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SECTION: BIODIVERSITY, ECOLOGY AND NATURE CONSERVATION

Palynological studies and Holocene ecosystem dynamics in north western Khyber Pakhtunkhwa Province of Pakistan in the Hindu Kush Himalayan region

Dissertation zur Erlangung des Doktorgrades der

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vorgelegt von

M.Phil.

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Charsadda, Khyber Pakhtunkhwa, Pakistan

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Dissertation submitted for the award of Doctoral Degree at the faculty of

Mathematics and Natural Sciences

Georg-August-University of Göttingen

By

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Dedication

Dedicated with love to one human family, who share the same genetic material with all other organisms, whose atomic bodies are composed of the cosmic stardust of the universe and who evolved over millions of generations on this tiny planet, mother Earth, which came into being almost 4.6 billion years ago. My Pakhtun (Afghan) people are an integral part of this human family who are living between the Oxus and Indus for the last 5000 years in the historical land of Afghanistan and from Chitral to Bolan in the recently created Pakistan since 1947. For having carried and transmitting their DNA safely to my body, I especially dedicate my PhD thesis to my Pakhtun Afghan forefathers including Amir Karorr Baba, Shahab Uddin Ghouri, Sher Shah Suri, Khushal Khan Khattak, Gaju Khan, Ahmad Shah Baba, Mir Wais Khan Baba, Aman Ullah Khan, Ghazi Ayub Khan, Malaley of Maiwand, Zarghona Nya, Pir Rokhan, Abdul Ghaffar Khan-Bacha Khan, Khan Shaheed Abdul Samad Khan Achakzai, Darya Khan, Mirzali Khan Faqir Api, Aimal Khan, Haji sb Turangzai, Ghani Khan (poet philosopher), Eejab Khan (My great grandfather), Khudai Khidmatgaar Ghulam Sarwar Khan (my grandfather) Abdul Wakil Khan (my sweet father) and Siraja (my sweet mother).

پیرزو نه

ده یو انسانی کوړمي په نامه چه ده هر ژوندي شي سره یو شان شریکه جنسی خټه لری او په ملینونو نسلونو په دې نړۍ اباد دی، کومه چه خواوشا ۴۰۶بیلینونه کاله زړه نړۍ ده. ځما پښتون افغان نژاد د انسانانو د دغه کوړمي یوه تړلي برخه ده څوک چه ده پښتنو په پلارني وطن افغانستان کښ ده امو تر اباسینه ده تیرو ۵۰۰۰ کالو راهسې او ده تیرو ۶۷کالو راهسې پاکستان کې ده چتراله تر بولانه ژوند ژواک کوی .د دې خبرې له کبله چه ځما وجود ته یې ده یو پښتون افغان خټه راوبخښله، زه د خپلې پی-ایچ-ډی مقاله د خپلو مالومو او نامالومو اتلانو په نا مه او ده خپلو پښتنو افغانانو پلرونو نیکونو په نامه پیرزو کوم چه پکښ په ځانګړی توګه امیر کروړبا با،شهاب الدین غوري ،شیرشاه سوری ، ستر خوشال بابا، ګجو خان بابا، احمدشاه بابا، میرویس نیکه، غازی امان الله خان،غازی ایوب خان، ملالۍ انا میوندی،زرغونه انا، پیر روښان،عبداغفار خان باچاخان ، خان شهیدعبدالصمد خان اچکزئی ،دریا خان ،میرزالی خان فقیر ایپی، ایمل خان ،صنوبرحسین کاکاجی،د ترنګزو حاجي صیب ،غنی خان ،ایجاب خان (ځما کرنیکه)، خدایی خدمتګارغلام سرور خان (ځما نیکه) ،عبدالوکیل خان (ځما خوږ پلار) او سراجه (ځما خوږه مور) شامل دی

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Farooq Jan (Muhammadzai Afghan)

Summary

Khyber Pakhtunkhwa (31°49´N, 70°55´E to 35°50´N, 71°47´E) is located in north western Pakistan in south Asia. The Hindu Kush mountains of Afghanistan lies to its west, Indian Himalayas to the northeast and Karakorum Mountains south of Tibetan Plateau of China, lying to its north. This PhD-project comprises of three separate studies conducted along a 200 km elevational gradient in Khyber Pakhtunkhwa starting from the sedimentary basin plains of Peshawar valley (275 m a.s.l.) reaching high up to Malam Jabba Hills in Swat (2600 m a.s.l.).

The first study based on a specific dataset of 160 Poaceae species reveal trends showing that C3 and C4 polyploid Poaceae species have larger grain sizes than their respective diploid species. C4 species have larger grain size than C3 species in our specific dataset. Grasslands dominated by C3 or C4 Poaceae species from different regions and habitats can be separated by studying the pattern of trend in their increasing or decreasing pollen grain sizes. Polyploidy is more common in C4 than in C3 Poaceae species in our dataset. The method used can be applied on Poaceae pollen grains deposited in environmental archives to reconstruct past climate and to assess the dynamics of past grassland ecosystems. This study will not only help the ongoing palaeo-ecological studies in unravelling aspects such as changes in vegetation composition and shifts in biomes of past grassland ecosystems but also provide useful insights for future predictions.

The second study deals with modern pollen spectra from surface samples and their relationship with the surrounding vegetation which provide useful data for the interpretation of future Holocene pollen records. Along the 200 km gradient four distinct elevational zones are defined by dominating plant families, which are reflected in the pollen assemblages by different proportions, indicating a significant correlation between pollen rain and vegetation in families like Poaceae, Asteraceae, Cyperaceae, Verbenaceae, Acanthaceae and Euphorbiaceae. Pollen assemblages also vary considerably from the associated vegetation composition and major discrepancies are caused by large differences in pollen and vegetation proportions in Boraginaceae, Saxifragaceae, Apiaceae, Balsaminaceae and Rubiaceae families. The establishment of a modern pollen rain – vegetation relationship, at least on family level, is necessary for calibration and interpretation of fossil pollen record from such sites.

The third study deals with fossil pollen record from Kabal Swat area, providing a detailed history of the vegetation and climate of the Hindu Kush Mountains since the last 3300 years of the late Holocene. From 3300 to 2400 cal yrs BP, the subtropical semiarid herbaceous vegetation represented by Cyperaceae and Poaceae species was dominant in the valley. They were replaced by mixed coniferous forests of *Taxus, Pinus, Juglans,* Poaceae and Cyperaceae from 2400 to 900 cal yrs BP, suggesting a comparatively moderate climatic variability during the late Holocene. The decrease in Poaceae from 2400 to 1500 cal yrs BP and again an increase from 1500 to 1200 cal yrs BP show that Kabal Swat went through respective wet-cooler and dry-warmer periods. Conifers mostly occur in the mixed coniferous forests at higher altitudes in the Alpine area today. Further high resolution Holocene pollen records of the Hindu Kush are needed, to allow a more elaborate comparison with other South and Central Asian palaeo-archives, providing more detailed and applicable knowledge for management and conservation issues.

Zusammenfassung

Khyber Pakhtunkhwa (31 ° 49'N, 70 ° 55'E bis 35 ° 50'N, 71 ° 47'E) liegt im Nordwesten Pakistans im Süden Asiens. Das Hindukusch-Gebirge in Afghanistan liegt im Westen, dem indischen Himalaya im Nordosten und die Karakorum Berge südlich vom tibetischen Hochland auf der Nordseite. Diese Arbeit besteht überwiegend aus drei separaten Studien entlang eines 200 km langen Transekts mit einem Höhengradienten ausgehend von den Sedimentbecken im Peshawar Tal (275 m ü.M.) bis hinauf zu den Malam Jabba Hills im Swat-Tal (2600 m ü.M.).

Die erste Studie, die auf einer Datengrundlage von 160 Poaceae Arten beruht, zeigt Trends, dass polyploide C3- und C4-Poaceae-Arten größere Pollenkkörner als die jeweiligen diploiden Arten haben. In diesem Datensatz haben alle C4-Arten größere Pollenkörner als die C3-Arten. Ob Grassländer von C3 oder C4 Arten dominiert werden kann in verschiedenen Regionen und Lebensräumen durch die Untersuchung der Muster des Trends von zu- oder abnehmenden Pollenkorngrößen ermittelt werden. In unserem Datensatz ist Polyploidie bei C4-Gräsern häufiger als bei den C3 Arten. Die verwendete Methode kann auf Poaceae-Pollenkörner in Umweltarchiven angewendet werden, um das Klima der Vergangenheit zu rekonstruieren und die Dynamik der früheren Graslandökosysteme zu bewerten. Dieser Ansatz wird nicht nur bei laufenden paläoökologischen Studien helfen aufzuklären, wie die Änderungen der Vegetations-zusammensetzung und die Veränderungen in Biomen vergangener Graslandökosysteme zu entschlüsseln sind, sondern auch nützliche Erkenntnisse für die Vorhersage zukünftiger Entwicklungen ermöglichen.

Die zweite Studie befasst sich mit modernen Pollenspektren aus Oberflächenproben und ihre Beziehung zu der umgebenden Vegetation, die nützliche Daten für die Interpretation von holozänen Pollenprofilen bietet. Dabei konnten entlang eines 200 km langen Höhengradienten vier verschiedene Höhenstufen unterschieden werden, wo die dominierenden Pflanzenfamilien, Poaceae, Asteraceae, Cyperaceae, Verbenaceae, Acanthaceae und Euphorbiaceae eine signifikante Korrelation mit dem gefunden Pollenniederschlag hatten, während sich bei anderen Familien, den Boraginaceae, Saxifragaceae, Apiaceae, Balsaminaceae und Rubiaceae große Unterschiede zu der zugehörigen Vegetationszusammensetzung ergaben. Für die Kalibrierung und Interpretation fossiler Pollendaten sollte also immer auch die aktuellen Beziehungen von Pollenniederschlag und Vegetationsdaten zumindest auf der Familienebene berücksichtigt werden.

Die dritte Studie befasst sich mit einem Pollenprofil aus der Kabal Swat-Region, welches eine detaillierte Geschichte der Vegetation und des Klimas des Hindukuschs der letzten 3300 Jahre, also dem späten Holozäns enthält. Von 3300 bis 2400 cal BP, war eine subtropische semiaride krautige Vegetation hauptsächlich durch Cyperaceae- und Poaceae-Arten vertreten. Sie wurde ersetzt von gemischten Nadelwäldern mit *Taxus, Pinus*, sowie *Juglans*, Poaceae und Cyperaceae während der Zeit von 2400 bis 900 cal BP, was auf eine vergleichsweise moderate Klimaschwankung während des späten Holozäns weist. Der Rückgang der Poaceae von 2400

bis1500 cal BP und eine erneute Zunahme von 1500 bis 1200 cal BP Jahre zeigen, dass das Kabal Swat nass-kühlere und trocken-wärmere Phasen durchmachte. Nadelbäume in den gemischten Nadelwäldern treten heute bei größeren Höhe im alpinen Bereich auf. Weitere hochauflösende holozäne Pollenprofile des Hindukusch sind notwendig, um einen ausführlicheren Vergleich zu anderen süd- und zentralasiatischen Paläo-Archiven zu ermöglichen, die auch ein detaillierteres und anwendbares Wissen für Management und Naturschutzfragen ergeben.

Chapter 1

Introduction

South Asia comprising of eight countries i.e., Pakistan, Afghanistan, Bangladesh, Bhutan, India, Maldives, Nepal and Sri Lanka, is home to more than one fifth of the world's population and is known to be the most disaster prone region in the world due to its increasing urbanization, climate change and global warming (UNEP, United Nations Environment Program 2003). South Asia has gained a considerable attention for scientific research in the field of tropical and subtropical ecology in the past few decades. In this context the study of vegetation under changing environmental conditions is of prime importance also within the science of palynology and palaeoecology.

Palynology- The bridge between Ecology and Palaeoecology

The term "palynology" can be defined as "the study of scattered dust" (from the Greek: παλύνειν/palynein - to scatter) or the study of strewn particles (Faegri and Iversen, 1989). Simply, palynology is the study of pollen, spores, dinoflagellates and other microscopic palynomorphs or in a more pragmatic sense, it is the study of microscopic organic structures that are resistant to treatments with hydrochloric or hydrofluoric acid. The basic assumption of palynology is that the number of pollen grains deposited per unit time, at a given point, is directly related to the abundance of the associated species in the surrounding vegetation (Dimblebly, 1957; Davis, 1963). Pollen grains are the reproductive units of seed plants because they contain the microgametophytes needed for fertilization. It is useful to think of pollen analysis as a remote sensing instrument, which records the past and present composition of vegetation (Schüler, 2012). Most of pollen grains when deposited in sediments may be preserved as fossils. At this points palaeoecology comes into play as the fossil grains may be extracted from sediments and identified down to family, genus and species level. The stratigraphic level at which grains are extracted corresponds with particular periods in the past. The starting point of palynology, then dealing with ecology is that, 'ecosystems are dynamic and have a history' (Willis & Birks, 2006; Birks, 2012). Assemblages of the fossil pollen can provide a record of the palaeoenvironment since each biological species require particular environmental conditions for

regeneration,establishment and growth. Hence, palynology serves as a bridge between the science of palaeoecology and ecology (Mercuri et al., 2013). To a certain extent Quaternary palaeoecology has now converged with plant ecology.

Rationale for palynological and palaeoecological investigation in Khyber Pakhtunkhwa

Palynological work in Pakistan has mainly been focused on fresh plants and a list of pollen Flora of a number of angiosperm families from different parts of the country have been published by Perveen and Qaiser from Karachi University. The potential prospects for palaeoecological studies in the plains and mountains of Pakistan and Afghanistan have hitherto not received any attention. This area is susceptible to the Indian monsoon system and is subject to the changing vegetation cover, ongoing desertification, forcing atmosphere system and climate change (Claussen et al., 2003). Significant progress in the investigation of Holocene climate in the monsoon regions of India and China have been made during the last few decades, based on evidence from cave deposits, speleothems, lake sediments, peat deposits and loess–palaeosol sequences (An et al. 2000; Hong et al. 2005; 2010; Trivedi and Chauhan 2009; Wang et al. 2010; Chen et al. 2014). Palynological and palaeoecological studies have so far been concentrated on tropical deciduous and evergreen forests in South India, Sri Lanka (Anupama et al. 2000; Barboni & Bonnefille 2001), northeast India (Basumatary & Bera 2007), foothills of Himalaya (Gupta & Yadav 1992), Madhya Pradesh (Quamar & Chauhan 2007) and tropical deciduous scrub vegetation in the north western desert in India (Singh et al. 1973).

Despite the availability of an abundant wealth of information received from the numerous geological studies conducted in Pakistan (Waagen, 1882-1885; Noetling, 1901; Balme, 1970; Pakistani–Japanese Research Group, 1985; Wardlaw & Pogue, 1995; Mertmann, 2003; Sultan, 2004; Alam, 2008; etc.), yet only a few palaeoecological studies have been conducted so far e.g. in Karachi coast near the Arabia Sea (von Rad et al. 1995; Schulz et al. 1996; Ivory and Lézine, 2009) and in the salt range of Pakistan (Schneebeli-Hermann et al. 2014). Till date there is scarcity of information with respect to palaeoecological studies to unravel the past vegetation and climate changes in the Hindu Kush Himalayas. The present work is the third overall palaeoecological study of the area after Badgley and Behrensmeyer (1980) who focused on the Neogene mammal faunas of northern Pakistan. Miehe et al. (2009) traced the early human impact in the upper forest ecotone of Hindu Kush- Himalaya at Shukan site (36°23' N/73°07' E),

which is located in a small tongue basin of a side valley of the Nazbar River in the western upper catchment of the Gilgit River near Karakorum. To our knowledge there is no published work on the vegetation history of the neglected north western side of Pakistan. With its varying range of different climate and vegetation zones, montane forest and alpine vegetation above the tree line and the center of Gandhara civilization (Peshawar valley 1500-500 BC), Khyber Pakhtunkhwa is the most suitable area for studying the influence of climate change on vegetation and for reconstructing the vegetation dynamics of the past. Our project based on the palynology and palaeoecology dealing with the Holocene ecosystem dynamics of this area is an effort in this direction.

Plant biodiversity is necessary for regulation of overall system in the Hindu Kush Himalayas which is the birth place for 10 largest rivers in Asia and a huge carbon sink. Hence, ecological changes in the Hindu Kush Himalayas affect global climate by bringing changes in temperature and precipitation patterns of the world (Khan et al, 2012).Vegetation of the area is diverse and range from tropical evergreen species in the south to alpine species in the north western parts (Behera and Kushwaha, 2007). These mountains are extremely sensitive to global climatic change and the continuous melting of snow on the peaks might result in dangerous rise in world sea-levels (Xu et al., 2009). The glaciers in the Himalayas are receding faster than in any part of the world and the likelihood of their disappearance by the year 2035 is very high at its current rate (Husnain et al., 2005). This impact will be observed more in the Himalayas and Hindu Kush as the contribution of snow to the runoff of major rivers on the western side is about 60 - 70% compared to only 10 % on the eastern side (IPCC, 2001).Monsoons in the subtropical Hindu Kush and Himalayan montane are the result of the seasonally reversing tropical winds bringing dry and wet seasons. Monsoon rains on these montane systems resulted in rich flora famous for its unique endemic and threatened biodiversity, forests, wildlife and immense unexplored genetic resources, due to which the western Himalayan moist temperate Ecoregion has been included in the Global-200 priority ecoregion for conservation (Ahmad, 2014).

Study region

Geography

Pakistan is located in southwest Asia just above the tropic of cancer, extending northeast to southwest from latitude 37° N to 23° N and longitude 60° E to 75° E. Khyber Pakhtunkhwa is

one of the four provinces of Pakistan, located in the north west of the country (31°49´N, 70°55´E to 35°50´N, 71°47´E). The study area comprises a 200 km-long elevational gradient starting from the lowest elevated sedimentary basin plains of Peshawar valley (275–315 m a.s.l.) reaching up to the highest elevated upper montane Malam Jabba Hills (2600 m a.s.l.). Swat valley is located between the Hindu Kush Mountains of Afghanistan to the west, Indian Himalayas to the east and Karakorum Mountains towards the north separating Pakistan from the Tibetan Plateau of China (Khan et al.2012). The Swat River emerges from the highest elevated Hindukush montane in the north, flowing down the gradient to Swat valley crossing Malakand zone, entering the Kabul River located down in the plain zone of Charsadda in the south. The exact point of the coring site is located in the western part of Swat valley in Kabal village (34°66'N 72°13'E) at an elevation of 750m a.s.l. Kabal is a Tehsil in district Swat lying between the foothills of Hindu Kush and the Himalayas in the north western Khyber Pakhtunkhwa Province. Kabal is located 10 km northwest of Mingora city of Swat, covering an area of about 40026 ha between latitude 34°45'N to 35°55'N and longitude 72°08'E to 72°50'E.

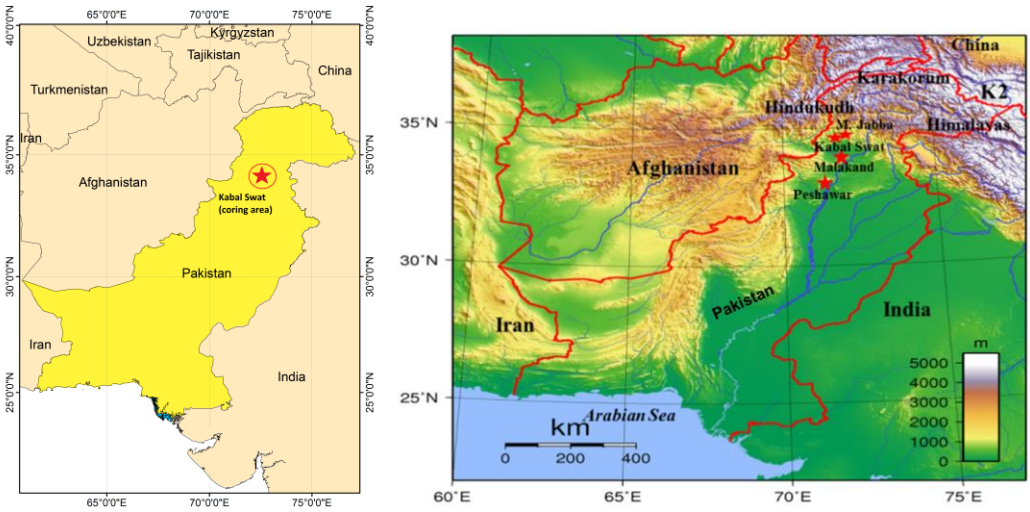

Figure 1: a) Pakistan is located in southwest Asia extending northeast to southwest from latitude 37° N to 23° N and longitude 60° E to 75° E just above the tropic of cancer, **b)** Topographic map of Pakistan; the research area shown by red stars (comprises a 200 km long elevational gradient starting from the plain Peshawar valley (275 m) crossing Malakand hills (500 m a.s.l) and Kabal Swat valley (750 m) reaching up to Malama Jabba (2600m). The area is surrounded by China in the north, Afghanistan on the west, Iran on the south west, Arabian Sea in the south and India on the east. The northwestern and northeastern part of the country consists of the lofty mountain ranges the Hindu Kush (Malam Jabba 3000 m) Himalayas and Karakorum with highest peak, K-2 (8475m.These ranges separates the Indo-Gangetic Plain from the Tibetan Plateau of China. Sources: www.worldofmaps.net/en/asia/map-pakistan (Fig.1a), Sadalmelik, 2007 (Fig. 1b).

Geology

The research area starting from the low elevated Nisatta Charsadda in Peshawar valley (275 m) shows strong edaphic and biotic disturbance up to the lower mountains of Malakand (500 m). Lithologically, the area is composed of quartz to dolerite, schist and granite, sandstones, mudstones and conglomerates (Hussain et al. 1993). Geologically, it consists of sedimentary and metamorphic rocks of Ordovician and Devonian origin and the geomorphologic features include piedmont plains, rolling sand plains, loess plains, infilled basins, cover flood plain and local fans

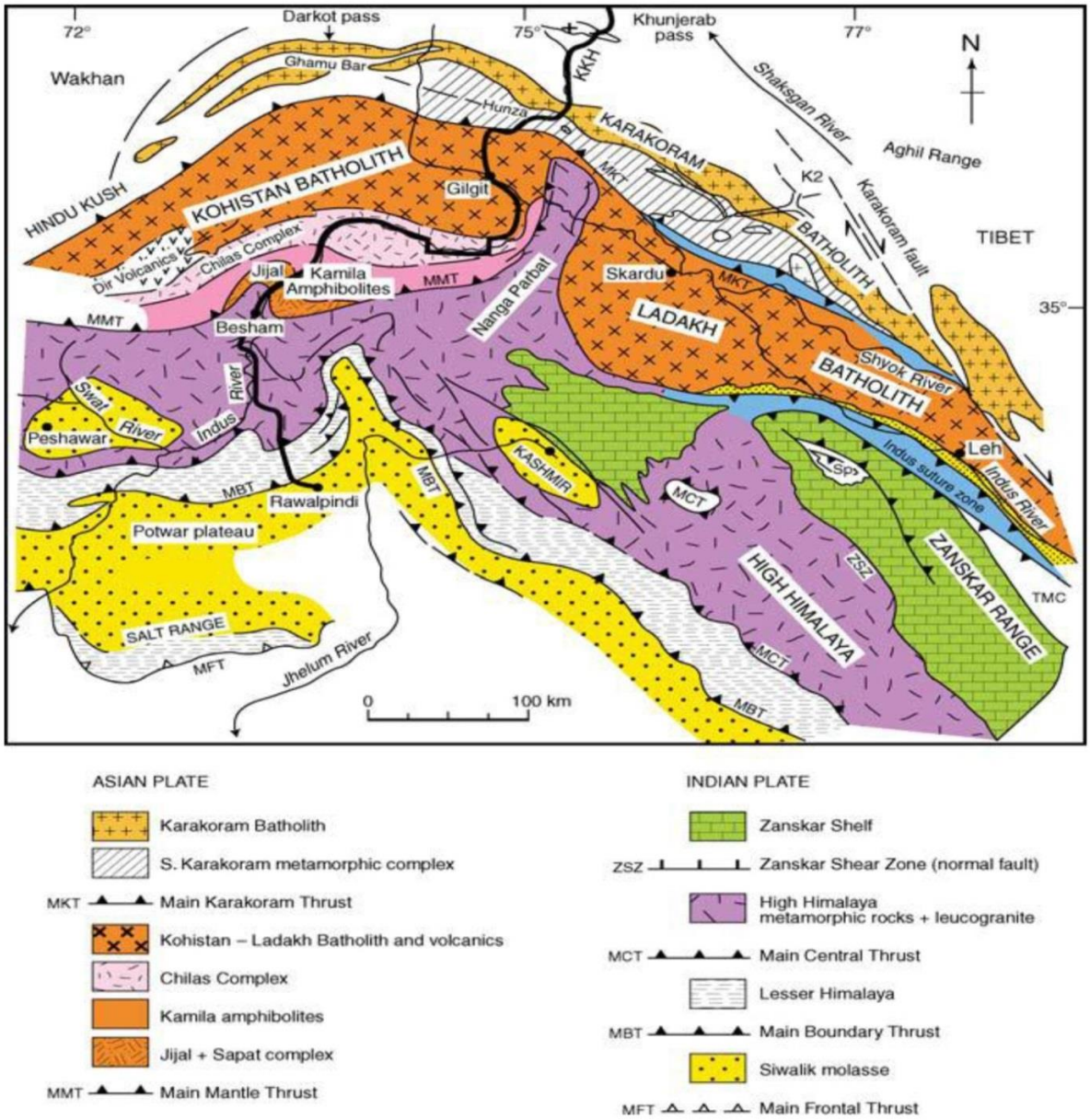

Figure 2: Geological map of the western Himalaya, Kohistan and Karakorum (after Searle et al., 1999).

