn, P., 2008. Can levoglucosan be used to characterize and quantify char/charcoal black carbon in environmental media? Organic Geochemistry 39 (10), 1466–1478.

Kuo, L. J., Louchouarn, P., Herbert, B. E., Brandenberger, J. M., Wade, T. L., Crecelius, E., 2011. Combustion-derived substances in deep basins of Puget Sound: Historical inputs from fossil fuel and biomass combustion. Environmental Pollution 159 (4), 983–990.

Nakahara, K., Kitamura, Y., Yamagishi, Y., Shoun, H., Yasui, T., 1994. Levoglucosan dehydrogenase involved in the assimilation of levoglucosan in Arthrobacter sp. I-552. Bioscience, biotechnology, and biochemistry 58 (12), 2193–2196.

Norwood, M. J., Louchouarn, P., Kuo, L. J., Harvey, O. R., 2013. Characterization and biodegradation of water-soluble biomarkers and organic carbon extracted from low temperature chars. Organic Geochemistry 56 (2013), 111–119.

OECD, 1992. Guidelines for the Testing of Chemicals - Test No. 301: Ready Biodegradability.

Schüpbach, S., Kirchgeorg, T., Colombaroli, D., Beffa, G., Radaelli, M., Kehrwald, N. M., Barbante, C., 2015. Combining charcoal sediment and molecular markers to infer a Holocene fire history in the Maya Lowlands of Petén, Guatemala. Quaternary Science Reviews 115, 123–131.

Sheridan, P. P., Miteva, V. I., Brenchley, J. E., 2003. Phylogenetic analysis of anaerobic psychrophilic enrichment cultures obtained from a Greenland glacier ice core. Applied and Environmental Biology 69 (4), 2153–2160.

Sikes, E. L., Medeiros, P. M., Augustinus, P., Wilmshurst, J. M., Freeman, K. R., 2013. Seasonal variations in aridity and temperature characterize changing climate during the last deglaciation in New Zealand. Quaternary Science Reviews 74, 245–256.

Simoneit, B. R. T., Elias, V. O., 2000. Organic tracers from biomass burning in atmospheric particulate matter over the ocean. Marine Chemistry 69 (3-4), 301–312.

Warren, N., Allan, I. J., Carter, J. E., House, W. a., Parker, A., 2003. Pesticides and other micro-organic contaminants in freshwater sedimentary environments-A review. Applied Geochemistry 18 (2), 159–194.

Xie, H., Zhuang, X., Bai, Z., Qi, H., Zhang, H., 2006. Isolation of levoglucosan-assimilating microorganisms from soil and an investigation of their levoglucosan kinases. World Journal of Microbiology and Biotechnology 22 (9), 887–892.

Yao, P., Schwab, V. F., Roth, V. N., Xu, B., Yao, T., Gleixner, G., 2013. Levoglucosan concentrations in ice-core samples from the Tibetan Plateau determined by reverse-phase high-performance liquid chromatography-mass spectrometry. Journal of Glaciology 59 (216), 599–612.

Zangrando, R., Barbaro, E., Zennaro, P., Rossi, S., Kehrwald, N. M., Gabrieli, J., Barbante, C., Gambaro, A., 2013. Molecular markers of biomass burning in Arctic aerosols. Environmental Science and Technology 47 (15), 8565–8574.

Zennaro, P., 2013. Fire in ice: 120,000 years of fire activity inferred from levoglucosan analyses on the Greenland NEEM ice core. Phd thesis, University of Ca' Foscari Venice.

Zennaro, P., Kehrwald, N., Mcconnell, J. R., Schüpbach, S., Maselli, O., 2014. Fire in ice: two millennia of Northern Hemisphere fire history from the Greenland NEEM ice core. Climate of the Past 10, 809–857.

Zhang, X. F., Yao, T. D., Tian, L. D., Xu, S. J., An, L. Z., 2008. Phylogenetic and physiological diversity of bacteria isolated from Puruogangri ice core. Microbial Ecology 55 (3), 476–488.

