Università
Ca'Foscari
Venezia

Scuola Dottorale di Ateneo
Graduate School

Dottorato di ricerca
in Science and Management of Climate Change
Ciclo 28°
Anno di discussione 2016

The Early Impact of Agriculture
How Humans Have Been Affecting Climate for Thousands of Years

SETTORE SCIENTIFICO DISCIPLINARE DI AFFERENZA: CHIM/01
Tesi di Dottorato di Elena Argiriadis, matricola 825854

Coordinatore del Dottorato	**Tutore del Dottorando**
Prof. Carlo Barbante	**Prof. Carlo Barbante**

Study the past if you would define the future.

-Confucius-

Through all ages the use of fire has perhaps been the most important skill to which man has applied his mind. Fire gave to man, a diurnal creature, security by night from other predators… The fireside was the beginning of social living, the place of communications and reflection.

-Carl Sauer, *Agricultural Origins and Dispersals* (1969)-

CONTENTS

PREMISE

Climate change has become one of the biggest issues of modern times. Scientists of all fields are working hard to answer the key questions that are arising from evidence and research. Whether and to what extent this phenomenon is imputable to the human species is probably the most urgent and challenging one. The present work aims to contribute to the answer by investigating the origins of human-induced environmental changes, starting from the hypothesis that such modifications might date back to a much earlier period than believed.

Climate science is a fascinating and complicated field, shaped day by day by the contribution of several disciplines, apparently very distant from one another. The study of past changes, called *paleoclimatology*, builds upon the solid bases of geology, chemistry, physics and ecology, and is supported by archaeological findings and historical knowledge. In the framework of the PhD course in Science and Management of Climate Change, the tool of choice for reconstructing past changes in this work is analytical chemistry.

Specific markers of human presence and of its influence on local and regional scales were selected and tested. These markers are recorded in valuable and informative archives such as lake sediments. The reconstruction and interpretation of trends provide insight into the mechanisms that may or may not have driven intense transformations in the past, and a powerful instrument to support and integrate paleoecological information.

This work is part of the Early Human Impact project, funded as an Advanced Grant from the European Research Council under the European Union's Seventh Framework Programme (FP7/2007-2013) / ERC Grant agreement n°267696 – EARLYhumanIMPACT.

ABSTRACT

Humans have started shaping the environment since their very first agricultural activities. Whether this influenced climate before the industrial era is object of a lively scientific discussion. Climate changes in the Late Pleistocene – Holocene transition allowed an unprecedented geographic expansion and population growth due to the possibility of producing food. Although centers of early origin and expansion of agriculture are well documented worldwide, researchers highlight the lack of instruments and data for assessing the local and regional effects of deforestation and farming activities on climate. In this work, specific molecular markers for tracing past fire events and evaluating human and animal presence are selected. Namely, polycyclic aromatic hydrocarbons (PAHs), monosaccharide anhydrides and fecal sterols are used as source specific and stable indicators in lacustrine environments. In the first part, specific analytical methods for detecting and quantifying the selected tracers in lake sediment cores are developed. In the second part, the proposed methods are tested and applied on target locations.

New Zealand is known to represent a particular site for the study of the ecological impacts of human settlement. It was occupied by Polynesian only 700-800 y BP and resulted in abrupt and huge landscape modifications. Here, the molecular marker methods were applied on samples from two lakes and results were compared with existing paleoecological records, proving their validity in order to confirm human presence and correlate it with intensity and frequency of fires. Results showed a dramatic increase in the fluxes of both fire and human tracers soon after the Māori arrival, consistent with intensive anthropogenic land clearance, as previously hypothesized from charcoal and pollen evidence. The European 19th century colonization is also evident in the flux of fecal sterols, that rapidly increased following the population growth.

Molecular tracers were further analyzed on test sample batches from Lake Victoria (Uganda) and Flinders Island (Tasmania). In Africa, increased fire activity is observed in correspondence with the drier periods documented by paleolimnological proxies. The influence of the eastward migration of the Bantu speaking populations in the last 2000 years is visible in the sterol record, that shows good correspondence with changes in vegetation and fire regimes. In Tasmania, fecal sterols vary in accordance with the

human presence, that was intermittent along the last 10,000 years, although higher resolution would be required in order to draw more precise conclusions.

All results are presented, compared with literature data and interpreted according to paleoecological, anthropological and archaeological evidence, where possible.

This work provided a reliable and objective instrument for future studies oriented to the creation of a spatial and temporal high resolution database of the early human impact on landscape and climate.

1. INTRODUCTION

1.1. When did the Anthropocene start?

The moment humans started to actively alter climate is generally thought to coincide with the beginning of the industrial era and the consequent release in the atmosphere of significant amounts of greenhouse gases, namely carbon dioxide (CO_2) and methane (CH_4). This period is sometimes called "Anthropocene", a term coined by Crutzen and Stoermer in 2000 [1] to refer to the time span between 1780 AD and the present day. Some authors, though, criticize this view and argue that humans may have had a relevant role as a climate driver since their very first active modifications of the landscape a few thousands of years ago [2, 3]. The debate about the beginning of the so-called new epoch has been so wide that it resulted in the establishment of a new dedicated journal in 2013, *Anthropocene*. Foley et al. (2013) recently tried to settle the matter by introducing an additional term that indicates the transitional period before the industrial revolution in which human effects are small but recognizable at a regional level, though not globally: *Paleoanthropocene* [3]. The Paleoanthropocene would encompass both the Holocene and part of the Pleistocene, starting with the first appearance of humans (see figure 1.1).

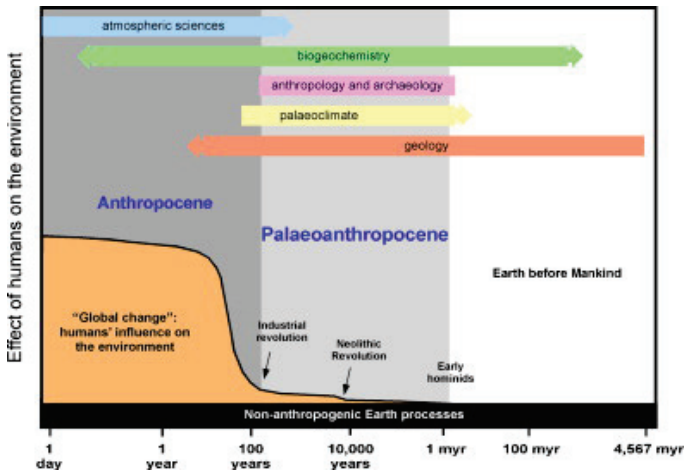

Figure 1.1 Logarithmic scale of Earth's history and Paleoanthropocene placement in time. *From Foley et al. (2013)* [3].

Smith and Zeder (2013) proposed further interpretation that refers to the Anthropocene not as a proper new epoch but as a period coeval to the Holocene, delineating the whole time span in which humans have influenced climate and environment first at a small, negligible scale, then at regional and finally global levels. Indeed, there is no geological evidence supporting the idea of a neat transition from the Holocene to the Anthropocene, although a very recent historical event such as the nuclear explosions starting in 1945 was addressed as most suitable marker [4]. However, several alternative boundaries have been proposed that are identifiable as huge changes in the characteristics of one environmental compartment such as the atmosphere - with the rise in CO_2 and CH_4 concentrations - or the pedosphere with human-induced agricultural transformations (see fig. 1.2.) [5]. As summarized in figure 1.2, even this evidence does not indicate a univocal date for the hypothetical transition: chemistry, for instance, identifies at least three steps of the *global atmospheric change* at 8,000-5,000 years BP [2], at 5,000-4,000 yr BP [6] and, more intuitively, in AD 1750-1800 as a consequence of the industrial revolution [1].

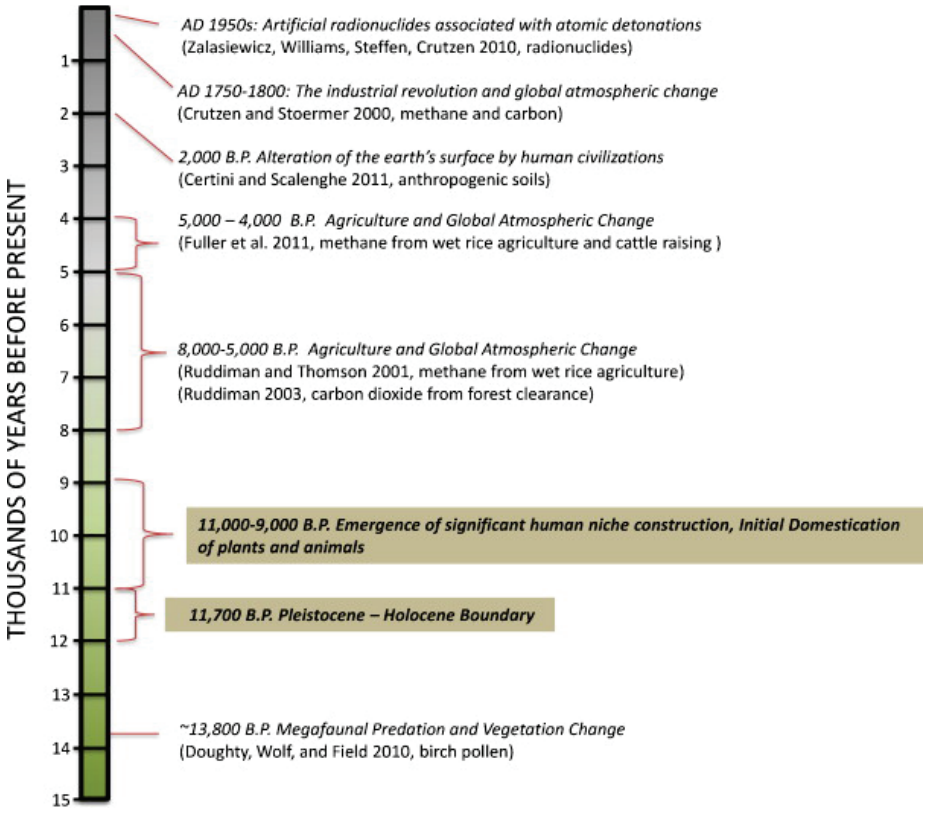

Figure 1.2. Different proposed Holocene-Anthropocene boundaries. *From Smith and Zeder (2013)* [5].

Regardless of all the various definitions arising from different assumptions and backgrounds typical of the diverse disciplines involved in the investigation of paleoclimate, the core problem is *whether, when and to what extent humans started to affect climate*. The paleoclimatologist William F. Ruddiman, in particular, has long concerned himself with this question, and his work resulted in a series of papers published between 2003 and 2015: the fundamental recurring concept is called the *Early Anthropogenic Hypothesis* and states that humans may have started affecting climate thousands of years ago with the spread of agriculture and rice cultivation all over the world [2, 7–12]. The assumption is based on the observation that CH_4 and CO_2 values in Antarctic ice cores start deviating from orbitally driven predictable trends around 5,000 and 8,000 years ago, respectively [13].

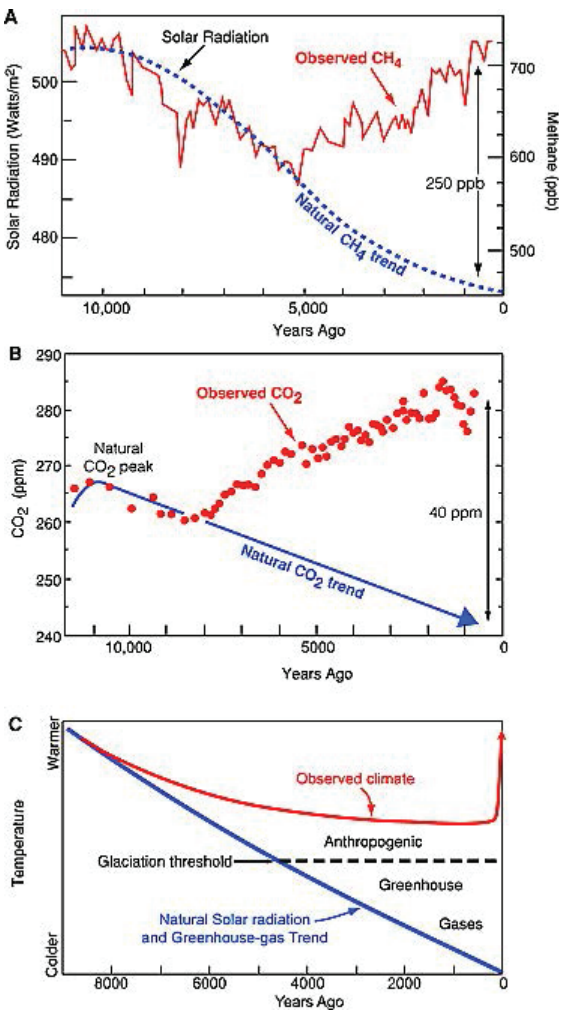

Figure 1.3 Deviation of observed CO_2 and CH_4 from orbitally driven predictable trends. *From Ruddiman (2007)* [8].

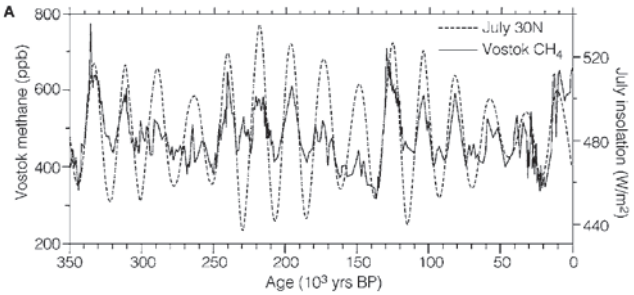

Figure 1.4 Insolation *vs.* CH_4 trend in the Vostok ice core. *From Ruddiman (2003)* [2].

The possible explanation proposed for the 250 ppb methane anomaly is quite linear and simple. Atmospheric CH_4 follows the monsoon cycle forced by insolation with a 23,000 year periodicity. The predictable pattern is respected along all the record until about 5 ky ago, when the projected decreasing trend is contradicted by observed increasing values. Ruddiman identifies the only possible source in early rice cultivation, which appeared first in China approximately 10,000 years ago [14] and by c. 8,5 kyr BP had spread from East to South Asia [15].

By 5,000 years BP, extensive irrigation had begun [2]. Rice paddies produce methane only when fields are flooded and anoxic conditions are favored (see figure 1.6), so only in a part of the cultivation cycle [16]. Nevertheless, huge amounts of methane and other greenhouse gases are produced even nowadays by intensive farming (see figure 1.5). The uncontrolled release of CH_4 in the atmosphere is a cause of concern and mitigation strategies, and guidelines for compiling inventories areregularly included in the IPCC (Intergovernmental Panel on Climate Change) reports [17, 18].

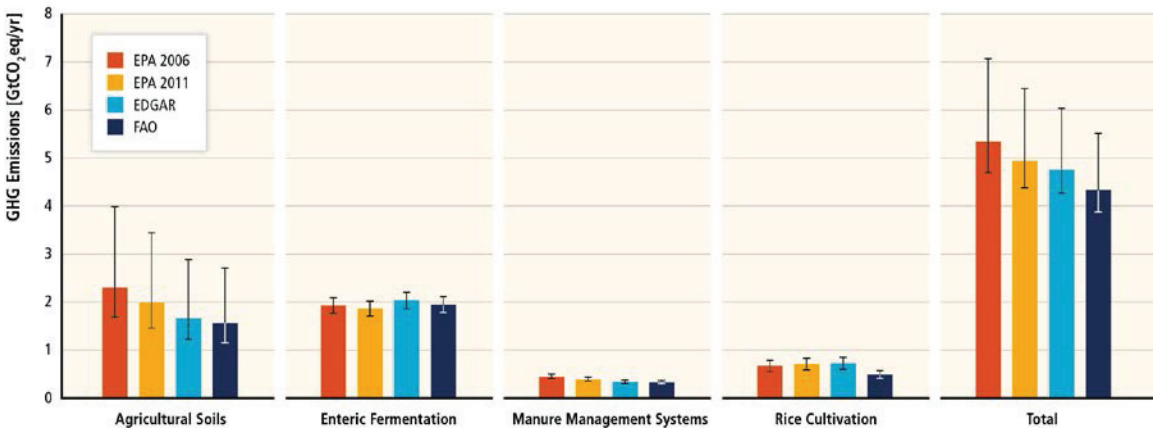

Figure 1.5 The role of agriculture in the production of GHG emissions. *From IPCC 5th assessment report (2014)* [18].

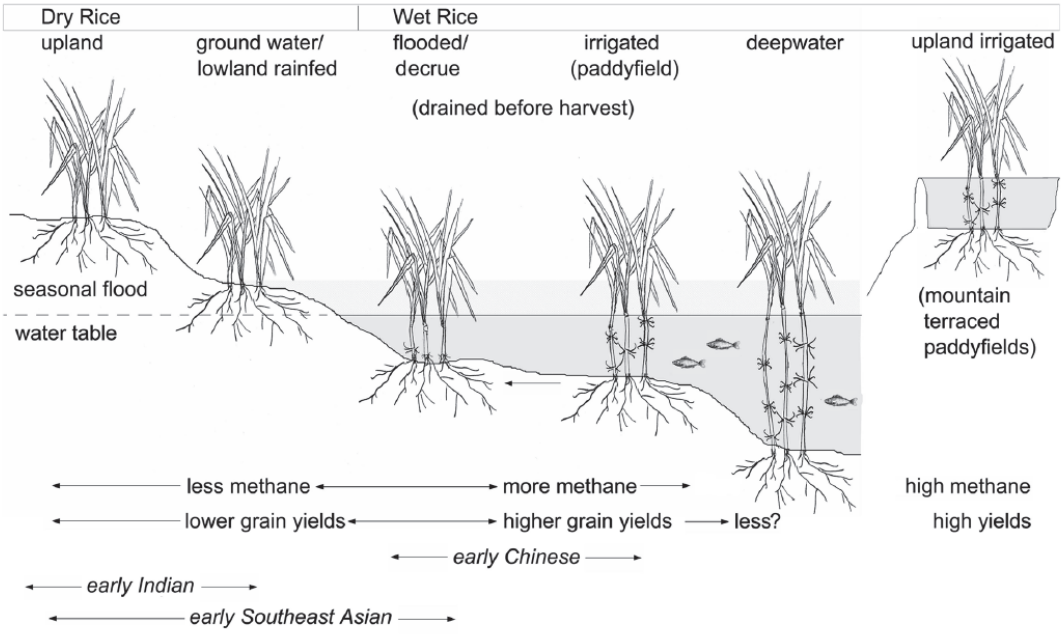

Figure 1.6 Schematic view of the rice cultivation system related to methane emissions. *From Fuller et al. (2011)* [6].

It is thought, therefore, that inefficient early irrigation techniques could have caused the observed increase 5 kyr ago. Even though it appears disproportionate if compared to the population density of the time, the quick expansion of irrigation from China and the spread of agriculture with the consequent deforestation can justify such rapid and huge transformations [2, 9].

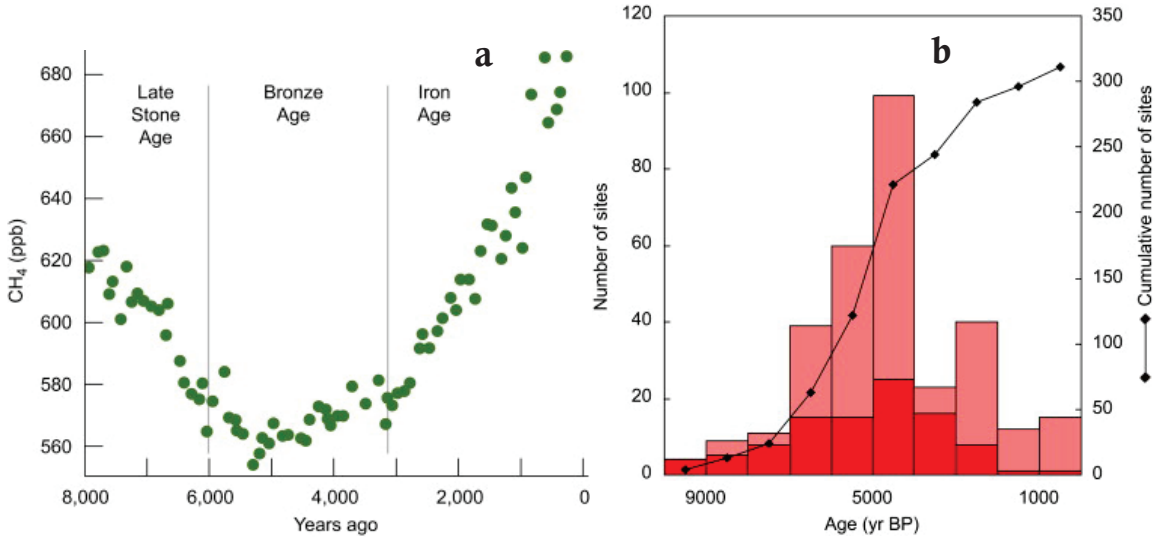

Figure 1.7 Holocene methane trend (a) and appearance of rice sites since 10,000 years ago (b). *From Ruddiman et al. (2008)* [9].

Other sources of greenhouse gases exist, that are directly related to agriculture and land use and contribute to the overall balance: one could mention biomass burning for forest clearance and waste elimination, livestock tending, human and animal waste. Figures 1.7a and 1.7b show the last 8,000 years methane trend (EPICA Dome C) in relation to the main stages of human evolution in China and the appearance of rice cultivation sites in the last 10,000 years [9]. It is evident that the reversal of the methane curvecoincides with the appearance of the highest number of rice sites in the Holocene. Once humans had assimilated how to control land and produce food, a rapid diffusion of these practices occurred, followed by a continuous increase in population that produced the consequences on the environment and climate that are the object of this work.

A similar trend is observed even earlier for CO_2, starting about 8,000 years ago. The possible explanation here is more complex, as natural variations in the CO_2 trend are

subject to all three orbital periods and a large number of processes concur to its release in the atmosphere. It is clear, though, that the Holocene rise is unprecedented if compared to previous interglacials. Several hypotheses have been proposed in order to identify the natural drivers of this rise in carbon dioxide [10 and references therein]. The main critics of the anthropogenic explanation claim that human population at the time was too small to produce such dramatic changes [19]. Broecker et al. (1999 and 2006) proposed thatthe regrowth of forests and bogs after the glaciation could have resulted in a disequilibrium in the ocean CO_2-$CaCO_3$ system, followed by readjustment andrecovery to a sufficiently large extent as to justify the increase in atmospheric CO_2 [19, 20]. A second valid idea, referred to as the "coral-reef hypothesis", states that an acceleration in the building of coral reefs enriched the ocean with CO_2 that was subsequently released into the atmosphere [21].

Ruddiman and his co-authors, though, provide evidence to confute these theories point by point: they use mass balances to prove that previous hypotheses are not sufficient to explain the effective amount of CO_2 released in the atmosphere. With the support of models,the observed imbalance of 330 GtC (corresponding to 23 ppm) can be ascribed to anthropogenic deforestation as a consequence of agricultural diffusion, despite the major criticism about population density [11]. The key concept to be considered here is the importance of *land use per capita*. All previous theories assume that the individual amount of land consumed for human settlement and agricultural activitiesincreased linearly with population. On the contrary, it seems reasonable to think that a small population, in the moment of major expansion and diffusion of agriculture, would occupy and exploit as much land as possible, also taking into account the inefficiency of early cultivation techniques. Once population density increased and agriculture became more intensive, less land was used per capita [22].

The model approach discussed by Kaplan et al. (2011) and later by Ellis et al. (2013) shows how, depending on the initial assumptions on the relationship between land use and population density, early land use in the Holocene can be pictured both as a major change at a global level or, conversely, as almost insignificant before industrial times. The authors used two models called HYDE and KK10, starting from two very similar population datasets. The first model does not include land-use intensification over time, while the second is based on a non-linear relationship between population and land use.

Outputs looked very distant from each other, as visible in figure 1.8: HYDE simulated a world where, except for more developed countries, no significant anthropogenic impact occurs before industrial times; on the contrary, results from KK10 showed relevant land use in Europe and Asia by 5000 years ago, achieved also in the rest of the world by 1000 years ago [22, 23].

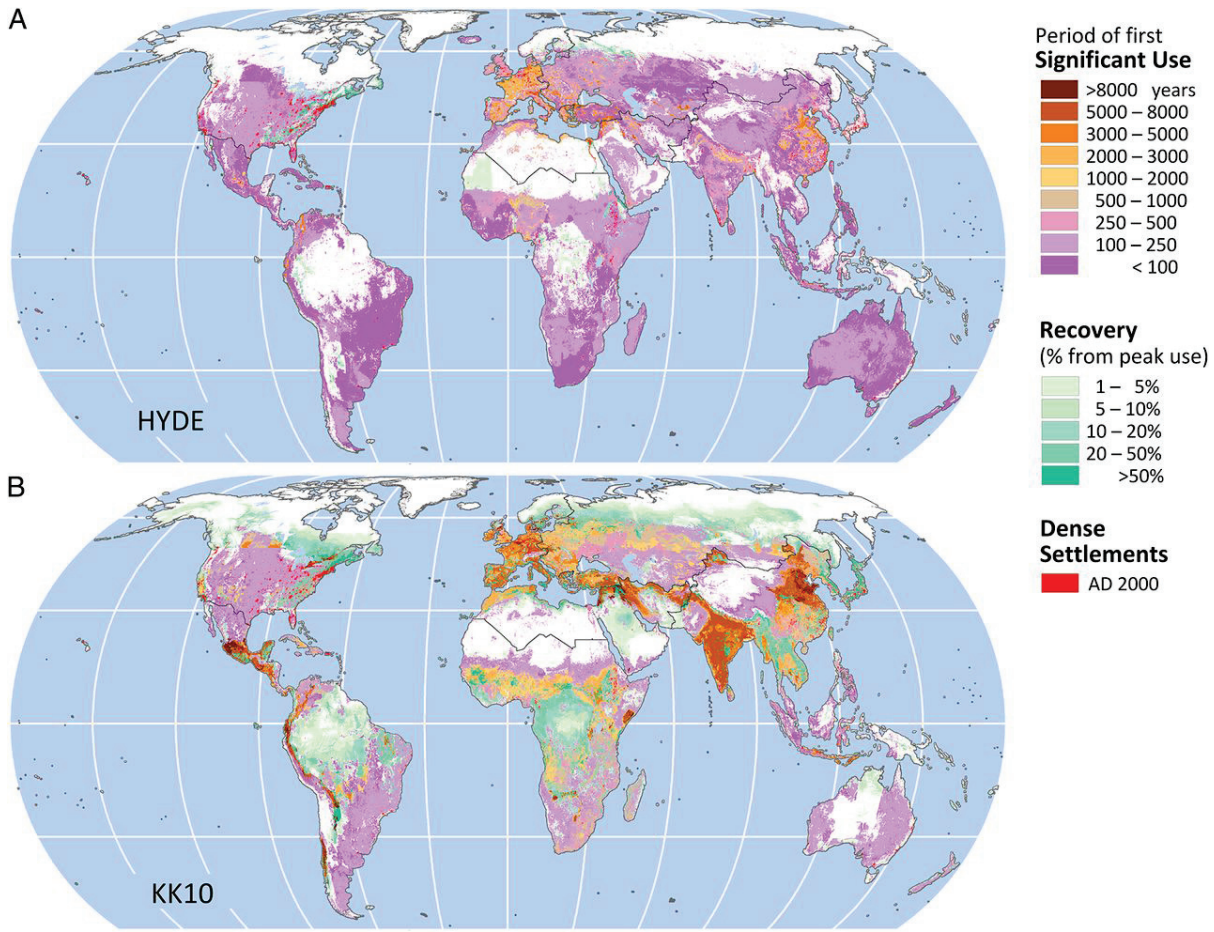

Figure 1.8 Time period of first significant land use as reconstructed from HYDE and KK10. *From Ellis et al. (2013)* [22].

It must be stressed, though, that models tend to suffer from a strong limitation deriving from the scarcity and heterogeneity of existing data about land use. Measures exist only for contemporary times while, going back in time, datasets become more and more indirect, discontinuous and empirical. Temporal and spatial coverage are therefore insufficient to provide a high-resolution portrait of the past, and simulations are based on hindcasted geographical patterns, which certainly affects the accuracy of the

reconstruction [22]. Moreover, the study of regional and short-time changes and of the influence of humans before the industrial era is a recent field and the adequate tools are still under development. Up to now, more effort has been put in developing instruments for the study of global, long-scale phenomena both for reconstructing the past and for modeling future scenarios. Thus, there is a strong need to improve them and to collect more data in order to delineate changes that occurred before the Anthropocene [22].

What is certain is that no existing model at present is able to produce accurate predictions on a global scale for the Holocene. For all the above reasons, research needs to focus on establishing common methods and indicators for a high-resolution description of the Holocene.

1.2. The birth and spread of agriculture

The moment people began *producing* rather than *collecting* food dates back to around 11,000 years ago, when climatic conditions after the end of the last ice age started to favor the growth of edible species. Tundra and steppe left space to forests, and changes occurred in the composition of plant and animal populations at all latitudes. The change in temperatures and habitat affected the body size of large mammals, that became smaller and less numerous [14, 24–26]. Many species disappeared completely and by 10,000 years ago the number of previously existing megafauna genera had decreased by two thirds, something unprecedented during earlier deglaciations. Several authors believe that hunting by humans played a major role in the so-called megafaunal extinction: climate responded differently to deglacial changes at different latitudes, so it would be difficult to explain all the extinctions only with climatic shifts [27].

The long debated "overkill hypothesis", originally proposed by the paleoecologist Paul Martin in 1984, identifies the main cause of this massive event, that interested mainly the Americas and Australia, in human hunting [28]. Again, critics claim that people were too few to cause such a dramatic loss of species. Moreover, some species that were not hunted went extinct as well [26, 27]. Therefore, the most likely explanation could reside in the simultaneous effect of both factors. Environmental changes caused a disequilibrium in the size of habitats and in prey-predator relationships, at the expense of highly specialized and sensitive species [26]. Hunting was probably simplified by more favorable climatic conditions, and human hunting skills progressively improved: this resulted in population growth of and in the depletion of large preys, which made the search for an alternative food sources mandatory [24, 29]. The relatively warm and wet new climate made possible in many areas something that was unattainable in the Pleistocene: agriculture [24, 30]. This process started as soon as the high-frequency variability and the extremeness of the Ice Age left space to the more stable Holocene. According to Richerson et al. (2001) and Diamond (2002), therefore, the "agricultural revolution" could not have occurred before the end of the Pleistocene although human capacities might have been mature before this date and *Homo sapiens* had already colonized all the continents (figure 1.9). Environmental changes triggered an unavoidable chain of events that led to enormous historical and social consequences [24, 30].

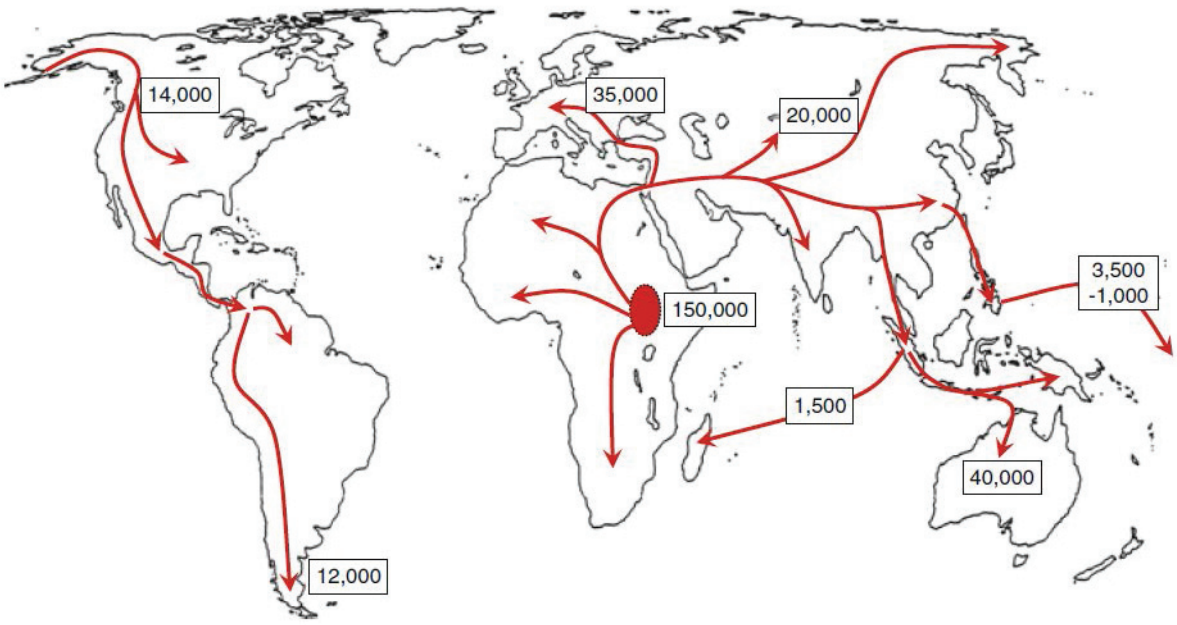

Figure1.9 The spread of *Homo sapiens* from Africa around the world (years BP). *From Martin and Sauerborn (2013)* [14].

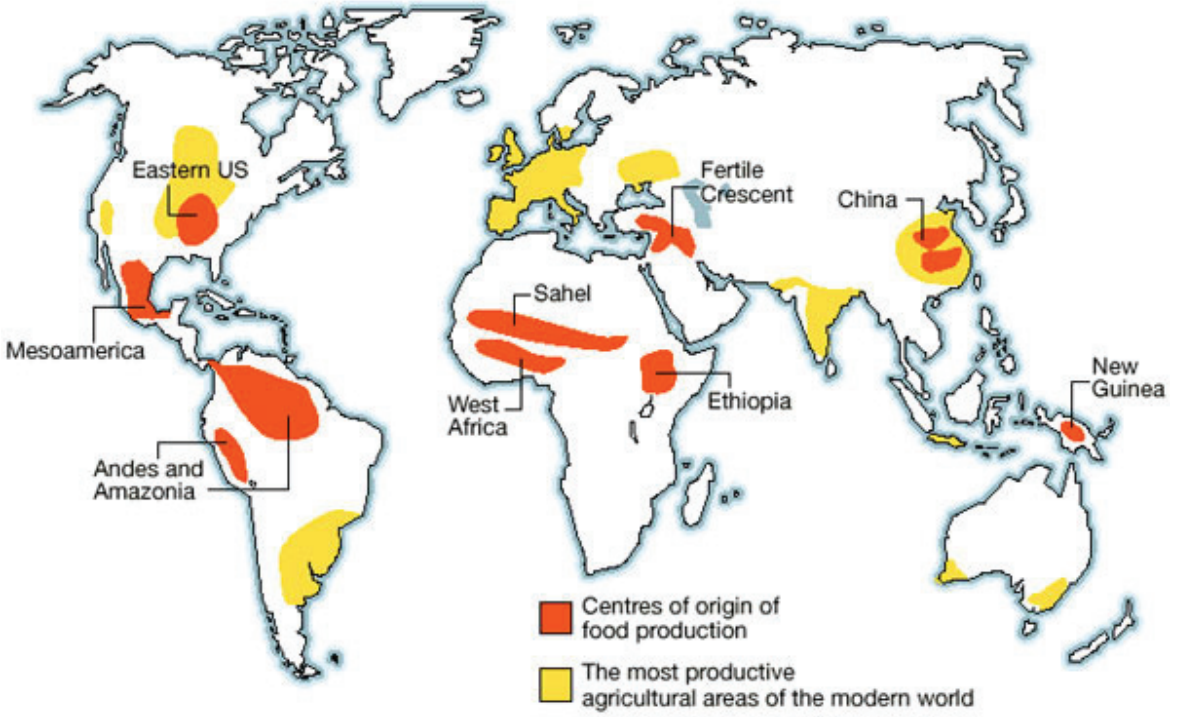

Figure1.10 Ancient and modern centers of agriculture. *From Diamond (2002)* [24].

But stepping into this totally new way of living was not a rapid and linear process. Rather, it was a slow and gradual transition from hunter-gatherers to farmers, during which the two strategies coexisted for a long time [24, 31]. Farming was not preferable over foraging in terms of energies spent, number of people employed and nutritional value gained, so a strong constraint must have acted as discriminating factor [25, 32]. In addition to the climatic issue, population pressure was probably an important trigger. Agriculture emerged and spread once wild plants and animals started to be insufficient and climate variations made more domesticable wild species available: food production allows to feed more people per area unit, by intensifying land use.

Classic theories identified in the Fertile Crescent - a limited region located in southwest Asia between the modern Turkey, Syria and Iraq - the center of origin of agriculture from where plant domestication would have spread all over the world. More recent findings, though, proved that agriculture originated independently in several parts of the world and in some cases at very different times [31, 33]. Based on sedimentary and archaeological evidence, independent centers of origin of agriculture were highlighted in several "hot spots" on each continent, as visible in figure 1.10. Curiously, they do not coincide with the current most productive areas, but of course with the areas where the largest number of domesticable crops and livestock were present. The regions and approximate timing for the first appearance of domesticated plant and animal species are the following, according to references 13, 24 and 33:

- **Southwest Asia (Fertile Crescent)**
  - ~11,500 years, plants: *emmer and einkorn wheat, barley, pea, chickpea, flax*
  - ~10,500 years, animals: *sheep, goat, pig*
- **China**
  - ~10,000 years, plants: *rice, millet, soybean*
  - ~ 10,000 years, animals: *chicken, pig*
- **Mesoamerica**
  - ~9,000 years, plants: *winter squash, corn*
  - ~2000 years, animals: *turkey*

- **South America**

  ~10,000 years, plants: *potato, peanut, common bean* (Andes); *squash, pumpkin, chili, pepper, pineapple, sweet potato, cassava, avocado, cotton* (tropical lowlands)

  ~6000 years, animals: *guanaco, guinea pig*

- **Southeast Asia (New Guinea)**

  ~10,000 years, plants: *banana, sugar cane, taro, yam*

- **Africa**

  ~10,000-8000 years, animals: *cattle*

  ~6000 years, plants: *coffee, finger millet, teff* (Ethiopia);*sorghum, pearl millet, African rice* (northern Africa savannas), *oil palm, cowpea, African yam* (western Africa savannas)

- **Eastern North America**

  ~4,000 years, plants: *sumpweed, pigweed, sunflower*

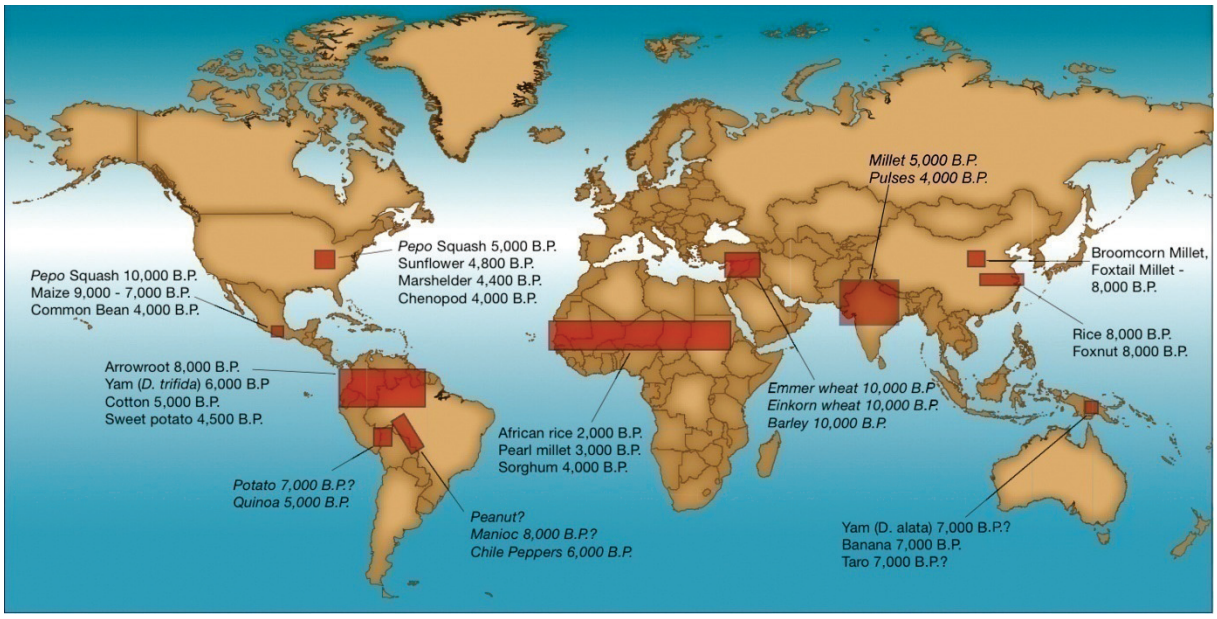

Figure 1.11 Major centers of domestication and dates for earliest plants and animals. *From Price and Bar-Yosef (2011)* [34].

Figure 1.11 provides a detailed picture of first agricultural areas and cultivated species. Dates in the text refer to the latest findings reported in literature, which for many locations pushed back the time of the first appearance of domesticated species [35]. This

would explain the discrepancy between dates in the figure [34] and those reported in the text [14].

Once adopted in the original regions, cultivation techniques, seeds and instruments started to travel and spread in neighbouring zones. Although not the only one, the Fertile Crescent was certainly the first and major repository of the crops and livestock on which today's intensive food production is based too, such as wheat, pig, sheep and cattle. From there, domestication could rapidly reach the whole Mediterranean basin through colonialism, the adoption of farming techniques and further domestication of local wild species. Similarly, the newly acquired knowledge proceeded eastward towards the Indian subcontinent. By about 7000 years ago, agriculture was diffused along all the coasts of the Mediterrenean and in most regions of Central Europe, as shown in figure 1.12 [14, 35]. It took some thousand years to reach Northern Europe and alpine zones, where animal husbandry was long privileged over land cultivation. By 4000-3500 years ago, east-western Euroasiatic exchanges had brought to Europe crops and domestic animals from Central and Southeast Asia [35].

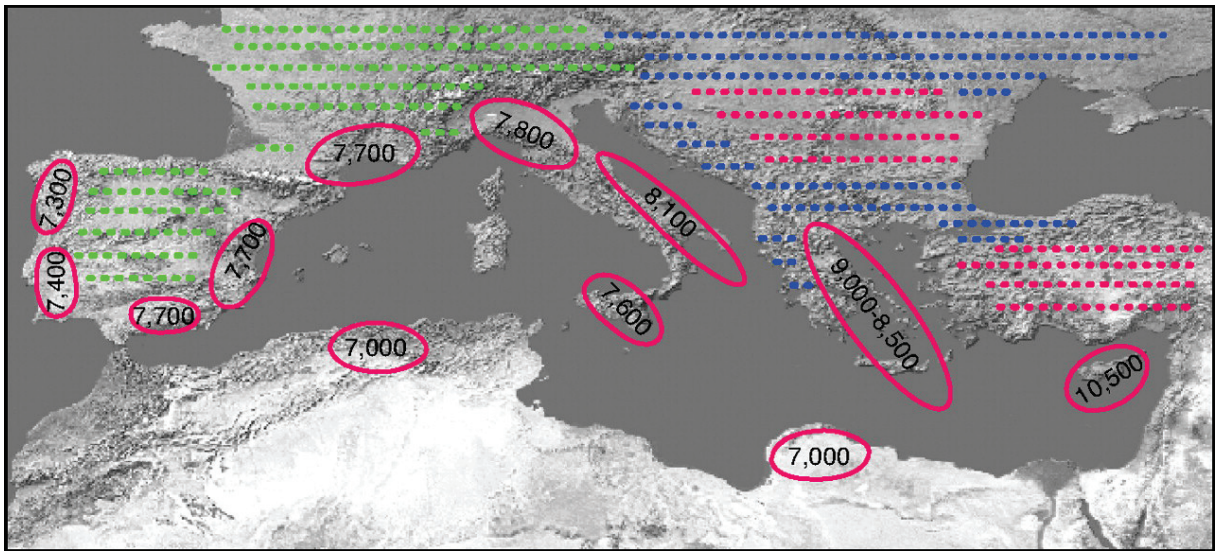

Figure 1.12 Diffusion of agriculture in the Mediterranean basin. Red ellipses indicate colonist enclaves with approximate dates in cal years BP. Red dots: colonist farmers; green dots: farming adopted by local populations; blue dots: colonist farmers integrated with indigenous foragers. *From Zeder (2008)* [35].

However, domestication followed different pathways and dynamics according to the environmental, climatic and social contexts. Humans adopted the strategies that proved most profitable and best suited to their life and habits. In the arid North Africa, for

example, food production started with herding: the first farmers were nomadic hunter-gatherers domesticating cattle since about 10,000-8000 years ago; the first domestic sheep and goats, probably imported from Asia, appeared around 7000 years ago. Plant domestication occurred only after 4000 years ago, with wide differences between the north and the south of the continent, where wild domesticable species were scarce, and the diffusion was anything but homogeneous [24, 36]. Similarly, eastern North America started domestication only around 4000 years ago, about six thousand years later than in southwest Asia. In North America, the availability of indigenous domesticable wild seeds was limited and, as argued by Smith (2011), climatic conditions became more stable and favorable to the occupation of riversides only in the Mid-Holocene. Native Americans kept relying on wild animals and plants until the emergence of food-producing economies around 2500 years ago [37, 38].

Hunting-gathering and farming are therefore not necessarily mutually exclusive, and each region could tell its own history of domestication and cultivation. Nor does agriculture automatically imply sedentism: villages in the Fertile Crescent existed before people began cultivating plants, and nomadic hunter-gatherers practiced farming in China, North America and Mesoamerica [31].

1.2.1. Impact and consequences of agriculture

Figure 1.13 shows the increase in world human population starting from the appearance of the genus *Homo* to the present day. As previously mentioned, population growth was both a driver and a major consequence of the Neolithic agricultural revolution. A relatively abrupt increase in human birth rate is observed in Asia, Europe, Africa and North America in correspondence with the transition from foraging to farming, regardless of the specific moment in which it occurred [39, 40]. This phenomenon is named *Agricultural Demographic Transition* (ADT) and is believed to result from an increase in fertility rather than from a decrease in mortality, as a consequence of changes in diet (introduction of legumes and carbohydrates) and smaller energy expenditure required by sedentary life [25, 40, 41]. It is estimated that human population grew from

~5 to ~50 million between 10 and 5 thousand years ago, doubled in the following 2000 years and reached 250 million between 3000 and 1000 years ago. These enormous increases were made possible mainly in Asia by the adoption of aquatic rice growing systems and only later in the Middle Ages with the introduction of fallow and animal-drawn plow in Europe [42].

The demographic shift is the prerequisite for the construction of a complex, stratified society as we know it today: once technological advances allowed it, food surpluses were stored to feed non-farmer individuals, who covered political, religious and economic roles [25].

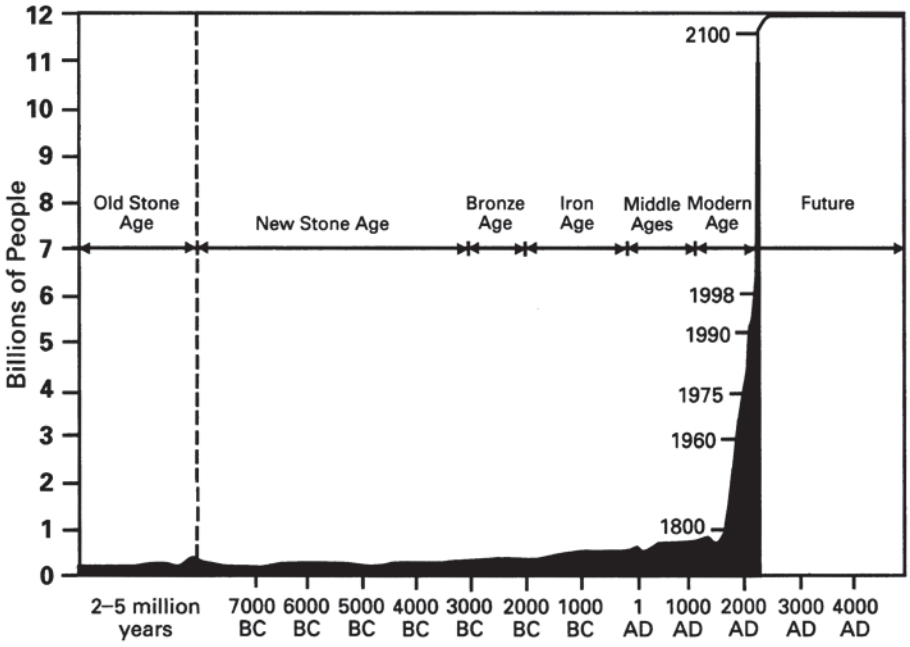

Figure 1.13 World population growth since the birth of agriculture. *From McFall (1991)* [43].

Beside social consequences, which are beyond the scope of this work, the increase in population density meant progressive intensification of land use with growing pressure on the environment. Since its very beginning, the agricultural revolution has had undeniable effects on the biosphere, atmosphere and pedosphere that cannot be disregarded and still wait to be accurately quantified.

Impacts on soil

At first, agriculture was practiced on unforested areas. The exponential rise in population, though, required continuous expansion and increasing yield per land unit. Cleared land therefore soon ran out and Neolithic farmers had to turn to forests and grasslands: *slash-and-burn* agriculture then became the most common practice for managing land cultivation. Available stone tools allowed cutting trees and shrubs, but were useless for working the soil and clearing it from grasses, so farming developed preferably on forested areas rather than grasslands, where pastoralism was privileged. The slash-and-burn technique requires cutting smaller trees, leaving the plant material to dry on the ground, burning it, then letting the ash fertilize the soil and sow seeds. Cleared areas are then dedicated to one or two cultivations at most, before being abandoned, slowly reforested and cleared again after some decades. Farmers therefore need to keep shifting and clearing new land patches around their settlements, expanding the area of influence. If the idle period is shortened, as in the case of population growth resulting in intensive productive pressure, the soil becomes overexploited and depleted in nutrient minerals, with a consequent loss in fertility. Thus, the forest has no time to recover and the higher deforestation rate also increases the risk of erosion and extreme events connected to intense runoff, such as floods and landslides. Finally, the smaller retaining capacity of water in soil and plant biomass in wide areas can cause the climate to dry out. Some environments are more sensitive than others to the consequences of deforestation. This is the case of Mediterranean areas and in particular Mesopotamia, where erosion and nutrient loss brought to a decline in soil productivity and the consequent need to integrate farming with animal breeding and to devote depleted areas to grazing [29, 42].

Impacts on biodiversity

The use of deforested areas as pasture for livestock close to cultivated zones was a common practice that further exploited an already depleted soil. Grazing, indeed, tends to leave only inedible species on land, operating an unintended selection that affects biodiversity [29]. As mentioned above, by the time agriculture started its unstoppable diffusion, humans had already acted significantly on the number and variety of mammals by hunting. Furthermore, the elimination of forest by fire caused an immediate loss in

biodiversity and destroyed the habitat of many animal species. More sensitive species were therefore destined to local or total extinction [44].

The most important negative effects on biodiversity, however, are ascribable to the domestication and selection of both wild animals and plants. Only a handful of animal breeds and crops actually had all the characteristics required for domestication and selection generation after generation, and this long, difficult process undoubtedly produced several unexpected effects. For example, the worldwide diffusion of agriculture often resulted in the intentional or accidental introduction of non-native species that rapidly spread in absence of natural competitors and regulation systems, disrupting the equilibrium of ecosystems and population dynamics [42, 45].

Human necessities often tend to privilege features that have no evolutionary advantage in nature, and properties that natural selection would eliminate. Many domesticated species therefore lost the capacity to disperse and reproduce and became fully dependent on agricultural mechanisms [45]. Genetic variability guarantees resistance to pests and diseases, while the base of domestication is the reduction of the degree of mutations and the selection of advantageous ones. This process creates pools of individuals that are genetically similar and more vulnerable than the wild ancestors they replaced [42, 44]. Biodiversity loss and intensive land use also facilitate the diffusion of germs: one significant example of indirect consequences is the spread of endemic plagues that killed millions of people in the course of history. Such epidemics could not have developed prior to the increase in human and animal population density that followed the spread of agriculture [24, 25].