(Hussain et al., 1993). The lower montane Malakand hills are a chromite rich mineralized area containing the biogeochemical distribution of enzyme-bound metals in the plants and soil (Kfayatullah et al., 2001). The Swat valley and adjoining areas of northwestern Pakistan consist of rock units of Precambrian-Cambrian basement representing the Kohistan arc sequence, the Tethys oceanic lithosphere and the Indo-Pakistan plate sequence (Arif et al., 2011). The Kohistan arc sequence consists essentially of late Jurassic-Cretaceous and Tertiary plutonic and metamorphic plutonic, volcanic and sedimentary rocks (Arif et al., 2011). The Kohistan arc terrane is widely regarded as one of the most complete exposed sections from the deep root to the volcanic edifice of an island arc (Searle et al., 1999). From south to north, the KAS consists of the (i) Jijal Complex, (ii) Kamila Amphibolites, (iii) Chilas Complex, (iv) Kohistan Batholith and Gilgit gneisses, (v) Chalt-Ghizar, Sharman and Utror volcanics and (vi) Yasin meta sediment. The ultramafic-mafic Jijal Complex represents the upper mantle to lower crust transition (Arif et al, 2011).The basement rocks in the Swat valley are overlaid unconformably by Phanerozoic metasedimentary rocks of the Alpurai group (DiPietro, 1991; DiPietro and Lawrence, 1991; DiPietro et al., 1993; Arif et al., 2011).

Climate

Pakistan lies in the northern Hemisphere just above the tropic of Cancer in the western part of the monsoon climate zone with subtropical climate, experiencing extreme temperature variations. The overall climate of the research area is given in Table 1. The data is based on the 30 years average (1971-2000) of daily mean annual temperatures (MAT) in C°, average rainfall in mm (ARF) and relative humidity in percentage (RH), obtained from the federal meteorological department Islamabad (Meteorological department of Pakistan. ISO 9001: 2008; certified provider of aviation Meteorological service. http://www.pmd.gov.pk/).

Pakistan experiences four well defined seasons i.e. 1) Cold season from November to February 2) Pre-Monsoon dry hot season from March to mid June 3) Humid Monsoon from mid-July to mid-September 4) Post Monsoon from mid-September to October. Summer season is extremely hot and the relative humidity ranges from 25% to 50%.The day time temperature in summer remains 40°C and beyond in plain areas (Qamar-uz-Zaman et al., 2009).The average temperatures in winter range from 4°C to 20°C.Temperature sometimes falls well below freezing point in northern parts of the country. The major part of Pakistan is arid to semiarid with large spatial variability in the temperature (Chaudhry and Rasul, 2004).

Table 1
Meteorological data of the four elevational zones along the 200 km elevational gradient KP Province.
LMM (Lower montane Malakand hills, UMM (Upper montane Malam Jabba Hills)

Plain zone	**coordinates**	**Elevation**	**ARF(mm)**	**RH %**	**MAT(°C)**
Nisatta	34°01'N 71°35′E	315m a.s.l	> 127	45-50	> 20
Charsadda	34°80'N 71°43′E	276 m a.s.l	> 127	45-50	> 20
Mardan	34°12'N 72°20'E	285 m a.s.l	> 127	45-50	> 20
LMM zone	**coordinates**	**Elevation**	**ARF**	**RH**	**MAT**
Malakand	34° 33'N71° 55'E	500 m a.s.l	> 889	55-60	> 20
Batkhela	34°10′N 71°53′E	650 m a.s.l	> 889	55-60	> 20
Thana	34° 38′N72° 40'E	700 m a.s.l	> 889	55-60	> 20
Swat zone	**coordinates**	**Elevation**	**ARF**	**RH**	**MAT**
Kabal hill	34°66'N 72°13'E	950m a.s.l	> 1016	60-65	> 15
Fiza gut hill	34°79'N 72°38'E	1150 m a.s.l	> 1016	60-65	> 15
UMM zone	**coordinates**	**Elevation**	**ARF**	**RH**	**MAT**
Malam Jabba	34°81'N 72°57'E	2600m a.s.l	> 1016	60-65	> 15

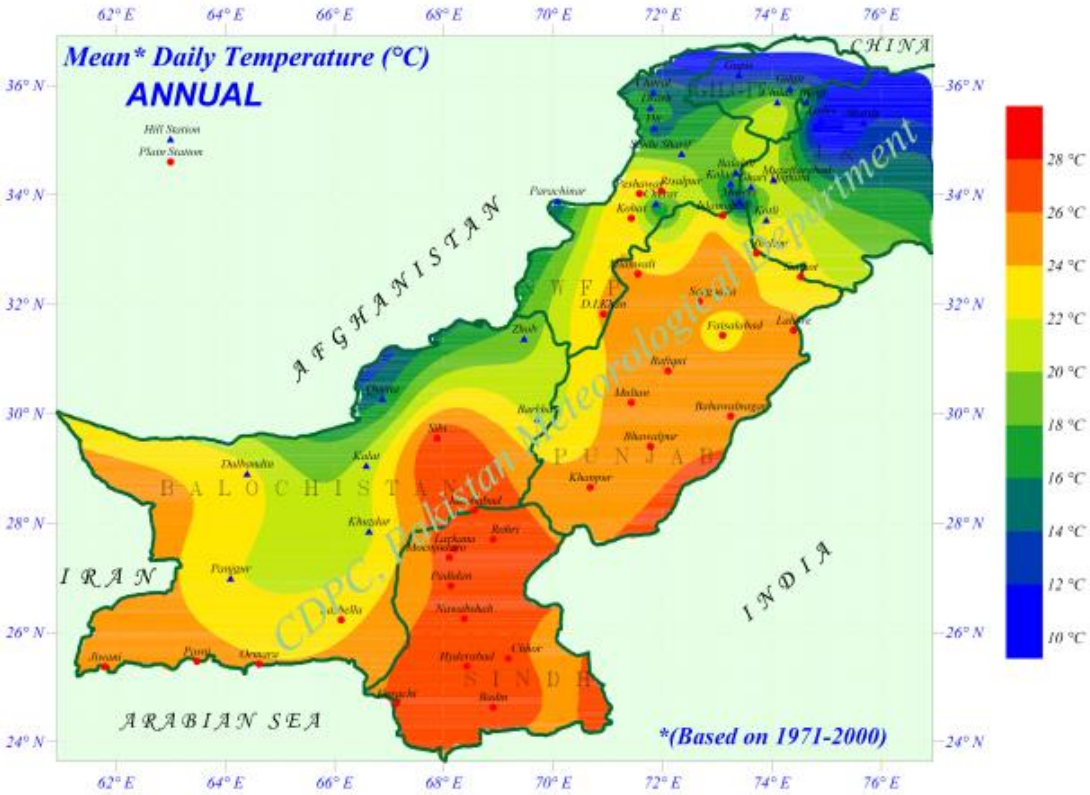

Figure 3: a) Mean annual temperature of Pakistan**,** Source: Pakistan meteorological department Islamabad.

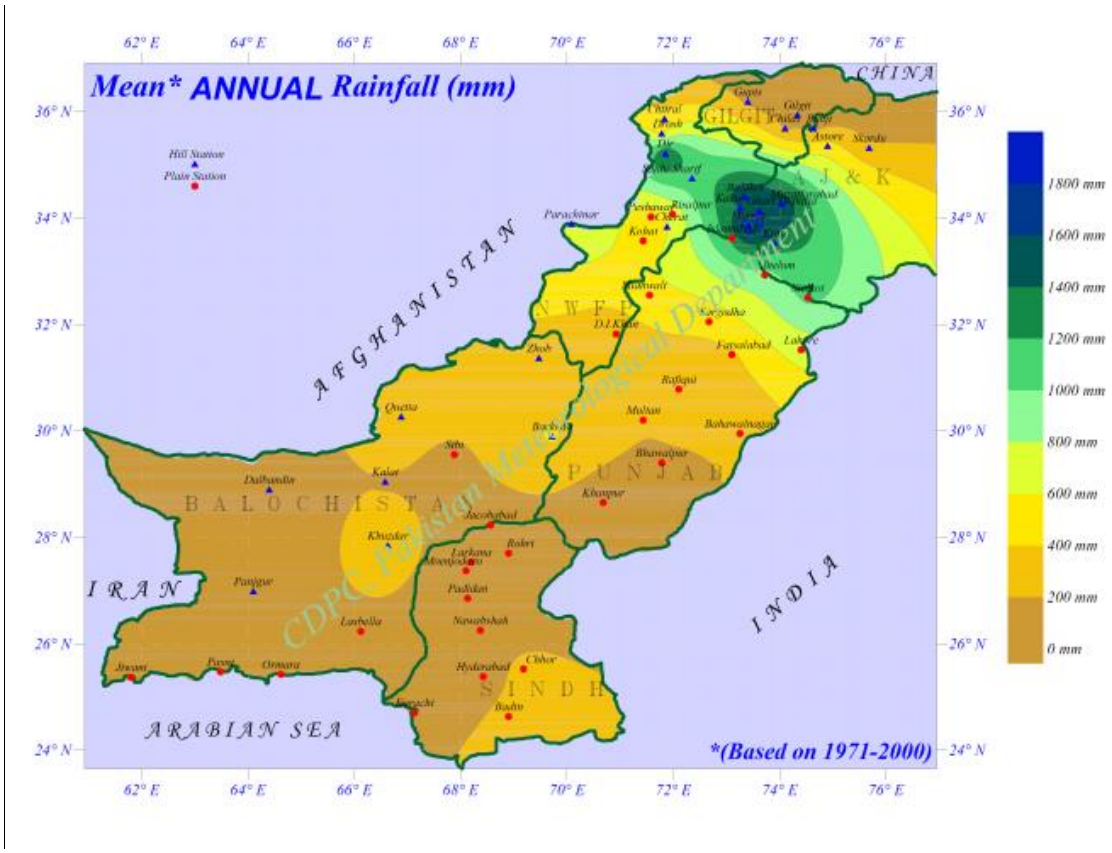

Figure 3: b) Mean annual rainfall of Pakistan. Source: Pakistan meteorological department Islamabad.

Glaciers in the Himalayas are receding faster than in any part of the world and the likelihood of their disappearance by the year 2035 is very high at its current rate (Husnain et al., 2005). This impact will be observed more in the western Himalayas as the contribution of snow to the runoff of major rivers on the western side is about 60 - 70% compared to only 10 % on the eastern side (IPCC, 2001). The high mountain region in Himalayas and Hindu Kush is mostly winter rain dominated, whereas the sub mountain region is summer rain dominated. The winter rains fall from December to March due to the western disturbances passing along the path between 30 - 60°N, whereas monsoon rains are caused by lows and depressions developing in the Arabian Sea and Bay of Bengal during July to September (Sheikh and Manzoor, 2004). The eastern part of the Hind Kush becomes more similar to the Himalayas in terms of climate and flora thus most biogeographers call it the Hindu Kush-Himalaya.

In June and July the equatorial trough of the Inter Tropical Convergence Zone (ITCZ) moves northward. The trade winds swing around as it crosses the equator becoming the south west Monsoon into Asia. Monsoons are the most intense in Asia which has the largest land mass

building up an intense low pressure system each summer (Sarfaraz, 2007). The Himalayan montane causes the ITCZ to move much further north than anywhere else in the world. As the air flows northward it picks moisture from the Indian Ocean. So the Monsoon brings torrential rain to Asia in July and August. Monsoons in the subtropical Hindu Kush and Himalayan montane are the result of the seasonally reversing tropical winds bringing dry and wet seasons.

Vegetation

The vegetation of the 200 km-long elevational gradient, starting from Nisatta Charsadda in Peshawar valley up to Malam Jabba Mountains in Swat, presents an overall reflection of the changing topography of the landscape. The drastic variation in the elevational gradient and changing floristic composition affect the overall physiognomy and layering of ecosystems across each elevational zone from lowland to highlands. The gradient starts at the lowest elevated sedimentary basin plain zone (275–315 m a.s.l.) passing through Peshawar valley, Charsadda and Mardan-Swabi road. This zone represents grassy meadows, occasional dry deciduous forests in vanishing condition and agricultural lands showing biological disturbance and interference of human activities resulting in secondary vegetation (Beg, 1978; Hussain et al., 1993). Different plant species frequently observed in this zone are *Cynodon dactylon, Eucalyptus camaldulensis, Cannabis sativa, Euphorbia prostrata, Populus ciliata, Acacia modesta, Morus alba* and *Salix species*. Six dominant herbal species namely *Parthenium hysterophorus*, *Cannabis sativa*, *Cynodon dactylon*, *Coronopus didymus*, *Cyperus rotundus* and *Euphorbia helioscopia* are very common in the plains of Peshawar valley and surrounding areas of Charsadda, Mardan and Swabi (Khan et al. 2014).

The second zone is the lower montane Malakand hills (500-900 m a.s.l.), which starts from Dargai area in the foothills of Malakand pass and ends at the top of a fertile valley surrounded by hills including Batkhela and Thana areas. This zone is represented by subtropical forests including the planted fuel wood *Eucalyptus camaldulensis* and *Dodonaea viscosa* which merge with subtropical pine and temperate forests towards the Swat valley. The most important species of this zone are *Dodonaea viscosa, Pinus roxburghii, Eucalyptus camaldulensis, Cynodon dactylon, Cannabis sativa, Pinus walichiana, Justicia adhathoda and Berberis lyceum*. Overuse and unmanaged cutting of the forest resources has put the vegetation under severe pressure which badly affects the wildlife and plants habitat (Barkatullah and Ibrar, 2011). Biotic and

Figure 4a) Secondary grassland (Nisatta Charsadda)

Figure 4b) Panorama (lower Malakand)

abiotic disturbances have almost depleted the original plant cover (Hussain et al. 1989; 2005; Barkatullah and Ibrar, 2011).

Above the lower montane Malakand Pass, surrounding areas of the upper plain Swat valley (950-1450 m a.s.l.) represent the third elevational zone. This zone experiences overgrazing, deforestation, logging, and clearing of land for terrace cultivation, which are the major threats responsible for the overall degradation of forests (Hussain et al. 1997; Sher et al. 2010; 2011). The most important taxa of this zone are *Dodonaea viscosa*, *Pinus roxburghii*, *Dalbergia sissoo*, *Pinus walichiana*, *Polygonum plebjum*, *Otostegia limbata*, *Prunus sp.*, *Plectranthus rugosus* and *Duchesnea indica etc*

Figure 5: a) Panorama of upper Malakand

Figure 5: b) A view of river Swat

The highest elevated upper montane Malam Jabba Hills (1550 –2600 m a.s.l.) represent our fourth elevational zone. Malam Jabba hills are comprised of natural coniferous forests of *Pinus*, *Abies*, *Cedrus* and *Picea* species which are also under the heavy social and economic pressure of tree felling (Siddiqui et al. 1999). The most essential taxa of this zone are *Pinus roxburghii*,

Pinus walichiana, Quercus incana, Abies pindrow, Cedrus deodara, Picea smithiana and different ferns. We also encountered occasional species of *Quercus semecarpifolia, Viburnum grandiflorum and Indigofera heterantha.* Besides deforestation and uncontrolled logging Malam Jabba hills experience occasional smuggling of wood by wood thieves which are the major threats responsible for the overall degradation of forests in this zone (Iqbal and Hamayun, 2005; Sher et al., 2010; 2011).

Holocene ecosystem dynamics in Kabal Swat, Khyber Pakhtunkhwa Pakistan

Kabal Swat is the site along the 200 km elevational gradient in research area, lying at mid altitude (750 m a.s.l.) between the low elevated Peshawar valley (275 m a.s.l.) and high elevated Malam Jabba hills (2600 m a.s.l.). The present day climate and vegetation of Kabal and surrounding areas of Qalagai hills in Swat is of temperate mountain type (Champion et al., 1965; Beg, 1975; Ilyas et al., 2012). Because of the marked differences in edaphic, physiographic and local climatic conditions in different slopes at different elevations, they support different plant communities (Ahmad, 1986; Ilyas et al., 2012).

Figure 6a) Dargai Malakand Sept, 2012)

Figure 6b) Kabal Swat Core (Photos by Qadeem Khan)

The Kabal area in Swat is surrounded by mountains and majority of the pollen and spores are most likely to have originated from vegetation growing on the adjacent mountain system. Hence the pollen assemblage in Kabal area of Swat valley is likely to originate from plants having a broad spectrum of altitudes. Consequently, changes in past climate must have left a notable effect on the altitudinal occurrence of taxa and these changes are likely to be represented in the pollen record. As a result, the natural vegetation around Kabal area in Swat valley should be a

reasonably sensitive indicator of the climatic conditions on the surrounding mountains at any period of time. Our results based on the pollen diagram and multivariate data analysis reveal changes in the vegetation and environmental conditions of Kabal area in Swat during the last 3300 cal yrs BP. The core derived from Kabal area of Swat reflects local and regional vegetation dynamics since the early late Holocene. Two bulk samples taken at the depth of 116 cm and 148 cm recorded time up to 5660 and 8100 cal yrs BP. respectively. But since no pollen or negligible pollen grains were found from 76 cm downwards, probably due to drier climatic conditions, therefore we relied on the samples till 76 cm which recorded the vegetation history up to 3300 cal yrs BP.

Objectives of the project

This project deals with the present and past vegetation, plant diversity patterns, their responses to climate and land use changes in the Hindu Kush, north western Khyber Pakhtunkhwa province of Pakistan. Monsoon rains in this area have resulted in rich flora famous for its unique endemic and threatened biodiversity, forests, wildlife and immense unexplored genetic resources, due to which the Hindu Kush Himalayan ecoregion has been included in the Global-200 priority ecoregion for conservation (Ahmad, 2014). Considering the immense potential and importance of this area regarding biodiversity, extreme vulnerability to climate change in the form of ever increasing floods, deforestation and natural disasters, this area should receive special attention in terms of conservation. Keeping these aspects in view, three (03) goals have been set for this project.

1. One goal of this PhD-project is to assess the dynamics of grassland ecosystems in this area. For understanding modern grassland ecosystems, their transformations due to human impact and their response to global change, the history of grassland ecosystems needs to be studied. Of particular interest is the composition of C3 and C4 Poaceae species in different grasslands because they can be interpreted as climate proxies for past temperature, atmospheric CO_2 concentration and precipitation variation (Schüler and Behling, 2011). Since the long-term dynamics of grasslands are rather difficult to access using modern Poaceae pollen due to their uniform monoporate pollen type, this project investigates as to what extent differences in the pollen grain size of C3 and C4 Poaceae species exist and how those differences can be interpreted. For this purpose C3 and C4

Poaceae species collected from the research area have been compared with Poaceae species from South America, Europe and Africa.

2. As the accurate palaeoecological and palaeoclimate reconstructions, using palynology, require a broad spectrum approach in which the study of present day vegetation becomes a prerequisite. Hence the second goal of this project is to bring to limelight the modern pollen rain vegetation relationship in this unexplored area for gaining the focus of public attention. The idea is to link past and present ecological questions related to palynological patterns of Khyber Pakhtunkhwa region in the Hindu Kush. It is well known that past shifts in the distribution of vegetation types are reconstructed from fossil pollen assemblages, the exact relationship between modern vegetation and modern pollen compositions is necessary for calibration of the fossil pollen records. Such calibration studies which quantitatively relate pollen with elevation or climatic parameters are still lacking in South Asia. Hence the second goal of this project is to establish exact pollen-vegetation and pollen-climate relationships, from which transfer functions can be developed for detailed reconstruction of past vegetation and palaeoclimate from fossil pollen records.

   To meet this goal the following three questions have been addressed:

   a) Whether the plant diversity patterns of the existing vegetation along successive elevational zones from lowland to highland are reflected in modern pollen-rain?
   b) Whether the modern pollen rain represents a reflection of each elevational zone in the form of vegetation boundaries and if so what are the key taxa which reflect the different elevational zones?
   c) Whether the land use and consequently the secondary vegetation are reflected in the modern pollen rain?

3. The third goal of this project is to explore the past biodiversity patterns and to understand the role of climate change and human impact in shaping vegetation change in the diverse ecosystems of Khyber Pakhtunkhwa, so as to link palaeoecology to conservation plans. To meet this goal, following questions have been addressed:

   a) How did the vegetation respond to past environmental changes?

b) What changes in biodiversity can be observed?

c) How did the floristic composition of the different vegetation types change in space and time?

Brief outline of chapters

The chapters have been prepared as manuscripts for submission to peer-reviewed journals. They are as follows:

Chapter 1 deals with introduction of the project

Chapter 2 deals with assessing the dynamics of grassland ecosystems. This study shows that grasslands dominated by C3 and C4 Poaceae species from different regions and habitats can be separated by studying the pattern of trends in their pollen grain sizes.

Chapter 3 deals with modern pollen spectra from surface samples and their relationship with the surrounding vegetation provide useful data for the interpretation of future Holocene pollen records. This study demonstrate that the establishment of a modern pollen rain – vegetation relationship, at least on family level, is necessary for calibration and interpretation of fossil pollen record from such sites.

Chapter 4 unravels new and interesting aspects providing a detailed history of the vegetation and climate of the Hindu Kush mountains in Pakistan since the last 3300 years.

Chapter 5 deals with the most important outcomes of this research and fundamental conclusions are drawn.

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CHAPTER 2

Trends of pollen grain size variation in C3 and C4 Poaceae species using pollen morphology for future assessment of grassland ecosystem dynamics

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Abstract

This pilot study aims to find whether C3 and C4 Poaceae species can be separated by their pollen grain size. One hundred and sixty Poaceae species were prepared for pollen grain size analyses using standard methods. The whole dataset included 70 species from northern Pakistan, 35 species from Tanzania, 25 from South America and 30 from Germany. We measured four pollen grain parameters, i.e. pollen grain length, pollen grain width, pore diameter and annulus width. Results of the statistical tests and multivariate analyses performed on our specific dataset confirmed that C3 polyploid and C4 polyploid species show trends of larger grain size than the respective diploid species. Results of our fresh Poaceae grains should be applied on fossil pollen embedded in environmental archives for reconstructing past climate, for detecting shifts between their C3/C4 Poaceae compositions and assessing the dynamics of grassland ecosystems.

