NEOTROPICAL MOUNTAIN LAKES RESPOND WITH NOVEL DIATOM ASSEMBLAGES TO INDIGENOUS AND POST-EUROPEAN OCCUPATION

Abstract

Evidence indicates that Indigenous peoples and European settlers transformed terrestrial ecosystems in the American tropics, but far less is known about how aquatic ecosystems were affected. We re-examined diatom records from sediment cores originally collected for paleoclimate studies in mountain lakes of Guatemala, Panama and Colombia. Our goal was to explore long-term human influences on the ecology of these lakes. We focused on two aspects of beta diversity in identified diatom zones, species turnover and variation in relative abundances of species. All records displayed a single diatom zone or cluster of zones with assemblages that had no close analogues in the past. The ages of these distinctive assemblages varied from post-European to 5000 cal yr BP. Most novel assemblages were made up of moderately motile, non-planktonic, single-celled diatoms, associated with disturbed, productive environments. Archaeological data and pollen records point to the onset or intensification of human activities as the main driver behind the diatom changes. Maximum depth exerted control on how diatom communities responded to anthropogenic stressors. In shallower lakes, diatom assemblages transitioned gradually over time into their current novel composition. In deeper lakes, assemblages displayed slight fluctuations before shifting to their current condition. Differences between the two lake groups, might have been a consequence of more complete water-column mixing and higher rates of nutrient cycling in the shallow lakes, which resulted in diatoms responding more rapidly to environmental changes. The shift to novel recent diatom assemblages in all the study lakes suggests the water bodies lost their capacity to be resistant or resilient in the face of environmental stress, and hence now display ecosystem characteristics with no past analogues.

Key words: Anthropocene, Novel diatoms assemblages, European colonization, Holocene, Lake depth, Neotropics,

1. Introduction

There is growing consensus that modern ecosystems are the product of millennia of human-environment interactions (Dodson, 2010; Power et al. 2018). Prior to European settlement in the tropical Americas in the 1400s, landscapes had already been highly modified by Indigenous peoples (Anselmetti et al. 2007; Castilla-Beltrán et al. 2018; Erickson 2008; Flantua et al. 2016; Hooghiemstra et al. 2018; Metcalfe et al. 2007; Sluyter 2001). Forests were cleared for agriculture and construction, and landscapes were altered to the extent that it has been suggested that the well-documented, protracted droughts associated with the 9th-century Late Classic Maya cultural collapse in southern Mexico and Central America could have been a consequence of widespread vegetation removal that altered the regional precipitation regime (Cook et al. 2012). It was also suggested that when areas that had been deforested and or cultivated by Indigenous peoples were abandoned in the early 16th century, largely as a consequence of introduced European diseases, i.e. the “Great Dying,” widespread forest re-growth and consequent absorption of atmospheric CO_2 led to the Little Ice Age (Koch et al. 2019; Loughling et al. 2018). It is also thought that the biogeography and enormous biodiversity of the Amazon Basin are not solely the result of natural processes, but rather, arée the consequence of human-environment interactions that occurred over thousands of years, during which local Indigenous peoples managed the forests, water, and soils, using sophisticated techniques that included construction of irrigation canals, land terracing, and production of *terra preta* (Amazon dark soil) (Erickson 2008; McMichael et al. 2012). After the “Great Dying,” European-style agriculture was introduced, and many areas in the Americas were again cleared for pasture, row crops, and mineral extraction, with profound consequences for terrestrial ecosystems (Etter and van Wyngaarden 2000; Hooghiemstra et al. 2018; Loughlin et al. 2018;). Native forests were altered and new ecological dynamics emerged as a consequence of the introduction of exotic plants and large herbivores. For instance, cattle were new to the Americas and were capable of eating young trees, thereby preventing forest re-growth in some areas, leading to the development of open forests and treeless grasslands across many regions of Central and South America (Caetano-Andrade 2019; Castilla-Beltrán et al. 2018; Etter et al. 2008; Hooghiemstra et al. 2018).

Paleolimnological studies carried out in North America, Central America, and the Caribbean, indicate that lakes and rivers that were subjected to human influence received greater sediment and nutrient inputs as a consequence of deforestation and soil erosion, and that the additional sediment input reduced water clarity and altered natural biogeochemical cycles, e.g. nitrogen and phosphorus (Brugam and Munoz 2018; Castilla-Beltrán et al. 2018; Clement and Hoorn, 2002; Dubois et al. 2018; Frisch et a. 2014; Hooghiemstra et al. 2018; Metcalfe et al. 1991; Sowers and Brush 2014; Vélez et al. 2011). Very few paleolimnological studies in southern Central and northern South America, however, have focused on understanding the responses of lakes themselves to human activities. Whereas there is abundant evidence for the profound influence of pre-Hispanic cultures and European settlers on terrestrial ecosystems in these tropical areas, little is known about how aquatic ecosystems were affected by humans. Nevertheless, a few studies suggest that aquatic organisms responded differently depending on the degree of human influence. For instance, a plant macrofossil study in Lake Fúquene, Colombia, today, a highly eutrophic water body dominated by water hyacinths (*Eichhornia crassipes*) and Brazilian elodea (*Egeria densa*), showed that increases in nutrient load and reductions in water levels from hydrologic diversion for irrigation, resulted in the loss of native aquatic plants at the expense of the invasive, exotic species, and dominance (homogenization) by a few competitive aquatic macrophyte species (Salgado et al. 2019). In contrast, diatom assemblages in a suite of less human-influenced, highland lakes in Ecuador showed little compositional change over the last 150 years (Benito et al. 2019; Giles et al. 2018).

We used statistics to re-examine previously studied diatom records from a suite of shallow and deep Neotropical mountain lakes that ranged in degree of past human influence. Our goals were to: (1) elucidate how Neotropical mountain lakes changed in response to long-term (centuries to millennia) anthropogenic impacts in their respective watersheds, and (2) determine if diatom responses differed according to lake's depth. These original records had not been analyzed statistically and had been used solely to address questions about past local climate and environmental change. This study thus presents new information about the response of Neotropical lakes to human activities. We have three working hypotheses: (1) that the lakes were affected by human activities such as controlled fires, water diversion for irrigation, and deforestation for agriculture in their respective watersheds. Such disturbances would have changed water supply and lake levels, increased runoff and associated sediment and nutrient inputs to the lakes (Anderson 2014) thereby affecting the diatom community through increased water turbidity, shading and eutrophication, and habitat alteration (i.e. changes in both littoral and pelagic areas). (2) That these human-mediated changes in the watersheds would have led eventually to different and more homogeneous diatom assemblages similar to what has been observed for aquatic macrophytes in culturally eutrophic lakes (Salgado et al. 2018). In these new diatom assemblages, fewer species, mainly motile, single celled forms tolerant of the new conditions, would be expected to dominate. Motile diatoms have the ability to move away from disturbed areas in the lake, and for example can become dominant in water bodies that receive high inputs of detrital particles (Jones et al. 2014). They are also known to compete well for nutrients in eutrophic waters (Passy 2007). And, (3) that the trajectory to a more homogenous diatom community would differed between shallow and deep lakes. Shallow lakes (z_{max}< 7 m). Shallow lakes are characterized by regular water and nutrient mixing, and macrophyte cover in shallow basins often extends across much of the lake bottom, thereby reducing water turbidity. In contrast, in deeper lakes (z_{max}>14 m), macrophyte cover is restricted to the shallow littoral zone, and nutrient and water circulation are limited by density stratification.

2. Methods

Diatom data from sediment cores collected to study long-term climate and environmental variation in mid- and high-elevation lakes in the Neotropics were re-analyzed using several statistical approaches. We chose the diatom datasets using several criteria. We sought records: (1) from shallow (z_{max} < 7 m) and deep (z_{max} > 14 m) lakes at middle to high elevations (all >780 m above sea level (asl)). Records from lowland lakes can be affected by complex hydrologic dynamics that make identification of specific environmental stressors difficult; (2) that extend back several millennia prior to European arrival; (3) with reliable radiocarbon chronologies (Flantua et al. 2016); and (4) from lakes with a modern gradient of human influence in their watersheds. Statistical analyses were performed on diatom relative abundances in samples, for which a minimum of 400 valves had been counted. All species names were updated using the websites Diatom.org, and AlgaeBase.org. Details on taxonomic classification and paleoenvironmental interpretation are provided in Supplementary Information (SI) Table 1. In this study, we further classified the diatoms according to their motility (section 2.3).

Mountainous water bodies in the Neotropics range from ephemeral pools and bogs to shallow and deep lakes. Thus, assessments of the effects of humans on aquatic ecosystems in the region can be challenging. In an attempt to identify how lake depth influences diatom responses to human influence, we divided our lake dataset into shallow ($z_{max} < 7$ m) and deep ($z_{max} > 14$ m) water bodies (Table 1). Selected deep lakes included Amatitlán in Guatemala, San Carlos in Panama, and Ubaque, Pedro Palo, and La Cocha in the Eastern Cordillera of Colombia (Fig. 1). Shallow lakes included Santurbán-Berlín, Fúquene, and Siscunsí, all in the Eastern Cordillera of Colombia (Table 1, Fig. 1).

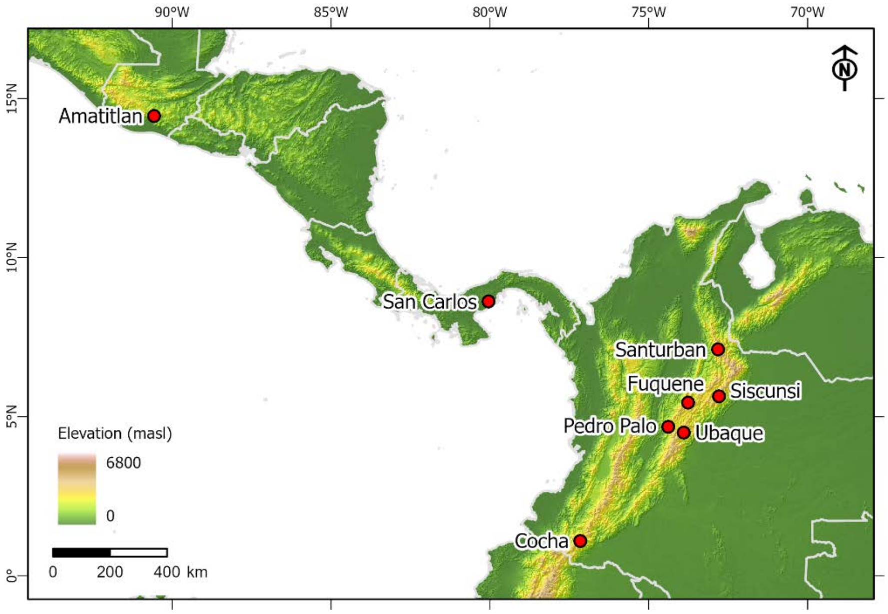

Figure 1. Geographic locations of the study lakes.

Lake type	**Record**	**Country**	**Max age (cal yr BP)**	**Altitude (m asl)**	**Surface area (km^2)**	**Max depth (m)**	**Vegetation cover**	**Current trophic status**	**Original reference**
Deep (z_{mx}>14 m)	Amatitlán	Guatemala	2400	1,186	15.2	33	Forest	Hypereutrophic	Vélez et al. 2011
	San Carlos	Panama	1080	780	0.04	8	Forest	Eutrophic	Correa-Metrio et al. 2016
	Ubaque	Colombia	4100	2,179	0.10	14	Forest	Eutrophic	Bird et al. 2017
	Pedro Palo	Colombia	8000	2,000	0.2	20	Forest	Mesotrophic	Vélez et al. submitted
	La Cocha	Colombia	13,700	2,780	40.5	75	Forest/Páramo	Oligotrophic	González-Carranza et al. 2012
Shallow (z_{mx}<7 m)	Fúquene	Colombia	23,770	2,540	30	7	Forest/Páramo	Hypereutrophic	Vélez et al. 2003
	Santurbán-Berlín	Colombia	27,600	3,800	0.04	<1	Páramo	Oligotrophic	Patiño et al. submitted
	Siscunsí	Colombia	2860	3,687	0.5	4.2	Páramo	Mesotrophic	Temoltzin-Loranca 2018

Table 1. Names, locations and morphometric characteristics of the selected study lake types (deep or shallow), along with maximum age for each sediment record, local vegetation cover, tropic status and citation for the original publication in which the diatom records were published.

Most of the diatom records and sediment lithologic descriptions were published previously (Table 1), whereas manuscripts about the records from Santurbán-Berlín and Pedro Palo are under review. The record from Siscunsí appeared in a MSc thesis. Selected study lake characteristics are presented in Table 1. Sediment records from Lakes Pedro Palo and La Cocha contain only pre-European deposits because uppermost sediments from these lakes were not recovered.

2.1. Core chronology

We used published chronologies and age-depth models based on Bayesian statistics that employed Bacon software (Blaauw and Christen 2011) for Lakes San Carlos, Ubaque, Siscunsí, Santurbán-Berlín and Pedro Palo (Table 2 SI; Table 1). Previous age models for cores from Lakes Amatitlán, Fúquene and La Cocha were developed with linear interpolation, and we produced new chronologies using Bacon and the ^{14}C dates from the original publications (SI Table 2). All radiocarbon dates are reported in calibrated years BP (cal yr BP), rounded to the nearest decade.

2.2. Statistical analyses

Sediment samples that lacked diatoms were removed prior to statistical analyses and diatom counts were square-root-transformed to weight the relative importance of rare *vs.* abundant species (Legendre and Gallagher 2001). Major zones of diatom change, reflecting characteristic diatom assemblages, were identified in each core by Constrained Hierarchical Clustering analysis on chord distances, performed using the *chclust* function (*coniss* method) of the *rioja* package (Juggins 2019) in R (R Development Team 2019). Clusters per each record are presented in Supplementary Information SI Figure 1.

