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### Morphometrics of modern and fossil Poaceae pollen from South America

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**Publication date**  
2023

[Link to publication](#)

#### **Citation for published version (APA):**

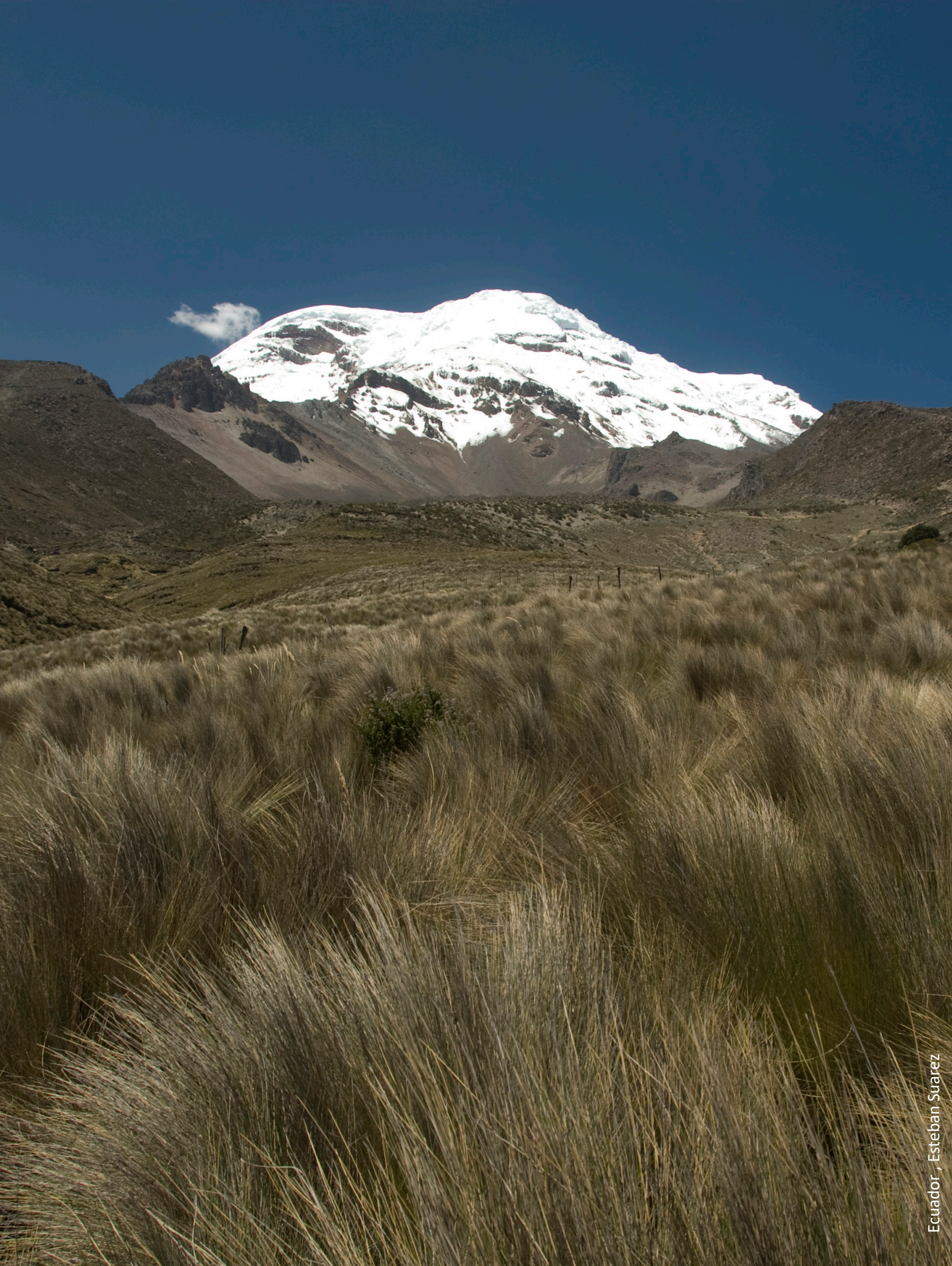
Wei, C. (2023). *Morphometrics of modern and fossil Poaceae pollen from South America*. [Thesis, fully internal, Universiteit van Amsterdam].