Keywords: *pollen grain size, C3 and C4 Poaceae species, ploidy, Pakistan, Europe, Africa, South America*

Introduction

The Poaceae family has about 700 genera and 11 000 species of grasses worldwide (Chen et al. 2006). There are 158 genera with 492 species of Poaceae in Pakistan (Cope 1982; Perveen 2006). Poaceae species are cosmopolitan covering 20% of the Earth's land surface (Shantz 1954; Kellogg 2001). Grassland ecosystems cover different landscapes forming ecosystems such as savannas, páramos, pampas and steppes on different continents. For understanding modern grassland ecosystems, their transformations due to human impact and their response to global change, the history of grassland ecosystems needs to be studied. Since long-term dynamics of grasslands are rather difficult to access using modern Poaceae pollen due to their uniform monoporate pollen type, we investigate to what extent differences in the pollen grain size of C3 and C4 Poaceae species exist and how we can interpret those differences. Of particular interest is the composition of C3 and C4 Poaceae species in different grasslands because they can be interpreted as climate proxies for past temperature, atmospheric CO_2 concentration and precipitation variation (Schüler & Behling 2011b). C4 grasses are mostly dominant in regions with warm seasonal precipitation, while C3 grasses prefer cool seasonal precipitation (Epstein et al. 1997; Sage 2004). C3 Poaceae species grow under cool moist conditions, preferring high-

elevated habitats. The percentage of C3 grasses increases steadily above 2000 m above sea level (a. s. l.) in Ecuador replacing the C4 grass species, which are dominant at lower elevations (Moinuddin et al.1994). There is a competitive advantage of the C3 metabolism in wetter and cooler areas in contrast to the one of C4 species, which shows higher water use efficiency in more arid and warmer habitats (Boom et al. 2001).

Despite numerous studies on the complex taxonomy and ecology of Poaceae, to our knowledge, there is no published work that compares the pollen morphology of C3 and C4 species, especially on continental scale. Uniform morphology of Poaceae grains poses problems to palaeo-ecologists in the reconstruction of past grassland ecosystems (Andersen 1979). Our aim is to explore whether C3 and C4 Poaceae species from different regions can be separated by their pollen grain size. Spatial variations of tropical and subtropical grassland ecosystems, as well as changes in vegetation composition, locations and boundary shifts play an important role in past climate reconstruction (Prieto 1996; Haberle & Maslin 1999; Behling & Hooghiemstra 2001; Behling 2002; Behling et al. 2004; Behling & Pillar. 2007; Brunschön & Behling 2009).

Schüler and Behling (2011a) used Poaceae pollen grain size as a tool to distinguish past grasslands in South America demonstrating that a distinction between grassland ecosystems is possible based on measurable Poaceae pollen grain parameters. Schüler and Behling (2011b) used characteristics of Poaceae pollen grains as a tool to assess palaeo-ecological grassland dynamics in South America concluding that with the help of Poaceae grain characteristics, it is possible to get additional information on the specific grassland characteristics, their development on a temporal and spatial scale and their response to climate change as well as human impact.

Other studies have attempted to use morphology to distinguish different types of Poaceae pollen. Perveen and Qaiser (2012) described the pollen grain morphology of 54 species belonging to 30 genera of the family Poaceae from Pakistan, using light and scanning electron microscopy (SEM). They recognized five distinct pollen grain types, based on the exine ornamentation, stating that grain morphology does not correspond with tribe and genus classification, but is significantly helpful at the species level. Ahmad et al. (2011) described the pollen grain morphology of five Poaceae species belonging to three genera of the tribe Chlorideae, stating that grain characters are important in delimiting the Poaceae family at species, genus and tribe levels. The pore diameter as well as the annulus shape and size are the principle characteristics and can be used for distinction between Poaceae species (Beug 1961). The author suggested an

intraspecific wide range of grain sizes, but did not report the exact annulus size of Poaceae species.

Dickson (1988) distinguished cereal pollen grains from wild grass pollen grains and reported that *Secale* pollen grains are easily identified, but there are some problems with *Hordeum*, *Avena* and *Triticum* as the pollen grains are swollen with acetoysis and glycerol mounting. *Hordeum* grains can be distinguished by their scabrate sculpturing from that of *Avena* and *Triticum*, which have verrucate grains. Poaceae taxa seem to display a rather variable grain size range at species level (Salgado-Labouriau & Rinaldi 1990a; 1990b). These authors confirmed that the variation in the grain size of cultivated grasses is higher than the wild grass species. Kohler and Lange (1979) used SEM to distinguish cereal pollen and wild grass pollen. They used the fine sculpture of the exine for this distinction. Mander et al. (2013) used SEM images of 240 grains from twelve species within Poaceae and classified these species by developing algorithmic features that quantify the size and density of sculptural elements on the pollen surface.

Ploidy appears to have a significant effect on grain size. Pollen grain size increases with ploidy level for example in *Avena* (Katsiotis & Forsberg 1995) and *Rumex* (Den Nijs et al. 1980). The authors found that in *Avena sativa* L. the pollen grain size increases with increasing ploidy levels. Our morphometric approach of studying the pollen grains of C3 and C4 species is an attempt to distinguish the uniform monoporate pollen grains within Poaceae family.

Material and methods

Collection of Poaceae pollen

Twelve field visits were made to the central and northern districts of the Khyber Pakhtunkhwa province of Pakistan during March through April 2010 and August through September 2012, and fresh Poaceae species were collected along with flowers. We selected a 200 km long elevational transect from Charsadda to Malam Jabba Swat in Khyber Pakhtunkhwa, investigating grasslands for Poaceae species in the plains of Peshawar Valley, Charsadda, Nowshera and Mardan (280–350 m a. s. l.). We collected Poaceae species from mid-elevated grasslands at the Malakand pass, in the Dargai, Batkhela, Thana areas (350–750 m) reaching up to the high altitudes areas of Kabal, Fizagut and Malam Jabba Hill top (750–2600 m) collecting a total of 70 Poaceae species. The plants were dried and preserved using standard herbarium techniques (Bridson & Forman 1992). Identification was done using reproductive characteristics of flowers and with the help of

available literature (Köhler & Lange 1979; Cope 1982; Liu et al. 2004; Andersen & Bertelsen 1972; Ullah et al. 2011; Perveen & Qaiser 2012), with the help of very experienced taxonomists (D. Hojsgaard, Z. Ullah, M Schwerdtfeger, N. Akhtar) and online research (e.g. www.efloras.org). In order to look for pattern of differences in the pollen grain size within Poaceae, we used additional species representing diverse geographical habitats including 35 species from Tanzania (Africa), 25 from South America including three species each from Brazil and Venezuela and 19 species from Ecuador. We further collected 30 species from the Botanical Garden of the University Göttingen, Germany (European species), represented by ten species each from the genera *Poa*, *Hordeum* and *Bromus*. Hence, the whole data set comprised 160 species including 98 C4 and 62 C3 species (see Appendix Table A1).

Sample preparation for measurement

For analyses of pollen grain size, ten species were processed per day with identical treatment according to the standard method described by Fægri and Iversen (1989). To avoid any swelling of the pollen grains on prepared slides, they were measured on the same day when pollen grains were mounted in glycerine (Cushing 1961; Hamilton 1972; Moore & Webb 1978; Mäkelä 1996; Schüler & Behling 2011a). Pollen residues were safely kept in distilled water after processing for future use. Identical chemical treatment avoids alteration of pollen grain size allowing the comparison of pollen grain size variation. All measurements were made using a Leica Photo Microscope and the accompanying image analyses software Leica QWin. We measured to the nearest 0.01 μm under 400 × magnifications considering the following four parameters (Figure 1):

1. Pollen grain length (PGL) is the longest axis of the grain.
2. Pollen grain width (PGW) is the axis of the grain perpendicular to PGL.
3. Pore diameter (PD) excluding the annulus.
4. Annulus width (AW) is the distance between pore and outline of the annulus.

Figure 1. The four parameters of a Poaceae pollen grain considered in the study. 1, Pollen grain length (PGL) is the longest axis of the grain. 2, Pollen grain width (PGW) is the axis of the grain perpendicular to PGL. 3, Pore diameter (PD) excluding the annulus width. 4, Annulus width (AW) is the distance between the pore and the outline of the annulus.

Measurement setting

Prior to performing the measurements, leading to a differentiation between grass grain size distributions, we determined how many grains had to be measured to get a representative result for the average grain length per species. Hence, we observed the stability of the average grain length within one species with an increasing number of measured pollen grains. This was done for all datasets, which were later pooled for further analyses. Forty-five pollen grains were measured per species. As not all grain size distributions showed a Gaussian distribution, the average grain length is given by the median. The following equations were used to calculate the development of the median grain length with increasing numbers of measured pollen grains:

$$X_{med} = \sum_{1}^{n} X_{n/2+1}/n \qquad \text{for all odd } n$$

$$X_{med} = \sum_{1}^{n} \tfrac{1}{2} (X_{n/2} + X_{n/2+1})/n \qquad \text{for all even with } n = \text{number of measured grains}$$

Statistical analysis

The R software package (The R Foundation for Statistical Computing 2013) was used for the statistical analyses. We obtained median values for all four pollen parameters and applied the Shapiro test to both C3 (diploid, polyploid) and C4 (diploid, polyploid) datasets to estimate if the average measures of the four pollen grain parameters were normally distributed. In case our data

set was normally distributed, we applied an *F*-test to test the variances and subsequently applied a Student *t*-test to compare the mean of pollen grain measurements. If the data set did not show a normal distribution, we applied the WILCOXON *U*-Test for differences in mean pollen grain measurements.

Pearson correlation analyses

Pearson correlation coefficient values were calculated as a measure of linear correlation for determining the strength of relationship among the four pollen grain parameters.

Linear discriminant analysis (LDA)

LDA was performed to discriminate *a priori* defined C3/C4 groups and diploid/polyploid groups as dependent variables, and the four pollen grain parameters as independent variables. Assuming that the groups are linearly separable, the aim was to look for a linear combination of variables that best explains the difference between the respective groups. The prior probability percentages of C3/C4 or diploid/polyploid, out of the total dataset of 160 species, were simply determined based on the number of samples in each group. The mean values and coefficients of linear discriminants for the pollen grain parameters were determined. The percentage of overlaps in C3/C4 and diploid/polyploid scores was estimated by class prediction.

Non metric multidimensional scaling (NMDS)

Based on the scores of the four pollen grain parameters, we performed separate NMDSs both for the C3/C4 group and diploid/polyploid groups (using the meta-MDS function available in the vegan package of R; Oksanen et al. 2013).The NMDS scores of the four pollen grain parameters were plotted for C3/C4 species and diploid/polyploid species separately.

Ploidy levels of Poaceae species

For determining the ploidy levels of the species under study, we relied on already published data (e.g. Cope 1982; Ahsan et al. 1994; Morrone et al. 2006; Hojsgaard et al. 2008; Peichoto et al. 2011; www.efloras.org). The whole dataset of 160 species was divided into small datasets of C3 diploid, C3 polyploid species and C4 diploid, C4 polyploid species.

Results

Measurement setting

The average grain length (given as median) still varies a lot within the first 30–35 measured pollen grains (Figure 2). However, it stabilizes after about 40 pollen grains. Above this number, the median remains stable with a variation between 0.1 and 0.8 µm.

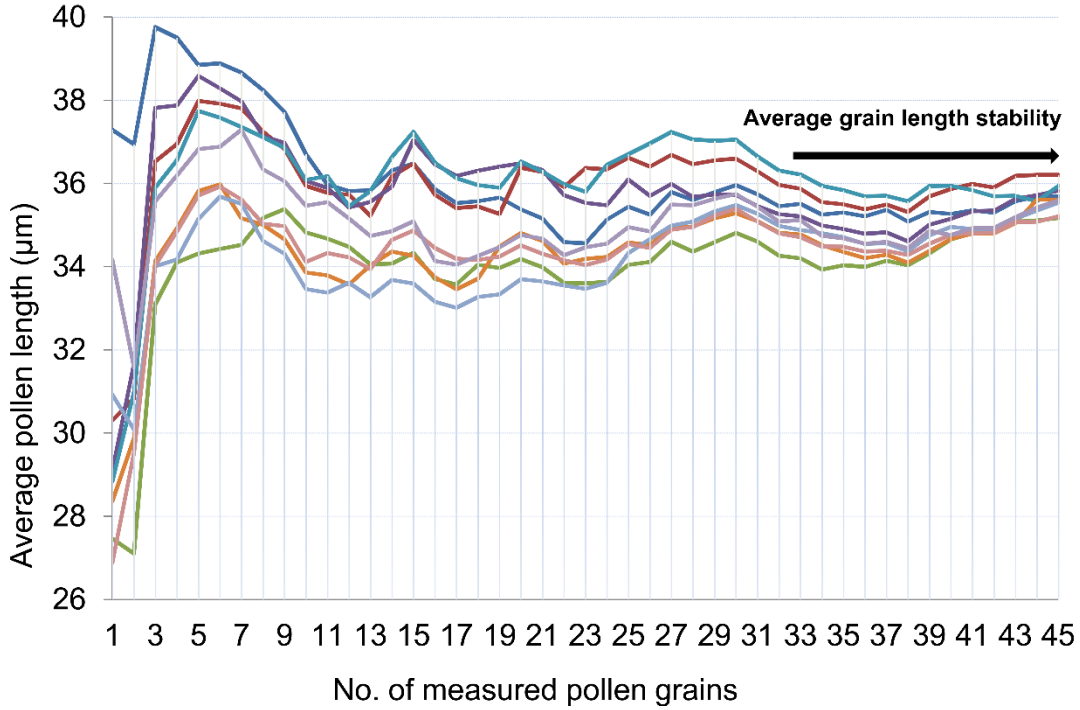

Figure 2. Stability graph showing pollen grain length (PGL) as the representative pollen grain parameter for determining the number of pollen grains, required to be counted to reach stable averages. Stability in PGL is achieved, once the number of counted pollen grains reaches to30. The X-axis is showing the number of measured pollen grains and the Y-axis is showing the mean value for PGL in micrometres. The same procedure was followed for all the four pollen grain parameters.

Measurement of the four pollen grain parameters

Frequency distribution histograms of the measurements for the four pollen grain parameters along with their measurement range, maximum frequency, average values and their differences (in µm) are given in Table I. Values of average pollen grain measurements are larger in C4 than in C3 species and larger in polyploid than in diploid species. The pollen grain length of C3

diploid species (maximum frequency) are in the range of 26–28 µm, while those of C3 polyploid species are 32–34 µm, showing that, on average, C3 polyploids species have larger a pollen grain length than C3 diploids (Figure 3A, B). The pollen grain length of C4 diploid species (maximum frequency) are in the range of 30–38 µm, while those of C4 polyploid species are 38–40 µm, showing that, on average, C4 polyploids have a larger pollen grain length than C4 diploid species (Figure 3C, D).

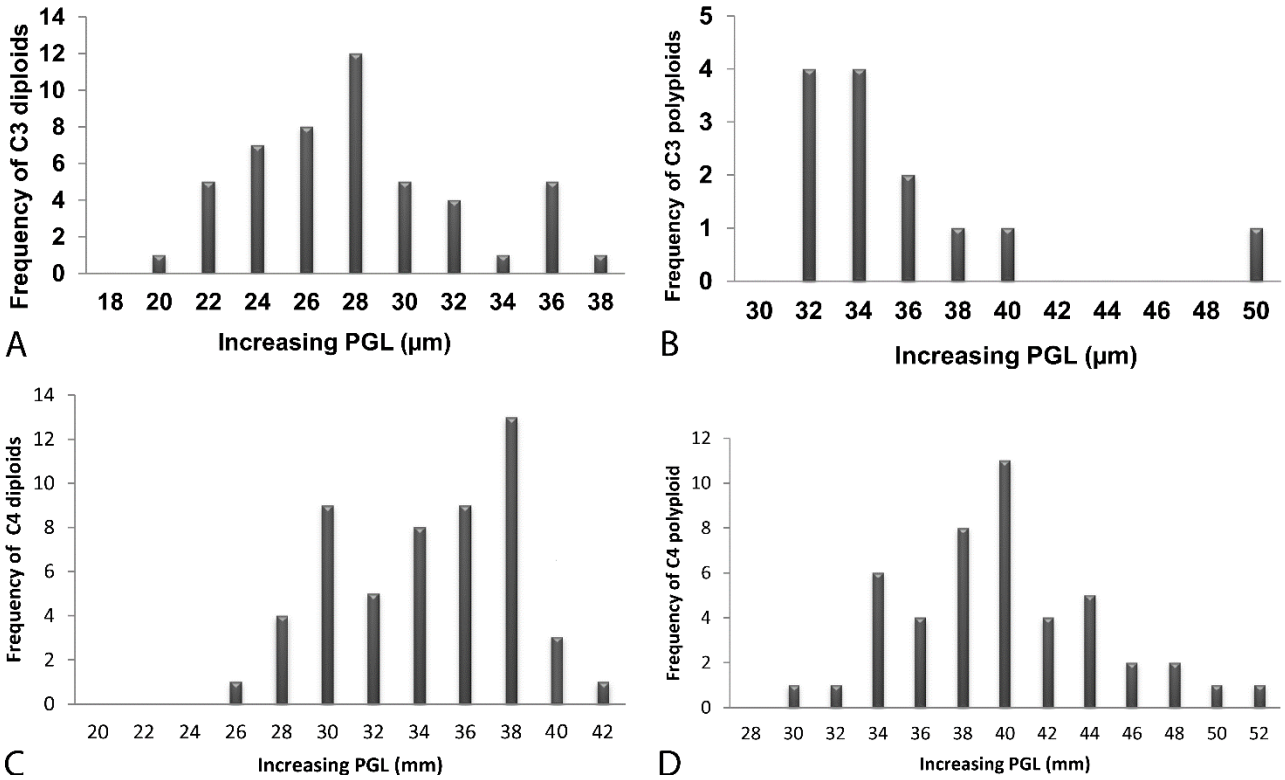

Figure 3. Frequency distribution histograms of the average measures of 62 C3 and 98 C4 Poaceae species. In all the cases, the X-axis is showing the measures of the pollen grain length (µm) while the Yaxis is showing the number of C3 or C4 species, whose respective pollen grain length fall in a certain measurement range. A, B. Pollen grain length of C3 diploid and polyploids, respectively. C, D. Pollen grain length of C4 diploids and polyploids, respectively.

Statistical analysis

Box plots for both C3 and C4 datasets (diploid, polyploid), based on the four pollen grain parameters, show that the median values are higher in polyploid species than in diploid species (Figure 4A–H) except for the pore diameter and annulus width of C3 dataset (Figure 4C, D). The significance of difference in all the four pollen grain parameters between C3, C4 (diploid,

polyploid) datasets is evident from the *p*-values as revealed by the Shapiro-Wilk normality test, *F*-test, *t*-test and Wilcoxon *U*- tests (Table II).

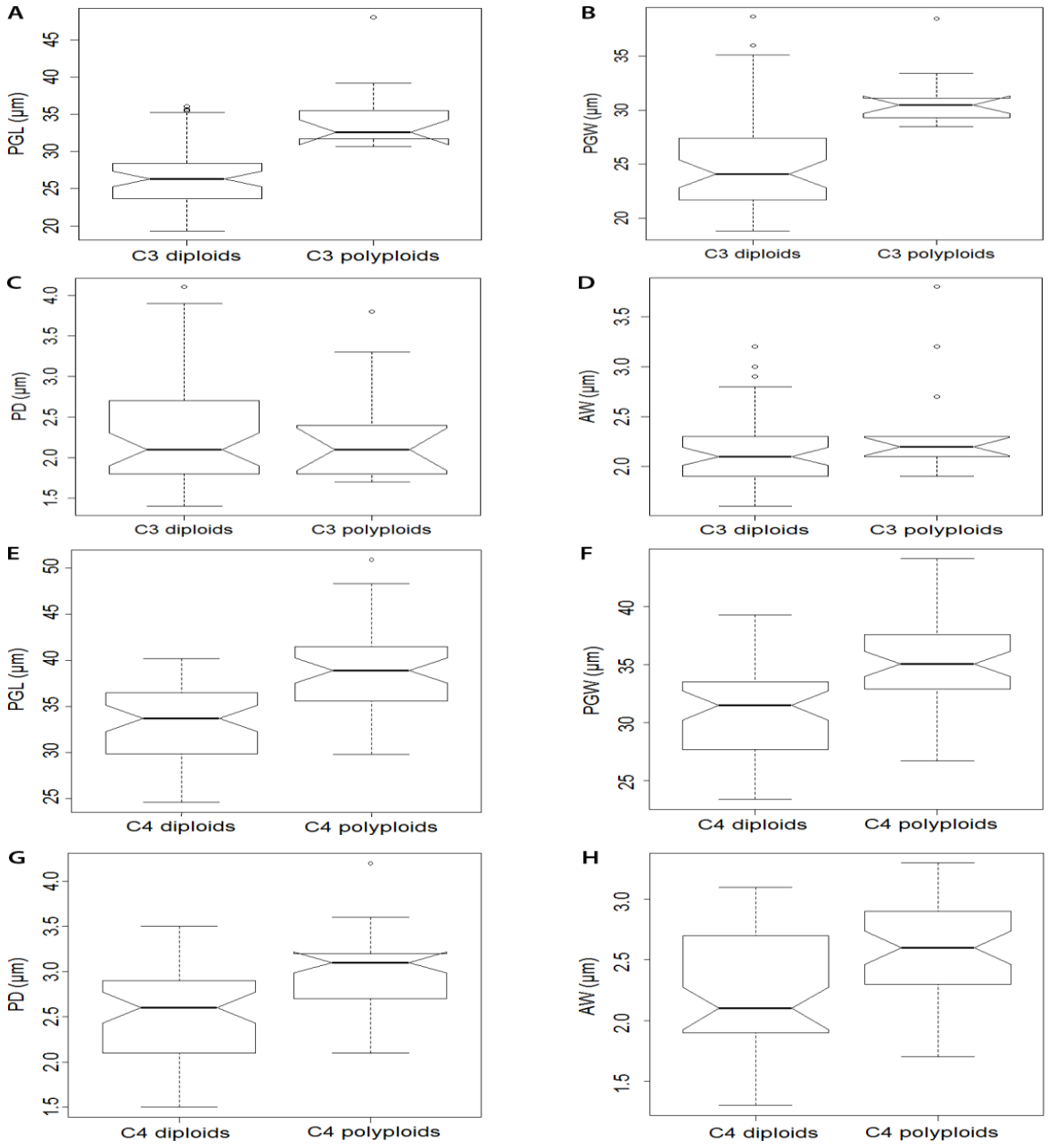

Figure 4. Boxplots showing the four pollen grain parameters of Poaceae species on the Y-axis and their photosynthetic pathway (A–D) and ploidy levels (E–H) on the X-axis. A. Pollen grain length (PGL) in relation to anatomy. B. Pollen grain width (PGW) in relation to anatomy. C. Pore diameter (PD) in relation to anatomy. D. Annulus width (AW) in relation to anatomy. E. PGL in relation to ploidy levels F. PGW in relation to ploidy. G. PD in relation to ploidy. H. AW in relation to ploidy.

Table I. Measurement of the four pollen grain parameters of C3 and C4 Poaceae species in micrometers.

S.No	Anatomy	Ploidy	Parameter	Range	Max. freq	Avg	Difference
1	C3	diploids	PGL	19.3 - 38.7 µm	26 - 28 µm	26.9	
2	C3	diploids	PGW	18.8 - 36.1 µm	22 - 26 µm	25.2	
3	C3	diploids	PD	1.4 - 4.1 µm	1.8 - 2.8 µm	2.3	
4	C3	diploids	AW	1.6 - 3.2 µm	2.0 - 2.2 µm	2.1	
5	C3	Polyploids	PGL	30.7 - 48.1 µm	32 - 34 µm	34.7	C3 poly 7.8 µm > C3 dip
6	C3	Polyploids	PGW	28.5 - 38.5 µm	31 - 32 µm	31	C3 poly 5.8 µm > C3 dip
7	C3	Polyploids	PD	1.7 - 3.8 µm	1.9 - 2.1 µm	2.3	No difference
8	C3	Polyploids	AW	1.9 - 3.8 µm	2.0 - 2.2 µm	2.4	C3 poly 0.3 µm > C3 dip
9	C4	diploids	PGL	24.6 - 40.2 µm	30 - 38 µm	33.4	
10	C4	diploids	PGW	23.4 - 39.3 µm	26 - 34 µm	30.8	
11	C4	diploids	PD	1.5 - 3.5 µm	1.9 - 2.2 µm	2.5	
12	C4	diploids	AW	1.3 - 3.1 µm	2.0 -2.8 µm	2.2	
13	C4	Polyploids	PGL	29.8 - 50.9 µm	38 - 40 µm	38.9	C4 poly 5.5 µm > C3 dip
14	C4	Polyploids	PGW	26.7 - 44.1 µm	33 - 37 µm	35.4	C4 poly 4.6 µm > C3 dip
15	C4	Polyploids	PD	2.1 - 4.2 µm	2.8 - 3.2 µm	2.9	C4 poly 0.4 µm > C3 dip
16	C4	Polyploids	AW	1.7 - 3.3 µm	2.7 - 2.9 µm	2.6	C4 poly 0.1 µm > C3 dip

Note: Maximum range of measurement results given by the frequency of values most often given by the pollen grain parameters. PGL, pollen grain length; PGW, pollen grain width; PD, pore diameter; AW, annulus width.