Temporal shifts in abundance and species composition (Beta diversity; Whittaker 1960) have often been shown to display greater variation in biological communities in response to human-driven environmental change, than have measurements of species richness alone (Anderson et al. 2011; Legendre and De Cáceres 2013). Although different measures of beta diversity have been proposed to express that variation (Anderson et al. 2011; Legendre and De Cáceres 2013; Tuomisto et al. 2010a,b), recent analyses suggest that when possible, beta diversity studies should focus on two complementary aspects of community structure (Anderson et al. 2011), *turnover* - the directional change in assemblage composition from one sampling unit to another along a temporal gradient-, and *assemblage heterogeneity* -the variation in assemblage composition arising from shifts in species identities and abundances among groups of sampling units over time-. Use of these two measures of beta diversity in concert helps reveal the underlying nature of patterns in β diversity that arise simultaneously from presence/absence data and relative abundance information (Anderson et al. 2011). We therefore focused our statistical analyses on these two complementary aspects of beta diversity by undertaking the combined approaches of Principal Coordinate Analysis (PCoA), Homogeneity Analysis of Multivariate Dispersions (HMD; Anderson 2006) and Permutational Analysis of Variance (PERMANOVA; Anderson 2001). PCoA enabled visual assessment of trajectories in the diatom assemblages (turnover) between samples at each lake. HMD analysis was suitable for assessing the significance of assemblage heterogeneity attributed to variation in relative abundances of species, and PERMANOVA enabled assessment of the significance of the compositional heterogeneity attributed to variation in the identity of species (turnover) present.

HMD analysis is a non-parametric method that compares variability of mean distance to a centroid (dispersion) within temporally predefined groups, to variability in this distance between the predetermined groups in a PCoA. Variation in assemblage heterogeneity was thus defined in our study as the distance to the spatial median of the dissimilarities in diatom species relative abundances among sediment samples, grouped respectively, within the major temporal zones of change (groups) detected by hierarchical cluster analysis. A zone with higher values of mean distance to the group median was assumed to be characterized by greater variability (greater multivariate dispersion) in diatom species abundance between the sediment samples, and hence, greater temporal assemblage heterogeneity (Anderson et al. 2006; Salgado et al. 2018). Conversely, low multivariate dispersion (lower mean distance to the group median) indicates a more homogenous assemblage structure. We conducted an overall HMD analysis for each lake, treating each zone as an independent group. Differences between zone pairs were then tested *post hoc* using the Tukey honest test (499 permutations, $p < 0.05$) to determine specifically which zones differed in diatom assemblage heterogeneity from one another. HMD analyses were performed using the *betadisper* function in the vegan package (Oksanen et al. 2013) and results were plotted using boxplots and PCoA plots.

Although HMD analysis provides a robust measure of compositional heterogeneity, it does not discriminate between samples differing in the identity of species composition only, i.e. two zones of diatom change could be equally heterogeneous/homogenous, but differ significantly in species composition (Anderson et al. 2011). Therefore, we used PERMANOVA (*adonis* function in *vegan* package R; Oksanen et al. 2019) to explore species compositional differences between assemblages. PERMANOVA is a non-parametric method for multivariate analysis of variance that compares the variability of average dissimilarity within groups, versus the variability among groups, using the ratio of the F-statistic through permutational tests.

2.3. Diatom responses to human activity

To understand the response of diatom communities to human activities, we also grouped individual diatom species from each lake into two broad functional groups based on their motility. Table 1 SI presents the classification for dominant species. If a given diatom could not be classified at the species level, we classified it according to its genus, following Berthon et al. (2011). *Non-motile* (NM) diatoms lack movement and include mainly planktonic forms. This group was further subdivided into: (i) non-motile diatoms that form long chains (NM-Ch), such as *Aulacoseira* spp., (ii) non-motile, single-cell diatoms that do not form chains, like *Cyclotella* and *Discostella* spp. (NM-S), and (iii) diatoms that may or may not form long chains, but are tychoplanktonic, that is, live in both the plankton and benthos (NM-TYC). These include pioneer diatoms such as *Fragilaria brevistriata, F. construens* and *F. pinnata* (Biskaborn et al. 2012). NM-Ch and NM-TYC are often associated with productive environments (Passy 2007), whereas NM-S are generally associated with disturbances associated with water currents and highly productive environments (Passy 2007).

The second functional group, *Motile diatoms* (M), comprises diatoms with active movement. When more information on species was available, they were subdivided into Slightly Motile (SM), Weakly Motile (WM), Moderately Motile (MM), and Highly Motile (HM), following the ecology presented in Diatoms.org. These include several species of *Nitzschia, Gomphonema*, *Encyonema*, *Eunotia* and *Pinnularia*. When possible, we also noted if the species grew prostrate, that is, on the substrate surface with the entire valve attached (*Motile Prostrate* [M-PRO]), as is the case for *Achnanthidium minutissimum* and *Cocconeis placentula* (Passy 2007; Berthon 2011), or if the species was aerophilous (*Motile Aerophilous* [M-AER]), such as

Diploneis elliptica. These species grow in soils and are adapted to periods of desiccation. M-PRO can tolerate currents and other disturbances.

Under increasing anthropogenic activity in the watershed and associated soil erosion, an increase in detrital sediments and nutrients would be expected in the lakes. These physical perturbations would favor the increase or even dominance of motile and non-motile single-celled diatoms. Motile species can avoid physical disturbances such as erosion-generated turbidity, shading, and burial (Jones et al. 2014), while also seeking favorable nutrient-rich habitats like those generated by input of soil nutrients (Passy 2007). Single-celled diatoms are common in environments with perturbations generated by currents, and in habitats with abundant macrophytes (Berthon et al. 2011), like those that result from eutrophication and or a decrease in water level.

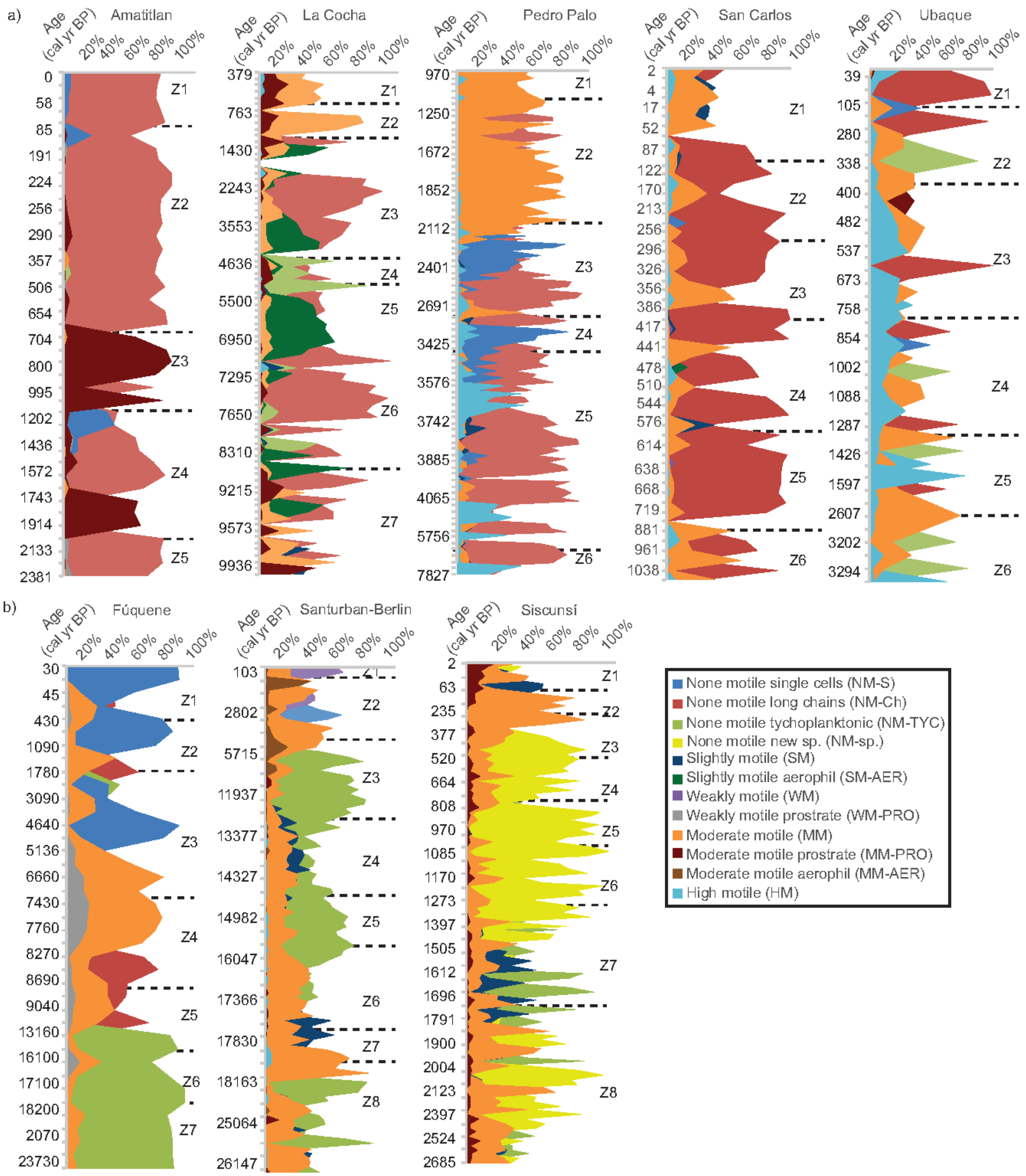

Figure 2. Relative proportions of motile and non-motile diatoms in each record, along with the zones determined by cluster analysis (black dashed lines) for (a) deep and (b) shallow lakes.

2.4. Deep lakes

2.4.1. Lake Amatitlán, Guatemala (1186 m asl)

Cluster analysis identified five diatom zones of community change spanning the last ca. 2400 years (Fig. 2a). Zones 5-4 (2380-1330 cal yr BP) and zone 2 (700-190 cal yr BP) were composed of non-motile planktonic *Aulacoseira granulata*, *Fragilaria delicatissima*, planktonic single-celled *Discostella stelligera* and moderately motile, prostrate *Nitzschia amphibia* and *N. palea*, and spanned the Pre-Classic and Classic Maya archaeological periods. Zone 3 (1200-750 cal yr BP) corresponded to the Terminal Classic and part of the Postclassic, the period associated with the Maya "collapse" and is dominated by moderately motile, prostrate *N. palea* and *N. amphibia var. rostrata.* Zone 1 (110 cal yr BP-Present) is dominated by *A. granulata*, with significant increases in non-motile single-celled *Cyclotella meneghiniana*, tychoplanktonic *Ulnaria ulna*, *F. delicatissima*, and the first appearance of *Fragilaria crotonensis*.

HMD analysis showed that temporal shifts in diatom assemblages were accompanied by a significant ($p = 0.005$; F = 4.5157) gradual decline in assemblage heterogeneity over time (Fig. 3a). More heterogeneous assemblages (greater mean distance to the centroid) occurred in zone 5, whereas recent zone 1 was more homogenous (Fig. 3a). The Tukey test revealed that the following zones were significantly different: zones 5 and 1 ($p = 0.02$), and zones 5 and 2 ($p = 0.004$). Diatom assemblages in zone 3 appeared more distant from zones 5, 4 and 2 in the multivariate PCoA space, by clustering on the right-hand side of PCoA axis 1. Similarly, diatoms from zone 1 were clustered away from the more historical zones, along the negative portion of PCoA axis 2. PERMANOVA showed that with the exception of zones 5 and 4, all remaining zones were significantly different from one another with respect to diatom species composition (Fig. 3a; Table 3 SI).

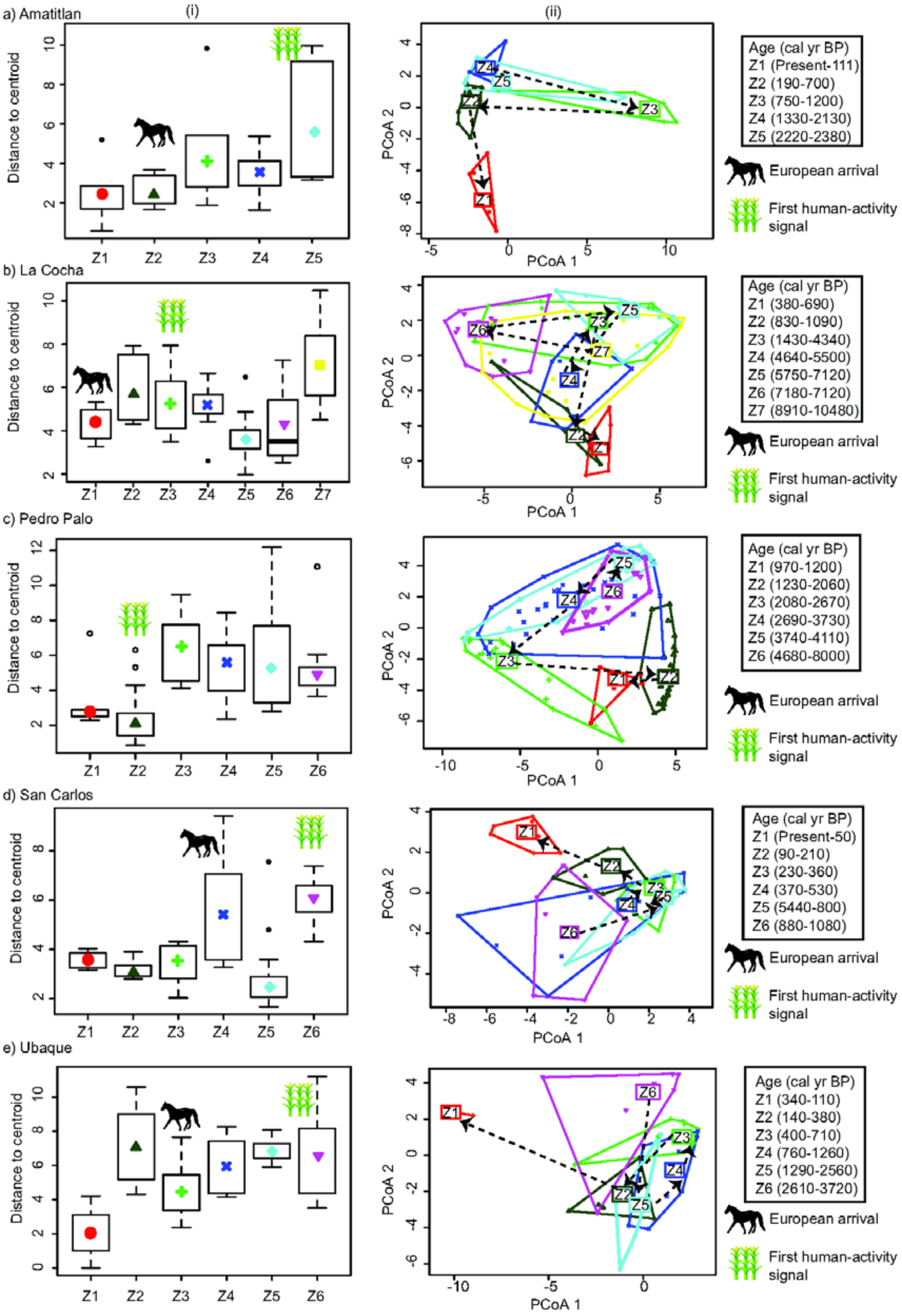
a) Amatitlan
(i)
(ii)
Distance to centroid
Z1 Z2 Z3 Z4 Z5
PCoA 1
PCoA 2
Age (cal yr BP)
Z1 (Present-111)
Z2 (190-700)
Z3 (750-1200)
Z4 (1330-2130)
Z5 (2220-2380)
European arrival
First human-activity signal
b) La Cocha
Z1 Z2 Z3 Z4 Z5 Z6 Z7
Age (cal yr BP)
Z1 (380-690)
Z2 (830-1090)
Z3 (1430-4340)
Z4 (4640-5500)
Z5 (5750-7120)
Z6 (7180-7120)
Z7 (8910-10480)
European arrival
First human-activity signal
c) Pedro Palo
Z1 Z2 Z3 Z4 Z5 Z6
Age (cal yr BP)
Z1 (970-1200)
Z2 (1230-2060)
Z3 (2080-2670)
Z4 (2690-3730)
Z5 (3740-4110)
Z6 (4680-8000)
European arrival
First human-activity signal
d) San Carlos
Z1 Z2 Z3 Z4 Z5 Z6
Age (cal yr BP)
Z1 (Present-50)
Z2 (90-210)
Z3 (230-360)
Z4 (370-530)
Z5 (5440-800)
Z6 (880-1080)
European arrival
First human-activity signal
e) Ubaque
Z1 Z2 Z3 Z4 Z5 Z6
Age (cal yr BP)
Z1 (340-110)
Z2 (140-380)
Z3 (400-710)
Z4 (760-1260)
Z5 (1290-2560)
Z6 (2610-3720)
European arrival
First human-activity signal