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# Chapter 1

General introduction

## 1 Introduction

The grasses (Poaceae) are one of the most diverse angiosperm families on the Earth, comprising 12 subfamilies and close to 12,000 species (Dahlgren et al., 1984; Soreng et al., 2022). The grassland and graminoid dominated habitats, form one of Earth's major biomes, occurring on all continents and covering over 40% of the planet's land surface (White et al., 2000; Gibson, 2009; Blair et al., 2014; Buisson et al., 2022).

Grassland and grasses have played an important role in human evolution and development of agricultural societies, where they stand as the paramount crop plants (domesticated grasses, such as rice, corn, cereals, wheat) and supply humans with crucial building material (i.e. bamboo) and biofuels (i.e. switchgrass) (Strömberg, 2011; Blair et al., 2014; Strömberg and Staver, 2022). The ecology and economics of grasses provide critical ecosystem goods and services at local, regional, and global scales (Blair et al., 2014; Jaramillo, 2023), and as such have received considerable attention to understand their evolutionary history and geographical expansion (Edwards, 2010; Strömberg, 2011; Kirschner and Hoorn, 2020; Jaramillo, 2023).

The study of grass microfossils, including pollen and phytoliths (plant silica bodies), have been regarded as direct records that can be used to reconstruct the past grasses and grasslands of the past (i.e. Leopold et al., 1992; Bush, 2002; Strömberg, 2011). Phytolith studies have confirmed that the grasses date back to at least the Cretaceous with grass phytoliths extracted from mammal coprolites found in India and from the dentitions of duck-billed dinosaur in China, suggesting that the grasses had evolved by 100 million years ago (Ma) (Prasad et al., 2005; Wu et al., 2018). However, phytoliths are often better preserved in dry grassland sediments, hence they are not always present in the fossil record (Prasad et al. 2005).

Pollen has been regarded as an effective and direct tool to understand past biomes and vegetation. In general, pollen morphology offers important information on taxonomic boundaries and affinities between different taxa (Erdtman, 1952). Grasses

are wind-pollinated and thus have a relatively high pollen production compared with some other families (Duffin and Bunting, 2008), this is supported by the high abundance of grass pollen in the sedimentary record. Together these factors should make them ideal candidates to reconstruct past vegetation (Linder, 1987; Leopold et al., 1992; Bush, 2002; Strömberg, 2011). However, the traditional study of Poaceae pollen under light microscopy is presented by a significant challenge, as their pollen has a relatively stenopalynous morphology (principally monoporate spheroidal grains) (Page, 1978; Beug, 2004; Halbritter et al., 2018). Consequently, it is difficult to use pollen as a palaeoecological indicator to understand past grasses and vegetation below the family level.

In term of grass palynology, different methods have been proposed to improve taxonomic identification, but it remains unclear if they can be successfully applied to long-term palynological records (i.e. Schüler and Behling, 2011; Mander et al., 2013; Jan et al., 2015; Mander and Punyasena, 2015). In this thesis, I focus on evaluating the proposed proxies and developing advanced techniques to provide insights into grass pollen and its applicability in palaeoecology studies. Furthermore, I link extant pollen data to (a)biotic factors to determine if there is a relationship. I also conduct morphometric analysis of fossil and extant pollen to explore the developmental history of grass pollen and the underlying mechanisms.

I fill the knowledge gap by studying a series of extant and fossil grass pollen from South America. South America is an ideal location for the study of grasses because it encompasses a diverse phylogenetic range of grasses, a wide variety of climates and environments, as well as rich grass pollen records since the Neogene (Hoorn et al., 2010; Cheng et al., 2013; Kirschner and Hoorn, 2020). Firstly, I generate biometric data from a range of extant pollen types under light microscopy and test the relationship between pollen size and various abiotic and biotic variables by fitting different statistical models. Secondly, I combine high-resolution imaging of extant pollen surface ornamentation under scanning electron microscopy and

computational image analysis, to test if the morphotypes identified by human-eyes can be supported by computational analysis, and if these morphotypes related to abiotic and biotic variables. Lastly, I analyse grass pollen evolution by applying scanning electron microscopy and computational image analysis on fossil pollen that ranges in age from the early Miocene to the Present. The resulting pollen surface ornamentation data are used to create quantitative features to reveal grass pollen development through continental space and geological time.