Pearson correlation analyses

The Pearson correlation coefficient values reveal the strongest relationship of pollen grain length with pollen grain width (r = 0.85), followed by pollen grain width with pore diameter (r = 0.60) and pore diameter with annulus width (r = 0.39; Figure 5).

Results of the LDA

LDA results for dependent variables, i.e. C3/C4 groups (Figure 6A) and diploid/polyploid groups (Figure 6B) are shown as frequency distribution histograms on the Y-axis; the independent variables, i.e. the four pollen grain parameters describing these groups, are shown on the X-axis. In the case of C3/C4 groups, the LDA range on the X-axis varies from –2 to 4 with a threshold value of 0 in between. All scores < 0 on the X-axis represent C3 species, while all scores > 0 represent C4 species (Figure 6A). For the C3 group, the frequency distribution is concentrated in the negative range of the threshold value 0 (41/62 = 66%) having an overlap with C4 (21/62 = 33%). The prior probabilities of the groups revealed C3 = 38.75% and C4 = 61.25% with an area under the curve = 77 %. For the C4 group, the frequency distribution is concentrated on the right of the threshold value 0 (86/98 = 88%) having an overlap with C3 (12/98 = 12%). The same is the case for the diploid/polyploid group (Figure 6B). The prior probabilities of the groups

revealed diploid = 63.75% and polyploid = 36.25% with an area under the curve = 68.55%.The coefficient of linear discriminants, values of the mean pollen grain parameters and the percent overlap estimated by class prediction for both C3/C4 and diploid/polyploid groups are given in Table III.

Table II. Results of the statistical tests.

Shapiro-Wilk normality results for C3 and C4 datasets				
Dataset	**PGL**	**PGW**	**PD**	**AW**
C3 diploids	P = 0.01	P = 0.0002	P = 0.0001	P = 0.005
C3 polyploids	P = 0.001	P = 0.004	P = 0.01	P = 0.001
C4 diploids	P = 0.11	P = 0.21	P = 0.08	P = 0.007
C4 polyploids	P = 0.39	P = 0.43	P = 0.27	P = 0.09
***F*-test for variances between diploids and polyploids**				
Dataset	**PGL**	**PGW**	**PD**	**AW**
In C3 dataset	P = 0.31	P = 0.01	P = 0.37	P = 0.03
In C4 dataset	P = 0.09	P = 0.39	P = 0.08	P = 0.04
***U*-test for differences in means of C3 diploids and C3 polyploids**				
Dataset	**PGL**	**PGW**	**PD**	**AW**
In C3 dataset	P = 1.259e-05	P = 0.0001	P = 0. 94	P = 0.21
Student *t*-test to compare the means of C4 diploids and C4polyploids				
Dataset	**PGL**	**PGW**	**PD**	**AW**
In C4 dataset	p = 5.92e-09	p = 1.66e-07	p = 6.32e-06	p = 0.0002

NMDS

Results of the NMDS of the four pollen grain parameters based on Euclidean distance, with the dataset square root transformed, centered scaling, PC rotation and standardization, revealed a stress equal to 0.041, procrustes equal to 8.601e-05 and maximum residuals value equal to 0.0005 for the C3/C4 group. The observed dissimilarity is shown on a scale of – 0.15 to +0.15 along NMDS axis 1 and the ordination distance on the scale of – 0.04 to +0.04 along NMDS axis 2. The NMDS based on the four pollen grain parameters does not show strong differences in the scores of C3 and C4 species along NMDS axis 1, as revealed from the *t*-test (p = 0.3; Figure 7A). Similarly NMDS for the diploid/polyploid group revealed a stress = 0.042, procrustes = 4.134e-05 and maximum residuals value = 0.0003. The NMDS based on the four pollen grain parameters does not show strong differences in the scores of diploid/polyploid species along NMDS axis 1, as revealed from the *t*-test (p = 0.5; Figure 7B).

Table III: Results of LDA

Group	PGL	PGW	PD	AW
C3	28.6	26.43	2.3	2.23
C4	35.9	32.91	2.75	2.37
Diploid	30.3	28.14	2.42	2.2
Polyploid	38	34.38	2.84	2.52
Coefficient Of LD for C3,C4	0.1	0.098	-2.327	2.27
Coefficient Of LD for dip, poly	0.21	0.008	2.492	-2.788
Class prediction	**C3**	**C4**	**Overlap %**	
C3	41	21	33	
C4	12	86	12	
Class prediction	**dip**	**poly**	**Overlap %**	
diploid	85	17	16	
polyploid	18	40	31	

Note: LD, linear discriminants. "dip" means diploid and "poly" means polyploid.

Diploid and polyploid species in the dataset

Out of the total 98 C4 species, 52 species (53%) are diploid and 46 species (47%) are polyploid. Out of the total 62 C3 species, 49 species (79%) are diploid, while 13 species (21%) are polyploid. Hence, in our specific data set of 160 species, polyploidy is more prevalent in C4 species, while diploidy is more prevalent in C3 species.

Discussion

This pilot study dealing with a small dataset of 160 species is certainly limited and the results are specific to our dataset. Hence, the number of Poaceae species needs to be substantially increased for replicating the study in future. Pollen grain studies of paired C3/C4 taxa would allow a more robust comparison. The main results revealed by different analyses are discussed below.

Identical chemical treatment allows us to compare the minute variations of Poaceae grains representing grasslands of diverse habitats. Due to the swelling effect of glycerine on the grain

size, we recommend to prepare only a limited number of samples that can be measured on the same day or shortly after sample preparation. As chemical treatment has a clear effect on the size of pollen grains, we recommend standardizing the sample preparation to avoid minute alterations in the size of grains due to different chemical treatments (Schüler & Behling 2011a).

The evaluation of changes in median with an increasing number of measured pollen grains revealed that the median grain length per species remains stable after about 40 measured pollen grains. This becomes very important when trying to compare data sets from different habitats and different periods. The median is certainly a rough measure for an analysis of differences in pollen grain length and their composition. However, it serves well as a first approach to get an impression on the dataset of our species. Despite the stabilization of the median value above 40 measured pollen grains, we recommend measuring at least 60 pollen grains per species to be completely sure about the representation of the median for the data set.

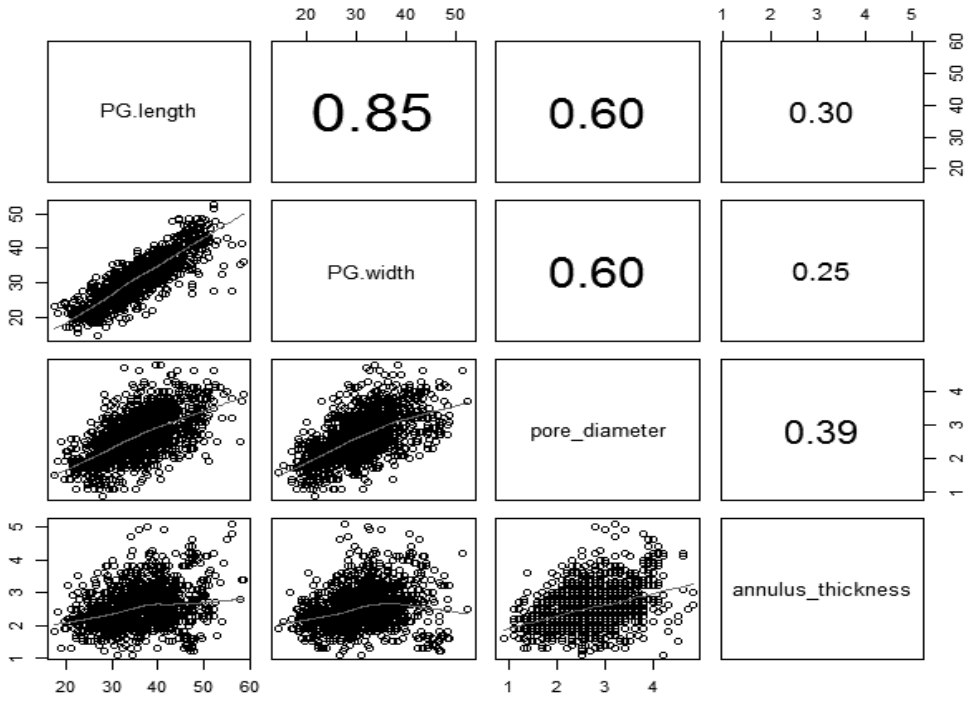

Figure 5. Pearson correlation coefficient values showing the strength of relationship among the four pollen grain parameters in descending order from top to bottom.

Schüler and Behling (2011a) described a strong correlation between pollen grain length, pollen grain width and pore diameter, while distinguishing South American past grasslands. Their

assertion is based on the studies by Katsiotis and Forsberg (1995), who described a correlation between pollen grain length and pollen grain width in *Avena sativa*. Results of this study are in agreement with the previously obtained results. The correlation analysis revealed a strong relationship between the four pollen grain parameters.

Our results demonstrate that the average measures of pollen grain length, pollen grain width and annulus width are clearly larger in C3 polyploids as compared to C3 diploid species. However, no considerable difference was found in their pore diameter. All measured pollen grain parameters are larger in C4 polyploids than C4 diploid species. Although we used four pollen grain parameters for distinguishing C3 and C4 Poaceae species, it appears sufficient to use the two most prominent parameters 'pollen grain length' and 'pollen grain width' for determining the grain size. Differences in the measures of pore diameter and annulus width can be neglected due to minor differences.

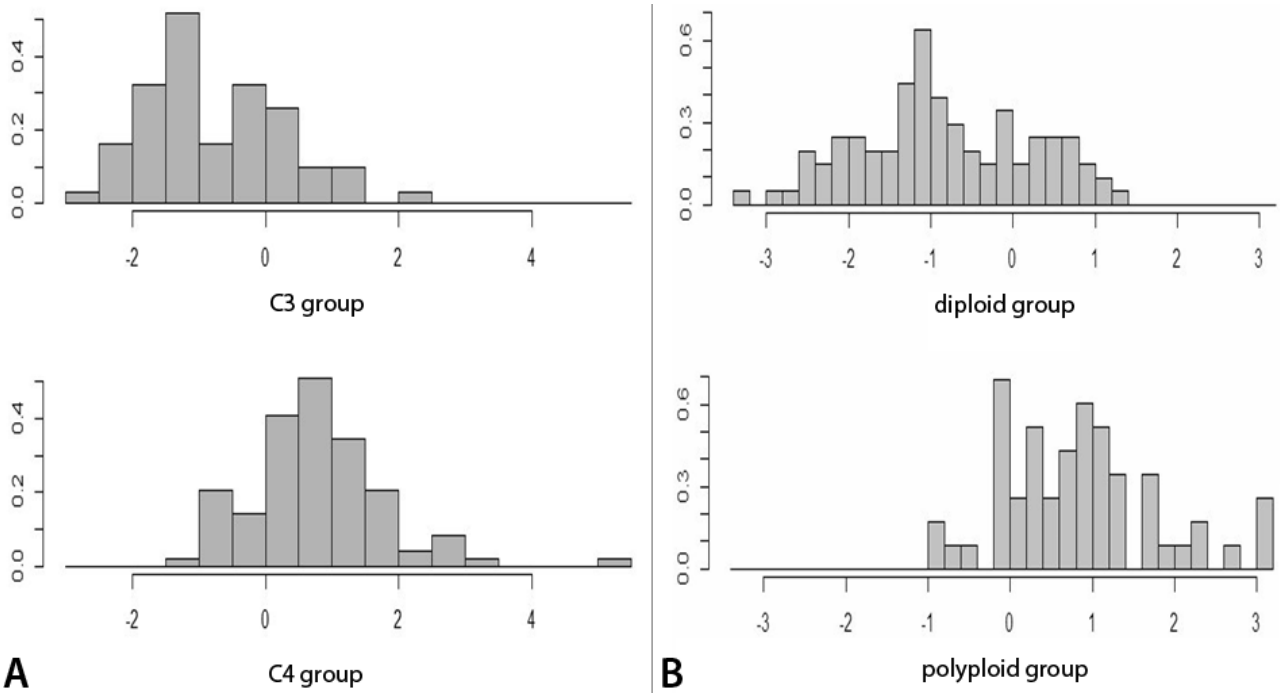

Figure 6. Results of the linear discriminant analysis (LDA). A. Comparison of the C3/C4 group. B. Comparison of the diploid/polyploid group.

Due to overlaps, the results of our LDA and NMDS suggest that neither the scores of C3/C4 Poaceae species nor those of diploid/polyploid species are clearly separable using these four

pollen grain measurements. However there are clear trends showing that the pollen grain sizes are larger in C4 as compared to C3 species and are larger in polyploids as compared to diploid species. Despite these overlaps, the average difference in the pollen grain sizes counted from a set of samples along a core may indicate shifts between C3 and C4 Poaceae composition of grasslands during the past. Unlike the clear and distinctly identified Poaceae species used in our dataset, in which the stabilization of the median value for a single species is achieved after about 40 measured pollen grains, a fossil assemblage will always be a mix of unidentified taxa having more variability.

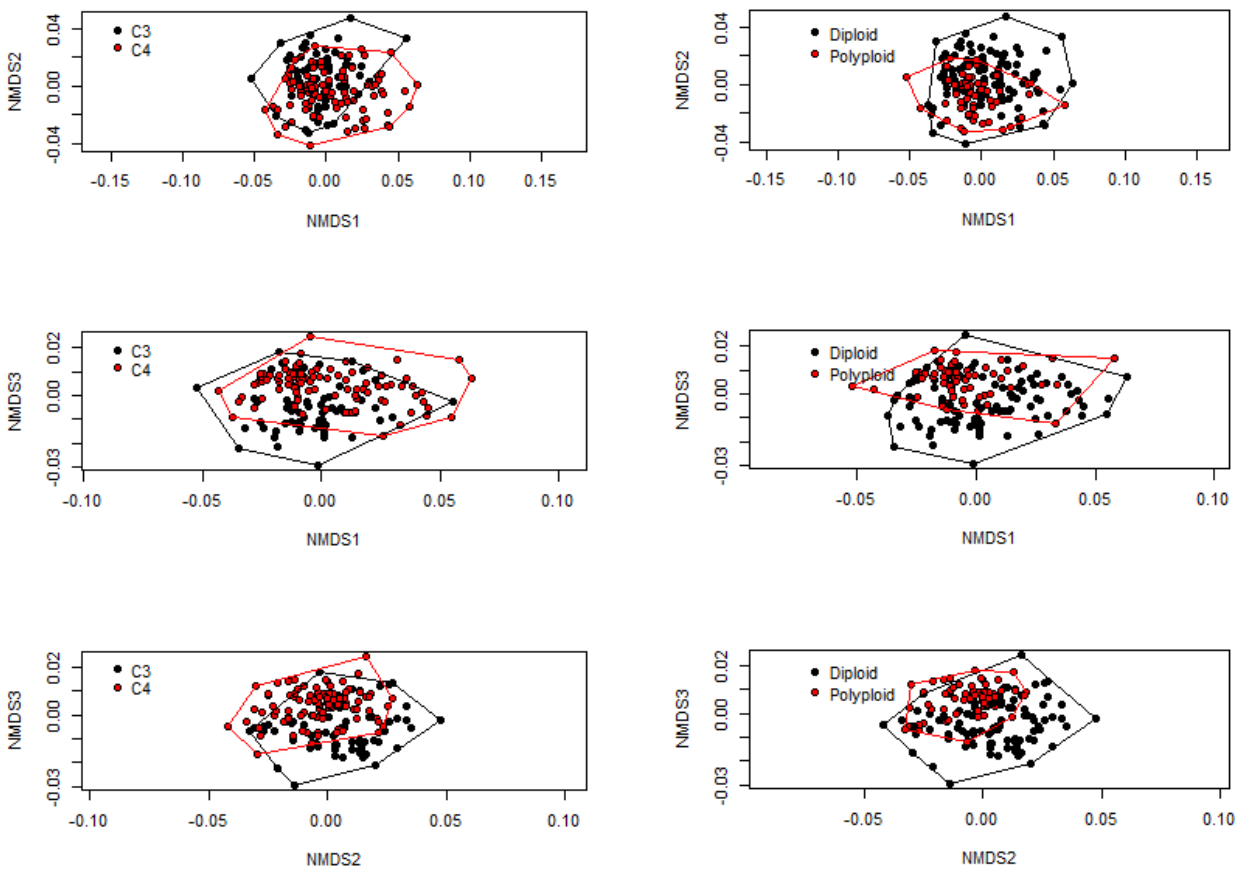

Figure 7. A. Non-metric multidimensional scaling (NMDS) results of the first two axes based on the separation of C3 and C4 species scores despite overlaps. C4 scores (red) and C3 scores (black). B. NMDS scores of polyploid species (red) and diploid species (black).

To cope with this problem when working with palaeo-samples, the count number of grains needed to reach a stable median value would need to be assessed heuristically each time from

sample to sample. Regarding the relative abundance of C3 and C4 Poaceae species, our developed method provides a useful tool allowing new insights into the dynamics of past grassland ecosystems. Changes may also be tested by the carbon isotopic analysis of Poaceae grains or deposited phytoliths.

Beside the photosynthetic pathway, several factors might contribute to the variation in grain size such as ploidy levels, specific taxa compositions, phylogenetic relatedness and environmental stress. In plants, increases in pollen grain and guard cell sizes are used as surrogate evidence for polyploidy (Tate & Simpson 2004). The percentage of polyploidy in our 98 C4 species (47%) is greater than in 62 C3 species (21%). Similarly, we observed that out of the 70 Pakistani species, polyploidy is higher in C4 species (65.4%) than in C3 species (34.6%). The larger pollen grain size coupled with higher percentage of polyploidy in C4 species, in comparison to the smaller grain size coupled with higher percentage of diploidy in C3 species, indicate a link between ploidy and grain size. It is difficult to assign a specific range size to a particular Poaceae taxon as the pollen grains of Poaceae show variation in size (Joly et al. 2007; Schüler & Behling 2011a). In our dataset, the general pattern of larger grain size of C4 vs C3 species is contradicted by some specific taxa compositions worth mentioning, of which are the four Ecuadorian C3 *Chusquea* species (Bambusoideae), i.e. *C. dombeyena*, *C. falcata*, *C. leonardium* and *C. neurophylla*, which had much larger grains than many of the C4 species. Some studies have found that in arid climates under higher desiccation stress, pollen grains become larger and more spherical (Ejsmond et al. 2011). Consequently, phenotypic plasticity with in Poaceae may pose a problem when applied to fossil samples, e.g. some C3 species of Pooideae are found in very dry habitats indicating the correlation of grain size variation more to their characteristic ecological envelope than to the photosynthetic pathway of species in the community. In such cases, the composition and dynamics of existing grasslands based on the grain size analysis of fresh Poaceae species can be studied but interpretation of the palaeo-samples for detecting the composition of past grasslands becomes complicated. Hence, the interpretation of signals from fossil samples for detecting the composition of past grasslands shall be managed with caution. It also cannot be ruled out that pollen size is simply a product of phylogenetic relatedness (Little et al. 2010). Members of the monophyletic PACMAD clade (Panicoideae, Arundinoideae, Chloridoideae, Micrairoideae, Aristidoideae and Danthonioideae; Duvall et al. 2007; Strömberg & McInerney 2011), where C4 photosynthesis evolved 22–24 times from their C3 ancestors,

might have larger pollen grains than pooids, can potentially provide taxonomic information about past grasslands, i.e., which clades were present, but not about the photosynthetic pathway as all PACMADs are not C4. However, phytolith assemblages can indicate the proportion of C3 and C4 grasses in the flora, if supported by evidence of isotopic studies (Wang et al. 1994).

C4 grasses are mostly dominant in regions with warm season precipitation, while C3 grasses prefer cool season precipitation (Epstein et al. 1997; Sage 2004; Urban et al 2010). C4 Poaceae species with their specialized Kranz anatomy perform faster photosynthesis under high temperature, sufficient light preferring low elevated arid plain habitats. In this regard, our findings fit well with C4 grasses having larger grain size as some C4 species are associated with the drier climates of the low elevated Peshawar, Charsadda, Mardan areas at 275–300 m a. s. l. in northern Pakistan, while some C3 species having smaller grains are associated to the moist climate of the highly elevated Swat region (2200–2600 m) in northern Pakistan.

Conclusion

We demonstrate that in our specific dataset of 160 Poaceae species, C3 and C4 (diploid and polyploid species) are not clearly separable based on the measurement results of their pollen grain length, pollen grain width, pore diameter and annulus width. However, the measurement results and statistical analysis performed on these specific Poaceae species reveal trends showing that C3 polyploids have larger grain sizes than C3 diploid species, and C4 polyploids have larger grain size than C4 diploid species. Our study, even though it is based on a limited dataset (1.45% of all Poaceae species), shows a pattern in the trend of pollen grain size variation between modern C3 and C4 Poaceae species. C4 species have a larger grain size than C3 species in our specific dataset. Grasslands dominated by C3 and C4 Poaceae species from different regions and habitats can be separated by studying the pattern of trends in their pollen grain sizes. In our dataset, polyploidy is more common in C4 than in C3 species. This method can be applied on Poaceae pollen grains deposited in environmental archives to reconstruct past climate and assess the dynamics of past grassland ecosystems. This approach will not only help the ongoing palaeo-ecological studies in unravelling aspects such as changes in vegetation composition and shifts in biomes of past grasslands ecosystems but also provide useful insights for future predictions. The average difference in the pollen grain sizes counted from samples along a core in future studies may well indicate shifts between C3 and C4 Poaceae composition of grasslands during the past.

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Table I. Measurements of the four pollen grain parameters of the 160 species dataset showing their anatomy and ploidy.