Figure 3. Results of HMD analyses for the deep lakes: a) Amatitlán, b) La Cocha, c) Pedro Palo, d) San Carlos, and e) Ubaque. (i) boxplots of the variation in diatom assemblages and the distance to mean value at each zone determined by cluster analysis, (ii) variation of the diatom assemblages and their distance to spatial median in the ordination space (PCoA). Zones, determined by cluster analysis, are identified by specific symbols: zone 1 (most recent; red circle), zone 2 (triangle), zone 3 (cross), zone 4 (x), zone 5 (diamond), zone 6 (inverted triangle), and zone 7 (square). Ages of the zones and legend for the onset of human activities and European arrival are presented on the right.

2.4.2. Lake La Cocha, Colombia (2780 m asl)

Cluster analysis identified seven diatom zones extending back to the early Holocene (Fig. 2a). The early and middle Holocene are represented in zones 7 to 4 (10,480-4640 cal yr BP), the late Holocene in zone 3 (4340-1430 cal yr BP), and the last 1430 years in zones 2 and 1. Zones 7-3 (10,480-1430 cal yr BP) are characterized by non-motile species *A. ambigua,* aerophilous *Orthoseira roeseana* and *Humidophila contenta,* and tychoplanktonic *S. brevistriata*. Zones 2 and 1 (the last 1430 years) display an increase in the moderately motile aerophilous *Diploneis elliptica* and in moderately motile species of *Cymbella*, *Frustulia*, *Pinnularia* and *Eunotia* (Table 1 SI).

HMD analysis showed that the temporal shifts in diatom assemblages were accompanied by significant ($p < 0.001$; F = 6.7508) variation in assemblage heterogeneity over time. More heterogeneous assemblages occurred in zones 7 and 2, whereas more homogenous assemblages characterized zones 5 and 1 (Fig. 3b). The Tukey test revealed that the following zone pairs are significantly different: zones 1 and 7 ($p = 0.005$), zones 4 and 5 (p = 0.02), zones 5 and 7 ($p < 0.001$), and zones 6 and 7 ($p < 0.001$). The PCoA plot (Fig. 3b) showed that from 10,480 to 1430 cal yr BP (zones 7 to 3), diatom assemblages were relatively more similar in composition and thus appeared closer in PCoA space. Zones 2-1 clustered together, but were distant from previous zone 3, in a similar fashion to Amatitlán. PERMANOVA indicated that all zones differed significantly from one another in terms of diatom species composition (Fig. 3b; Table 3 SI).

2.4.3. Lake Pedro Palo, Colombia (2000 m asl)

Cluster analysis identified six diatom zones in the record spanning the last 8000 cal yr BP (Fig. 2a). Zone 6 (8000-4680 cal yr BP) is dominated by planktonic *A. granulata* var. *angustissima*, with prostrate, epiphytic *C. placentula;* zones 5 to 3 (4110-2080 cal yr BP) are composed of planktonic, single-celled *Discostella stelligera, D. pseudostelligera* and chain-forming *F. crotonensis*, and zones 2 and 1 (2060-970 cal yr BP) are composed of moderately motile and epiphytic *Encyonema minutum* and planktonic *A. granulata* var. *angustissima* (Table 1 SI).

HMD analysis showed that temporal shifts in the diatom assemblages were accompanied by a significant ($p < 0.001$; F= 13.979) reduction in community heterogeneity in zones 2 and 1 (Fig. 3c). Accordingly, the Tukey test revealed these two more recent zones differ significantly ($p < 0.05$ in all cases) from zones 6-3. The PCoA plot indicates that the diatom community started to transition into a new compositional association after zone 3 by moving away from the other historical zones (6-4) along PCoA axis 1 and 2. PERMANOVA indicated that all zones differed significantly from one another in terms of diatom species composition (Fig. 3c; Table 3 SI).

2.4.4. Lake San Carlos, Panama (780 m asl)

Cluster analysis identified six main diatom zones spanning the last ~1000 cal yr BP (Fig. 2a). Zones 6 to 3 (1080-230 cal yr BP) have similar diatom assemblages (Fig. 3d), dominated by planktonic *F. crotonensis,* with peaks in *A. granulata,* and moderately motile species such as *Navicula lanceolata, Gomphonema* spp. and *Eunotia* spp. Zone 2 (210-90 cal yr BP) contains the first peak in planktonic, single-celled *D. stelligera*. In zone 1 (50 cal yr BP-Present) the composition is significantly different and includes slightly motile *Achnanthidium exiguum* var. *constricta, A. minutissimum* (formerly *Achnanthes exigua* and *A. minutum,* respectively), and benthic, moderately motile *Encyonema silesiacum* (formerly *Cymbella silesiaca*) and *N. amphibia* (Table 1 SI).

HMD analysis showed that temporal shifts in the diatom assemblages were accompanied by a significant ($p < 0.001$; F = 6.2169) gradual reduction in community heterogeneity over time, with more heterogenous assemblages occurring in zones 6 and 4 and more homogenous assemblages characterizing the remaining zones (Fig. 3d). The Tukey test revealed that the following zone pairs were significantly different: zones 1 and 6 ($p = 0.03$), zones 2 and 6 ($p = 0.01$), zones 3 and 6 ($p < 0.016$), and zones 5 and 6 ($p < 0.001$). The PCoA plot indicates that the diatom assemblage in zone 1 is more distant from the previous historical zones along the positive portion of PCoA axis 2. PERMANOVA indicated that diatom species composition among zones 2-4, 3-4, 3-5, 4-5, 4-6 were highly similar and that zone 1 was significantly different from all other zones (Fig. 3d; Table 3SI).

2.4.5. Ubaque Lake, Colombia (2179 masl)

Cluster analysis identified six main diatom zones spanning the last ca. 3700 cal yr BP (Fig. 2a). Zones 6-3 (3,720-400 cal yr BP) are composed of peaks of planktonic *A. ambigua* and variable appearances of non-motile tychoplanktonic *Staurosirella pinnata*, *Pseudostaurosira elliptica*, and planktonic *Fragilaria tenera, F. crotonensis* and *C. meneghiniana*. Zone 2 (380-140 cal yr BP) is dominated mainly by *S. pinnata* and zone 1 (110 cal yr BP-Present) includes significant peaks of planktonic *A. ambigua* and a peak in *C. meneghiniana*, with increases in abundance of prostrate valves of colonizer *Achnanthidium* and *Planothidium* species (Figs. 2a, 3e, Table 1 SI).

HMD analysis showed that temporal shifts in the diatom community were accompanied by a significant ($p = 0.007$; $F= 3.8537$) gradual reduction in community heterogeneity over time, with more heterogeneous assemblages in zones 6 and 2 and more homogenous assemblages in zones 5, 4, 3 and 1 (Fig. 3e). The Tukey test revealed that the following zone pairs are significantly different: zones 1 and 2 ($p = 0.02$), zones 1 and 5 ($p = 0.02$), and zones 1 and 6 ($p = 0.03$). The PCoA plot indicates that the diatoms in zone 1 are more distant from the previous zones, along PCoA axis 2, and in turn that they are more similar between themselves. PERMANOVA indicated that diatom composition differed significantly among all zones (Fig. 3e; Table 3 SI)

2.5. Shallow lakes

2.5.1. Lake Fúquene, Colombia (2540 m asl)

Cluster analysis identified seven main diatom zones spanning the last 14,550 cal yr BP (Fig. 2b). Zones 7 and 6 (14,550-10,020 cal yr BP) correspond to the last glacial/Holocene climate transition and are composed of non-motile tychoplanktonic species like *Pseudostaurosira brevistriata* and *Staurosirella pinnata* (formerly *Fragilaria brevistriata* and *F. pinnata,* respectively). Zones 5 and 4 (~9830-5340 cal yr BP) correspond to the early and middle Holocene, and are composed of planktonic *Aulacoseira ambigua*, with benthic and motile species *Encyonospis microcephala* (formerly *Cymbella microcephala*) and *N. amphibia*. Zones 3 to 1 (last ca. 5000 cal yr BP) are marked by the dominance of planktonic *D. stelligera* and isolated peaks in the abundance of other planktonic species such as *A. distans* cf. *laevisima* and *C. meneghiniana* (Fig. 3a; Table 1 SI).

HMD analysis showed that temporal shifts in the diatom assemblages were accompanied by significant ($p < 0.001$; F = 6.0227) variation in community heterogeneity over time, with more heterogenous assemblages in zone 3 and 2 and more homogenous assemblages in zones 7-5 (Fig. 4a). The Tukey test revealed that the following zone pairs are significantly different: zones 3 and 5 ($p = 0.02$), zones 1 and 5 ($p < 0.001$), and zones 3 and 7 ($p < 0.001$). The PCoA plot indicates that diatom assemblages transitioned over time into new associations (zones 3, 2 and 1), located more distant from earlier zones along the negative portion of PCoA axis 1. PERMANOVA indicated that the diatom composition in zones 3, 2 and 1 was highly similar (Fig. 4a; Table 3SI).

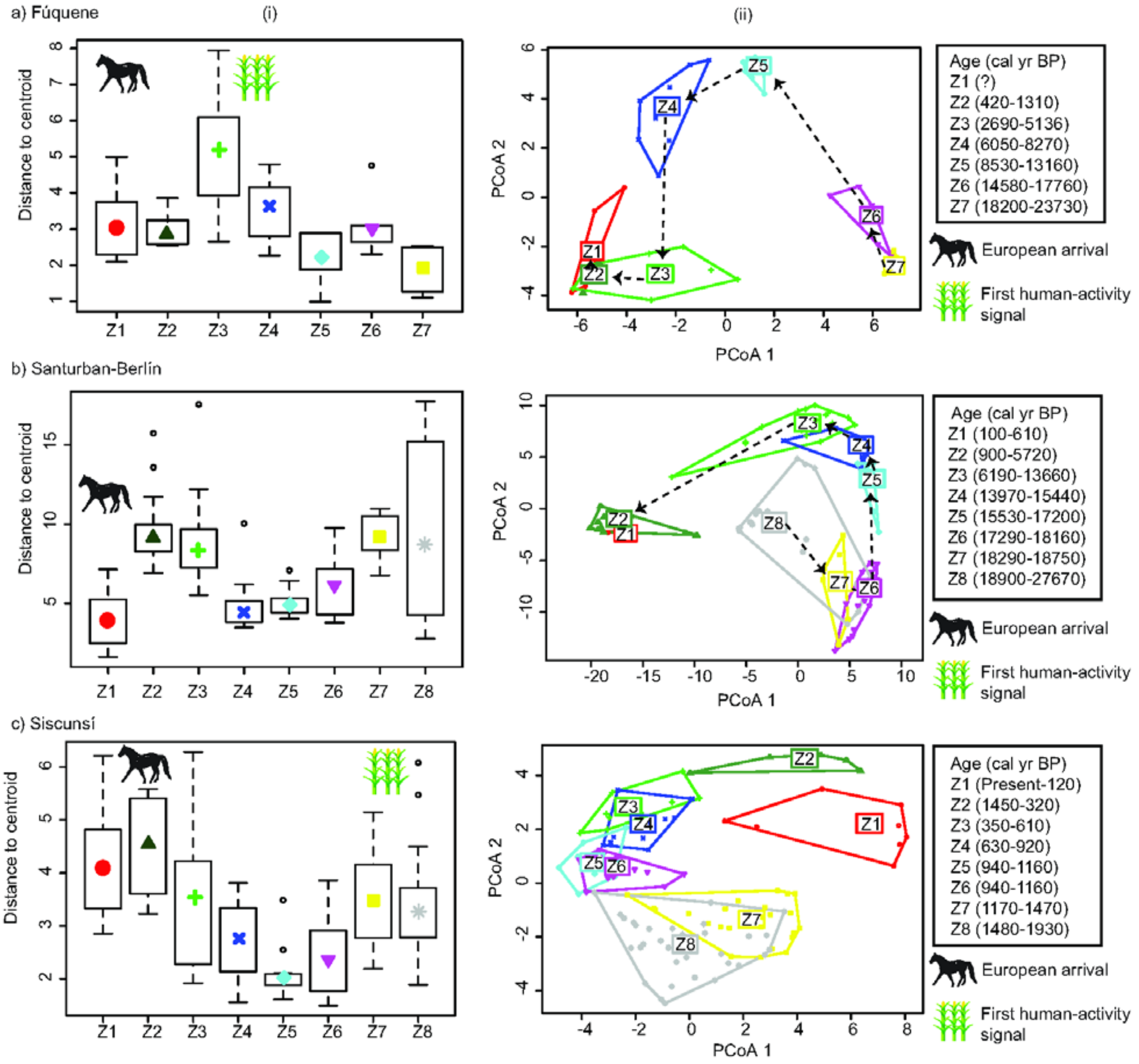
a) Fúquene
(i)
(ii)
Distance to centroid
Z1 Z2 Z3 Z4 Z5 Z6 Z7
PCoA 1
PCoA 2
Age (cal yr BP)
Z1 (?)
Z2 (420-1310)
Z3 (2690-5136)
Z4 (6050-8270)
Z5 (8530-13160)
Z6 (14580-17760)
Z7 (18200-23730)
European arrival
First human-activity signal
b) Santurban-Berlín
Distance to centroid
Z1 Z2 Z3 Z4 Z5 Z6 Z7 Z8
PCoA 1
PCoA 2
Age (cal yr BP)
Z1 (100-610)
Z2 (900-5720)
Z3 (6190-13660)
Z4 (13970-15440)
Z5 (15530-17200)
Z6 (17290-18160)
Z7 (18290-18750)
Z8 (18900-27670)
European arrival
First human-activity signal
c) Siscunsí
Distance to centroid
Z1 Z2 Z3 Z4 Z5 Z6 Z7 Z8
PCoA 1
PCoA 2
Age (cal yr BP)
Z1 (Present-120)
Z2 (1450-320)
Z3 (350-610)
Z4 (630-920)
Z5 (940-1160)
Z6 (940-1160)
Z7 (1170-1470)
Z8 (1480-1930)
European arrival
First human-activity signal