## **2 Current understanding of Poaceae history by means of palynology**

### **2.1 Challenges in Poaceae palynology**

The high abundance of Poaceae pollen presented in the sedimentary record is of special interest when applying palynology to reconstruct grass diversification history and the drivers leading to their global dominance (i.e., Retallack, 2001; Bonnefille, 2010; Strömberg, 2011). However, the floristic composition of past vegetation is only known at a broad taxonomic scale based on grass palynomorphs. The grass pollen, with relatively stenopalynous morphology (monoporate spheroidal grains), has been recognized as a notoriously difficult problem, making it challenging to identify grass pollen even at the subfamily level (Page, 1978; Beug, 2004; Halbritter et al., 2018). This challenge has undoubtedly hindered the study of the evolutionary history of Poaceae. Nevertheless, scientists have devoted a significant amount of effort to develop the ways to overcome the taxonomic identification barrier (Andersen and Bertelsen, 1972; Page, 1978; Köhler and Lange, 1979; Mander et al., 2013; Jan et al., 2015; Mander and Punyasena, 2015; Julier et al., 2016; Jardine et al., 2021).

Previous research demonstrated considerable variation in grass pollen morphology when observed under different microscope types, including quantifiable variation in grass pollen size seen under light microscopy (Salgado-Labouriau and Rinaldi, 1990; Beug, 2004; Holst et al., 2007; Jan et al., 2015; Radaeski et al., 2016, 2020) and pollen surface ornamentation seen under scanning electron microscopy (Andersen and Bertelsen, 1972; Page, 1978; Köhler and Lange, 1979; Mander and Punyasena,

2015). These findings provide a promising avenue into grass pollen classification and understanding their global diversification. However, as yet there is no evaluation showing that they can be applied over broad spatial and deep temporal scales.

## **2.2 Palynological studies on Poaceae pollen size**

Mature Poaceae pollen grains range in size from ~20 to >100  $\mu\text{m}$  (Ferguson et al., 1977; Beug, 2004). Their considerable variation in pollen size has previously been suggested as a proxy to reconstruct the past vegetation and climates. For example, pollen grain size has long been used to differentiate between domesticated and wild grasses in Holocene pollen records (Salgado-Labouriau and Rinaldi, 1990; Beug, 2004; Holst et al., 2007). More recently, grain size has been suggested to be a proxy for distinguishing grassland types in South America, such as the Páramo, Campos and Pampa ecosystem (Schüler and Behling, 2011), C3 and C4 dominated grassland (Jan et al., 2015), as well as arboreal forest and herbaceous forest (Radaeski et al., 2016, 2020).

In spite of this, the application of pollen size to reconstruct past grassland and climate has been deemed controversial. For instance, the study on *Nothofagus* pollen is suggested to use grain size as a tool to reconstruct moisture availability in the fossil record (Griener and Warny, 2015), but a re-evaluation of the data showed that this application is premature as the impacts of genome size variations on pollen size are not well understood (Jardine and Lomax, 2017). Nevertheless, it remains uncertain whether variations in pollen size can be applied on a broader spatial scale and which abiotic and/or biotic factors might have shaped pollen size variation over time.

## **2.3 Palynological studies on Poaceae pollen micro-ornamentation**

Previous studies have revealed a considerable degree of variability in grass pollen surface ornamentation under scanning electron microscopy (SEM) (Andersen and Bertelsen, 1972; Page, 1978). Scientists have attempted to classify grass pollen into

different groups based on the variation in pollen micro-ornamentation. These efforts mainly involve morphotypes established by human-eyes using descriptive terminology (i.e. Köhler and Lange, 1979; Mander and Punyasena, 2015) and computational analysis algorithmized on high-resolution images (Mander et al., 2013).

Köhler and Lange (1979) first proposed that the micro-ornamentation of grass pollen surface can be classified into different morphotypes on the basis of human-eye level determination. Much later, a review survey of published SEM images of grass pollen surface ornamentation reported a total of six morphotypes from 99 grass species (0.89% of the total grass species), specifically *Hordeum*-type, *Triticum*-type, *Avena*-type, *Setaria*-type, *Pariana*-type, and *Stipa*-type (Mander and Punyasena, 2015). Nevertheless, the rarity or prevalence of known morphotypes and the specific (a)biotic factors that shaped the pollen ornamentation variation have remained unclear, as only low numbers of documented specimens with a clear surface pattern have been reported.

With the continuous improvement in modern computing and imaging hardware and software, analysing pollen morphology under high resolution microscopies with computational image analysis/deep learning methods is now a developing tool that can quantify a range of pollen morphological features (Mander et al., 2013; Holt et al., 2014; Romero et al., 2020). The implementation of advanced techniques hold promise for gaining further insights into the variation between grass pollen on the basis of ornamentation. Yet, these techniques have been not tested at a broader scale in extant grasses, or within fossil records.