S.NO	Species	Anatomy	PGL	PGW	PD	AW	Ploidy	Country
1	*Avena fatua* L.	C3	26.8	22.2	2.1	2.3	2	Pakistan
2	*Bromus catharticus* Vahl	C3	28.1	24.2	2.8	2.6	2	Pakistan
3	*Bromus pectinatus*Thunb	C3	30.2	25.7	2.7	2.1	2	Pakistan
4	*Bromus unioloides* Kunth	C3	28.7	27.4	2.8	2.9	2	Pakistan
5	*Dactylis glomerata* L.	C3	25.3	22.1	3.1	2.2	2	Pakistan
6	*Koeleria macrantha* (Ledeb.) Schult.	C3	27.1	21.2	1.4	2.2	2	Pakistan
7	*Phalaris paradoxa* L.	C3	27.2	21.4	2.4	2.6	2	Pakistan
8	*Phleum paniculatum* Huds.	C3	26.3	22.4	1.9	2.1	2	Pakistan
9	*Piptatherum munroi* (Stapf) Mez	C3	28.4	25.3	2.2	2.0	2	Pakistan
10	*Poa annua* L.	C3	25.2	20.2	1.8	2.2	2	Pakistan
11	*Polypogon fugax* Nees ex Sted.	C3	27.8	22.0	2.2	2.3	2	Pakistan
12	*Polypogon monspeliensis (*L.) Desf.	C3	30.7	27.7	2.6	2.8	2	Pakistan
13	*Stipa brandisii* Mez	C3	25.7	24.9	2.8	2.6	2	Pakistan
14	*Stipa capensis* Moraldo	C3	27.4	25.2	2.8	1.6	2	Pakistan
15	*Agropyron pilosula* Schur	C3	26.2	21.2	2.2	2.1	2	Pakistan
16	*Agrostis gigantean* Roth	C3	20.9	26.7	2.5	2.4	2	Pakistan
17	*Ampelodesmos mauritanicus* (Poir.) T. Durand & Schinz	C3	21.3	20.6	2.2	2.6	2	Venezuela
18	*Stipa carpensis* L.	C3	35.3	35.1	2.7	2.0	2	Venezuela
19	*Isachne species*	C3	26.1	24.9	1.9	1.6	2	Ecuador
20	*Holcus lanatus* L.	C3	23.7	23.7	2	1.7	2	Ecuador
22	*Chusquea dombeyana* Kunth	C3	33.2	36	3.3	3	2	Ecuador
23	*Chusquea falcata* L.G.Clark	C3	35.5	33.5	3.8	2.9	2	Ecuador
24	*Chusquea leonardium* L.G.Clark .	C3	35.2	33	3.9	2.8	2	Ecuador
25	*Chusquea neurophylla* L.G.Clark	C3	35.5	38.7	3.1	2.3	2	Ecuador
26	*Neurolepis aristata (Munro) Hitchc*	C3	35.7	33.6	4.1	3.2	2	Ecuador
27	*Neurolepis elata* (Kunth) Pilg.	C3	22.8	28.8	2.7	2.2	2	Ecuador
28	*Chusquea species*	C3	27.2	25.9	1.7	1.9	2	Ecuador
29	*Bromus squarrosus* L.	C3	30.2	29.6	2.1	2.1	2	Germany
30	*Bromus japonicas* Thunb.	C3	27.3	26.2	1.9	2.1	2	Germany
31	*Bromus rubens* L.	C3	28.4	25.3	2.2	1.8	2	Germany
32	*Bromus carinatus* Hook. & Arn.	C3	27.9	23.4	1.8	2.3	2	Germany
33	*Bromus willdenowii* Kunth	C3	28.1	25.1	2	1.9	2	Germany
34	*Bromus macrostachys* Desf.	C3	22.8	21.2	1.9	2	2	Germany
35	*Hordeum spontaneum* K. Koch	C3	25.4	22.7	2.4	2.1	2	Germany
36	*Hordeum distichon* L.	C3	22.5	21.7	1.8	1.9	2	Germany
37	*Hordeum caespitosum* Scribn.	C3	21.8	29.6	1.7	1.9	2	Germany
38	*Hordeum compressum* Griseb.	C3	22.6	20.2	1.8	1.9	2	Germany
39	*Hordeum glaucum* (Steud.) Tzvelev	C3	30.8	28.7	1.8	2.1	2	Germany
40	*Poa supine* Schrad.	C3	22.6	20.8	1.6	1.8	2	Germany
41	*Poa trivialis* L.	C3	19.3	18.8	1.7	2.1	2	Germany
42	*Poa pratensis* L.	C3	21.2	20.4	1.8	1.9	2	Germany
43	*Poa angustifolia* L.	C3	20.8	20.1	1.7	1.7	2	Germany
44	*Poa annua* L.	C3	25.1	22.3	1.8	1.9	2	Germany
45	*Poa infirma* Kunth	C3	23.5	20.6	1.9	2.1	2	Germany
46	*Poa chaixii* Vill.	C3	24.1	22.3	1.8	2.2	2	Germany
47	*Poa compressa* L.	C3	24.6	21.9	2.1	2.2	2	Germany
48	*Poa palustris* L.	C3	26.2	24.1	1.8	2.1	2	Germany

49	*Poa bulbosa* L.	C3	25.1	23.3	1.7	1.9	2	Germany
50	*Hordeum murinum* L.	C3	37.9	33.4	3.2	2.7	3	Pakistan
51	*Leersia oryzoides* Hedw. ex Batsch	C3	35.5	28.5	2.4	2.2	3	Pakistan
52	*Phalaris minor* Retz.	C3	39.2	32.5	3.3	3.8	3	Pakistan
53	*Aulonemia patula* (Pilg.) McClure	C3	48.1	38.5	3.8	3.2	3	Ecuador
54	*Bromus errectus* "Hudson Smith" ex Moris	C3	31.7	30.5	2.1	2.3	3	Germany
55	*Bromus rigidus* Roth	C3	31.3	28.6	1.7	1.9	3	Germany
56	*Bromus diandrus* Roth	C3	32.6	29.8	2.1	2.2	3	Germany
57	*Bromus arvensis* Lam.	C3	33.4	30.6	1.8	2.2	3	Germany
58	*Hordeum marinum* Huds.	C3	32.2	30.5	1.7	2.1	3	Germany
59	*Hordeum vulgare* L.	C3	30.7	29.3	2.4	2.2	3	Germany
60	*Hordeum murinum* L.	C3	34.2	29.3	1.9	2.1	3	Germany
61	*Hordeum bulbosum* L.	C3	32.4	31.1	2.1	2.2	3	Germany
62	*Hordeum jubatum* L.	C3	31.7	30.6	1.8	1.9	3	Germany
63	*Acrachne racemosa* Wight & Arn. ex Chiov.	C4	33.7	31.4	1.9	2.5	2	Pakistan
64	*Apluda mutica* L.	C4	34.8	32.2	2.7	2.7	2	Pakistan
65	*Chrysopogon gryllus* (L.) Trin.	C4	32.7	29.9	2.4	2.4	2	Pakistan
66	*Dactyloctenium aegyptium* (L.) Willd.	C4	31.7	27.4	2.0	2.7	2	Pakistan
67	*Helectrotrichon virescens* Nees ex Steud.	C4	37.9	35.4	3.5	2.7	2	Pakistan
68	*Imperata cylindrica* (L.) P. Beauv.	C4	32.5	28.9	2.8	2.2	2	Pakistan
69	*Microstegium nudum* (Trin.) A.Camus	C4	38.1	36	2.9	2.4	2	Pakistan
70	*Setaria viridis* (L.) P. Beauv.	C4	37.2	31.6	3.2	3.1	2	Pakistan
71	*Themeda anathera* (Nees ex Steud.) Hack.	C4	34.6	32.0	3.3	3.1	2	Pakistan
72	*Brachiaria reptans* (L.) C.A. Gardner & C.E. Hubb.	C4	36.8	34.9	2.9	2.7	2	Pakistan
73	*Chrysopogon aucheri* (Boiss.) Stapf	C4	29.8	28.5	2.9	2.6	2	Pakistan
74	*Desmostachya bipinnata* (L.) Stapf	C4	35.4	33.6	2.4	2.9	2	Pakistan
75	*Imperata Cylindrica* (L.) P. Beauv	C4	31.9	29.1	2.8	2.1	2	Pakistan
76	*Panicum antidotale* Freckmann & Lelong	C4	39.5	38.7	2.9	2.7	2	Pakistan
77	*Paspalum paspalodes* (Michx.) Scribn.	C4	36.5	35.8	3.2	3.1	2	Pakistan
78	*Pennisetum flaccidum* Griseb. ex Roshev.	C4	38.9	36.8	3.2	3.1	2	Pakistan
79	*Pennisetum glaucum* (L.) R. Br.	C4	36.5	35.5	2.9	2.8	2	Pakistan
80	*Saccharum griffithii* Munro ex Aitch	C4	33.3	32.5	2.8	2.6	2	Pakistan
81	*Saccharum rufipilum*	C4	37.2	32.1	2.8	2.6	2	Pakistan
82	*Stipa capensis* Moraldo	C4	36.9	35.7	2.8	1.5	2	Pakistan
83	*Aristida adscensionis* L.	C4	35.6	33.2	3	1.9	2	Tanzania
84	*Bothriochloa insculpta* (Hochst. Ex A.Rich.)	C4	36.9	33.5	2	1.5	2	Tanzania
85	*Brachiaria brizantha* (Hochst. ex A. Rich.) Stapf	C4	34.7	32.7	2.7	1.9	2	Tanzania
86	*Chloris roxburghiana* Schult.	C4	31.4	33.7	2.5	2	2	Tanzania
87	*Cymbopogon caesius* (Nees ex Hook. & Arn.) Stapf	C4	27.1	28.6	2.1	2.2	2	Tanzania
88	*Dactyloctenium aegyptium* (L.) Willd.	C4	32.9	25.7	2.1	1.6	2	Tanzania
89	*Digitaria dragonalis* L.	C4	32.1	29.8	2.2	1.6	2	Tanzania
90	*Digitaria abyssinica* (Hochst. ex A. Rich.) Stapf	C4	35.6	29.5	2	1.5	2	Tanzania
91	*Diheteropogon Amplectens* (Nees) Clayton	C4	37.5	33.5	3	1.9	2	Tanzania
92	*Elionurus muticus* (Spreng.) Kuntze	C4	29.9	25	2.7	2.1	2	Tanzania
93	*Enneapogon cenchroides (Licht. ex Roem. & Schult.)*	C4	29.2	25.1	2.2	2.1	2	Tanzania
94	*Enteropogon macrostachyus* K. Schum. ex Engl.	C4	34.4	30.9	2.8	1.8	2	Tanzania
95	*Eragrostis schweinfurthii* Chiov.	C4	31.7	30.8	2	1.7	2	Tanzania
96	*Eragrostis cilianensis* (All.) Link ex Vignolo	C4	26.2	24.3	2.4	1.6	2	Tanzania
97	*Eragrostis kinensis* Wolf	C4	36.3	24.5	1.7	1.6	2	Tanzania
98	*Eragrostis superba* Peyr	C4	33.2	32.3	3.3	2.7	2	Tanzania

99	*Eustachys paspaloides* (Vahl) Lanza & Mattei	C4	28.8	30.5	3.3	2.7	2	Tanzania
100	*Leptothrium senegalense* (Kunth) Clayton	C4	31.2	27.6	3.1	2.1	2	Tanzania
101	*Oropetium minimum* (Hochst.) Pilg.	C4	29.2	26.2	1.9	1.8	2	Tanzania
102	*Panicum coloratum* L.	C4	28.8	27.9	1.5	1.3	2	Tanzania
103	*Sehima nervosum* (Rottler ex Roem. & Schult.) Stap	C4	40.2	39.3	2.5	1.9	2	Tanzania
104	*sporobolus junceus* (P. Beauv.) Kunth	C4	28.7	24.4	2.1	1.9	2	Tanzania
105	*Sporobolus pellucidus* Hochst.	C4	27.4	24.4	2.1	1.9	2	Tanzania
106	*Sporobolus tenuissimus* (Mart. Ex Schrank) Kuntze	C4	28.9	24.7	2.6	1.9	2	Tanzania
107	*Tetrapogon cenchroides* (A. Rich.) Clayton	C4	37.7	26.3	2.3	1.5	2	Tanzania
108	*Tetrapogon tenellus* (J. Koenig ex Roxb.) Chiov.	C4	36.4	31.5	1.7	2.1	2	Tanzania
109	*Tragus berteronianus* Schult.	C4	27.9	37.4	3.1	2.6	2	Tanzania
110	*Trachypogon spicatus* (L. f.) Kuntze	C4	35.7	35	2.0	2.7	2	Venezuela
111	*Paspalum urvillei* Steud.	C4	28.6	27.7	1.9	1.8	2	Brazil
112	*Sporobolus indicus* (L.) R. Br.	C4	24.6	23.4	2.3	2	2	Brazil
113	*Andropogon species*	C4	35.2	32.5	2.1	1.9	2	Ecuador
114	*Melinis minutiflora* P. Beauv.	C4	32.8	32.2	2.3	1.9	2	Ecuador
115	*Rhypidocladum species*	C4	36.5	32.3	3.1	3.1	2	Ecuador
116	*Arthraxon prinoides* P. Beauv.	C4	44.9	41.6	2.9	3.0	3	Pakistan
117	*Cenchrus ciliaris* L.	C4	41.1	35.5	3.1	2.3	3	Tanzania
118	*Cymbopogon jawarancosa* Spreng.	C4	39.8	34.4	2.7	2.2	3	Pakistan
119	*Cynodon dactylon* (L.) Pers.	C4	37.7	33.5	2.3	2.3	3	Pakistan
120	*Dichanthium annulatum* (Forssk.) Stapf	C4	33.8	31.3	2.7	2.4	3	Pakistan
121	*Digitaria ciliaris* (Retz.) Koeler	C4	42.3	35.8	3.2	2.5	3	Pakistan
122	*Echinochloa crus gallii* (L.) P. Beauv.	C4	39.7	33.9	3.1	2.7	3	Pakistan
123	*Eleusine indica* (L.) Gaertn	C4	33.3	33.3	3.5	3.1	3	Pakistan
124	*Eragrostis cilianensis* (All.) Link ex Vignolo	C4	29.8	27.7	2.7	2.8	3	Pakistan
125	*Heteropogon contortus* (L.) P.Beauv. ex Roem. & Schult.	C4	39.1	32.2	3.1	2.7	3	Pakistan
126	*Paspalidium flavidum* (Retz.) A. Camus	C4	44.2	37.2	3.2	2.8	3	Pakistan
127	*Pennisetum orientale* Rich.	C4	36.8	35.4	3.4	2.9	3	Pakistan
128	*Pennisetum typhoideum* Rich.	C4	41.1	37.5	3.5	2.9	3	Pakistan
129	*Saccharum rufipilum* Steud.	C4	37.4	31.8	3.1	2.7	3	Pakistan
130	*Cenchrus pennisetiformis* Hochst. & Steud	C4	37.5	36.4	3.2	2.8	3	Pakistan
131	*Bothriochloa ischaemum* (L.) Keng	C4	38.7	34.1	2.9	2.6	3	Pakistan
132	*Brachiaria deflexa* (Schumach.) C.E. Hubb.ex Robyns	C4	37.5	35.6	3.1	2.8	3	Pakistan
133	*Brachiaria eruciformis* (Sm.) Griseb.	C4	34.7	33.6	2.9	2.7	3	Pakistan
134	*Brachiaria ramose* Stapf	C4	33.6	32.9	2.8	2.6	3	Pakistan
135	*Chrysopogon gryllus* (L.) Trin.	C4	32.4	28.8	2.4	2.4	3	Pakistan
136	*Isachne himalaica* Hook. f.	C4	35.2	33.8	2.9	2.6	3	Pakistan
137	*Panicum maximum* L.	C4	36.8	35.4	3.1	2.9	3	Pakistan
138	*Panicum repens* L.	C4	37.5	35.3	2.8	2.5	3	Pakistan
139	*Panicum turgidum* Nees	C4	33.1	31.5	2.6	2.5	3	Pakistan
140	*Paspalum dilatatum* Poir.	C4	35.6	34.6	3.1	3	3	Pakistan
141	*Pennisetum flavidum* Griseb. ex Roshev.	C4	47.8	42.9	3.4	3	3	Pakistan
142	*Saccharum spontaneum* L.	C4	37.6	31.4	3.4	2.7	3	Pakistan
143	*Setaria intermedia* Roem. & Schult.	C4	41.5	40.3	3.1	3.1	3	Pakistan
144	*Setaria verticillata* (L.) P. Beauv.	C4	42.5	40.6	3.2	2.9	3	Pakistan
145	*Setaria pumila* (Poir.) Roem. & Schult.	C4	48.3	41.9	3.2	2.8	3	Pakistan
146	*Sorghum bicolor* (L.) Moench	C4	39.8	37.6	3.1	2.9	3	Pakistan
147	*Sorghum halepense* (L.) Pers.	C4	39.1	34.5	3	2.5	3	Pakistan
148	*Urochloa panicoides* P. Beauv.	C4	40.5	34.7	2.7	3.3	3	Pakistan

149	*Brachiaria deflexa* (Schumach.) C.E. Hubb. ex Robyns	C4	31.2	32.8	3.2	2.6	3	Tanzania
150	*Digitaria milanjiana* Henrard	C4	42.5	38.7	4.2	3.1	3	Tanzania
151	*Heteropogon contortus* (L.) P.Beauv. Ex Roem. & Schult	C4	50.9	44.1	3.4	1.7	3	Tanzania
152	*Panicum maximum* Jacq.	C4	38.6	26.7	2.4	1.8	3	Tanzania
153	*Paspalum scorbiculatum* L.	C4	39.2	34.8	2.3	1.8	3	Tanzania
154	*Rhynchelytrum repens* Nees	C4	42.2	36.4	2.8	2.3	3	Tanzania
155	*Themeda triandra* Forssk.	C4	42.5	40.6	3.5	2.1	3	Tanzania
156	*Paspalum notatum* Alain ex Flüggé	C4	34.1	31.1	2.3	2.1	3	Brazil
157	*Axonopus compressus* (Sw.) P. Beauv	C4	47.8	42.7	2.8	2.1	3	Ecuador
158	*Eleusine species*	C4	38.1	35.4	2.1	1.9	3	Ecuador
159	*Panicum species*	C4	39.5	38.4	3.6	2.5	3	Ecuador
160	*Paspalum paniculatum* L.	C4	33.3	32.3	2.6	1.8	3	Ecuador

CHAPTER 3

Vegetation and pollen along a 200 km transect in Khyber Pakhtunkhwa Province, north western Pakistan

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Abstract

This paper presents the first study on the relationship between vegetation and modern pollen rain along a 200 km elevational gradient (275-2600 m a.s.l.), in the Khyber Pakhtunkhwa Province of north western Pakistan. A vegetation survey of 24 plots (6 plots of 10x10 m from four elevational zones) was carried out following the Braun-Blanquet´s method. Percent cover of all taxa was documented and species were assigned to their respective families. Twenty four surface samples (each taken from the same plots from which the vegetation data recorded), were processed according to standard methods for retrieving pollen grains. Based on a count of 300 pollen grains per sample, the percent abundance of taxa in the pollen assemblages was compared to the corresponding percentage of cover abundance in the vegetation, at family level. Results of the constrained incremental sum of squares (CONISS) derived from the pollen data reveal that the natural vegetation zonation is well reflected in pollen assemblages despite alteration of the vegetation by human activities. The identification of key taxa for the different vegetation zones improves our confidence to draw vegetation boundaries and distinguish the elevational zones along the gradient. The Spearman's rank correlation coefficient at $P < 0.05$ indicates a significant correlation between vegetation cover and pollen rain in Poaceae, Asteraceae, Cyperaceae, Verbenaceae, Acanthaceae and Euphorbiaceae, whereas weak correlations are observed in Boraginaceae, Saxifragaceae, Apiaceae, Balsaminaceae and Rubiaceae. Results based on the comparison of vegetation and the pollen spectra at the family level, and their transfer factors (TF) reveal that large-sized Poaceae/cereal pollen grains > 45 µm, Myrtaceae, Polygonaceae, Brassicaceae, Asteraceae, Chenopodiaceae/Amaranthaceae and Cannabaceae reflect the proximity of cultivated land and human habitation in the lower three vegetation zones of Peshawar valley, lower montane Malakand hills and Swat valley. Contrarily the abundance of the Pinaceae, Pteridaceae, Dryopteridaceae and certain spores reflect natural vegetation in the upper montane Malam Jabba zone.

Keywords: north western Pakistan, Khyber Pakhtunkhwa Province, elevational gradient, lowlands to montane, vegetation – pollen rain relationship, secondary vegetation

Introduction

Studies of modern pollen rain/vegetation relationships provide important background information for the interpretation of fossil pollen records to reconstruct past vegetation changes (Niemann et al. 2010). They also provide the necessary basis for more Quantitative views on past environmental conditions (Giesecke et al. 2010; Urrego et al. 2011; Jantz et al. 2014). Modern pollen analogues are essential tools for palaeoecological reconstructions (Weng et al. 2004; Huntley et al. 2011; Schüler et al. 2014). In Quarter - nary palynology this type of study is also known as modern pollen rain studies, or modern pollen deposition pattern, or Response Transfer Function (Quamar & Beraa 2014). These studies serve as modern analogues for the accurate explanation of pollen sequences generated from the sedimentary beds in a region, and in terms of the past vegetation and climate in chronological order during the Quaternary Period, especially the Holocene and/or late Pleistocene epochs (Chauhan & Quamar 2012; Quamar & Beraa 2014). Modern pollen deposition studies have shown that pollen assemblages reflect general patterns in the vegetation (Weng et al. 2004; Fontana 2005; Niemann et al. 2010; Urrego et al. 2011). Nonetheless, pollen representation is biased by several factors such as differences in pollen production, dispersal and preservation of taxa (Prentice 1988). Hence, pollen abundance cannot be directly translated into plant abundance in the vegetation when interpreting pollen assemblages from the past (Fontana 2005).

Studies of modern pollen rain/ vegetation relationships in south Asia are still scarce and are concentrated on tropical deciduous and evergreen forests in South India, Sri Lanka (Anupama et al. 2000; Barboni & Bonnefille 2001; Trivedi & Chauhan 2011), northeast India (Basumatary & Bera 2007), foothills of Himalaya (Gupta & Yadav 1992), Eastern Himalayan foothills in the Indo-Burma Range (Basumatary et al. 2014), Madhya Pradesh (Quamar & Chauhan 2007; Chauhan 2008) and tropical deciduous scrub vegetation in the north western desert in India (Singh et al. 1973). However the potential prospects for such studies in the plains and montane regions of Pakistan and Afghanistan have not received any attention.

The present study explores the modern pollen rain/ vegetation relationship of the central and northern districts of Khyber Pakhtunkhwa province in north western Pakistan. The relationship is studied along a 200 km elevational gradient in four distinct elevational zones extending successively upwards from the lowest elevated disturbed plains of

Charsadda (275 m a.s.l.) and reaching high above the Swat valley (950 m a.s.l.) to the upper montane Malam Jabba hills (2600 m a.s.l.). Vegetation boundaries are inherent features of landscapes that play more than one functional role in terrestrial ecosystem dynamics (Hansen & Di Castri 1992). The delineation of ecotones or boundary areas, either sharp or gradual, between adjacent vegetation communities depend on the spatial and temporal resolution of the data available, e.g., field data, aerial photographs and remotely sensed images (Fortin & Edwards 2001). Due to a lack of the availability of any such data for our study area, the division of the entire 200 km long gradient into four distinct elevational zones, despite the overlaps of habitats, is based on our observation of the area during fieldwork. Considering the drastic variation in the physiographic landscape along this 200 km elevational gradient, the aim of the study was to investigate the relationship between the present vegetation and the modern pollen rain from lowlands to the progressively upward highlands (275 to 2600 m a.s.l.). We addressed the following three questions: 1. Do plant diversity patterns of the existing vegetation along successive elevational zones from lowland to highland reflect the modern pollen-rain? Does the modern pollen rain represent each elevational zone in the form of vegetation boundaries and, if so, what are the key taxa which reflect the different elevational zones? 3. Do land use and, consequently, the secondary vegetation reflect the modern pollen rain?

Palynologists working in different ecosystems around the world are striving to answer the unaddressed bottleneck involved in establishing a statistically sound relationship between the biodiversity patterns and their pollen representation. Various approaches have been applied to explore the qualitative and quantitative relationships between vegetation and its pollen representation. Scatter plots showing the relationship between modern pollen and plant percentages/abundances have been widely applied (Fontana 2005). Schüler et al. (2014) applied non-metric multidimensional scaling (NMDS) to detect the adequate taxonomic level of pollen grain identification for reliable results on the corresponding afromontane vegetation (Kilimanjaro montane Tanzania, Africa) and concluded that it is possible to analyse the pollen rain at the plant family level, in order to derive the forest zonation of the surrounding vegetation. Plant family level is also sufficient for palaeo-environmental reconstruction across the range of several different tropical regions such as the Neotropics (Punyasena 2008). Jantz et al. (2014) found that taxonomic surrogacy at the family level is a good tool for comparing presence–absence

patterns of plant and pollen data in tropical regions with high tree diversity. The authors asserted that, on a family basis, pollen presence–absence data represent the corresponding tree vegetation data, but uncertainties increase with decreasing altitude which can partly be explained by wind patterns, local abundance of shrubs and herbs and differences in evenness.