Figure 4. Results of HMD analyses for the shallow lakes: a) Fúquene, b) Santurbán-Berlín and c) Siscunsí. (i) boxplots of the variation in diatom assemblages and the distance to mean value at each zone determined by cluster analysis. (ii) variation of the diatom assemblages and their distance to spatial median in the ordination space (PCoA). Zones (determined by cluster analysis) are denoted by specific symbols: zone 1 (most recent; red circle), zone 2 (triangle), zone 3 (cross), zone 4 (x), zone 5 (diamond), zone 6 (inverted triangle), zone 7 (square) and zone 8 (star). Ages of the zones and legend for the onset of human activities and European arrival are presented on the right.

2.5.2. Lake Santurbán-Berlín, Colombia (3800 m.asl)

Cluster analysis identified eight diatom zones in the record spanning the last ca. 28,000 cal yr BP (Fig. 2b). Zones 8 to 3 (27,670-6190 cal yr BP) are composed of variable abundances of non-motile, tychoplanktonic *S. pinnata*, *S. construens* and *Gomphonema* cf. *minutum.* Zones 2 and 1 (5720 cal yr BP-Present) are dominated by aerophilous diatoms and weakly motile species rarely present in previous zones, *Eunotia monodon* (dominant) and *A. alpigena*. The upper 10 cm (covering the last 900 years) contains few valves of planktonic and phosphorus-loving *Stephanodiscus oreganicus* and *S. reimeri* (Fig. 4b; Table 1 SI).

HMD analysis showed that temporal shifts in the diatom assemblages were accompanied by a significant ($p < 0.001$; $F = 6.8936$) variation in community heterogeneity over time, with more heterogeneous assemblages in zones 8, 6, 3, and 2, and more homogenous assemblages in zones 5, 4, and 1 (Fig. 4b). The Tukey test revealed that the following zone pairs differ significantly: zones 2 and 4 ($p < 0.001$), zones 2 and 5 ($p = 0.005$), zones 2 and 6 ($p = 0.009$), zones 3 and 4 ($p = 0.003$), zones 3 and 5 ($p = 0.02$), zones 3 and 6 ($p = 0.04$), zones 4 and 7 ($p = 0.03$), zones 4 and 8 ($p = 0.002$), zones 5 and 8 ($p = 0.02$) and zones 6 and 8 ($p = 0.03$). The PCoA plot indicates that diatom assemblages transitioned over time into new associations in zones 2 and 1, more distant from the other historical zones along the negative portion of PCoA axis 1. PERMANOVA indicated that diatom composition differed significantly among all zones (Fig. 4b; Table 3SI).

2.5.3. Lake Siscunsí, Colombia (3687 m asl)

Cluster analysis identified eight main diatom zones spanning the last ca. 3000 cal yr BP (Fig. 2b). Zones 8 to 3 (2860-350 cal yr BP) are dominated by non-motile *Staurosira* sp. nov and variable abundances of non-motile, tychoplanktonic *S. construens*, and *Punctiastriata mimetica*, and prostrate, epiphytic *Cocconeis placentula*. Moderately motile species such as *Stauroneis anceps* and *Sellaphora pupula* are more abundant in zones 6 to 1. In zones 2 and 1, planktonic *Fragilaria tenera*, and moderately motile and prostrate *Gomphonema truncatum, G. acuminatum* and tube-forming *Encyonema silesiacum* appear for the first time; other species that increase significantly at the expense of *Staurosira* sp. nov include prostrate *C. placentula*, and moderately motile, benthic *Planothidium frequentissimum, S. pupula* and *G. affine* (Figs. 2b and 4c; Table 1 SI).

HMD analysis showed that temporal shifts in the diatom community were accompanied by a significant ($p < 0.001$; F= 7.873) variation in community heterogeneity over time, with more heterogeneous assemblages in zones 1 and 2 and more homogenous assemblages in zone 5. The Tukey test revealed that the following zone pairs are significantly different: zones 1 and 4 ($p = 0.02$), zones 1 and 5 ($p < 0.001$), zones 1 and 6 ($p < 0.001$), zones 2 and 4 ($p = 0.003$), zones 2 and 5 (p < 0.001), zones 2 and 6 ($p < 0.001$), zones 2 and 8 ($p < 0.001$), zones 3 and 5 ($p = 0.01$), zones 5 and 7 ($p = 0.006$), zones 5 and 8 ($p < 0.001$) and zones 6 and 7 ($p = 0.04$). The PCoA plot indicates that diatom communities gradually shifted over time into new associations in zones 2 and 1, which are more distant from the other historical zones along the positive portion of PCoA axis 1. PERMANOVA indicated that diatom species composition differed significantly among all the zones (Fig. 4c; Table 3SI).

3. Discussion

Our combined analyses show that in each lake, diatom species shifted significantly into a recent, more homogenous and distinct assemblages with no past analogues in the sediment record. The trajectory of the assemblages in PCoA space shows that in both shallow and deep lakes, more recent assemblages are separated significantly from previous assemblages (Figs. 3-4). PERMANOVA and HDM also indicate that these new assemblages are compositionally different and more homogenous, except in Lake Siscunsí.

In all lakes, the most recent diatom assemblage zones include taxa that tolerate physical disturbance (e.g. turbidity and or alteration of water level), such as single-celled, motile prostrate and moderate motile and some non-motile forms that prefer nutrient-rich waters, like chain-forming diatoms (Figs. 3 and 4). The timing of the switch to these recent diatom assemblages varied between lakes, but with the exception of Lake Santurbán-Berlín, coincided with evidence for human activities (e.g. fire, deforestation, agriculture, and soil erosion) in their respective watershed, as inferred from charcoal, pollen, and/or sediment lithology (Table 2). In the Santurbán-Berlín record there is no evidence for agriculture. The high altitude and dry climate around the lake could have prevented Indigenous peoples from settling there. Therefore the diatom assemblages in zones 2-1 probably reflect a change from lacustrine to mire conditions, as inferred from multi-proxy analysis, and were most likely driven by climate variability (Patiño et al. in review).

Table 2. Timing of the shift to a novel diatom assemblage, total time span of the record, and the date of the onset of human occupation in the watershed, as indicated by other sediment variables, e.g. changes in lithology. Right column indicates if the change to the novel assemblage was gradual over time (shallow lakes), i.e. if the assemblages varied over time among relatively similar associations before shifting to the current novel assemblage, or distinct (deep lakes), i.e. diatoms changed little before switching to the new assemblage (Fig. 5) (Time in thousands of years before present [ka]).

Lake's depth	**Lake**	**Date of shift to novel diatom assemblage (zone[s])**	**Time span of the record**	**Proxy for human activity and date of onset**	**Depth and date of lithological change**	**Change to novel assemblage**
Deep	Amatitlán	0.1 ka; zone 1	2.4 ka	Throughout the record	NA	Distinct
	La Cocha	1.1 ka; zones 2-1	14 ka	Charcoal (fire) 1.4 ka	260 cm (~1.4 ka); light to dark brown peat	Distinct
	Pedro Palo	2 ka; zones 2-1	8 ka	Charcoal (fire) 4.0 ka	N/A	Distinct
	San Carlos	0.05 ka; zone 1	1 ka	Pollen (agriculture) Throughout the record	30 cm (0.035 ka) Peat to light clay	Distinct
	Ubaque	0.1 ka; zone 1	3.7 ka	Throughout the record	51 cm (~0.4 ka) Sand to silt	Distinct
Shallow	Fúquene	4.9 ka; zones 3-1	17 ka	Pollen (deforestation) ~6.3 ka	30 cm organic-detrital to peat	Gradual
	Siscunsí	0.32 ka; zones 2-1	2.8 ka	Chl-a (eutrophication) 1.5 ka	51 cm (0.35 ka) silt increases	Gradual
	Santurbán-Berlín	5.7 ka; zones 2-1	27.6 ka	No evidence (pollen)	35 cm (~4 ka) silt to organic mud	Gradual

3.1. Novel diatom assemblage and lake's depth

We divided our study lakes into two groups, those with z_{max} < 7 m (shallow) and those with z_{max} > 14 m (deep). Diatom assemblages in the shallow lakes (Fúquene, Siscunsí and Santurbán) gradually transitioned over time into new compositional associations (Fig. 5; Table 2), whereas older assemblages in each of the deeper lakes (Amatitlán, La Cocha, Pedro Palo, San Carlos and Ubaque) showed little change until shifting towards the more recent novel assemblage (Fig. 5).

3.1.1. Deep lakes

In Lake Amatitlán, diatom assemblages prior to European arrival were composed of species indicative of high nutrient concentrations, most likely a consequence of soil erosion and physical disturbance from human activities in the watershed (e.g. non-motile planktonic *A. granulata* and *F. delicatissima,* motile *N. amphibia* and *N. palea,* and non-motile, single-celled *D. stelligera*). During the Maya Terminal Classic and part of the Postclassic (1200-750 cal yr BP), Lake Amatitlán was pushed into a slightly modified limnological condition (*Nitzschia* spp dominated zone 3) indicative of intense physical disturbance, but the diatom assemblage evidently did not cross a threshold, and later recovered by returning to conditions that prevailed throughout the Preclassic and Classic, ca. 2000 BCE-900 CE (Figs. 3-4; 5). After European arrival, *F. crotonesis*, a species associated with cyanobacteria blooms in other Guatemalan lakes (Perez et al. 2013) and with anthropogenic eutrophication in lake Juanacatlan, Mexico (Davies et al. 2018), appeared for the first time in the sediment record, along with an increase in *C. meneghiniana,* a diatom that is tolerant of turbid conditions (Hassan 2013). Taken together, the results indicate that Lake Amatitlán experienced a long history of anthropogenic influence, in the form of human-mediated nutrient loading, organic pollution and harsh physical conditions such as turbidity, alterations to littoral areas and water level. Nevertheless, in the last century, the lake has become hypereutrophic as a consequence of intensified human activities, driven in part by rapid population growth and urban expansion of the Guatemalan capital, which lies ~25 km from the lake, and contributes nutrients and sediment to the lake via the inflowing Villalobos River (Vélez et al. 2011).

In Lakes La Cocha, San Carlos and Ubaque, post-European times were characterized by an increase in prostrate and moderately motile species (*Achnanthidium, Cymbella/Encyonema, Diploneis* species). This functional diatom association suggests that in more recent times, physical perturbations (e.g. changes in water level, increased erosion and delivery of detrital sediments) increased in importance. In Lake La Cocha for instance, the last ~1430 cal yr BP have been characterized by severe anthropogenic activities, e.g. deforestation by fire (González-Carranza et al 2012), initially by the Protopasto Indigenous peoples and later on by the Pastos (Rappaport 2011). Increases in water runoff and fine sediment inputs caused by land clearance have been shown to increase both turbidity and nutrient availability (Jones et al. 2014).

In Lake Pedro Palo highly motile and non-motile single cells diatoms increase significantly during the most intense period of anthropogenic activity (zones 5-3; Table 2), after which, moderately motile species increase at the expense of non-motile single cells (zones 2-1). This change likely reflects a decrease in turbidity and nutrient rich waters that allowed the expansion of macrophytes (zones 2-1), after the most intense period of human occupation diminished (Vélez et al. submitted).

Fluctuations in water level alter the extent of the pelagic and littoral areas, thereby changing the abundance and distribution of macrophytes (Talling 1966), and thus conditions for motile and non-motile diatom taxa. For example, the disappearance of non-motile planktonic diatoms and the increase in motile aerophilous diatoms in the novel assemblage of Lake La Cocha (Fig. 3b), likely reflects fluctuations in the lake's water level and consequent expansion of littoral vegetation. The increase in non-motile, planktonic, chain-forming and single-celled taxa, e.g. *A. ambigua* and *C. meneghiniana*, respectively, and in prostrate species (e.g. *A. minutissimum*) in Lake Ubaque (Fig. 3e), indicates that European arrival affected the lake's water quality, mainly by increasing nutrient concentrations and turbidity.

Although our approach sought to assess the influence of lake depth on diatom community response to stressors, other morphometric factors, as well as hydrology (e.g. water residence time), and the presence/extent of floating or submerged vegetation, as currently observed in the shallow areas of Lake Ubaque, probably also influenced the response of the diatom communities in both shallow and deep lakes (Dubois 2018; Talling 1966).

Diatom compositional shifts in deeper tropical lakes are probably related to changes in water level, expansion and or contraction of the littoral zone and thermal stratification of the water column (Michelutti et al. 2003; Talling 1966). Thermal stratification restricts movement of algae between the epilimnion and deeper water layers, contributes to oxygen depletion in the hypolimnion, and if permanent (more likely at high-elevation lakes), prevents nutrient cycling from deep waters, which only occurs with a breakdown of density stratification, normally under cooling conditions (Michelutti et al. 2003; Talling 1966).