Impacts on atmospheric chemistry and climate

According to the latest IPCC assessment report, about 25% of today's total anthropogenic greenhouse gases emissions are ascribable to *agriculture, forestry and other land use* [46]. Without any doubt, deforestation occupies an important place in the global carbon cycle. As mentioned above, biomass burning was (and still is today) largely employed in forest clearing [47]. Combustion releases in the atmosphere huge amounts of water vapor and CO_2 as primary products, both active as greenhouse gases. This release

is rapid and immediate, whereas it took some time to store atmospheric CO_2 in biomass in the first place. In the same way, a burned forest would take long to grow again and readjust its balance: meanwhile, CO_2 is accumulated in the atmosphere where it exerts its warming potential. In a shifting agriculture, where forest is cleared and after a relatively short usage period the land is abandoned and left to natural reforestation, the CO_2 released by burning is rapidly taken up by re-growing vegetation. When land is permanently cleared, though, the agricultural crop or grasses that replace the former forest cannot store the same amount of CO_2 released, resulting in a net increase of the atmospheric burden [48].

Along with water vapor and CO_2, many other trace gases and substances associated to particulate matter are produced by biomass burning, mainly as a result of incomplete combustion. Carbon monoxide (CO) is produced by the oxidation of carbon compounds together with CO_2, and relative amounts vary according to the characteristics of fire and the oxygen supply. Burning in conditions of oxygen depletion produces methane (CH_4) and many other hydrocarbons, together with a variety of organic compounds that will be treated extensively below. Compounds containing nitrogen and sulfur also contribute with the production of nitrous oxide (N_2O), nitrogen oxides (NOx) and sulfur oxides (SOx) [48]. Table 1.1 shows an estimate of the amount of carbon (as CO_2, CO, CH_4 and other hydrocarbons) released at the present day in the tropics by various practices associated to agriculture and deforestation and a list of other substances with the relative contribution to the total emission [49].

Aerosols with such composition act on local and regional climate by absorbing or reflecting radiation. Particulates and sulfur dioxide (SO_2) somehow contrast the effect of greenhouse gases by scattering solar radiation and acting as cloud condensation nuclei, thus affecting cloud cover [50]. On a large scale, changes in land cover also alter the albedo. Agricultural land has higher albedo than forest: deforestation therefore increases the amount of reflected radiation, resulting in reduced evapotranspiration and thus in reduced rainfall, which can seriously alter the local hydrological equilibrium, with consequences for soil and erosion patterns [51, 52].

Table 1.1 Carbon emitted through biomass burning in the tropics and comparison of emitted gases and particulates to estimated total sources. [a]Nitriles: $(CN)_2$+HCN+CH_3CN+C_2H_5CN+C_2H_4CN; [b]Carbonyl sulfide; [c] Total Particulate Matter; [d] Particulate Organic Carbon; [e] Elemental Carbon. *From Crutzen and Lelieveld (2001)* [49].

Sources	**Amount (Tg year $^{-1}$)**		
Slash and burn agriculture	500-1000		
Forest clearing	200-700		
Savanna grass fires	300-1600		
Wood burning	300-600		
Agricultural wastes	500-800		
Total	1800-4700		
Compound	**Amount**	**Emissions from Biomass Burning (Tg year^{-1})**	**Total emissions by all sources (Tg year^{-1})**
All carbon		1800-4700	
CO_2		1600-4100	
CO	43±13%	400-700	780-1960
CH_4	6.6±5%	10-70	520-680
H_2		5-16	36
CH_3Cl		0.5-2	2
NOx	18.1±11.3%	3-13	30-73
RCN[a]	3.4±2.5%	0.5-1.7	>0.4
NH_3	3.8±3.2%	0.5-2.0	20-60
N_2O	0.7±0.3%	0.1-0.3	12-14
SO_2	2.2±1.3%	1.0-4.0	70-170
COS[b]	0.01±0.0005%	0.04-0.20	0.6-1.5
TPM[c]	30±15 g/kg C	36-154	≈1500
POC[d]	20±10 g/kg C	24-102	≈180
EC[e]	5.4±2.7 g/kg C	6.4-28	20-30

In all these processes, however, it is important to consider the scale factor: the impact of agriculture described so far can have local, regional or global effects depending on the spatial and temporal scales involved. Slash-and-burn traditional agriculture, practiced on a small scale and with long recovery periods, allowed the carbon budget to balance naturally. On the contrary, intensive land use and irreversible land use changes have caused and are still causing the huge imbalance we observe [51]. At some point in history, therefore, the intensity of human activities crossed this limit and the rate of resources consumption became too high to be counterbalanced without consequences for the climate. Whether this happened 8000 years ago as proposed by Ruddiman or only

250 years ago with the industrial revolution is still the object of an open discussion [1, 11, 53, 54].

1.2.2. Fire and humans: a brief history

Fire appeared on Earth as soon as fuel and oxygen were available, that is when land plants emerged about 420 million years ago (Mya). Natural fires were triggered by lightning, volcanic material or meteorite impacts. Charcoal data show how fire activity increased with the increase in atmospheric oxygen ~345 Mya, as shown in figure 1.14. The oxygen percentage in the atmosphere reached 31% in that period, 10% more than the present 21% [55, 56]. Fire frequency then followed oxygen fluctuations, and increased progressively after its stabilization to current values in the Tertiary [55].

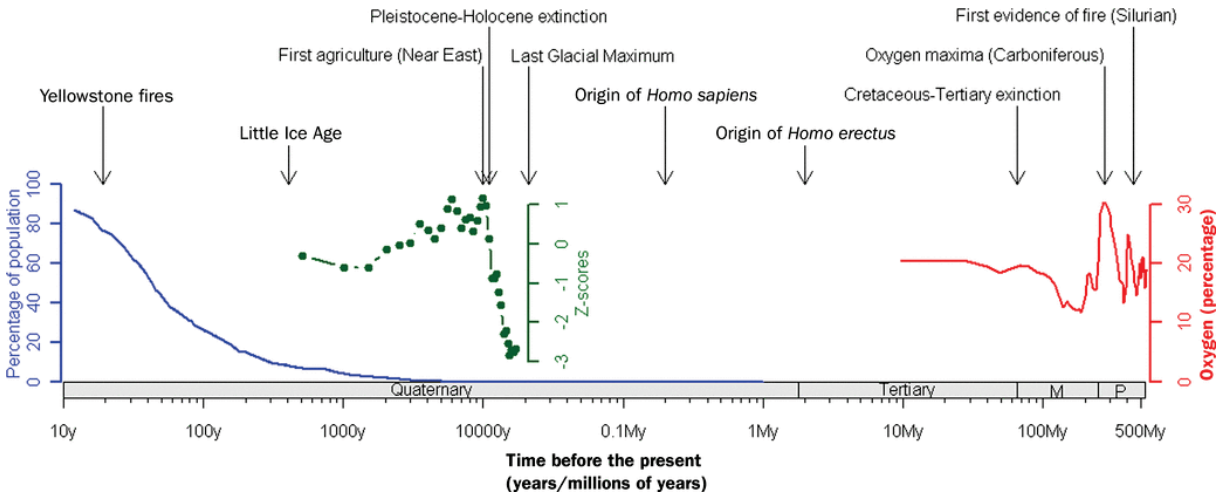

Figure 1.14 Oxygen percentage from 500 to 10 Mya (%, red), global charcoal values in the interval 4000-100 y BP (Z scores, green) and human population since 1 Mya (% relative to 2006, blue). Time is in logarithmic scale. *From Pausas and Keeley (2009)* [55].

It must be stressed that a wildfire is not necessarily a catastrophe, as we are used to think. To the contrary, it has a specific ecologic function and significant effects on the selection and evolution of plants [45]. Adaptation to fire has brought to the development of particular strategies involving bark thickness, the ability to resprout after a fire and germination. Some plants, in fact, are so well-adapted to fire that they actually need it for

their reproductive cycle: a typical example is the north American jack pine, whose cones are able to open only if subjected to temperatures higher than 120 °C, or some angiosperm species, in which seeds germinate in response to chemicals contained in smoke or heat shocks [29, 45, 55]. Such adaptations confer an enormous advantage to plants that are able to rapidly spread in post-burn landscapes, at the expense of the previous population. Moreover, as farmers have always known, fire exterminate parasites and post-fire levels of nutrients are higher due to the accelerated decomposition of organic layers, increasing the subsequent net primary productivity [29, 45].

The intensity and frequency of natural fires are regulated by precise laws as are other natural phenomena, such as earthquakes [57]. It is the use of fire by humans that introduced a new variable in the natural equilibrium. The mastery of fire is something exquisitely human, something that distinguishes the genus *Homo* from the rest of animals even more than the elaboration of languages, the use of tools or the upright position. Through history, people used fire for multiple applications: clearing vegetation for hunting and, later, for agriculture; keeping off enemies, dangerous animals and pests; providing heat and light; cooking; creating tools and pottery; producing charcoal; burning the dead [45, 58].

Although contested, the first record of man consciously using fire appears to date back to approximately 1.6 Mya in East Africa, while evidence is better established starting 790 thousand years ago (kya) in Israel and more or less synchronously in China 780-680 kya. European sites show more robustness but raise a question: indeed, it was thought that fire had been decisive in the expansion towards Europe during the Pleistocene 1.2-1.1 Mya. However, fire is not recorded in Europe before 400 kya and tracks of extensive and regular use of fire are visible only from 125 kya. Early occupants therefore should have managed to live without the knowledge of fire, although they needed it to reach higher latitudes and survive during glacial periods [56, 59].

The introduction of fire in human lives allowed huge evolutionary steps: the timing of fire discovery coincides with the emergence of *Homo erectus*. It is believed that their success over other species was made possible by the use of fire. Consuming cooked food improved health conditions and decreased the chance of diseases; clearing land enhanced the hunting success rate; and the knowledge of fire allowed northward expansion: all

these advantages guaranteed higher survival and reproduction possibilities, thus allowing population growth and physical development [55, 58]. The uses of fire are collectively described as fire-stick farming, and characterize the way in which fire was used during the Paleolithic and the Mesolithic. The Neolithic revolution, as mentioned above, needed fire to open up landscape and this likely became necessary after the megafaunal extinction, as huge grazers disappeared and control on natural fires and forest expansion was no longer exerted [55].

Not much is known about the effective intensity and extent of anthropogenic fires throughout the Holocene, and just as little about the relative importance of humans as a fire driver compared to climate fluctuations. We know from archaeology and anthropology that humans used fire for modifying the environment and for a wide variety of scopes, but understanding if and when human control of fire ever overcame climate control requires adequate instruments that are still under investigation [60].

Sedimentary charcoal is the best known and most widely employed among fire tracers. It is a reliable and powerful instrument for reconstructing past fire intensity and frequency and for inferring sources and spatial scales. Important charcoal studies represent the base of knowledge about Holocene fire regimes worldwide. The Global Charcoal Database has grouped and compared all available charcoal data from sites all over the world, in order to obtain a portrait of paleofire regimes since the Last Glacial Maximum (LGM), centered 21,000 years ago [60]. Along the whole transition from the Late Pleistocene to the Holocene, fire patterns globally followed climate change and reflected insolation cycles: cool and dry conditions prevented the accumulation of fuel and fire frequency was limited up to 16,000-15,000 years ago, when a significant increase is observed in Australia and slight increases, still limited by fuel availability, occurred in South America and Europe. Early Holocene recorded the increase in forest cover at latitudes previously covered by ice: temperature and insolation conditions favored generally higher fire frequencies in North and South America and Europe, followed by a new global decrease after 9000 years BP. From 6000 years BP, the combined effect of climate and human control on vegetation started providing conditions for a widespread increase, so that by 3000 years ago fire regimes resembled present ones [60].

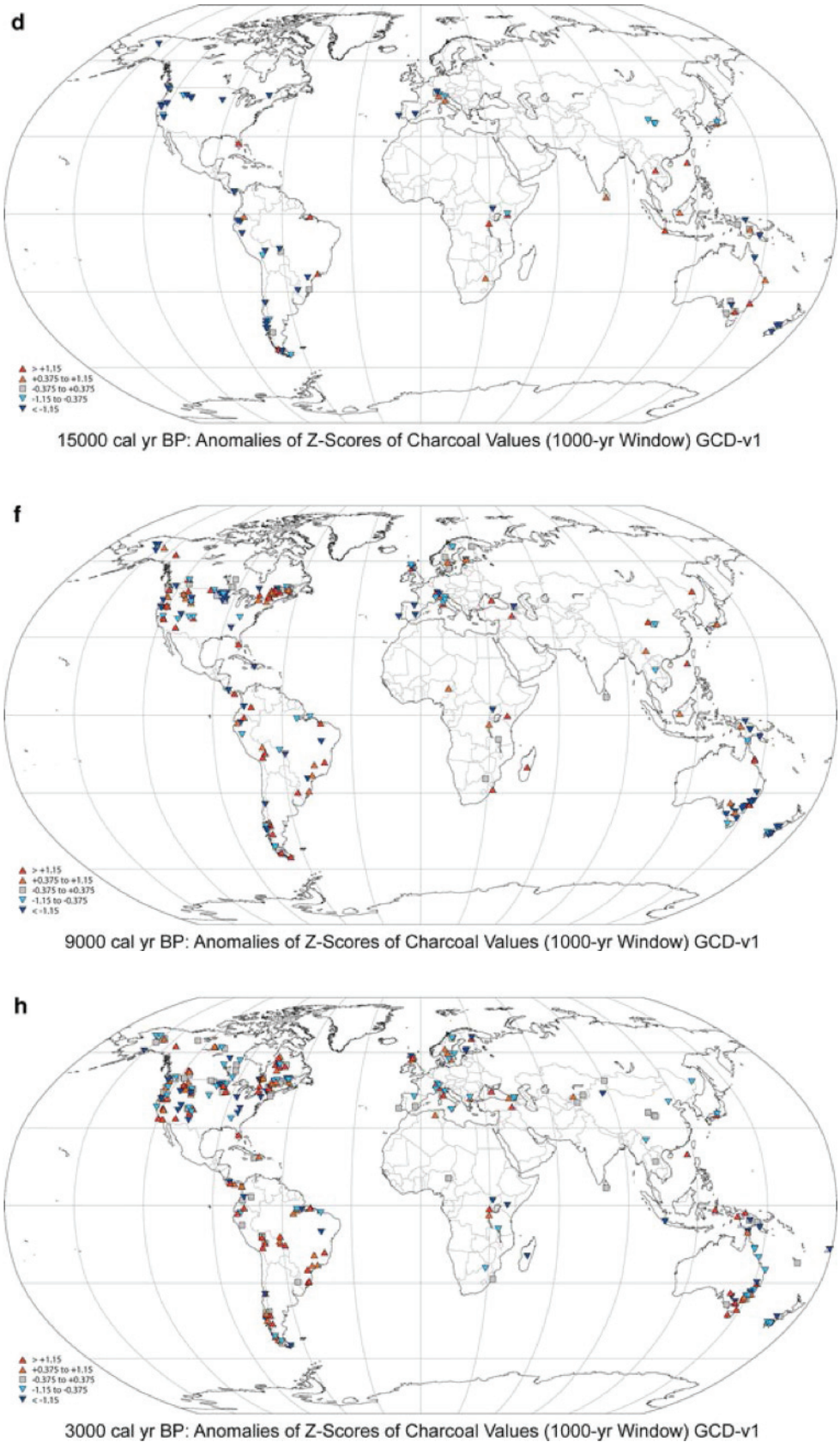

Figure 1.15 Global map of positive (red) and negative (blue) anomalies in charcoal (Z scores) since the LGM. *From Power et al. (2008)* [60].

Beside this general pattern, changes occurred differently at different latitudes and anthropogenic influence developed heterogeneously: in order to better assess the relative importance of the anthropic influence, high-resolution studies of local and regional changes are needed [61]. Despite the huge effort that has been put in reconstructing Holocene fire regimes, one of the biggest issues in Holocene fire science remains the individuation of methods for disentangling natural and anthropogenic fires [54, 60–63].

1.2.3. Fire as a climate driver

Fire regimes are therefore closely connected to climate variability, and climate is in turn strongly influenced by fires. Combustion makes the mass transfer between different environmental compartments immediate, accelerating all oxidation processes that would otherwise occur on long time scales and abruptly modifying the composition of the atmosphere. As previously discussed, biomass burning plays an important role in the carbon cycle and regulates part of the radiative forcing by releasing significant amounts of greenhouse gases and aerosols. It is estimated that CO_2 from all sources of fire is about 50% of that produced by fossil fuels. The production of greenhouse gases and black carbon results in a warmer climate, enhancing the probability of fire events that would lead to further warming. Fire can therefore be considered as the trigger of a positive feedback acting globally, but is also involved in the surface albedo feedback [64], as discussed in paragraph 1.2.1.

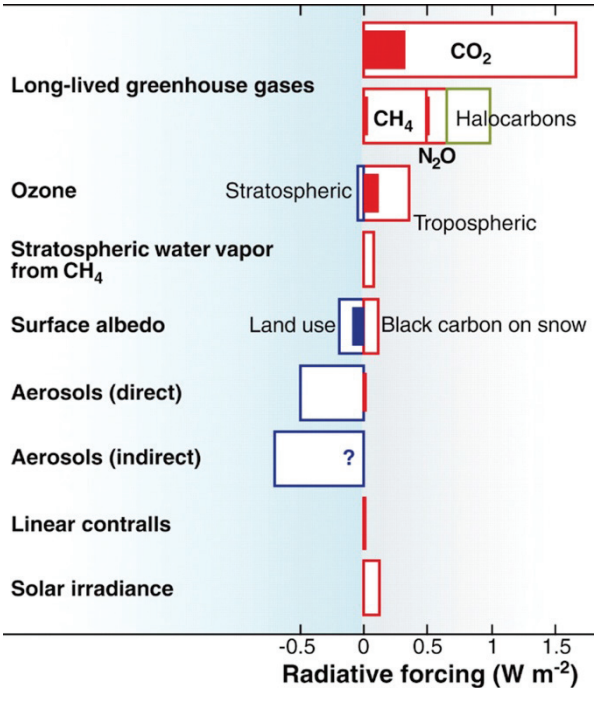

Figure 1.16 Estimated effects of fire associated to deforestation on radiative forcing according to *Bowman (2009)* [64].

Direct and indirect effects of burning thus involve and connect atmosphere, biosphere, hydrosphere, pedosphere and anthroposphere and must occupy an important place in climate studies. The complexity of interactions and feedbacks, together with the scarcity

of data, make the accurate quantification and prediction of both global and local effects rather difficult [46]. For these reasons, the understanding of past dynamics and of the role of humans in actively shaping the present needs to be better assessed by further research.

1.3. Tracing past environmental changes

Up to this point, we have introduced the birth of agriculture and the use of fire by humans in the past with its possible consequences. Landscape modifications, though, leave durable traces in environmental archives. Individuating appropriate methods for measuring these traces leads to the reconstruction and interpretation of past phenomena.

Biomass is composed mainly of carbon, therefore its intermediate oxidation products are for the most part organic compounds at different chemical complexity levels. Combustion processes occur as the result both of biomass burning and of respiration in the metabolism of animals. In the first case, the reaction takes place rapidly and at high temperatures, with a huge energy release. In uncontrolled fires, therefore, thousands of different products may be generated in variable amounts according to the characteristics of fuel and burning conditions. In the case of respiration, reactions occur more slowly, at low temperature and the range of possible products is more limited, although still very complex. In the present work, the results of both processes are considered: tracers produced by fire and tracers produced by human and animal metabolism. This choice arises from the already discussed need to find methods for disentangling natural fires from anthropogenic ones. If the moment of human settlement and the level of anthropic pressure in a region could be estimated from a direct tracer, then this information could be coupled to the intensity and frequency of fires in order to better understand cause-effect relationships and the degree of human influence on natural processes.

1.3.1. Paleolimnology and proxies

The information needed for this ambitious purpose is stored in sedimentary archives, a highly instructive repository capable of recording changes from both terrestrial and atmospheric processes. For the scope of this work, lacustrine environments constitute a useful target. Lakes are diffused worldwide and in very diverse environmental contexts, in elevation or at sea level. They can originate from depressions created by former glaciers, or on bedrocks shaped by erosion, tectonic crust deformation or volcanism and by river depositional activity [65]. Depending on the origin, age and location of the lake,

its dimensions and hydrological conditions can vary considerably, and so can the catchment area. In any case, the continuous deposition of material on the lake bottom guarantees the registration of local and regional changes, that can be reconstructed more or less directly through a large number of physical, biological and chemical proxies [66]. Sedimentary material is produced by a wide variety of processes, including atmospheric deposition and precipitation, input from rivers, erosion and biological activity, as illustrated in figure 1.17 [67]. Sediments therefore integrate a huge amount of information which, provided that deposition and layering are relatively undisturbed, may be conserved for a long time. For the above reasons, lately the research interest in lake sediments has grown considerably, giving rise to the research field called *paleolimnology* [68].

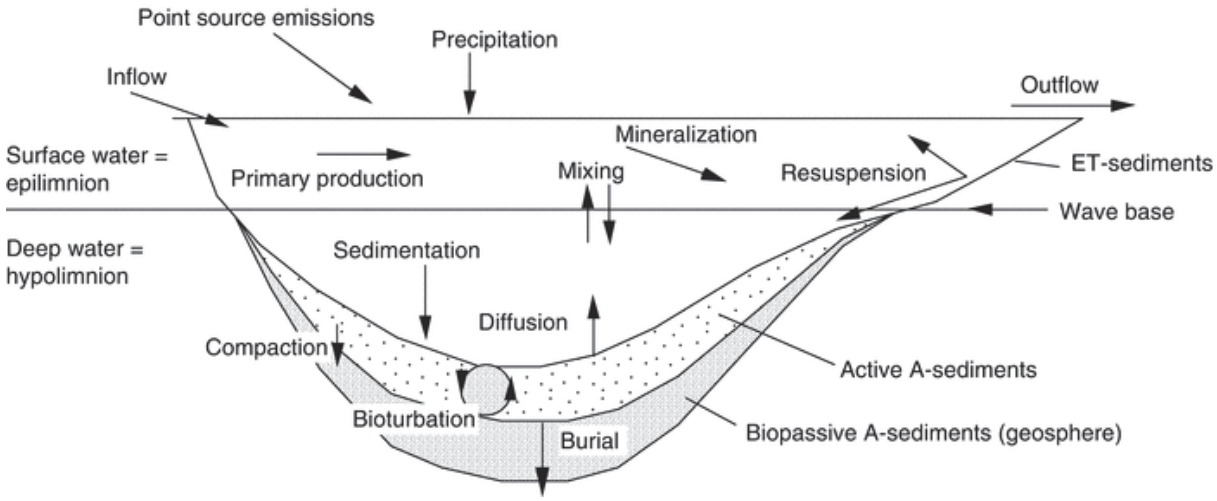

Figure 1.17 Scheme of transport processes involved in lake sedimentation. *From Herschy (2012)* [67].

The first approach to the study of lake sediments obviously focuses on geological aspects and stratigraphic data: a macroscopic description of the sedimentary material and layering provides information on sedimentation processes and deposition rate. This is especially true in the case of *varved* sediments, seasonally and annually laminated layers occurring in low-energy systems when a scarce oxygen availability prevents bioturbation, particularly useful in high-resolution studies [68–70]. Other classical approaches to paleoenvironmental indicators in lake sediments include biotic indicators, such as diatoms or chironomids, used for reconstructing the variation of physical and chemical

characteristics of the water that reflect environmental and climatic variations such as pH, temperature or the concentration of nutrients. The number of indicators has grown significantly in time and could virtually include any proxy, provided that suitable measuring methods are developed. Hundreds of geochemical markers, among which isotopes, elements and several organic molecules indicative of different processes have been introduced in order to obtain a multivariate description of environmental changes, certainly more complex but more reliable than the use of a single index.

Fire and fire tracers

In the field of paleoecological studies, plant remains, in particular pollen, are widely employed for describing the variations in plant population, ascribable to climate changes or to local phenomena such as fires. Pollen analysis, often coupled to the analysis of charcoal, is used to identify past fires by individuating abrupt shifts in the composition of plant community, typically occurring after the elimination of non fire-resisting species [68, 71]. As mentioned earlier, charcoal is the main descriptor of paleofires in sediments. As a direct and abundant product of organic matter combustion between 280 and 500 °C, it provides clear evidence of biomass burning in sedimentary archives, mainly lake sediments and peat [71, 72]. The deposition of charcoal occurs primarily by atmospheric transport, but also as a result of erosion, run-off and redeposition after sediment mixing. Based on the source, it is therefore appropriate to distinguish between *primary* and *secondary* charcoal. Figure 1.18 illustrates a schematic view of the ways through which charcoal can reach sediments [71]. Primary charcoal is transported to the lake in the short time span immediately following the fire event, while secondary charcoal can be introduced mainly by run-off and post-depositional processes in periods without fire activity [71]. The charcoal signal thus gives indications about local to regional fires according to the characteristics of fire (fuel, size, intensity, temperature) that affect both charcoal production and transport by air. It was observed that the size of charcoal particles is representative of the distance covered: macrocharcoal (particles >100-200 μm) cannot be lifted much and are soon deposited near the source, thus reflecting local fires, while microcharcoal (10-200 μm) can reach great heights and cover long distances, therefore being more suitable for regional studies [71, 72].

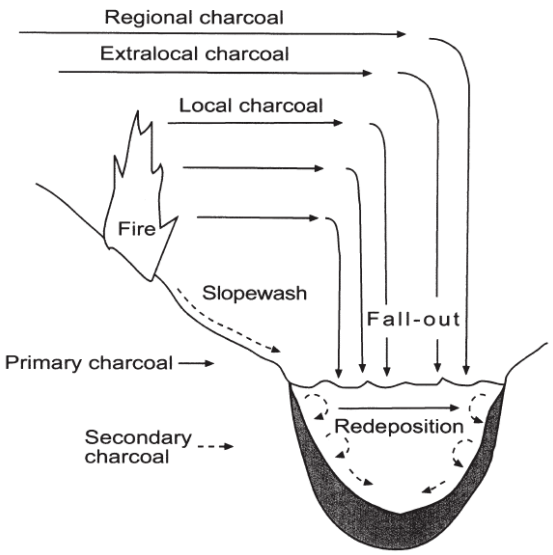

Figure 1.18 Schematic view of primary and secondary charcoal sources. *From Whitlock and Larsen (2001)* [71].

Apart from charcoal, fires produce large geochemical evidence from erosion and direct alteration of soil minerals and from the incomplete combustion of biomass due to oxygen scarcity. The second process results in aerosols composed of a complex mixture of gases and particles, whose composition varies depending on the fuel and the temperatures reached during the fire. Typically, a burning event is characterized by distinct moments, as summarized in figure 1.19: during heating, the components of biomass start to be hydrolyzed, oxidized and lose water reaching pyrolization, which leads to the formation of char (a solid mixture rich in carbon), tar (composed of intermediate molecular weight species) and volatile compounds. At the ignition temperature of tarry and volatile substances, the proper exothermic reaction of combustion begins. Two separate phases can be described: until flammable substances sustain fire, the flaming combustion occurs, converting them to fully oxidized final molecules such as water vapor, CO_2, N_2, N_2O, NO, SO_2 or to intermediates as CO, CH_4, aliphatic and aromatic hydrocarbons and particulate. Char continues to be produced during both flaming and the subsequent smoldering combustion, which can go on for days or even more after a forest fire [73, 74]. Other volatile products and partially oxidized substances continue to be released during smoldering: the amount of organic intermediates varies with the flaming/smoldering ratio, that characterize each fire depending on the fuel, size, temperature and duration [74].

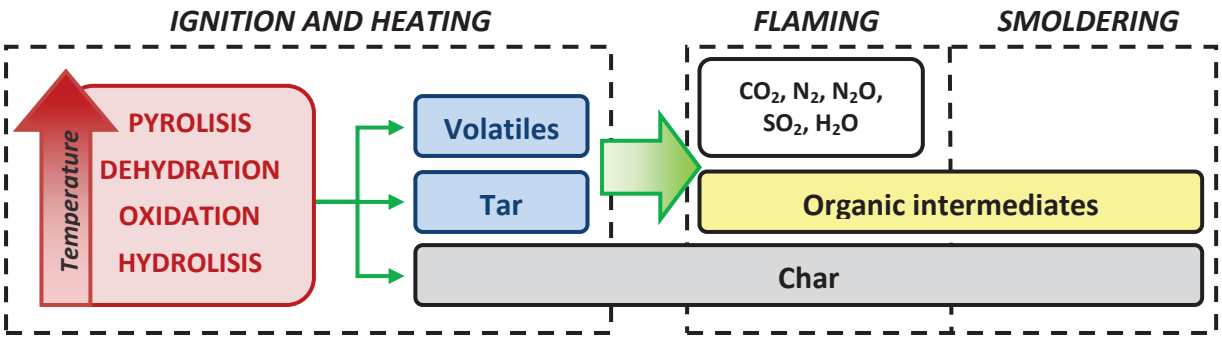

Figure 1.19 Schematic view of the main biomass combustion steps.

All the steps occur and may coexist during any fire, but depending on the type of fuel and the amount of moisture one phase may be privileged over the other, so that the composition of final products can vary considerably. Relative emission factors for each product can vary much among savanna or grassland, tropical forest, boreal forest and agricultural residues, as reviewed by Andreae and Merlet (2001) [74]. Due to the complexity of the combustion process, the scarcity of data and the uncontrolled nature of wildfires, though, it is quite hard to compile an accurate register. For this reason, many investigations have focused on the study of particulates produced by known fuel in controlled temperature conditions, in order to identify products individually and calculate emission factors for all products.

1.4. Proxies

A series of three papers published between 2001 and 2006 by Daniel Oros and Bernd R.T. Simoneit, in particular, presents a detailed (but still not exhaustive) list of molecular tracers in smoke particulates from conifers, deciduous trees and grasses respectively [75–77]. Hundreds of organic compounds are considered, divided by chemical properties as follows:

- *(i)* homologous compounds series (*n*-alkanes, *n*-alkenes, *n*-alkanoic acids, *α,ω*-alkanedioic acids, *n*-alkanones, *n*-alkanols);
- *(ii)* molecular biomarkers (terpenoids, monosaccharide derivatives, methoxyphenols, steroids, wax esters);
- *(iii)* polycyclic aromatic hydrocarbons;
- *(iv)* lignin pyrolisis products (phenols);
- *(v)* monosaccharides;
- *(vi)* organic and elemental carbon
- *(vii)* unknown/unresolved compounds

A huge number of different molecules are therefore released to the atmosphere, as volatiles or in association with charcoal and soot particles, and reach the surface of water bodies by direct deposition or run-off. Eventually, particulates sink down to the bottom and become part of the sediment as previously described. As it enters the environment, any chemical substance is naturally affected by physical, chemical and biological processes, starting with transport and adsorption on particles, followed by oxidation, dissolution and microbially-mediated transformations [78]. Low molecular weight, water-soluble and photolabile molecules are generally more prone to degradation. In order to be suitable for use as a molecular marker, a molecule must be source-specific and have a certain level of persistence in the environment, at least for the time scales considered [78].

The present work focuses on three categories of molecular markers that meet the aforementioned requirements. As mentioned above, monosaccharide anhydrides and polycyclic aromatic hydrocarbons from lake sediments are used as indicators of past fire

events while a particular subset of sterols is employed for the reconstruction of human presence. The three groups are described in detail below.

Monosaccharide anhydrides

Plant biomass is composed primarily of water (up to 60%), biopolymers containing cellulose and hemicelluloses (50-70% of the dry weight), lignin (15-35%), minerals (up to 10%). The remnant is distributed among proteins, amino acids and diverse volatile compounds such as alcohols, terpenes and aldehydes [74]. Cellulose and hemicelluloses constitute the fibrous structure of wood, whose rigidity is due to the infiltration of lignin within the fibers. A cellulose molecule contains thousands of β-D-glucopyranose (D-glucose) monomers, linearly organized to formlong-chain structures that interact through hydrogen bonds between layers. Hemicellulose is made of 5- and 6-carbon sugar monomers (arabinose, galactose, glucose, mannose, xylose) organized in groups of 100-200. Lignin is derived mainly from the polymerization of *p*-coumaril, conyferil and sinapyl alcohols, as shown in figure 1.20 [79–81].

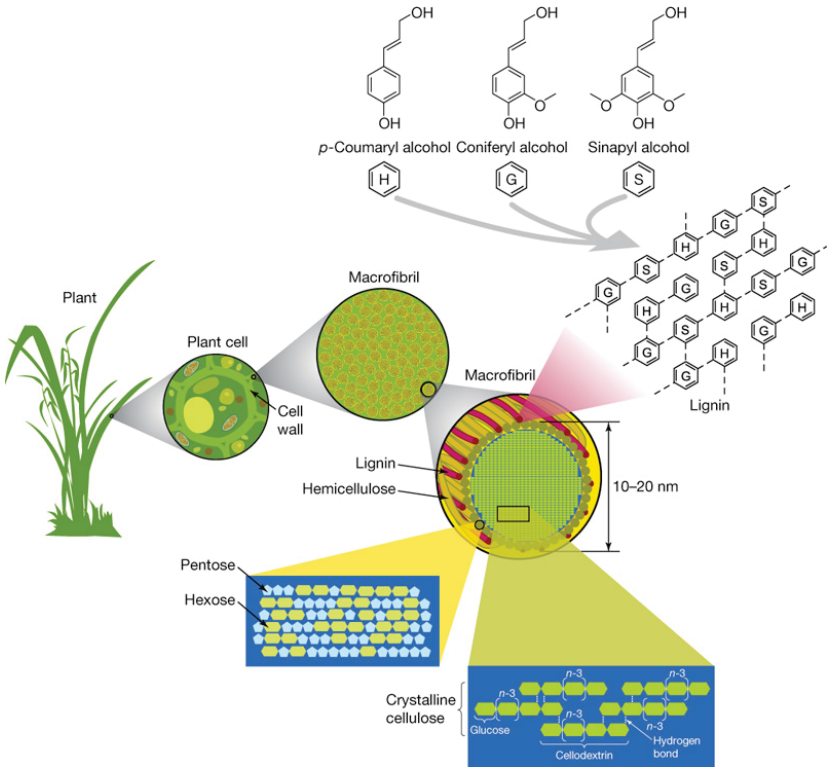

Figure 1.20 Cellulose, hemicellulose and lignin structures**.** *From Rubin (2008)* [79].

When wood tissue burns, therefore, the composition of products reflects the major components of the cell structure. Depending on the temperature, two different pathways can be followed. Below 300 °C, the combustion of cellulose results mainly in the formation of char after polymer decomposition, loss of water and oxidation. Above 300 °C, cellulose undergoes bond breaking, transglycosylation and redox reactions that result in the production of tarry and volatile substances. Tar is composed of anhydrous sugars, mainly 1,6-anhydro-β-D-glucopyranose, also called ***levoglucosan***, and 1,6-anhydro-β-D-furanose. Similar reaction mechanisms lead also to the formation of the two levoglucosan isomers 1,6-anhydro-β-D-mannopyranose (*mannosan*) and 1,6-anhydro-β-D-galactopyranose (*galactosan*) from the combustion of hemicellulose, as shown in figure 1.21. In this work, levoglucosan, mannosan and galactosan are referred to as *monosaccharide anhydrides* (MAs).

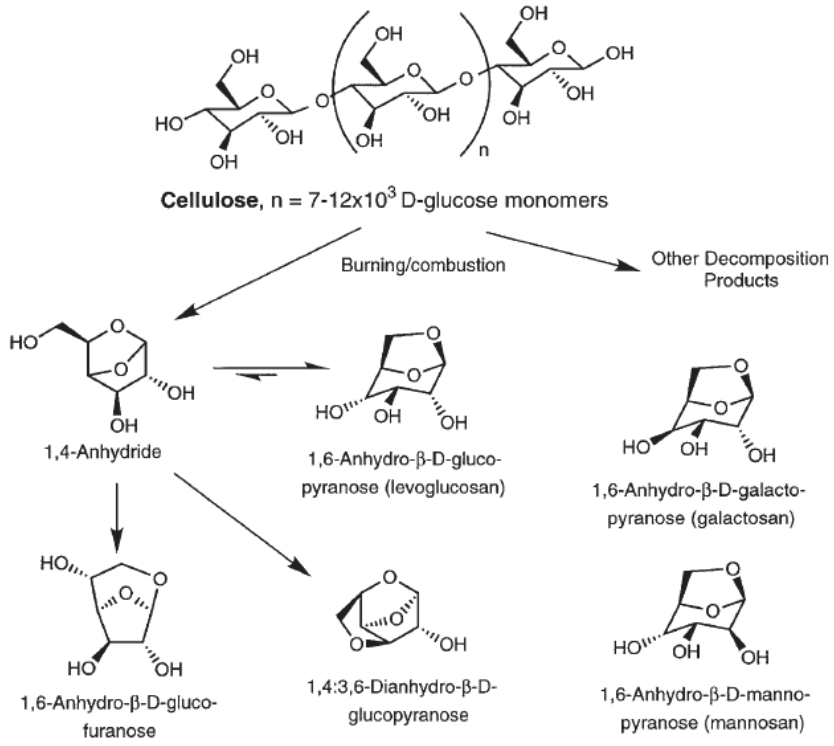

Figure 1.21 Main decomposition products of cellulose combustion. From *Simoneit (2002)* [73].

Due to its abundance in the smoke particulate phase and its source-specificity, levoglucosan represents a good molecular marker for biomass combustion processes. It was observed, indeed, that no other kind of combustion produces levoglucosan, which

has an emission rate of 40-2000 mg kg^{-1} for wood and is quite stable in the atmosphere [80, 82]. Simoneit (1999) found large amounts of levoglucosan in smoke from a large number of gymnosperms, angiosperms and grasses from diverse areas in the world [80]. The ratio of levoglucosan to its less abundant isomers can be employed in the discrimination of sources, due to the differences in the composition of hemicelluloses between softwood and hardwood [83, 84].

The use of levoglucosan as a major tracer of biomass burning in atmospheric particulate phase has become well established and is described in several research papers [82, 85–88]. More recent works investigated its application in the reconstruction of past fires from ice cores, where levoglucosan proved a valid tracer, preferable to less specific indicators such as ammonium, potassium or oxalate [89–91]. Lately, this approach was successfully applied also to sedimentary archives, in order to reconstruct local and regional fire regimes, often in association to charcoal or other fire tracers[92–95]. A review of existing methods is presented in the methods section.

Polycyclic aromatic hydrocarbons

Among combustion products, polycyclic aromatic hydrocarbons (PAHs) have perhaps received the greatest attention in relation to their toxic potential. Indeed, some congeners were recognized as carcinogenic and included in a priority group of 17 molecules [96]. PAHs are widespread as atmospheric contaminants both in the gas and particulate phases, and are therefore extensively monitored worldwide. They result from the incomplete combustion of both fossil fuels and vegetation: in the first case, the main mechanism of formation is the pyrosynthesis from simple linear hydrocarbons, while pyrolysis can occur as biomass burns in conditions of oxygen depletion [97]. PAHs are the result of two or more aromatic rings fused together: during combustion stages, the increase in temperature favors the increase in the number of rings, generating higher molecular weight congeners [98].

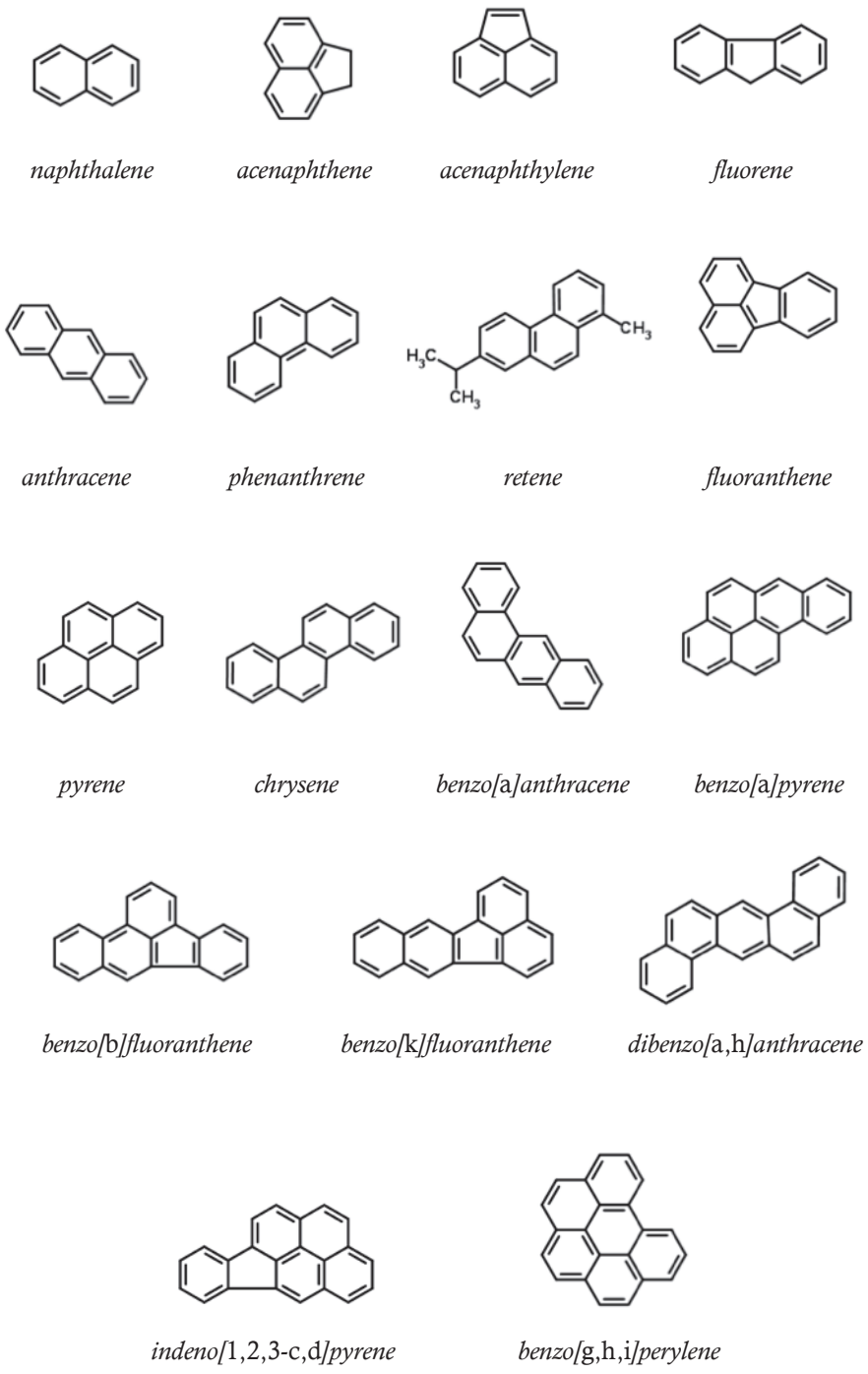

Figure 1.22 Structure of the PAHs considered in this work.

The formation of PAHs starts from free radicals produced by the fragmentation of organic compounds contained in the fuel. Different pathways have been proposed for the formation of the first aromatic ring, although it was observed that the production of PAHs is significantly enhanced if benzenic rings are already present, as in the case ofthe main fossil fuels and aromatic compounds contained in biomass. Further reactions with small molecules - such as acetylene - lead to the progressive growth of multi-ring structures. PAHs are among the major constituents ofparticulate matter, and are involved in the formation of soot from the nucleation of higher molecular weight structures [99, 100].

The formation of PAHs from the pyrolysis of cellulose, lignin and chlorogenic acid is believed to occur mainly during the high temperature cracking of primary gas products, above 600 °C. The formation of different PAHs was shown to strongly depend on the temperature and duration of the combustion process, rather than on the fuel. The increase in the temperature of secondary pyrolysis of primary chars leads to an increase in the PAH yield. Under controlled conditions, no PAHs are observed in the tar produced below 300 °C; 3-ring congeners are observed above 400 °C, while 4-ring PAHs appear at 500 °C; 5- and 6-ring compounds start forming between 600 and 650 °C and increase with further heating [101, 102].

The concentrations and characteristics of single PAHs produced by biomass burning can therefore help in reconstructing the temperature and intensity of fires [101, 103], making these tracers particularly valuable in order to complement the information obtained by more specific markers such as monosaccharide anhydrides [104] or paleoecological records such as pollen and charcoal [72]. PAH profiles replicate known fire events without visible secondary deposition effects: this characteristic could be very useful to solve interpretation problems deriving from charcoal particles deposited a long time after fires [103]. Although PAHs are not source specific, they are persistent in the environment and prior to the industrial era it is reasonable to ascribe their presence in climatic archives to biomass burning events. Due to their lipophilic nature, PAHs deposited onto water bodies tend to precipitate and be stored in sediments, where the association to particles prevent them from biodegradation [100, 105]. Moreover, particles originated from forest fires are quite coarse, therefore they cannot cover large distances and their size better protects PAHs from degradation, providing a good conservation of the geochemical

fingerprint of past fires [105]. The scarcity of oxygen and the strong interaction with the sedimentary material ensure the stability of PAHs in deep sediments [106]. Available sedimentary PAH records, though, usually cover only few centuries and are therefore employed to track the effects of industrialization rather than as climate proxies. However, fluctuations in the PAH trend reflect changes in climate and population, which have enhanced or reduced the probability of fires [107]: climatic conditions (humidity, seasonality) can be inferred from a combination of combustion-derived PAHs and aromatic plant markers [108]. Recently, the analysis of PAHs together with charcoal and other inorganic proxies in sediment cores was proposed as a paleoclimatic application. The validity of PAHs as past fire tracers is thus demonstrated, showing how the increase in pyrogenic PAHs is unequivocally correlated to the development of productive and agricultural activities [92, 107].

Fecal and plant sterols

Besides fire tracers, the novel character of the present work is mainly represented by the use of sterols for the purpose of paleoclimatic reconstruction. The validity of fecal remains in the reconstruction of human and animal presence is well established in archaeological applications. A macroscopic examination of evidence, however, is not always possible and hardly allows the discrimination of sources [109]. In this sense, the analysis of molecular biomarkers can reveal "invisible" evidence or reliably complement archeological findings, when present [110]. Until recently, fecal sterols have been employed especially as tracers of fecal contamination from sewage in water bodies and for assessing the efficiency of water treatment plants [111–115]. In recent years, though, some applications for paleoenvironmental and archaeological studies have been proposed [110, 116–118]. In the present study, we test the utility of fecal and plant sterols and of their degradation products for assessing the importance of human and animal influence and its correlation with the fire activity reconstructed from monosaccharide anhydrides and PAHs.

The compounds considered for the purposes of this work are *cholesterol* (cholest-5-en-3β-ol), *cholestanol* (5α-cholestan-3β-ol) *coprostanol* (5β-cholestan-3β-ol), *epicoprostanol*(5β-cholestan-3α-ol), β-*sitosterol* (24α-ethyl-cholest-5-en-3β-ol), *stigmastanol* (24α-ethyl-5α-

cholestan-3β-ol), shown in figure 1.24. Figure 1.23 illustrates the main chemical mechanisms that convert Δ^5-sterols into their reduced degradation products. Briefly, the microbially mediated reduction of cholesterol and other 5β-sterols in the intestine of mammals produces preferentially 5β-stanols, such as coprostanol, rather than 5α-stanols [119]. Once released in the environment, further conversion of the 5β-stanols to epi-5β-stanols (epimerization) can occur in soil and sediment, again as a result of bacterial activity.

Following this scheme, the six compounds listed above were chosen as a representative subset of substances present in the dietary intake (cholesterol, β-sitosterol), their digestion products (cholestanol, coprostanol, stigmastanol) and their environmental degradation products (epicoprostanol). Moreover, sterols have shown a good stability in aquatic systems [120], a characteristic required for their use as molecular markers.

Depending on the diet, different sterols profiles are produced by different animals. The main distinction is between herbivores and omnivores: among selected compounds, stigmastanol occurs mainly in the first case, while coprostanol is present only in small amount in cow, horse and sheep if compared to human ones, where coprostanol constitutes about the 60% of the total sterols [113, 119]. Leeming et a. (1996) observed a very significant concentration of coprostanol in other omnivore (cats, dogs) and pig dejections as well, but still around one order of magnitude below the concentrations contained in human feces [113]. Thus, the occurrence of coprostanol and its epimer epicoprostanol denotes a specific source, and their detection in water and sedimentary systems provides a powerful instrument for tracing human presence back in time. The evaluation of specific ratios between different congeners, usually applied to current contamination surveys [112], may also help in the reconstruction of past animal husbandry practices and in the assessment of relative population sizes.

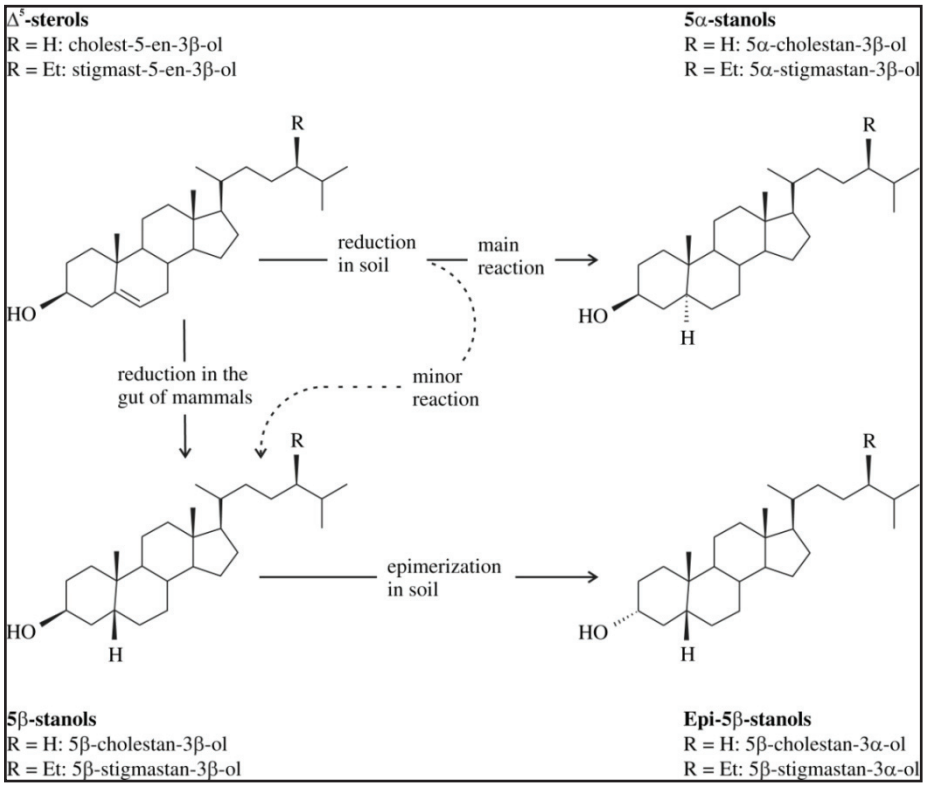

Figure 1.23 Reduction and epimerization of Δ^5-sterols in the gut of mammals and in soil. From *Birk et al. (2011)* [117].

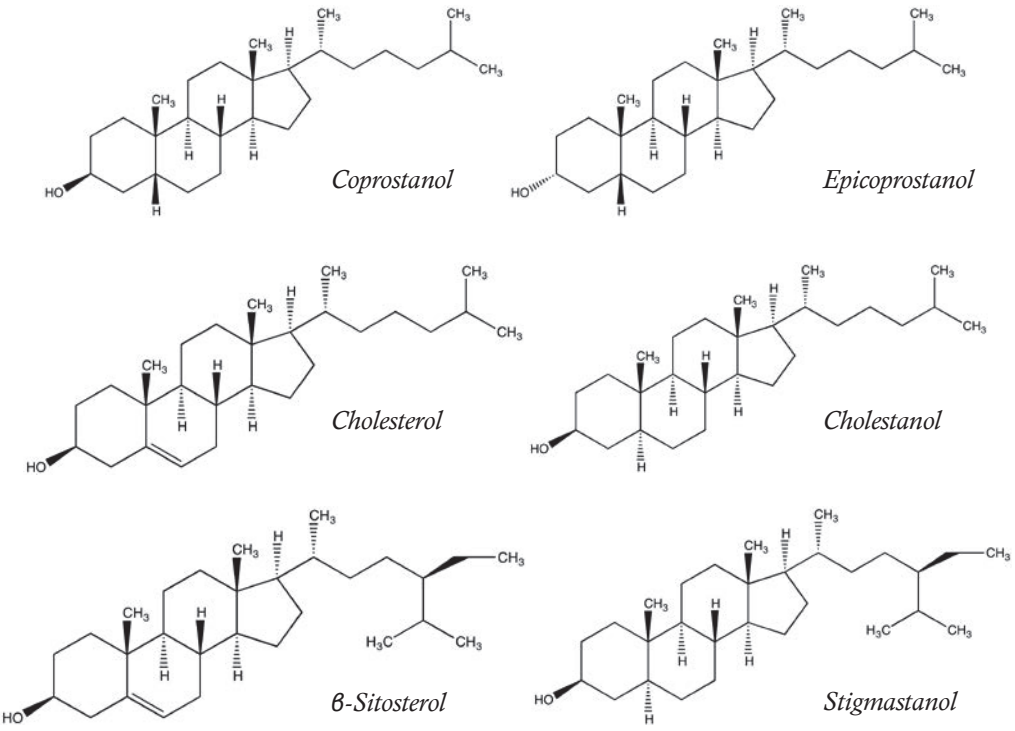

Figure 1.24 Structure of the fecal and plant sterols and stanols considered in the present work.