In this study the vegetation survey along the elevational gradient was carried out in order to compare the percentage cover of taxa in the vegetation to those in the pollen assemblages at family level. Our aim was to look for the biodiversity patterns and to establish a relationship between vegetation and modern pollen rain. The degrees to which the different plant families representing the vegetation can be distinguished by their pollen assemblages were analysed and key taxa were recorded. The comparison between dominating plant families representing the vegetation cover and pollen spectra was carried out by frequency analysis. The representativeness of plant families in the modern pollen rain was quantified using a transfer factor (TF). This factor can be used to enable calibration of palaeo-pollen data in future studies, based on the relationship between the plant family abundance in the modern pollen rain and the surrounding vegetation (Schüler et al. 2014). These authors concluded that comparison of the transfer factors of the different forest zones also allows for further ecological conclusions concerning pollen production and dispersal. These factors can also be applied to fossil pollen data (Marchant & Taylor 2000) and compensate for differences in representativeness of pollen (Schüler et al. 2014). To determine the human impact and the composition of secondary vegetation, we analysed the plant families in the pollen assemblages from each elevational zone to know if the floristic diversity of cultivated or cultural plants is reflected in the data.

Study area

Khyber Pakhtunkhwa is one of the four provinces of Pakistan, located in the north of the country (31°49´N, 70°55´E to 35°50´N, 71°47´E) covering an area of 74,521 km² (Figure 1-3). Our study area comprises a 200 km-long elevational gradient starting from the lowest elevated sedimentary basin plains of Peshawar-Charsadda, Nowshera and Mardan areas (275–315 meters above sea level), entering the lower montane Malakand pass, Dargai, Batkhela and Thana areas (315–500 m a.s.l.), crossing the upper Swat valley (500-900 m a.s.l.) and reaching up to the highest elevated upper montane Malam

Jabba Hills (1550–2600 m a.s.l.). The Swat River emerges from the highest elevated Hindukush montane in the north, flowing down the gradient to Swat valley, crossing Malakand zone, and entering the Kabul River located down in the plain zone of Charsadda in the south. The lowest elevated plain zone of Peshawar valley, up to the lower montane Malakand (275-500 m a.s.l.), shows strong edaphic and biotic disturbance. Lithologically, the area is composed of quartz to dolerite, schist and granite, sandstones, mudstones and conglomerates (Hussain et al. 1993). Geologically, it consists of sedimentary and metamorphic rocks of Ordovician and Devonian origin and the geomorphologic features include piedmont plains, rolling sand plains, loess plains, infilled basins, cover flood plain and local fans (Hussain et al. 1993). The lower montane Malakand hills are a chromite-rich mineralized area containing the biogeochemical distribution of enzyme-bound metals in the plants and soil (Kfayatullah et al. 2001). The Swat valley and adjoining areas of north western Pakistan consist of rock units of Precambrian-Cambrian basement representing the Kohistan Arc sequence, the Tethys oceanic lithosphere and the Indo-Pakistan plate sequence (Arif et al. 2011). The Kohistan Arc sequence consists essentially of late Jurassic-Cretaceous and Tertiary plutonic and metamorphic plutonic, volcanic and sedimentary rocks (Arif et al. 2011). The basement rocks in the Swat valley are overlaid unconformably by Phanerozoic metasedimentary rocks of the Alpurai group (DiPietro 1991; DiPietro & Lawrence 1991; DiPietro et al. 1993; Arif et al. 2011).

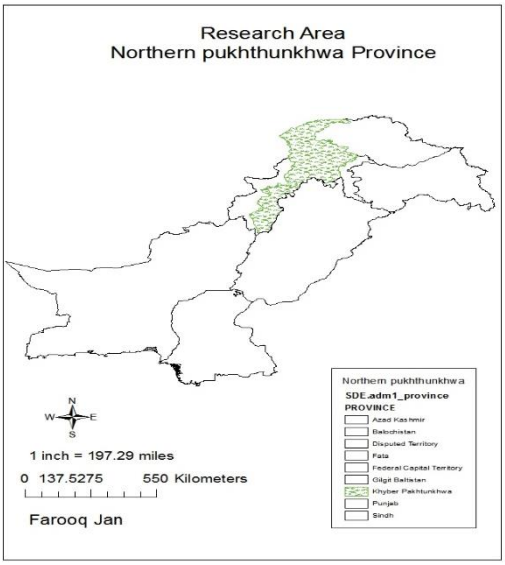

Figure 1. Map of Pakistan showing the north western Khyber Pakhtunkhwa province in green dotted.

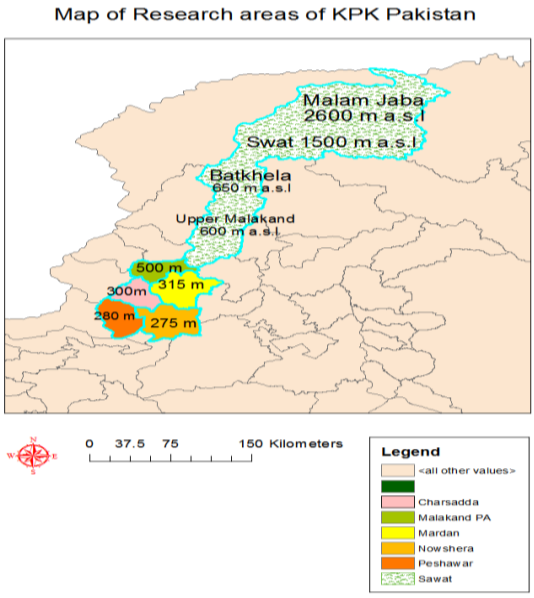

Figure 2. Shows the lower two elevational zones in colored segments and the upper Two zones as green dotted

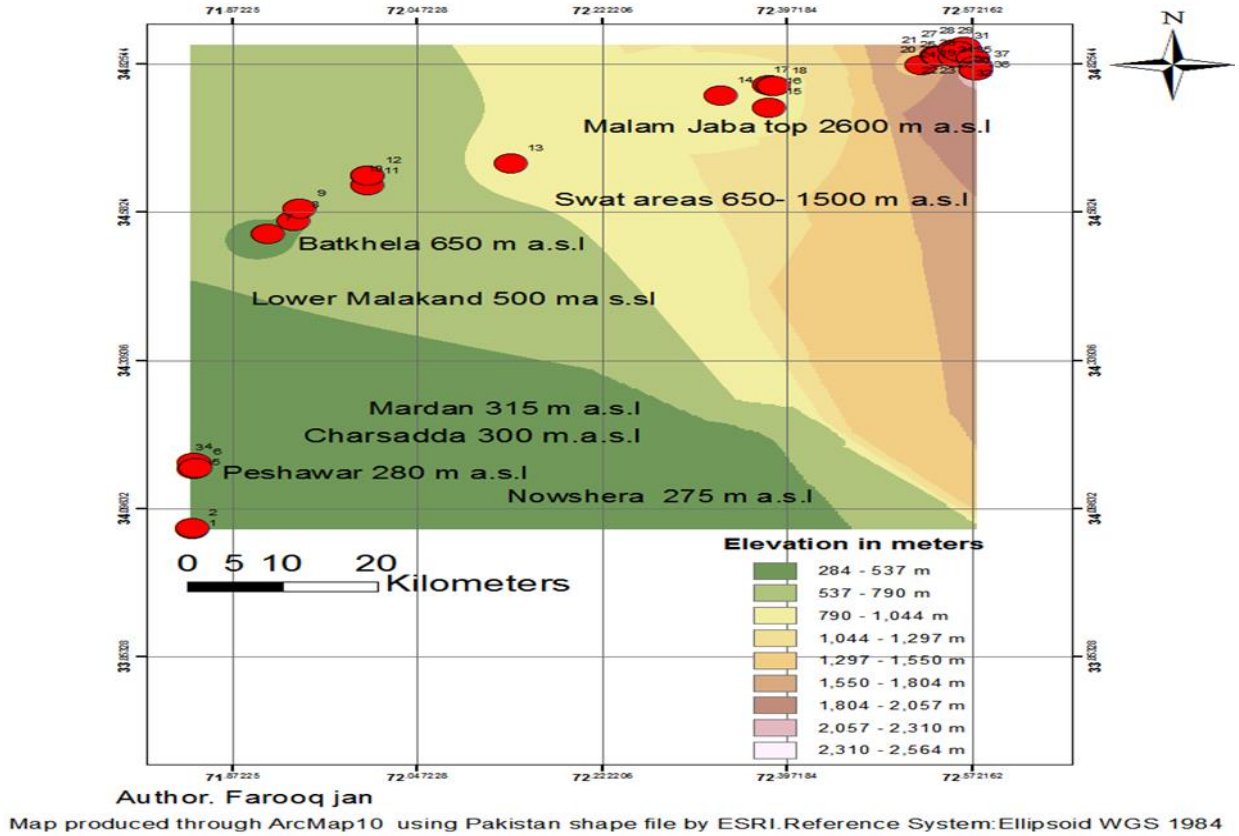

Figure 3. Digital elevation model of the entire 200 km long gradient from 275 to 2600 m a.s.l. Names of the areas and their respective elevations where plots were exactly established for vegetation analysis and collecting the surface samples are shown in red Dotted.

Climate

Pakistan lies in the northern Hemisphere just above the Tropic of Cancer in the western part of the monsoon climate zone, with subtropical climate experiencing extreme temperature variations (Khan et al. 2010). The overall climate of the research area is given in Table 1. The data are based on the 30 years average (1981-2010) of daily mean annual temperatures (MAT) in C°, average rainfall in mm (ARF) and relative humidity in percentage (RH), obtained from the federal meteorological department, Islamabad (Meteorological Department of Pakistan. ISO 9001: 2008; certified provider of aviation Meteorological service. http://www.pmd.gov.pk/).

Vegetation

The vegetation of this 200 km-long gradient presents an overall reflection of the changing topography of the landscape. We divided the entire gradient into four

Table 1

Meteorological data of the four elevational zones along the gradient in KP Province.

Plain belt	coordinates	Elevation	ARF(mm)	RH %	MAT(°C)
Nisatta	34°01'N 71°35′E	315m a.s.l	> 127	45-50	> 20
Charsadda	34°80'N 71°43′E	276 m a.s.l	> 127	45-50	> 20
Mardan	34°12'N 72°20'E	285 m a.s.l	> 127	45-50	> 20
LMM belt	coordinates	Elevation	ARF	RH	MAT
Malakand	34° 33'N71° 55'E	500 m a.s.l	> 889	55-60	> 20
Batkhela	34°10′N 71°53′E	650 m a.s.l	> 889	55-60	> 20
Thana	34° 38′N72° 40'E	700 m a.s.l	> 889	55-60	> 20
Swat belt	coordinates	Elevation	ARF	RH	MAT
Kabal hill	34°66'N 72°13'E	950m a.s.l	> 1016	60-65	> 15
Fiza gut hill	34°79'N 72°38'E	1150 m a.s.l	> 1016	60-65	> 15
UMM belt	coordinates	Elevation	ARF	RH	MAT
Malam Jabba	34°81'N 72°57'E	2600m a.s.l	> 1016	60-65	> 15

elevational zones to classify the vegetation. The drastic variation in the elevational gradient and changing floristic composition affect the overall physiognomy and layering of ecosystems across each elevational zone from lowland to highlands. The gradient starts at the lowest elevated sedimentary basin plain zone (275–315 m a.s.l.) passing through Peshawar valley, Charsadda and the Mardan-Swabi road. This zone represents grassy meadows, occasional dry deciduous forests in vanishing condition and agricultural lands showing biological disturbance and interference of human activities resulting in secondary vegetation (Beg 1978; Hussain et al. 1993). Different plant species frequently observed in this zone are *Cynodon dactylon, Eucalyptus camaldulensis, Cannabis sativa, Euphorbia prostrata, Populus ciliata, Acacia modesta, Morus alba* and *Salix* species. Six dominant herbal species namely *Parthenium hysterophorus*, *Cannabis sativa*, *Cynodon dactylon*, *Coronopus didymus*, *Cyperus rotundus* and *Euphorbia helioscopia* are very common in the plains of Peshawar valley and surrounding areas of Charsadda, Mardan and Swabi (Khan et al. 2014).

The second zone is the lower montane Malakand hills (500-900 m a.s.l), which starts from Dargai area in the foothills of Malakand pass and ends at the top of a fertile valley surrounded by hills including Batkhela and Thana areas. This zone is represented by subtropical forests including the planted fuel wood *Eucalyptus camaldulensis* and *Dodonaea viscosa* which merge with subtropical pine and temperate forests towards the Swat valley. The most important species of this zone are *Dodonaea viscosa, Pinus roxburghii, Eucalyptus camaldulensis, Cynodon dactylon, Cannabis sativa, Pinus walichiana, Justicia adhathoda* and *Berberis lyceum*. Overuse and unmanaged cutting of the forest resources has put the vegetation under severe pressure which badly affects

the wildlife and plants habitat (Barkatullah & Ibrar 2011). Biotic and abiotic disturbances have almost depleted the original plant cover (Hussain et al. 1989, 2005; Barkatullah & Ibrar 2011).

Above the lower montane Malakand Pass, the surrounding areas of the upper plain Swat valley (950-1450 m a.s.l.) represent the third elevational zone. This zone experiences overgrazing, deforestation, logging, and clearing of land for terrace cultivation, which are the major threats responsible for the overall degradation of forests (Hussain et al. 1997; Sher et al. 2010, 2011). The most important taxa of this zone are *Dodonaea viscosa, Pinus roxburghii, Dalbergia sissoo, Pinus walichiana, Polygonum plebjum, Otostegia limbata, Prunus sp., Plectranthus rugosus* and *Duchesnea indica etc*.

The highest elevated upper montane Malam Jabba Hills (1550 –2600 m a.s.l.) represent our fourth elevational zone. Malam Jabba hills are comprised of natural coniferous forests of *Pinus, Abies, Cedrus* and *Picea* species which are also under the heavy social and economic pressure of tree felling (Siddiqui et al. 1999). The most essential taxa of this zone are *Pinus roxburghii, Pinus walichiana, Quercus incana, Abies pindrow, Cedrus deodara, Picea smithiana* and different ferns. We also encountered occasional species of *Quercus semecarpifolia, Viburnum grandiflorum* and *Indigofera heterantha.* Besides deforestation and uncontrolled logging, Malam Jabba hills experience occasional smuggling of wood by wood thieves which are the major threats responsible for the overall degradation of forests in this zone (Iqbal & Hamayun 2005; Sher et al. 2010, 2011).

Material and methods

Vegetation survey

The vegetation survey was carried out in the central and north western districts of Khyber Pakhtunkhwa Province Pakistan (August-September 2012). Four sampling areas along the 200 km elevational gradient, representing the four distinct vegetation zones from plain areas up to the montane top, were selected. A total of 24 plots covering a wide variety of vegetation types were established, such that each of the four distinct elevational zones was represented by 6 plots (each 10x10 m). The exact location and altitude of each sampling site was recorded by GPS. The traditional phytosociological approach of preferential sampling (Braun-Blanquet 1964) was applied to track and

sample the full range of floristic variation in vegetation of the study area. Due to disturbance in the secondary vegetation of the lowest plain zone, plots were established in the relatively undisturbed grasslands at the junction of Nisatta, Nowshera and Mardan areas. Keeping in view the ecological representativeness, plots for the rest of the zones were established respectively at Malakand pass, Malakand hill base, Malakand hill start, Batkhela, Thana, Chakdara, Qambar, Kabal, Fizagut, Fizagut hill basin, mid Fizagut hill ,Manglawar Keshowra, Khona, Shaltalu-Asharrey, Speeney Oba, Qala Patey and Malama Jabba hill top . All species present in the plots were recorded and their cover abundance values were estimated based on Braun-Blanquet´s cover abundance scale (Braun-Blanquet 1964). The species richness index S (total number of taxa) and species constancy (SPC % - number of plots in which a given species occurs) was calculated as a measure of floristic diversity in each sampling plot.

Modern pollen rain sampling and laboratory methods

In total, 24 surface samples, a mixture of four individual subsamples (24 x 4 = 96), collected as the upper two centimetres (cm) of surface soil, preferably mosses, were taken within each 10x10 m plot. The samples were processed according to standard laboratory methods as described by Faegri & Iversen (1989). Sequential chemical treatments with 10% Hydrochloric acid, 10% Potassium hydroxide, 70% Hydrofluoric acid (left for 3 days due to the large silicate contents) and acetolysis were applied. Samples were sieved through a 150 µm mesh to remove plant fragments and coarse debris. The pollen residue obtained was filtered using a 10 µm sieve and kept in distilled water until mounted into glycerol jelly on slides for pollen analysis. For each sample we counted at least 300 pollen grains. The calculated pollen sum includes trees, shrubs and herbs. Fern spores were also counted and expressed as percentages of the pollen sum. Pollen and spore identification was based on large reference collections at the Department of Palynology and Climate Dynamics, Göttingen. This was supported by morphological descriptions provided in different atlases such as Asian environmental history (Fujiki et al. 2005), Pollen of wet evergreen forests of the Western Ghats India (Tissot et al. 1994), Pollen of Maharashtra State, India (Nayar 1990) and other relevant publications (Huang 1972; Seetharam 1985; Bonnefille 1999). The pollen percentage diagram was compiled with the program TILIA (TILIA 1.7.16, Grimm 2011) illustrating the most abundant and ecologically important taxa. Pollen taxa were

assigned to their respective vegetation groups such as herbs and crops, trees/shrubs, conifers and are depicted as pollen sums.

Representation of plant families in the modern pollen rain

Due to difficulties in identifying all pollen types to their respective genera, the pollen types were pooled on family level for data analysis. The percentages of plant families found in the vegetation cover were compared to their respective percentages in modern pollen rain for all four elevational zones. To enable calibration according to the relationship between the percentage of the families in surface samples and the surrounding vegetation, we generated a transfer factor (TF = % taxon in vegetation / % taxon in pollen rain (Schüler et al. 2014)).

Correlation of plant families

Using scatterplots, the abundance of the most common families in the vegetation from each elevational zone were plotted against their pollen proportion in the surface samples. Spearman's rank correlation coefficient was calculated for each data set to indicate the degree to which both variables are related. The percentages of the different plant families are expressed as proportions of the total cover of their representative species within the 10x10 m plots.

Results

Vegetation survey

The vegetation of the 200 km-long elevational gradient in north western Khyber Pakhtunkhwa Province of Pakistan is largely characterized by Poaceae, Pinaceae, Myrtaceae, Sapindaceae, Fabaceae, Euphorbiaceae, Cyperaceae and Cannabaceae species. Complete details of these families and their species along with their percentage cover abundance on Braun Blanquet scale and their constancy in each plot are given in Table 2. The average percentage of the dominant species along the gradient, in all four zones, which occur in more than 40% of the plots are *Cynodon dactylon* (68%), *Pinus roxburghii* (55%), *Poa annua* (50%), *Eucalyptus camaldulensis* (45%), *Cyperus* species (45%) and *Dodonaea viscosa* (41%).

Plain zone

The low elevated plain zone of Peshawar valley has disturbed vegetation including patches of secondary grasslands. The average representation of the dominant families found in all six plots of this zone are: Poaceae (10%) mostly as *Cynodon dactylon* and *Poa annua,* Myrtaceae (6%) mostly as *Eucalyptus camaldulensis,* Cannabaceae (5%) mostly as *Cannabis sativa*, Euphorbiaceae (4%) mostly as *Euphorbia prostrata*, Salicaceae (4%) mostly as *Populus ciliata,* Fabaceae (4%) mostly as *Acacia modesta* and Amaranthaceae (3%) mostly as *Achyranthes aspera*.

Lower montane Malakand zone

The average representation of the dominant families found in all the six plots of the second zone of the lower montane Malakand are: Sapindaceae (8%) mostly as *Dodonea viscosa,* Pinaceae (7%) mostly as *Pinus roxburghii* and *Pinus walichiana*, Myrtaceae (6%) mostly as *Eucalyptus camaldulensis*, Poaceae (5%) mostly as *Cynodon dactylon,* Cannabaceae (5%) mostly as *Cannabis sativa*, Lamiaceae (3%*)* mostly as *Mentha viridis,* Asclepediaceae (3%) mostly as *Calotropis procera,* and Acanthaceae (2%) solely as *Justicia adhatoda*.

Swat valley zone

The average representation of the dominant families found in all the six plots of the Swat valley zone are: Asteraceae (8%) mostly as *Parthenium hysterophorus,* Sapindaceae (6%) mostly *Dodonea viscosa*, Pinaceae (7%) mostly *Pinus roxburghii* and *Pinus walichiana*, Fabaceae (4%) mostly *Dalbergia sissoo*, Poaceae *(*4%) mostly *Cynodon dactylon,* Polygonaceae (2%) mostly *Polygonum plebjum,* Lamiaceae (2%) mostly *Otostegia limbata*, Myrtaceae (2%) mostly *Eucalyptus camaldulensis*, and Rosaceae (2%) mostly *Prunus* species.

Upper montane Malam Jabba zone

The average representation of the dominant families found in the six plots of the top most upper montane zone of Malam Jabba are: Pinaceae (20%) mostly *Pinus roxburghii*, *Abies pindrow*, *Cedrus deodara* and *Picea smithiana*, Asteraceae (7%) mostly Bidens, Conyza and Artemisia species, Fagaceae (6%) mostly *Quercus incana*,

Dryopteridaceae (4%), *Adiantum incisum* 3%, *Pteris* species (3%) and Cyperaceae species (3%).

Modern pollen rain

Pollen percentage data of the surface samples (taken from the same 24 plots from which the vegetation data recorded) reveal a total of 101 pollen and 6 spore types representing 64families. The pollen percentage diagram (Figure 4) of modern pollen rain summarizes the average percentage values of the most abundant pollen types, their sums as well as the ecologically distinctive taxa grouped into the four elevational zones.

Plain zone

Pollen assemblages from the plain zone are dominated by Poaceae pollen grains which are of two types: small Poaceae grains < 45 µm (15%) and large sized Poaceae grains > 45 µm (6%). Other herbal taxa present are: Amaranthaceae (8%), Polygonaceae (6%), Brassicaceae (5%), Rutaceae (5%), Euphorbiaceae (4%), Asteraceae (4%) and others. Tree taxa mainly represent Salicaceae (6%) and Myrtaceae (4%). Pollen percentages for the individual taxa vary considerably among the samples.

Lower montane Malakand zone

Sapindaceae and Pinaceae (11% each) and Asteraceae (7%) are the dominant taxa in the pollen spectra of this zone. The pollen representation of these taxa varies greatly between the sites. Pollen percentages of further taxa are Cannabaceae (6%), Solanaceae (5%), Fabaceae (5%), Rananculaceae (5%), Berberidaceae (4%), Asclepiadaceae (4%), Poaceae (4%), *Acacia* (3%), Verbenaceae (3%), Rosaceae (3%), Piceaceae (3%), Cyperaceae (2%) and others.

Swat valley zone

Oleaceae (7%) and Boraginaceae (6%) are the dominant taxa in the pollen spectra of this zone. The pollen representation of these taxa differs between the sites. Percentages of further families are Lamiaceae (5%), Oxalidaceae (5%), Plantaginaceae (5%), Caryophyllaceae (5%), Moraceae (5%), Rubiaceae (4%), Bignoniaceae (4%), Cucurbitaceae (3%), fern spores (3%) and others.