3.1.2. Shallow lakes

In shallow Lakes Fúquene and Siscunsí, earlier times were characterized by non-motile tychoplanktonic species (*Staurosira, Pseudostaurosira, Staurosirella, Aulacoseira*), a functional profile commonly associated with mesotrophic (i.e. intermediate nutrient levels) environments and littoral conditions (Passy 2007; Berthon et al. 2011). A combination of non-motile, single-celled taxa such as *Discostella* and *Cyclotella* species, non-motile planktonic *F. tenera*, moderately motile benthic *Gomphonema* spp., and prostrate *C. placentula, Achnanthidium*, and *Planothidium* subsequently appeared or increased in the more recent diatom assemblage(s), after evidence of human activity. These diatom species are often associated with factors such as shading, turbidity, erosion and nutrient increase, suggesting that conditions in these lakes had become more eutrophic and there was greater physical perturbation.

Shallow lakes are often more productive than deeper lakes and are characterized by long-term macrophyte successional dynamics both spatially and temporally, more complete and frequent water-column mixing (especially in the tropics where this can occur year round), high rates of nutrient cycling, and smaller water volumes to dilute incoming nutrients (Scheffer 2001). Therefore, shallower systems often respond more quickly than deeper lakes to climate and environmental changes, catchment degradation and cultural eutrophication (Scheffer 2001).

Our results concur with previous studies that showed shallow lakes respond earlier and more sensitively to human-related limnological changes than do larger, deeper lakes (Scheffer 2001). They are, however, at odds with recent findings, which indicated that deep lakes in the tropical Andean páramos are more responsive to environmental changes than small shallow water bodies (Giles et al., 2008)

3.2. Timing of the change to the novel diatom assemblage

The shift to a novel diatom assemblage in Colombian Lakes Fúquene, Santurbán-Berlín, Pedro Palo and La Cocha, occurred in the middle to late Holocene. Except the relatively undisturbed Lake Santurbán-Berlín, human-mediated deforestation within these lake catchments began in the middle to late Holocene (Table 2). The Indigenous population in what is today Colombia numbered at least five million when Europeans arrived, and some three million of them lived in the Andean region where the lakes are located (Etter and van Wyngaarden 2000). The region known as the Altiplano Cundiboyacense, where Lakes Siscunsí, Ubaque, Pedro Palo and Fúquene are located, was densely populated and intensively cultivated since ~5000 cal yr BP (Etter et al. 2008; Delgado 2016; Reichel-Dolmatoff 1965; Rodriguez-Gallo 2019), with constructed raised terraces (*camellones*) surrounded by water-filled channels, which provided food year-round (Rodriguez-Gallo, 2019; Van der Hammen 2003). The estimated Indigenous population density around Lake Fúquene was 16-33 people km^2, but the human density subsequently rose to 39-78 km^2 after European arrival (Etter 2015). Human activities would likely have altered lake water levels, and increased nutrient and sediment loads, contributing to increased turbidity and eutrophication (Anderson 2014; Jones et al. 2012), which in turn would have made diatom assemblages more homogenous (Salgado et al. 2018).

In records from lakes where Indigenous activities are documented throughout the entire sediment record, i.e. Siscunsí, Ubaque, Amatitlán (Guatemala) and San Carlos (Panama) (Table 2), the shift to the novel diatom assemblage occurred after European arrival (Figs. 3 and 4). This suggests that prior to European settlement, diatom communities may have had the ability to resist environmental stress stemming from human activities, while also responding to natural climate disturbances, e.g. changes in precipitation and/or temperature (Correa-Metrio et al. 2016; Vélez et al. 2011, 2003).

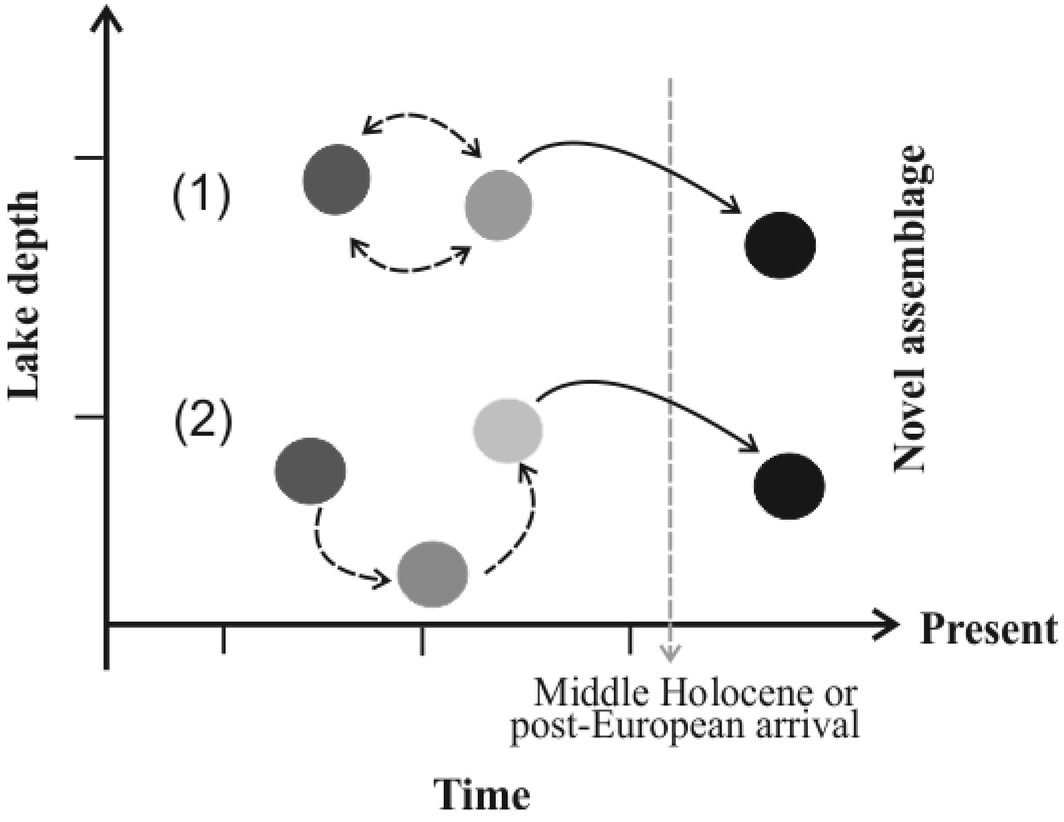

Figure 5. Conceptual representation of the shift from historical heterogeneous diatom assemblages (grey circles) to recent, more homogenous novel assemblages (black circles): (1) in deeper lakes (Amatitlán, San Carlos, La Cocha and Ubaque), historical diatom communities were grouped under similar compositional assemblages, but then shifted to a new, unique diatom assemblage; (2) in shallow lakes (Fúquene, Santurbán-Berlín and Siscunsí) diatom communities transitioned over time until they reached the current, different assemblage.

3.3. Caveats

Our ability to generalize about diatom community responses to human influence in Neotropical mountain lakes has some limitations. Differences in the time range and temporal sampling resolution among cores from lakes, together with uncertainties in the core chronologies, confound efforts to link shifts in the sediment records with archaeological and historical changes in the watersheds (the latter also subject to dating uncertainties). Furthermore, differential diatom preservation and taxonomic approaches among the published and unpublished diatom records we utilized could also introduce disparities among paleoenvironmental inferences from lacustrine records. Nevertheless, the multiple independent lines of evidence for drivers of change in diatom communities show general agreement, especially with respect to the development of a novel, recent diatom zone (or zones), that lacks an older analogue. The magnitude and timing of changes observed in our records coincide with what is known about past human occupation of the watersheds, as well as with what is known about past climate changes in the region. By combining the diatom species from each lake into functional groups, we were able to reduce the potential influence of taxonomic discrepancies between records. Thus, despite the potential limitations of the paleoecological approach, the data provide important insights into past human-mediated changes in the studied Neotropical lakes.

3.4. Implications for lake management in the "Anthropocene"

The shift to a novel diatom assemblage in the lakes, suggests that they have lost or are losing the capacity to resist change or recover from stressors including human interference and climate change. Implementation of new technologies and intensification of deforestation, agriculture, and urbanization in the catchments were likely the main causes of diatom community change (Table 2). Similar to the other lakes, Lake Siscunsí developed a novel diatom assemblage after European arrival, however unlike the other lakes, the recent diatom assemblages in Siscunsí became more heterogeneous. This could reflect a change in land use that occurred after European arrival. According to Temoltzin-Loranca (2018), by 1500 cal yr BP, at the time of the Muisca culture, the lake had become eutrophic (Table 2). The more heterogeneous assemblages that characterized the period following European arrival may indicate subsequent abandonment of the lake catchment and that the lake did not lose its resilience.

The shift in recent times to an unprecedented, more homogenous diatom assemblage indicates that the lakes have surpassed, or will soon surpass, an environmental threshold (Olden et al. 2004). For the last several decades, Lake Amatitlán has been hypereutrophic and plagued with massive cyanobacteria blooms (Perez et al. 2013). Similarly, Lake Fúquene was shown to be in a precarious state after losing many native submerged macrophyte species (Salgado et al. 2019). The current dominance of invasive plant species, in conjunction with increased eutrophication, is making it increasingly unlikely, from both ecological and economical perspectives, that it will be possible to restore the lake to anything resembling its pre-disturbance condition (Salgado et al. 2019). Homogenization is now considered one the most prominent forms of biotic impoverishment in freshwater ecosystems worldwide (Rahel 2002; Olden et al. 2004) and as indicated by the diatom assemblages in the Neotropical mountain lakes we studied, might play a significant role in influencing lake food-web structure (Beisner et al. 2003), rates of non-native species spread and community resistance to future invasions (Olden et al. 2004), and loss of resilience in the face of further eutrophication (Salgado et al. 2018) and or climate change (Salgado et al. 2018; Michelutti et al. 2003).

4. Conclusions

We explored how diatom assemblages in Neotropical mountain lakes changed in response to human activities in their watersheds (hypothesis 1), the composition of the diatom assemblages relative to historical assemblages found throughout the lake's history (hypothesis 2) and if diatom response differed according to lake depth (shallow vs. deep) (hypothesis 3). We found that human activities, both before and after European arrival, pushed the lakes into a more recent condition, characterized by diatom assemblages that were different in species composition and more homogenous (Beta diversity) than the assemblages that inhabited the lakes in earlier times. Lake Santurbán-Berlín was least affected by human activities, and thus the shift to a new, more homogenous diatom community reflects changes in the climate and local environment.

We also found that the lakes responded differently, depending on their maximum water depth. In shallow lakes ($z_{max} < 7$ m), the diatom community changed gradually towards a novel diatom assemblage, whereas in deeper lakes ($z_{max} > 14$ m), diatom assemblages fluctuated, but maintained similar species composition, then subsequently shifted to a different assemblage type. The differential response of diatom communities in shallow and deep lakes may be explained by greater submersed vegetation cover and a more complete water-column mixing and high rates of nutrient cycling in shallow lakes, which means biota in these systems respond more quickly to catchment degradation, sediment input and cultural eutrophication. Other factors that may contribute to the differential response of diatoms in shallow and deep lakes include differences in lake volume, water residence times and climate change (Giles et al. 2018; Michelutti et al. 2003).

For lakes with diatom records that spanned the Holocene, or more (lakes La Cocha, Fúquene and Pedro Palo), the shift to the novel diatom assemblage occurred in the middle to late Holocene, with the onset or intensification of human activities. Lakes that yielded shorter records, and were subject to human impacts throughout the time span of the recovered sequence

(Amatitlán, San Carlos, Ubaque) showed a pronounced change after European settlement, reflecting the intensification of post-colonial human activities.

Although paleoenvironmental inferences are limited by the time span, sampling resolution, age model, and preservation of fossils in the sediment records, they nonetheless provide invaluable insights into past environments and enable us to explore ecosystem processes that occur on time scales longer than funding cycles and even human life-times. Diatom assemblages from our study lakes indicated that human activities had a profound influence on the ecology of Neotropical aquatic ecosystems. Furthermore, they revealed that in most cases, the shift to a novel diatom assemblage occurred long before 1950 CE, a date proposed by some to mark the beginning of the Anthropocene (Zalasiewicz et al. 2017).

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Conflict of Interest

The authors declare no conflict of interest.