# A Appendix

## A.1 Declaration of Contributions

- **Study I: Method for the determination of specific molecular markers of biomass burning in lake sediments** (Organic Geochemistry 2014, 71, 1-6.)

  **Authorship:** Co-Authorship: predominant work; Contributions: T.K. (55%) concept, laboratory work, data evaluation, discussion, writing the manuscript; S.S. (30%) laboratory work, data evaluation, comments on the manuscript; N.K. (5%) comments on the manuscript; D.B.MW. (5%): sampling, comments on the manuscript; C.B. (5%): comments on the manuscript

  **Paper Type:** Full research paper; peer reviewed; **Status:** Published; **Journal:** Organic Geochemistry; **Impactfactor** (2013): 2.8

- **Study II: Combining charcoal sediment and molecular markers to infer a Holocene fire history in the Maya lowlands of Petén, Guatemala.** (Quaternary Science Reviews 2015. 115, 123-121.)

  **Authorship:** Co-Authorship: equal contribution; Contributions: S.S. (35%) concept, laboratory work, data evaluation, discussion, writing the manuscript; T.K. (35%) laboratory work, data evaluation, discussion, writing the manuscript; C.D. (10%) comments on the manuscript; G.B. (5%): laboratory work; M.R. (5%): laboratory work; N.K. (5%) comments on the manuscript; C.B. (5%): comments on the manuscript.

  **Paper Type:** Full research paper; peer reviewed; **Status:** published; **Journal:** Quaternary Science Reviews; **Impactfactor** (2013): 4.6

- **Study III: Late holocene record of human and fire at lake Trasimeno, Italy: An organic molecular marker approach**

  **Authorship:** Co-Authorship: predominant work. **Paper status:** unpublished manuscript.

- **Study IV: Biodegradation of levoglucosan, mannosan and galactosan: Implications for paleoclimate research.**

  **Authorship:** Co-Authorship: predominant work. **Paper status:** unpublished manuscript.

## A.2 Curriculum Vitae

**About**

| | |
|---|---|
| Name: | Torben Kirchgeorg |
| Born: | 06.02.1984 |
| Place of Birth: | Wildeshausen, Germany |

**Education and Work**

| | |
|---|---|
| since 11/2011 | Cotutelle PhD student at Ca' Foscari University Venice (Italy) and Leuphana University Lüneburg (Germany) |
| 12/2010-05/2010 | Research assistant at the Department of Environmental Chemistry, Helmholtz-Zentrum Geesthacht, Germany |
| 10/2004-11/2010 | Environmental science studies, focusing on environmental chemistry and sustainability management. Leuphana University Lüneburg, Germany |
| | Diploma Thesis: Perfluorinated Compounds, Polybrominated Diphenyl Ethers and Polycyclic Aromatic Hydrocarbons in a High Alpine Ice Core |
| | Academic degree: Diplom Umweltwissenschaftler<br>Grade point average (German): 1.1 |
| 2001-2003 | Diploma from German secondary school (qualifying for university admission), Gymnasium Wildeshausen, Germany |

## A.3  List of Publications (not included in this thesis)

- Barbaro, E.; **Kirchgeorg, T.**; Zangrando, R.; Vecchiato; M.; Piazza, R.; Barbante, C.; Gambaro, A.: Sugars in Antarctic Aerosol. Atmospheric Environment 2015. under review. *

- Spolaor, A.; Opel, T.; McConnell J.R.; Maselli, O.J.; Spreen, G.; Varin, C.; **Kirchgeorg, T.**; Fritzsche, D; Vallelonga, P. Halogen-based reconstruction of Russian Arctic sea ice extent from Akademii Nauk ice core (Severnaya Zemlya) The Cryosphere 2015. under review. *

- Kehrwald, N.; Zennaro, P.; Schüpbach, S.; **Kirchgeorg, T.**; McConnell, J.R.; Zangrando, R.; Gambaro, A.; Barbante, C. Two thousand years of boreal biomass burning recorded in the NEEM ice cores. PAGES Magazine 2015. 28. 14-15.**