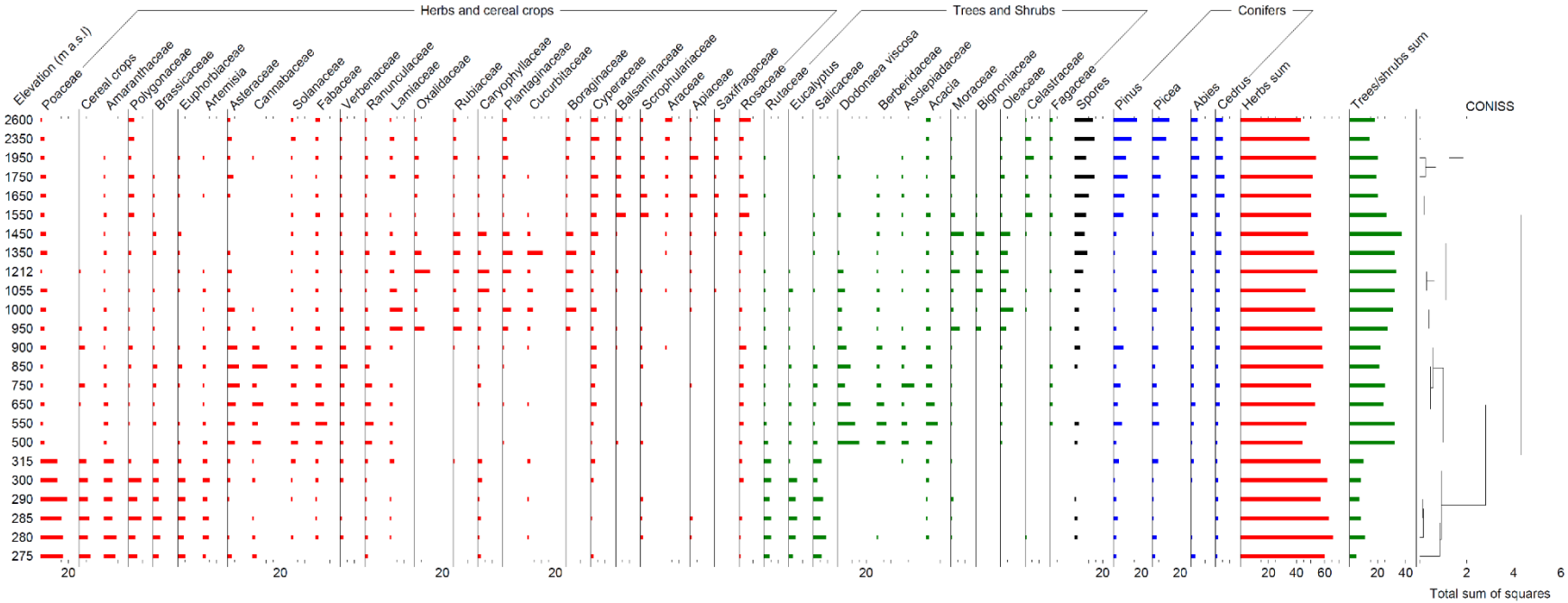

Figure 4. Pollen percentage diagram of modern pollen rain.The CONISS is showing zonation of the ecological groups and their sums along the 200 km gradient from 275 to 2600 m a.s.l in Khyber Pakhtunkhwa province of north western Pakistan.

Upper montane Malam Jabba zone

Conifers, along with ferns, are the dominant taxa in the pollen spectra of this zone. Percentages of the different families are Pinaceae (36%, including *Pinus* 20%, *Picea* 8%, *Abies* 6% and *Cedrus* 2%), fern spores (12%), Cyperaceae (5%), Rosaceae (5%), Balsaminaceae (5%), Celastraceae (3%), Polygonaceae (3%), Scrophulariaceae (3%), Apiaceae (3%), Saxifragaceae (2%), Araceae (2%) and others.

Representativeness

The comparison of modern pollen rain with the surrounding vegetation for each elevational zone reveals that different plant families reflect the plant diversity patterns along the 200 km elevational gradient as shown in Figure 5-8 and summarized as transfer factors (TF) in Table 3. In the plain zone, the percentage of Poaceae is almost double in the pollen record (21%) what it was in the vegetation (10%) resulting in a low TF (0.5). In contrast the pollen percentages of Moraceae (1%) and Rubiaceae (2%) are lower than in the present-day vegetation (3% and 5%, respectively) resulting in high TF values (3.6 and 3.3, respectively). In the lower montane Malakand zone, the pollen record shows percentages for Sapindaceae and Pinaceae (11% each), Asteraceae (7%) and Fabaceae (5%) whereas for Polygonaceae, Acanthaceae and Euphorbiaceae (1% each), the pollen percentages are clearly lower than the family abundances in the vegetation. In the upper zone of Swat valley the pollen percentages are high for Pinaceae (10%), Oleaceae (7%) and Boraginaceae (6%) but lower for Asteraceae (5%), Sapindaceae and Fabaceae (3% each), Solanaceae (2%), Berberidaceae and Brassicaceae (1% each) than their respective percentages in the vegetation. Similarly, the pollen percentages in the upper montane Malam Jabba zone are higher for Pinaceae (36%), Rosaceae, Cyperaceae and Balsaminaceae (5% each) but lower for Poaceae (3%), Fagaceae, Moraceae (2% each), Brassicaceae and Berberidaceae (1% each) than their respective percentages in the vegetation.

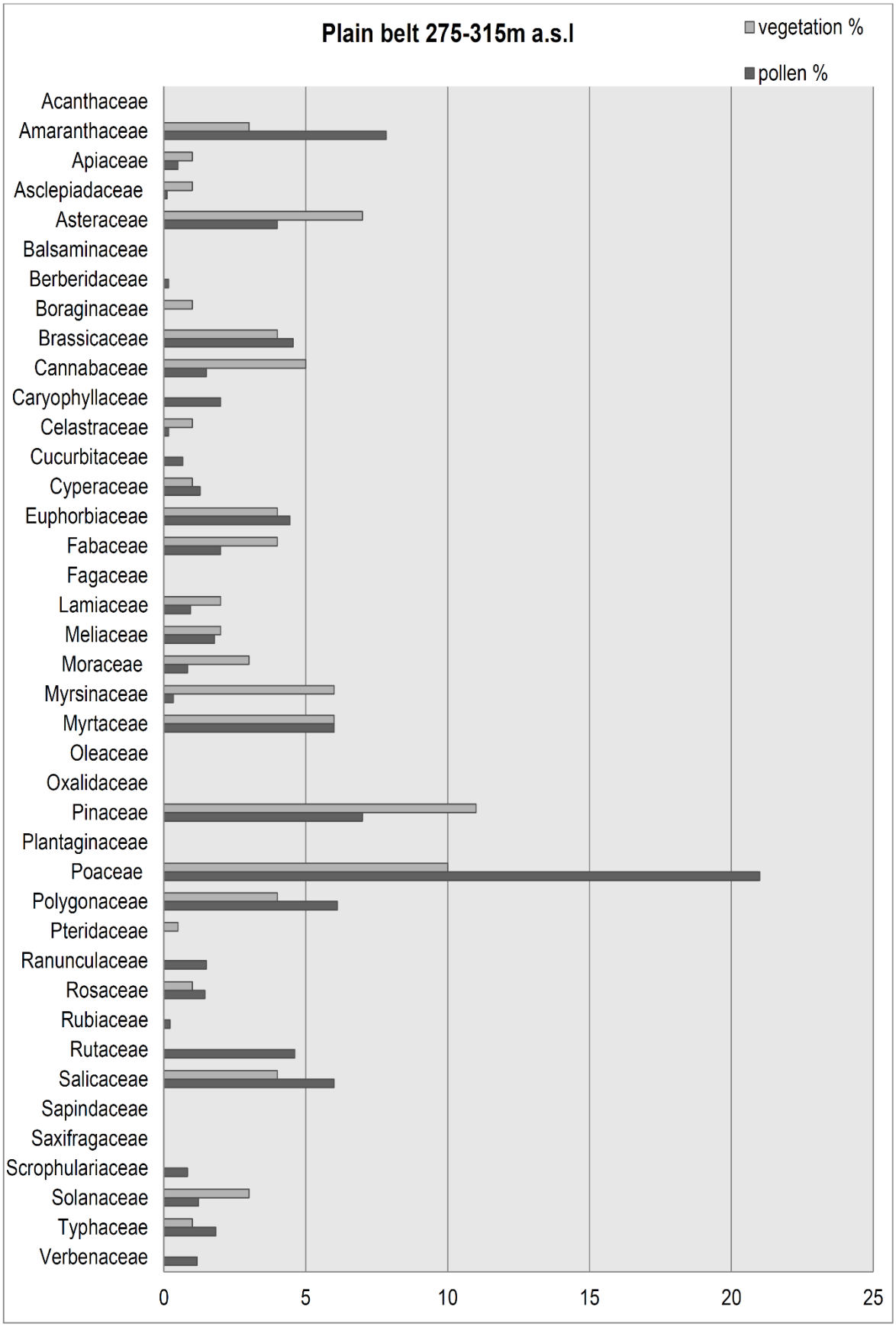

Figure 5. Showing histogram comparing percentage data of the modern pollen rain and plant taxa in vegetation plots on family level for the lowest plain zone (275-315m a.s.l).

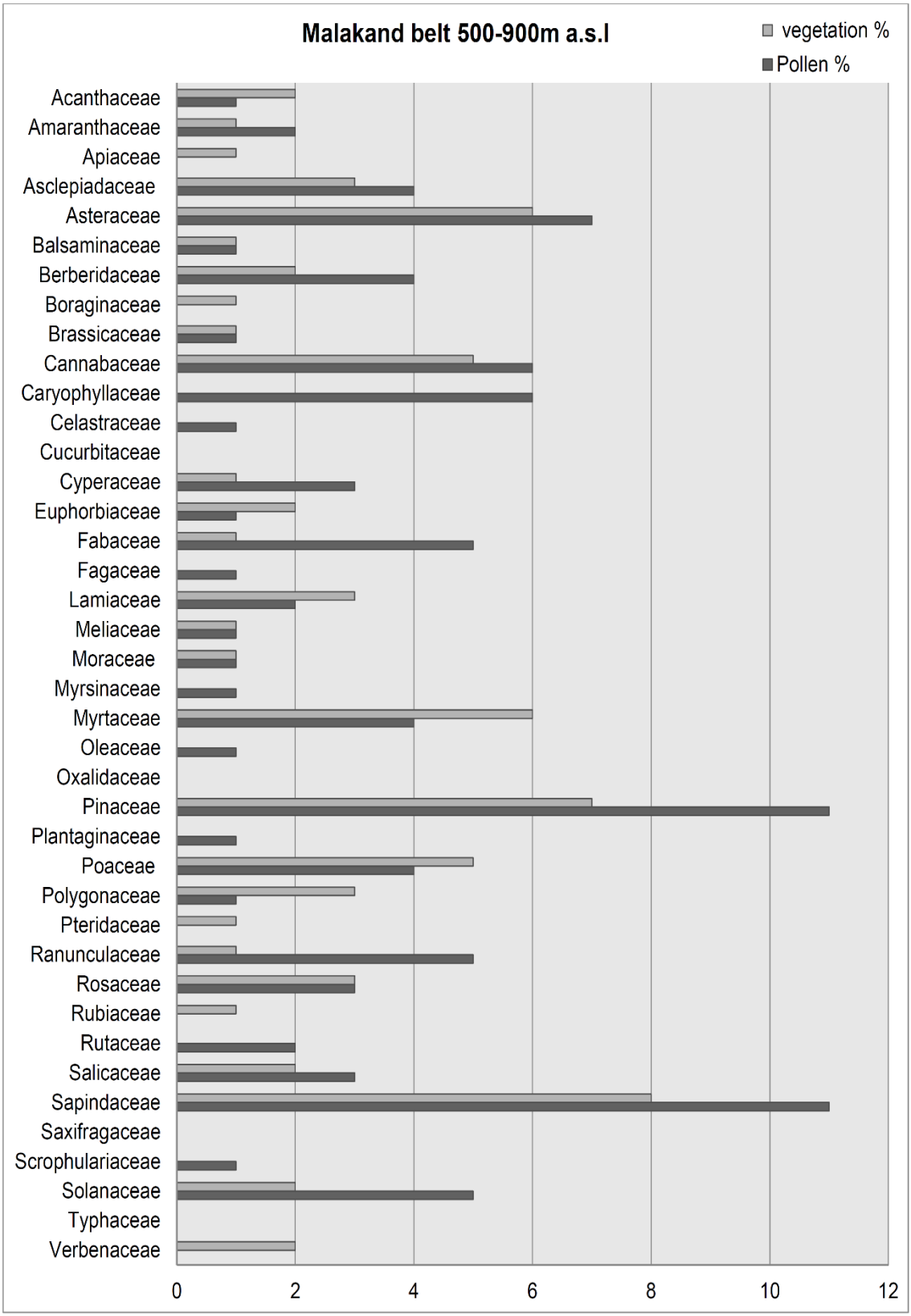

Figure 6. Showing histogram comparing percentage data of the modern pollen rain and plant taxa in vegetation plots on family level for the lowest plain zone (500-900 m a.s.l).

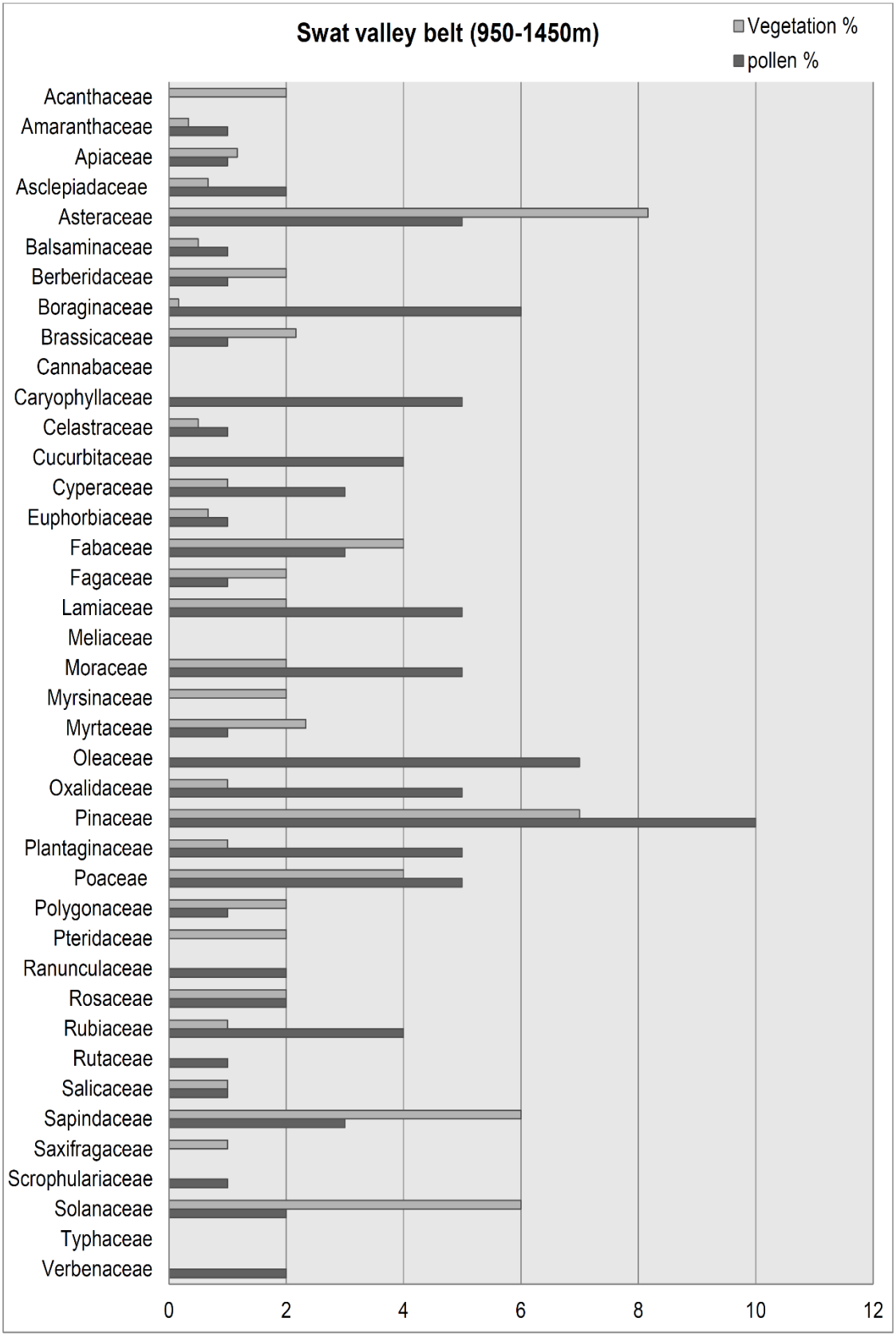

Figure 7. Showing histogram comparing percentage data of the modern pollen rain and plant taxa in vegetation plots on family level for Swat valley zone (950-1450 m a.s.l).

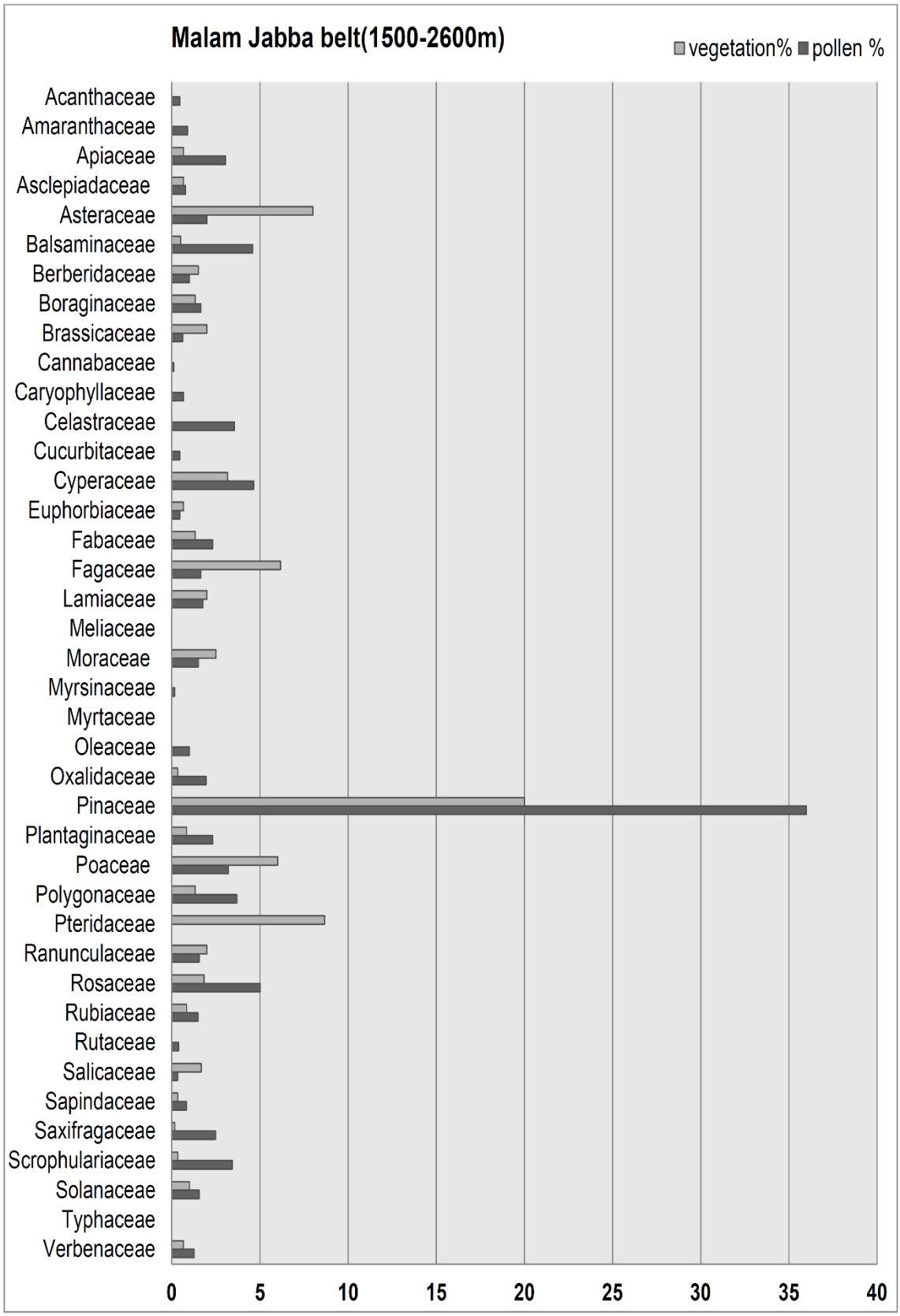

Figure 8. Showing histogram comparing percentage data of the modern pollen rain and plant taxa in vegetation plots on family level for Malam Jabba zone (1500-2600 m a.s.l).

Correlation

The relationships between plant cover and pollen (both in percentages) of the two most common taxa from each elevational zone are illustrated as scatter plots (Figure 9, 10). The Spearman's rank correlation coefficient (r_s value, with "0" no correlation to "1" strongest correlation) is indicated for each taxon. For all families the coefficient indicates a significant correlation at $P < 0.05$ except Boraginaceae ($r_s = 0.05$, $p = 0.79$), Saxifragaceae ($r_s = 0.01$, $p = 0.94$), Apiaceae ($r_s = 0.04$, $p = 0.85$), Balsaminaceae ($r_s = 0.18$, $p = 0.37$) and Rubiaceae ($r_s = 0.25$, $p = 0.22$). Strong correlation is found in Poaceae ($r_s = 0.54$, $p = 0.006$), Asteraceae ($r_s = 0.59$, $p = 0.002$), Cyperaceae ($r_s = 0.55$, $p = 0.004$), Verbenaceae ($r_s = 0.38$, $p = 0.06$), Acanthaceae ($r_s = 0.54$, $p = 0.006$) and Euphorbiaceae ($r_s = 0.51$, $p = 0.009$).

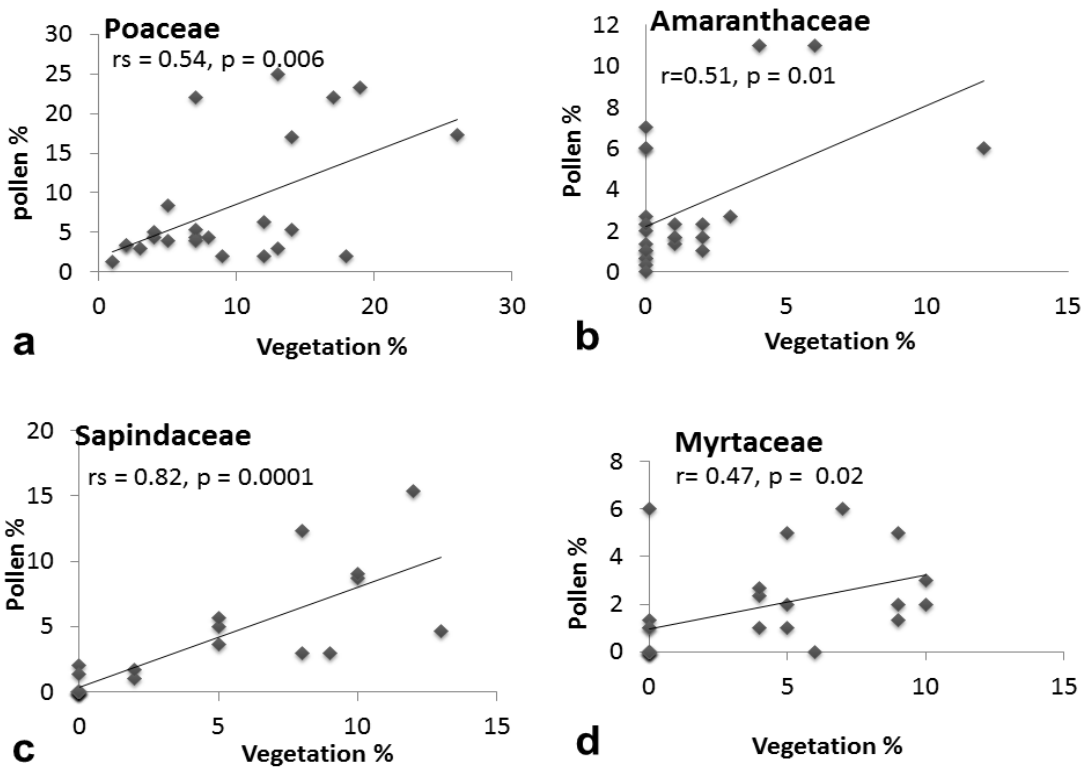

Figure 9. Scatter plots showing the relationship between plant cover percentages and pollen percentages for the two most common families (Figure 9A showing the lower two zones and Figure 9B showing the upper two zones). The Spearman's rank correlation coefficient (rs) and the significance value "p" are given for each taxon in brackets. Note the different scales.