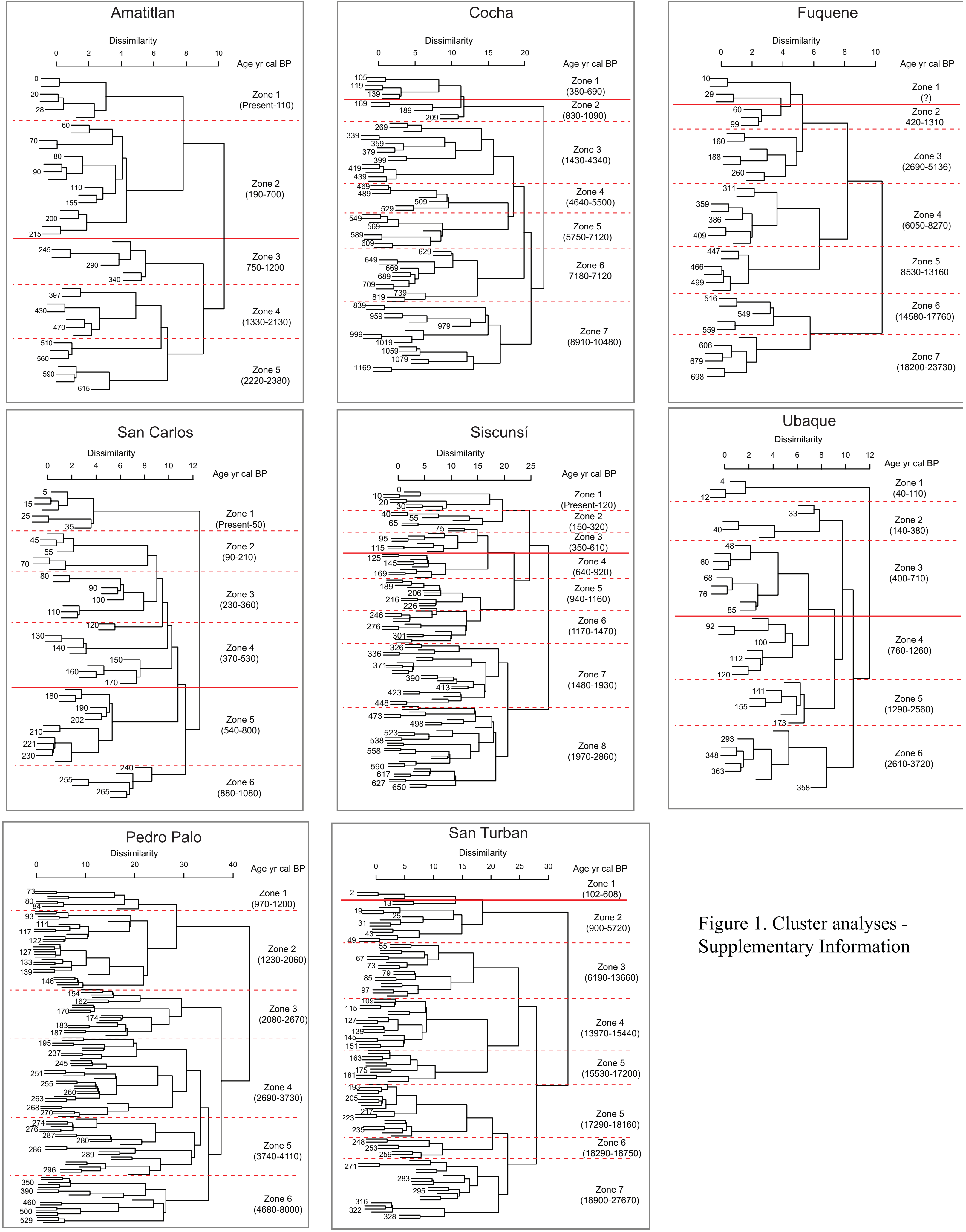


Figure 1. Cluster analyses - Supplementary Information

Table 1. Supplementary Information. Classification of diatoms according to motility

Species	Update name	Functional category	Other comments	Reference
Achnanthes exigua	Achanthidium exigum	Slightly motile; benthic		Diatoms.org
Achnanthes minustissima	Achnanthidium minutissim	Unattached-slighlty motile	Benthic	Diatoms.org
Achnanthes subhudsonis	Achnanthidium subhudsoni	Unattached-weakly motile		Diatoms.org
Planothidium frequentissimum	Planothidium	Motile		Berthon et al. 2011
Achnanthes inflata	Achnanthes inflata	Slightly motile; aerophil		Diatoms.org
Asterionella formosa		Non motile-Plank		Diatoms.org
Aulacoseira distans cf laevissima		Non motile-Plank		Diatoms.org
Aulacoseira granulata		Non motile-Plank		Diatoms.org
Aulacoseira granulata var angustissima		Non motile-Plank		Diatoms.org
Aulacoseira ambigua		Non motile-Plank		Diatoms.org
Aulacoseira alpigena		Non motile-Plank		Diatoms.org
Brachysira brebisonii		Moderate Motile		Diatoms.org
Craticula cuspidata		Highly Motile		Diatoms.org
Caloneis		Motile		Berthon et al. 2011
Cyclotella meneghiniana		Non motile single celled	Hyper-Eutrophic guatemalan	Perez 2013
		Non motile single celled	Increase in turbidity	Diatoms.org;Hassan 2013
Cyclotella stelligera	Discostella stelligera	Non motile single celled		Diatoms.org
Cyclotella pseudostelligera	Discostella pseudostelliger	Planktonic	Dominates today in Amatitla	Perez et al. 2013
Cymbella aspera		Slightly motile; vert att		Diatoms.org
Cymbella/Encyonema		Motile		Berthon et al. 2011
Cymbella minuta		Moderately motile-tube forming		Diatoms.org
Cymbella silesiaca	Encyonema silesiacum	Moderately motile-tube forming		Diatoms.org
Cymbella microcephala	Encyonospis microcephala	Moderately motile-tube forming		Diatoms.org
Cymbella cesatii	Encyonospis cesatii	Slightly motile		Diatoms.org
Cocconeis placentula		Non motile-weakly motile-prostrate		Diatoms.org
Diploneis elliptica		Aerophil; mod motile		Diatoms.org
Epithemia turgida		Mod motile-Prostrate		Diatoms.org
Eunotia preaerupta	Eunotia			Berthon et al. 2011
Eunotia spp		Slighly Motile		Berthon et al. 2011
Eunotia bilunaris		Prostrate-benthic moist habit		Diatoms.org
Eunotia monodon		Aerophil; weakly motile, prostrate		Diatoms.org
Eunotia naeglii		Weakly Motile-AERO		Diatoms.org
Eunotia triodon		Slighly Motile		Diatoms.org
Fragilaria brevistriata	Pseudostaurosira brevistria	Vertical attachemnt; non motile		Diatoms.org
Fragilaria crotonensis		Non-motile; planktonic	in guatemalan lakes with bloo	Perez et al. 2013; Diatoms.org
Fragilaria tenera		Non-motile; planktonic	acidic, low to circumnreutral;	Diatoms.org; da Silva-Lehmukuh et al. 2019
Fragilaria delicatissima		Non-motile; planktonic	in highland lakes of Guatemal	Perez et al. 2013; Diatoms.org;da Silva-Lehmukuh et al. 2019
Fragilaria elliptica	Pseudostaurosira elliptica	vertical-benthic		Diatoms.org
Fragilaria pinnata	Staurosirella pinnata	Non-motile-vertical-plank-benthic		Diatoms.org
Fragilaria construens	Staurosira construens	vertical-plank-benthic		Diatoms.org
Punctistriata mimetica		Non-motile unattached		Diatoms.org
Frustrulia spp		Mod motile-Tube forming		Diatoms.org
Gomphonema truncatum		Mod motile-Prostrate-vertical		Diatoms.org
Gomphonema acuminatum		Mod motile-Prostrate-vertical		Diatoms.org
Gomphonema parvulum		Highly motile		Stenger-Kovacks et al. 2013
Gomphonema gracile		Slighly Motile		Diatoms.org
Hantzchia amphioxis		Aerophil		
Halamphora veneta		Moderate Motile-Prostrate		Diatoms.org
Luticula mutica		Aerophil		see original pub. Table 1
Navicula contenta		Slightly motile; aerophil		Diatoms.org
Navicula gallica		Slighly Motile		Diatoms.org
Navicula radiosa		Mod motile-benthic		Diatoms.org
Navicula lanceolata		Mod motile; benthic		Diatoms.org
Nitzschia amphibia		Mod motile-Protstra-benthic		Diatoms.org
Nitzschia amphibia var rostrata		Mod motile-Protstra-benthic		Diatoms.org
Nitzschia palea		Mod motile-Protstra-benthic		Diatoms.org
Nitzschia linearis		Moderate Motile		Diatoms.org
Orthoseira roeseana		Aerophil; non motile unattached		Diatoms.org
Pinnularia maior		Mod motile		Diatoms.org
Pinnularia viridis		Mod motile		Diatoms.org
Pinnularia spp		Mod motile		Diatoms.org
Pinnularia borealis		Mod motile- AER		Diatoms.org
Rhoicosphenia abbreviata		Weakly Motile vertical att		Diatoms.org
Staurosira sp nov		Non-motile (araphid)		
Sellaphora pupula		Mod motile-benthic		Diatoms.org
Stauroenis anceps		Mod motile-benthic		Diatoms.org
Stauroneis acidoclinata		Mod motile-benthic		Diatoms.org
Stauroneis phoenicenteron		Mod motile-benthic		Diatoms.org
Synedra ulna	Ulnaria ulna	Non-motile; planktonic	Oligotrophic in Brazil reservo	da Silva-Lehmukuh et al. 2019
Tabellaria floculossa		Non-motile-Protrate		Diatoms.org

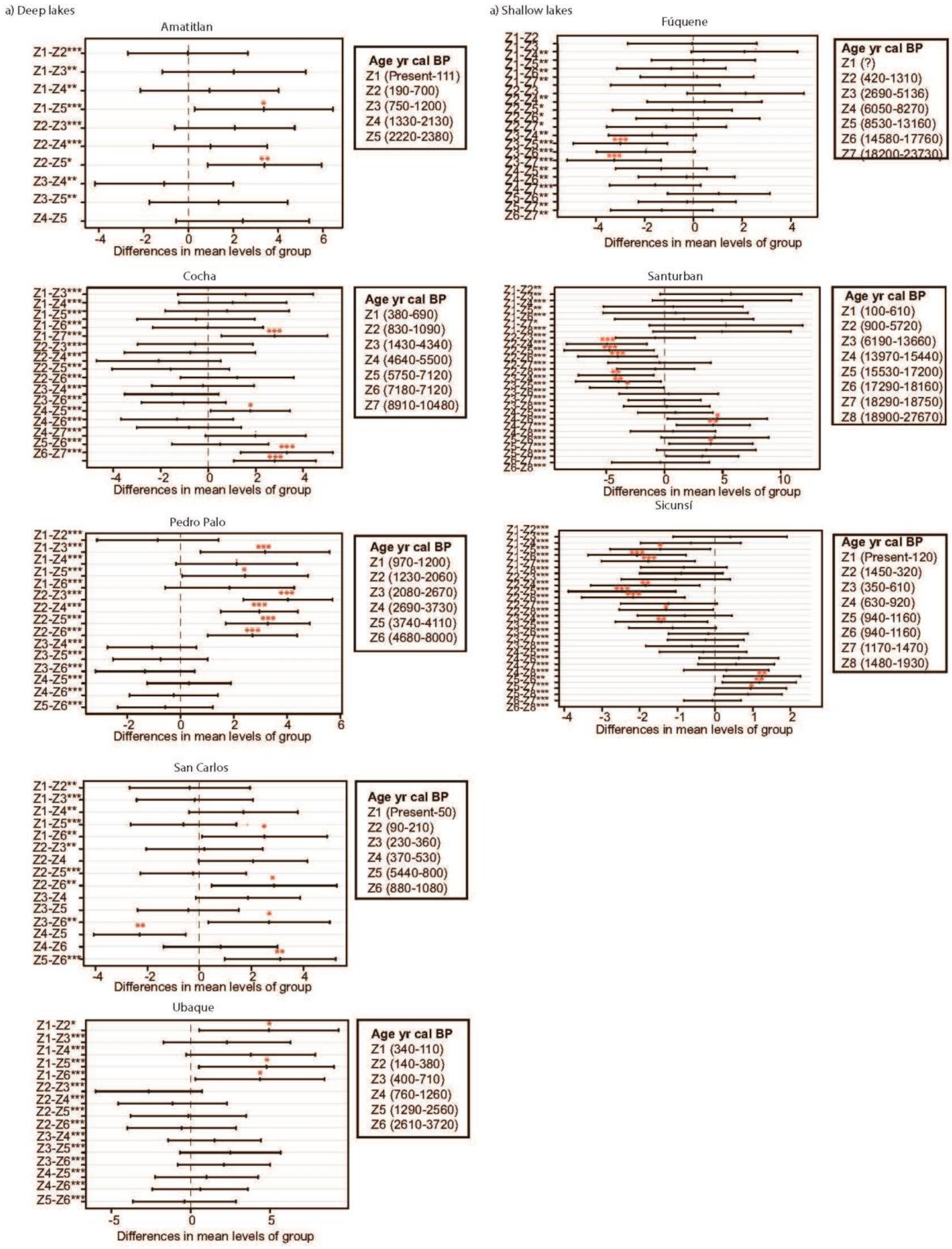
Supplementary Information

SI-Table 2. ^{14}C and calibrated ages of the studied records.

Amatitlán	**Depth (cm)**	**Age ^{14}C**	**Error**	**Calibrated**
	240	710	50	780
	440	1530	190	1612
	540	2010	100	2044
	610	2340	110	2358
	690	2570	40	2720
San Carlos	60	230	20	140
	100	255	20	300
	137.5	265	20	412
	222	690	20	670
	276	1210	20	1080
La Cocha	110	165	45	390
	194	695	35	712
	366	1915	25	1860
	525	2805	35	2930
	637	3520	30	3830
	720	4165	40	4780
	763	5200	30	5940
	801	5845	30	6660
	841	7005	30	7800
	898	7880	40	8660
	941	8325	40	9300
	989	8755	40	9740
	1029	9220	40	10170
	1060	9650	50	10390
	1079	10040	45	10540
	1119	11350	50	10840
	1174	12080	45	11240
	1198	12150	60	11420
Fúquene	349.5	6040	60	6890
	403.5	7070	50	7900
	448.5	7780	60	8560
	467.5	7850	70	8850
	481.5	7890	60	9130
	491.5	7970	60	9750
	504.5	8680	60	12140
	521.5	13110	120	15570

	543.5	14200	120	17140
	651.5	17990	60	21820
	699.5	19670	240	23770
Siscunsí	199.5	1310	120	1055
	207.5	1180	25	1090
	479	2020	40	2040
	580.5	2420	45	2490
Ubaque	43.5	500	120	520
	81.5	620	40	600
	115.5	1260	130	1170
	163.5	1660	70	1570
	203.5	1980	130	1940
	299.5	2495	15	2580
	335.5	3700	100	4050
	355.5	3095	30	3300
	369.5	3120	15	3350
	417.5	4030	60	4520
Santurbán-Berlín	41 .5	4170	30	4720
	53.5	5760	40	6550
	64.5	8330	40	9290
	84.5	10800	30	12740
	106.6	12020	30	13990
	152.5	12730	40	15410
	170.5	11740	40	16150
	185.5	14370	50	17180
	205.5	14570	40	17670
	244.5	14800	40	18220
	265.5	15580	50	18940
	279.5	21350	70	25290
	289.5	25740	100	25720
	307.5	21230	70	26280
	329.5	23530	80	27670
Pedro Palo	83	2225	30	2230
	162	2205	30	2230
	272	3465	30	3740
	385	4580	30	5300
	492	4665	30	5400
	602	8290	35	9310

Supplementary Information Table3.
Results from TUKEY Test and fromf homogeneity of multivariate dispersions and PERMANOVA



PERMANOVA
DEEP LAKES:

a) Amatitlan Lake

Homogeneity of multivariate dispersions

Average distance to median:

Z1	Z2	Z3	Z4	Z5
2.576	2.541	4.602	3.518	5.936

Eigenvalues for PCoA axes:

PCoA1	PCoA2	PCoA3	PCoA4	PCoA5	PCoA6	PCoA7	PCoA8
707.7013	270.1438	195.6974	100.1425	58.0642	32.0277	23.6668	22.3646

Anova

Analysis of Variance Table

Response: Distances

	Df	**Sum Sq**	**Mean Sq**	**F value**	**Pr(>F)**
Groups	4	67.401	16.8503	4.5157	0.004648 **
Residuals	36	134.334	3.7315		

\---

Signif. codes: 0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

Tukey multiple comparisons of means

95% family-wise confidence level

Group	**diff**	**lower**	**upper**	**p adj**
Z2-Z1	-0.03521336	-2.7139944	2.643568	0.9999995
Z3-Z1	2.02657122	-1.1751845	5.228327	0.3798273
Z4-Z1	0.94209947	-2.1431895	4.027388	0.9036107
Z5-Z1	3.36017790	0.2748889	6.445467	0.0270474*
Z3-Z2	2.06178458	-0.6169964	4.740566	0.1993445
Z4-Z2	0.97731284	-1.5611190	3.515745	0.8026368
Z5-Z2	3.39539126	0.8569594	5.933823	0.0041181**
Z4-Z3	-1.08447174	-4.1697608	2.000817	0.8494936
Z5-Z3	1.33360668	-1.7516823	4.418896	0.7278617
Z5-Z4	2.41807842	-0.5461714	5.382328	0.1553479

perMNOVA

	Df	**SumOfSqs**	**F**	**Pr(>F)**
Z2-Z1	1	156.49	19.561	0.001***
Z3-Z1	1	377.57	18.663	0.003**
Z4-Z1	1	14029.7	18.558	0.004**
Z5-Z1	1	187.44	6.5781	0.001***
Z3-Z2	1	459.08	33.772	0.001***
Z4-Z2	1	76.717	7.7345	0.001***
Z5-Z2	1	113.45	6.1425	0.011*
Z4-Z3	1	336.38	14.87	0.005**
Z5-Z3	1	2213.85	5.6045	0.01**
Z5-Z4	1	62.68	2.0903	0.188

b) Cocha Lake

Homogeneity of multivariate dispersions

```
Average distance to median:
  Z1    Z2    Z3    Z4    Z5    Z6    Z7
4.252 5.823 5.274 5.051 3.734 4.240 7.046
Eigenvalues for PCoA axes:
   PCoA1     PCoA2     PCoA3     PCoA4     PCoA5     PCoA6     PCoA7     PCoA8
1069.9151  584.2089  478.3129  209.9622  149.7861  114.2929  103.5510   93.4192
```

Anova

```
Analysis of Variance Table
Response: Distances
           Df   Sum Sq     Mean Sq          F value      Pr(>F)
Groups      6   96.666     16.1110          6.7508       1.365e-05 ***
Residuals  65   155.125    2.3865
```

Tukey multiple comparisons of means

```
   95% family-wise confidence level
Group        diff        lower       upper        p adj
Z2-Z1   1.5708139  -1.2753457  4.4169734   0.6320420
Z3-Z1   1.0220332  -1.2484167  3.2924830   0.8156808
Z4-Z1   0.7993320  -1.8156611  3.4143252   0.9662768
Z5-Z1  -0.5176308  -2.9948947  1.9596331   0.9953334
Z6-Z1  -0.0119273  -2.3317405  2.3078859   1.0000000
Z7-Z1   2.7939618   0.5619969  5.0259266   0.0055144
Z3-Z2  -0.5487807  -2.9759937  1.8784323  0.9928507
Z4-Z2   -0.7714818  -3.5236827  1.9807190  0.9780637
Z5-Z2  -2.0884447  -4.7101344  0.5332450  0.2055989
Z6-Z2  -1.5827412  -4.0561910  0.8907086  0.4578015
Z7-Z2   1.2231479  -1.1681044  3.6144002   0.7099307
Z4-Z3  -0.2227011  -2.3741955  1.9287932  0.9999150
Z5-Z3  -1.5396640  -3.5214751  0.4421471  0.2308040
Z6-Z3  -1.0339605  -2.8150479  0.7471269  0.5750670
Z7-Z3   1.7719286   0.1068742  3.4369830    0.0297270
Z5-Z4  -1.3169629  -3.6856802  1.0517545  0.6239807
Z6-Z4  -0.8112593  -3.0147835  1.3922649  0.9196291
Z7-Z4   1.9946297  -0.1162120  4.1054714    0.0760706
Z6-Z5   0.5057035  -1.5324737  2.5438807    0.9882947
Z7-Z5   3.3115926   1.3739910  5.2491942    0.0000433
Z7-Z6  2.8058891    1.0741278  4.5376503    0.0001180
```

PerMNOVA

```
          Df   SumOfSqs   F        Pr(>F)
 Z2-Z1    1    63.042     1.9271   0.02*
 Z3-Z1    1    261.00     8.9862   0.001***
 Z4-Z1    1    291.42     10.793   0.001***
 Z5-Z1    1    276.45     14.5     0.001***
 Z6-Z1    1    429.40     19.64    0.001***
```

Z7-Z1	1	226.02	4.7645	0.001***
Z3-Z2	1	173.63	5.0565	0.001***
Z4-Z2	1	220.41	6.068	0.001***
Z5-Z2	1	220.66	8.4313	0.001***
Z6-Z2	1	2.47.84	9.0557	0.001***
Z7-Z2	1	128.08	2.411	0.023*
Z4-Z3	1	310.301	9.8918	0.001***
Z5-Z3	1	72.78	2.7692	0.032*
Z6-Z3	1	317.36	11.753	0.001***
Z7-Z3	1	92.83	2.0994	0.056
Z5-Z4	1	236.29	10.23	0.001***
Z6-Z4	1	261.42	10.522	0.001***
Z7-Z4	1	293.38	6.0221	0.001***
Z6-Z5	1	367.76	18.479	0.001***
Z7-Z5	1	135.74	3.1857	0.015*
Z7-Z6	1	357.6	8.7341	0.001***

c) Pedro Palo

Homogeneity of multivariate dispersions

Average distance to median:

Z1	Z2	Z3	Z4	Z5	Z6
3.208	2.351	6.377	5.304	5.622	5.045

Eigenvalues for PCoA axes:

PCoA1	PCoA2	PCoA3	PCoA4	PCoA5	PCoA6	PCoA7	PCoA8
2199.8383	1373.8200	658.3963	544.4763	356.1054	231.5365	165.5512	109.6016

Anova

Analysis of Variance Table

Response: Distances

	Df	**Sum Sq**	**Mean Sq**	**F value**	**Pr(>F)**
Groups	5	275.53	55.106	13.979	7.264e-11 ***
Residuals	126	496.71	3.942		

Tukey multiple comparisons of means

95% family-wise confidence level

Group	**diff**	**lower**	**upper**	**p adj**
Z2-Z1	-0.8566211	-3.1352896	1.422047	0.8853742
Z3-Z1	3.1693430	0.7475624	5.591123	0.0031453**
Z4-Z1	2.0960205	-0.1753329	4.367374	0.0885031
Z5-Z1	2.4146213	0.0560629	4.773180	0.0414986
Z6-Z1	1.8373785	-0.5844020	4.259159	0.2471164
Z3-Z2	4.0259640	2.3517844	5.700144	0.0000000***
Z4-Z2	2.9526416	1.5045730	4.400710	0.0000005***
Z5-Z2	3.2712424	1.6898967	4.852588	0.0000003***
Z6-Z2	2.6939996	1.0198199	4.368179	0.0001155***
Z4-Z3	-1.0733225	-2.7375319	0.590887	0.4275768
Z5-Z3	-0.7547217	-2.5361101	1.026667	0.8233047
Z6-Z3	-1.3319645	-3.1962520	0.532323	0.3109616
Z5-Z4	0.3186008	-1.2521856	1.889387	0.9917404
Z6-Z4	-0.2586420	-1.9228515	1.405567	0.9976359
Z6-Z5	-0.5772428	-2.3586312	1.204146	0.9359139

perMNOVA

	Df	SumOfSqs	F	Pr(>F)
Z2-Z1	1	101.54	11.958	0.001***
Z3-Z1	1	284.60	7.7441	0.001***
Z4-Z1	1	267.03	9.4537	0.001**
Z5-Z1	1	339.35	9.7922	0.001***
Z6-Z1	1	308.39	12.336	0.001***
Z3-Z2	1	1162.4	53.843	0.001***
Z4-Z2	1	973.03	49.842	0.001***
Z5-Z2	1	794.81	36.82	0.001***
Z6-Z2	1	714.38	46.186	0.001***
Z4-Z3	1	484.14	13.213	0.001***
Z5-Z3	1	749.63	17.361	0.001***
Z6-Z3	1	720.22	19.248	0.001***
Z5-Z4	1	285.35	8.0373	0.001***
Z6-Z4	1	333.91	10.896	0.001***
Z6-Z5	1	232.4	6.4859	0.001***

d) San Carlos

Homogeneity of multivariate dispersions

Average distance to median:

Z1	Z2	Z3	Z4	Z5	Z6
3.570	3.199	3.392	5.257	2.959	6.067

Eigenvalues for PCoA axes:

PCoA1	PCoA2	PCoA3	PCoA4	PCoA5	PCoA6	PCoA7	PCoA8
466.6933	204.2074	132.8484	119.5199	72.2434	64.7015	60.2602	46.5531

Anova

Analysis of Variance Table

Response: Distances

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Groups	5	65.820	13.1639	6.2169	0.0001756 ***
Residuals	46	97.403	2.1175		

Tukey multiple comparisons of means

95% family-wise confidence level

Group	diff	lower	upper	p adj
Z2-Z1	-0.3711636	-2.68371727	1.9413901	0.9967350
Z3-Z1	-0.1779002	-2.41702063	2.0612203	0.9998923
Z4-Z1	1.6868732	-0.40491031	3.7786567	0.1784413
Z5-Z1	-0.6102743	-2.63851807	1.4179694	0.9458875
Z6-Z1	2.4972938	0.09031166	4.9042760	0.0379028*
Z3-Z2	0.1932634	-2.04585704	2.4323839	0.9998380
Z4-Z2	2.0580368	-0.03374671	4.1498203	0.0561686
Z5-Z2	-0.2391107	-2.26735448	1.7891330	0.9992562
Z6-Z2	2.8684574	0.46147525	5.2754396	0.0111551
Z4-Z3	1.8647734	-0.14552866	3.8750754	0.0831180
Z5-Z3	-0.4323742	-2.37647516	1.5117268	0.9852744

```
Z6-Z3  2.6751940  0.33867540  5.0117126 0.0163823*
Z5-Z4 -2.2971475 -4.06955448 -0.5247406 0.0045660**
Z6-Z4  0.8104206 -1.38530633  3.0061476 0.8799130
Z6-Z5  3.1075682  0.97228565  5.2428507 0.0010781**
```

perMNOVA

```
         Df   SumOfSqs    F        Pr(>F)
 Z2-Z1   1    100.97      7.4742   0.002**
 Z3-Z1   1    187.03      13.077   0.001***
 Z4-Z1   1    150.86      5.6153   0.003**
 Z5-Z1   1    243.71      19.005   0.001***
 Z6-Z1   1    130.59      4.5621   0.002**
 Z3-Z2   1    40.562      3.1318   0.008**
 Z4-Z2   1    41.95       1.6279   0.147
 Z5-Z2   1    61.12       5.1587   0.001***
 Z6-Z2   1    78.701      2.9116   0.002**
 Z4-Z3   1    47.76       1.628    0.143
 Z5-Z3   1    10.074      0.8073   0.613
 Z6-Z3   1    113.70      4.2483   0.002**
 Z5-Z4   1    54.88       2.5103   0.052
 Z6-Z4   1    69.81       1.8531   0.061
 Z6-Z5   1    132.36      6.1472   0.001***
```

e) Ubaque

Homogeneity of multivariate dispersions

```
Average distance to median:
  Z1    Z2    Z3    Z4    Z5   pZ6
2.073 7.001 4.353 5.850 6.840 6.435
Eigenvalues for PCoA axes:
  PCoA1    PCoA2    PCoA3    PCoA4    PCoA5    PCoA6    PCoA7    PCoA8
452.6176 297.9673 230.7813 202.8577 187.6076 130.1629 117.9511 111.3918
___
```

Anova

```
Analysis of Variance Table
Response: Distances
             Df      Sum    Sq Mean       Sq F value     Pr(>F)
Groups        5      76.395 15.2791       3.8537         0.007353 **
Residuals 33 130.839  3.9648
```

Tukey multiple comparisons of means

```
  95% family-wise confidence level
Group     diff          lower       upper      p adj
Z2-Z1   4.9285401  0.5318591 9.3252211 0.0207025*
Z3-Z1   2.2803416 -1.7332607 6.2939438 0.5302856
Z4-Z1   3.7775878 -0.2982445 7.8534202 0.0821589
Z5-Z1   4.7669539  0.5098859 9.0240220 0.0208903*
pZ6-Z1  4.3625916  0.2867592 8.4384240 0.0301898*
Z3-Z2  -2.6481985 -6.0062191 0.7098221 0.1910873
```

```
Z4-Z2  -1.1509522 -4.5831102 2.2812058 0.9099574
Z5-Z2  -0.1615861 -3.8071214 3.4839492 0.9999933
pZ6-Z2 -0.5659485 -3.9981065 2.8662095 0.9958864
Z4-Z3   1.4972463 -1.4281439 4.4226365 0.6372187
Z5-Z3   2.4866124 -0.6864188 5.6596436 0.1964399
pZ6-Z3  2.0822500 -0.8431402 5.0076402 0.2868469
Z5-Z4   0.9893661 -2.2620233 4.2407555 0.9385227
pZ6-Z4  0.5850038 -2.4251979 3.5952055 0.9911887
pZ6-Z5 -0.4043624 -3.6557518 2.8470271 0.9989265
```

perMANOVA

	Df	SumOfSqs	F	Pr(>F)
Z2-Z1	1	224.2	4.7686	0.024*
Z3-Z1	1	315.17	15.171	0.002**
Z4-Z1	1	317.02	9.2644	0.006**
Z5-Z1	1	266.24	6.1885	0.02*
Z6-Z1	1	250.97	5.829	0.005**
Z3-Z2	1	180.35	4.774	0.001**
Z4-Z2	1	117.60	2.337	0.002**
Z5-Z2	1	119.91	1.9739	0.012*
Z6-Z2	1	175.84	3.0552	0.005**
Z4-Z3	1	68.2	2.1348	0.001***
Z5-Z3	1	138.15	3.802	0.002**
Z6-Z3	1	162.36	4.3589	0.001***
Z5-Z4	1	77.25	1.619	0.046*
Z6-Z4	1	137.63	2.9241	0.001***
Z6-Z5	1	163.96	3.017	0.003**