2. METHODS

The interdisciplinary character of this work has required quite a long introduction in order to provide the reader with the necessary instruments for a better understanding of the research objectives. The work is divided into two parts of equal relevance: (1) the development of a method for the analysis of fecal sterols in sediment, then coupled to pre-existing methods for the analysis of PAHs and levoglucosan and (2) the analysis of molecular markers on real samples from different areas in the world, aimed at testing the validity of methods to be applied in paleoclimatic reconstructions. The novelty of the proposed approach resides in the simultaneous analysis of multiple tracers on the same sample - which has numerous advantages in terms of sample amount and data comparison - and, where possible, in the direct coupling to charcoal records from portions of the same samples.

Before approaching the detailed description of the work, it is worthwhile to list the main questions addressed by this research project:

a) *Are the selected molecular markers adequate indicators of past changes?*

b) *Do fire molecular markers agree with the charcoal trend and, if not, why?*

c) *Is there a temporal and spatial correspondence between human and fire signals?*

d) *To what extent can we distinguish between anthropogenic fire regimes and climate-driven ones?*

In order to search for the answers, advanced analytical techniques were employed as described below.

2.1. Instrumental equipment

This work was mostly conducted at the Environmental Analytical Chemistry laboratories of Ca' Foscari University of Venice, with the technical support of R&C Lab, Altavilla Vicentina (VI, Italy) and of the Paleoecology Lab of the Montana State University (Bozeman, MT, USA).

2.1.1. Sampling and dating

Subsamples from sediment cores used in the present work were provided by Dave B. McWethy (Montana State University) and Thomas C. Johnson (University of Minnesota). Cores from New Zealand and Tasmania were sampled with 7 cm polycarbonate tubes (Klein core) [121, 122]. The core portion obtained from Lake Victoria was sampled with a Kullenberg corer, a piston corer equipped with lead weights and a heavy duty winch for retrieving samples [123, 124].

Chronology for all cores was obtained by AMS (Accelerated Mass Spectrometry) ^{14}C dates, calibrated with the chronology models *BChron*, *OxCal*, *McAgeDepth* and *CalPal* [121–123].

2.1.2. Samples pre-processing

Wet samples (Lake Kirkpatrick, Lake Diamond, Flinders Island), were freeze dried (*Edwards Modulyo* freeze dryer) or dried in a glass desiccator in presence of silica gel up to constant weight. Dried samples were ball milled (*Retsch Mixer Mill 400*) or hand milled and homogenized in a ceramic mortar. Samples were conserved at room temperature in sealed vials until extraction.

2.1.3. Extraction

The extraction of organic analytes from the solid matrix was performed with Pressurized Liquid Extraction (*PLE*, FMS Fluid Management Systems, Inc.) at Ca' Foscari University Laboratories and with Accelerated Solvent Extraction (*ASE 200*, Dionex Thermo Fisher Scientific) at R&C Lab (Altavilla Vicentina, VI, Italy). The modes of operation of the two instruments are very similar: the sample, conveniently dispersed with inert materials, is introduced in a stainless steel vessel equipped with porous caps at both ends. The selected organic solvent or solvent mixture is then pumped into the vessel, which is kept at high pressure and temperature for a given time (static), so that the contact between the solvent and the sample is maximized. The vessel is then depressurized and the solvent discharged into a collection vial. This process can be repeated two or three times in order to obtain the maximum recovery of analytes.

2.1.4. Volume reduction

Sample extracts need to be reduced in volume in order to undergo the following passages. Volume reduction of extracts was obtained through a centrifugal evaporator (Genevac *EZ-2 Solvent Evaporator*) and through *Turbovap®* (Caliper Life Science). The first instruments uses vacuum and the centrifugal movement for evaporating the solvent, while in the second samples are introduced in a thermostatic bath and submitted to a gentle nitrogen flow until the desired volume is reached.

2.1.5. Clean-up

Sedimentary material is rich in organic matter, in particular lipids and plant remains. Undesired interferences must be eliminated from the sample before instrumental analysis, in order to avoid difficulties in recognizing and quantifying target compounds. Moreover, analytes with different chemical characteristics may need to be separated from one another. The clean-up and fractionation of samples were performed through SPE (Solid Phase Extraction) neutral silica tubes. Once the cartridge is conditioned with the solvent

of choice, the sample is loaded and eluted with increasing polarity solvents or mixtures. Interferences are retained on the silica, while analytes are eluted according to the relative polarity and chromatographic interaction with the stationary phase. Fractions can be collected separately, depending on analytical needs, and newly reduced in volume as described above.

2.1.6. Gas chromatography – mass spectrometry

The analyses of PAHs, fecal sterols and levoglucosan (Lake Victoria) were performed by gas chromatography coupled to mass spectrometry. The equipment used is an Agilent Technologies *7890A GC system* coupled to an Agilent Technologies *5975C inert MSD* quadrupole mass selective detector. The separation of analytes was obtained with an Agilent Technologies *HP5-MS* capillary column (60 m, 0.25 mm inner diameter, 0.25 μm film thickness), whose stationary phase is made from (5%-phenyl)-methylpolysiloxane. The electron impact source was operated at 70 eV, the mass spectrometer was set to positive mode and acquisition was obtained in selective ion monitoring (SIM) mode. Analytes were quantified through ^{13}C-labeled internal standards added to samples prior to extraction and data were corrected for the instrumental response factor.

2.1.7. Ion chromatography – mass spectrometry

For the analysis of levoglucosan (Lake Kirkpatrick) an ion chromatographic system (Dionex, *ICS 5000*, Thermo Fisher Scientific) coupled to a single quadrupole mass spectrometer (*MSQ Plus*, Thermo Fisher Scientific) was used. Separation was performed through a *CarboPac*™ *PA10* column (ethylvinylbenzene 55% cross-linked with divinylbenzene, 2 x 250 mm, Thermo Fisher Scientific) and an *AminoTrap*™ pre-column (polymeric resin, 2 x 50 mm, Thermo Fisher Scientific). The MS operated with electrospray ionization in the negative mode. Analytes were quantified through ^{13}C-labeled internal standards added to samples prior to extraction and data were corrected for the instrumental response factor.

2.2. Review of literature methods

One of the main objectives of the present work was to fill a research gap regarding quantitative methods for geochemical markers in lake sediments. The existing literature about chemical tracers in lacustrine environments rarely concentrates on analytical methods, privileging results and interpretation. In particular, only a few papers regard multi-analyte studies on complex and rich archives such as sediments. Conversely, a great number of papers exist which describe charcoal methods and applications, which are well established and have long been employed. In any case, the lack of standardized methodologies is evidenced by the relative heterogeneity of the techniques described in the literature.

A brief review of existing methods for quantifying charcoal (although it was not determined directly in this work) and for analyzing the target molecular markers (analyzed in the course of this project) in lake sediments is proposed below.

2.2.1. Charcoal

As pointed out in the review by Whitlock and Larsen (2001), a distinction is necessary between macroscopic and microscopic charcoal [71]. The importance of charcoal for tracing past fire events was highlighted back in 1941 by Iversen [125]. The occurrence of charcoal fragments in pollen slides encouraged its use as a fire proxy, and several applications were proposed [126–128]. Some authors argue that pollen slides contain mainly microcharcoal and therefore give an information on regional scale fires [127, 129]. Nevertheless, the method used for the preparation of pollen slides can affect size distribution by breaking charcoal particles in smaller portions, resulting in a misleading interpretation [71]. Alternative methods were elaborated in order to get a better idea of the represented spatial scale and/or a faster sample preparation. In the case of varved sediments, a rare occurrence, the thin section technique can be used: samples are dehydrated with acetone and embedded into epoxy resin, then measurements based on size classes are made using a light microscope [71, 130–132]. Although providing a high resolution record, this method is applicable only in limited cases and is quite time-

consuming [71].

The most widely applicable, cheapest and quickest technique is perhaps macroscopic sieving. This method does not affect the size of charcoal particles and is conservative also for macrofossils and other remains used for AMS dating [71]. The charcoal data used in the present work were obtained through the method described by Whitlock and Larsen (2001): the core is sub-sampled in portions between 1 and 5 cm^3 at 1 cm interval, then samples are disaggregated in presence of a deflocculant agent (typically sodium hexametaphosphate) for a few days. Samples are then sieved and washed through variable mesh sizes according to the target size interval, and charcoal amounts are quantified by counting under the stereomicroscope. Data are expressed as accumulation rates by dividing charcoal concentration (particles cm^{-3}) by the deposition time (yr cm^{-1}) [71, 133, 134].

2.2.2. Fecal sterols

The preferred instrumental analytical technique for the separation of the sterols considered in this work is gas chromatography, followed by mass spectrometry (GC-MS) [110, 117, 120] or flame ionization detection (GC-FID) [116, 135, 136]. Due to the higher selectivity of the mass selective detector, the GC-MS was preferred in this work in order to ensure a better discrimination of analytes from possible interferences. However, the scarce volatility of sterols entails the need for derivatization prior to gas chromatography. A derivatizing agent, usually N,O-Bis(trimethylsilyl)trifluoroacetamide (BSTFA), is therefore added to the sample, which is then heated in order to enhance the reaction speed. This reaction converts sterols in their more volatile trimethylsilyil ethers, more suitable for GC analysis [116, 136, 137].

Normally, an internal standard added to the sample before extraction is used for quantification. In the case of mass spectrometry, the best choice would be an isotopically labeled compound, such as ^{13}C-cholesterol or similar [138], although the use of α-cholestane [117, 118, 139] or other chemicals with very different characteristics from sterols is also reported in the literature [113, 140, 141].

With rare exceptions, the extraction of fecal sterols from sedimentary and soil matrices is performed with organic solvents, in particular dichloromethane or dichloromethane/methanol mixtures [137, 142]. Extraction techniques include the ultrasonic bath [117, 142], saponification with liquid extraction [113, 117] and Soxhlet [120, 143]. All these techniques present disadvantages in terms of time consumption, reproducibility and amount of solvent used, which can be avoided with the use of automated techniques such as PLE or ASE, proposed in a few applications [116, 138, 140, 144]. The clean-up and fractionation, where applied, are mostly achieved through column chromatography on silica gel [117, 118] or florisil [140, 144], sometimes in presence of alumina and sodium sulfate [139, 144].

The variety of proposed methodologies and the scarcity of paleoenvironmental applications led, in this work, to the development and validation of a dedicated method for the determination of fecal and plant sterols in freshwater sediments, subsequently applied on target locations, as will be illustrated in detail in next chapters [138].

2.2.3. PAHs

The general protocol used for the extraction of PAHs from sediment replicates the one used for sterols, with the exception of derivatization. Most of the classic methods for the determination of PAHs in sediments involve the following steps, which can vary slightly according to analytical requirements:

1) solvent extraction, performed with Soxhlet apparatus [145] or automated systems such as accelerated solvent extraction (ASE) [107, 146] or microwave assisted extraction [103, 147]. The most commonly used solvents are dichloromethane, *n*-hexane, toluene, acetone, methanol, also combined in mixtures [103];
2) purification and possible fractionation of analytes, obtained by eluting the extract along a chromatographic column usually prepared with silica gel [107, 145, 146] with the possible addition of alumina, sodium sulfate and copper [146] in order to guarantee the elimination of analytical interferences;

3) gas-chromatographic separation and mass spectrometric determination (GC-MS), based on the isotope dilution technique by using deuterated [107, 145, 146] or ^{13}C-labelled internal standards [148] for peak comparison.

PAHs from environmental samples are almost always analyzed by GC-MS: gas chromatographic separations and a wide range of mass spectrometric possibilities have been extensively reviewed by Poster et al. (2006) [149]. Nevertheless, alternative and successful methods based on different analytical techniques have been proposed, such as the high performance liquid chromatography with fluorescence detection (HPLC-FLD), which results in comparable or better sensitivity, selectivity and limits of detection [103].

2.2.4. Levoglucosan

Numerous methods have been proposed for the analysis of levoglucosan and its isomers in atmospheric aerosols and ice [86–88, 90, 150–153], but applications to sediments for paleofire reconstructions are relatively few [93–95, 154].

Generally, GC-MS is used after the reaction of analytes with BSTFA or other derivatizing agent in presence of pyridine [85, 86, 155, 156]. Recently, a couple of methods have been proposed that use liquid chromatography, best suited to the water-soluble nature of the analytes [94, 154]. The extraction procedures are generally carried out with automated extraction systems using methanol [94, 157], or dichloromethane/methanol mixtures (9:1, v/v) [92, 158] as solvents, or by ultrasonic bath with methanol [159]. Samples are finally evaporated to a few μL or dryness and re-dissolved in a convenient solvent prior to analysis [94, 95, 160]. When used, the internal standards reported in literature are sedoheptulosan [92], perdeuteroeicosanoic acid [159], ^{13}C labeled levoglucosan [94]. Alternative approaches use external standard curves for quantification [154, 161].

2.3. Method development

The first operative part of this work regarded the research of methods for an accurate determination of the selected analytes in sedimentary archives. Sediments are a complex matrix, rich in organic matter and possible interferences. The analyses of organic tracers are therefore time consuming and need several steps in sample preparation. Moreover, sediment cores are usually split for archive purposes and several other geological and paleoenvironmental applications, and the amount of available material is therefore often limited to a few grams or less. In order to get as much information as possible from one sample, reliable multi-proxy methods are needed. This way, directly comparable datasets are obtained. A considerable effort is required for optimizing each pre-analytical and analytical step, in order to obtain a good method performance. These considerations led to developing a method for the analysis of the aforementioned sterols described below. Subsequently, the method was combined with pre-existing methods for fire tracers, conveniently adapted, in order to obtain multi-proxy techniques that are proposed for application on different sedimentary archives.

2.3.1. Method for fecal sterols

The description of each step of the analytical method development and validation for the analysis of coprostanol (Cop), epicoprostanol (e-Cop), cholesterol (Chl), cholestanol (5α-Ch), β-sitosterol (Sit) and stigmastanol (5α-Sit) is provided in this section. All tests were performed on standard solutions prepared in dichloromethane from native compounds and ^{13}C-cholesterol (Sigma-Aldrich). The final method was the subject of the research paper entitled "*GC-MS method for determining faecal sterols as biomarkers of human and pastoral animal presence in freshwater sediments*" by Battistel et al. (2015), published in *Analytical and Bioanalytical Chemistry* [138]. The full text is included in the appendix.

Derivatization

The optimal derivatization temperature and time indicated in the literature are in the interval 60-70 °C and 30-60 min, respectively [137, 141, 162]. In order to ensure the complete reaction, the selected temperature was 70 °C. The derivatizing agent was BSTFA with 1% TMCS (trimethylchlorosilane) as a catalyst (Sigma-Aldrich). Derivatization time was tested in the interval of 1-4 h, as shown in figure 2.1a. The graph reports the relative variation in the area of selected compounds as a function of time: after 2 h, the yield in derivatized analytes is about 40% lower than after 1 h; after 3 and 4 h, the areas display a variation of ±10% with respect to the first value. Since no significant advantage was observed when heating samples for a longer time, the optimal derivatization time was set to 1h.

The stability of samples for GC-MS analysis after derivatization was tested as well by repeatedly injecting test solutions for eight days. Results are shown in figure 2.1b: there is an evident and dramatic drop in the amount of measured TMS derivatives drops dramatically after 3 days and the samples result stable only in a 2-day interval after derivatization. In order to ensure maximum yield and repeatability, therefore, samples need to be analyzed within 2 days from the derivatization reaction.

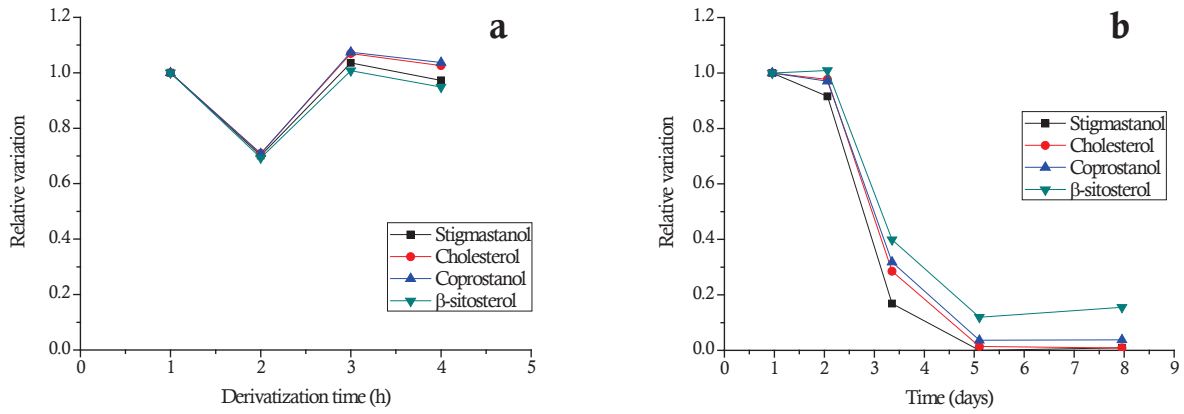

Figure 2.1. (a) Relative variation in the yield of analytes as a function of derivatization time. (b) Stability of TMS derivatives in time.

Gas Chromatography – Mass Spectrometry

Although many indications for the GC-MS analysis of sterols are reported in the literature, as discussed above, the major difficulty resides in fully separating epimers that differ only for the configuration of a –OH group, and therefore have a very similar chromatographic behavior - such as coprostanol and epicoprostanol. For this reason, several authors report some concentrations as sums (Cop+e-Cop, Chl+5α-Ch) rather than as single congeners, due to the lacking separation. For a complete understanding of post-sedimentation processes and a correct interpretation of data, however, a good resolution is required, especially for Cop and e-Cop [163–165].

Being very similar in structure, the considered sterols also have a similar fragmentation pattern in the mass spectrum, so that the produced m/z values may be common to different congeners. The retention time thus becomes very important for the discrimination of peaks, and so does the complete separation of analytes. Table 2.1 shows the relative retention times obtained for the analytes and the m/z ratios selected for quantification and confirmation.

Table 2.1 Relative retention times (RRT), quantification (I^Q) and confirmation ions (I^C) for the analytes.

Analyte	**RRT**	**I^Q**	**$I^C{}_1$**	**$I^C{}_2$**
Cop	0.926	370	215	257
e-Cop	0.932	370	215	257
Chl	1	368	353	460
5α-Chl	1.008	370	460	332
Sit	1.162	396	215	486
5α-Sit	1.172	473	488	215

From the full scan analysis of the single standard congeners in the range 50-550 m/z (mass/charge ratio), suitable ions to be used in the SIM analysis were identified. The chromatographic run was then optimized by analyzing a standard mixture containing all the analytes and the internal standard. In order to obtain full separation of Cop and e-Cop, it was found that a temperature ramp inferior to 3 °C min^{-1} is required with the available instrumental setup. The final optimal temperature program was set as follows:

70°C (2 min), 20 °C min^{-1} to 210°C, 3 °C min^{-1} to 300 °C (8 min), 305 °C for 15 min (postrun). The carrier gas was helium at 1 mL min^{-1} flow. The inlet temperature was 280 °C and samples were injected in splitless mode (split valve open after 1 min). The interface was kept at 280 °C, the ion source at 230 °C and the quadrupole at 150 °C. Figure 2.2 shows the effect of slowing down the temperature ramp on peak separation.

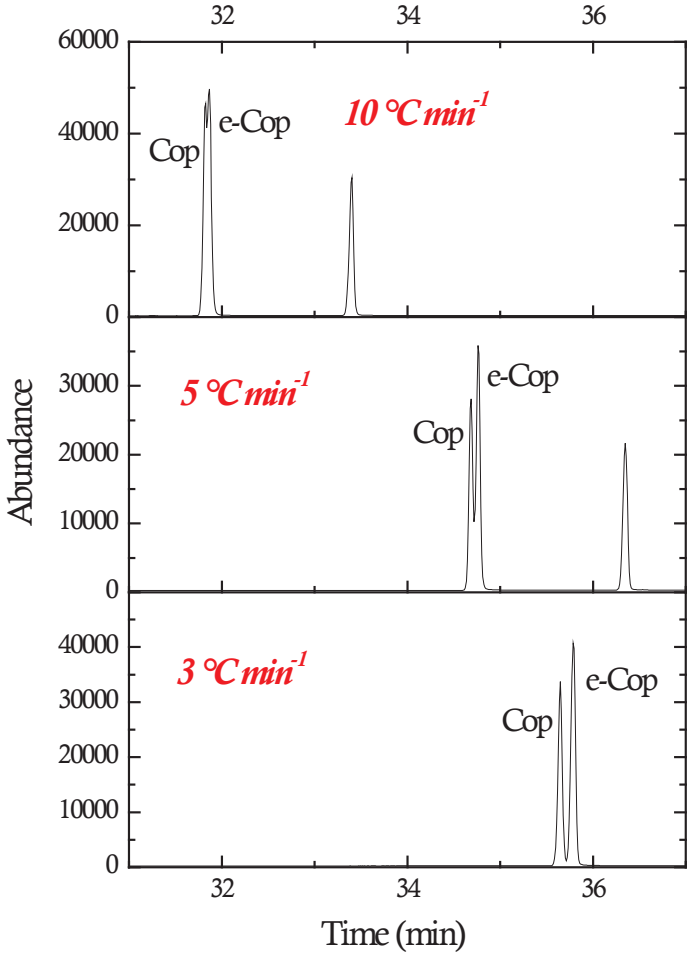

Figure 2.2 Effect of different temperature ramps on the separation of coprostanol and epicoprostanol.

Extraction

The extraction was performed with a PLE system, using a 20 mL stainless steel vessel. The extraction cell was filled with a small amount of Ottawa sand, diatomaceous earth (for blanks) or ~1 g of dried sample dispersed into diatomaceous earth and active copper.

Samples and blanks were spiked with known amounts of the internal standard solution (cholesterol-3,4-$^{13}C_2$) and extracted at 150 °C and 1500 psi according to reference [140].

In order to assess the optimal extraction conditions, four different solvent combinations were tested, namely: DCM, DCM:MeOH=9:1 (*v*/*v*), DCM:MeOH=2:1 (*v*/*v*) and DCM:Hex=1:1 (*v*/*v*). Extraction recovery for the different solvents was evaluated on aliquots of dried sediments (river Sile, Italy), spiked with cholesterol-25,26,27-$^{13}C_3$: results indicated DCM as the best solvent in terms of recovery and selectivity, resulting in 91.1% extraction yield with 5.2% RSD. DCM:MeOH=9:1 had similar yields but the increase in solvent polarity caused higher disturbance on the baseline. This effect was amplified when using DCM:MeOH=2:1 (see fig. 2.3), while the DCM:Hex=1:1 mixture did not allow a quantitative extraction (44.7%).

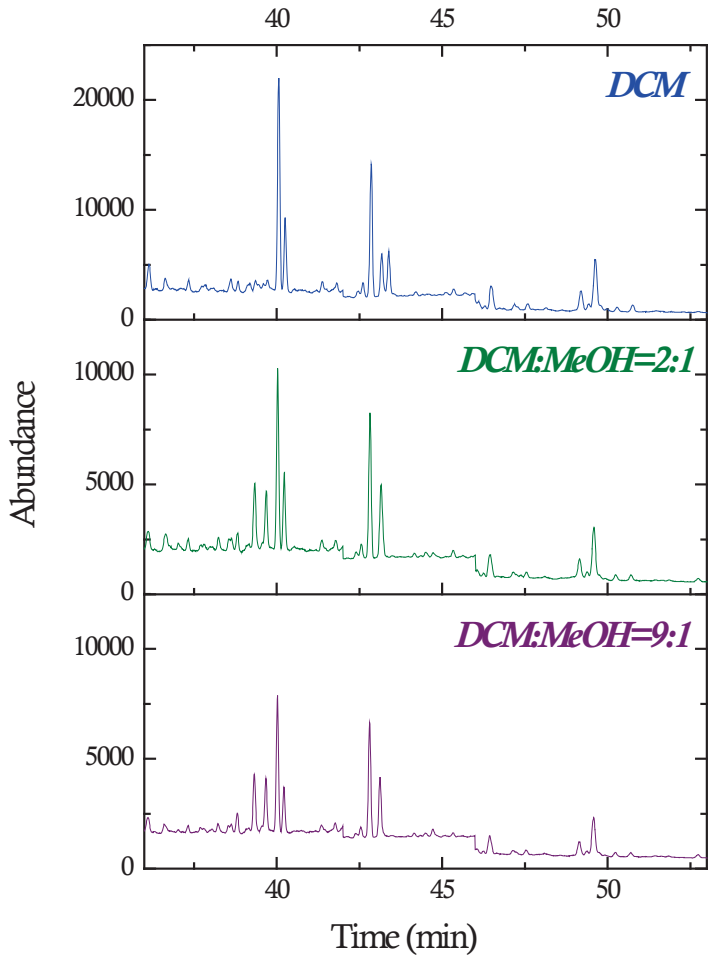

Figure 2.3 Total ion current (TIC) resulting from different polarity solvent mixtures.

The number of extraction cycles necessary to collect the totality of analytes was assessed by spiking blanks with a standard solution containing all native compounds and the internal standard, and by collecting the extract from each cycle separately. Although it was verified that one cycle was sufficient to collect the totality of analytes, two cycles (5 min static) were included in the method as a precaution to ensure complete extraction.

The extraction efficiency of PLE was also compared to the performance of the ultrasonic bath, which resulted in an extraction efficiency of 66% with 19% RSD, unsatisfactory if compared to PLE results.

Clean-up

The clean-up system of choice was selected for its velocity and reproducibility. SPE tubes were used (Discovery® DSC-Si SPE Tube, 2 g bed weight, 12 mL, Sigma-Aldrich), following the method employed by Isobe et al. (2002) with slight modifications [166]. The stationary phase was conditioned with DCM, and the amount of solvent needed for the complete elution of analytes was tested by collecting separate fractions. After conditioning, SPE tubes were spiked with 100 ng of the native sterols and eluted with DCM: ten fractions of 10 mL each were collected. Each fraction was then spiked with the internal standard. Results showed that all analytes are eluted within 70 mL. This value was therefore adopted as elution volume in the final method.

Method performance

The analytical performances of the final method were tested on spiked blanks, on a reference material and on real samples from river Piave. Since a standard reference matrix for sterols in freshwater sediments is not commercially available, the best approach was to employ a reference material certified for other analytes: ERM®-CC580 [167], an estuarine sediment certified for mercury (Hg) and methylmercury (CH_3Hg^+). All tests were run in replicate, in order to evaluate repeatability.

The recovery of the complete method was tested on blanks spiked with native and ^{13}C-labeled compounds simulating high (100 abs ng) and low (10 abs ng) concentrations of

sterols. As mentioned above, the best recovery was obtained for the extraction with DCM and ranged between 74 and 80% for all the analytes. The precision was tested on the reference matrix and on real samples. In the former, high amounts of sterols were detected (214-2549 ng g^{-1}) and the RSD ranged between 2.7 and 4.9%, while in the latter lower concentrations were measured (36-1752 ng g^{-1}) and the RSD was slightly higher (5.1-12.8%), denoting a strong dependence on the type of matrix and concentration level.

Instrumental detection (I-LOD) and quantification limits (I-LOQ) were identified as the concentrations resulting in peaks with an area of 3 and 10 times the signal to noise ratio, respectively. Laboratory detection (L-LOD) and quantification limits (L-LOQ) were identified as the mean values of procedural blanks plus 3 and 10 times the standard deviation, respectively. I-LODs ranged between 11 and 120 abs pg, I-LOQs between 37 and 399 pg. Only cholesterol (L-LOD: 120 pg; L-LOQ: 400 pg) and β-sitosterol (L-LOD: 202 pg; L-LOQ: 273 pg) were detected in blanks. The linearity was evaluated by repeatedly injecting solutions containing native standards in the range 0.01 to 20 ngμL^{-1} and the internal standard at 10 ngμL^{-1}. The instrumental response was found to be linear up to 10 abs ng for each compound (r^2=0.99).

All details about method development, validation and quality control are reported in the paper included in the appendix (tables 2 and 3) [138].

Final method scheme

The final scheme of the method for the analysis of coprostanol, epicoprostanol, cholesterol, cholestanol, β-sitosterol and stigmastanol in freshwater sediments is proposed in figure 2.4. The method will hereinafter be referred to as **method #1**.

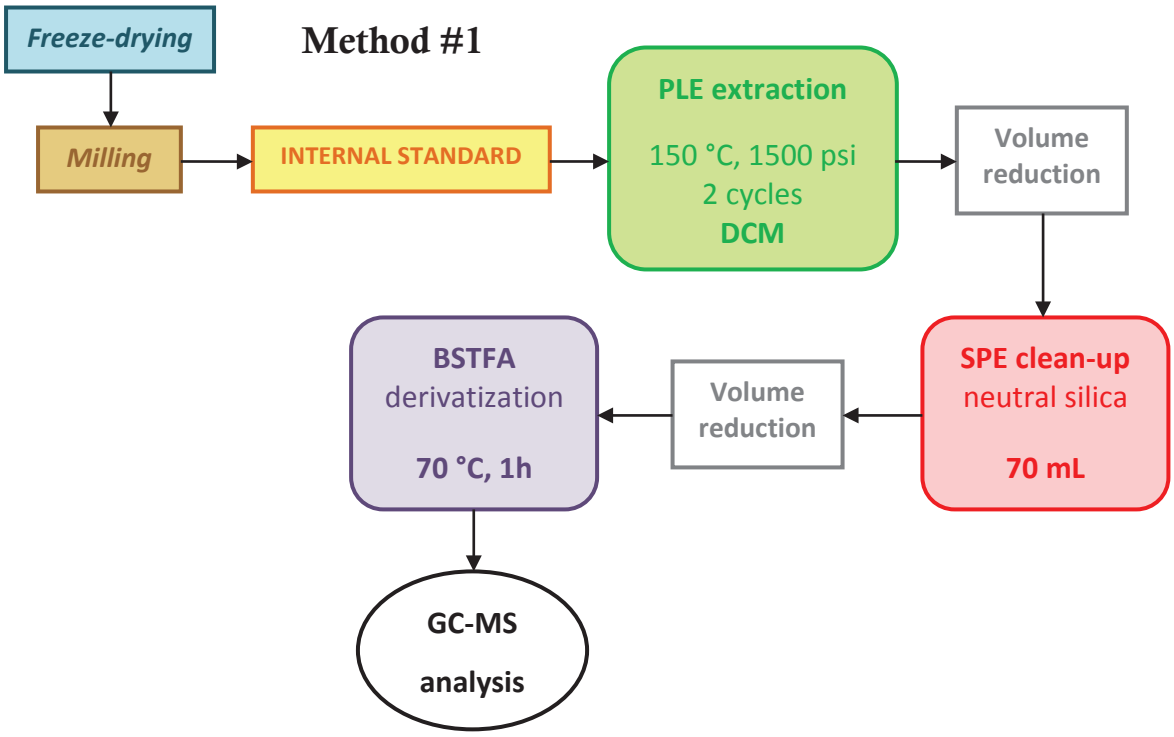

Figure 2.4 Summary of the final method for the analysis of sterols.

2.3.2. Multi-analyte methods

In the course of this work, the method for fecal and plant sterols was coupled to the pre-existing methods for the analyses of levoglucosan and PAHs, in order to obtain a multi-analyte technique capable of analyzing all three classes of compounds simultaneously on one sample. To achieve this goal, the method for fecal sterols published in the paper from Battistel et al. [138] was slightly modified to make it compatible with the other analytes.

Two multi-analyte methods were tested in this work, which differ mainly in the instrumental approach. In the first method (**method #2**), two different instrumental techniques are used, namely the GC-MS for sterols and PAHs and the IC-MS for levoglucosan. In the second method (**method #3**), the GC-MS was used for all the analytes, upon derivatization of the monosaccharide anhydrides. Both methods include the separation of analytes in fractions during the clean-up step.

The development of these methods was preceded by an application to a sediment core from Lake Trasimeno (central Italy), where levoglucosan and fecal sterols were analyzed on 72 samples and an interpretation of coupled records in the last 3000 years was proposed. The latter work was the object of the PhD dissertation by Kirchgeorg [168] and resulted in the submission of a manuscript to Geophysical Research Letters, entitled "*Late Holocene Record of Humans and Fire at Lake Trasimeno (Italy)*", by T. Kirchgeorg, D. Battistel, N. Kehrwald, E. Argiriadis, A. Polonia, L. Gasperini, D. Petheet, M. Radaelli, E. Bonatti, C. Barbante. The draft of the unpublished work is included in the appendix. Results will therefore not be discussed in the present work.

Method #2

Extraction

As reported in literature, levoglucosan requires a polar solvent or mixture to be efficiently extracted from sediments. The dichloromethane:methanol = 9:1 (*v*/*v*) mixture is often used as extraction solution for levoglucosan [92], PAHs [103] as well as for sterols [142]. As illustrated above, DCM:MeOH=9:1 was tested for sterols and results showed an extraction yield comparable to DCM, despite the higher matrix effect. It was therefore employed as the best compromise for the extraction of all analytes. The extraction instrumental conditions are the same as for Method #1. The internal standards added were a $^{13}C_6$-levoglucosan (Sigma-Aldrich) solution at 1 ng μL^{-1} in MeOH and a solution containing $^{13}C_6$-cholesterol (Sigma-Aldrich),$^{13}C_6$-acenaphthylene, $^{13}C_6$-phenanthrene and $^{13}C_4$-benzo[*a*]pyrene (Cambridge Isotope Laboratories), all at 1 ng μL^{-1} in DCM.

Clean-up

The SPE clean-up method was implemented for the separation of the analytes in two fractions, one destined to the GC-MS analysis and the second to the IC-MS. The same stationary phase as for Method #1 was used. The first fraction (F1), containing sterols and PAHs, was eluted with 70 mL of DCM and the second (F2), containing the monosaccharide anhydrides, was subsequently eluted with 20 mL of MeOH. F1 was concentrated to 100-200 µL and analyzed for PAHs. After the analysis, 100 µL of BSTFA + 1% TMCS were added to the samples, which were derivatized at 70 °C for 1 h.

After a stabilization period of 24 h, sterols were analyzed on samples. F2 was evaporated to dryness, redissolved in 0.5 mL of ultrapure water and centrifuged before IC-MS analysis, according to the method proposed by Kirchgeorg et al. (2014) [94].

GC-MS

The GC-MS method for sterols was further optimized and shortened with respect to method #1. The initial temperature was set at 150 °C (maintained for 1 min), then was brought to 220 °C at 30 °C min^{-1} (0 min), 300 °C at 2 °C min^{-1} (10 min) and finally at 315°C for 10 min (post run). The interface was kept at 300 °C while the other instrumental parameters were not changed. Such changes ensured a shorter run, a cleaner baseline and a better separation of isomers due to the further slowing of the ramp between 220 and 300 °C.

The chromatographic method for PAHs, already in use by the research group - and slightly modified from [169] - was as follows: initial temperature 70 °C (1.5 min), 150 °C at 10 °C min^{-1} (10 min), 300 °C at 3 °C min^{-1} (15 min), 305 °C for 30 min (postrun). Samples were injected in splitless mode (split valve open after 1.5 min), and the inlet and interface temperature was 300 °C. Helium was used as carrier gas at 1 mL min^{-1} flow.

IC-MS

The analysis of monosaccharide anhydrides by IC-MS followed the method by Kirchgeorg et al. (2014). Briefly, NaOH was used as a carrier solvent, with a flow of 0.250 mL min^{-1} and with the following gradient: 20 mM (0-15 min), 100 mM (15-40 min), 20 mM (40-60 min). The source, operated in negative mode, had a temperature of 350 °C, and the needle and cone voltages were -3.5 kV and -50 V. The target ion for quantification was *m/z* 161 and qualifiers were *m/z* 110 and 113 [94].

Method performance

The method was tested in replicate on several blanks spiked with native compounds and ^{13}C-labeled internal standards. The average extraction yield was 89% (16% RSD) for PAHs, 115% for sterols (17% RSD) and 99% (11% RSD) for MAs. Precision ranged between 2 and 23% for PAHs, 12-24% for sterols 19-33% for MAs. Sample results were corrected for the mean blank plus three times the standard deviation.

Final method scheme

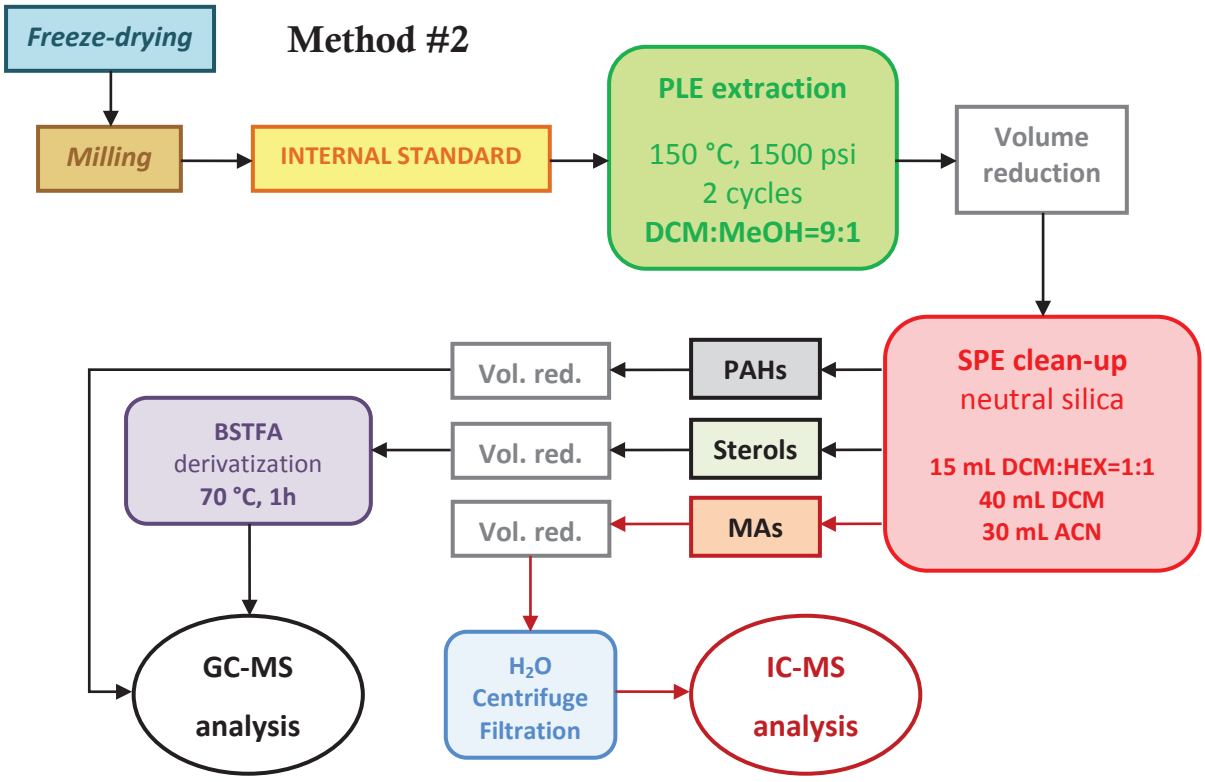

Figure 2.5 Summary of the final method #2 for the analysis of PAHs, sterols and MAs.

Method #3

Extraction

The extraction method was the same as for method #2.

Clean-up

As in methods #1 and #2, the clean-up procedure was performed through SPE neutral silica tubes. Here, three fractions and three different solvent combinations were required in order to separate the three classes of analytes. Suitable solvents and volumes were tested by conditioning columns with a DCM:HEX=1:1 (*v*/*v*) mixture and by spiking with a solution containing all the native analytes (concentration range 2-100 ng μL^{-1}). Several tests were run with different fractions and solvents. In general, the normal phase elution

on the selected stationary phase requires a sequence of growing polarity solvents to separate different polarity analytes. DCM:HEX=1:1 proved the most suitable eluent for PAHs [169], while DCM was already used for sterols, that are only slightly polar. Anhydrosugars, though, are highly polar compounds and therefore require a convenient solvent. Methanol (MeOH) and acetonitrile (ACN) were tested. Both proved suitable for the complete elution of levoglucosan and its isomers, but the higher polarity of MeOH affects the retaining capacity of the stationary phase, resulting in higher interference yields. The relatively low volatility of MeOH results in longer volume reduction time, associated to greater analyte loss (low recovery). Moreover, the characteristics of MeOH make it unsuitable for injection in GC due to high polarity and expansion volume, which can cause backflash and thus lower instrumental response [170]. For all the above reasons, ACN was preferred over MeOH as elution solvent for monosaccharide anhydrides.

The elution experiments showed that the following volumes, adopted as clean-up protocol, were sufficient to obtain a good recovery of the analytes: 15 mL of DCM:HEX=1:1 (F1); 40 mL of DCM (F2); 30 mL of ACN (F3). Each fraction was then concentrated and analyzed separately by GC. Results are shown in figure 2.6. F1 was directly injected for the analysis of PAHs, while F2 and F3 were derivatized as described below.

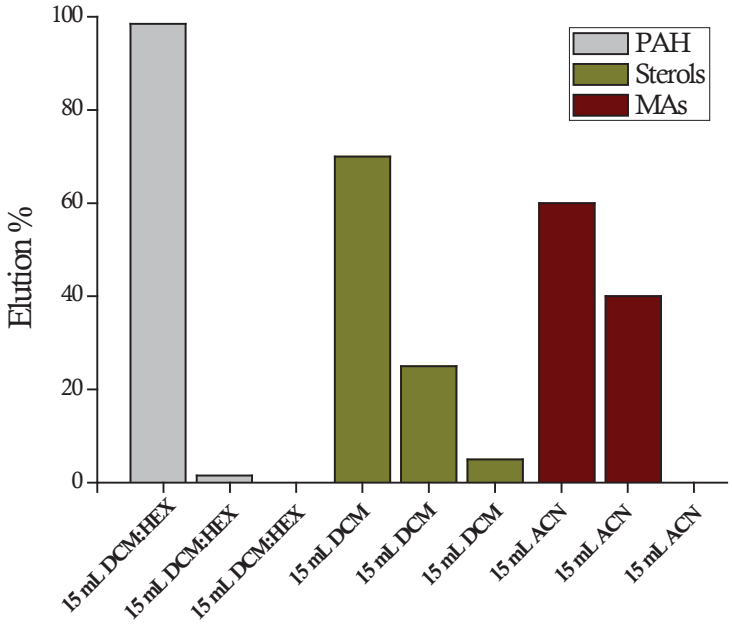

Figure 2.6 Eluted volumes and % of analytes recovered in each fraction.

Derivatization

For the fraction containing sterols (F2), the derivatization was conducted as discussed for method #2. Unlike sterols, anhydrosugars contain three –OH groups: the silylation reaction must therefore occur three times for a complete derivatization. In order to obtain fully silylated reaction products, the use of pyridine as a catalyst is often reported in literature [88, 171, 172]. The derivatization efficiency in presence of pyridine was therefore tested and compared to the use of BSTFA alone. Figure 2.7 displays the areas of peaks obtained in the two experimental conditions. The reaction yield with pyridine is on average 15 times higher, and the use of this catalyst is therefore essential to the success of the analysis.

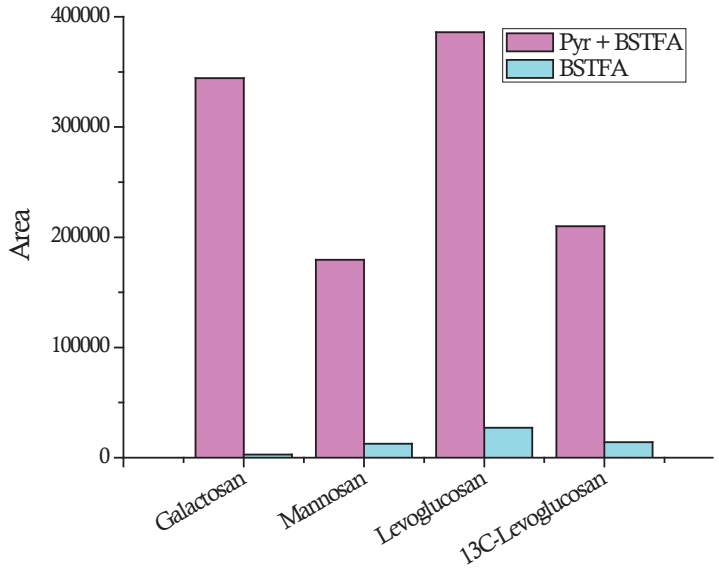

Figure 2.7 Comparison of derivatization yield for MAs with only BSTFA and in presence of pyridine.

Optimal reaction time and stability of derivatized samples were also evaluated. Standard solutions were evaporated to dryness and redissolved in 100 µL of pyridine, to which 100 µL of BSTFA were added. The reaction temperature was 70 °C and the time was tested in the interval 1-4 h. As for sterols, the highest instrumental response is obtained with 1 h derivatization. Test solutions were injected repeatedly for eight days in order to assess stability. Results showed that the instrumental response for silylated compounds reaches its maximum after two days, then starts to decrease quickly as shown in figure 2.8.

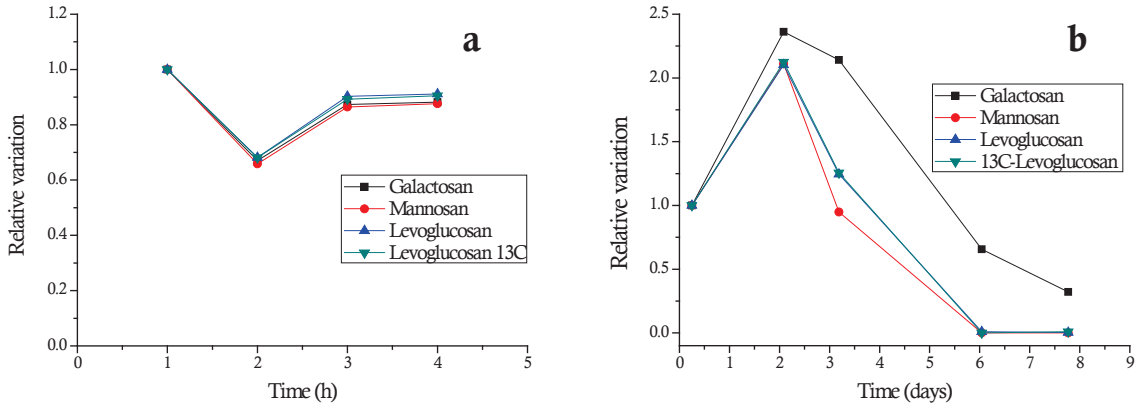

Figure 2.8 (a) Relative variation in the yield of analytes as a function of derivatization time. (b) Stability of TMS derivatives in time.

GC-MS

GC-MS analyses of PAHs and sterols were carried out as in method #2. The analysis of levoglucosan, mannosan and galactosan required an *ad-hoc* method, developed in the course of this work. The native compounds and the internal standard ($^{13}C_6$-levoglucosan) were derivatized as described above and analyzed individually in full scan for the identification of the characteristic fragmentation pattern. *m/z* ratios 204 and 217 (native compounds), 206 and 220 (internal standard) were selected as target ions. The final chromatographic method, which allows complete separation of the three isomers, is as follows: initial temperature 110 °C (5.5 min), 210 °C at 15 °C min^{-1} (0 min), 220 °C at 2 °C min^{-1} (5 min), 300 °C for 10 min (post run). The inlet and interface temperatures were 280 and 290 °C. Samples were injected in splitless mode (split valve open after 1.5 min). Helium was used as carrier gas at 1 mL min^{-1} flow.

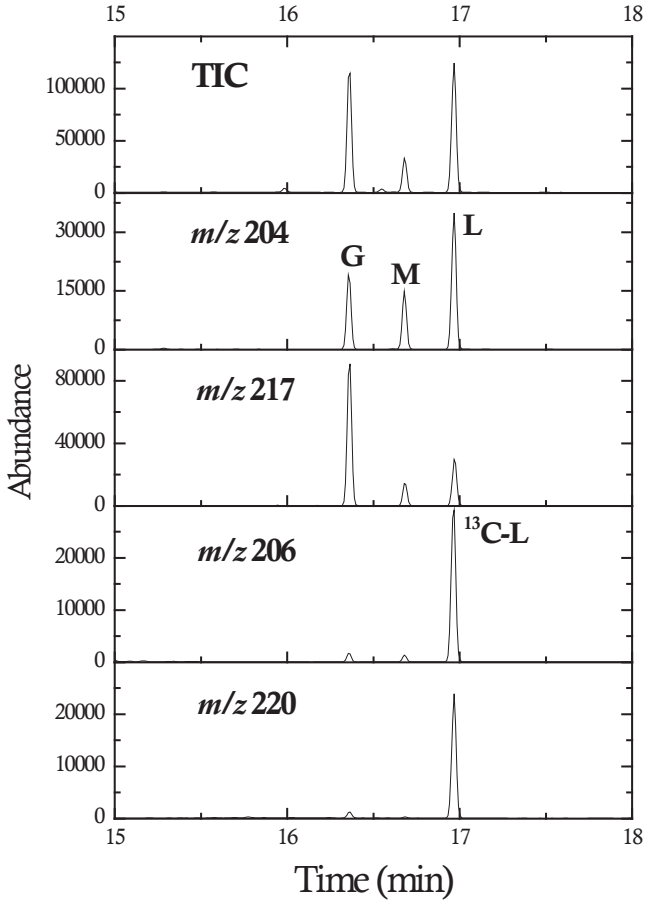

Figure 2.9 Total ion current (TIC) and SIM m/z ratios for galactosan (G), mannosan (M) and levoglucosan (L) in a sample chromatogram.

Method performance

The method was tested in triplicate on samples from river Sile - as in the validation of method #1 - spiked with a mixture of all the internal standards at 1 ng μL^{-1}. Results show an average recovery of the internal standard of 97.5% for PAHs (1.4% RSD), 97.2% for sterols (0.45% RSD) and 94.7% for MAs (0.61% RSD). The precision of the overall method, estimated as percent relative standard deviation, is 9-16% for PAHs, 12-18% for sterols and 13-16% for MAs. Sample results were corrected for the mean blank plus three times the standard deviation.

Final method scheme

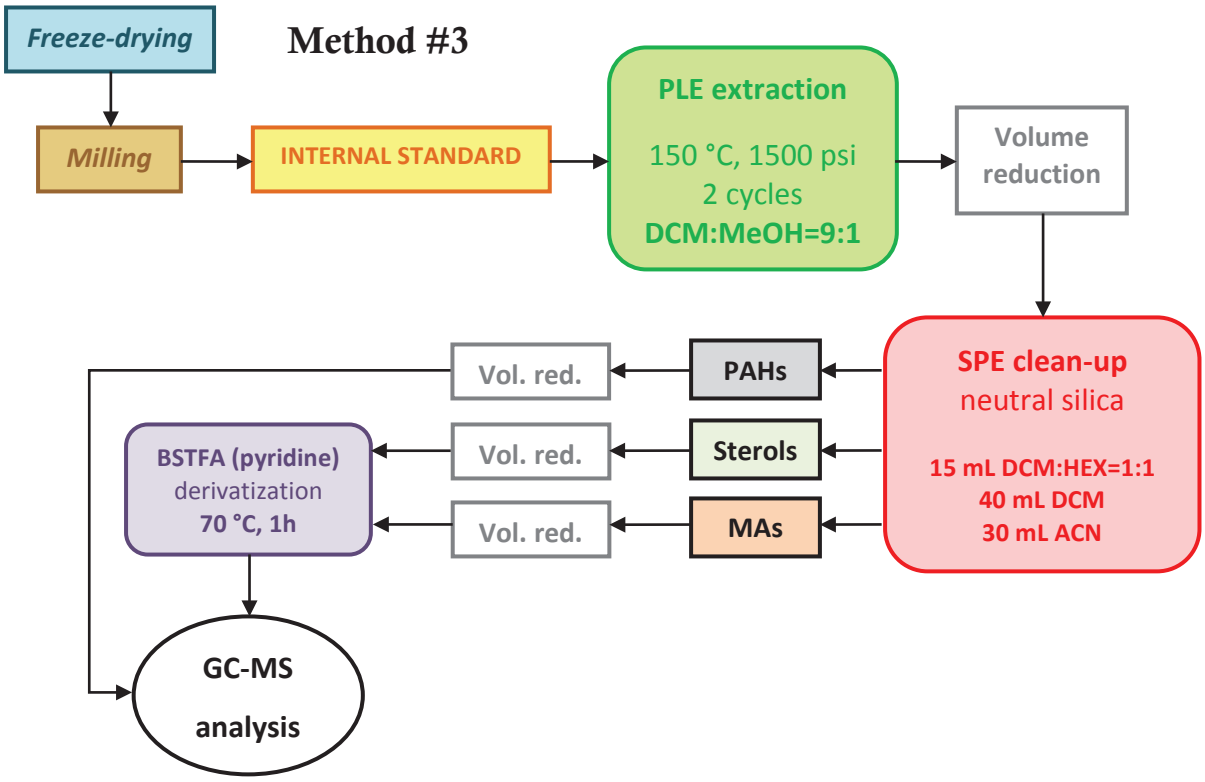

Figure 2.10 Summary of the final method #3 for the analysis of PAHs, sterols and MAs.