- Dreyer, A.; **Kirchgeorg, T.**; Weinberg, I.; Matthias, V. Particle size distribution of airborne poly- and perfluoralkyl compounds, Chemosphere 2015, 129, 142-149. doi:10.1016/j.chemosphere.2014.06.069 *

- Zennaro, P.; Kehrwald, N.; **Kirchgeorg, T.**; Zangrando, R.; Battistel, D.; Schüpbach, S.; Gambaro, A.; Barbante, C. Fire in ice and sediments: Looking for an early human impact, Italian Society for Climate Science / Conference proceeding. 2014.***

- Eǐerinskis, Z.; Spolaor, A.; **Kirchgeorg, T.**; Cozzi, G.; Vallelonga, P.; Kj, H.A.; Šapolaitė, J.; Barbante, C.; Druteikienė, R.: Determination of 129I in Arctic snow by a novel analytical approach using IC-ICP-SFMS, Journal of Analytical Atomic Spectrometry 2014, 29, 1827-1834. doi:10.1039/C4JA00179F *

- **Kirchgeorg, T.**; Dreyer, A.; Gabrieli, J.; Kehrwald, N.; Sigl, M.; Schwikowski, M.; Boutron, C.; Gambaro, A.; Barbante, C.; Ebinghaus, R. Temporal variations of perfluoroalkyl substances and polybrominated diphenyl ethers in alpine snow. Environmental Pollution 2013, 178, 367â374. doi:10.1016/j.envpol.2013.03.043 *

- Dreyer, A.; Thuens, S.; **Kirchgeorg, T.**; Radke, M. Ombrotrophic peat bogs are not suited as natural archives to investigate the historical atmospheric deposition of perfluoroalkyl substances. Environmental Science Technology 2012, 46, 7512â9. doi:10.1021/es204175y *

- **Kirchgeorg, T.**; Weinberg, I.; Dreyer, A.; Ebinghaus, R. Perfluorinated compounds in marine surface waters: data from the Baltic Sea and methodological challenges for future studies. Environmental Chemistry 2010, 7, 429. *

  *international peer reviewed journals ** editor reviewed *** not reviewed

## A.4 Conferences Contributions as Presenting Author

- **Kirchgeorg, T.**; Schüpbach, S.; Colombaroli D.; Beffa, G.; Radaelli, M., Kehrwald, N.; Barbante, C.; Combining charcoal sediment and molecular markers to infer a Holocene fire history in the Maya lowlands of Petén, Guatemala. Oral Presentation. EGU. Vienna, Austria. 2015. Oral Presentation

- **Kirchgeorg, T.**; Schüpbach, S.; Kehrwald, N.; McWethy, D.; Barbante, C. Determination of specific molecular markers of biomass burning in lake sediments. EGU. Vienna, Austria. 2014. Pico Session.

- **Kirchgeorg, T.**; Gabrieli, J., Dreyer, A., Xie, Z., Schwikowski, M., Barbante, C., Boutron, C., Ebinghaus, R. Perfluorinated Compounds in Ice Core Samples from the Alps. 21th SETAC Europe Annual Meeting. Milan, Italy. 2011. Poster.

- **Kirchgeorg, T.**; Xie, Z.; Dreyer, A.; Gabrieli, J.; Barbante, C.; Gabrielli, P.; Thompson, L.; Ebinghaus, R. Polyfluorierte Verbindungen und polybromierte Diphenylether in datierten Schneeproben aus den Alpen. 4th annual conference of the Working Group on Environmental Chemistry and Ecotoxicology of the German Chemical Society and the SETAC GLB. Dessau, Germany. 2011. Poster.

- **Kirchgeorg, T.**, Xie, Z.; Dreyer, A.; Gabrieli, J.; Barbante, C., Gabrielli, P.; Thompson, L.; Ebinghaus, R. Perfluorinated Compounds and Polybrominated Diphenyl Ethers in dated Snow Samples of the Alps. 20th SETAC Europe Annual Meeting. Seville, Spain. 2010. Poster.