DEEP LAKES

a) Fúquene

Homogeneity of multivariate dispersions

```
Average distance to median:
  Z1    Z2    Z3    Z4    Z5    Z6    Z7
3.022 2.979 5.119 3.439 2.124 3.173 1.877
```

```
Eigenvalues for PCoA axes:
  PCoA1    PCoA2    PCoA3     PCoA4    PCoA5   PCoA6   PCoA7   PCoA8
801.1564 460.4860 155.8276  110.7705  79.8751  40.4263  32.3226  25.9591
```

Anova

Analysis of Variance Table

Response: Distances

	Df Sum	Sq Mean	Sq F value	Pr(>F)
Groups	6 43.944	7.3241	6.0227	0.0002674 ***
Residuals	32 38.914	1.2161		

——

Tukey multiple comparisons of means

95% family-wise confidence level

```
Group   diff          lower        upper         p adj
Z2-Z1 -0.04376291 -2.69107431  2.60354849 1.0000000
Z3-Z1  2.09621056 -0.07631032  4.26873145 0.0643148
Z4-Z1  0.41623359 -1.70633761  2.53880478 0.9957871
Z5-Z1 -0.89812835 -3.13551484  1.33925814 0.8638753
Z6-Z1  0.15040226 -2.17475799  2.47556251 0.9999930
Z7-Z1 -1.14587395 -3.38326045  1.09151254 0.6772296
Z3-Z2  2.13997347 -0.25189330  4.53184025 0.1039561
Z4-Z2  0.45999650 -1.88659425  2.80658725 0.9957954
Z5-Z2 -0.85436544 -3.30529954  1.59656866 0.9248040
Z6-Z2  0.19416517 -2.33714869  2.72547903 0.9999808
Z7-Z2 -1.10211104 -3.55304515  1.34882306 0.7907047
Z4-Z3 -1.67997698 -3.47387705  0.11392310 0.0783980
Z5-Z3 -2.99433891 -4.92272355 -1.06595427 0.0005121***
Z6-Z3 -1.94580830 -3.97537456  0.08375795 0.0671599
Z7-Z3 -3.24208452 -5.17046916 -1.31369988 0.0001624***
Z5-Z4 -1.31436194 -3.18629378  0.55756990 0.3199819
Z6-Z4 -0.26583133 -2.24183757  1.71017492 0.9994906
Z7-Z4 -1.56210754 -3.43403938  0.30982430 0.1526989
Z6-Z5  1.04853061 -1.05032396  3.14738518 0.7012938
Z7-Z5 -0.24774560 -2.24892492  1.75343371 0.9996842
Z7-Z6 -1.29627622 -3.39513079  0.80257836 0.4695265
```

perMNOVA

	Df	**SumOfSqs**	**F**	**Pr(>F)**
Z2-Z1	1	403.8	0.5836	0.545
Z3-Z1	1	2408.1	1.6169	0.213
Z4-Z1	1	7408.4	13.288	0.005**
Z5-Z1	1	13025	25.884	0.003**
Z6-Z1	1	16376	24.062	0.008**
Z7-Z1	1	19835.5	38.152	0.008**
Z3-Z2	1	1966.6	1.4435	0.199
Z4-Z2	1	7572.3	22.196	0.005**
Z5-Z2	1	14230.6	65.499	0.011*
Z6-Z2	1	14731	39.128	0.014*
Z7-Z2	1	17316.7	73.278	0.014*
Z4-Z3	1	6102.9	6.0954	0.002**
Z5-Z3	1	14976	14.367	0.001***
Z6-Z3	1	13222	10.834	0.001***
Z6-Z3	1	16266	15.425	0.001***
Z5-Z4	1	5975.1	19.71	0.002**
Z6-Z4	1	11337.3	28.499	0.002**
Z7-Z4	1	15920	50.659	0.001***
Z6-Z5	1	12999.3	41.394	0.002**
Z7-Z5	1	18256.2	82.176	0.003**
Z7-Z6	1	4446.0	13.519	0.009**

b) Santurban-Berlin

```
Homogeneity of multivariate dispersions
Average distance to median:
   Z1    Z2    Z3    Z4    Z5    Z6    Z7    Z8
4.032 9.752 8.962 4.789 4.984 5.721 9.323 8.978

Eigenvalues for PCoA axes:
   PCoA1     PCoA2     PCoA3     PCoA4     PCoA5     PCoA6     PCoA7     PCoA8
7763.9527 4212.9455 1839.2522 1818.8003 1086.9580  718.7449  626.0589  579.0986
```

Anova

```
Analysis of Variance Table
Response: Distances
           Df   Sum Sq         Mean Sq      F value       Pr(>F)
Groups      7   461.69         65.955       6.8936        1.092e-06 ***
Residuals 100  956.75          9.568
---
```

Tukey multiple comparisons of means

```
   95% family-wise confidence level
Group    diff          lower       upper       p adj
Z2-Z1  5.72086499 -0.3718644 11.81359437 0.0819811
Z3-Z1  4.93063238 -1.0414369 10.90270170 0.1847851
Z4-Z1  0.75778389 -5.2393257  6.75489344 0.9999301
Z5-Z1  0.95264470 -5.2849783  7.19026767 0.9997506
Z6-Z1  1.68901352 -4.2830558  7.66108283 0.9875940
Z7-Z1  5.29127314 -1.3172197 11.89976600 0.2166231
Z8-Z1  4.94618348 -0.9830748 10.87544172 0.1746454
Z3-Z2 -0.79023262 -4.2028437  2.62237842 0.9963120
Z4-Z2 -4.96308111 -8.4193255 -1.50683674 0.0005832
Z5-Z2 -4.76822029 -8.6267488 -0.90969176 0.0053801
Z6-Z2 -4.03185148 -7.4444625 -0.61924044 0.0093655
Z7-Z2 -0.42959185 -4.8627036  4.00351993 0.9999886
Z8-Z2 -0.77468151 -4.1118068  2.56244381 0.9962543
Z4-Z3 -4.17284849 -7.4116578 -0.93403916 0.0030792
Z5-Z3 -3.97798768 -7.6430256 -0.31294972 0.0235673
Z6-Z3 -3.24161886 -6.4338242 -0.04941353 0.0438484
Z7-Z3  0.36064076 -3.9051230  4.62640456 0.9999955
Z8-Z3  0.01555111 -3.0958258  3.12692799 1.0000000
Z5-Z4  0.19486081 -3.5108394  3.90056105 0.9999998
Z6-Z4  0.93122963 -2.3075797  4.17003896 0.9863344
Z7-Z4  4.53348925  0.2327391  8.83423940 0.0312850
Z8-Z4  4.18839960  1.0292261  7.34757308 0.0020469
Z6-Z5  0.73636882 -2.9286691  4.40140677 0.9984981
Z7-Z5  4.33862844 -0.2916058  8.96886269 0.0832306
Z8-Z5  3.99353879  0.3986821  7.58839545 0.0185844
Z7-Z6  3.60225963 -0.6635042  7.86802342 0.1630690
Z8-Z6  3.25716997  0.1457931  6.36854685 0.0333686
Z8-Z7 -0.34508966 -4.5507088  3.86052945 0.9999964
```

perMANOVA

	Df	**SumOfSqs**	**F**	**Pr(>F)**
Z2-Z1	1	339.74	3.5115	0.008**
Z3-Z1	1	1485.7	17.422	0.002**
Z4-Z1	1	1820.7	67.853	0.001**
Z5-Z1	1	1725.21	61.307	0.008**
Z6-Z1	1	1855.49	51.186	0.001***
Z7-Z1	1	1416.36	16.618	0.011*
Z8-Z1	1	1110.4	11.212	0.001***
Z3-Z2	1	3495.6	35.452	0.001***
Z4-Z2	1	4613.4	73.48	0.001***
Z5-Z2	1	3867.8	53.096	0.001***
Z6-Z2	1	4882.9	72.286	0.001***
Z7-Z2	1	2660.6	25.03	0.001***
Z8-Z2	1	2833.9	26.535	0.001***
Z4-Z3	1	1581.0	26.221	0.001***
Z5-Z3	1	1062.4	15.552	0.001***
Z6-Z3	1	2983.8	46.207	0.001***
Z7-Z3	1	1958.7	20.583	0.001***
Z8-Z3	1	1678.0	16.843	0.001***
Z5-Z4	1	717.09	26.365	0.001***
Z6-Z4	1	2393.8	74.649	0.001***
Z7-Z4	1	1424.6	29.81	0.001***
Z8-Z4	1	1854.3	26.498	0.001***
Z6-Z5	1	1053.61	31.161	0.001***
Z7-Z5	1	1085.85	19.176	0.001***
Z8-Z5	1	1298.9	16.349	0.001***
Z7-Z6	1	758.1	13.87	0.001***
Z8-Z6	1	1522.3	20.641	0.001***
Z8-Z7	1	783.12	7.3931	0.001***

c) Siscunsí

Homogeneity of multivariate dispersions

Average distance to median:

Z1	Z2	Z3	Z4	Z5	Z6	Z7	Z8
4.195	4.593	3.546	2.739	2.123	2.417	3.365	3.295

Eigenvalues for PCoA axes:

PCoA1	PCoA2	PCoA3	PCoA4	PCoA5	PCoA6	PCoA7	PCoA8
1122.6538	575.7324	279.7083	158.7320	128.0314	98.8517	85.8268	72.6056

Anova
Analysis of Variance Table
Response: Distances

	Df	**Sum Sq**	**Mean Sq**	**F value**	**Pr(>F)**
Groups	7	45.157	6.4511	7.873	1.119e-07 ***
Residuals	107	87.675	0.8194		

Tukey multiple comparisons of means
95% family-wise confidence level

Group	**diff**	**lower**	**upper**	**p adj**
Z2-Z1	0.39825029	-1.11320613	1.90970670	0.9919563
Z3-Z1	-0.64870654	-1.97623392	0.67882084	0.8000430
Z4-Z1	-1.45534720	-2.78287459	-0.12781982	0.0212327
Z5-Z1	-2.07209071	-3.37252285	-0.77165857	0.0000818***
Z6-Z1	-1.77716213	-3.01754157	-0.53678270	0.0005885***
Z7-Z1	-0.82929257	-1.97184623	0.31326108	0.3344867
Z8-Z1	-0.89983206	-2.00610488	0.20644076	0.2006026
Z3-Z2	-1.04695683	-2.49218559	0.39827193	0.3369650
Z4-Z2	-1.85359749	-3.29882625	-0.40836873	0.0032304**
Z5-Z2	-2.47034100	-3.89072157	-1.04996043	0.0000122***
Z6-Z2	-2.17541242	-3.54102524	-0.80979960	0.0000823***
Z7-Z2	-1.22754286	-2.50495668	0.04987096	0.0689733
Z8-Z2	-1.29808234	-2.54315133	-0.05301336	0.0346052*
Z4-Z3	-0.80664066	-2.05824548	0.44496416	0.4917154
Z5-Z3	-1.42338417	-2.64621262	-0.20055572	0.0110597*
Z6-Z3	-1.12845559	-2.28721649	0.03030531	0.0621304
Z7-Z3	-0.18058603	-1.23396845	0.87279639	0.9994717
Z8-Z3	-0.25112552	-1.26504130	0.76279027	0.9944784
Z5-Z4	-0.61674351	-1.83957196	0.60608494	0.7730405
Z6-Z4	-0.32181493	-1.48057583	0.83694597	0.9889845
Z7-Z4	0.62605463	-0.42732779	1.67943705	0.5962390
Z8-Z4	0.55551514	-0.45840064	1.56943093	0.6911133
Z6-Z5	0.29492858	-0.83268909	1.42254625	0.9923066
Z7-Z5	1.24279814	0.22377443	2.26182185	0.0062948
Z8-Z5	1.17225865	0.19408688	2.15043042	0.0078251
Z7-Z6	0.94786956	0.00668588	1.88905325	0.0470799
Z8-Z6	0.87733008	-0.01946278	1.77412293	0.0599049
Z8-Z7	-0.07053949	-0.82626769	0.68518872	0.9999913

perMNOVA

	Df	**SumOfSqs**	**F**	**Pr(>F)**
Z2-Z1	1	162.22	7.0307	0.001***
Z3-Z1	1	369.96	20.621	0.001***
Z4-Z1	1	349.61	24.743	0.001***
Z5-Z1	1	476.72	40.841	0.001***
Z6-Z1	1	454.17	38.628	0.001***
Z7-Z1	1	252.00	17.471	0.001***
Z8-Z1	1	448.21	32.799	0.001***
Z3-Z2	1	205.37	10.613	0.001***
Z4-Z2	1	213.34	14.228	0.001***
Z5-Z2	1	364.75	30.015	0.001***
Z6-Z2	1	328.09	26.967	0.001***
Z7-Z2	1	262.97	17.675	0.001***
Z8-Z2	1	369.91	26.468	0.001***
Z4-Z3	1	38.738	3.1679	0.002**
Z5-Z3	1	79.85	7.8828	0.001***
Z6-Z3	1	63.966	6.1404	0.001***
Z7-Z3	1	268.76	20.153	0.001***
Z8-Z3	1	252.85	19.703	0.001***

Z5-Z4	1	107.73	15.568	0.001***
Z6-Z4	1	92.349	12.079	0.001***
Z7-Z4	1	260.32	22.774	0.001***
Z8-Z4	1	211.13	18.671	0.001***
Z6-Z5	1	25.147	4.115	0.004**
Z7-Z5	1	305.71	29.836	0.001***
Z8-Z5	1	223.97	21.622	0.001***
Z7-Z6	1	258.48	24.824	0.001***
Z8-Z6	1	187.22	17.853	0.001***
Z8-Z7	1	144.66	11.854	0.001***