2.4. Application sites

As mentioned above, the set of methods was tested on real sites. Below, the importance of the three locations selected for this work is briefly explained.

2.4.1. New Zealand

New Zealand, and in particular the South Island, is considered an excellent test site for studies about the impact of early human settlements. There are many reasons for this. The Polynesian settlement occurred only about 700-800 years ago and caused abrupt landscape modifications, with the loss of 40-50% of the pre-existing forest, which used to cover almost the 90% of New Zealand [121, 173–175]. A shift in fire regime and in the composition of vegetation is observed in charcoal and pollen records as a result of increased fire activity. Wildfires were infrequent before the advent of humans, and vegetation was not adapted to fire [122, 176, 177]. Such an abrupt, fast and well documented transition, together with the isolated nature of New Zealand, make it a perfect study site for the reconstruction of local scale phenomena and in particular for the purpose of this work. The existence of high-resolution paleoecological records allowed us to test the methods developed in this work and to implement the existing information about human presence. Moreover, although charcoal data give incontrovertibleevidence of some unprecedented fire events right after the arrival of the Māori, the significance of charcoal as a tracer for localand anthropogenic fire events has been questioned, stressing the need for new markers to confirm and completethe information about human presence and its effective impact [178, 179]. McGlone and Wilmshurst (1999), in particular, highlighted the lack of “specific human markers” and the importance of distinguishing between natural and anthropogenic fires [179]. The study of molecular markers proposed here provides a valid instrument for a more in-depth investigation of the nature of recorded fires, complementing and refining the reconstructions enabled by charcoal analysis.

The South Island is crossed for most of its length (500 km) by the Southern Alps, giving rise to many valleys and lakes of glacial origin.In this work, two small lakes in the south-west of the South Island were considered, namely Lake Kirkpatrick and Lake Diamond.

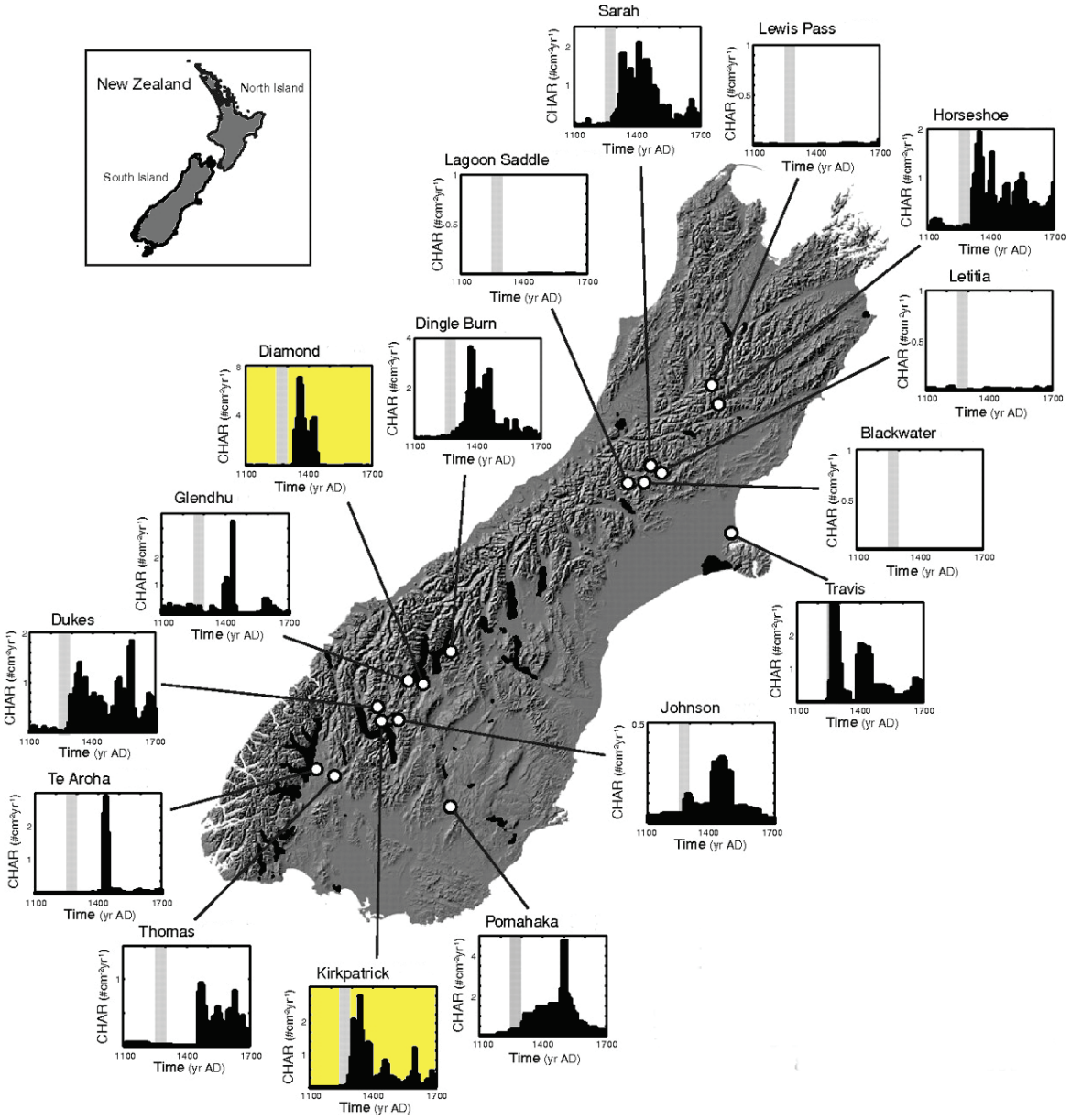

Figure 2.11 South Island, NZ. Charcoal accumulation rates in 16 sites, modified from *McWethy et al. (2010)* [180]. Target locations for this work are highlighted in yellow.

Figure 2.11 shows the location of sixteen sites in the South Island where charcoal analyses were carried out by McWethy et al. (2010). Lake Diamond and Lake Kirkpatrick are among the sites where clear spikes in the charcoal accumulation rate were revealed shortly after the arrival of the Māori. Only wetter, higher latitude locations did

not register this abrupt increase, indicative of a sudden change in the fire regime. Pollen records, where available,indicate that areas marked by low rainfall were affected more severely by the increased fire activity and were subject to a shift from forest trees to shrubs and grasses [180].

Lake Diamond (44.65° S, 168.96° E, ~1.6 km^2) is located in the Otago region at 380 m asl, in a dry site close to Lake Wanaka, the fourth largest lake in New Zealand (192 km^2) [180, 181]. Charcoal data document an intense fire activity in the period between AD 1300 and 1600, when four major peaks are registered, and one single peak around AD 1750, corresponding to the late Māori period. This was followed by a newly increased deforestation activity after the arrival of Europeans in the 19th century, accompanied by the introduction of alloctone plants. Pollen and limnobiotic data confirm an ecological shift in plant and planktonic species, in response to post-fire alteration of the forest and the watershed [180].

Lake Kirkpatrick (45.03° S, 168.57° E, ~0.04 km^2) is located southwest of Lake Diamond, at 570 m asl in a moderate rainfall context within the Lake Wakatipu basin, in Otago. The Wakatipu area was one of the main deposits of jade, found only in the South Island, extracted and traded by the Māori [122, 182]. Charcoal data are in agreement with Lake Diamond: they show two peaks at about AD 1367 and 1391, and a general increase in fire activity until 1600, followed by a second increase after the European colonization around 1800. Vegetation reacted with the decline of arboreal species in favor of grasses and bracken [122, 176].

Generally, the fire episodes of greatest magnitude appear to have occurred during the Māori deforestation, while in the European period smaller events are recorded, mostly as a consequence of the fuel composition change after the so-called initial burning period (IBP) [176]. It seems evident that the observed disruption in the fire regime is of anthropogenic origin, although to date there is no way of correlating the size and frequency of fires with the size of human population [183]. The possible role of climate change in the vegetation-fire feedback was also investigated by several authors, leading to the conclusion that it was a minor factor, unlike other locations in the world where Holocene fire regimes were the result of longer scale changes and reflected both natural and anthropogenic drivers [175, 180, 183]. In light of all these considerations, the

introduction of a direct marker of human presence, such as fecal sterols, provides an important contribution to the understanding of the observed phenomena.

2.4.2. Tasmania

Since islands are an ideal observatory for local paleoecological reconstructions, another insular location with a long colonization history was considered in this work. Flinders Island, the main island of the Furneaux Group, is located in the Bass Strait, 54 km north of the northeastern coast of Tasmania. This location provides a very different perspective if compared to New Zealand. Here, the climate of the Holocene played a fundamental role. The history of human settlement in this archipelago is still uncertain: human presence is reported in Tasmania and in the Bass Strait islands as early as 35,000 years ago and intensifies after about 20,000 years ago, although a certain discontinuity is evidenced from available records [184, 185]. The human presence on the Furneaux Group appears to be in strong connection with fluctuations in the sea level, which reached its minimum during the last glacial maximum (LGM) about 18,000 years BP.

Tasmania is defined as "the most extreme case of isolation", caused by the progressive rise in sea level which by 13,000 y BP had cut the land connection with Australia and among smaller islands by about 8000 y BP [184–186]. From that moment on, Tasmania and its population were isolated from external influence until the arrival of Europeans in the 19th century [184]. When the first explorers set foot on Flinders Island and King Island, they found no trace of people living there [187].

The majority of archaeological studies in Tasmania were conducted on limestone caves in the southwest region, extremely rich in human and animal remains [185, 188]. The sparser evidence available in the north and east indicates that humans were present at least 23,000 years ago in the Bass Strait islands [184, 185]. With the onset of the LGM, a general decline in human presence is observed in the southwest between 25,000 and 16,000 years ago, whereas islands show a continuative presence until 15,000 years ago, followed by scarce and scattered activity until 4500 years ago.

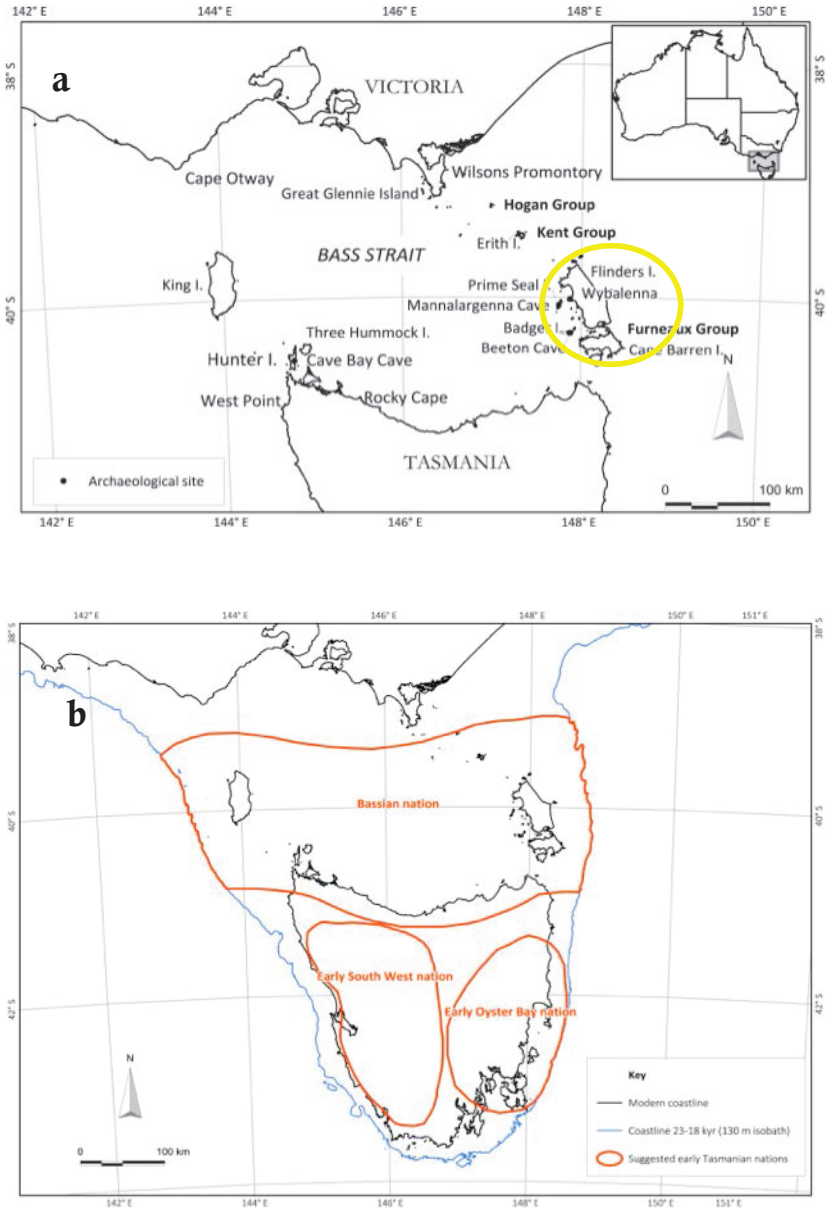

Figure 2.12 (a) Location of the Furneaux Group in Bass Strait, Flinders Island in the yellow circle. (b) Early coastline of Tasmania and suggested archaeological nations. *From Bowdler (2015)* [185].

It is believed that Aboriginals in the Bassian region conducted coastal life during glacial times and progressively retreated towards higher land following the sea level rise. For the islands in the northwestern sector, this meant definitive abandonment (apart from occasional visits recorded in some sites), while the Furneaux Group was inhabited for

several millennia after the formation of the Bass Strait. Flinders Island, in particular, was big enough to sustain a stable group of a few thousand individuals: according to archaeological evidence, though, no Aboriginal presence is documented on Flinders Island in the last 4500 years. The most plausible explanation is that these isolated people continued living there until they died out, rather than migrating after the introduction of watercrafts, which were not in use until 2500 years ago [185]. The reason for their extinction is probably linked to environmental changes: islands in the Pacific basin are highly influenced by El Niño Southern Oscillation (ENSO), which is believed to have triggered changes 5000-3000 years ago, resulting in a colder climate with drier summers that might have affected water resources [185, 187]. The long absence of humans on Flinders Island, therefore, makes it a privileged observatory for the study of the dynamics that link vegetation, fire and climate.

However, the reconstruction of fire regimes is crucial to understanding human influences on the environment, especially since archaeological evidence still leaves many unknowns about colonization patterns. Tasmanian pollen and charcoal data highlight a notable importance of fire in shaping the environment. The scarcity of ignition sources and the characteristics of the climate suggest that humans were the main cause of fires [189]. The Aboriginals are believed to have practiced fire-stick farming and to have strongly relied on seasonal hunting expeditions, using fire to open patches of landscape [185, 188]. Existing charcoal and pollen records mainly regard the southwestern region of Tasmania, where the actual moorland-dominated landscape appears to be the result of intense fire activity throughout the Holocene [189]. Human induced fire regimes until the arrival of Europeans were characterized by low intensity and high frequency. They were replaced by higher-intensity burning events after the 19th century occupation [189, 190].

The scarce data available for the northern and eastern parts of Tasmania do not provide sufficient instruments for the interpretation of the role of humans in fire regimes. On Hunter Island, located close to the northwestern tip of Tasmania, the increase in charcoal particles appears to coincide with its abandonment ~4500 years ago, suggesting an influence of the drier climate on the fire regime [191]. Probably the same changes that led to the disappearance of human population promoted an increase in natural fire activity. Killiecrankie and Middle Patriarch swamps on Flinders Island were the object of a pollen and charcoal survey covering the last 10,000 years by Ladd et al. (1992), who concluded

that the absence of humans caused no evident change in vegetation or in fire frequency. Fires were common and the vegetation was adapted to them, but the only significant change ascribable to human presence is visible in the last 200 years, when clearing for agricultural activities became extensive [186].

In the present work, samples from a wetland sediment core retrieved at Middle Patriarch Lagoon (39.99° S, 148.18° E, 15 m asl) were considered (McWethy et al., *unpublished data*). The fire history inferred from charcoal analyses is in agreement with the general biomass burning Holocene trend for the Australasian region: fire activity was high in the early Holocene, decreased significantly in the mid Holocene and then intensified again during the last 2000 years. The European presence in the last two centuries is marked by increased biomass burning [192, Mc Wethy et al., *unpublished data*]. The composition of vegetation, reconstructed from pollen data, reflects the transition from dry to wet periods influenced by large-scale oscillations. Moreover, periods of less favorable climate conditions may have resulted in an increased level of human control on landscape (Mc Wethy et al., *unpublished data*).

All the indications from archaeology and paleoecology underline the need for more direct indications of human influence. The use of molecular markers such as the fecal sterols considered in this work therefore appears highly attractive as it can provide further evidence of human settlements on the Tasmanian islands.

2.4.3. Lake Victoria

Lake Victoria, located in eastern Africa at 1135 m asl and bordered by Uganda, Tanzania and Kenya, occupies a plateau between the eastern and western branches of the Rift Valley, where it was formed by uplift activity approximately 400,000 years ago. With its 68,000 km^2 extension, it is the largest lake in Africa and the second in the world by area. It is also the source of the Nile River, constituting a freshwater reservoir of primary importance. Despite the wide occupied surface, Lake Victoria is quite shallow (maximum depth: 81 m) and its water balance is mainly determined by rainfall and evaporation rather than by rivers. The water inflow and outflow is limited to one main tributary, river Kagera, and one effluent, the Nile [193–195]. These characteristics make

it suitable for the reconstruction of regional paleofires and of anthropogenic land use, although relatively few paleoecological studies were carried out so far [196].

Considering the complex interactions among large scale and regional factors that drive present climate is particularly important in the case of East Africa [197]. Local conditions can be quite variable depending on the altitude and on the distance from lakes. The area surrounding Lake Victoria is mainly covered in wet savanna characterized by relatively high rainfall (700 - >1200 mm) due to the presence of the lake's huge water mass, while semi-arid zones of Kenya are characterized by drier savanna reflecting the low rainfall pattern (<200 mm). Despite these differences, a bimodal annual rainfall cycle affects the whole region, with precipitation peaks in March-May and in October-December [197]. On a wider scale, the climate is influenced by seasonal shifts in the Inter-tropical Convergence Zone (ITCZ) and the Congo Air Boundary (CAB): the main drivers are the dry southeast and northeast monsoons, and the humid Atlantic air coming from Congo, which causes rainfall in the northwest [196].

Throughout history, Lake Victoria and the other east African lakes registered severe droughts, resulting in significant lowering of the lake level and complete desiccation towards the end of the Pleistocene, as can be inferred from pollen sequences, diatoms and the stratigraphic analysis of sediments [195, 196, 198]. During the end of the last glaciations and the onset of the Holocene, warmer and wetter conditions were established, and the lake level began to rise rapidly, although this trend was punctuated by arid episodes in response to the main climate variations: two severe droughts are recorded in the Kilimanjaro ice record and in diatoms from Lake Victoria around 8000 and 5000 years ago, and a third abrupt event around 4000 years ago resulting in the most severe drought [196, 198, 199]. Pollen records reflect a general tendency to drier conditions in the region after 5000 years ago, with a shift towards more deciduous and grassy taxa in spite of the evergreen forest [196, 200].

A significant influence of humans is believed to have started during the middle Holocene, after 5000 years ago. Existing pollen records reveal a progressive loss of woodland species associated to land clearing after 4000 years ago, but highlight difficulties in disentangling a natural shift towards more arid conditions from direct human influence. Although tropical Africa is currently the major source of biomass burning [47], ancient

fire activity in the east of the continent is scarcely documented. Charcoal records are not very numerous in the area and, due to the scarcity of multi-proxy information, forest disturbance in the past 4000-4500 years has rarely been interpreted as induced by anthropogenic forest clearing [201].

The first evidence of food production in East Africa dates back to 4000 years ago in Kenya, and is attributed to a small group of herders migrating from Sudan and Ethiopia, which were becoming increasingly arid. Nevertheless, the diffusion of the herding lifestyle to southern Kenya and Tanzania was quite slow and became established only around 3000 years ago, when the onset of the actual rainfall patterns allowed to enhance productivity [36, 202]. This period, also called Pastoral Neolithic, saw the coexistence of highly mobile herders and hunter-gatherers: exchange and collaboration between the two groups provided a good strategy for facing drought periods and livestock losses [36].

Unlike in the other examined locations, in Africa animal breeding largely preceded crop production, as mentioned in the introduction. Possible explanations for this discrepancy include low rainfalls and the frequency of droughts, the uncertainty of harvest, and the nomadic attitude of herders [36, 203]. Only indirect evidence of crop production in this period was found in East Africa, mainly based on pottery and stone tools that suggest the cultivation of cereals in the Late Stone Age. The need for an alternative subsistence source might later have induced herders to start cultivating crops: the first stable settlements in the eastern Highlands date back to the Early Iron Age (c. AD 200), although agriculture expansion models are highly speculative due to the scarcity of data [203]. The only records of fire usage appear to be linked to the arrival of the Bantu 2000-1500 years ago: these peoples knew iron working, a technique that requires burning high amounts of wood. Some authors argued that the synchronous decline in woodland and the appearance of typical disturbance species in pollen series, associated to increased fire activity, were human-induced and possibly resulted from agricultural forest clearance, but without any further evidence [201, 204–207].

Most local cultivated crops, however, do not leave recognizable signals in the pollen record, and the reconstruction of agricultural activities must therefore be deduced from the sequences of wild plants, making it difficult to directly correlate data to anthropogenic land use [204, 206]. Also in the case of East Africa, despite the completely

different historical and geographical setting, the coupling of direct markers of fire and of human presence could contribute to fill archaeological, anthropological and environmental knowledge gaps in the framework of multi-proxy studies. The present study proposes the application of target proxies on a portion of the LV95-1P sediment core, retrieved in 1995 in the Ugandan sector of Lake Victoria (0.45° S, 33.42 E), as shown in fig. 2.13.

Figure 2.13 Map of Africa showing the location of Lake Victoria and the sampling site of core V95-1P.

3. RESULTS and DISCUSSION

3.1. New Zealand

As mentioned above, the analyses of charcoal and pollen performed by McWethy et al. (2010 and 2014) revealed a change in the natural fire regime corresponding to the arrival and settlement of humans on the South Island, consistent with a vegetation shift towards fire-adapted shrubs and grasses [121, 122]. As already discussed throughout this work, modified frequency and intensity of fires associated to deforestation are often interpreted as anthropogenic. In the case of New Zealand, where colonization is so recent, this interpretation leaves little room for doubt, which is why this location is ideal for testing new paleoenvironmental methods.

Although the evidence provided by charcoal and pollen gives incontrovertible proof of unprecedented burning events, the validity of these proxies has been questioned due to the lack of direct tracers of human presence. The main criticism regard the transport mechanisms of charcoal and pollen [178]. As discussed in the introduction, many transport and deposition artifacts can affect the size distribution and the interpretation of charcoal data. This problem is normally addressed by selecting precise dimension classes in order to account only for local phenomena (>125 μm) as was done for the charcoal data discussed here [121, 122]. However, pollen can travel great distances: it has been argued that part of the pollen species recognized as allochtonous in New Zealand sediment cores may originate from Australia and thus reflect wider scale processes [178].

For these reasons, the present work concentrated on the research of more direct instruments for the direct reconstruction of human influence on the environment. Results presented here demonstrate the validity of target proxies as powerful tools for paleoenvironmental studies.

3.1.1. Lake Kirkpatrick

Method #2 was applied on a sediment core from Lake Kirkpatrick in the 6-135 cm section. The time span represented is about 800 years (c. AD 1153 – 1961). The original 195 cm core was sampled in 2009; the chronology is based on thirteen radiocarbon dates obtained from twig charcoal and terrestrial plant macrofossils [122]. The age model obtained for Lake Kirkpatrick is shown in figure 3.1.

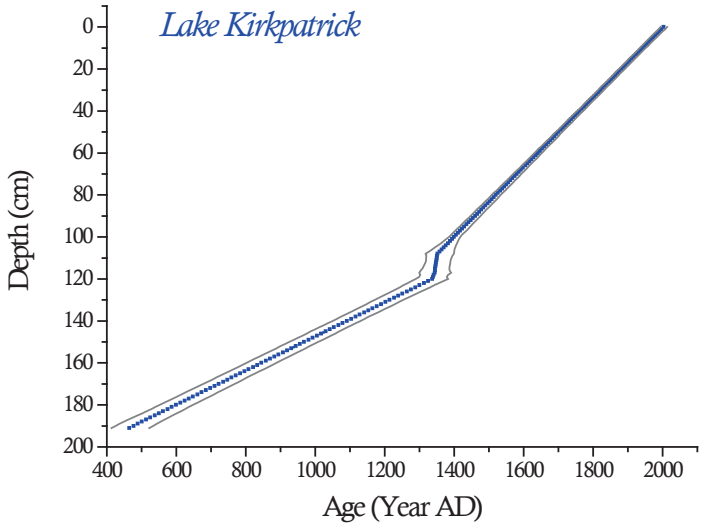

Figure 3.1 Age-depth model for Lake Kirkpatrick. *After McWethy et al. (2010)* [121].

The core was divided in subsamples of 1 cm thickness. Where the amount of material was too scarce for the analysis of molecular markers, it was necessary to merge two contiguous samples, thus obtaining a lower resolution than the charcoal record published for the same core [122]. The 72 samples obtained for this work had a water content ranging from 39% to 93% (79% on average), and the dry weight was 0.27-2.08 g. Results for PAHs, MAs and fecal sterols were calculated as ng g^{-1} dry sediment. Data are expressed here as fluxes (ng cm^{-2} yr^{-1}), calculated using the accumulation rate (cm yr^{-1}) derived from the age model and the dry density (g cm^{-3}) derived from water content as suggested in Menounos (1997) [208].

Polycyclic aromatic hydrocarbons (PAHs)

The analysis of PAHs showed a very marked difference among low-medium molecular weight and high molecular weight compounds. The former are included in the mass interval 166-228 g mol^{-1} and the latter in the range 252-276 g mol^{-1}. Two-ring compounds (naphthalene, acenaphthylene and acenaphthene) were scarcely represented in the whole core: due to their volatile nature, they tend to be associated to the gaseous phase and therefore are hardly ever deposited on water bodies in the particulate phase. The highest concentrations were measured for three and four-ring structures (fluorene, phenanthrene, anthracene, fluoranthene, pyrene, benzo[*a*]anthracene, chrysene, retene), often reported as tracers of biomass combustion [97, 107, 145, 209]. High molecular weight PAHs (benzo[*b*]fluoranthene, benzo[*k*]fluoranthene, benzo[*a*]pyrene, benzo[*ghi*]perylene, indeno[1,2,3-*cd*]pyrene, dibenzo[*ah*]antracene) are poorly represented and were found to occur in significant amounts only in a few samples. They are therefore not reported in this discussion. The observed pattern is consistent with the individual PAHs detected in the smoke from experimental wood burning, and in particular with results from hardwood [210]. The highest values were found for phenanthrene, ranging from 2 to 212 ng g^{-1}, which correspond to 0.2-180 ng cm^{-2} yr^{-1} fluxes, and for fluorene (concentration 0.2-148 ng g^{-1}, flux 0.01-125 ng cm^{-2} yr^{-1}). The observed distribution therefore showed a predominance of phenanthrene (Phe), followed by fluorene (Flu), fluoranthene (Fla) and pyrene (Pyr), as shown in figure 3.2.

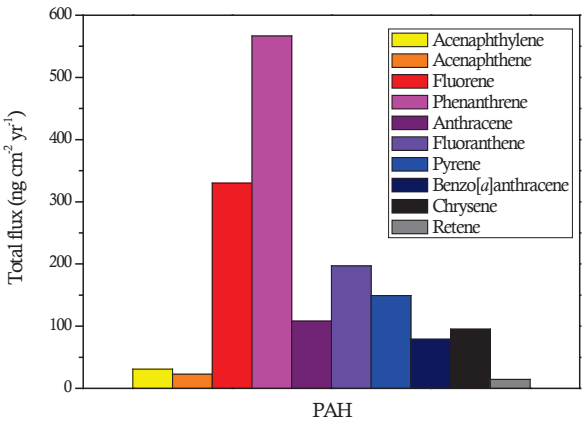

Figure 3.2 Distribution of detected PAHs in the core from Lake Kirkpatrick.

The higher molecular weight PAHs, not measured in the core, are generally reported as markers of petrogenic pollution. They are therefore related to fossil fuel consumption and are found in motor emissions. The detection of these compounds in sediments is usually limited to the portions corresponding to the post-industrial period. In our study, these PAHs showed no significant difference between the pre- and post-industrial sections of the core. The sampling site, located in an Alpine context far from urban settlements, therefore appears undisturbed by any signal of atmospheric pollution. This observation further confirms the validity of both the tracers and the site for paleoenvironmental surveys.

Figure 3.3 shows the fluxes of the most significant congeners in the considered time interval. All congeners show a dramatic and simultaneous increase at about AD 1350. This finding appears to mark a huge fire event or a cluster of intense and closely spaced events. Right after this peak, the levels of PAHs decrease and stabilize around more or less constant fluxes that rarely exceed 1 ng cm^{-2} yr^{-1} for the single congeners. Nevertheless, fluxes after AD 1350 are, on average, one order of magnitude greater than prior to the Initial Burning Period. In fig. 3.3, a logarithmic scale was used in order to better highlight this difference. The peak corresponds to a time shortly after the Māori settlement and is in agreement with charcoal data for Lake Kirkpatrick and several other lakes in the South Island [94, 176, 180]. Results suggest that after a rather short and very extreme burning period, the pre-existing fire regime shifted to small fires that kept occurring either as a result of human ignition, or naturally favored by the new composition of vegetation. The observed peak at c. AD 1350 must have resulted from huge amounts of fuel, capable of sustaining large fires and with high emission factors for PAHs. Once forest wood ran out and grasses and bracken took over, the new composition of fuel could sustain only smaller fires, which occurred spontaneously or were induced to maintain the landscape open. This interpretation is in agreement with the pollen diagram published in reference [122], where an abrupt vegetation shift at the expense of tall trees is reported after AD 1350.

The detected congeners are typical of the combustion of wood and are among the most frequent PAHs found in the few available literature records [107, 145, 211]. In burning experiments, they were shown to occur in secondary combustion at temperatures above 600 °C and the yield increases with increasing temperatures [101]. This observation

further validates the evidence of strong fires, which produced high amounts of char during both a flaming combustion and a prolonged smoldering one.

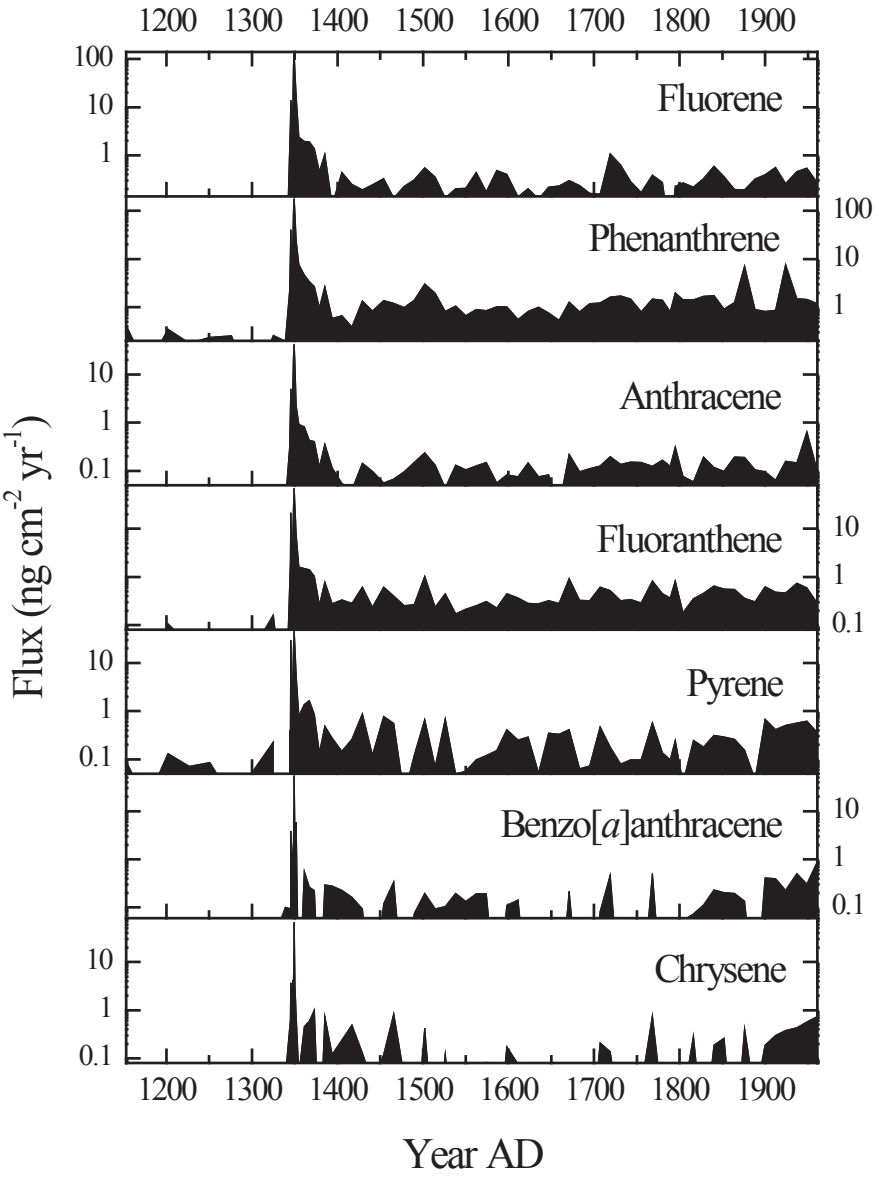

Figure 3.3 Fluxes (ng cm^{-2} yr^{-1}, log_{10} scale) of selected PAHs in the core from Lake Kirkpatrick.

Although PAH values provide important information on past changes, they suffer from a lack in source specificity, as already discussed in the introduction. Nevertheless, some congeners may be used as specific tracers. Retene, a mehtylated three-ring PAH, has

been proposed as tracer of gymnosperm (softwood) combustion [146, 212]. With respect to the other congeners reported in figure 3.3, retene is present in low amounts (<LOD – 2.3 ng cm^{-2} yr^{-1}) but follows the same trend, peaking at about 110 cm depth (fig. 3.4), corresponding to c. AD 1350. The small amounts detected could be representative of the conifers (Podocarpaceae) that were co-dominant together with the widespread *Nothofagus* (hardwood) at the time of the Māori settlement [122, 213]. This further confirms the validity of molecular fire tracers to replicate paleoecological proxies and the possibility to use them in a multi-proxy approach.

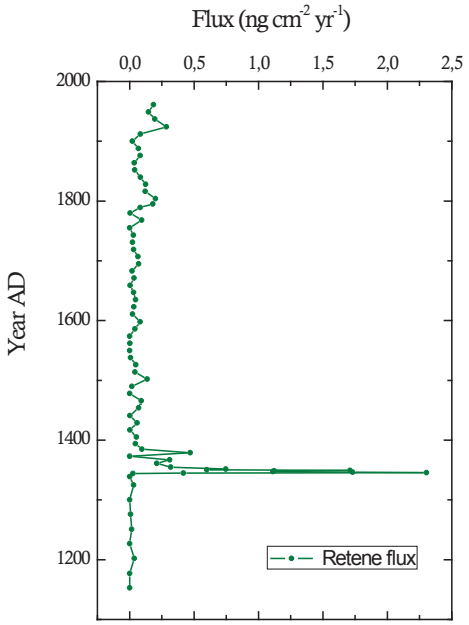

Figura 3.4 Retene flux variation with age in the core from Lake Kirkpatrick.

In order to further test the possible use of PAHs in a source apportionment analysis, some diagnostic ratios (DR) were calculated according to references [105, 214]. However, a word of caution is in order here: because DR are usually applied to aerosols or surface sediments. In the case of sediment cores the time variable plays an important role and transport, deposition and degradation processes may alter relative amounts of the considered PAHs. The application of DR in the present work is therefore only indicative and illustrative of a possible further use of PAH data. Furthermore, it must be

underlined that literature data provide highly variable and often contradictory interpretations of DR threshold values [97, 214, 215]. The ratio between fluoranthene and fluoranthene + pyrene was compared with the ratio of benzo[*a*]anthracene (BaA) and benzo[*a*]anthracene + chrysene (Chr), according to Yunker et al. (2002) who applied these ratios to river sediments in order to distinguish pyrogenic from petrogenic sources [105]. The resulting scatter plot is shown in fig. 3.5, where each quarter of the plot should be indicative of a different process. Such an approach does not appear very informative, and samples do not show much correlation, although they are mainly located in the grass/wood/coal combustion and the mixed source portion of the graph. This indication could be an effect of atmospheric partitioning or post depositional degradation. The ratio BaA/(BaA+Chr) therefore appears unsuitable to the application on lacustrine sedimentary archives, at least in this case, while the ratio Fla/(Fla+Pyr), when compared to the ratio Ant/(Ant+Phe), gives better results: samples show good correlation and are grouped in the area corresponding to wood/coal combustion (fig. 3.6). Finally, the ratios Flu/Pyr>1 and Phe/Ant<10, applied on a sedimentary record in China, are proposed as DR for the pyrogenic origin of PAHs [216]. Results for these ratios are shown in figure 3.7. Samples from Lake Kirkpatrick are mainly present in the pyrogenic zone. Although the use of DR has no other aim than to provide a general indication of sources, and despite the scarcity of applications on complex matrices, the DR proposed here appear to correctly identify the combustion origin of the PAH tracers.

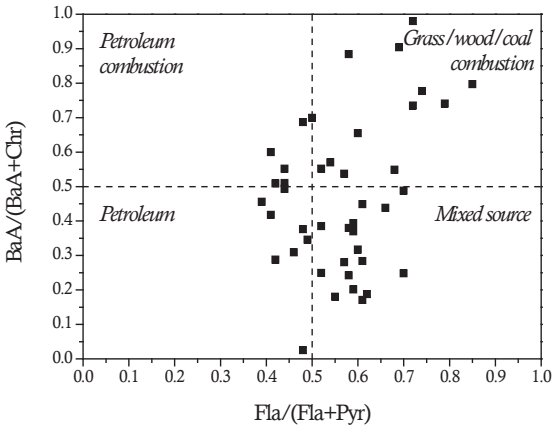

Figure 3.5 Fla/(Fla+Pyr) and BaA/(BaA+Chr) ratios for Lake Kirkpatrick.

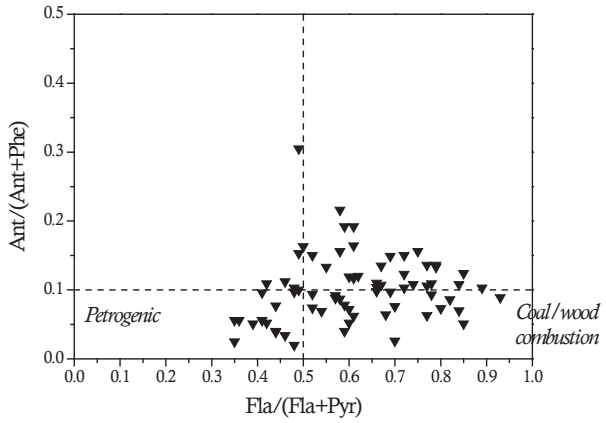

Figure 3.6 Fla/(Fla+Pyr) and Ant/(Ant+Phe) ratios for Lake Kirkpatrick.

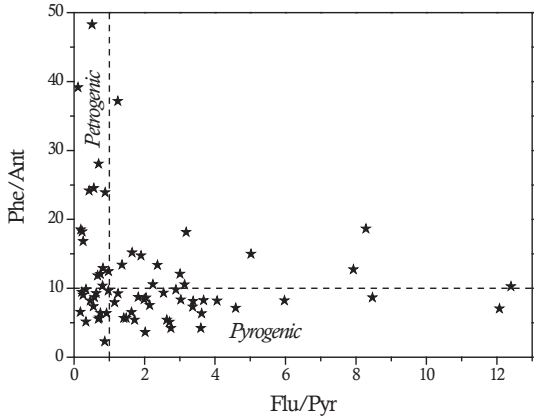

Figure 3.7 Flu/Pyr and Phe/Ant ratios for Lake Kirkpatrick.

Monosaccharide Anhydrides (MAs)

The scarce source-specificity of PAHs is compensated for by the analysis of further and more specific fire tracers. The source-specific nature of MAs was discussed in the introduction. Levoglucosan, mannosan and galactosan give unambiguous indications of biomass burning, in particular cellulose and hemicelluloses, above 300 °C [80]. The results for Lake Kirkpatrick replicate the ones obtained and discussed for PAHs. Figure 3.8 shows the temporal evolution of the MA fluxes (ng cm^{-2} yr^{-1}) in the sediment core. Also in this case, the record appears to be divided in three distinct periods. The first period, prior to the 14^{th} century, shows no significant fire activity, while a dramatic increase is registered in the short period starting around 1345, with a peak at c. AD 1349

and lasting until c. AD 1355. The peak fluxes correspond to 390, 278 and 66 ng cm^{-2} yr^{-1} for levoglucosan, mannosan and galactosan respectively. After the conclusion of the intense burning period, fire activity appears to adopt a new regime, characterized by frequent and small fires. Fluxes after AD 1360 almost never exceed 20 ng cm^{-2} yr^{-1}. The possible explanation follows the one proposed for PAHs and is in agreement with pollen and charcoal evidence: the huge fires following the settlement of the Māori resulted in a great forest loss, followed by the establishment of new vegetation, better adapted to fire [122].

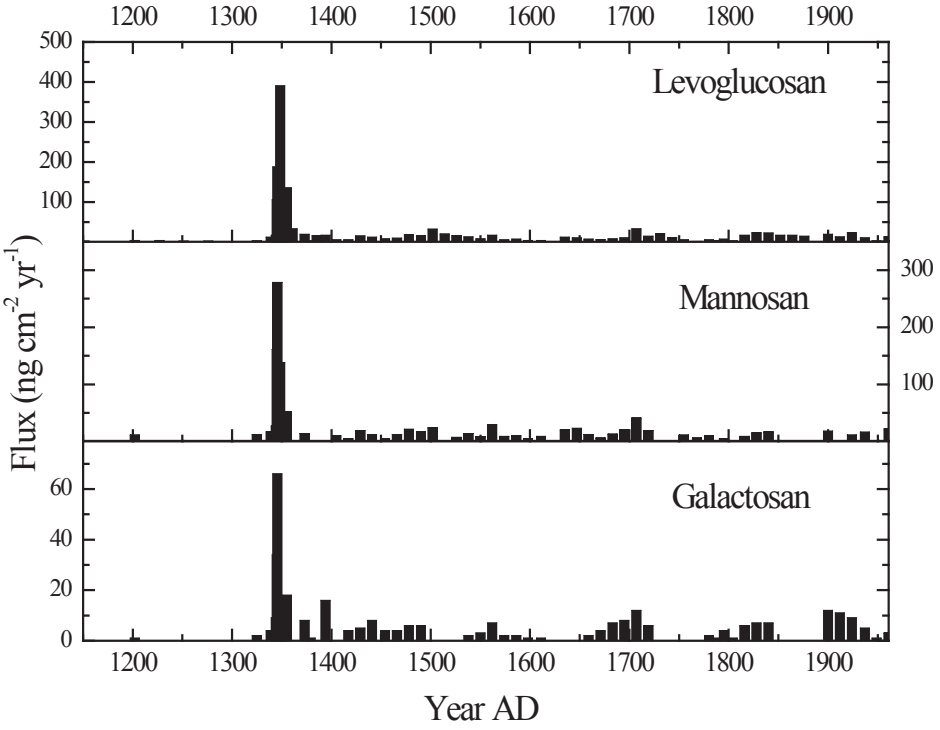

Figure3.8 Fluxes (ng cm^{-2} yr^{-1}) of selected MAs in the core from Lake Kirkpatrick.

The analysis of levoglucosan isomers can also help in the investigation of sources and burning conditions. The ratios between levoglucosan and mannosan (L/M) or between levoglucosan and the sum of mannosan and galactosan (L/M+G) have been proposed as diagnostic for distinguishing hardwood from softwood combustion, due to the different monosaccharide content of hemicelluloses [83, 84, 217]. Both ratios are reported to be smaller for softwood than for hardwood, but their validity was questioned in light of the observation that temperature and duration of the burning event affect the yields of mannosan and galactosan in chars [84]. Since the amount of levoglucosan produced does

not vary significantly, ratios should increase with higher temperature and longer lasting fires. From the comparison of L/M and L/(M+G) ratios, shown in figure 3.9, four such events can be highlighted, at about AD 1350, 1530, 1810 and 1950. The last three events are not clearly visible in the PAH and MA records, but could refer to single wood burning events of smaller size than the Māori forest clearance around AD 1350.

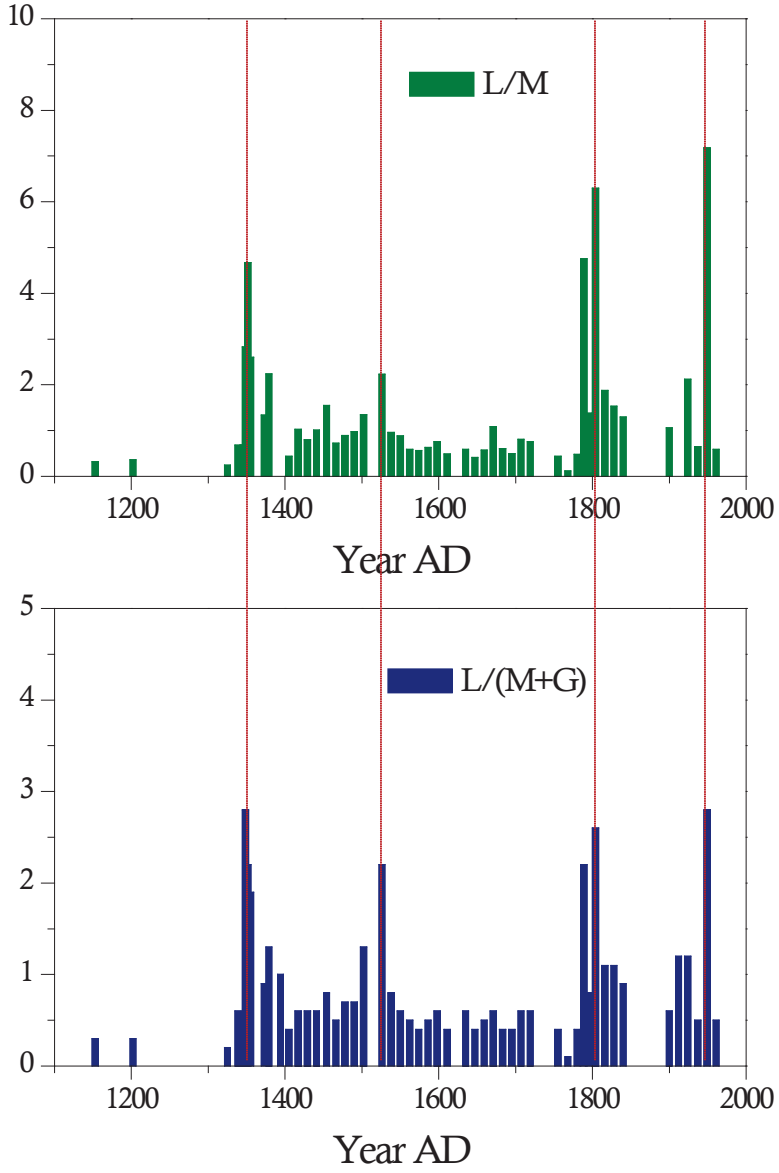

Figure 3.9 L/M and L/(M+G) ratios in the core from Lake Kirkpatrick.

The last two peaks in the L/M and L/(M+G) ratios coincide with the arrival of Europeans in the 19th century and modern times. The high values of the two ratios, especially in correspondence with the more recent events, could be related to the direct use of hardwood, more diffused than softwood, as fuel, rather than as forest clearing. It must be noted, however, that in this case also the published DR refer mainly to atmospheric particulate or to smoke produced in experimental conditions: results for sediments may therefore be affected by diagenesis and *in situ* degradation and must be evaluated carefully.

Fecal and plant sterols (FeSt)

Once the fire molecular tracers were tested as reliable proxies for the reconstruction of biomass burning, this work aimed to compare them with direct tracers of human presence. Results for fecal sterols in Lake Kirkpatrick confirm the importance of human influence after the colonization of the South Island. Coprostanol (Cop), epicoprostanol (e-Cop), cholestanol (5α-Chl), β-sitosterol (Sit) and stigmastanol (5α-Sit) were detected in the majority of samples. Cop, e-Cop,5α-Chl and 5α-Sit were never below the detection limit, while Sit showed higher variability. Despite its ubiquitous nature [112], cholesterol (Chl) was above the detection limit only in a few samples as a result of blank correction and will not be included in the discussion.

The general trend of FeSt replicates the PAH and MA trends, peaking at c. AD 1350 with high fluxes of Cop (70 ng cm^{-2} yr^{-1}), e-Cop (20 ng cm^{-2} yr^{-1}),5α-Chl (543 ng cm^{-2} yr^{-1}) and 5α-Sit (5334 ng cm^{-2} yr^{-1}). Prior to the arrival of humans, the total average flux was below 200 ng cm^{-2} yr^{-1}, which can be considered a background value due mainly to stigmastanol of plant origin or animal contribution. After the settlement of the Māori and the huge peak registered in the 14th century, values decrease again but maintain higher levels than before, about twice as much, until the beginning of the 18th century. This unexpected drop seems to support the idea of a partial abandonment of the area after the initial land clearance, possibly caused by the too intensive exploitation of local flora and fauna. With the arrival of the Europeans, however, the fluxes of all the considered congeners started increasing dramatically, as visible in figure 3.10.

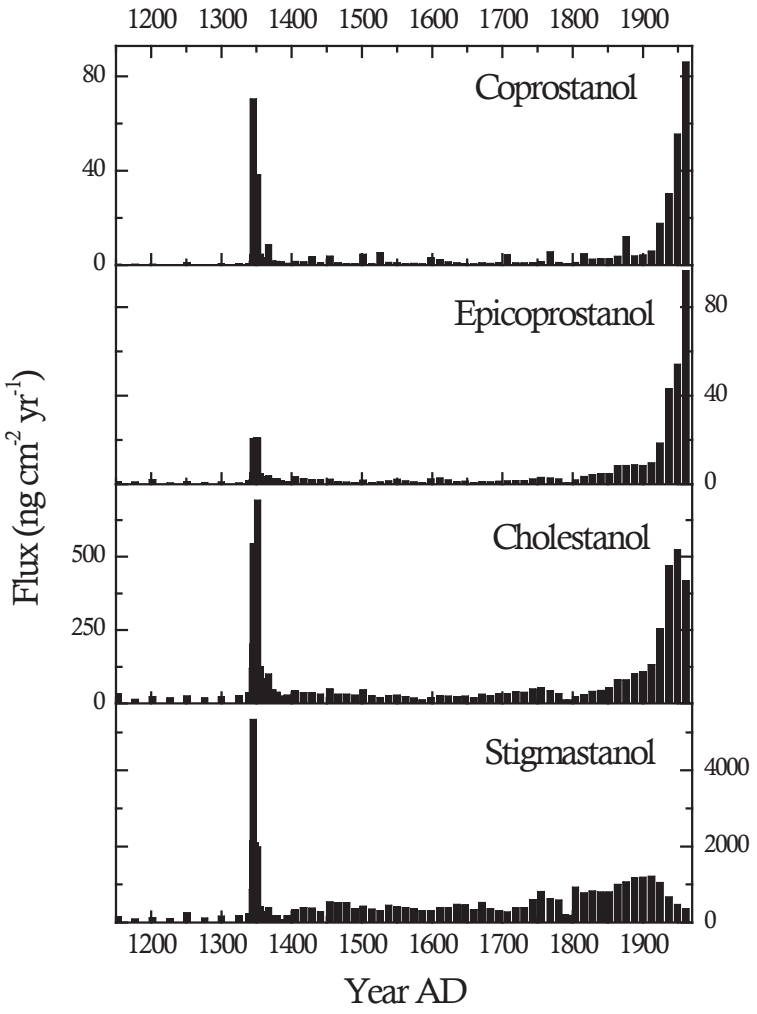

Figure 3.10 Fluxes (ng cm^{-2} yr^{-1}) of selected FeSt in the core from Lake Kirkpatrick.