### **3 Grasses and grasslands in South America**

#### **3.1 Why South America?**

Poaceae are highly diverse, geographically widespread, and form an important component of many terrestrial ecosystems. At present, the South American

continent encompasses multiple grass-dominated ecosystems with a wide variety of climates and environments, including high-elevation grasslands (páramo), savanna (cerrado), tropical, subtropical, and cold to temperate grasslands, and the floodplain environments (várzeas and igapós) (Burkart, 1975; Hoorn et al., 2010; Kirschner and Hoorn, 2020; Jaramillo, 2023). Additionally, Andean uplift and drainage readjustments as well as climate change have been suggested to affect species composition through time and space (Hoorn et al., 2010, 2023). Therefore, South America offers an ideal setting for exploring the mechanisms of grass expansion and diversification across (palaeo)biogeography and (palaeo)climate (Cheng et al., 2013; Kirschner and Hoorn, 2020).

### **3.2 Poaceae fossil records in South America**

In South America, fossil grass pollen was sourced from a wide range of habitats and environments, such as the paramo, the savannas and aquatic environments (i.e. Gosling et al., 2009; D’Apolito, 2016; Hoorn et al. 2017, 2022; Jaramillo et al. 2017). Palynological counting and the analysis of grass pollen percentages in fossil records gives strong evidence for changes in species diversity over time (i.e. Behling and Hooghiemstra, 2000; Gosling et al., 2018). The earliest grass pollen is represented by the form genus *Monoporites*, which occurred in the Maastrichtian to the Paleocene (c. 70–60 Ma) (Jacobs et al., 1999; GPWG II, 2012). Subsequently, grasses were modestly present in the Oligocene (c. 33–23 Ma) (Lorente, 1986) and became dominant from the Miocene to the Pleistocene (c. 23 Ma–11,700 years ago) (D’Apolito, 2016; Hoorn et al. 2017, 2022; Jaramillo, 2023). Although the history of grass-dominated biomes in South America goes back to c. 20 Ma (Strömberg and Staver, 2022), the mechanisms underlying grass species diversity and the drivers leading to their dominance in South America remain elusive.

### **3.3 Grassland dynamics and climate change in South America since c. 23 Ma**

The development of biodiversity is thought to be linked to tectonism (Kohn and Fremd, 2008), climate (Edwards et al., 2010), and the combination of landscape dynamics and climate (Hoorn et al., 2010, 2023; Jaramillo, 2023). The Neogene uplift of the Andes had significant impacts on regional climate and the evolution of biodiversity and species composition over time (Hoorn, 1993; Hoorn et al., 2010; Bicudo et al., 2019). Below I summarize the main changes in palaeogeography, paleoenvironmental and palaeoclimate factors in South America since the early Miocene.

### 3.3.1 Fluvial systems and the Pebas wetland (c. 23 to 16 Ma)

**Palaeogeography:** In the early Miocene, the western Amazon was dominated by fluvial systems that were predominantly sourced from the Amazon craton, with minor sediment input from the Andes (c. 23–10 Ma) (Salamanca et al., 2016; Hoorn et al., 2023). Poaceae pollen are rare in the sedimentary record, and vegetation is dominated by taxa from the fluvial floodplain and the Amazon craton (Hoorn, 1994a; Salamanca et al., 2016). Fluvial conditions and diverse Amazon forest transitioned into a lowland megawetland (the ‘Pebas system’), and this megawetland is hypothesized to have existed from c. 23 Ma up to c. 10 Ma (Wesselingh et al., 2006; Hoorn et al., 2010; Jaramillo et al., 2017). The wetland environment accommodated grasses on the lake shores (Hoorn et al., 2010, 2022, 2023; D’Apolito, 2016; Jaramillo, 2023). The Andes did not yet reach its modern elevation, however, at c. 14 Ma there are indications for the presence of a high montane forest and proto-paramo herb vegetation based on the presence of species such as *Acaena/Polylepis*, *Huperzia*, *Weinmannia*, *Puya*, *Sambucus* and many others (Hoorn et al., 2022).

**Global climate:** After a period of relatively stable global climate during the early Miocene, temperatures peaked in the Middle Miocene Climatic Optimum (MMCO) (c. 16.9–14.7 Ma), with a decline in global temperatures at the end of the Middle Miocene Climatic Transition (MMCT) (c. 14.7–13.8 Ma) (Westerhold et al., 2020).