The huge increase in FeSt in correspondence with the so-called "initial burning period" (IBP) probably originated from two different and strongly interconnected sources. The unequivocal presence of humans in the lake catchment area is demonstrated by the increase in the species more strictly related to mammalian feces, namely coprostanol, epicoprostanol and cholestanol [112]. The increase in stigmastanol, the most abundant compound with fluxes about one order of magnitude greater than the others, reflects the input of plant material to the lake, which must have been massive during and after the IBP as a consequence of direct transport and runoff.

β-Sitosterol, often considered an indicator of plant material too, but present also in the feces of some animal species [119], has a different trend, shown in figure 3.11. Sit peaks neither in correspondence to the IBP, nor shortly after as one may expect. Many samples resulted below the detection limit and only small, quite negligible peaks are observed before the European period. Fluxes started increasing sharply right after the beginning of the 19th century, probably as a result of intense land use by the Europeans. The difference between the records of stigmastanol and β-sitosterol could be interpreted as a difference in the represented spatial scale and transport processes, as β-sitosterol is a natural product observed in the smoke from grasses, conifers and deciduous trees [75–77]. A simpler interpretation could refer to the post-depositional degradation: as discussed in the introduction, it is highly likely for β-sitosterol to be bacterially reduced in sediments, and therefore to be converted to stigmastanol [117]. This could explain why significant amounts are detected only in the recent part of the core and reflect the increase in population and consequent land exploitation.

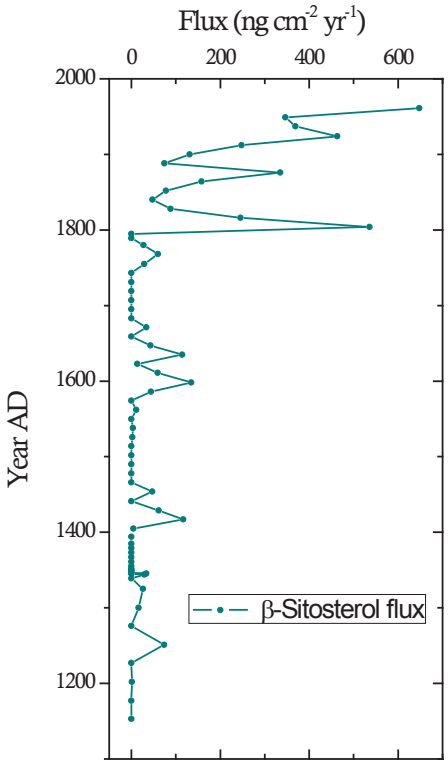

Figura 3.11 β-Sitosterol variation with time in the core from Lake Kirkpatrick.

The 19th century increase is easily explained if the record of Cop+e-Cop, the main tracers of human feces, is compared to the increase in the New Zealand population, as shown in figure 3.12. According to statistics, the population of the South Island is about 30-35% of the total New Zealand population and this proportion was preserved in the whole period considered [218]. Unfortunately, no census is available before 1850, and it is therefore impossible to compare these data with the population levels before the European occupation and with the peak at AD 1350.

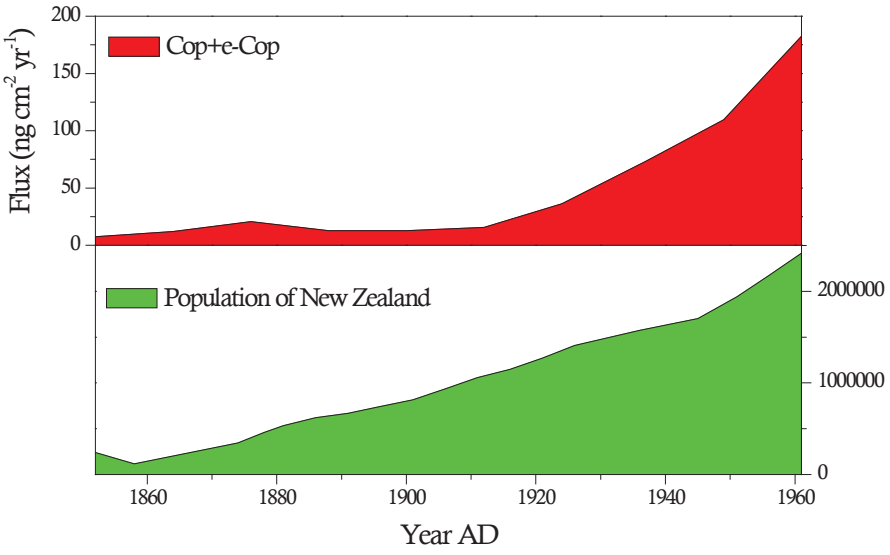

Figure 3.12 Flux of Cop+e-Cop in Lake Kirkpatrick and population increase in New Zealand in the 1852-1961 period [218].

Figure 3.13 shows the correlation between an estimate of the population growth since 1852 in the Queenstown district, where Lake Kirkpatrick is located, and the measured fluxes of Cop+e-Cop in the corresponding period. Obviously, this approach is applied here for the first time and should be considered as a further exploration into the potentialities of fecal tracers. However, an interesting logarithmic correlation was found with a r^2 value of 0.885. Although only about 200 years are considered here, the application of this kind of analysis to longer time series suggests the possibility of employing fecal sterols also for estimating the size of past populations in a lake's drainage basin.

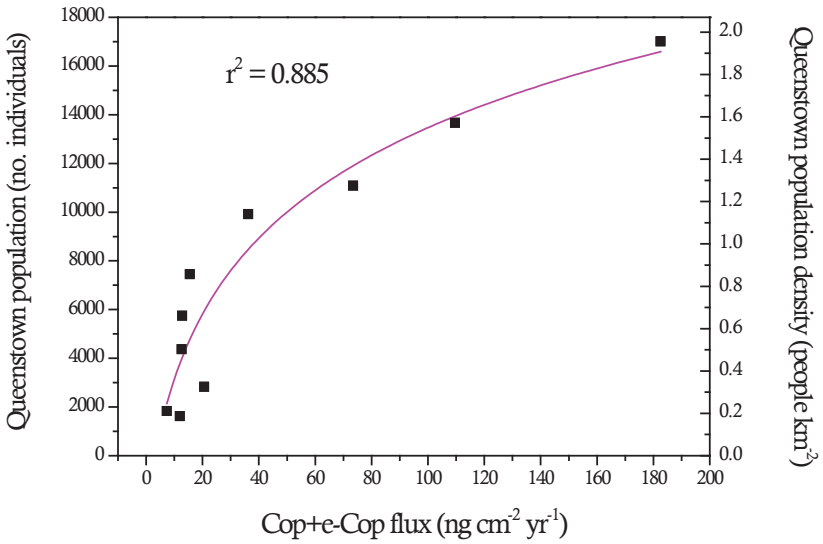

Figure 3.13 Logarithmic correlation between Cop+e-Cop values in Lake Kirkpatrick and the Queenstown population growth since 1852 [218].

Multi-proxy interpretation

From the observation of the age-depth model, it is evident that an abrupt change in the sedimentation rate occurred in the 108-118 cm interval, corresponding to the AD 1345-1355 period. The interpretation proposed here is that the high deforestation rate, inferred from paleoecological proxies and from the molecular tracers proposed in this work, resulted in an increased input of terrigenous and plant material to the lake. The increase observed in molecular markers coincides with this anomalous sediment input, thus corroborating the evidence of an unprecedented burning period.

Figure 3.14 displays the comparison between the total fluxes of the molecular markers measured in Lake Kirkpatrick in the course of this work and the charcoal flux measured by McWethy et al. (*personal communication*).

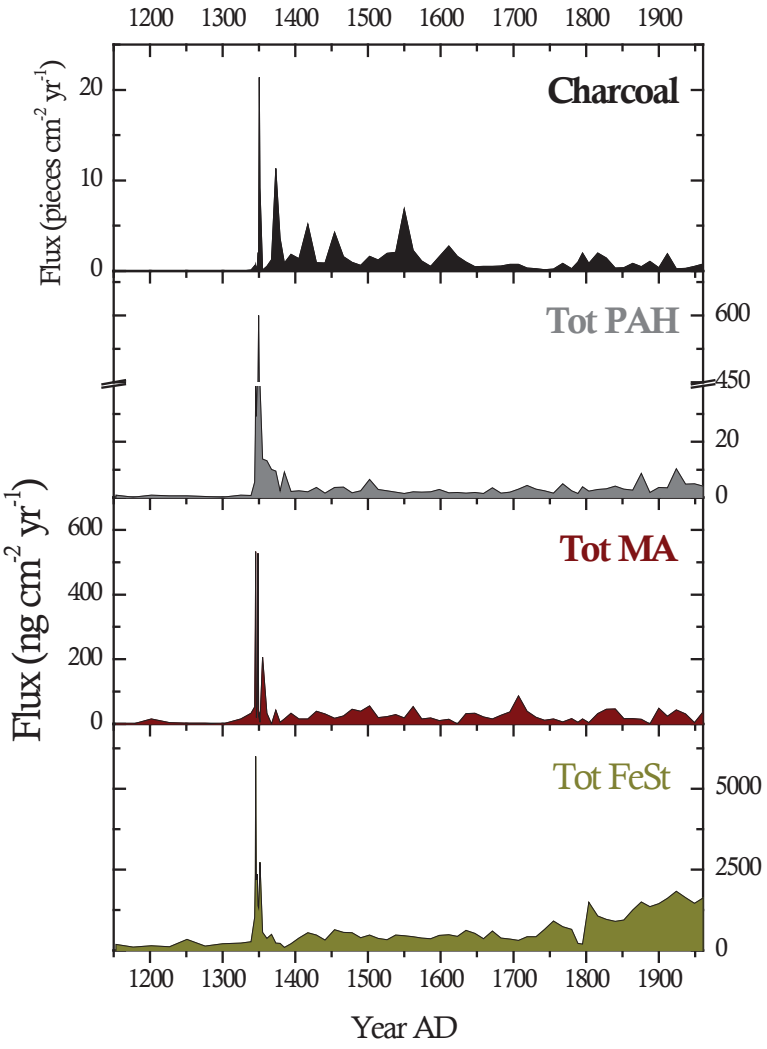

Figure 3.14 Total fluxes of charcoal (pieces cm^{-2} yr^{-1}), PAHs, MAs and FeSt (ng cm^{-2} yr^{-1}) in the core from Lake Kirkpatrick.

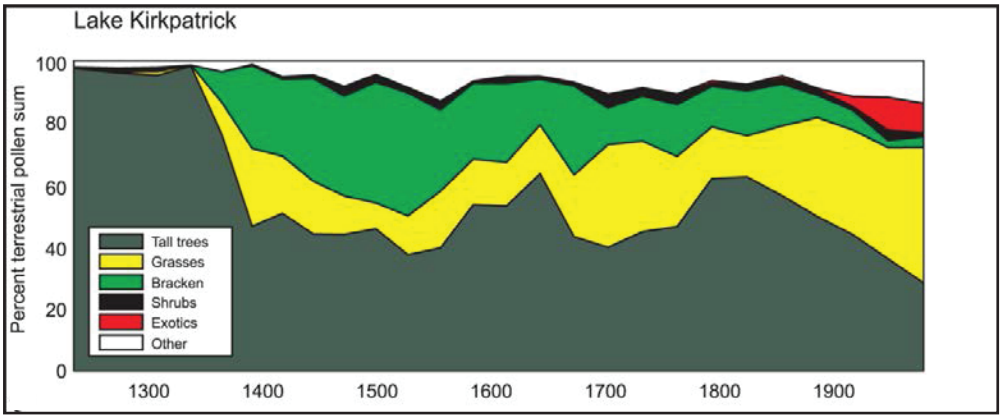

Figure 3.15 Pollen diagram for Lake Kirkpatrick. From McWethy et al. (2014) [122].

The correspondence among all the measured proxies in the 1345-1355 period is evident, marking the onset of a new fire regime after the arrival of first colonizers. The charcoal record, though, contains other peaks following that of 1350, indicating increased fire activity and quite intense fire events occurring every 50-100 years until c. AD 1600 [122]. The latter fire episodes do not result in clear peaks in the molecular markers records, even though all tracers register increased background values that point to increased fire activity and anthropogenic land use. However, two main factors must be considered in this analysis. First, the charcoal data refer to the >125 μm fraction, intentionally selected to trace fire events on a local scale, while fire molecular markers are transported by the atmosphere in association to the fine particulate matter. Therefore, molecular markers can cover longer distances and their signal may reflect a more regional scale, thus missing smaller local events. Indeed, it is widely documented that New Zealand, and in particular the Southern Alps, are interested by atmospheric depositions originating from southeastern Australia [219–222]. No data about organic fire tracers in these depositions are available, but it is reasonable to attribute part of the background levels measured to long-range transport, although the observed peaks are undoubtedly ascribable to local processes, as further confirmed by the anthropic signal. Second, as mentioned above, charcoal is reported to undergo secondary deposition after the fire, as an effect of runoff and erosion [71]: this phenomenon, which does not affect molecular markers [103], could explain part of the observed trend.

However, if the charcoal record is compared to the L/M and L/(M+G) ratios shown in figure 3.9, a correspondence can be observed between charcoal peaks and increased levoglucosan yields around AD 1550 and in the 19th century. As previously noted, this evidence could point to wood burning for use as fuel or in clearing operations, which would result in high production both of charcoal and levoglucosan.

The pollen diagram by McWethy et al. (2014), shown in fig. 3.15, confirms the important change in vegetation composition that occurred after c. AD 1350. An abrupt decline in tree species is observed, as testified by the decrease from 99% to 47% of the total terrestrial pollen from AD 1331 to 1391. The forest was converted to shrubland and the new fire regime with small but quite frequent fire events contributed to maintain the new composition of the vegetation [122]. Molecular markers records and in particular the plant input discussed above are consistent with this view.

Since the beginning of the 19th century, New Zealand was subject to a new colonization and rapid population growth whose effects are clearly reflected in the marked increase in FeSt starting in ~AD 1800. However, the expected significant increase in fire frequency or intensity could not be inferred from fire tracers. This apparent inconsistency only highlights the fitness of tracers for the reconstruction of local scale processes: the human presence in Lake Kirkpatrick's watershed is recorded in sediments, but this does not necessarily result in locally increased fire activity. As for the long-range transport mentioned above, anthropogenic fires in the European period may have occurred quite far from the lake, thus contributing only to background variations but not resulting in neat peaks. This pattern thus differs to what observed around AD 1350, when unequivocally local events disrupted the former fire regime.

The conclusion that can be drawn is that the multi-proxy approach applied here, coupling well established paleoecological proxies to somewhat new molecular markers, provided new insight into the processes that so deeply transformed a previously untouched environment. On the one hand, the new proxies proposed here showed the robustness of the charcoal indicator by confirming the observed shift and its anthropogenic nature. On the other hand, novel methods and instruments highlighted the possibility to investigate in more detail population dynamics and land use, suggesting new direct tracers and possible analytical approaches to be further researched in the future.

Moreover, the choice of New Zealand as a target location for testing the multi-proxy method elaborated in this thesis was advantageous due to the wealth of available data and the possibility of a detailed discussion and comparison. Such an approach further confirmed the validity of applying molecular markers to sedimentary archives and their utility in the understanding of fire events and human influence.

3.1.2. Lake Diamond

The other New Zealand site selected for this work was Lake Diamond, where only sterols were analyzed with method #1, in collaboration with the Paleoecology research group of MSU, who provided the samples. The 160 cm sediment core was retrieved in 2009, and subsampled at 1 cm intervals. The 49 samples analyzed in this work covered the time interval between 5 and 2134 cal years BP (5-147 cm). The sample amount was sufficient for the analysis of FeSt, so there was no need to merge samples in this case. Chronology and calibration procedures are described in reference [223]. The resulting age model is shown in figure 3.16. The sedimentation rate is quite low and linear throughout the record, showing only a slight increase (from an average rate of 0.06 cm yr^{-1} to about 0.2 cm yr^{-1}) between c. 370 and 270 cal yr BP.

Samples had high water content, ranging from 75 to 91% (89% on average). Dry weight was 0.45-4.39 g, averaging 1.74 g. The concentration of FeSt was measured (ng g^{-1}) and results are here presented as fluxes (ng cm^{-2} yr^{-1}) obtained using the accumulation rate (cm yr^{-1}) and dry density values (g cm^{-3}) as described for Lake Kirkpatrick.

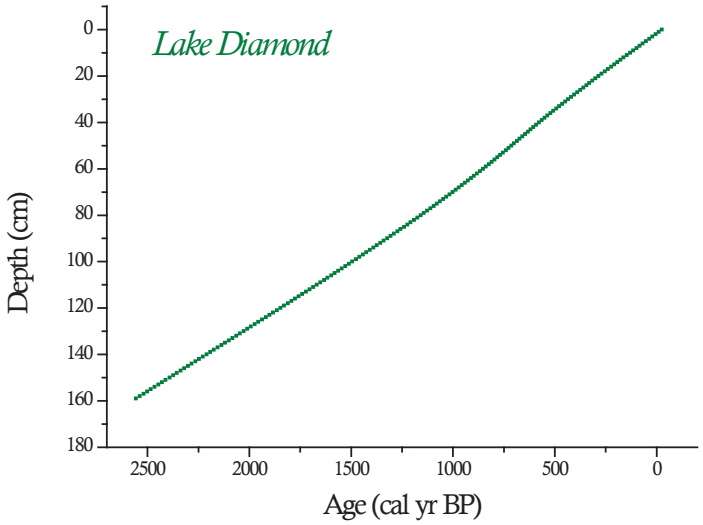

Figure 3.16 Age-depth model for Lake Diamond. *After McWethy et al. (2010)* [121].

Results for single sterols, focusing on the last 1000 years, are shown in figure 3.17. A distinct peak is observed, culminating at about AD 1340, in accordance with the data from Lake Kirkpatrick discussed above. This can undoubtedly be ascribed to the intense human influence on land following the arrival of the Māori. However, this record, where the most recent sample dates back to ~1945, does not show any increase in FeSt fluxes following the arrival of Europeans and the progressive increase in population. Unlike Lake Kirkpatrick, Lake Diamond seems to have experienced the most intense anthropogenic modifications of the last millennium in the 14th century.

The analysis of single congeners evidences some interesting details. As for Lake Kirkpatrick, stigmastanol has the highest flux throughout the record, with a 659 ng cm^{-2} yr^{-1}peak at c. AD 1338. Here, though, cholesterol and β-sitosterol are above the detection limit in all samples, and follow the very same trend as all other congeners, peaking right after the arrival of the Māori (Cop: 9 ng cm^{-2} yr^{-1}; e-Cop: 15 ng cm^{-2} yr^{-1}; 5α-Chl: 110 ng cm^{-2} yr^{-1}; Chl: 107 ng cm^{-2} yr^{-1}; Sit: 320 ng cm^{-2} yr^{-1}) and progressively decreasing afterwards, stabilizing again on background levels until the present. Markers of human presence here have similar trends to the vegetal and terrigenous input markers. Unexpectedly, the e-Cop values are two to three times higher than the Cop fluxes, displaying a reversed situation with respect to Lake Kirkpatrick. The two latter observations can lead to the conclusion that the biochemical conditions in the two lakes are somewhat different due to the different dimensions and input of organic matter. It is likely, therefore, that the conformation of Lake Diamond favors the oxygenation of the basin, and thus bacteria capable of converting Cop to e-Cop, while preventing the reduction of Sit, which is conserved in the record.

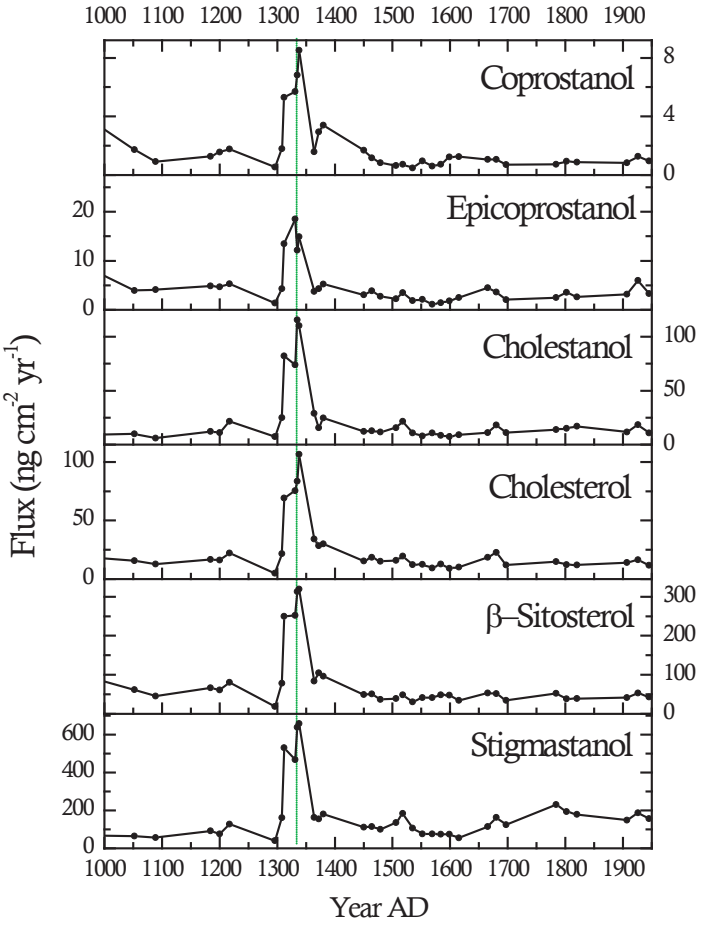

Figure 3.17 Fluxes (ng cm^{-2} yr^{-1}) of single FeSt in the core from Lake Diamond.

Figure 3.18 compares the charcoal record of Lake Diamond by McWethy et al. (*personal communication*) with the total FeSt flux measured in this work. As for the concentrations of FeSt, the charcoal flux is lower than for Lake Kirkpatrick, but shows the same increase, coincident with the IBP, starting at c. AD 1330 [176]. The peak coincides with the increase in the flux of all measured FeSt, culminating at c. AD 1338. During the following period, a second charcoal peak is observed at about AD 1420, with no correspondence in the FeSt record. The missing peak is most probably due to some differences between the sample subsets: for technical reasons, not all the samples analyzed for charcoal were analyzed for FeSt. In particular, the charcoal count was performed for each 1 cm portion, while no samples were available for FeSt analysis in the depth interval between 36 and 41 cm (AD 1380-1450), resulting in fewer data points.

Nevertheless, the comparison between the two records shows a correspondence between molecular markers of human presence and charcoal records for Lake Diamond as in the case of Lake Kirkpatrick. This further demonstrates the validity of the organic chemical approach to paleorecords.

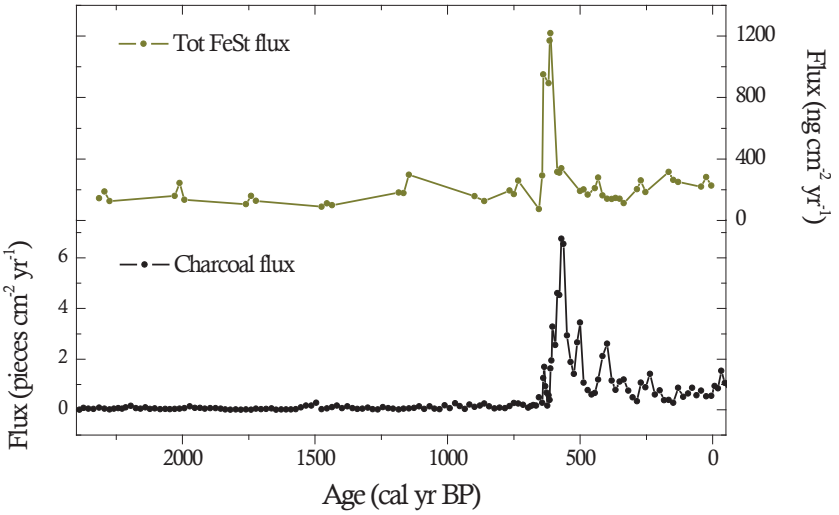

Figure 1.18 Total FeSt flux (ng cm^{-2} yr^{-1}) and charcoal flux (pieces cm^{-2} yr^{-1}) in the core from Lake Diamond.

Moreover, increased fire activity and a consequent shift in the vegetation composition in the same period can also be inferred from the pollen record, which is not reported here [176]. Much of the native species were replaced by grasses, bracken and shrubs, as a result of intense deforestation activities, as can also be inferred also from the increased input of plant sterols measured in this work.

3.1.3. Kirkpatrick VS Diamond

Figure 3.19 shows the FeSt and charcoal records measured for Lake Diamond and Lake Kirkpatrick. If plotted on the same time scale as in the figure, the differences between the two lakes are more evident. First of all, at Lake Kirkpatrick the two main peaks at c. AD 1350 are synchronous, as if the processes of settlement and land clearance were closer in

time than at Lake Diamond. Here the peak in FeSt corresponds to the beginning of fire activity, that appears more prolonged in time, suggesting slightly different dynamics in the occupation of the area by the Māori. The observed difference, however, is probably also ascribable to the spatial scale and the sedimentation factors discussed below.

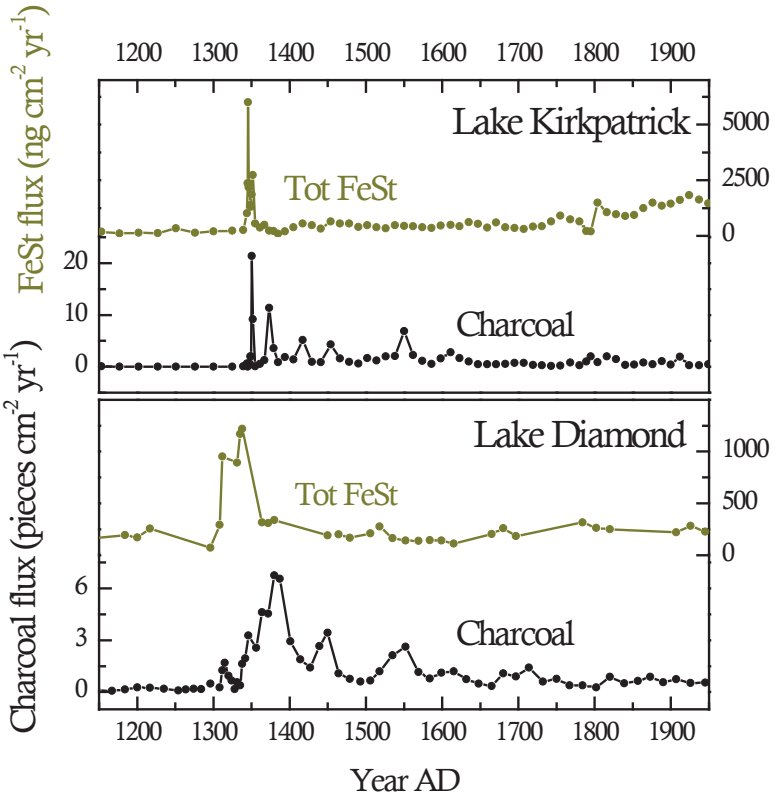

Figure 3.19 Comparison between charcoal and FeSt fluxes in Lake Kirkpatrick and Lake Diamond.

It must be noted that the sedimentation rate in Lake Diamond is on average four times lower than in Lake Kirkpatrick: the Lake Diamond core, indeed, covers a much longer time span than the Lake Kirkpatrick core. This results in a significant resolution difference between the two records: on average, one sample from Lake Diamond represents 16 years, while each sample from Lake Kirkpatrick is representative of about 6 years. A difference in the catchment and in the sedimentation dynamics between the two lakes, despite the similarities in the environmental setting, could explain the observed

discrepancies between the two records. Moreover, the fluxes measured for Lake Diamond are generally lower than for Lake Kirkpatrick, and so is the charcoal flux. A possible interpretation is related to the dimensions of the two lakes: Lake Diamond is somewhat larger than Lake Kirkpatrick (about 40 times in area), and this may result in a dilution effect on both molecular markers and charcoal particles. Therefore, the high concentrations registered for Lake Kirkpatrick could partly be a consequence of its limited extension.

As mentioned above, the different dimensions of Lake Diamond and Lake Kirkpatrick could also result in different mixing and therefore in different oxygen content in the two lakes. The almost absolute absence of Chl and the scarcity of Sit in favor of 5α-Sit and 5α-Chl in the sediments of Lake Kirkpatrick highlights reducing conditions that also have the effect of scarcely promoting the bacterial epimerization of Cop. On the other hand, sediments from Lake Diamond have conserved significant amounts of Chl and Sit, and the Cop to e-Cop conversion appears to have been efficient along the whole record, suggesting a better oxygenation of the basin. These observations suggest that such biomarkers could have the secondary use proposed by Vane et al. (2010) [115], also for inferring the chemical conditions of sediments in the case of paleorecords.

Another factor that justifies the difference in the most recent part of the records is related to the modern anthropic pressure on the two sites. Lake Diamond is located far from any urban area, apart from two small settlements (Glenorchy and Kinloch) on the northern shore of Lake Wakatipu, accounting for a few hundred inhabitants. As mentioned above, Lake Kirkpatrick is close to Queenstown and the minor cities in its district, which counts about 32,400 inhabitants [218]. The observed increase in Lake Kirkpatrick fecal sterols starting in the 19th century, not revealed for Lake Diamond, may thus reflect the development of this inhabited area since the European colonization.

3.2. Tasmania

3.2.1. Flinders Island

Method #1 for the analysis of sterols was applied on a small exploratory subset of samples from Middle Patriarch Lagoon (MPL), provided by the MSU Paleoecology research group. 15 samples were picked up from a 177 cm core, retrieved in 2012 and subsequently divided into 1 cm thickness portions. The accumulation rate is very low, ranging from 0.004 to 0.054 cm yr^{-1}, thus the core covers a very long time span, from ~-5 to ~11,800 cal years BP. Three changes in the sedimentation rate are observed along the core, respectively at ~10,400, ~7800 and ~2800 cal years BP. The number of years represented by one sample therefore varies between 18 and 225. The preliminary age-depth model for MPL (McWethy et al., *unpublished data*) is shown in figure 3.20.

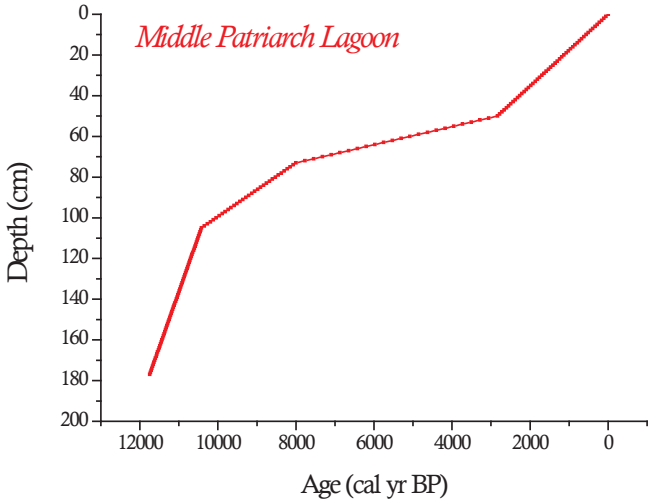

Figure 3.20 Preliminary age-depth model for MPL. *From McWethy et al. (unpublished data).*

Figure 3.21 displays the fluxes of the single sterols analyzed. The trends detected here must be interpreted only as general indication of past processes, due to the extension of the time interval and the poor resolution of the subset. Archaeological surveys on Flinders Island found no indication of stable human settlement after 4500-4000 years ago, as discussed above [185]. Charcoal and pollen data agree with this view, showing

increased fire activity during the Early Holocene, supposedly driven by climate fluctuations, and a neat decrease in frequency and intensity of fires since 6000-7000 yr BP (McWethy et al., *unpublished data*).

In general, no significant FeSt fluxes were measured in the Early and Middle Holocene samples. Coprostanol and epicoprostanol fluxes, however, were never below the detection limit. Relatively high values of epicoprostanol were found at 12,000-10,000 cal yr BP. These values have no correspondence in the coprostanol record. Due to the antiquity of samples, it is likely that Cop was fully converted to e-Cop. This evidence suggests that people lived close to MPL in the Early Holocene. Significant charcoal peaks are observed in the same time period, showing a correspondence with human presence. Since the number of samples analyzed for FeSt was small, however, data must be interpreted cautiously: confirming the cause-effect relationships between the two data series will require higher-resolution analyses.

Chl, 5α-Chl, Sit and 5α-Sit all show a similar trend, with values close to zero in the Early and Middle Holocene and a progressive increase starting about 2500 cal yr BP, approximately when charcoal recorded a second regime shift, this time towards small but frequent fires (McWethy et al., *unpublished data*). A small increase is observed also in Cop and e-Cop with the same timing. A possible explanation could reside in the Aboriginal episodic visits to abandoned islands. Archaeologists hypothesized that such visits likely occurred after the introduction of watercrafts around 3000-2500 years ago [185].

All sterols show the highest fluxes in the latest part of the record, coinciding with the 19th century European colonization and relative land exploitation, also confirmed by the increase in biomass burning revealed by charcoal fluxes (fig. 3.22). However, levels of Cop and e-Cop increase only slightly with respect to the other congeners, with fluxes of 3 and 8 ng cm^{-2} yr^{-1} at ~165 cal years BP. Historical records report that the Furneaux Group was frequented by sealers since the late 18th century, who intensively exploited seals for food and skin trading, leading to a quick decline in seal population. Many sealers then chose to settle in the Furneaux, dedicating themselves to farming and muttonbirding [224–226]. Measured fluxes of Cop and e-Cop for this period are consistent with intermittent human presence or small population.

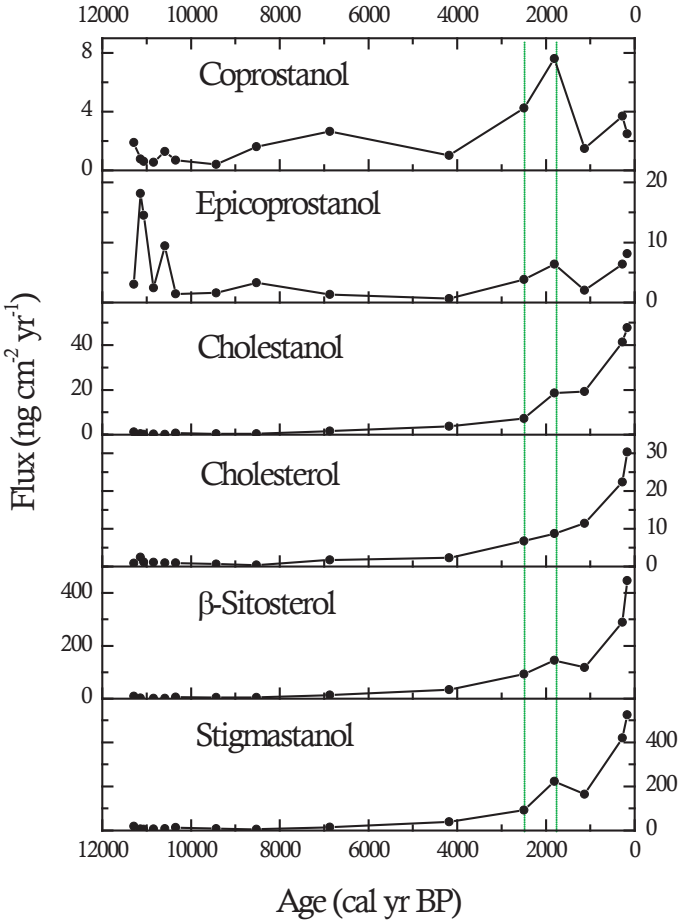

Figure 3.21 Fluxes (ng cm^{-2} yr^{-1}) for single FeSt in the core from MPL. Green lines indicate the beginning and the peak of the increasing trend.

Higher levels of Chl and 5α-Chl, peaking respectively at 30 and 48 ng cm^{-2} yr^{-1}, could be explained by the simultaneous presence of people and cattle since the introduction of farming in the 19th century. Sit and 5α-Sithave high fluxes in the last 2000 cal years BP, with peaks of 447 and 525 ng cm^{-2} yr^{-1} at ~165 cal years BP. These values agree with the Late Holocene increase in fire activity (McWethy et al., *unpublished data*), and in particular with increased biomass burning and consequent input of plant material. Fluctuations in terrigenous input along the whole record could be a result of the alternation of dry and wet conditions, reflected also in the observed shifts in the sedimentation rate. Unfortunately, no samples representing the modern period were available for tracking further increase of fecal sterols with population growth. As mentioned above, future investigations regarding molecular markers in the Bass Strait

islands should involve a higher resolution set and include fire tracers in order to obtain a detailed picture of human and natural changes.

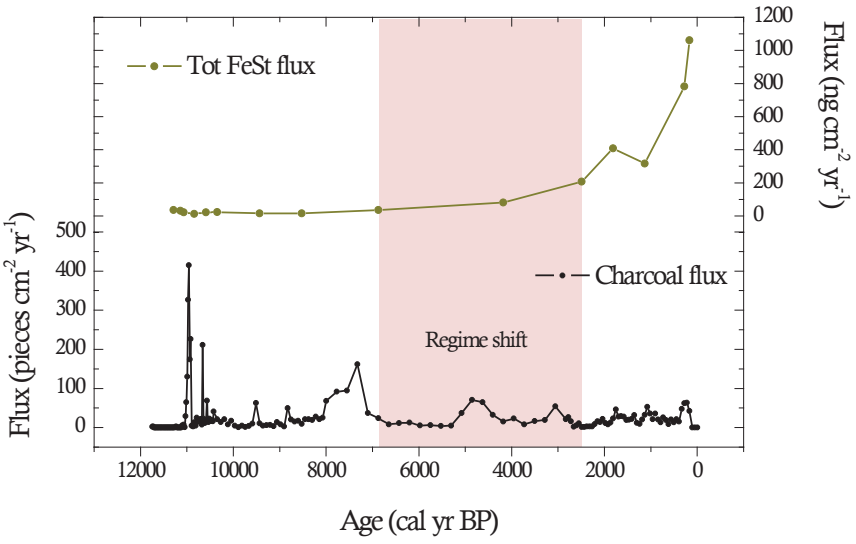

Figure 3.22 Total FeSt flux (ng cm^{-2} yr^{-1}) and charcoal flux (pieces cm^{-2} yr^{-1}, McWethy et al., unpublished data) in the core from MPL. The pink area corresponds to the period of observed fire regime shift according to McWethy et al. (*unpublished data*).

3.3. East Africa

3.3.1. Lake Victoria

A section of V95-1P core, retrieved in 1995 during the IDEAL (International Decade of East African Lakes) drilling campaign [227], was provided by T.C. Johnson (University of Minnesota) for the multi-proxy analysis of molecular markers. Method #3 was applied on 20 samples, previously dried and milled. The dry weight of samples was 0.075-0.453 g. The time interval covered by the 0-45 cm section was ~1257-2139 cal years BP: the upper portion of the sediment core, equivalent to about 1000 years, was not recovered due to technical issues during sampling [123]. The age-depth model for the considered samples, inferred from the chronology published by Johnson et al. (2000) [195], is shown in figure 3.23. The sedimentation rate appears constant, without any relevant change in slope. The humidity of samples, not directly provided, was reasonably assumed to be similar to that of core V95-2P, whose average water content was 80%, with a linear trend and almost constant values along the whole section [195]. These approximations were used for calculating the dry density (g cm^{-3}) and accumulation rate (cm yr^{-1}) in order to obtain fluxes (ng cm^{-2} yr^{-1}) for the target analytes.

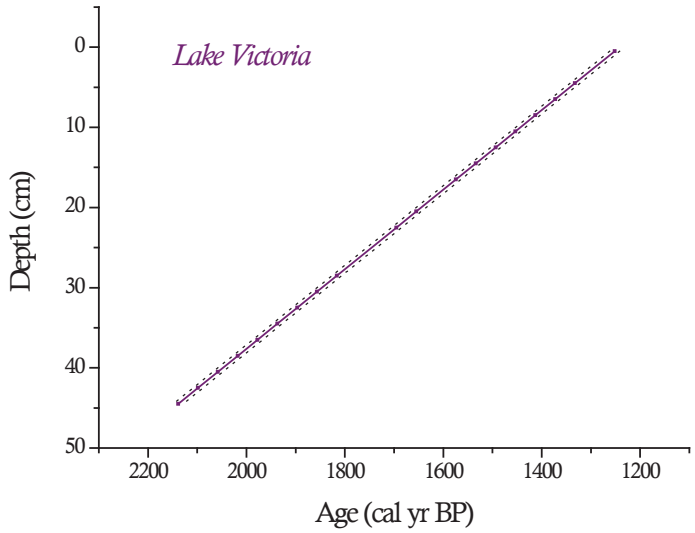

Figure 3.23 Age-depth model for Lake Victoria, derived from *Johnson et al. (2000)* [195].

Polycyclic Aromatic Hydrocarbons (PAHs)

In general, PAH fluxes measured for Lake Victoria are significantly lower than those discussed for New Zealand. Values for all congeners are around a few ng cm^{-2} yr^{-1} in the whole record. Not all PAHs were detected, and variations occur in a small range. Thus individuating trends and peaks in the considered periods is no straightforward operation. The presence of low fluxes despite the intense fire activity of East Africa is likely due to the spatial scale. The huge dimensions of the lake and the large scale atmospheric circulation involved in the climate of the region amplify the dispersion effect of tracers, both in the air and water media, affecting concentrations in sediments. Small alpine lakes, on the contrary, act as efficient collectors of runoff and atmospheric contributions, as observed for Lake Kirkpatrick and Lake Diamond. On the other hand, Lake Victoria proved an excellent archive for the reconstruction of long-term regional climate fluctuations [123, 196, 199]. To date, no fire records exist for Lake Victoria. The tracers examined here could therefore provide a better understanding of regional biomass burning dynamics, supported by the comparison with existing data for other lakes in the area.

The observation of cumulative fluxes of PAHs in the record examined here allowed us to identify periods of more intense burning and trends on a centennial scale. As immediately visible from fig. 3.24, the represented PAHs are mainly three- and four-ring congeners, in particular acenaphthene (ACE), fluorene (FLU) and phenanthrene (PHE). Maximum fluxes for these three congeners are 8, 12 and 12 ng cm^{-2} yr^{-1}, respectively, at about 1550 cal yr BP. A similar trend is revealed for all other congeners, although poorly represented. The predominance of light to medium molecular weight PAHs could be a result of atmospheric fractionation: lighter compounds, associated to the gas phase and fine particles, have the chance to travel longer distances than heavy ones [97]. However, the pattern of detected congeners fits the gas and particulate phase pattern produced by savanna fires, and is therefore consistent with biomass burning [228]. The most intense burning period appears to have occurred between c. 1650 and c. 1350 cal yr BP, with peaks at about 1550 and 1450 cal yr BP. A less intense increase in fire is centered at ~1900-1850 cal yr BP.

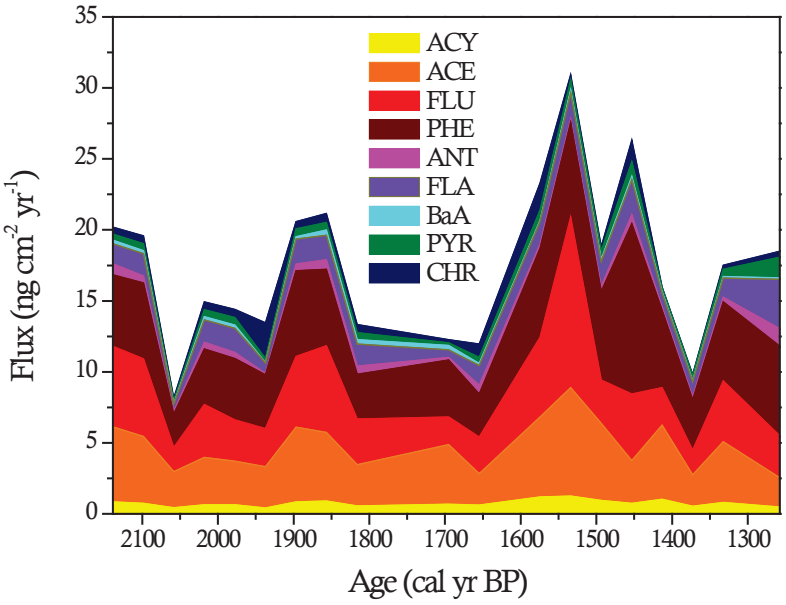

Figure 3.24 Fluxes (ng cm^{-2} yr^{-1}) of all the PAHs detected in the core from Lake Victoria.

As previously reported for LK, a particular source is attributed to retene, which is produced in large amounts by the combustion of softwood [75, 212]. In the record from Lake Victoria, the flux of retene shows a substantial increase from background values starting between ~1650 and 1570 cal yr BP (fig. 3.25). Although this fact is apparently counterintuitive, some conifers are part of the actual and past typical Afromontane vegetation, namely *Podocarpus* and *Juniperus procera*. A significant presence of these two species, which have been described as characteristic of a fire-adapted ecosystem, is reported in the pollen records of the entire Holocene [196, 204]. *Podocarpus*, in particular, is often shown to increase in concomitance with increased aridity episodes [196]. It is therefore likely that the retene signal results from increased burning of forest trees, ascribable either to a natural shift towards drier, fire-promoting conditions, or to anthropogenic use of wood for land clearing and iron smelting following the Bantu expansion to East Africa [204].

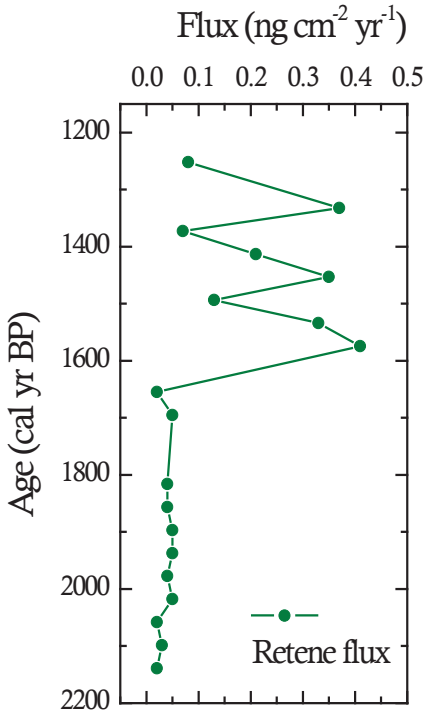

Figure 3.25 Retene flux (ng cm^{-2} yr^{-1}) in the core from Lake Victoria.

The application of DR to Lake Victoria PAHs gave unsatisfactory results due to the low concentrations of single congeners, although the comparison of the Fla/(Fla+Pyr) and Ant/(Ant+Phe), not reported here, shows high correlation among samples. Such a correlation is strongly indicative of wood combustion as a common source for all the samples.

Monosaccharide Anhydrides (MAs)

Unexpectedly, a neat predominance of galactosan (G) over levoglucosan (L) results from the GC-MS analysis of silylated compounds. Mannosan (M) was below the detection limit in all samples. Part of the explanation likely resides in the analytical method: the chromatographic yield of derivatized mannosan is poorer than that of the other two isomers, as visible in figure 2.9 (paragraph 2.3.2), a problem that affects small concentrations. However, values are always corrected for response factors in order to account for differences in the instrumental response. Another factor that may have

contributed on long time scales is the biodegradation of MAs. During the deposition process along the water column and before burial in anoxic sediments, MAs are likely to have undergone bacterial degradation. No study is currently available about the stability of mannosan and galactosan in aquatic environments, and only one research paper was published describing in detail the degradation of levoglucosan in the atmosphere [229]. However, Kirchgeorg (2015, *unpublished data*) tested the biodegradation rate of the three MAs over a 28-day period in a mineral medium: although preliminary, the results showed that the biodegradation of galactosan was significantly lower than that of levoglucosan and mannosan (44% to 85% and 86%) [168]. If confirmed, the biodegradation hypothesis could explain the fluxes measured for Lake Victoria. The biodegradation effect could probably be considered negligible for the small spatial and short temporal scales involved in LK, but could assume a significant importance for larger systems. The observed trends of levoglucosan and galactosan could therefore reflect the combustion of large amounts of biomass on a regional scale, as discussed for PAHs, and be affected by atmospheric transport and biodegradation within the water body and surface sediments.

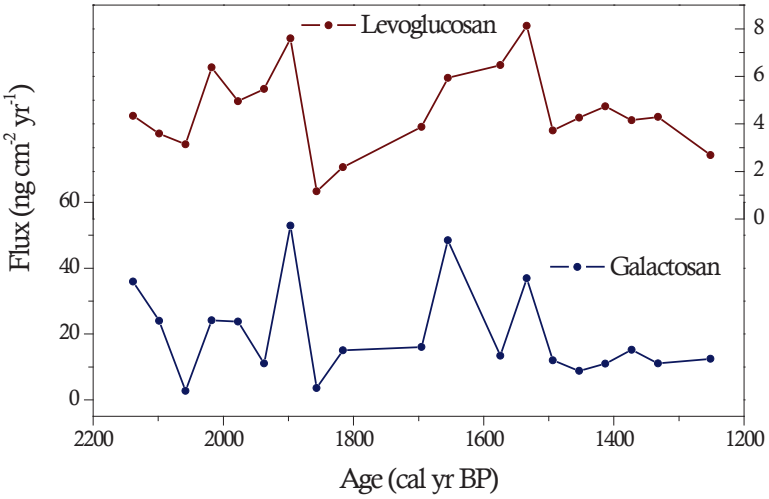

Figure 3.26 Levoglucosan and galactosan fluxes (ng cm^{-2} yr^{-1}) in the core from Lake Victoria.

Fluxes of L and G, shown in figure 3.26, replicate the trend observed for PAHs, with two main increases in fire activity centered at about 1550 and 1900 cal yr BP. This further

confirms that PAH trends are the result of biomass burning in a period of increased aridity [230].

Fecal and plant sterols (FeSt)

The fluxes of all measured sterols in Lake Victoria are shown in figure 3.27. As for the other tracers, levels are significantly lower than for LK. Again, low concentrations are likely ascribable to the large dimensions of the lake and the consequent dilution of dissolved substances. However, one peak culminating at c. 1450 cal yr BP is visible for all FeSt, likely indicating the simultaneous presence of humans and cattle, together with increased β-sitosterol associated with increased plant input that could result from anthropogenic land use change.

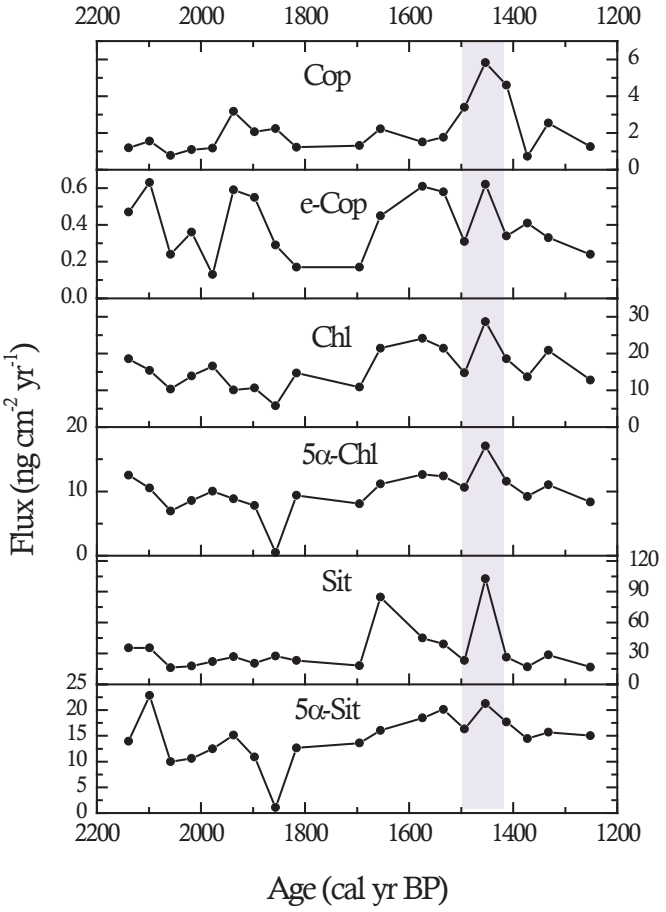

Figure 3.27 Fluxes (ng cm^{-2} yr^{-1}) for single FeSt in the core from Lake Victoria. The grey area corresponds to the major peak detected for all FeSt.

Other maxima of fecal sterols are visible earlier in the record. The eastward migration of the Bantu speaking people, whose presence is documented at least since 2000 cal yr BP [201], could explain the observed trend. The major peaks observed at 1500-1400 cal yr BP may result from the establishment of settlements and the consequent intensification of land clearance, as documented by pollen records for Lake Masoko and Lake Tanganyika [204, 205].

Multi-proxy interpretation

Unfortunately, no charcoal records are available for Lake Victoria, but a direct comparison of data is possible with other lakes in the Rift Valley. Late Holocene paleoclimate and paleofire reconstructions exist for Lake Edward, Lake Masoko, Lake Katinda and Lake Tanganyika, all located in equatorial East Africa (fig. 3.28) [205, 206, 230, 231].

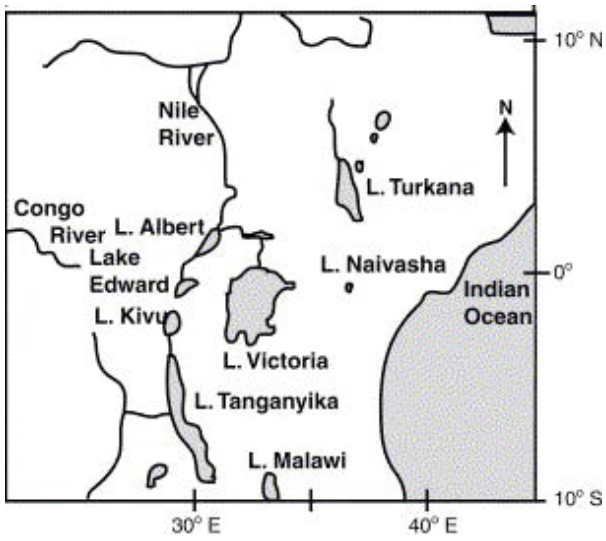

Figure 3.28 Great Lakes system. *Modified from Russell and Johnson (2005)* [231].

A general intensification of droughts is registered in the last 2000 cal yr BP, with a severe event at about 1850 cal yr BP, inferred from Mg concentrations and biogenic silica at Lake Edward and confirmed by high carbonate content in Lake Katinda. A vegetation transition from forest to more open savanna woodland is also reported for Lake Katinda area in the period from 2170 to 1850 cal yr BP, associated with more frequent local fires [230]. Interestingly, the pollen and charcoal records from Lake Masoko indicate an opening of landscape, synchronous with local fires, around 1560 cal yr BP: authors have hypothesized an anthropogenic influence on fire regimes in this period, which coincides with the arrival of the Bantu speaking people in the region [204, 206].

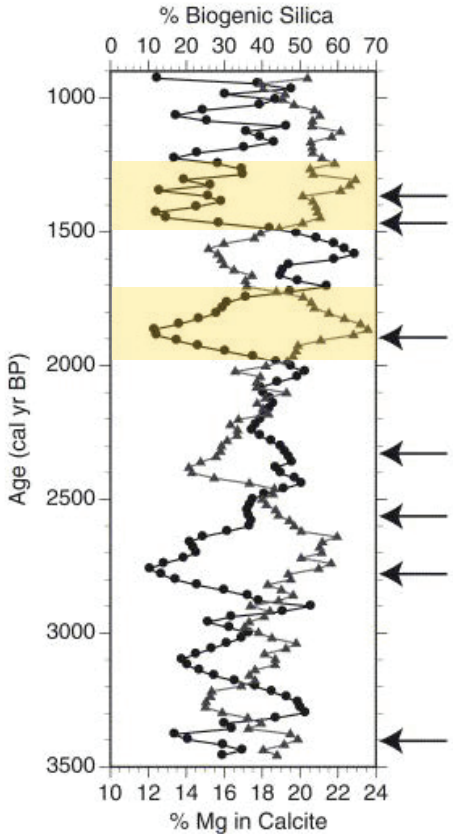

Figure 3.29 % Biogenic silica (circles) and % Mg (triangles) in Lake Edward. Colored areas highlight periods of drought evidenced by the two proxies in the last 2000 years, focusing on the period considered in the present work. *Modified from Russell and Johnson (2005)* [231].

The comparison among results for all the molecular markers considered in the present work is shown in figure 3.30. Human contribution is reported as the ratio between the sum of coprostanol and epicoprostanol and the sum of all sterols. This normalization is suggested for a correct assessment of the human contribution to the total amount of sterols [119]. Two moments of increased human presence are identified by applying either this ratio or any other suggested in the literature [112, 119], indicating the robustness of the fecal tracers. The FeSt record produced in the present work shows a first increase between ~2000 and ~1800 cal yr BP, which displays a good correspondence with increased biomass burning and a second one between ~1500 and ~1400 cal yr BP, lagging the peak in fire activity by 50-100 years. Chronology shifts in paleoenvironmental records of lakes in the Rift Valley region are observed frequently and attributed to differences in local climates and vegetation [204, 206, 230]. However, the time

discrepancy between FeSt and fire frequency in the same record could be related to the difference in depositional processes.

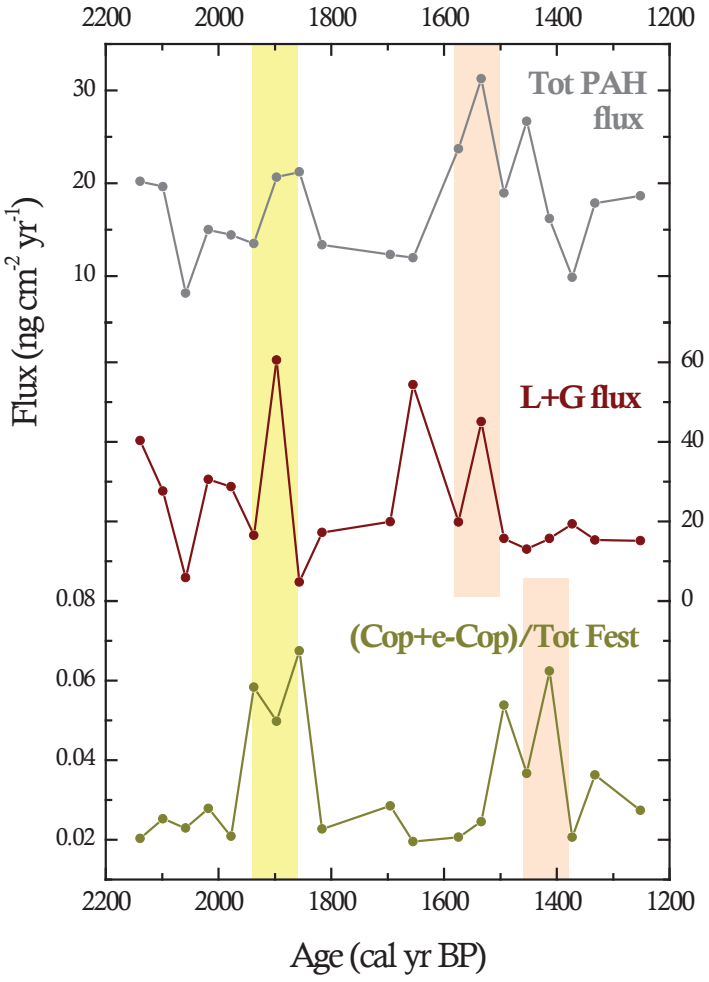

Figure 3.30 Fluxes (ng cm^{-2} yr^{-1}) of total PAHs, MAs and Cop normalized for total FeSt in the core from Lake Victoria.

As formerly illustrated, PAHs and MAs reach sediments mainly by air transport and deposition, particularly in the case of Lake Victoria, highly dependent on the atmospheric input. Indeed, there are differences also between the signals of these two classes of fire tracers, as occurred also at Lake Kirkpatrick. One would expect the two records to present very similar trends. However, differences in mass, polarity, partitioning characteristics and atmospheric lifetime affect the adsorption of tracers onto particulate matter and thus their transport potential. Two records returning exactly the

same signal would be of little use, and differences allow us to complement information and to formulate a more articulate interpretation of the data.

FeSt reach lake sediments through runoff and fluvial inflow. On a spatial scale, fire tracers may thus represent a regional signal, modulated by several burning sources. FeSt are the result of human activities limited to the drainage basin of the lake, which in the case of Lake Victoria extends for 190,000 km^2 [193]. On a temporal scale, the frequency of droughts and the particular rainfall pattern that characterize equatorial East Africa may have influenced runoff with a different timing with respect to the atmosphere, which responds more quickly to changes and actually drives climatic fluctuations in this area.

The three records elaborated here are in agreement with the indications inferred from pollen and charcoal data, that suggest increased aridity and increased anthropogenic disturbance in the Great Lakes region in the last 2000 years [204–206, 230]. Data suggest human influence in the region since about 2000 cal yr BP, coincident with pulses of increased fire activity. This evidence agrees with the hypothesis of progressive settlement of the Bantu since the Early Iron Age, and the intense forest clearing for farming and iron smelting, that would require considerable amounts of biomass with high caloric power in order to reach required temperatures. The mastery of iron probably led to progress in agriculture due to the availability of adequate tools, now visible from trends in land use markers. It is also possible that the observed time shift between fecal sterols and fire tracers is due to natural fires that opened the landscape and made settlement and farming easier, or to repeated human-set fires aimed at land clearing that subsequently favored human settling.

4. CONCLUSIONS

The present study focused on the research and application of novel techniques for the investigation of the early human impact on landscape. New analytical methods were developed and tested, along with multi-proxy records that provide deep insight into the processes that transformed our environment in the past. It was shown that molecular proxies are in good agreement with existing paleoecological records such as pollen and charcoal, and have the potentiality of confirming or confuting hypotheses based on inference by means of direct evidence. Of course, the applications illustrated here highlight the absolute need for good chronologies and high resolution archives.

This work is meant as an exploratory survey in the complex world of paleoenvironmental studies. Further work is needed to refine techniques and interconnect analytical chemistry with the other disciplines that actually hold the expertise in paleoscience. As anticipated in the premise, the interdisciplinary character of this field is both its most fascinating and its most complicated aspect. What emerges from this work is that three main elements are required for obtaining high quality datasets: good analytical methods - whose importance is sometimes underestimated in the literature on molecular markers in paleorecords - good resolution and good skills in data handling and interpretation. The latter would be impossible without a deep knowledge of environmental processes and climate dynamics. For these reasons, future collaboration with scientists from other fields is highly desirable.

Three distant and different locations were chosen in order to test methods in very different historical and environmental conditions. Data were compared to existing information, where possible, and four factors proved particularly important: (1) the spatial scale represented by tracers, or the source area; (2) the information on the physical and chemical processes that affect the distribution and environmental fate of all the considered proxies; (3) the resolution, in terms of number of samples per core section, and (4) large scale climate fluctuations. In the case of Lake Kirkpatrick and Lake Diamond, a good knowledge of the recent past exists and a short time span with a good resolution was considered. The sampling sites offered the possibility of testing methods against solid paleoecological evidence, and results confirmed the fitness of molecular

tracers for the purpose, also providing new elements for the interpretation of existing records. The small sample set from Flinders Island, then, shed a little more light on the scarce information provided by archaeology, by indicating some human presence during the Late Holocene despite the lack of any other evidence on the island. It also underlined the importance of high resolution for revealing small fluctuations and causal links among different processes. Finally, Lake Victoria showed the effect of a large spatial scale and a long time scale on molecular proxies, which may lose part of their significance. All data obtained by the multi-proxy approach testified to the importance of knowing the dynamics that precede and follow the deposition in sediments. However, proposed tracers were in good agreement with existing data, demonstrating the robustness of techniques and proxies for paleoenvironmental use.

Table 4.1 offers a summary of the main findings discussed in the present work.

Table 4.1 Summary of the main results and findings proposed in the present work for each of the considered locations.

Study region	Location	Lake	Proxy	Main results	New findings
New Zealand	*South Island*	*Kirkpatrick*	▪ *PAHs* ▪ *MAs* ▪ *FeSt*	*Sharp peak of all tracers after Māori arrival, then decrease to background values. Huge increase in FeSt in the European period .* *FeSt pattern suggests a reducing environment.*	▪ *Results suggest abrupt and brief human impact in the Māori period.* ▪ *Possible correlation of FeSt with past population size.* ▪ *Use of FeSt as indicators of past and present chemical conditions.*
		Diamond	▪ *FeSt*	*Peak of human signal at ~ AD 1338.*	▪ *FeSt peak anticipates charcoal peak by a few decades.* ▪ *No FeSt increase observed in European period, suggesting little human influence in the lake's watershed.*

Study region	Location	Lake	Proxy	Main results	New findings
Tasmania	*Flinders Island*	*Middle Patriarch Lagoon*	▪ *FeSt*	*Peaks of e-Cop at 12-10 kyr BP indicate human presence and total degradation of Cop. Increase in Cop and e-Cop fluxes around 2000 cal yr BP.*	▪ *FeSt suggest return of humans starting ~2500 yr BP, not documented previously.*
East Africa	*Uganda*	*Victoria*	▪ *PAHs* ▪ *MAs* ▪ *FeSt*	*Fire tracers indicate increased biomass burning in dry periods peaking at ~1900 and ~1550 cal yr BP. Increase in retene since ~1600 cal yr BP. Cop and e-Cop peaks at ~1900 and ~1450 cal yr BP indicate human presence following periods of higher fire activity.*	▪ *First existing fire and human records for Lake Victoria.* ▪ *Results suggest that environmental changes may have promoted human settlement.* ▪ *Possible increased use of wood as fuel since ~1600 cal yr BP inferred from retene record.*

As one can see from the table, in many cases the research presented here set the basis for future work, which should be oriented to enhance the robustness of the dataset and of the interpretations proposed. An important issue to be considered is the occasionally imperfect correspondence between the different proxies in terms of time. As observed in the results chapter, there is a discrepancy between fire tracers and fecal sterols, and sometimes also between PAHs and MAs, both in New Zealand and in East Africa. As multi-proxy methods have the advantage of preventing any time mismatch among proxies caused by sampling and dating artifacts, such a lack of perfect correspondence should be attributed only to environmental factors.

Based on the literature about the environmental dynamics of biomarkers and the analytical aspects, it seems reasonable to ascribe this discrepancy to a few main factors, which could be summarized as follows: (1) the different processes that originate proxies, and also the variability within the same process, such as fuel and combustion conditions in the case of fire tracers; (2) transport processes, that affect the patterns observed in sediments and the spatial scales represented by markers; (3) residence time in atmosphere

and water; (4) differences in stability; (5) the relationship between humans and fire, and between climate and fire.

The last element, investigated in the present work, is probably responsible for the differences in the behavior of proxies between New Zealand and East Africa. In the first case, more pronouncedly at Lake Diamond than at Lake Kirkpatrick, the human signal precedes the increase in fire activity, as if the settlement occurred slightly before the beginning of land clearance. In Lake Victoria, the opposite situation occurs: the increase in FeSt generally lags behind the fire signal, suggesting a different dynamics. Here, a natural increase in fire activity seems to have promoted landscape opening and the subsequent settlement of humans, who in turn used fire for further land clearance, metallurgy and other activities. Although FeSt are representative of more local events, while fire tracers could reflect also regional changes, it must be pointed out that large scale environmental changes may affect human behavior and habits also on the small scale while, on the other hand, a small group of individuals is able to cause wide landscape fire-mediated modifications.

One could still argue that the aforementioned discrepancies challenge the validity of proxies. However, in all the cases the interpretation is consistent with existing evidence, where available. Data in New Zealand satisfyingly fit the charcoal record and confirm the close interdependence between human presence and the observed changes in the fire regime, despite the evident differences in the represented processes and areas (which should be further investigated). On the contrary, no other fire records exist for Lake Victoria, making any comparison quite hard. However, a general correspondence with paleolimnological and paleoecological proxies from nearby sites was observed, although obvious stratigraphic discrepancies affect the timing of events. This point could be clarified only by analyses at higher resolution and broader spectrum.

Where no multi-proxy analysis was performed, namely at Lake Diamond and Flinders Island, only a tentative interpretation was given, consistently with major climate changes, fire activity and archaeological evidence. The analysis of fecal markers in paleorecords is something quite new, and so is the interpretation of results. However, outcomes are encouraging and should stimulate the continuation of research in this sense.

More than a weakness, therefore, the observed differences among proxies and locations represent a useful instrument to better understand the cause-effect relationship between humans, fire and climate. Although the knowledge in this field is far from being

exhaustive, this work provided a first example of how biomarkers can be used to assess whether the human or the climatic factor prevailed on a local scale and to fill the gap in existing data. Without any doubt, each of the aspects considered here should be analyzed more in depth in future research, in order to provide robust and broad datasets to be used in modeling, source identification and extension to new tracers of paleoenvironmental relevance.

This work and all the data-based observations had the purpose to answer, at least in part, the set of questions proposed in the method section:

a) *Are the selected molecular markers adequate indicators of past changes?*

Yes. Selected markers showed good correspondence with paleolimnological and paleoecological findings, even when derived from different locations. The ability to reproduce past changes reliably is therefore undeniable

b) *Do fire molecular markers agree with the charcoal trend and, if not, why?*

Where possible, data were compared with charcoal records, and results showed good correlation for local and regional fires. When the correspondence is missing, the reason resides in the intrinsic physical differences among tracers, that influence transport and deposition mechanisms, or simply to a difference in the resolution of examined records.

c) *Is there a temporal and spatial correspondence between human and fire signals?*

For Lake Kirkpatrick and Lake Diamond the correspondence was full, thanks to the suitability of the two locations for the scope of the analysis and to a recent target time interval. In the case of Middle Patriarch Lagoon, a general good correspondence is shown, but due to the low number of samples any certain conclusion would be incautious. For Lake Victoria, charcoal records were not available but molecular markers

show a good agreement with the ones existing for other lakes in the region. However, a partial correspondence is observed between the measured fire and fecal markers, despite small fluxes and a slight time shift, imputed to depositional processes or partly unknown anthropic settlement patterns.

d) *To what extent can we distinguish between anthropogenic fire regimes and climate-driven ones?*

This question was at the basis of the Early Human Impact project, in which the present work is involved. Nevertheless, no conclusive answer could be given even in ideal circumstances. The use of FeSt coupled to fire tracers surely showed the potential of the approach used here, giving surprising results especially in the case of New Zealand. However, the interdependence between environment and humans has always been so close, that it is almost impossible to tell which one caused changes in the other.

Overall, we can say that the present work fulfilled its purposes satisfyingly, although new efforts in sampling, analyzing and interpreting data are certainly required.

The final and hardest question to answer was posed at the very beginning of this work and is *whether, when and to what extent humans started to affect climate*. This is also the starting question of the Early Human Impact project, and that this PhD work aimed to address it at least in part. The question is complicated and we are still far from an exhaustive answer. However, this work provided valuable instruments for completing the picture that other disciplines have delineated. The presence of humans very soon became the third actor in the interplay between climate and environment. Thousands of years later, the signal they left with their activities is still visible in paleorecords. The best answer, for the moment, is probably that each location examined tells a different story, depending on its sensitivity to climate variability and on its ability to recover after huge disturbances. Humans learnt how to benefit from natural changes and how to control the size of fires and the composition of vegetation to their advantage. The history of anthropogenic climate change is therefore a complex mosaic of social evolution,

geographical expansion and human-environment relationships. For us to position a new tile is above all a matter of spatial and temporal scales, that strongly affect the level at which we can see and understand changes.

5. ACKNOWLEDGEMENTS

I wish to say thank you, from the deepest of my heart, to:

Carlo Barbante and all the environmental analytical chemistry research group of the University of Venice, for believing I could do this and giving me the chance to try

Dario, Natalie, Torben, Marco V., Alice, Maria, Marco R. for their invaluable help

Cathy Whitlock, Dave McWethy and all the Paleoecology lab staff of Montana State University, for making this research possible

Daniela Almansi, for her support and her careful review

Anna, Gloriana and all the staff of the R&C Lab

Fabio, Irene, Giovanni, Daniele, Michela, Giuliano, my great PhD mates

Chiara, Stefano, Enrico, who I am missing more than ever

Maddalena, Elettra, Sara, Matteo and the pink elephants

"Thank you" will never be enough also for:

Marco and our son Pietro, the Loves of my life

my Mom, my Dad and Gabriella, who support me every day

my Grandmother, who survived another battle

my Godfather Symeon, who never gave up on me

my Sisters Anna and Mary, who where there for me all the time

my old and new Friends

all the great people who passed away and now watch over me.

6. REFERENCES

1. Crutzen PJ, Stoermer EF (2000) The "Anthropocene." IGBP Glob Chang Newsl 17–18.
2. Ruddiman WF (2003) The anthropogenic greenhouse era began thousands of years ago. Clim Change 61:261–293. doi: 10.1023/B:CLIM.0000004577.17928.fa
3. Foley SF, Gronenborn D, Andreae MO, Kadereit JW, Esper J, Scholz D, Pöschl U, Jacob DE, Schöne BR, Schreg R, Vött A, Jordan D, Lelieveld J, Weller CG, Alt KW, Gaudzinski-Windheuser S, Bruhn K-C, Tost H, Sirocko F, Crutzen PJ (2013) The Palaeoanthropocene – The beginnings of anthropogenic environmental change. Anthropocene 3:83–88. doi: 10.1016/j.ancene.2013.11.002
4. Waters CN, Syvitski JPM, Gałuszka A, Hancock GJ, Zalasiewicz J, Cearreta A, Grinevald J, Jeandel C, McNeill JR, Summerhayes C, Barnosky A (2015) Can nuclear weapons fallout mark the beginning of the Anthropocene Epoch? Bull At Sci 71:46–57. doi: 10.1177/0096340215581357
5. Smith BD, Zeder MA (2013) The onset of the Anthropocene. Anthropocene 4:8–13. doi: 10.1016/j.ancene.2013.05.001
6. Fuller DQ, van Etten J, Manning K, Castillo C, Kingwell-Banham E, Weisskopf A, Qin L, Sato Y-I, Hijmans RJ (2011) The contribution of rice agriculture and livestock pastoralism to prehistoric methane levels: an archaeological assessment. The Holocene 21:743–759. doi: 10.1177/0959683611398052
7. Ruddiman WF (2005) The early anthropogenic hypothesis a year later. Clim Change 69:427–434.
8. Ruddiman WF (2007) The early anthropogenic hypothesis: challenges and responses. Rev Geophys 45:RG4001. doi: 10.1029/2006rg000207
9. Ruddiman WF, Guo Z, Zhou X, Wu H, Yu Y (2008) Early rice farming and anomalous methane trends. Quat Sci Rev 27:1291–1295. doi: 10.1016/j.quascirev.2008.03.007
10. Vavrus S, Ruddiman WFF, Kutzbach JEE (2008) Climate model tests of the anthropogenic influence on greenhouse-induced climate change: the role of early human agriculture, industrialization, and vegetation feedbacks. Quat Sci Rev 27:1410–1425. doi: 10.1016/j.quascirev.2008.04.011
11. Ruddiman WF, Kutzbach JE, Vavrus SJ (2011) Can natural or anthropogenic explanations of late-Holocene CO_2 and CH_4 increases be falsified? The Holocene 21:865–8879. doi: 10.1177/0959683610387172
12. Ruddiman WF, Ellis EC, Kaplan JO, Fuller DQ (2015) Defining the epoch we live in. Science 348:38–39. doi: 10.1126/science.aaa7297
13. Epica Community Members (2004) Eight glacial cycles from an Antarctic ice core. Nature 429:623–628. doi: http://www.nature.com/nature/journal/v429/n6992/suppinfo/nature02599_S1.html
14. Martin K, Sauerborn J (2013) Agroecology. Agroecology. doi: 10.1007/978-94-007-5917-6
15. Melieres M-A, Marechal C (2015) The past 12,000 years: the warm Holocene. In: Clim. Chang.

Past, Present Futur. Wiley-Blackwell Publishing, pp 201–224

16. Wassmann R, Papen H, Rennenberg H (1993) Methane emission from rice paddies and possible mitigation strategies. Chemosphere 26:201–217. doi: 10.1016/0045-6535(93)90422-2
17. Yan X, Akiyama H, Yagi K, Akimoto H (2009) Global estimations of the inventory and mitigation potential of methane emissions from rice cultivation conducted using the 2006 Intergovernmental Panel on Climate Change guidelines. Global Biogeochem Cycles 23:20–23. doi: 10.1029/2008GB003299
18. IPCC (2014) Climate Change 2014: Mitigation of Climate Change. IPCC Working Group III Contribution to AR5.
19. Broecker WS, Stocker TF (2006) The Holocene CO_2 rise: anthropogenic or natural? Eos (Washington DC) 87:PAGE 27. doi: 200610.1029/2006EO030002
20. Broecker W., Clark E, McCorkle DC, Peng T-H, Hajdas I, Bonani G (1999) Evidence for a reduction in the carbonate ion content of the deep sea during the course of the Holocene. Paleoceanography 14:744–752.
21. Ridgwell AJ, Watson AJ, Maslin M a., Kaplan JO (2003) Implications of coral reef buildup for the controls on atmospheric CO_2 since the Last Glacial Maximum. Paleoceanography 18:n/a–n/a. doi: 10.1029/2003PA000893
22. Ellis EC, Kaplan JO, Fuller DQ, Vavrus S, Klein Goldewijk K, Verburg PH (2013) Used planet: a global history. Proc Natl Acad Sci 110:7978–7985. doi: 10.1073/pnas.1217241110
23. Kaplan JO, Krumhardt KM, Ellis EC, Ruddiman WF, Lemmen C, Goldewijk KK (2011) Holocene carbon emissions as a result of anthropogenic land cover change. The Holocene 21:775–791. doi: 10.1177/0959683610386983
24. Diamond J (2002) Evolution, consequences and future of plant and animal domestication. Nature 418:700–707. doi: 10.1038/nature01019
25. Diamond J (1999) Guns, germs and steel: the fates of human societies. Norton & Co., New York
26. Gibbons R (2004) Examining the extinction of the Pleistocene megafauna. Stanford Undergrad Res J 3:22–27.
27. Ruddiman WF (2008) Earth's climate. Past and future, Second edi. W. H. Freeman and Company, New York
28. Martin PS (1989) Quaternary extinctions: a prehistoric revolution. University of Arizona Press
29. Simmons IG (1996) Changing the face of the Earth. Culture, environment, history, Second Edi. Blackwell Publishers Ltd, Oxford
30. Richerson PJ, Boyd R, Bettinger RL, Vavilov N (2001) Was agriculture impossible during the Pleistocene but mandatory during the Holocene? A climate change hypothesis. Am Antiq 66:387–411.
31. Pringle H (1998) The slow birth of agriculture. Science 282:1446–1446. doi: 10.1126/science.282.5393.1446
32. Weisdorf JL (2005) From foraging to farming: explaining the Neolithic Revolution. J Econ Surv 19:561–586. doi: 10.1111/j.0950-0804.2005.00259.x

33. Brown TA, Jones MK, Powell W, Allaby RG (2009) The complex origins of domesticated crops in the Fertile Crescent. Trends Ecol Evol 24:103–109. doi: 10.1016/j.tree.2008.09.008
34. Price TD, Bar-Yosef O (2011) The origins of agriculture: new data, new ideas. Curr Anthropol 52:S163–S174. doi: 10.1086/659964
35. Zeder MA (2008) Domestication and early agriculture in the Mediterranean basin: origins, diffusion, and impact. Proc Natl Acad Sci 105:11597–11604. doi: 10.1073/pnas.0801317105
36. Marshall F, Hildebrand E (2002) Cattle before crops: the beginning of food production in Africa. J World Prehistory 16:99–143.
37. Smith BD (1989) Origins of agriculture in Eastern North America. Science 246:1566–1571. doi: 10.1126/science.246.4937.1566
38. Smith BD (2011) The cultural context of plant domestication in Eastern North America. Curr Anthropol 52:S471–S484. doi: 10.1086/659645
39. Bocquet-Appel J-P (2011) The agricultural demographic transition during and after the agriculture invention. Curr Anthropol 52:S497–S510. doi: 10.1086/659243
40. Bocquet-Appel J-P (2011) When the World 's population took off: the springboard of the Neolithic Demographic Transition. Science 333:560–561.
41. Gage TB, DeWitte S (2009) What do we know about the agricultural demographic transition? Curr Anthropol 50:649–655. doi: 10.1086/605017
42. Mazoyer M, Roudart L (2006) A history of World agriculture. From Neolithic age to the current crisis, First edit. Earthscan, London
43. McFall JA (1991) Population: a lively introduction. Popul Bull 46:1–43.
44. Ellis EC (2013) Land-use and land-cover change. http://www.eoearth.org/view/article/51cbee4f7896bb431f696e92.
45. Goudie A (1990) The human impact on the natural environment, Third Edit. Basil Blackwell Ltd, Oxford
46. IPCC (2013) Climate Change 2013: The Physical Science Basis.
47. Mouillot F, Field CB (2005) Fire history and the global carbon budget: a 1°× 1° fire history reconstruction for the 20th century. Glob Chang Biol 11:398–420. doi: 10.1111/j.1365-2486.2005.00920.x
48. Andreae MO (1991) Biomass burning: Its history, use and distribution and its impact on environmental quality and global climate. In: Levine JS (ed) Glob. biomass Burn. Atmos. Clim. Biosph. Implic. MIT Press, Cambridge, pp 3–21
49. Crutzen PJ, Lelieveld J (2001) Human impacts on atmospheric chemistry. Annu Rev Earth Planet Sci 29:17–45.
50. Lohmann U, Lesins G (2002) Stronger constraints on the anthropogenic indirect aerosol effect. Science 298:1012–1015.
51. Tinker PB, Ingram JSI, Struwe S (1996) Effects of slash-and-burn agriculture and deforestation on climate change. Agric Ecosyst Environ 58:13–22. doi: 10.1016/0167-8809(95)00651-6
52. Olofsson J, Hickler T (2008) Effects of human land-use on the global carbon cycle during the last

6,000 years. Veg Hist Archaeobot 17:605–615. doi: 10.1007/s00334-007-0126-6

53. Salinger MJ (2007) Agriculture's influence on climate during the Holocene. Agric For Meteorol 142:96–102. doi: 10.1016/j.agrformet.2006.03.024

54. Carcaillet C, Almquist H, Asnong H, Bradshaw RHW, Carrión JS, Gaillard MJ, Gajewski K, Haas JN, Haberle SG, Hadorn P, Müller SD, Richard PJH, Richoz I, Rösch M, Sánchez Goñi MF, von Stedingk H, Stevenson AC, Talon B, Tardy C, Tinner W, Tryterud E, Wick L, Willis KJ (2002) Holocene biomass burning and global dynamics of the carbon cycle. Chemosphere 49:845–863. doi: http://dx.doi.org/10.1016/S0045-6535(02)00385-5

55. Pausas JG, Keeley JE (2009) A burning story: the role of fire in the history of life. Bioscience 59:593–601. doi: 10.1525/bio.2009.59.7.10

56. Glikson A (2013) Fire and human evolution: the deep-time blueprints of the Anthropocene. Anthropocene 3:89–92. doi: 10.1016/j.ancene.2014.02.002

57. Malamud BD, Turcotte DL (2006) An inverse cascade explanation for the power-law frequency-area statistics of earthquakes, landslides and wildfires. Geol Soc London, Spec Publ 261:1–9. doi: 10.1144/GSL.SP.2006.261.01.01

58. Takács-Sánta A (2004) The major transitions in the history of human transformation of the biosphere. Hum Ecol Rev 11:51–66.

59. Attwell L, Kovarovic K, Kendal JR (2015) Fire in the Plio-Pleistocene : the functions of hominin fire use, and the mechanistic, developmental and evolutionary consequences. 93:1–20. doi: 10.4436/jass.93006

60. Power MJ, Marlon J, Ortiz N, Bartlein PJ, Harrison SP, Mayle FE, Ballouche a., Bradshaw RHW, Carcaillet C, Cordova C, Mooney S, Moreno PI, Prentice IC, Thonicke K, Tinner W, Whitlock C, Zhang Y, Zhao Y, Ali a. a., Anderson RS, Beer R, Behling H, Briles C, Brown KJ, Brunelle a., Bush M, Camill P, Chu GQ, Clark J, Colombaroli D, Connor S, Daniau a.-LL, Daniels M, Dodson J, Doughty E, Edwards ME, Finsinger W, Foster D, Frechette J, Gaillard M-JJ, Gavin DG, Gobet E, Haberle S, Hallett DJ, Higuera P, Hope G, Horn S, Inoue J, Kaltenrieder P, Kennedy L, Kong ZC, Larsen C, Long CJ, Lynch J, Lynch E a., McGlone M, Meeks S, Mensing S, Meyer G, Minckley T, Mohr J, Nelson DM, New J, Newnham R, Noti R, Oswald W, Pierce J, Richard PJH, Rowe C, Sanchez Goñi MF, Shuman BN, Takahara H, Toney J, Turney C, Urrego-Sanchez DH, Umbanhowar C, Vandergoes M, Vanniere B, Vescovi E, Walsh M, Wang X, Williams N, Wilmshurst J, Zhang JH (2008) Changes in fire regimes since the Last Glacial Maximum: an assessment based on a global synthesis and analysis of charcoal data. Clim Dyn 30:887–907. doi: 10.1007/s00382-007-0334-x

61. Olsson F, Gaillard M-JM-J, Lemdahl G, Greisman A, Lanos P, Marguerie D, Marcoux N, Skoglund P, Wäglind J (2010) A continuous record of fire covering the last 10,500 calendar years from southern Sweden - The role of climate and human activities. Palaeogeogr Palaeoclimatol Palaeoecol 291:128–141. doi: http://dx.doi.org/10.1016/j.palaeo.2009.07.013

62. Marlon JR, Bartlein PJ, Carcaillet C, Gavin DG, Harrison SP, Higuera PE, Joos F, Power MJ, Prentice IC (2008) Climate and human influences on global biomass burning over the past two

millennia. Nat Geosci 1:697–702. doi:
http://www.nature.com/ngeo/journal/v1/n10/suppinfo/ngeo313_S1.html

63. Moore J, Rhodes N (2000) Introduction to fire and the palaeoenviroment. Palaeogeogr Palaeoclimatol Palaeoecol 164:vii–xi.
64. Bowman DMJS, Balch JK, Artaxo P, Bond WJ, Carlson JM, Cochrane MA, D'Antonio CM, Defries RS, Doyle JC, Harrison SP, Johnston FH, Keeley JE, Krawchuk MA, Kull CA, Marston JB, Moritz MA, Prentice IC, Roos CI, Scott AC, Swetnam TW, van der Werf GR, Pyne SJ (2009) Fire in the Earth system. Science 324:481–4. doi: 10.1126/science.1163886
65. Day T (2006) Lakes and rivers. Chelsea House, New York
66. Wohlfarth B (2012) Paleolimnology. Encycl. Lakes Reserv.
67. Herschy RW (2012) Lake sediments. Encycl. Lakes Reserv.
68. Birks HJB, Lotter AF, Juggins S, Smol JP (2012) Tracking environmental change using lake sediments. Data handling and numerical techniques. Springer
69. Jones PD, Briffa KR, Osborn TJ, Lough JM, Vinther BM, Luterbacher J, Wahl ER, Zwiers FW, Mann ME, Schmidt GA, Ammann CM, Buckley BM, Cobb KM, Esper J, Goosse H, Graham N, Jansen E, Kiefer T, Kull C, Küttel M, Overpeck JT, Riedwyl N, Schulz M, Tudhope AW, Villalba R, Wanner H, Wolff E, Xoplaki E (2009) High-resolution palaeoclimatology of the last millennium : a review of current status and future prospects. The Holocene 19:3–49.
70. Schnurrenberger D, Russell J, Kelts K (2003) Classification of lacustrine sediments based on sedimentary components. J Paleolimnol 29:141–154. doi: 10.1023/A:1023270324800
71. Whitlock C, Larsen C (2001) Charcoal as a fire proxy. In: Track. Environ. Chang. using lake sediments. pp 75–97
72. Conedera M, Tinner W, Neff C, Meurer M, Dickens AF, Krebs P (2009) Reconstructing past fire regimes: methods, applications, and relevance to fire management and conservation. Quat Sci Rev 28:555–576. doi: 10.1016/j.quascirev.2008.11.005
73. Simoneit BR. (2002) Biomass burning — a review of organic tracers for smoke from incomplete combustion. Appl Geochemistry. doi: 10.1016/S0883-2927(01)00061-0
74. Andreae MO, Merlet P (2001) Emissions of trace gases and aerosols from biomass burning. Global Biogeochem Cycles 15:955–966.
75. Oros DR, Simoneit BR. (2001) Identification and emission factors of molecular tracers in organic aerosols from biomass burning Part 1. Temperate climate conifers. Appl Geochemistry 16:1513–1544. doi: 10.1016/S0883-2927(01)00021-X
76. Oros DR, Simoneit BRT (2001) Identification and emission factors of molecular tracers in organic aerosols from biomass burning Part 2. Deciduous trees. Appl Geochemistry 16:1545–1565.
77. Oros DR, Abas MR Bin, Omar NYMJ, Rahman NA, Simoneit BRT (2006) Identification and emission factors of molecular tracers in organic aerosols from biomass burning: Part 3. Grasses. Appl Geochemistry 21:919–940. doi: 10.1016/j.apgeochem.2006.01.008
78. Eganhouse RP (1997) Molecular markers and environmental organic geochemistry: an overview. In: Mol. Markers Environ. Geochemistry. pp 1–20

79. Rubin EM (2008) Genomics of cellulosic biofuels. Nature 454:841–845.
80. Simoneit BRT, Schauer JJ, Nolte CG, Oros DR, Elias VO, Fraser MP, Rogge WF, Cass GR (1999) Levoglucosan, a tracer for cellulose in biomass burning and atmospheric particles. Atmos Environ 33:173–182. doi: 10.1016/S1352-2310(98)00145-9
81. Jenkins BM, Baxter LL, Miles TR Jr, Miles TR (1998) Combustion properties of biomass. Fuel Process Technol 54:17–46. doi: http://dx.doi.org/10.1016/S0378-3820(97)00059-3
82. Fraser MP, Lakshmanan K (2000) Using Levoglucosan as a molecular marker for the long-range transport of biomass combustion aerosols. Environ Sci Technol 34:4560–4564. doi: 10.1021/es991229l
83. Fabbri D, Torri C, Simoneit BRT, Marynowski L, Rushdi AI, Fabiańska MJ (2009) Levoglucosan and other cellulose and lignin markers in emissions from burning of Miocene lignites. Atmos Environ 43:2286–2295. doi: 10.1016/j.atmosenv.2009.01.030
84. Kuo LJ, Louchouarn P, Herbert BE (2011) 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. doi: 10.1016/j.chemosphere.2011.06.074
85. Zdráhal Z, Oliveira J, Vermeylen R, Claeys M, Maenhaut W (2001) Improved method for quantifying levoglucosan and related monosaccharide anhydrides in atmospheric aerosols and application to samples from urban and tropical locations. Environ Sci Technol 36:747–753. doi: 10.1021/es015619v
86. Simpson CD, Dills RL, Katz BS, Kalman DA (2004) Determination of levoglucosan in atmospheric fine particulate matter. J Air Waste Manag Assoc 54:689–694. doi: 10.1080/10473289.2004.10470945
87. Dye C, Yttri KE (2005) Determination of monosaccharide anhydrides in atmospheric aerosols by use of high-performance liquid chromatography combined with high-resolution mass spectrometry. Anal Chem 77:1853–1858.
88. Schkolnik G, Rudich Y (2006) Detection and quantification of levoglucosan in atmospheric aerosols: a review. Anal Bioanal Chem 385:26–33. doi: 10.1007/s00216-005-0168-5
89. Kehrwald N, Zangrando R, Gabrielli P, Jaffrezo JL, Boutron C, Barbante C, Gambaro A (2012) Levoglucosan as a specific marker of fire events in Greenland snow. Tellus, Ser B Chem Phys Meteorol 64:1–9. doi: 10.3402/tellusb.v64i0.18196
90. 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. Anal Chem 80:1649–55. doi: 10.1021/ac701655x
91. Kawamura K, Izawa Y, Mochida M, Shiraiwa T (2012) Ice core records of biomass burning tracers (levoglucosan and dehydroabietic, vanillic and p-hydroxybenzoic acids) and total organic carbon for past 300 years in the Kamchatka Peninsula, Northeast Asia. Geochim Cosmochim Acta 99:317–329. doi: http://dx.doi.org/10.1016/j.gca.2012.08.006
92. Kuo L-J, Louchouarn P, Herbert BE, Brandenberger JM, Wade TL, Crecelius E (2011)

Combustion-derived substances in deep basins of Puget Sound: historical inputs from fossil fuel and biomass combustion. Environ Pollut 159:983–90. doi: 10.1016/j.envpol.2010.12.012

93. Schüpbach S, Kirchgeorg T, Colombaroli D, Beffa G, Radaelli M, Kehrwald NM, Barbante C (2015) Combining charcoal sediment and molecular markers to infer a Holocene fire history in the Maya Lowlands of Petén, Guatemala. Quat Sci Rev 115:123–131. doi: 10.1016/j.quascirev.2015.03.004
94. Kirchgeorg T, Schüpbach S, Kehrwald N, McWethy DB, Barbante C (2014) Method for the determination of specific molecular markers of biomass burning in lake sediments. Org Geochem 71:1–6. doi: 10.1016/j.orggeochem.2014.02.014
95. Elias VO, Simoneit BRT, Cordeiro RC, Turcq B (2001) Evaluating levoglucosan as an indicator of biomass burning in Caraja's, Amazonia: a comparison to the charcoal record. Geochim Cosmochim Acta 65:267–272.
96. Services H, ATSDR (1995) Toxicological profile for polycyclic aromatic hydrocarbons (PAHs).
97. Ravindra K, Sokhi R, Vangrieken R, Van Grieken R (2008) Atmospheric polycyclic aromatic hydrocarbons: source attribution, emission factors and regulation. Atmos Environ 42:2895–2921. doi: 10.1016/j.atmosenv.2007.12.010
98. McGrath T, Sharma R, Hajaligol M (2001) An experimental investigation into the formation of polycyclic-aromatic hydrocarbons (PAH) from pyrolysis of biomass materials. Fuel 80:1787–1797. doi: 10.1016/S0016-2361(01)00062-X
99. Richter H, Howard J. (2000) Formation of polycyclic aromatic hydrocarbons and their growth to soot—a review of chemical reaction pathways. Prog Energy Combust Sci 26:565–608. doi: 10.1016/S0360-1285(00)00009-5
100. Lima ALC, Farrington JW, Reddy CM (2005) Combustion-Derived Polycyclic Aromatic Hydrocarbons in the Environment - A Review. Environ Forensics 6:109–131. doi: 10.1080/15275920590952739
101. McGrath TE, Chan WG, Hajaligol MR (2003) Low temperature mechanism for the formation of polycyclic aromatic hydrocarbons from the pyrolysis of cellulose. J Anal Appl Pyrolysis 66:51–70. doi: 10.1016/S0165-2370(02)00105-5
102. Hajaligol M, Waymack B, Kellogg D (2001) Low temperature formation of aromatic hydrocarbon from pyrolysis of cellulosic materials. Fuel 80:1799–1807. doi: 10.1016/S0016-2361(01)00063-1
103. Denis EH, Toney JL, Tarozo R, Scott Anderson R, Roach LD, Huang Y (2012) Polycyclic aromatic hydrocarbons (PAHs) in lake sediments record historic fire events: validation using HPLC-fluorescence detection. Org Geochem 45:7–17. doi: 10.1016/j.orggeochem.2012.01.005
104. Simoneit BRT (1999) A review of biomarker compounds as source indicators and tracers for air pollution. Environ Sci & Pollut Res 6:159–169.
105. Yunker MB, Macdonald RW, Vingarzan R, Mitchell RH, Goyette D, Sylvestre S (2002) PAHs in the Fraser River basin: a critical appraisal of PAH ratios as indicators of PAH source and composition. Org Geochem 33:489–515. doi: 10.1016/S0146-6380(02)00002-5
106. Page DS, Boehm PD, Douglas GS, Bence AE, Burns WA, Mankiewicz PJ (1999) Pyrogenic

polycyclic aromatic hydrocarbons in sediments record past human activity: a case study in Prince William Sound, Alaska. Mar Pollut Bull 38:247–260. doi: 10.1016/S0025-326X(98)00142-8

107. Musa Bandowe B a., Srinivasan P, Seelge M, Sirocko F, Wilcke W (2014) A 2600-year record of past polycyclic aromatic hydrocarbons (PAHs) deposition at Holzmaar (Eifel, Germany). Palaeogeogr Palaeoclimatol Palaeoecol 401:111–121. doi: 10.1016/j.palaeo.2014.02.021
108. Jiang C, Alexander R, Kagi RI, Murray AP (1998) Polycyclic aromatic hydrocarbons in ancient sediments and their relationships to palaeoclimate. Org Geochem 29:1721–1735. doi: 10.1016/S0146-6380(98)00083-7
109. Linseele V, Riemer H, Baeten J, De Vos D, Marinova E, Ottoni C (2013) Species identification of archaeological dung remains: a critical review of potential methods. Environ Archaeol 18:5–17. doi: 10.1179/1461410313Z.00000000019
110. Shillito LM, Bull ID, Matthews W, Almond MJ, Williams JM, Evershed RP (2011) Biomolecular and micromorphological analysis of suspected faecal deposits at Neolithic Çatalhöyük, Turkey. J Archaeol Sci 38:1869–1877. doi: 10.1016/j.jas.2011.03.031
111. Grimalt JO, Fernandez P, Bayona JM, Albaiges J (1990) Assessment of fecal sterols and ketones as indicators of urban sewage inputs to coastal waters. Environ Sci Technol 24:357–363. doi: 10.1021/es00073a011
112. Reeves a. D, Patton D (2005) Faecal sterols as indicators of sewage contamination in estuarine sediments of the Tay Estuary, Scotland: an extended baseline survey. Hydrol Earth Syst Sci 9:81–94. doi: 10.5194/hess-9-81-2005
113. Leeming R, Ball a., Ashbolt N, Nichols P (1996) Using faecal sterols from humans and animals to distinguish faecal pollution in receiving waters. Water Res 30:2893–2900. doi: 10.1016/S0043-1354(96)00011-5
114. Noblet J a., Young DL, Zeng EY, Ensari S (2004) Use of fecal steroids to infer the sources of fecal indicator bacteria in the lower Santa Ana River Watershed, California: sewage is unlikely a significant source. Environ Sci Technol 38:6002–6008. doi: 10.1021/es049799v
115. Vane CH, Kim a W, McGowan S, Leng MJ, Heaton THE, Kendrick CP, Coombs P, Yang H, Swann GE a (2010) Sedimentary records of sewage pollution using faecal markers in contrasting peri-urban shallow lakes. Sci Total Environ 409:345–56. doi: 10.1016/j.scitotenv.2010.09.033
116. D'Anjou RM, Bradley RS, Balascio NL, Finkelstein DB (2012) Climate impacts on human settlement and agricultural activities in northern Norway revealed through sediment biogeochemistry. Proc Natl Acad Sci 109:20332–7. doi: 10.1073/pnas.1212730109
117. Birk JJ, Teixeira WG, Neves EG, Glaser B (2011) Faeces deposition on Amazonian Anthrosols as assessed from 5β-stanols. J Archaeol Sci 38:1209–1220. doi: 10.1016/j.jas.2010.12.015
118. Machado KS, Froehner S, Sánez J, Figueira RCL, Ferreira PAL (2014) Assessment of historical fecal contamination in Curitiba, Brazil, in the last 400 years using fecal sterols. Sci Total Environ 493:1065–72. doi: 10.1016/j.scitotenv.2014.06.104
119. Bull ID, Lockheart MJ, Elhmmali MM, Roberts DJ, Evershed RP (2002) The origin of faeces by means of biomarker detection. Environ Int 27:647–654. doi: 10.1016/S0160-4120(01)00124-6

120. Benfenati E, Cools E, Fattore E, Fanelli R (1994) A GC-MS method for the analysis of fecal and plant sterols in sediment samples. Chemosphere 29:1393–1405. doi: 10.1016/0045-6535(94)90273-9
121. McWethy DB, Whitlock C, Wilmshurst JM, McGlone MS, Fromont M, Li X, Dieffenbacher-Krall A, Hobbs WO, Fritz SC, Cook ER (2010) Rapid landscape transformation in South Island, New Zealand, following initial Polynesian settlement. Proc Natl Acad Sci 107:21343–21348. doi: 10.1073/pnas.1011801107
122. McWethy DB, Wilmshurst JM, Whitlock C, Wood JR, McGlone MS (2014) A high-resolution chronology of rapid forest transitions following Polynesian arrival in New Zealand. PLoS One 9:e111328. doi: 10.1371/journal.pone.0111328
123. Berke MA, Johnson TC, Werne JP, Grice K, Schouten S, Sinninghe Damsté JS (2012) Molecular records of climate variability and vegetation response since the Late Pleistocene in the Lake Victoria basin, East Africa. Quat Sci Rev 55:59–74. doi: 10.1016/j.quascirev.2012.08.014
124. LacCore (University of Minnesota National Lacustrine Core Facility) (2010) Kullenberg. http://lrc.geo.umn.edu/laccore/kullenberg.html.
125. Iversen J (1941) Land occupation in Denmark's stone age : a pollen-analytical study of the influence of farmer culture on the vegetational development. Reitzels, Koebenhavn
126. Tinner W, Conedera M, Ammann B, Gaggeler HW, Gedye S, Jones R, Sagesser B (1998) Pollen and charcoal in lake sediments compared with historically documented forest fires in southern Switzerland since AD 1920. Holocene 8:31–42. doi: 10.1191/095968398667205430
127. Carcaillet C, Bouvier M, Fréchette B, Larouche a. C, Richard PJH (2001) Comparison of pollen-slide and sieving methods in lacustrine charcoal analyses for local and regional fire history. The Holocene 11:467–476. doi: 10.1191/095968301678302904
128. Asselin H, Payette S (2005) Detecting local-scale fire episodes on pollen slides. Rev Palaeobot Palynol 137:31–40. doi: 10.1016/j.revpalbo.2005.08.002
129. Finsinger W, Tinner W (2005) Minimum count sums for charcoalconcentration estimates in pollen slides: accuracy and potential errors. The Holocene 15:293–297. doi: 10.1191/0959683605hl808rr
130. Clark JS (1988) Stratigraphic charcoal analysis on petrographic thin sections: Application to fire history in northwestern Minnesota. Quat Res 30:81–91. doi: 10.1016/0033-5894(88)90089-0
131. Lamoureux SF (1994) Embedding unfrozen lake sediments for thin section preparation. J Paleolimnol 10:141–146. doi: 10.1007/BF00682510
132. Tinner W, Hu FS (2003) Size parameters, size-class distribution and area-number relationship of microscopic charcoal: relevance for fire reconstruction. The Holocene 13:499–505. doi: 10.1191/0959683603hl615rp
133. Millspaugh SH, Whitlock C (1995) A 750-year fire history based on lake sediment records in central Yellowstone National Park, USA. The Holocene 5:283–292. doi: 10.1177/095968369500500303
134. Rhodes AN (1998) A method for the preparation and quantification of microscopic charcoal from terrestrial and lacustrine sediment cores. The Holocene 8:113–117. doi: 10.1191/095968398671104653
135. Brown RC, Wade TL (1984) Sedimentary coprostanol and hydrocarbon distribution adjacent to a

sewage outfall. Water Res 18:621–632. doi: 10.1016/0043-1354(84)90213-6

136. Piironen V, Lindsay DG, Miettinen TA, Toivo J, Lampi A-M (2000) Plant sterols: biosynthesis, biological function and their importance to human nutrition. J Sci Food Agric 80:939–966. doi: 10.1002/(SICI)1097-0010(20000515)80:7<939::AID-JSFA644>3.0.CO;2-C
137. Saim N, Mohammad M, Hamid RD, Osman R (2008) Simultaneous extraction, derivatization and clean-up of sterols from soil using accelerated solvent extraction. Malaysian J Anal Sci 12:500 – 504.
138. Battistel D, Piazza R, Argiriadis E, Marchiori E, Radaelli M, Barbante C (2015) GC-MS method for determining faecal sterols as biomarkers of human and pastoral animal presence in freshwater sediments. Anal Bioanal Chem. doi: 10.1007/s00216-015-8998-2
139. Wu J, Hu R, Yue J, Yang Z, Zhang L (2009) Determination of fecal sterols by gas chromatography-mass spectrometry with solid-phase extraction and injection-port derivatization. J Chromatogr A 1216:1053–1058. doi: http://dx.doi.org/10.1016/j.chroma.2008.12.054
140. Li D, Dong M, Shim WJ, Kannan N (2007) Application of pressurized fluid extraction technique in the gas chromatography-mass spectrometry determination of sterols from marine sediment samples. J Chromatogr A 1160:64–70. doi: http://dx.doi.org/10.1016/j.chroma.2007.05.036
141. Szűcs S, Sárváry A, Cain T, Ádány R, Szucs S, Sárváry A, Adány R (2006) Method validation for the simultaneous determination of fecal sterols in surface waters by gas chromatography-mass spectrometry. J Chromatogr Sci 44:70–76. doi: 10.1093/chromsci/44.2.70
142. Holtvoeth J, Vogel H, Wagner B, Wolff G a. (2010) Lipid biomarkers in Holocene and glacial sediments from ancient Lake Ohrid (Macedonia, Albania). Biogeosciences 7:3473–3489. doi: 10.5194/bg-7-3473-2010
143. Wisnieski E, Bícego MC, Montone RC, Figueira RCL, Ceschim LMM, Mahiques MM, Martins CC (2014) Characterization of sources and temporal variation in the organic matter input indicated by n-alkanols and sterols in sediment cores from Admiralty Bay, King George Island, Antarctica. Polar Biol 37:483–496. doi: 10.1007/s00300-014-1445-6
144. Burkhardt MR, ReVello RC, Smith SG, Zaugg SD (2005) Pressurized liquid extraction using water/isopropanol coupled with solid-phase extraction cleanup for industrial and anthropogenic waste-indicator compounds in sediment. Anal Chim Acta 534:89–100. doi: 10.1016/j.aca.2004.11.023
145. Elmquist M, Zencak Z, Gustafsson O (2007) A 700 year sediment record of black carbon and polycyclic aromatic hydrocarbons near the EMEP air monitoring station in Aspvreten, Sweden. Environ Sci Technol 41:6926–32.
146. Lima ALC, Eglinton TI, Reddy CM (2003) High-resolution record of pyrogenic polycyclic aromatic hydrocarbon deposition during the 20th century. Environ Sci Technol 37:53–61.
147. Jautzy J, Ahad JME, Gobeil C, Savard MM (2013) Century-long source apportionment of PAHs in Athabasca oil sands region lakes using diagnostic ratios and compound-specific carbon isotope signatures. Environ Sci Technol 47:6155–63. doi: 10.1021/es400642e
148. Argiriadis E, Rada EC, Vecchiato M, Zambon S, Ionescu G, Schiavon M, Ragazzi M, Gambaro A