### **3.3.2 Formation of the Amazon drainage basin and transition to the modern landscape (c. 13 to 0.5 Ma)**

**Palaeogeography:** The Pebas wetland transitioned into a fluvial landscape and culminated with the birth of the transcontinental Amazon River and the formation of the Amazon drainage basin. From the late Miocene onwards (c. 10 Ma), uplift in the Andes accelerated followed by extensive erosion (Hoorn et al., 2010; 2023), and deep alluvial canyon incision in the Andes (Lease and Ehlers, 2013) and Amazon lowlands (Irion and Kalliola, 2009). Particularly in the Pleistocene this led to a vast increase in sediment supply into the Amazon submarine fan (Figueiredo et al., 2009). The forest biomes expanded until c. 5 Ma, with a high abundance of *terra firme* rainforests although this is thought to have been less diverse than present (Hoorn et al., 1995, 2010; 2017; Jaramillo et al., 2017). In the Pleistocene, Poaceae are very abundant in the Amazon submarine fan record. Their source cannot be established, but it was hypothesized that they are derived from Poaceae on the Andean slopes, in particular from vegetation on alluvial fan system on the lower slopes (Hoorn et al., 2017; Kirschner and Hoorn, 2020).

**Global climate:** Global cooling was initiated due to Antarctic ice sheet expansion during the late Miocene, followed by a warm period in the late Pliocene (Westerhold et al., 2020).

### **3.3.3 Extensive fluvial floodplains and savanna development (c. 0.5 Ma to Present)**

**Palaeogeography:** At the end of the Pleistocene the western Amazon was still dominated by erosion and alluvial fan systems. By the Holocene (last 11,700 years), however, the Andes had already obtained its modern elevation and the Amazon was covered by rainforest (Gregory-Wodzicki, 2000), with extensive savanna vegetation in its periphery (Simon et al., 2009).

**Global climate:** In the Pleistocene, the climate was characterized by phases of intense cooling, and glacial and interglacial and cycles. In the Holocene, global temperature rose and the modern climate was reached (Westerhold et al. 2020).

#### **4 Research questions**

In this thesis, I focus on advancing techniques in Poaceae palynology and further exploring the mechanisms underlying the evolution of grass pollen in South America since c. 23 Ma. In particular, I investigate fossil and extant grass pollen from South America and cover three major themes. The first two themes focus on exploring the variation of grass pollen in extant species across different microscopic levels and assessing their applicability in the context of fossil pollen. The third theme focuses on improving the advanced techniques based on extant studies and applying them to fossil records, further parameterizing and comparing the morphological space (morphospace) occupancy of fossil and extant pollen to understand the underlying factors driving grass pollen evolution and development.

Firstly, I assess whether grass pollen size variation can be regarded as a robust proxy in reconstructing past vegetation, and which abiotic and/or biotic factors relate to pollen size and may be responsible for shaping their size variation through time. Secondly, I explore the variation in grass pollen based on surface ornamentation, evaluate whether the morphotypes grouped using descriptive terminology by the human eyes are supported by computational analysis, and investigate the potential (a)biotic factors that cause variation in pollen ornamentation. Thirdly, I compare the morphometrics of modern and fossil Poaceae pollen from South America and exploring the drivers leading to their evolution in selected ecosystems.

## Chapter 1

The specific research questions are:

### ***Size variability in Poaceae pollen (Chapter 2):***

- I. Do Poaceae pollen vary in size under light microscopy at different taxonomic levels? If this is the case, does such variation occur at genus or among/within species level?
- II. Does sample processing and storage time affect pollen size? And if so, how?
- III. Is grass pollen size related to abiotic and biotic variables? And is grass pollen size a promising proxy for fossil records?

### ***Variability in the surface ornamentation of Poaceae pollen (chapter 3):***

- I. How many grasses pollen morphotypes are there, and will more morphotypes appear if increasing numbers of specimens and species are analysed? Does pollen surface ornamentation vary at intraspecific and/or interspecific levels?
- II. Can pollen surface ornamentation be quantified through computational analysis, and which features can be extracted? Are the morphotypes supported by quantitative analysis?
- III. How are the morphotypes related to (a)biotic variables and distributed across the grass phylogeny?

### ***The morphometrics of modern and fossil Poaceae pollen (Chapter 4):***

- I. Can the technique of combining high-resolution imaging and computational analysis be applied to fossil grass pollen? If so, how can the techniques be improved?
- II. Are there differences in the pollen morphometrics between fossil and extant pollen groups? If so, how did the morphospace occupation of pollen change through time?
- III. What are the mechanisms underlying grass pollen evolution since c. 23 Ma?