(2014) Assessing the influence of local sources on POPs in atmospheric depositions and sediments near Trento (Italy). Atmos Environ 98:32–40. doi: 10.1016/j.atmosenv.2014.08.035

149. Poster DL, Schantz MM, Sander LC, Wise SA (2006) Analysis of polycyclic aromatic hydrocarbons (PAHs) in environmental samples: a critical review of gas chromatographic (GC) methods. Anal Bioanal Chem 386:859–81. doi: 10.1007/s00216-006-0771-0
150. Schkolnik G, Falkovich a H, Rudich Y, Maenhaut W, Artaxo P (2005) New analytical method for the determination of levoglucosan, polyhydroxy compounds, and 2-methylerythritol and its application to smoke and rainwater samples. Environ Sci Technol 39:2744–52.
151. 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. Anal Bioanal Chem 398:2253–2264. doi: 10.1007/s00216-010-4151-4
152. Zangrando R, Barbaro E, Zennaro P, Rossi S, Kehrwald NM, Gabrieli J, Barbante C, Gambaro A (2013) Molecular markers of biomass burning in Arctic aerosols. Environ Sci Technol 47:8565–8547.
153. You C, Yao T, Gao S, Gong P, Zhao H (2014) Simultaneous determination of levoglucosan, mannosan and galactosan at trace levels in snow samples by GC/MS. Chromatographia 77:1–6. doi: 10.1007/s10337-014-2702-0
154. Hopmans EC, dos Santos R a. L, Mets A, Sinninghe Damsté JS, Schouten S (2013) A novel method for the rapid analysis of levoglucosan in soils and sediments. Org Geochem 58:86–88. doi: 10.1016/j.orggeochem.2013.02.003
155. Pashynska V, Vermeylen R, Vas G, Maenhaut W, Claeys M (2002) Development of a gas chromatographic/ion trap mass spectrometric method for the determination of levoglucosan and saccharidic compounds in atmospheric aerosols. Application to urban aerosols. J Mass Spectrom 37:1249–57. doi: 10.1002/jms.391
156. Hsu C, Cheng C-Y, Lee C-T, Ding W (2007) Derivatization procedures and determination of levoglucosan and related monosaccharide anhydrides in atmospheric aerosols by gas chromatography-mass spectrometry. Talanta 72:199–205. doi: 10.1016/j.talanta.2006.10.018
157. Schouten S, Hopmans EC, Sinninghe Damsté JS (2013) The organic geochemistry of glycerol dialkyl glycerol tetraether lipids: a review. Org Geochem 54:19–61. doi: 10.1016/j.orggeochem.2012.09.006
158. Kuo L, Herbert BE, Louchouarn P (2008) Can levoglucosan be used to characterize and quantify char/charcoal black carbon in environmental media? Org Geochem 39:1466–1478. doi: 10.1016/j.orggeochem.2008.04.026
159. Elias VO, Simoneit BRT, Cordeiro RC, Turcq B (2001) Evaluating levoglucosan as an indicator of biomass burning in Caraja´s, Amazonia: A comparison to the charcoal record. Geochim Cosmochim Acta 65:267–272.
160. Medeiros PM, Simoneit BRT (2007) Gas chromatography coupled to mass spectrometry for analyses of organic compounds and biomarkers as tracers for geological, environmental, and

forensic research. J Sep Sci 30:1516–1536. doi: 10.1002/jssc.200600399

161. Otto A, Gondokusumo R, Simpson MJ (2006) Characterization and quantification of biomarkers from biomass burning at a recent wildfire site in Northern Alberta, Canada. Appl Geochemistry 21:166–183. doi: 10.1016/j.apgeochem.2005.09.007
162. Elhmmali MM, Roberts DJ, Evershed RP (1997) Bile acids as a new class of sewage pollution indicator. Environ Sci Technol 31:3663–3668. doi: 10.1021/es9704040
163. Chou C-C, Liu Y-P (2004) Determination of fecal sterols in the sediments of different wastewater outputs by GC-MS. Int J Environ Anal Chem 84:379–388. doi: 10.1080/03067310410001680019
164. Truong TT, Marriott PJ, Porter NA, Leeming R (2003) Application of comprehensive two-dimensional gas chromatography to the quantification of overlapping faecal sterols. J Chromatogr A 1019:197–210. doi: http://dx.doi.org/10.1016/S0021-9673(03)00766-0
165. Daughton CG (2012) Real-time estimation of small-area populations with human biomarkers in sewage. Sci Total Environ 414:6–21. doi: 10.1016/j.scitotenv.2011.11.015
166. Isobe KO, Tarao M, Zakaria MP, Chiem NH, Minh LY, Takada H (2002) Quantitative application of fecal sterols using gas chromatography − mass spectrometry to investigate fecal pollution in tropical waters : Western Malaysia and Mekong Delta, Vietnam. Environ Sci Technol 36:4497–4507. doi: 10.1021/es020556h
167. Institute for Reference Materials and Measurements (2005) Certificate of Analysis ERM® - CC580.
168. Kirchgeorg T (2015) Specific molecular markers in lake sediment cores for biomass burning reconstruction during the Holocene. Università Ca' Foscari Venezia
169. Piazza R, Gambaro A, Argiriadis E, Vecchiato M, Zambon S, Cescon P, Barbante C (2013) Development of a method for simultaneous analysis of PCDDs, PCDFs, PCBs, PBDEs, PCNs and PAHs in Antarctic air. Anal Bioanal Chem 405:917–932. doi: 10.1007/s00216-012-6464-y
170. Agilent Technologies. Backflash and its impact on GC analysis. https://www.agilent.com/cs/library/Support/Documents/a16183.pdf.
171. Little JL (1999) Artifacts in trimethylsilyl derivatization reactions and ways to avoid them. J Chromatogr A 844:1–22. doi: 10.1016/S0021-9673(99)00267-8
172. Fabbri D, Marynowski L, Fabiańska MJ, Zatoń M, Simoneit BRT (2008) Levoglucosan and other cellulose markers in pyrolysates of Miocene lignites: geochemical and environmental implications. Environ Sci Technol 42:2957–63.
173. Wilmshurst JM, Anderson AJ, Higham TFG, Worthy TH (2008) Dating the late prehistoric dispersal of Polynesians to New Zealand using the commensal Pacific rat. Proc Natl Acad Sci U S A 105:7676–80. doi: 10.1073/pnas.0801507105
174. Perry GLW, Wilmshurst JM, McGlone MS, McWethy DB, Whitlock C (2012) Explaining fire-driven landscape transformation during the Initial Burning Period of New Zealand's prehistory. Glob Chang Biol 18:1609–1621. doi: 10.1111/j.1365-2486.2011.02631.x
175. McGlone MS (1989) The Polynesian settlement of New Zealand in relation to environmental and biotic changes. N Z J Ecol 12:115–129.
176. McWethy DB, Whitlock C, Wilmshurst JM, McGlone MS, Li X (2009) Rapid deforestation of

South Island, New Zealand, by early Polynesian fires. The Holocene 19:883–897. doi: 10.1177/0959683609336563

177. Bowman DMJS, Haberle SG (2010) Paradise burnt: how colonizing humans transform landscapes with fire. Proc Natl Acad Sci U S A 107:21234–5. doi: 10.1073/pnas.1016393108
178. Butler K (2008) Interpreting charcoal in New Zealand's palaeoenvironment - What do those charcoal fragments really tell us? Quat Int 184:122–128. doi: 10.1016/j.quaint.2007.09.026
179. McGlone MS, Wilmshurst JM (1999) Dating initial Maori environmental impact in New Zealand. Quat Int 59:5–16.
180. McWethy DB, Whitlock C, Wilmshurst JM, McGlone MS, Fromont M, Li X, Dieffenbacher-Krall A, Hobbs WO, Fritz SC, Cook ER (2010) Rapid landscape transformation in South Island, New Zealand, following initial Polynesian settlement. Proc Natl Acad Sci 107:21343–21348. doi: 10.1073/pnas.1011801107
181. McKinnon M (2015) Otago places - Lakes Wānaka and Hāwea. Te Ara - Encycl. New Zeal.
182. Keane B (2012) Pounamu – jade or greenstone - Pounamu – several names. Te Ara - Encycl. New Zeal.
183. McGlone MS, Wilmshurst JM (1999) A Holocene record of climate, vegetation change and peat bog development, east Otago, South Island, New Zealand. J Quat Sci 14:239–254. doi: 10.1002/(sici)1099-1417(199905)14:3<239::aid-jqs438>3.0.co;2-9
184. Cosgrove R (1999) Forty-Two Degrees South: the Archaeology of Late Pleistocene Tasmania. J World Prehistory 13:357–402. doi: 10.1023/A:1022310029016
185. Bowdler S (2015) The Bass Strait Islands revisited. Quat Int 385:206–218. doi: 10.1016/j.quaint.2014.07.047
186. Ladd PG, Orchiston DW, Joyce EB (1992) Holocene vegetation history of Flinders Island. New Phytol 122:757–767.
187. Sim R, West D (1999) Prehistoric human occupation in the Bass Strait region, southeast Australia: an Aboriginal and an archaeological perspective. Archaeol Anthropol Landsc Shap Your Landsc 423–438.
188. Pike-Tay A, Cosgrove R, Garvey J (2008) Systematic seasonal land use by late Pleistocene Tasmanian Aborigines. J Archaeol Sci 35:2532–2544. doi: 10.1016/j.jas.2008.04.007
189. Fletcher MS, Thomas I (2010) A Holocene record of sea level, vegetation, people and fire from western Tasmania, Australia. The Holocene 20:351–361. doi: 10.1177/0959683609351903
190. Marsden-Smedley JB, Kirkpatrick JB (2000) Fire management in Tasmania's Wilderness World Heritage Area: Ecosystem restoration using Indigenous-style fire regimes? Ecol Manag Restor 1:195–203. doi: 10.1046/j.1442-8903.2000.00052.x
191. Hope G (1999) Vegetation and fire response to late Holocene human occupation in island and mainland north west Tasmania. Quat Int 59:47–60. doi: 10.1016/S1040-6182(98)00071-8
192. Mooney SD, Harrison SP, Bartlein PJ, Daniau AL, Stevenson J, Brownlie KC, Buckman S, Cupper M, Luly J, Black M, Colhoun E, D'Costa D, Dodson J, Haberle S, Hope GS, Kershaw P, Kenyon C, McKenzie M, Williams N (2011) Late Quaternary fire regimes of Australasia. Quat Sci

Rev 30:28–46. doi: 10.1016/j.quascirev.2010.10.010
193. Herschy RW (2012) Victoria Lake. Encycl. Lakes Reserv.
194. Saundry P (2012) Lake Victoria. Encycl. Earth
195. Johnson TC, Kelts K, Odada E (2000) The Holocene history of Lake Victoria. Ambio 29:2–11.
196. Kiage LM, Liu K -b. (2006) Late Quaternary paleoenvironmental changes in East Africa: a review of multiproxy evidence from palynology, lake sediments, and associated records. Prog Phys Geogr 30:633–658. doi: 10.1177/0309133306071146
197. Nicholson SE (1996) A review of climate dynamics and climate variability in Eastern Africa. Limnol Climatol Paleoclimatology East African Lakes 25.
198. Johnson TC, Scholz C a., Talbot MR, Kelts K, Ricketts RD, Ngobi G, Beuning K, Ssemmanda I, McGill JW (1996) Late Pleistocene desiccation of Lake Victoria and rapid evolution of Cichlid fishes. Science (80-) 273:1091–1093. doi: 10.1126/science.273.5278.1091
199. Verschuren D, Johnson TC, Kling HJ, Edgington DN, Leavitt PR, Brown ET, Talbot MR, Hecky RE (2002) History and timing of human impact on Lake Victoria, East Africa. Proc R Soc B Biol Sci 269:289–294. doi: 10.1098/rspb.2001.1850
200. Kendall RL (1969) An Ecological History of the Lake Victoria Basin. Ecol Monogr 39:121–176.
201. Hamilton A, Taylor D, Vogel JC (1986) Early forest clearance and environmental degradation in South-West Uganda. Nature 320:164–167. doi: 10.1038/320164a0
202. Marshall F (1990) Origins of specialized pastoral production in East Africa. Am Anthropol 92:873–894.
203. Robertshaw PT, Collett DP (1983) The identification of pastoral peoples in the archaeological record: an example from East Africa. World Archaeol 15:67–78.
204. Vincens A, Williamson D, Thevenon F, Taieb M, Buchet G, Decobert M, Thouveny N (2003) Pollen-based vegetation changes in southern Tanzania during the last 4200 years: climate change and/or human impact. Palaeogeogr Palaeoclimatol Palaeoecol 198:321–334. doi: 10.1016/S0031-0182(03)00473-5
205. Msaky ES, Livingstone D, Davis OK (2005) Paleolimnological investigations of anthropogenic environmental change in Lake Tanganyika: V. Palynological evidence for deforestation and increased erosion. J Paleolimnol 34:73–83. doi: 10.1007/s10933-005-2398-0
206. Thevenon F, Williamson D, Vincens A, Taieb M, Merdaci O, Decobert M, Buchet G (2003) A late-Holocene charcoal record from Lake Masoko, SW Tanzania: climatic and anthropologic implications. The Holocene 13:785–792. doi: 10.1191/0959683603hl665rr
207. Finch J, Marchant R (2011) A palaeoecological investigation into the role of fire and human activity in the development of montane grasslands in East Africa. Veg Hist Archaeobot 20:109–124. doi: 10.1007/s00334-010-0276-9
208. Menounos B (1997) The water content of lake sediments and its relationship to other physical parameters: an alpine case study. The Holocene 7:207–212. doi: 10.1177/095968369700700208
209. Jenkins BM, Jones AD, Turn SQ, Williams RB (1996) Emission Factors for Polycyclic Aromatic Hydrocarbons from Biomass Burning. Environ Sci Technol 30:2462–2469. doi:

10.1021/es950699m

210. Bari MA, Baumbach G, Kuch B, Scheffknecht G (2009) Wood smoke as a source of particle-phase organic compounds in residential areas. Atmos Environ 43:4722–4732. doi: 10.1016/j.atmosenv.2008.09.006

211. Muri G, Wakeham SG, Faganeli J (2003) Polycyclic aromatic hydrocarbons and black carbon in sediments of a remote alpine lake (Lake Planina, northwest Slovenia). Environ Toxicol Chem 22:1009–16.

212. Ramdahl T (1983) Retene - a molecular marker of wood combustion in ambient air. Nature 306:580–582.

213. Ogden J, Basher L, McGlone M (1998) Fire, forest regeneration and links with early human habitation: Evidence from New Zealand. Ann Bot 81:687–696. doi: 10.1006/Anbo.1998.0637

214. Tobiszewski M, Namieśnik J (2012) PAH diagnostic ratios for the identification of pollution emission sources. Environ Pollut 162:110–119. doi: 10.1016/j.envpol.2011.10.025

215. Katsoyiannis A, Sweetman AJ, Jones KC (2011) PAH molecular diagnostic ratios applied to atmospheric sources: a critical evaluation using two decades of source inventory and air concentration data from the UK. Environ Sci Technol 45:8897–906. doi: 10.1021/es202277u

216. Liu GQ, Zhang G, Li XD, Li J, Peng XZ, Qi SH (2005) Sedimentary record of polycyclic aromatic hydrocarbons in a sediment core from the Pearl River Estuary, South China. Mar Pollut Bull 51:912–21. doi: 10.1016/j.marpolbul.2005.02.038

217. Engling G, Lee JJ, Tsai Y-W, Lung S-CC, Chou CC-K, Chan C-Y (2009) Size-resolved anhydrosugar composition in smoke aerosol from controlled field burning of rice straw. Aerosol Sci Technol 43:662–672. doi: 10.1080/02786820902825113

218. Statistics New Zealand (2014) Population. http://www.stats.govt.nz/browse_for_stats/population.aspx.

219. Marx SK, Kamber BS, McGowan HA (2005) Estimates of Australian dust flux into New Zealand: quantifying the eastern Australian dust plume pathway using trace element calibrated 210Pb as a monitor. Earth Planet Sci Lett 239:336–351. doi: 10.1016/j.epsl.2005.09.002

220. McGowan HA, Kamber B, McTainsh GH, Marx SK (2005) High resolution provenancing of long travelled dust deposited on the Southern Alps, New Zealand. Geomorphology 69:208–221. doi: 10.1016/j.geomorph.2005.01.005

221. Marx SK, Kamber BS, McGowan HA (2005) Provenance of long-travelled dust determined with ultra-trace-element composition: A pilot study with samples from New Zealand glaciers. Earth Surf Process Landforms 30:699–716. doi: 10.1002/esp.1169

222. Marx SK, McGowan HA, Kamber BS (2009) Long-range dust transport from eastern Australia: a proxy for Holocene aridity and ENSO-type climate variability. Earth Planet Sci Lett 282:167–177. doi: 10.1016/j.epsl.2009.03.013

223. Whitlock C, Higuera PE, Mcwethy DB, Briles CE (2010) Paleoecological perspectives on fire ecology: revisiting the fire-regime concept. Open Ecol J 3:6–23. doi: 10.2174/1874213001003020006

224. Flinders Council (2016) Furneaux History. http://www.flinders.tas.gov.au/furneaux-history.
225. Kirkwood R, Warneke RM, Arnould JPY (2009) Recolonization of Bass Strait, Australia, by the New Zealand fur seal, Arctocephalus forsteri. Mar Mammal Sci 25:441–449. doi: 10.1111/j.1748-7692.2008.00250.x
226. Ling J (1999) Exploitation of fur seals and sea lions from Australian, New Zealand and adjacent subantarctic islands during the eighteenth, nineteenth and twentieth centuries. Aust Zool 31:323–350. doi: 10.7882/AZ.1999.036
227. Cohen AS, Scholz CA, Johnson TC (2000) The International Decade of East African Lakes (IDEAL) drilling initiative for the African Great Lakes. J Paleolimnol 24:231–235.
228. Masclet P, Cachier HH, Liousse C, Wortham H (1995) Emissions of polycyclic aromatic hydrocarbons by savanna fires. J Atmos Chem 22:41–54. doi: 10.1007/BF00708180
229. Hoffmann D, Tilgner A, Iinuma Y, Herrmann H (2010) Atmospheric stability of levoglucosan: a detailed laboratory and modeling study. Environ Sci Technol 44:694–699. doi: 10.1021/es902476f
230. 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 Chang Biol 20:2903–2914. doi: 10.1111/gcb.12583
231. Russell JM, Johnson TC (2005) A high-resolution geochemical record from Lake Edward, Uganda Congo and the timing and causes of tropical African drought during the late Holocene. Quat Sci Rev 24:1375–1389. doi: 10.1016/j.quascirev.2004.10.003

APPENDIX

Page I-X:

GC-MS method for determining faecal sterols as biomarkers of human and pastoral animal presence in freshwater sediments

Page XI-XXV:

Late Holocene Record of Humans and Fire at Lake Trasimeno (Italy)

Anal Bioanal Chem
DOI 10.1007/s00216-015-8998-2

RESEARCH PAPER

GC-MS method for determining faecal sterols as biomarkers of human and pastoral animal presence in freshwater sediments

Dario Battistel[1,2] · **Rossano Piazza**[1,2] · **Elena Argiriadis**[1] · **Enrico Marchiori**[1] · **Marta Radaelli**[2] · **Carlo Barbante**[1,2]

Received: 12 July 2015 / Revised: 17 August 2015 / Accepted: 20 August 2015

Abstract In order to determine sterols and stanols in freshwater sediments to reconstruct the past presence of humans and pastoral animals, we developed an analytical method based on pressurised liquid extraction (PLE), clean-up performed using solid phase extraction (SPE) and sterol determination using gas chromatography–mass spectrometry (GC-MS) analysis. PLE extraction conditions were optimised using dichloromethane (DCM) and DCM/methanol mixtures. Clean-up was performed with 2 g silica SPE cartridges, and the concentrated extracts were eluted with 70 mL DCM. Extraction yield was evaluated using an in-house reference material spiked with ^{13}C-labelled cholesterol and aged for 10 days. In comparison with pre-extraction, where the sediment is extracted and then spiked with a known analyte concentration, this approach preserves the original composition of the sediment. DCM and DCM/methanol mixtures resulted in high extraction yields ranging from 86 to 92 % with good reproducibility (relative standard deviation (RSD) 5–8 %). PLE extraction yields obtained with DCM as the extracting solvent were about 1.5 times higher than extractions using an ultrasonic bath. The solvent extraction mixture and matrix composition strongly affected the solvent extraction composition where higher overall recoveries (70–80 %) for each compound were obtained with DCM. The extraction mixture and matrix composition also affected the analyte concentrations, resulting in a method precision ranging from 1 to 18 %. Diatomaceous earth spiked with 10 to 100 ng of sterols, and environmental samples fortified with suitable amounts of sterols provided apparent recovery values ranging from 90 to 110 %. We applied the method to environmental samples both close to and upstream from sewage discharge zones, resulting in substantially higher faecal sterol (FeSt) concentrations near the sewage. In addition, we also applied the method to a 37-cm freshwater sediment core in order to evaluate its applicability for obtaining vertical sterol profiles.

Keywords Faecal sterols · GC-MS · Freshwater sediments · Pressurised liquid extraction (PLE) · Biomarkers

Introduction

Faecal biomarkers are steroids that include stanols and sterols, which are commonly examined together as faecal sterols (FeSt). 5β-stanols are produced by the reduction of cholest-5-en-3β-ol (cholesterol) and other congeners (sitosterol, campesterol and stigmasterol) [1]. The microbial reduction of sterols in the intestine of higher mammals leads to the formation of 5β(H) rather than 5α(H) stereoisomers [2, 3]. Thus, cholesterol and phytosterols are almost completely converted into the corresponding hydrogenated stanols such as coprostanol, sitostanol and stigmastanol [1]. Coprostanol represents ~60 % of the total sterols in human faeces and therefore indicates the presence of humans [4, 5], whilst stigmastanol is mainly produced by ruminants, including domesticated pastoral animals, and therefore indicates the presence of grazing animals [1, 6]. Moreover, 5α-isomers can be produced in situ from sterols by biohydrogenation [7].

✉ Dario Battistel
dario.battistel@unive.it

[1] Department of Environmental Science, Informatics and Statistics, University Ca' Foscari of Venice, Via Torino, 155, 30172 Venezia Mestre, Venice, Italy

[2] Institute for the Dynamics of Environmental Processes, IDPA/CNR, Via Torino, 155, 30172 Venezia Mestre, Venice, Italy

Published online: 05 September 2015

Although sterols and stanols are relatively stable over timescales as long as thousands of years, and across a wide range of environmental and anaerobic conditions [7–10], aerobic bacteria can degrade coprostanol in situ and form the epimer 5β-cholestan-3α-ol (epicoprostanol) [1, 11]. The degradation rate strongly decreases with burial depth as anoxic conditions inhibit degradation [5, 12]. The highest average biodegradation rates occur in sediments from non-aerated coastal waters and amount to 0.438 μg g^{-1} day^{-1} [13]. This relative stability [14–16] suggests that these compounds can be successfully employed as molecular tracers in archaeological contexts or integrated into paleoclimatic reconstructions [17, 18]. Recently, D'Anjou et al. [18] proposed supplementing climatic information obtained from lacustrine sediments with the unequivocal signature of anthropogenic activity provided by faecal biomarkers. Due to the hydrophobic nature of these compounds, faecal biomarkers preferentially associate with particulate matter, hindering their removal in aquatic environments [1], and therefore can be preserved in sediments for millennia. The ratios of diverse FeSts such as coprostanol or phytostanol can differentiate between the past presence of humans and/or other mammals in the areas surrounding the lake sediments [19–25].

The literature contains methods for determining faecal sterols in water, sediments and soil using various extraction, clean-up and derivatisation procedures, followed by gas chromatography–mass spectrometry (GC-MS) or gas chromatography–flame ionization detector (GC-FID) analyses [17, 20, 26–45]. These methods involve various extraction, clean-up and derivatisation procedures, followed by GC-MS or GC-FID analyses. Conventional extraction methods include the following: ultrasonic bath [37–39] which results in relatively fast extractions and does not require expensive instrumentation; saponification followed by liquid extraction [40]; and Soxhlet [15, 20, 41–46] that, although time consuming, offers high extraction yields. Scientists developed pressurised liquid extraction (PLE) in order to improve the extraction selectivity of target compounds, increase the extraction efficiency as well as reduce time and solvent consumption [47]. The elevated temperatures and pressures when using PLE as an extraction method increase the overall extraction capabilities [47, 48]. Researchers successfully employed PLE to extract sterols in polluted marine sediments [30, 38, 45] and biological matrices [49], where sterol concentrations range between 1 and 100 μg g^{-1} dry weight [6, 9, 14, 15]. PLE has never been tested in environmental archives such as in deep freshwater sediment cores where sterol concentrations are generally lower.

Despite the recognised advantages of PLE extraction, environmental matrices still often result in dirty extracts [50]. In freshwater sediment samples, where the organic matter content is generally high, removing the matrix background is often challenging and requires post-extraction steps. Solid phase extraction (SPE) cartridges or home-made purification columns filled with silica [17, 36] or florisil [30, 50] as their stationary phase can help clean the extracted samples. These SPE methods are attractive because they are rapid, efficient and use much less solvent than other techniques.

The quantification and recovery of sterols are commonly performed via the internal standard method, using deuterated standards such as cholesterol-2,2,3,4,4,6-d_6 [6, 39] and 17β-estradiol-2-4-16-16-d_4 [9], or native compounds that are assumed to be absent from the sample (e.g., 5β-pregnan-3-ol [15, 17], 5α-cholestane [29, 51], 5α-androstan-3β-ol [14, 16]). Deuterium-hydrogen exchange may occur with highly deuterated compounds, although these reactions are mainly activated by adjacent carbonyl groups or aromatic systems [52]. Although rare, such deuterium-hydrogen exchange can occur in sterols [53].

We therefore examine coprostanol, epicoprostanol, cholesterol, cholestanol, sitosterol and stigmastanol as biomarkers for reconstructing the presence of humans and pastoral animals as archived in freshwater sediment cores. As sterol concentrations are considerably lower in deep freshwater sediment samples than in modern and/or polluted sediments [45], the high extraction efficiency achievable with PLE is essential for determining FeSt concentrations in deep freshwater sediments.

Here, we propose a PLE extraction method, followed by SPE clean-up and GC-MS analysis, to determine the previously mentioned six faecal sterols in freshwater sediments. We evaluated the PLE extraction efficiency, matrix background interference and overall recovery using four different solvent mixtures. We also compared the PLE extraction yield which was also compared with extractions using an ultrasonic bath in the same conditions. This method employs ^{13}C-labelled cholesterols as internal and surrogate standards that, to the best of our knowledge, were never tested in GC-MS steroid analysis. Analytical performance was evaluated in terms of limit of detection (LOD) and quantification (LOQ), recovery, apparent recovery and precision. We also tested the method both in potentially polluted environmental samples and in deep riverine sediment samples to encompass a possible range of FeSt concentrations.

Materials and methods

Standards and reagents

Pesticide grade methylene chloride (DCM), *n*-hexane (Hex), acetone and methanol (MeOH) (Romil Ltd., Cambridge, UK) were used. Anhydrous Na_2SO_4 (Carlo Erba Reagenti S.p.A., MI, Italy) was oven-dried at 150 °C for 24 h, washed with both DCM and *n*-hexane and then stored with *n*-hexane. Metallic copper (Carlo Erba Reagenti S.p.A., MI, Italy) was

activated by rinsing it 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. 5β-cholestan-3β-ol (CoP), 5β-cholestan-3α-ol (*e*-CoP), cholest-5-en-3β-ol (Chl), 5α-cholestan-3β-ol (5α-Ch), 24-ethyl-5α-cholestan-3β-ol (5α-Sit) and 24-ethyl-cholest-5-en-3b-ol (Sit), ^{13}C-labelled cholesterol-25,26,27-$^{13}C_3$ ($^{13}C_3$-Chl) and cholesterol-3,4-$^{13}C_2$ ($^{13}C_2$-Chl) were used as surrogate and internal standards, and the derivatisation reagent *N*,*O*-bis(trimethylsilyl)trifluoroacetamide (BSTFA) with 1 % trimethylchlorosilane (TMCS) was purchased from Sigma-Aldrich. A mixture of working standards, containing 1 and 10 μg mL^{-1} of each compound, and surrogate and internal standards, each containing 1 μg mL^{-1} ^{13}C-labelled cholesterols, were prepared in DCM. All the standard solutions were stored at −20 °C. All the tools and glassware were washed with an aqueous 5 % (*v*/*v*) Contrad® solution (Decon Laboratories Limited, UK), dried and rinsed with dichloromethane.

We tested the method with the European Reference Material for estuarine sediment ERM®-CC580. The use of ERM®-CC580, although certified for mercury and methylmercury, guarantees the availability of a sediment sample for inter- and/or intra-laboratory comparison.

Sediment samples

We collected surface sediments from the Sile River close to Casier di Treviso (45° 38′ N, 12° 17′ E) in northeastern Italy. The sediments were dried and weighed in order to obtain a 50-g sample. River sediments were sieved (<2 mm) and finely ground using a mortar.

We also collected surface river sediments (RPS) from the Piave River (45° 38′ N, 12° 32′ E) in northeastern Italy both close to a sewage drainage area (RPS1 and RPS3) and approximately 6 km upstream from the sewage site (RPS2). We collected a 37-cm sediment core using a manual plexiglass piston corer (6 cm diameter) at RPS3 and divided this core into five 1-cm-thick samples at depths of 0–1, 10–11, 20–21, 30–31 and 36–37 cm. These depths were labelled RPS3^{i}, where *i* corresponds to the depth of collection. All the samples were dried at room temperature until reaching a constant weight. River sediments were sieved (<2 mm) and finely ground in a mortar.

Extraction

Samples were extracted via a PLE system (FMS, Inc., Watertown, MA, USA) equipped with stainless steel extraction cells (20 mL). The system was cleaned twice with dichloromethane at 100 °C and 2,000 psi before processing each sample. The extraction cells were prepared by first placing ~5 mm of Ottawa sand at the bottom of the cell and then adding ~1 g of dried sample, dispersed in diatomaceous earth and spiked with 100 μL of surrogate standard solution at a concentration of 1 μg mL^{-1}. The cell was then filled with diatomaceous earth and topped with ~2 g of activated copper and a small amount of Ottawa sand. We tested PLE extractions using different solvent mixtures (DCM; DCM/MeOH 9:1 *v*/*v*, DCM/MeOH 2:1 *v*/*v* and DCM/Hex 1:1 *v*/*v*) at temperatures of 150 °C and pressures of 1,500 psi. The clean extracts were then concentrated to ~0.5 mL under a nitrogen stream by using a TurboVap II® system (Caliper Life Science, Hopkinton, MA, USA).

In order to determine the extraction yield, 25 g of dried Sile River sediments was wet with DCM and spiked with 100 μL of 1 μg mL^{-}of $^{13}C_3$-Chl. The sample was left for 10 days at room temperature until the DCM completely evaporated and then the sample was homogenised. This spiked sediment will be afterwards indicated as WS-13C.

Sterols naturally interact with organic matter present in sediments within 12 h [54]. Pre-extracting sterols from sediments by using DCM for sterol decontamination could alter the original sediment composition. We argue that introducing a ^{13}C-labelled sterol that is not present in the original matrix does not alter the sediment composition and is therefore a better option when evaluating sterol extraction performances in sediments.

Clean-up

Normal SPE cartridges (DSC-Si Tube; 12 mL; 52657 Supelco, Sigma-Aldrich) packed with 2 g silica gel (particle size 50 μm) were topped with anhydrous Na_2SO_4, and the column was preconditioned with DCM. The concentrated lipid extract was then transferred onto the top of the cartridge. The fraction containing steroids was eluted with 70 mL DCM, without separating sterols and stanols, and concentrated to about 0.1 mL by TurboVap II®. The residue was gently dried under a stream of nitrogen and then re-dissolved in 100 μL of internal standard solution (1 μg mL^{-1} $^{13}C_2$-Chl). In order to determine the extraction yield, we added the internal standard ($^{13}C_2$-Chl) immediately after the extraction step.

Derivatisation

We derivatised samples by adding 100 μL BSTFA 1 % TMCS to the concentrated fraction containing sterols and stanols, leading to the silylation of the steroids. This solution was heated at 70 °C for 1 h. The solution was cooled at room temperature, and 2 μL of solution was injected into the GC-MS system.

Instrumentation

The silylated steroids were analysed via gas chromatography–mass spectrometry with a 6,890-N GC system coupled to a quadrupole mass spectrometer 5973 (Agilent Technologies, Santa Clara, CA, USA). The chromatographic analysis was carried out using a capillary column (HP 5 ms, 60 m length, 0.25 mm inner diameter and 0.25 μm film thickness; Agilent Technologies, Santa Clara, CA, USA). He flow (1 mL min^{-1}) was used as a carrier. The inlet temperature was set to 280 °C, and 2 μL was injected in splitless mode (1 min splitless time). The solvent delay was 8 min. For the analysis of sterol and stanol derivatives, the temperature programme used was as follows: 70 °C (held for 2 min) to 210 °C at 20 °C min^{-1} then to 300 °C at 3 °C min^{-1} and held for 8 min. The interface temperature was 280 °C, the mass selective detector (MSD) ion source temperature was 230 °C and MSD quadrupole was 150 °C. Electron ionisation was performed at 70 eV. Measurements in full scan mode identified peaks, whilst measurements in selected ion monitoring (SIM) were performed for quantification. We report the quantification (I^Q) and confirmation (I_i^C) ions in Table 1. FeSt quantification was performed by comparing the native compound peak areas with that of ^{13}C-labelled cholesterols (surrogate and internal standards). Results were corrected for the instrumental response factor, which was checked periodically with a DCM solution containing 1 μg mL^{-1} of each FeSt (including both surrogate and internal standards).

Total organic carbon (OC) analyses were performed using TOC-5050A with SSM 5000A (Shimadzu). OC content was determined by following the procedure reported in the Italian certification for analysing organic carbon in sediments, UNI-EN 13137[55]. Briefly, 100 mg of dry sediment was treated with 2.5 mol L^{-1} HCl in order to remove inorganic carbonates and bicarbonates at 120 °C for 30 min. An aliquot of treated sediment (5–10 mg) was analysed, and the amount of OC was normalised by the corrected weight, thereby taking into account the mass loss due to interactions with HCl. Calibration curves were obtained by using glucose standard solutions at different concentrations.

Sonic bath extraction was performed using a Dentsply Neytech 57 H Cleaner (335 W).

Limit of detection, limit of quantification and linearity

Instrumental limit of detection (I-LOD) and quantification (I-LOQ) for each FeSt were determined as the sample concentrations resulting in a peak area with a signal-to-noise ratio of at least 3:1. LOQ was the lowest concentration that could be determined with a signal-to-noise ratio of at least 10:1 [56]. Laboratory limit of detection (L-LOD) and quantification (L-LOQ) were defined as the mean concentrations of the procedural blanks plus three and ten times the standard deviations, respectively [56]. The linearity of the detector response was determined by analysing a mixture of working standards, containing FeSt concentrations from 0.01 to 20 μg mL^{-1} and a constant amount of $^{13}C_2$-Chl (10 μg mL^{-1}) in order to correct the possible loss of solvent during derivatisation.

Results and discussion

Gas chromatography–mass spectrometry

Typical total ion current (TIC) and selected ion monitoring (SIM) chromatograms of FeSt standard mixture (1 μg mL^{-1} of each compound) are reported in Fig. 1. The run time was 47 min, and all the sterols were fully separated. It is worth noting that, although CoP and *e*-CoP have very similar structures, the HP-5ms capillary column possesses a suitable stationary phase to separate of these compounds (TIC, top panel of Fig. 1). However, we were not able to achieve a complete separation when the temperature ramp slope exceeded 3 °C min^{-1} between 210 and 300 °C. This separation is crucial in order to distinguish human faecal inputs from those of other mammals [5, 23], as previously discussed. The retention times

Table 1 Target (T) and qualifier (Q) ions of *m/z* applied to the GC-MS quantitation and their corresponding ratios

Analyte	Abbreviation	t_R (min)	I^Q	I_1^C	I_2^C	I^Q/I_1^C	I^Q/I_2^C
5β-cholestan-3β-ol	CoP	36.18	370	215	257	1.05	2.56
5β-cholestan-3α-ol	e-CoP	36.45	370	215	257	0.52	1.75
cholest-5-en-3β-ol	Chl	39.09	368	353	460	1.56	3.33
5α-cholestan-3β-ol	5α-Ch	39.41	370	460	332	1.12	5.02
24-ethyl-cholest-5-en-3β-ol	Sit	45.41	396	215	486	2.43	3.85
24α-ethyl-5α-cholestan-3β-ol	5α-Sit	45.82	473	488	215	1.69	0.11
Cholest-5-en-3β-ol-3,4$^{13}C_2$[a]	$^{13}C_2$-Chl	39.09	370	355	–	1.44	–
Cholest-5-en-3β-ol-25,26,27$^{13}C_3$[b]	$^{13}C_3$-Chl	39.09	332	371	–	1.49	–

[a] Internal standard

[b] Surrogate standard

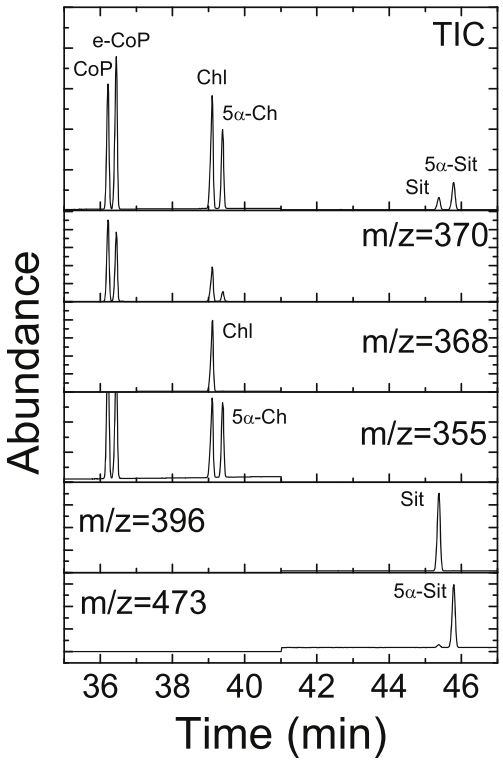

Fig. 1 TIC (top) and SIM chromatograms for sterols and stanols derivatives in a standard mixture (1 μg mL^{-1})

(t_R), determined from the chromatograms and the I^Q/I^C ratios, are also reported in Table 1.

In order to determine sterols and stanols by gas chromatography, a prior derivatisation step is generally required to obtain more volatile compounds, and thereby improving chromatographic separation and detection limits. This step often involves silylation using BSTFA and TMCS as a catalyst. The reaction usually takes place at 60–70 °C and provides quantitative results within 1–2 h [15, 17, 26, 28, 57]. The stability of the silylated products and the reproducibility of our method (i.e., 70 °C for 1 h) were assessed by analysing five replicates (n=5) of silylated standard solutions (1 μg mL^{-1}) that were injected every 1–2 days into the GC-MS for 2 weeks after derivatisation. The reproducibility, in terms of relative standard deviation (RSD), of the ratios between each sterol/stanol and $^{13}C_2$-Chl peak areas ranged between 4 and 6 %. Furthermore, RSD values did not vary significantly within at least 3 days of the derivatisation step. However, for all the investigated FeSts, RSD increased significantly (>10 %) after 5–6 days. Several authors suggest evaporating the excess of the silylation reagent and re-dissolving the derivatives in other solvents such as toluene or *n*-hexane [9, 17, 36]. In our experience, there are no drawbacks to directly inject the solution containing BSTFA in the GC-MS system. The stability of the stationary phase of the column was not compromised after several hundreds of injections, and we did not observe any interferences in the instrumental blanks.

PLE extraction

Generally, faecal sterols are partially excreted in esterified forms. Esterified coprostanol, for instance, comprises 8–15 % of total sterols in sewage [58]. For this reason, saponification reactions are sometimes employed to increase the extraction efficiency [17]. Nevertheless, Isobe et al. [36] found that this precaution led to an increase in yields below 20 % for most of the analysed FeSts and discouraged the saponification step. In this work, we omitted the saponification step as it hinders analysing a large number of samples in a shorter amount of times [36, 58].

In order to evaluate the best extraction conditions, 1 g of WS-13C was preliminarily extracted in a 2:1 *v*/*v* DCM/MeOH mixture at 150 °C and 1,500 psi, with 5 min of static time and two cycles of extraction, following the experimental conditions reported in the literature for marine sediments [30]. The extraction was performed in triplicate (n=3) where the extracts were immediately spiked with 100 ng g^{-1} $^{13}C_2$-Chl as an internal standard. The amount of effectively extracted native sterols was determined using $^{13}C_2$-Chl as internal standard (see Table 2). The extraction yield was determined quantifying the amount of $^{13}C_3$-Chl compared to $^{13}C_2$-Chl with respect to the 100-ng g^{-1} initially spiked into WS-13C. Although these conditions result in a high extraction yield (86.9 %) and reproducibility (RSD=7.4 %) (Table 2), the I^Q/I^C ratio of some sterols, such as 5α-cholestanol and sitosterol, strongly differs from the ratios obtained in standard solutions (I^Q/I^C ratios in Tables 1 and 2) and could be attributed to the matrix effect.

In order to overcome this drawback and to optimise the method, we evaluated the performances of different extraction solvent mixtures. The extraction yield did not significantly change when using DCM or DCM/MeOH mixtures but strongly decreased for the DCM/Hex mixture (Table 2). The interferences observed when extracting with DCM/MeOH mixtures for 5α-cholestanol and sitosterol are not observed in DCM and DCM/Hex (Tables 1 and 2). These results suggest that, at least for this sediment sample, the increase in polarity promotes the extraction of compounds that produce a fragmentation pattern that could interfere with the monitoring of selected ions. On the other hand, the decrease of the polarity of the extraction mixture that limited the interference effect was associated with a decrease in the extraction yield to 44.7 %. In light of these results, FeSt extractions using only DCM provide the best extraction yield with only a negligible matrix effect.

We compared the extraction yield of each compound using different solvent mixtures with the sterol amounts extracted with only DCM. In all cases, the extraction yields were lower compared to extraction yields using only DCM, and, in particular, DCM/MeOH mixtures were less selective for

Table 2 FeSt concentrations in WC-13C extracted with different solvent mixtures and their corresponding I^Q/I^C ratios

Sterols extracted (ng g^{-1})						I^Q/I^C ratios							
Sample	Analyte	DCM	DCM/MeOH 9:1	DCM/MeOH 2:1	DCM/Hex 1:1	DCM		DCM/MeOH 9:1		DCM/MeOH 2:1		DCM/Hex 1:1	
						$I^Q/I_1{}^C$	$I^Q/I_2{}^C$	$I^Q/I_1{}^C$	$I^Q/I_2{}^C$	$I^Q/I_1{}^C$	$I^Q/I_2{}^C$	$I^Q/I_1{}^C$	$I^Q/I_2{}^C$
WS-13C	CoP	272.5	262.1	246.3	186.4	1.04	2.41	1.01	2.64	0.97	2.78	0.99	2.45
WS-13C	e-CoP	68.1	67.3	66.0	50.5	0.50	1.62	0.45	1.68	0.56	1.78	0.48	1.80
WS-13C	Chl	379.8	363.7	361.6	201.9	1.49	3.37	1.42	3.45	1.52	3.61	1.43	3.52
WS-13C	5α-Ch	365.0	348.1	346.7	162.6	1.08	5.31	0.52	5.61	0.44	6.02	1.11	5.22
WS-13C	Sit	186.9	170.0	144.1	111.9	2.35	3.94	1.78	3.82	1.19	3.81	2.32	3.78
WS-13C	5α-Sit	268.1	216.1	190.1	115.6	1.74	0.15	1.80	0.18	1.78	0.20	1.71	0.14
WS-13C	$^{13}C_3$-Chl	Extraction yield % (RSD)											
		91.1 (5.2)	87.2 (6.7)	86.9 (7.4)	44.7 (6.7)								
WS-13C	$^{13}C_3$-Chl	Recovery % (RSD)											
		71.3 (6.0)	46.2 (7.8)	36.5 (9.1)	13.0 (8.5)								

Extraction yield and recovery evaluated for $^{13}C_3$-Chl

phytosterols, where this lack of selectivity depends on the amount of methanol present in the mixture (Fig. 2).

Moreover, in order to compare PLE extraction system with an ultrasonic bath, we also extracted 1 g of WS-13C three times by sonicating with DCM (20 mL and 10 min for each cycle). The extracts were immediately spiked with an internal standard of 100 ng $^{13}C_2$-Chl. This experiment was repeated in triplicate (n=3). Extraction efficiency, determined using the previously described methods, was 66 % which is considerably less than the extraction efficiency in samples using the PLE system. Moreover, the reproducibility of the ultrasonic bath extractions was poor (RSD=19 %) with respect to the reproducibility of extractions using PLE techniques (Table 2).

Clean-up

The clean-up procedure used SPE cartridges to purify stanols and sterols in sediment extracts, utilising the Isobe et al. method [36] with small modifications. The SPE cartridge containing H_2O-deactivated silica gel was initially conditioned with DCM until the stationary phase appeared to be homogeneous and translucent (corresponding to solvent volumes of ~20 mL). In order to investigate compound elution, we pipetted 100 ng of all native sterols mixed with 500 μL DCM. The analytes were eluted with DCM, and we collected ten fractions of 10 mL each. After elution, 100 ng $^{13}C_3$-Chl was spiked in each fraction and analysed by GC-MS. The elution volumes obtained in these operative conditions showed that all the FeSt completely eluted in 50 mL. However, the volume required for eluting all the analytes also depends on their concentrations. The volume required for the complete elution of the analytes was ~70 mL when pipetting 2 μg of native sterols in 500 μL DCM. In this method, we propose using 70 mL DCM for elution, as 2 μg of FeSt corresponds to a final concentration of 10 μg mL^{-1} before injection and therefore falls within the range of linearity evaluated in this paper.

Recovery

The recovery of the overall method was evaluated using high (100 ng) and low (10 ng) amounts of all native FeSt and surrogate standards ($^{13}C_3$-Chl) spiked on to diatomaceous earth and labelled as D-10 and D-100. After extraction, preconcentration and clean-up, the extract volume was reduced to dryness and re-dissolved in 100 μl of a solution containing 1.0 or 0.1 μg mL^{-1} C_2-Chl before derivatisation, for D-10 and D-100, respectively. The recovery of the method was evaluated by quantifying FeSt using $^{13}C_2$-Chl as an internal standard and comparing the results with 100 or 10 ng of initially spiked sterols (Table 3). In order to evaluate the matrix effect on the recovery, we also analysed the environmental sample WS-

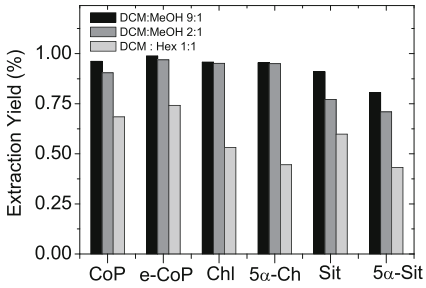

Fig. 2 Extraction yield for each compound obtained using DCM/MeOH 9:1, DCM/MeOH 2:1 and DCM/Hex 1:1

Table 3 Recovery%, RSD, apparent recovery and concentrations (C) in D-10, D-100 with different extraction mixtures and environmental samples

			CoP	e-CoP	Chl	5α-Ch	Sit	5α-Sit
D-10	DCM	Recovery %	70.2	71.3	n.d.	n.d.	n.d.	73.2
		RSD	12.4	14.3	n.d.	n.d.	n.d.	18.4
		Apparent recovery	89.4	96.2	n.d.	n.d.	n.d.	111.2
	DCM/MeOH 9:1	Recovery %	46.5	46.8	n.d.	n.d.	n.d.	48.4
		RSD	21.5	22.8	n.d.	n.d.	n.d.	27.4
		Apparent recovery	87.6	97.2	n.d.	n.d.	n.d.	117.2
D-100	DCM	Recovery %	74.1	74.5	77.8	78.0	78.8	79.6
		RSD	2.7	2.5	5.4	6.8	6.4	4.7
		Apparent recovery	94.2	98.7	100.4	102.2	105.8	110.3
	DCM/MeOH 9:1	Recovery %	52.6	52.4	55.5	56.5	61.1	59.8
		RSD	0.9	2.0	6.2	8.7	7.8	3.1
		Apparent recovery	89.9	100.0	102.3	104.5	110.5	116.6
	DCM/MeOH 2:1	Recovery %	45.5	47.1	47.5	48.1	50.2	52.7
		RSD	4.2	8.6	9.1	10.8	9.9	7.5
		Apparent recovery	84.1	95.2	98.7	103.6	110.7	118.5
	DCM/Hex 1:1	Recovery %	22.2	21.8	24.5	28.5	28.4	32.3
		RSD	14.2	15.5	8.9	12.2	15.8	17.3
		Apparent recovery	95.4	101.2	103.3	105.8	107.7	110.8
ERM®-C580	DCM	C (ng g^{-1} d.w.)	1,442.3	551.2	2,549.1	2,067.6	1,734.1	214.3
		RSD	3.4	2.7	3.6	3.4	4.2	4.9
RPS1	DCM	C (ng g^{-1} d.w.)	503.3	36.3	505.2	110.3	247.2	119.5
		RSD	5.3	6.9	10.8	6.4	12.8	6.6
RPS2	DCM	C (ng g^{-1} d.w.)	53.4	19.6	169.3	132.0	1,752.1	1,110.1
		RSD	9.5	8.9	7.8	7.5	6.4	5.1
$RPS2^F$	DCM	C (ng g^{-1} d.w.)	160.5	128.8	–	–	–	–
		RSD	5.8	6.2	–	–	–	–
		Apparent recovery	107.1	109.2	–	–	–	–

Concentrations below procedural blank values are considered to be non-determinable

n.d. non-determinable

13C ($n=3$) where 100 ng $^{13}C_2$-Chl was added before derivatisation (Table 2).

The recovery of sterols is higher for both diatomaceous earth and in WS-13C when using DCM (Table 3). However, although the presence of MeOH in the mixture did not significantly decrease the extraction yield, MeOH resulted in a decrease of the recovery. This decrease is probably due to the pre-concentration step, where the presence of methanol slows down the evaporation process and promotes the stripping of the analytes. The presence of the matrix certainly enhanced this analyte loss (Tables 2 and 3). Comparing sterols demonstrates that recoveries were slightly higher for phytosterols than for CoP and *e*-CoP where this difference is probably due to the lower volatility of phytosterols. Low recovery values obtained from a DCM/Hex 1:1 mixture are mainly due to the previously discussed low extraction yield. As extracting FeSt with only DCM results in elevated extraction efficiency, higher recoveries and negligible interference, DCM is the preferred extracting solvent condition, and we use DCM in the following experiments.

Linearity LOD, LOQ and blanks

Linearity, instrumental and laboratory LOD and LOQ were determined as described in the "Materials and methods" section. Calibration curves were obtained by correcting the concentration values of each FeSt using the internal standard response factor. The concentrations compared to the chromatographic signal are linear (r^2 was 0.999 for CoP, 0.999 for *e*-CoP, 0.998 for Chl, 0.997 for 5α-Ch, 0.997 for Sit, 0.997 for 5α-Sit) when using up to 10 ng of each compound on the column (oc). I-LODs and I-LOQs) were 11 (37)pg oc for CoP, 13 (43)pg oc for *e*-CoP, 22 (73)pg oc for Chl, 120 (399)pg oc for 5α-Ch, 86 (286)pg oc for Sit and 25 (83)pg oc for 5α-Sit.

The contribution of the solvents, PLE extraction system, inert materials, SPE cartridges and the laboratory environment to the samples was evaluated by extracting a 20-mL vessel filled with diatomaceous earth and Ottawa sand and then spiking this material with 10 ng $^{13}C_3$-Chl as a surrogate standard to create six procedural blanks. L-LOD and L-LOQ for each FeSt were calculated following the procedure described in the "Materials and methods" sections. FeSt concentrations in procedural blanks were less than three times the *S*/*N* ratio except for in the cases of Chl and Sit. The L-LOD was 120 and 202 pg oc for Chl and Sit and L-LOQ 400 and 673 pg oc, respectively. The presence of Chl and Sit may be due to the contamination from atmospheric aerosols that could contain these sterols as by-products of biomass burning, especially in urban environments [59].

Precision study

The method precision, in terms of RSD, was first evaluated by repeatedly measuring (n=4) D-10 and D-100. For higher concentrations (100 ng), the method provides RSD <7 % for each compound extracted in DCM as reported in Table 3. For lower concentrations (10 ng), RDS values are lower when extracting with DCM (12–18 %) with respect to DCM/MeOH 9:1 (21–28 %).

The precision of the method was also tested in certified estuarine sediment (ERM®-CC580) and in sediments collected in the Piave River, where these sediments were only extracted in DCM, where our preference for DCM is discussed above. We extracted four replicates (n=4) from 1 g of ERM®-CC580, where Fig. 3A demonstrates a typical TIC chromatogram obtained from ERM®-CC580. The concentrations and RSD values obtained for ERM®-CC580 (Table 3) demonstrate that the RSD for each compound is comparable with the values obtained for spiked diatomaceous earth. Possible matrix interferences are negligible due to the high concentrations of each FeSt. The fact that the I^Q/I^C values did not statistically differ from those obtained in standard solutions further supports the idea that the matrix did not interfere with the FeSt concentrations in the certified estuarine sediment.

Due to the high concentrations of each FeSt in ERM®-CC580, we also tested the method on two RPS samples located near a sewage discharge location (RPS1) as well as ~6 km upstream (RPS2) from the sewer discharge in the Piave River (Fig. 3B, C). The matrix interference was negligible in these samples, and the I^Q/I^C values agreed with previously obtained values. In contrast to ERM®-CC580, the absolute amounts of FeSt are generally lower in the Piave River, and the RSD is slightly higher (Table 3). The precision of the method ranged between 0.9 and 17 % for all sterols and strongly depends on the kind of extracted sediment and the FeSt concentrations.

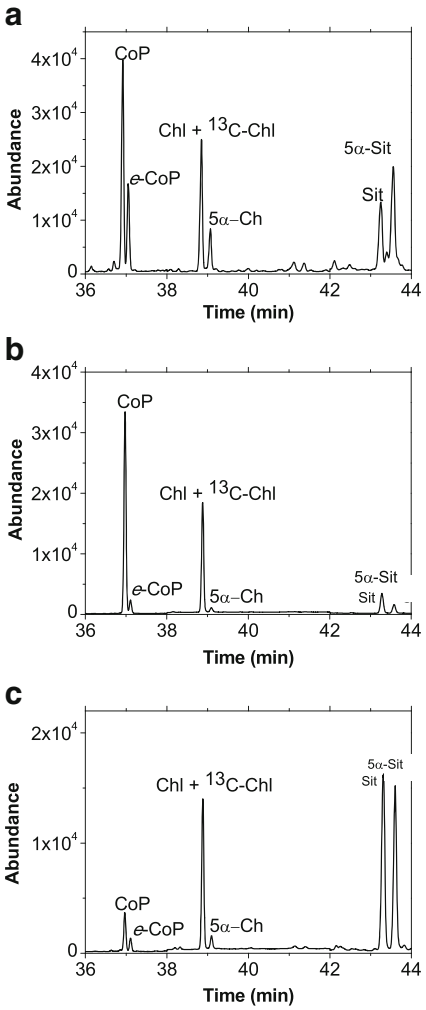

Fig. 3 Total ion chromatogram for sterol and stanol derivatives for ERM®-CC580 (**A**), RPS1 (**B**) and RPS2 (**C**)

Apparent recovery

The apparent recovery, as defined by IUPAC [60], was determined in D-10 and D-100 and represented a measure of the accuracy of the method. For D-100, the apparent recovery was determined for all considered solvent mixtures. Apparent recovery values for DCM ranged from 94.2 to 110.3 % of the expected value for every FeSt (Table 3). In particular, the apparent recovery values increase from the most volatile compound (CoP) to the least volatile (5α-Sit), following the chromatographic elution order (Fig. 1). Since the surrogate standard ($^{13}C_3$-Chl) elutes at retention times between the times of these two latter compounds, this occurrence leads to a respective progressive underestimation/overestimation of the most/less volatile compounds. For the other solvent mixtures employed in D-100, the apparent recovery covered a wider range from 84.1 to 118.5 % depending on the solvent mixture and the FeSt. For D-10, apparent recoveries did not significantly differ from the values obtained for higher

Table 4 FeSt concentrations and major index for RPS3 samples collected in the Piave River

	Depth (cm)	CoP (ng mg^{-1}_{OC})	e-CoP (ng mg^{-1}_{OC})	Chol (ng mg^{-1}_{OC})	5α-Chl (ng mg^{-1}_{OC})	Sit (ng mg^{-1}_{OC})	5α-Sit (ng mg^{-1}_{OC})	Cop/*e*-CoP	5β-index	CoP/Chl	Cop/5α-Sit	Sit/Chol
$RPS3^{1}$	0–1	575.3	43.8	316.0	46.8	601.31	217.9	13.12	0.93	1.82	2.65	1.90
$RPS3^{11}$	10–11	258.6	21.2	208.0	20.8	248.9	83.4	12.22	0.93	1.24	3.10	1.20
$RPS3^{21}$	20–21	354.4	68.5	319.1	49.7	768.5	246.5	5.17	0.89	1.11	1.44	2.41
$RPS3^{31}$	30–31	27.7	11.7	44.4	22.3	264.1	269.3	2.36	0.64	0.62	0.10	5.94
$RPS3^{37}$	36–37	4.9	2.4	17.8	5.2	233.0	148.9	2.04	0.59	0.28	0.03	13.06

5β-index=(CoP+*e*-CoP)/(5α-Ch+CoP+*e*-CoP) [23]

concentrations when using DCM or DCM/MeOH 9:1. However, when analysing low concentrations, cholesterol, 5α-cholestanol and sitosterol could not be determined since they resulted below the L-LOQs and/or instrumental LOQs.

Due to the small concentrations of COP (53.3 ng g^{-1} d.w.) and e-COP (19.6 ng g^{-1} d.w.) in RPS2 and the importance of these compounds in the evaluation of anthropogenic inputs, the accuracy was evaluated by fortifying the RPS2 sediment (n=4) with 100 ng of COP and e-COP (hereinafter labelled $RPS2^{F}$). The accuracy, expressed in terms of apparent recovery subtracted by the value obtained for RPS2, and the recalculated RSD that includes the error propagation and RSD obtained in RPS2 are reported in Table 3. The method provided an apparent recovery of about 107 and 109 % for CoP and e-CoP, respectively.

Faecal sterols in a shallow sediment core in the Piave River

We also tested the method throughout the vertical section of a shallow sediment core (37 cm deep, RPS3) collected near RPS1. The values of the six FeSt analysed at different depths were normalised by the amount of OC (Table 4). The maximum concentrations of CoP, e-CoP, Chl and 5α-Ch were generally observed in the most superficial portion and decreased to a minimum in sample $RPS3^{37}$.

Parameters such as the Cop/Chl ratio are widely used for assessing anthropogenic faecal pollution. In particular, Cop/Chl ratios higher than 1.0 indicate a sewage source, whilst ratios below 1.0 indicate biogenic (animal) sources. However, any value higher than 0.2 indicates the presence of faecal matter [61, 62]. Cop/Chl ratios in RPS3 sediment samples were always above 0.2, suggesting a major input of sterols from biogenic sources. Moreover, in the most recent samples (up to about 20 cm), the values were greater than 1.0, suggesting urban sewage inputs which is consistent with 5β-index results. Although an age-depth model of the core is not available, this trend may be related to the first urban settlement in this area (~1970–1980) and to the first sewage plants, which still currently drain faecal matter into the river.

Small values (0.25) of the parameter Cop/5α-Sit are diagnostic of ruminant faecal input [1, 18]. In RPS-3, this parameter falls down to values of about 0.1. This ratio is consistent with the fact that, before the first local human settlement (~1970–1980), the area was extensively used for grazing animals, especially sheep, whilst this pastoralism currently does not occur in the local area. However, the dominant phytosterol present in the core is sitosterol. The high concentrations of this compound and the Sit/Chl ratio are potentially due to terrestrial organic matter input and may even be due to the flood of 1966, which transported trees into the river basin and where their presence and decomposition is still notable in the Piave River.

Conclusions

Extracting sterols from freshwater sediments using PLE results in extraction yields of ~90 %. The best extraction conditions were obtained when using only DCM as a solvent leading to recoveries of ~70 % and a negligible interference contribution in GC/MS analysis in a variety of laboratory and environmental samples. The PLE extraction system is especially an advantage when processing a large number of samples—as is often the case when analysing riverine or lacustrine sediment cores—due to the relatively quick extraction times.

This method therefore can reconstruct the past presence of humans in a particular area as archived in lacustrine sediment cores, with this possibility of extending these reconstructions back thousands of years. In addition to such past reconstructions, this method can also determine the different pollution sources even in environments that are considered pristine, such as Antarctic ice-free areas [63].

Acknowledgments This work was financially supported by the Early Human Impact ERC Advance Grant of the European Commission's VII Framework Programme, grant number 267696, contribution no. 14. We would also like to thank Dr. Daniela Almansi and Dr. Natalie M. Kehrwald for the revision of our manuscript.

Conflict of interest The authors declare that they have no competing interests.

References

1. Bull ID, Lockheart MJ, Elhmmali MM, Roberts DJ, Evershed RP (2002) Environ Int 27:647–654
2. Murtaugh JJ, Bunch RL (1967) J Water Pollut Control 39:404–409
3. Hatcher PG, McGillivary PA (1979) Environ Sci Technol 13:1225–1229
4. Leeming P, Ball R, Ashbolt A, Jones N, Nichols G (1984) Aust J Chem 61:434–435
5. Daughton CG (2012) Sci Total Environ 414:6–21
6. Vane CH, Kim W, McGowan S, Leng MJ, Heaton THE, Kendrick CP, Coombs P, Yang H, Swann GE (2010) Sci Total Environ 409: 345–356
7. Tse TJ, Codling G, Jones PD, Thoms K, Liber K, Giesy JP, Wheater H, Doig LE (2014) Chemosphere 103:299–305
8. Wen-Yen H, Meinschein W (1976) Geochim Cosmochim Acta 40: 323–330
9. Dureth S, Herrmann R, Pecher K (1986) Water Air Soil Pollut 28: 131–149
10. Chapman PM, Wang F, Janssen C, Persoone G, Allen HE (1998) Can J Fish Aquat Sci 55:2221–2243
11. Wardroper AMK, Maxwell JR, Morris RJ (1978) Steroids 32:203–221
12. Canuel EA, Martens CS (1996) Geochim Cosmochim Acta 60: 1793–1806
13. Bachtiar T, Radjasa OK, Sabdono A (2004) J Coastal Environ 8: 17–25
14. Froehner S, Maceno M, Martins RF (2010) Environ Monit Assess 170:261–72
15. Sojinu SO, Sonibare OO, Ekundayo O, Zeng EY (2012) Sci Total Environ 441:89–96
16. Cordeiro LGSM, Carreira SM, Wagener ALR (2008) Org Geochem 39:1097–1103
17. Birk JJ, Dippold M, Wiesenberg GLB, Glaser B (2012) J Chromatogr A 1242:1–10
18. D'Anjou RM, Bradley RS, Balascio NL, Finkelstein DB (2012) Proc Natl Acad Sci U S A 109:20332–7
19. Tyagi P, Edwards DR, Coyne MS (2007) Water Air Soil Pollut 187: 263–274
20. Takada H, Farrington JW, Bothner MH, Johnson CG, Tripp BW (1994) Environ Sci Technol 28:1062–1072
21. Grimalt JO, Fernandez P, Bayona JM, Albaiges J (1990) Environ Sci Technol 24:357–363
22. Venkatesan MI, Santiago CA (1989) Mar Biol 102:431–437
23. Hughes K (2004) Environ Pollut 127:315–321
24. Bethell PH, Goad LJ, Evershed RP, Ottaway J (1994) J Archaeol Sci 21:619–632
25. Evershed RP, Bethell PH (1996) ACS Symp Ser 625:157–172
26. Kawakami SK, Montone RC (2002) J Brazil Chem Soc 13:226–232
27. Ogura K, Machihara T, Takada H (1990) Org Geochem 16:805–813
28. Szucs S, Sárváry A, Cain T, Adány R (2006) J Chromatogr Sci 44: 70–76
29. Noblet JA, Young DL, Zeng EY, Ensari S (2004) Environ Sci Technol 38:6002–6008
30. Li D, Dong M, Shim WJ, Kannan N (2007) J Chromatogr A 1160: 64–70
31. Writer JH, Leenheer JA, Barber LB, Amy GL, Chapra SC (1995) Wat Res 29:1427–1436
32. Nichols PD, Leeming R, Rayner MS, Latham V (1996) J Chromatogr A 733:497–509
33. Benfenati E, Cools E, Fattore E, Fanelli R (1994) Chemosphere 29: 1393–1405
34. Jayasinghe LY, Marriott PJ, Carpenter PD, Nichols PD (1998) J Chromatogr A 809:109–120
35. Nichols PD, Leeming R, Rayner MS, Latham V (1993) J Chromatogr A 643:189–195
36. Isobe KO, Tarao M, Zakaria MP, Chiem NH, Minh LY, Takada H (2002) Environ Sci Technol 36:4497–4507
37. Readman JW, Preston MR, Mantoura RFC (1986) Mar Pollut Bull 17:298–308
38. Saeed T, Al-Shimmari F, Al-Mutairi A, Abdullah H (2015) Mar Pollut Bull 307–317
39. Derrien M, Arcega Cabrera F, Velasquez Tavera NL, Kantun Manzano CA, Capella Vizcaino S (2015) Sci Tot Environ 511: 223–229
40. Peng X, Zhang G, Mai B, Min Y, Wang Z (2002) Mar Pollut Bull 45:295–299
41. Green G, Skerratt JH, Leeming R, Nichols PD (1992) Mar Pollut Bull 25:293–302
42. Venkatesan MI, Mirsadeghi FH (1992) Mar Pollut Bull 25:328–333
43. Venkatesan MI, Kaplan IR (1990) Environ Sci Technol 24:208–214
44. Laureillard J, Saliot A (1993) Mar Chem 43:247–261
45. Martins CC, Aguiar SN, Wisnieski E, Ceschim LMM, Figueira RCL, Montone RC (2014) Mar Pollut Bull 78:218–223
46. Wisnieski E, Bicego MC, Montone RC, Figueira RCL, Ceschim LMM, Mahiques MM, Martins CC (2014) Polar Biol 37:483–496
47. Osman R, Saim N (2013) J Chem 2013, ID 357252
48. Baultz H, Polzer J, Stieglitz L (1998) J Chromatogr A 215:231–241
49. Boselli E, Velazco V, Caboni MF, Lercker G (2001) J Chromatogr A 917:239–244
50. Burkhardt MR, ReVello RC, Smith SG, Zaugg SD (2005) Anal Chim Acta 534:89–100
51. Grilo CF, Neto RR, Vicente M, de Castro EVR, Figueira RCL, Carreira RS (2013) Appl Geochemistry 38:82–91
52. Rychlik M (2011) Fortification of foods with vitamins. Wiley-VCH Verlag GmbH & Co. KGaA
53. Kwok WH, Leung DKK, Leung GNW, Tang FPW, Wan TSM, Wong CHF, Wong JKY (2008) Rapid Commun Mass Spectrom 22:682–686
54. Zhou JL, Liu YP (2000) Mar Chem 71:165–176
55. UNI - EN 13137 (2001) Characterization of waste—determination of total organic carbon (TOC) in waste, sludges and sediments
56. Sanagi MM, Ling SL, Nasir Z, Hermawan D, Ibrahim WA, Abu NA (2009) J AOAC Int 92:1833–1838
57. Wu J, Hu R, Yue J, Yang Z, Zhang L (2009) J Chromatogr A 1216: 1053–1058
58. McCalley DV, Cooke M, Nickless G (1981) Water Res 15:1019–1025
59. Radzi bin Abas M, Oros DR, Simoneit BRT (2004) Chemosphere 55:1089–1095
60. Burns DT, Danzer K, Townshend A (2002) Use of the terms 'recovery' and 'apparent recovery' in analytical procedures IUPAC, 74:2201–2205
61. Leeming R, Ball A, Ashbolt N, Nichols P (1996) Water Res 30: 2893–2900
62. Patton D, Reeves AD (1999) Mar Pollut Bull 38:613–618
63. Vecchiato M, Zambon S, Argiriadis E, Barbante C, Gambaro A, Piazza R (2014) Microchem J 120:26–33

Late Holocene Record of Humans and Fire at Lake Trasimeno (Italy)

Torben Kirchgeorg[a,b*], Dario Battistel[a], Natalie Kehrwald[a,c], Elena Argiriadis[a], Alina Polonia[d], Luca Gasperini[d], Dorothy Peteet[e], Marta Radaelli[a],Enrico Bonatti[d,e], Carlo Barbante[a,f,g]

[a]Department of Environmental Sciences, Informatics and Statistics, University Ca'Foscari, Venice, 30170, Italy

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

[c]Geosciences and Environmental Change Science Center, U.S. Geological Survey, Lakewood, CO 80225, USA

[d]Institute of Marine Sciences – CNR-ISMAR, Bologna, 40129, Italy

[e]Lamont-Doherty Earth Observatory-Columbia University, Palisades, NY, USA

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

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

*Corresponding author: Torben Kirchgeorg, kirchgeorg@unive.it

Keypoints:

- Levoglucosan and fecal sterols in lake sediment cores as proxies for fire activity and human occupation
- Proxy data demonstrate the Roman occupation and pre-Roman fire activity at Lake Trasimeno

Abstract

Specific organic molecular markers record a Late Holocene history of human impact and regional fire activity in a sediment core from Lake Trasimeno in central Italy. Levoglucosan, a tracer for biomass burning, 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. We analyzed fecal sterols as a proxy for human presence near the lakeshore. Although levoglucosan does not always correlate with the analysed fecal sterols, which are a proxy for the presence of humans near the lakeshore, regional fire activity may still largely relate to human activity. The most prominent human impact on the lake is marked by peak fecal sterol concentrations 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 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 practices. Roman and pre-Roman impactsdominate the fire and fecal sterol profiles throughout the Late Holocene, even surpassing modern values.

Index Terms:4323, 0420, 6221

Keywords: Biomass Burning, Late Holocene, Human Impact

1. Introduction

People have lived on the shores of Lake Trasimeno in central Italy since at least the Bronze Age (Angelini et al., 2014). Archeological and historical evidence demontrates human activity in this region for millennia while pollen stratigraphy in the nearby Lake dell'Accesa demonstrate a human impact on vegetation types beginning as early as 8000 years before present (Drescher-Schnieder et al., 2007). Charcoal records demonstrate that people burned regional forests to clear land for agriculture (Vannière et al., 2008 and references within) where this region then became integral to the formation of the Roman Empire. The human history of this region is therefore closely tied to fire history. Sediment studies provide essential information regarding past land use change, yet until recently analytical techniques were unable to directly determine the presence of humans.Here, we apply innovative analytical techniqiues to examine a late Holocene record of interactions between people, grazing animals and associated fire activity in the Lake Trasimeno region.

Recent developments allow detecting specific molecular markers that indicate fire activity as well as the presence of livestock and humans. We analyze monosaccharide anhydrides and

particularly levoglucosan 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 only generated in high concentrations during cellulose combustion and is thus a specific proxy for biomass burning.Other pyrolysis markers (e.g. polycyclic aromatic hydrocarbons) can also originate from other combustion sources and therefore are not as specific as levoglucosan (Simoneit, 2002). The stability of levoglucosan is still debatedas at least a portion of dissolved levoglucosan in the water phase degrades during laboratory experiments (Norwood et al., 2013). However, many studies detect levoglucosan in sediments up to 20 kyr before present suggesting that at a minimum sediments archive particulate-bound levoglucosan over long time scales (Elias et al., 2001; Kirchgeorg et al., 2014; Kuo et al., 2011; Schüpbach et al., 2015).

The potential influence of humans on fire activity is difficult to determine 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 the presence of humans and livestock in a lake catchment to reconstruct population history on a Norwegian island (D'Anjou et al., 2012). These FeSt include 5β-cholestan-3β-ol (coprostanol) and stigmastanol (Stg), where coprostanol (CoP) 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 5β-cholestan-3α-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). We use a multi-proxy extraction method to analyze FeSt and levoglucosan in a Lake Trasimeno sediment core to consider the interactions between human activity, pastoralism and fires in this region during the late Holocene.

2. Study Site

Lake Trasimeno is located in the Umbria region of central Italy(Figure S1)with a diameter of 10 km and a surface area of ~120 km^2. Although Lake Trasimeno is one of the largest lakes in Italy, the maximum water depth is less than ~6 m (Bortoluzzi et al., 2005; Gasperini et al., 2011). Precipitation and minor streams comprise the major water input to the lake. The

shallow depth and the limited inflowled to periodical changes in lake water levels due to variations in the precipitation-evaporation balance (Dragoni et al., 2011). Such floods and droughts resulted in early water management approaches by Etruscans and Romans to regulate the lake levels (Burzigotti et al., 2003).The modern lake level is regulated by an artificial outflow located along the southeastern shore resulting in lower lake levels relative to other periods in the late Holocene.

3. Materials and Methods

3.1. Sampling and Sample Preparation

A 3.6 m long core (TR13_01, Fig. 1) was collected with a gravity corer in a water depth of 4.4 m near the northern shore of the lake close to Tuoro sul Trasimeno in 2013. The coring site was chosen based on the analysis of high-resolution seismic profiles (Fig. S2) previously collected in the area (Bortoluzzi et al., 2005; Gasperini et al. 2011). The core was transported and stored at CNR-ISMAR (Bologna, Italy). We subsampled the core at 5 cm intervals, 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.

3.2. Age Depth Model

We focus on the uppermost section of the sedimentary sequence (0 – 235 cm, 46 samples) encompassing the most recent ~3100 years. The chronology in this upper core section is based on the ^{14}C AMS date from woody fragments at 235 cm (2970 ±20 uncalibrated). Visual core descriptions and high-resolution geophysical data analyses (Gasperini et al. 2011) indicate continuous deposition in a low-energy environment during the time interval considered in this work (Figure S2). Radiometric ages and a depth-age model (Figure S3) were calculated and calibrated with CLAM Version 2.2 (Blaauw, 2010) using the Northern Hemisphere terrestrial calibration curve (Reimer, 2013). The ratio between penetration of the coring pipe (610 cm) and the core length (360 cm) suggests a compression factor of about 50%. Radiometric dating and the assumption of continuous sedimentation during the Late Holocene, allow estimating an age for each analyzed core section. The core interval 0-235 cm thus corresponds to 3140 ±65 cal yr BP (1 cm = ~13.6 yr) where the 5 cm sampling resolution is ~70 years. If we consider the 50% compression of the sediments during coring, these rates are in agreement with the average deposition rate estimated using independent methods (Gervasi et al., 2003).

3.3. Extraction of FeSt and Levoglucosan

Extractions were performed with a 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 ($^{13}C_3$-Cholesterol and $^{13}C_6$-levoglucosan with concentrations of 1 µg mL^{-1}) and activated copper was added to the samples in order to remove sulfur interference. 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 a 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 trace water in the samples, where the cartidges were cleaned and conditioned with 40 mL DCM. The pre-concentrated sample was loaded onto the cartridge and the FeSt fraction was eluted with 80 mL DCM followed by 20 mL of MeOH for the levoglucosan fraction. The FeSt fraction was evaporated to dryness and the residues were dissolved in DCM. Prior tothe 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 µL ultrapure water and centrifuged when solid residues were visible. The samples were stored at 4 °C prior to HPAEC-MS analyses.

3.4. 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) and increased to 210 °C with a rate of 20 °C min^{-1} and then increased to 300 °C at 3 °C min^{-1} and held for 8 min. An electron ionization source was used for mass detection while the single ion monitoring mode quantified the concentrations based on target ions and qualifiers. All instrumental details are reported in Battistel et al. (2015).

Levoglucsoan determination followed 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^{TM} 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. Levoglucosan and the $^{13}C_6$-levoglucosan were detected in single ion monitoring mode (m/z 161 and 167, respectively).

3.5. 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 levoglucosan 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 clean-up procedure blanks were created 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: CoP (L3: 7 %); e-CoP (L3: 8%); 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 parentheses): CoP: 0.4 ng mL^{-1} (±0.6); e-CoP: 0.5 ng mL^{-1} (±0.5); Stg 22 ng mL^{-1}(±18) and for levoglucosan: 15 ng abs (±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.

4. Results and Discussion

4.1. Fires and Human Activity

Levoglucosan is a proxy for regional fire activity at Lake Trasimeno while FeSt concentrations mark the presence of humans and/or pastoral animals. The two proxies do not significantly correlate (Figure 1) suggesting that the presence of humans does not necessarilyresult in an increase in fire activity. However, each proxy documents changes in different total catchments. Levoglucosan records regional fire activity as levoglucosan can be atmospherically transported over long distances and deposited on the lake surface.Fecal sterols are washed directly into the lake from run-off and are therefore a local marker. Here, we chronologically examine the combination of levoglucosan fluxes, fecal sterols, and historical documents to help decipher if humans impacted local to regional fire activity.

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 along 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 (Angelini et al., 2014). 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). While the population of these settlements is not well defined, the presence of cereal and legume pollen in conjuction with elevated micro-charcoal concentrations in regional lake cores, demonstrate intensive land use and cultivation (Sadori et al., 2004). Ecological and anthropological studies suggest that humans strongly altered the regional landscape during this period (Valsecchi et al., 2006) and thus the high fire activity during this period may reflect anthropogenic activities.Levoglucosan flux and thus fire activity peaked between 2500±53 and 3000 ±63 cal yr BP, although this fire activity was highly variable (Figure 2).Climate conditions may have also contributed to the high fire activity as low lake levels suggest dry climate conditions ~3000 cal yr BP.

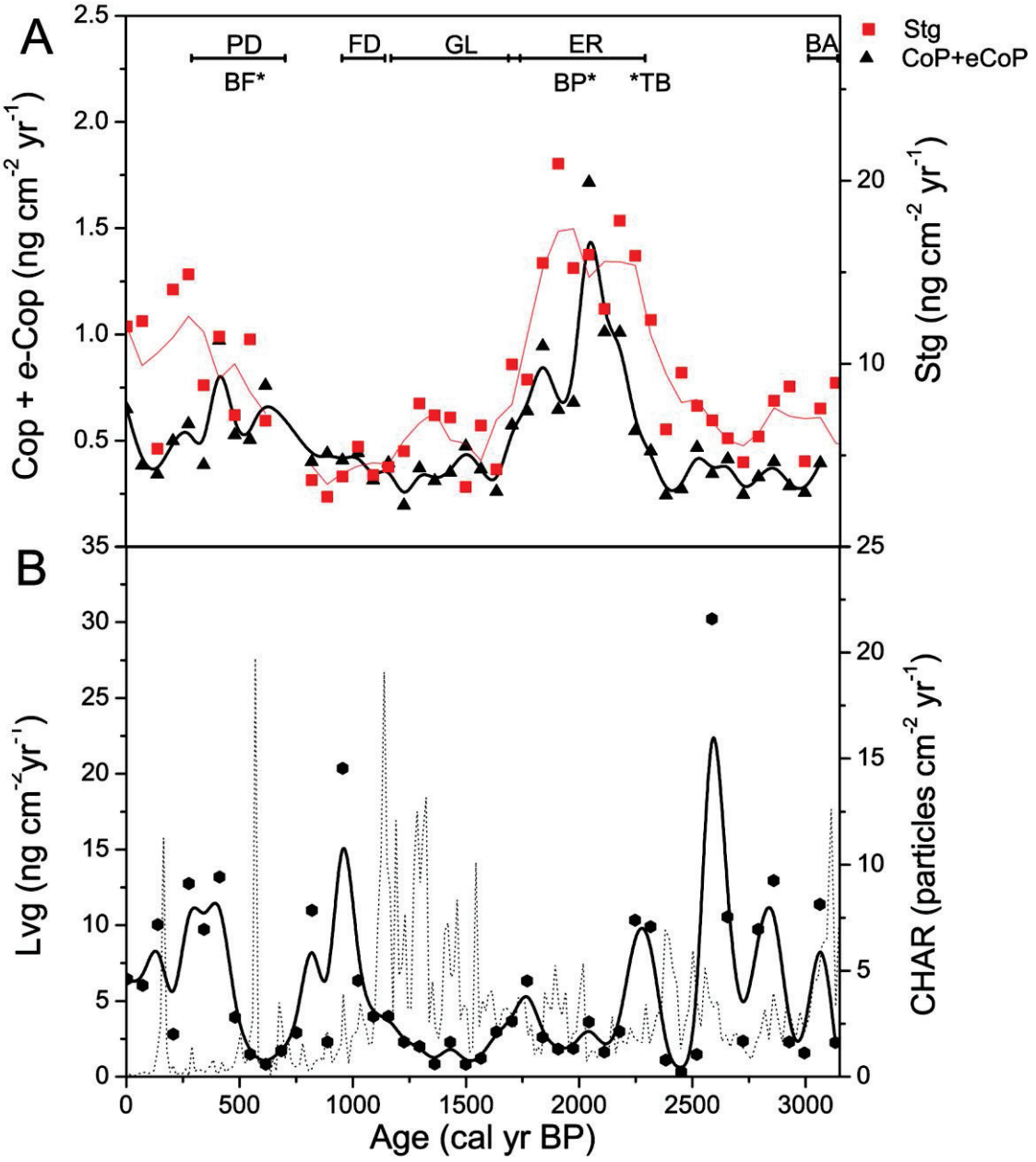

Figure 2: A: Cop+e-Cop (black triangles; black-line: 3-point moving average) and Stg fluxes (red squares; red-line: 3-point moving average). Historical periods are marked as follows: Late Bronze Age settlements (BA), Etruscan-Roman Period (ER), Gothic and Longobard Domination (GL), Franks Domination (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).

Humans substantially impacted Lake Trasimeno during the late Etruscan and early Roman period between 2500±53 and 1750 ±37 cal yr BP as demonstrated by the highest FeSt concentrations over the entire core. However, Etruscan and Roman era fire activity demonstrates more complex trends. Levoglucosan peaks at ~2750±57 cal yr BP, followed by

lower peaks at ~2250±47 cal yr BP and at ~1750 ±37 cal yr BP (Figure 2B). The major levoglucosan peak at 2750±57 cal BP occurs during the late Bronze Age and early Etruscan period (Angelini et al., 2014). The Bronze Age biomass burning decimated forests in order to open the landscape for agriculture, and suchlarge scale burning was no longer possible or necessary after this initial landscape clearance. The low fire activity centered around ~2000 cal yr BP during the Roman era in Lake dell'Accesa and Lake Trasimeno might result from Roman land management practices including reducing fire risk by removing dead biomass from the forests (Tinner et al.,1998). Synthesis studies of European biomass burning also demonstrate a decrease in total burned biomass (Vanniere et al. 2015).

Low fire activity between 1600±34 and 1250 ±27 cal yr BP may result from a depopulation of the region during the post Roman period as supportedby low FeSt fluxes (Figure 2). Fire activity increases again from 1250±27 cal yr BP until reaching its maximum at ca. 1000±21 cal yr BP. This fire peak may correspond to peaks of micro- and macroscopic charcoal at Lake dell'Accesa and Lake Massaciuccoli, located north of Lake Trasimeno, where local fire maxima occur slightly earlier at 1300-1000 cal yr BP (Vannière et al., 2008), but where this difference may be related to dating uncertainties in the Lake Trasimeno core. These studies assume that agricultural activities and associated land clearance due to an increase in popluation are the major reasons for the increased charcoal concentrations at these lakes(Colombaroli et al., 2007; Vannière et al., 2008). Fire frequency peaks during this time period across much of Europe, due in part to such a regional population increase (Vanniere et al. 2015). Historical records also demonstrate that agricultural land near the lake shore flooded during this time due to higher lake levels. People living near Lake Trasimeno responded tot he flooding by using fire to open new landscapes forcultivation(Gallorini, 1992) whichmay explain the corresponding levoglucosan increase (Figure 1).

The final period of high fire activity begins ~500±12 cal yr BP, reaching a maximum at ~300 ±8 cal yr BP before slightly decreasing until the present. Maps from this time period demonstrate several settlements around this lake suggesting a local and regional a medieval population increase (Burzigotti et al., 2003 and references within). Historical documents also describe the annual burning the cane thicket around the lake to maintain traditional fishing practices. It is unclear when this thicket burning started, although it continued until the 1980s. This annual burning may have resulted in an additional input of levoglucosan to the lake surface, due to the fact that the cane thicket is located in the shallow lake border.

4.2. Human Impacts and Livestock

Although archeological findings demonstrate that humans lived near Lake Trasimeno during the Bronze Age (Angelini et al., 2014; Ramrath et al., 2000; Sadori et al., 2004), fluxes of the human-specific fecal sterols CoP + e-CoP are not significantly higher in this related core sectionperiod(Figure 2A). The low CoP + e-CoP values may be due to the low population density during this period or to the fact that the Bronze Age pile dwelling excavation site was on the opposite side of the lake from our sampling location (Ramrath et al., 2000; Sadori et al., 2004). This Bronze Age archeological and chemical evidence is compatable as fecal material transported into the lake is deposited close to the sourcedue to the size and shallow water of the lake, and therefore only produces a local signal

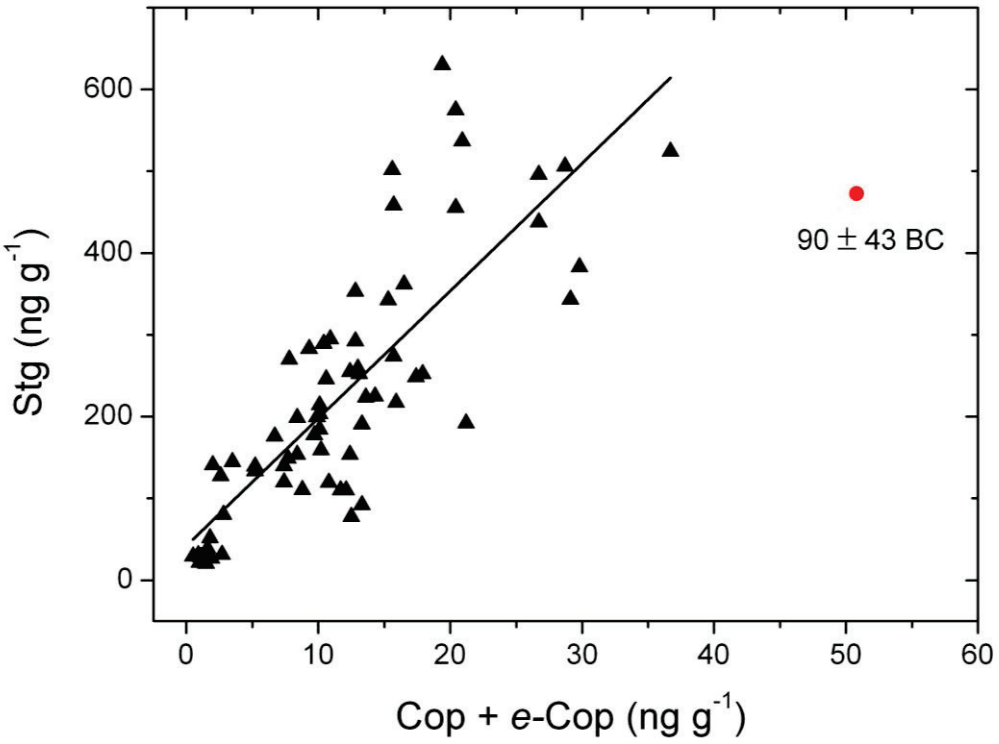

Figure 3: Correlation between Stg and Cop + e-Cop concentrations. Outlier (marked red) correspond to 90±43 BC.

Our results clearly demonstrate human occupation of the area centered around 1750 ±37 cal yr BP to 2250±47 cal yr BP as demonstrated by the highest CoP + e-CoP flux of the entire core and corresponding with the Etruscan - Roman period. Historical documents suggest that Lake Trasimeno was an important military and economic center for the Romans (Colacicchi and Bizzani, 2008 and references within). The most famous example is the Battle of Trasimeno (217 BC) between Hannibal's army and the Romans where ~30,000 Roman

soldiers camped on the northern lake shores near the sampling location. Lake Trasimeno was also a stategic location for Roman troops during the Perusine War between 40-42 BC.

Although the military camps were only temporary settlements, the impact of human fecal matter on the aquatic system may have significantly increased during this period and may correspond to the concentration peaks in the record. Pastoral grass-eating animals produce the fecal sterol Stg, where the presence of Stg in lake sediments suggests the presence of livestock. A significant correlation between CoP+e-CoP and Stg ($r^2 \geq 0.7$ 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. However, the correlation between CoP + e-CoP and Stg demonstrates an outlier during this period (Figure 3; 90 ±43BC), which may correspond to one of these military events.During the military phase a high number of humans were present compared to the number of livestock in the region. Traiano and Constanzo Empire coins (98-306 AD) demonstrate that Romans remained in this region even after the military occupation (Feruglio, 1966) and CoP + e-Cop values remain relatively high, especially when compared to pre-Roman times.

In the time period after the fall of the Roman Empire between 750±17 – 1650±35 cal yr BP, CoP + e-CoP concentrations decrease and remain low for several centuries (Figure 2). The Gothic and Longobard Domination of the region surrounding Lake Trasimeno resulted in a general demographic decline during this time period (Colacicchi and Bizzari , 2008) when other Italian regions also were subject to major rural depopulation (Di Pasquale et al., 2014).The corresponding decline in the Stg flux,suggests that livetock numbers decreased along with the general human population density.

Historians actively debate if regional population increased after ~750 yr BP to the present (Campano, 1992; Gambini, 1995). Our record suggests a strong increase in both human and pastoral animal populations as indicated by increased FeSt, Stg and CoP + e-CoP fluxes.Fire activity also increased during this period, which may be due to the pressures of a rapidly increasing population that opened additional landscapes by fire.

The most recent sample (5 ±2 cal yr BP) demonstrates the highest concentrations of Stg and may correspond to modern agriculture. Interestingly, the FeSt that is most closely reclated to humans, CoP+e CoP, only slowly increases in this period, even with the major modern increase in population, perhaps due to improvements in modern sewagetreatment conditions where human feces are currently not directly discharged into the lake. Recent outcries over

raw sewage reaching the lake either due to overflows or illegal activity (*Corriere Dell'Umbria* 9 April 2015; *Il Messaggero* 26 February 2014; *Giornale Dell'Umbria*11 April 2012) suggest that such instances are anomalous, and that wastewater is usually treated before returning to the lake.

5. Conclusions

Fecal sterol and levoglucosan concentrations from the Lake Trasimeno sediment core demonstratesubstantial human impacts on the lake system during the Etruscan-Roman Period. This time period encompasses the famous Battle of Trasimeno against Hannibal where almost 30,000 soldiers camped directly at the lakeshore, corresponding with a major increase in CoP+e CoP concentrations. Fire intensity did not significantly increase during the Roman era, suggesting either that the landscape was already successfully cleared in previous periods or Roman land management suppressed fire activity. The presence of livestock, as determined from Stg concentrations, strongly correlates with the presence of humans during the Etruscan-Roman Period. Fire reconstructions indicate 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 postive correlation between the presence of humans and peaks in fire activity does not always occur. The Lake Trasimeno sediment core demonstrates that organic molecular markers in lake sediments are a suitable tool to provide information about fire, human presence and pastoralism in lake catchments.

Acknowledgements

The research leading to these results received funding from the European Research Council under the European Union's Seventh Framework Program (FP7/2007-2013) / ERC Grant agreement No. 267696 – "EARLYhumanIMPACT". This is EARLYhumanIMPACT contribution ###. The authors would like to thank Maria del Carmen Villoslada Hidalgo, Marco Roman and Alice Callegaro as well the team of the R&C Lab for providing the ASE and their valuable help in the laboratory. Giuseppa Toscano, Samulee Ranucci and Maria Cappelletti provided helpful discussions of the local history at Lake Trasimeno. Furthermore, we would like to thank ELGA Lab water, High Wycombe, UK, for supplying the pure water systems used in this study. Dorothy Peteet sampled the woody fragmentsused for ^{14}C AMS dating (Grant CSEF CP91,Columbia University, Lamont Doherty Earth Observatory). Lorenzo Borgia organized and led the coring operations using two APM Perugia boats, while the Comune di Tuoro provided coring permission. Enrico Dalpasso and Flavio Priore

supported the coring operation and preliminary sample analysis. Ermanno Gambini provided important insights in deciphering the complex historical chronicles of the Trasimeno area.Finally, we would like to thank Robert D'Anjou and the anonymous reviewer for their suggestions to improve the manuscript.The Dataset is included in the supporting information (DataSet S4).

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., Argiriadis, E., Marchiori, E., Radaelli, M., Barbante, C. GC-MS Method for Determining Faecal Sterols as Biomarkers of Human and Pastoral Animal Presence in Freshwater Sediments, Analytical Bioanalytical Chemistry, 2015, in press.

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

Bortoluzzi, G., Gasperini, L., Bellucci, L.G., Ligi, M., 2005. Ricerche Geofisiche nel Lago Trasimeno, ISMAR-CNR Bologna, Rapporto Tecnico N.97, Novembre 2005, Bologna, pp.49.

Brizzi, G., Gambini, E., 2007. Di nuovo sulla battaglia del Trasimeno: qualche ulteriore considerazione. Rivista storica dell'antichita', 37, 77-100.

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.

Colacicchi, R., and Bizzarri, R., 2008. Correlation between environmental evolution, historical settlement and cultural heritage upgrading in Valle Umbra (central Italy). Geografia Fisica e Dinamica Quaternaria 31, 107-118.

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

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

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

404 Dragoni, W., Melillo, M., Giontella, C., 2011. Bilancio idrico del lago Trasimeno. In: Tutela
405 Ambientale del lago Trasimeno, Libri Arpa Umbria, Perugia, pp.69-83.

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

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

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

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

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

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

422 Gasperini, L., Barchi, M.R., Bellucci, L.G., Bortoluzzi, G., Ligi, M., Pauselli, C., 2010.
423 Tectonostratigraphy of Lake Trasimeno (Italy)and the geological evolution of the
424 Northern Apennines. Tectonophysics, 492, 164-174.

425 Gasperini, L., Bellucci, L.G., Delbianco, F., 2011. Il rilievo Geofisico-Geologico del lago
426 Trasimeno. In: Tutela Ambientale del lago Trasimeno, Libri Arpa Umbria, Perugia,
427 pp.125-130.

428 Gervasi L., Sciurpi G., Tenti P., Vignali S., Galinari W., Spaccapelo D., Pallotti D., Merlini
429 S., Lorenzoni M., Murgia S.M., Dragoni W. (2003). Piano per la riduzione degli apporti
430 solidi al lago Trasimeno.Provincia di Perugia –CIPLA, Tecnical Report, pp. 32.

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.

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. Radio- carbon 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

Vannière, B., Blarquez, O., Rius, D., Doyen, E., Brücher, T., Colombaroli, D., Connor, S., Feurdean, A., Hickler, T., Kaltenrieder, P., Lemmen, C., Leys, B., Massa, C., Olofsson, J., 2015. 7000-year human legacy of elevation-dependent European fire regimes Quaternary Science Reviews. In press. 1–7.

Estratto per riassunto della tesi di dottorato

Studente: ELENA ARGIRIADIS matricola: 825854

Dottorato: Science and Management of Climate Change

Ciclo: 28°

Titolo della tesi : The Early Impact of Agriculture. How Humans Have Been Affecting Climate for Thousands of Years.

Abstract:

Gli esseri umani iniziarono a modificare l'ambiente sin dalle primissime attività agricole. Se questo ebbe un'influenza sul clima ben prima dell'epoca industriale è oggetto di una vivace discussione in ambito scientifico. I cambiamenti climatici avvenuti durante la transizione dal tardo Pleistocene all'Olocene consentirono un'espansione geografica e una crescita della popolazione senza precedenti, dovute alla possibilità di produrre cibo. Nonostante i luoghi in cui l'agricoltura ebbe origine e dai quali si espanse siano ben noti, la ricerca ha evidenziato la mancanza di strumenti e dati per valutare gli effetti della passata deforestazione e delle attività agricole sul clima a livello locale e regionale. In questo lavoro sono stati selezionati specifici *marker* molecolari per tracciare gli incendi del passato e valutare la presenza umana e animale. In particolare, idrocarburi policiclici aromatici (IPA/PAHs), monosaccaridi anidri e steroli fecali sono stati usati come indicatori sorgente-specifici e stabili negli ambienti lacustri. Nella prima parte, si sono sviluppati metodi analitici specifici per determinare e quantificare i traccianti selezionati in sedimenti lacustri. Nella seconda parte, i metodi proposti sono stati testati e applicati nei siti scelti come obiettivo dell'indagine.

La Nuova Zelanda rappresenta un sito particolare per lo studio degli impatti dei primi insediamenti umano sull'ambiente. Fu occupata dai Polinesiani soltanto 700-800 anni fa e ciò ebbe come risultato improvvise e ingenti modificazioni dell'ambiente naturale. Qui i metodi sviluppati per l'analisi dei *marker* molecolari sono stati applicati su campioni provenienti da due laghi, e i risultati sono stati confrontati con i *record* paleoecologici esistenti, che ne hanno provato la validità nel confermare la presenza umana e nel correlare quest'ultima con l'intensità e la frequenza degli incendi. I risultati mostrano un marcato aumento nei flussi dei traccianti di fuoco e dei traccianti di presenza umana poco dopo l'arrivo dei Māori, coerente con un'intensa attività antropica di deforestazione, come precedentemente ipotizzato sulla base dei dati pollinici e di *charcoal*. La colonizzazione europea del diciannovesimo secolo è allo stesso modo evidente dai flussi di steroli fecali, che aumentano rapidamente in seguito alla crescita della popolazione.

I traccianti molecolari sono stati testati anche su due gruppi di campioni provenienti dal Lago Vittoria (Uganda) e da Flinders Island (Tasmania). In Africa, un aumento nella frequenza degli incendi si osserva in corrispondenza dei periodi aridi documentati dai *proxy* paleolimnologici. L'influenza della migrazione verso est delle popolazioni Bantu negli ultimi 2000 anni è visibile nel record di steroli fecali, che mostra una buona corrispondenza con i cambiamenti nella vegetazione e nel regime dei fuochi. In Tasmania, gli steroli fecali variano contestualmente alla presenza umana, che è stata intermittente durante gli ultimi 10000 anni, tuttavia una maggiore risoluzione sarebbe auspicabile per trarre conclusioni più precise.

Tutti i risultati sono presentati, confrontati con dati di letteratura e interpretati sulla base delle evidenze paleoecologiche, antropologiche e archeologiche, ove possibile.

Questo lavoro ha fornito un valido e oggettivo strumento per studi futuri che siano orientati alla creazione di un *database* ad alta risoluzione spaziale e temporale dei primi impatti antropici sul paesaggio e sul clima.

Abstract:

Humans have started shaping the environment since their very first agricultural activities. Whether this influenced climate before the industrial era is object of a lively scientific discussion. Climate changes in the Late Pleistocene – Holocene transition allowed an unprecedented geographic expansion and population growth due to the possibility of producing food. Although centers of early origin and expansion of agriculture are well documented worldwide, researchers highlight the lack of instruments and data for assessing the local and regional effects of deforestation and farming activities on climate. In this work, specific molecular markers for tracing past fire events and evaluating human and animal presence are selected. Namely, polycyclic aromatic hydrocarbons (PAHs), monosaccharide anhydrides and fecal sterols are used as source specific and stable indicators in lacustrine environments. In the first part, specific analytical methods for detecting and quantifying the selected tracers in lake sediment cores are developed. In the second part, the proposed methods are tested and applied on target locations.

New Zealand is known to represent a particular site for the study of the ecological impacts of human settlement. It was occupied by Polynesian only 700-800 y BP and resulted in abrupt and huge landscape modifications. Here, the molecular marker methods were applied on samples from two lakes and results were compared with existing paleoecological records, proving their validity in order to confirm human presence and correlate it with intensity and frequency of fires. Results showed a dramatic increase in the fluxes of both fire and human tracers soon after the Māori arrival, consistent with intensive anthropogenic land clearance, as previously hypothesized from charcoal and pollen evidence. The European 19th century colonization is also evident in the flux of fecal sterols, that rapidly increased following the population growth.

Molecular tracers were further analyzed on test sample batches from Lake Victoria (Uganda) and Flinders Island (Tasmania). In Africa, increased fire activity is observed in correspondence with the drier periods documented by paleolimnological proxies. the influence of the eastward migration of the Bantu speaking populations in the last 2000 years is visible in the sterol record, that shows good correspondence with changes in vegetation and fire regimes. In Tasmania, fecal sterols vary in accordance with the human presence, that was intermittent along the last 10,000 years, although higher resolution would be required in order to draw more precise conclusions.

All results are presented, compared with literature data and interpreted according to paleoecological, anthropological and archaeological evidence, where possible.

This work provided a reliable and objective instrument for future studies oriented to the creation of a spatial and temporal high resolution database of the early human impact on landscape and climate.

Firma dello studente

_____