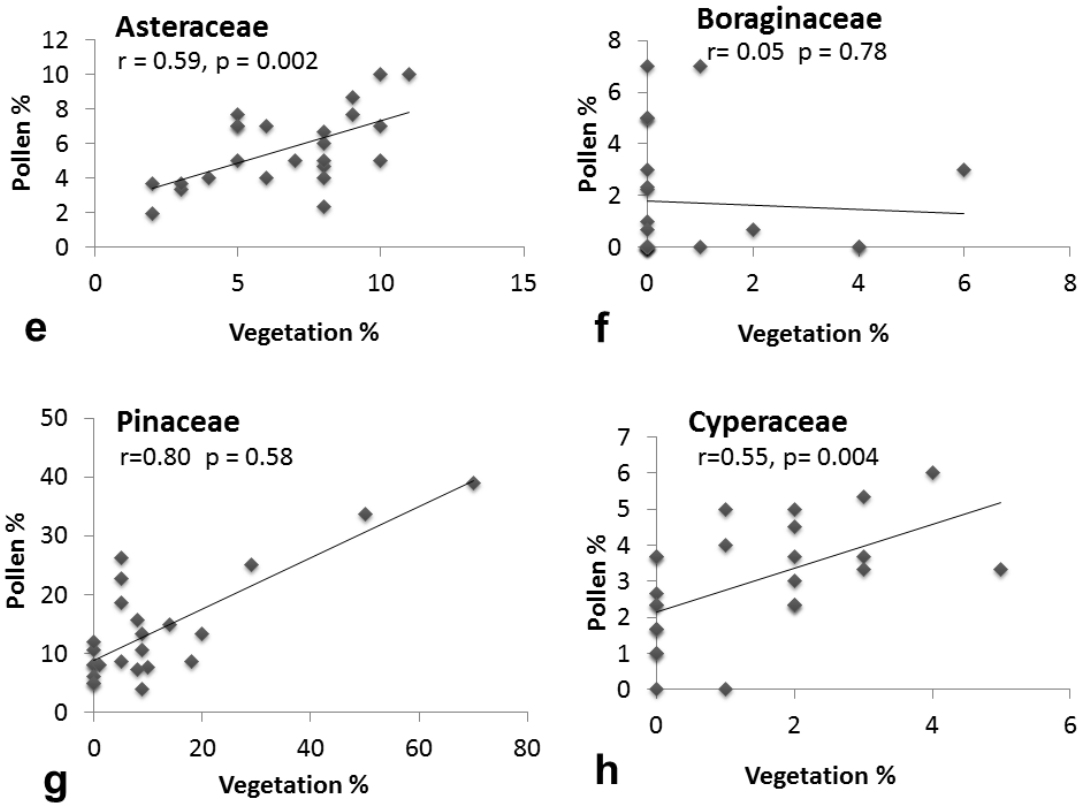

Figure 10. Scatter plots showing the relationship between plant cover percentages and pollen percentages for the two most common families (Figure 10 A showing the lower two zones and Figure 10B showing the upper two zones). The Spearman's rank correlation coefficient (rs) and the significance value "p" are given for each taxon in brackets. Note the different scales.

Vegetation boundaries and key taxa

The pollen diagram (Figure 11) shows the key taxa of all the four elevational zones. The pollen signal retrieved from all the surface samples reveals the possibility of demarcating vegetation boundaries along the gradient. Potential vegetation boundaries can be seen in the pollen sums for all the four elevational zones. The plain zone (275-315 m a.s.l.) is represented by families like Poaceae (including cereals as 6% pollen grains are > 45 µm), Polygonaceae, Amaranthaceae, Rutaceae, Euphorbiaceae, Brassicaceae, Salicaceae and Myrtaceae. The lower montane Malakand zone (500-900 m a.s.l.) is represented by families like Sapindaceae, Asteraceae, Cannabaceae, Solanaceae, Berberidaceae, Fabaceae, Rananculaceae, Asclepediaceae and Verbenaceae. The upper zone of the Swat valley (950-1450 m a.s.l.) is represented by families like Oleaceae, Boraginaceae, Lamiaceae, Oxalidaceae, Plantaginaceae, Rubiaceae, Moraceae, Caryophyllaceae, Cucurbitaceae and Bignoniaceae. The topmost

zone of upper montane Malam Jabba hills (1550-2600 m a.s.l.) is dominated by Pinaceae members like *Pinus, Picea, Abies* and *Cedrus* species. Others families are Cyperaceae, Rosaceae, Balsaminaceae, Celastraceae, Scrophulariaceae and Araceae.

Discussion

Pollen rain - vegetation relationship

When comparing modern pollen rain and vegetation, the taxonomic level of identification from family to species level needs to be considered. Often pollen grains can only be determined to genus or family level and sometimes to joint family levels e.g. Moraceae/Urticaceae or Melastomataceae/Combretaceae. We recommend the use of higher-ranked systematic levels such as genera or families instead of species for unexplored remote areas like north western Pakistan. Taxonomic surrogacy at family level seems to be the right choice for a comparison of pollen and plant taxa in the tropics (Odgaard 2001; Jantz et al. 2014).

To address our first research question, i.e., whether the plant diversity patterns of the existing vegetation, along successive elevational zones from lowland to highland are reflected in modern pollen rain, we can state that the vegetation of the secondary grassland and meadows in the plain zone and the *Pinus* forests in the upper montane zones are very well reflected in the pollen rain.

The comparison of modern pollen rain with the surrounding vegetation for each elevational zone reveals that different plant families reflect the plant diversity patterns along the 200 km elevational gradient shown in Figure 5-8 and summarized as transfer factors (TF) in Table 3A. A TF value close to 1 means that, the percent abundance of a plant family in the pollen record is almost equal to that in the vegetation. TF values < 1 indicate over-representation in the pollen record and TF values > 1 indicate under representation (Schüler et al. 2014). Our results based on the transfer factor clearly reveal that there is a strong relationship between the diversity of plant taxa at the family level. Along the whole gradient the over-represented and well represented families (TF < 1) include Poaceae, Amaranthaceae, Rutaceae, Rananculaceae, Salicaceae, Pinaceae, Sapindaceae, Solanaceae, Boraginaceae, Plantaginaceae, Rubiaceae, Cucurbitaceae and Oleaceae. The under-represented families (TF > 1) include Myrsinaceae, Myrtaceae, Asteraceae, Rubiaceae, Verbenaceae, Fagaceae and Pteridaceae. Comparisons at family level have previously been proven to be reliable for explaining the patterns of biodiver -

sity (La Torre-Cuadros et al. 2007; Leal et al. 2010) as well as for the reconstruction of beta diversity (Terlizzi et al. 2008). However, care should be taken while applying family level comparison because higher taxonomic levels as Linnean ranks are a product of phylogeny as well as historical determination processes that change over time (Bertrand et al. 2006) which makes each case unique. Moreover, complexities in determination increase while working with fossil pollen types (Jantz et al. 2014).

Correlation analysis

Despite significant correlation coefficients calculated from the scatter plots for some families, some data sets are still highly influenced by one or two samples with either high plant or pollen values e.g. Boraginaceae, Saxifragaceae, Apiaceae and Rubiaceae. Others show a good spread of data points approximating a linear trend e.g. Poaceae, Asteraceae, Cyperaceae, Verbenaceae and Euphorbiaceae. One reason for the rather poor correspondence and weak correlation between plant and pollen abundance may lie in the varying proportion of un-vegetated area in the different sampling plots along the gradient. The absolute amount of pollen coming may thus be affected by the local vegetation and hence influence the representation of pollen from beyond the sampling plot. High vegetation frequencies of Poaceae species in the grasslands of lowest elevated plain areas correspond to high pollen percentages showing a significant correlation (r_s =0.54, p = 0.006). Poaceae pollen grains which are found in higher frequencies in the surface samples of the plain zone probably come from the secondary grasslands and meadows in the surroundings. These grasslands comprise wild Poaceae species like *Cynodon dactylon*, *Poa annua*, *Brachiaria eruciformis*, *Desmostachya bipinnata*, *Cenchrus ciliaris* and *Dichanthium annulatum* while the large sized pollen grains (> 45µm) probably come from the cereal crops of *Zea mays* fields in the faraway peripheries. While entering the second zone of lower montane Malakand areas, the pollen and vegetation percentages of Poaceae decrease gradually but increase again in the Swat valley and finally decrease again in upper montane Malam Jabba hills. Amaranthaceae in pollen samples and vegetation are also significantly correlated (r_s = 0.51 p = 0.01) in the plain zone. Salicaceae species (r_s = 0.73, p = 0.00003) have an average cover of 4% in the vegetation which is comparable to the 6% in pollen abundance. *Eucalyptus camaldulensis* is present with 6% cover in the vegetation plots of the plain zone and the abundance of Myrtaceae family is also reflected by 6% in the pollen spectra. This probably corresponds to the fact that *Eucalyptus camaldulensis* is

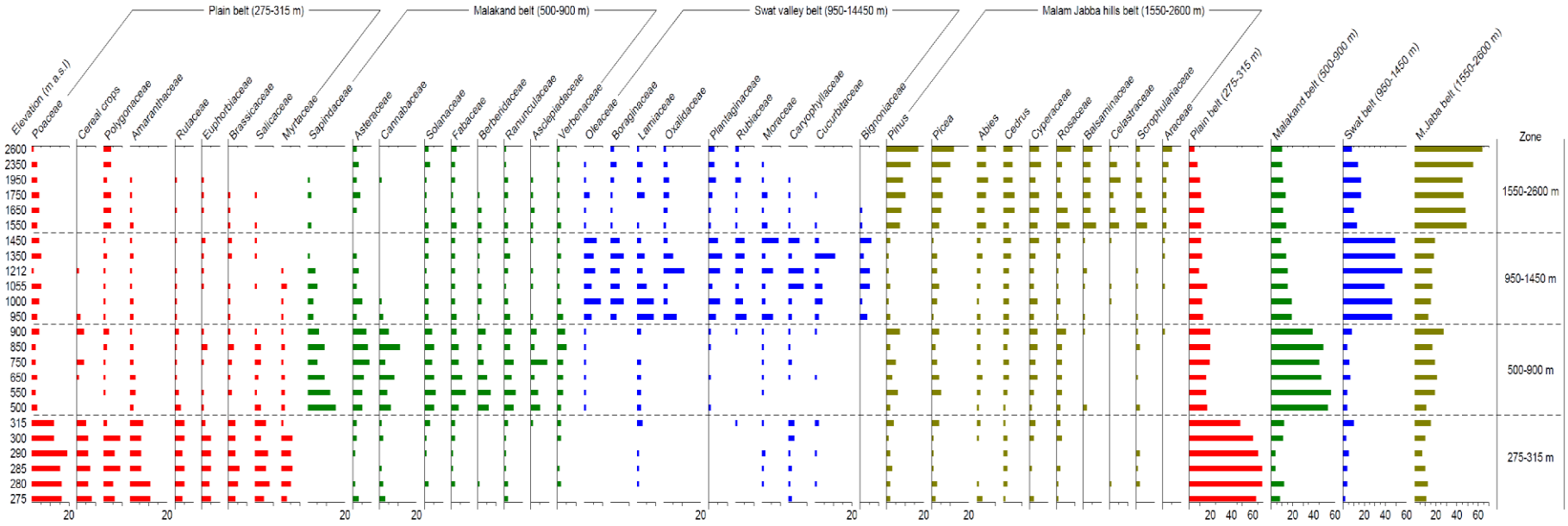

Figure 11. Pollen percentage diagram of the modern pollen rain showing the key taxa (families) and their sums, making four distinct vegetation zones along the 200 km-long elevational gradient. The diagram shows that it is possible to distinguish between the elevational zones based on the pollen rain assemblage.

Table 3. Information given for all plant families having considerable representation both in vegetation and in surface samples. Transfer factors (TFs) values are given when the family is present both in vegetation survey and in pollen record.

Families	**PZ**	**LMZ**	**SVZ**	**UMZ**
Verbenaceae	x	st	x	0.5
Typhaceae	0.5			
Solanaceae	2.5	0.4	3.0	0.6
Scrophulariaceae	x	x	x	x
Saxifragaceae			st	x
Sapindaceae		0.7	2.0	x
Salicaceae	0.7	0.7	1.0	st
Rutaceae	x	x	x	
Rubiaceae		st	0.3	0.6
Rosaceae	0.7	1.0	1.0	0.4
Ranunculaceae	x	0.2	x	1.3
Pteridaceae	st	st	st	st
Polygonaceae	0.7	3.0	2.0	0.4
Poaceae 0.5	1.3	0.8	1.9	
Plantaginaceae		x	0.2	0.4
Pinaceae1.6	0.6	0.7	1.1	
Oxalidaceae			0.2	x
Oleaceae		x	x	x
Myrtaceae	1.0	1.5	2.3	
Myrsinaceae	st	x	st	
Moraceae	3.6	1.0	0.4	1.7
Meliaceae	1.1	1.0		
Lamiaceae	2.1	1.5	0.4	1.1
Fagaceae		x	2.0	3.7
Fabaceae	2.0	0.2	1.3	0.6
Euphorbiaceae	0.9	2.0	0.7	st
Cyperaceae	0.8	0.3	0.3	0.7
Cucurbitaceae	x		x	
Celastraceae	st	x	0.5	x
Caryophyllaceae	x	x	x	x
Cannabaceae	3.3	0.8		
Brassicaceae	0.9	1.0	2.2	3.3
Boraginaceae	st	st	x	0.8
Berberidaceae		0.5	2.0	1.5
Balsaminaceae		1.0	0.5	0.1
Asteraceae	1.8	0.9	1.6	4.0
Asclepiadaceae	st	0.8	0.3	0.9
Apiaceae	2.0	st	1.2	0.2
Amaranthaceae	0.4	0.5	x	x
Acanthaceae		2.0	st	

an abundant pollen producer, because the lower correlation value within Myrtaceae family (r_s = 0.47, p = 0.02) corresponds to the presence of other Myrtaceae species in the surroundings of the plot areas.

Pollen and vegetation are significantly correlated in Sapindaceae (r_s = 0.82, p = 0.000001) and Myrtaceae (r_s =0.47, p = 0.02) families, in the lower montane Malakand zone. One reason for this may be the thick vegetation of *Dodonea viscosa* and *Eucalyptus camaldulensis* in this zone. Asteraceae (r_s = 0.59, p = 0.002) are well reflected in the modern pollen of the Swat valley. One reason for this may be the diversity of Asterac - eae species in this zone including abundant pollen producers like *Parthenium hysterophorus*, *Conyza aegyptiaca*, *Tagetes minima*, *Artemesia japonica*, *Tussilago farfara*, *Calandula arvensis*, *Silybum marianum* and *Senecio chrysanthe- moides.* Boraginaceae (r_s = 0.05, p = 0.79) show a very weak correlation here which may be due to the presence of only two species in the plots i.e. *Heliotropium europaeum* and one *Cynoglossum* species. Pinaceae (r_s = 0.80, p = 0.58) and Cyperaceae (r_s = 0.55, p= 0.004) are well reflected in the upper montane Malam Jabba hills due to their high abundance. We observed that *Pinus* trees are generally very rare or almost absent from the plain zone. Furthermore no single tree of *Picea*, *Abies* or *Cedrus* was found in the vegetation of the three lower zones. However, their pollen grains were present by up to 2% each in the pollen spectra. This most likely corresponds to their long distance wind dispersal across 200 km from the upper montane Malam Jabba hills down to the areas in the plain zone.

Natural vs secondary vegetation

Variation in species diversity and vegetation zonation along elevational and environmental gradients has been a major topic of ecological studies in recent years and has been explained with reference to biotic interaction, habitat heterogeneity, changing climate and productivity (Currie & Francis 2004; Shaheen & Shinwari 2012; Khan et al. 2013). In answer to our second question, i.e., whether or not the modern pollen rain represents a reflection of each elevational zone in the form of vegetation boundaries, we can state that there is a definite zonation resulting in observable vegetation boundaries due to distinct key taxa found in each elevational zone. The pollen diagram (Figure 11) reveals clear vegetation boundaries that can be seen along the altitudinal zones in terms of the key taxa as inferred from the pollen rain. The key families of the plain zone are Poaceae, Polygonaceae, Amaranthaceae, Rutaceae, Euphorbiaceae, Brassicaceae, Salicaceae and Myrtaceae. Key families of the lower montane Malakand zone are Sapindaceae, Asteraceae, Cannabaceae, Solanaceae, Fabaceae, Berberidaceae,

Rananculaceae, Asclepiadaceae and Verbenaceae. The upper plain Swat valley is represented by key families like Oleaceae, Boraginaceae, Lamiaceae, Oxalidaceae, Plantaginaceae, Rubiaceae, Moraceae, Caryophyllaceae, Cucurbitaceae and Bignoniaceae. Similarly key taxa of the upper montane Malam Jabba hills are *Pinus*, *Piceae*, *Abies*, *Cedrus*, Cyperaceae, Rosaceae, Balsaminaceae, Celestraceae, Scrophulariaceae and Araceae.

The secondary vegetation along the 200 km elevational gradient reflects the biodiversity pattern of existing vegetation despite the human impact; moreover the modern pollen rain still reflects the elevational zones (see the CONISS dendrogram Figure 4 and compare with vegetation in Table 2). Due to severe anthropogenic activities as a result of over population in the lower three zones, the entire vegetation along this gradient presents a secondary vegetation spectrum. Altitudinal variation along this 200 km-long elevational gradient has exerted marked impact over vegetation cover and species composition. Social and economic factors determine landscape and plant community dynamics in rural areas dominated by human activities (Hietel et al. 2005; Pueyo & Alados 2007). Unlike the secondary vegetation in the lower three elevational zones where disturbance regimes decrease natural vegetation frequency, the upper montane Malam Jabba zone is dominated by natural vegetation with conifers like species of *Pinus*, *Abies*, *Cedrus* and *Picea* along with Pteridaceae and Dryopteridaceae ferns, mosses and bryophytes. However, vegetation here is transforming at an alarming speed from natural to secondary as a result of severe biotic pressure and indiscriminate deforestation besides overgrazing. The inhabitants are primarily dependent on the plant resources of the area and they utilize wood mainly as fuel and cut trees to make more land available for agriculture. Moreover collection of medicinal plants in Malam Jabba zone has threatened the existence of some indispensable and valuable medicinal plants like *Valeriana jatamansi*, *Acorus calamus*, *Paeonia emodi* and *Podophylum hexandrum* (Iqbal & Hamayun 2005). Mosses, ferns and bryophytes were either absent from the highly disturbed lower two zones (275-900 m a.s.l.) or less frequent in the third zone of Swat valley (950-1450 m a.s.l.) but more frequent in the comparatively undisturbed upper montane Malam Jabba hills (1550-2600 m a.s.l.). Biodiversity of plants was observed as rich in the upper montane Malam Jabba hills which gradually decreases along the gradient and is lowest in the plains revealing its definite relation with

topography and altitude. The difference of the overall plant cover was observed as lower in disturbed sites as compared to the undisturbed sites.

Vegetation survey indicate strong human impact

The vegetation survey of the lower three ascending zones indicates a strong human impact that changed the entire vegetation along this 200 km-long elevational gradient. The impact of human activities in the lower three zones is evident from the secondary vegetation documented. The abundance of cereal crops like *Triticum aestivum*, *Zea mays* and domesticated forage Poaceae species like *Cynodon dactylon*, *Poa annua*, fuel wood tree species of Myrtaceae like *Eucalyptus camaldulensis* and of Salicaceae such as *Populus ciliata*, weed herbs like *Cannabis sativa* and *Euphorbia helioscopia* are the principal components. The frequent encounter of these pollen grains along with cultivated Poaceae species such as *Oryza sativa*, as well as Brassicaceae, Fabaceae, Moraceae Chenopodiceae/Amaranthaceae, and Amaranthaceae reflects the proximity of cultivated land and human habitation. All these species reflect secondary vegetation due to the strong human impact.

Conclusion

The modern pollen spectra from surface samples and their relationship with the surrounding vegetation provide useful data for the interpretation of future Holocene pollen records from north western Khyber Pakhtunkhwa province in Pakistan, as well as for similar environments in different geographical areas of South Asia. Our study reveals that despite some distortions, the pollen rain signal in the surface samples reflects well the plant diversity pattern corresponding to the existing vegetation along the 200 km-long elevational gradient. Four distinct elevational zones are defined by dominating plant families which are reflected in the pollen assemblages by different proportions, indicating a significant correlation between pollen rain and vegetation in families like Poaceae, Asteraceae, Cyperaceae, Verbenaceae, Acanthaceae and Euphorbiaceae. Pollen assemblages also vary considerably from the associated vegetation composition and major discrepancies are caused by large differences in pollen and vegetation proportions in Boraginaceae, Saxifragaceae, Apiaceae, Balsaminaceae and Rubiaceae families. Moreover there is a considerable proportion of non-local pollen in every spectrum e.g. conifer pollen in the lowland zones. Our results

demonstrate that relationships between pollen rain and the surrounding vegetation are comparable at their family level. Results based on the comparison of vegetation and the pollen spectra at family level and their transfer factors (TF) revealed that large sized Poaceae cereal pollen grains > 45 µm, Myrtaceae, Polygonaceae, Brassicaceae, Asteraceae, Chenopodiaceae/Amaranthaceae and Cannabaceae reflect the proximity of cultivated land and human habitation in the lower three zones including Peshawar valley, lower montane Malakand hills and Swat valley. Similarly Pinaceae, Pteridaceae, Dryopteridaceae and other spores reflect natural vegetation in the upper montane Malam Jabba zone. The establishment of a modern pollen rain – vegetation relationship, at least at the family level, is necessary for calibration and interpretation of the fossil pollen record from such sites. The differences in the representation of plant families along an elevational gradient recorded from the vegetation survey and the pollen signal are measurable as transfer factors. Such transfer factors can be applied to calibrate palaeorecords in future studies enabling us to quantitatively reconstruct the past climate and vegetation of this remote and neglected part of north western Pakistan in South Asia.

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Table 2 Floristic composition of the 24 plots in percent cover along the four belts of altitudinal gradient in northern Pakistan

Elevation belts	**Plain zone (275-315 m)**						**Malakand zone (500-900m)**						**Swat valley zone (950-1450m)**						**M. Jabba zone (1550-2600m)**						
Plot´s No	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**	**17**	**18**	**19**	**20**	**21**	**22**	**23**	**24**	
Elevation (m a.s.l)	**275**	**280**	**285**	**290**	**300**	**315**	**500**	**550**	**650**	**750**	**850**	**900**	**950**	**100 0**	**105 0**	**121 0**	**135 0**	**145 0**	**155 0**	**165 0**	**175 0**	**195 0**	**235 0**	**260 0**	
Species richness (S)	**30**	**28**	**37**	**39**	**34**	**29**	**33**	**29**	**19**	**31**	**44**	**44**	**31**	**46**	**40**	**36**	**47**	**45**	**39**	**32**	**23**	**34**	**26**	**16**	**%F**
Poaceae																									
Cynodon dactylon	12	14	4	5	15	10	5	7	5	5	4	3	4	2	3	2	2	.	1	2	1	.	.	.	83
Brachiaria eruciformis	2	1	.	.	.	.	2	2	.	1	2	1	.	.	1	.	.	.	.	.	.	.	.	.	33
Desmostachya bipinnata	2	.	.	1	.	.	.	2	.	.	1	.	.	.	1	.	.	.	.	.	.	.	.	.	21
Cenchrus ciliaris	.	2	1	.	1	.	2	.	.	.	2	.	1	.	2	3	.	.	.	.	.	.	2	.	38
Cenchrus pennisetiformis	.	.	.	.	.	.	.	.	.	.	.	.	.	1	1	.	.	1	1	.	2	.	.	.	21
Poa annua	2	.	.	5	5	2	2	1	2	.	.	.	2	.	5	2	.	3	.	6	.	2	.	1	58
Dichanthium annulatum	.	.	.	2	3	.	.	3	.	.	1	1	.	.	1	.	2	2	.	.	2	.	.	.	38
Hordeum murinum	.	.	1	.	1	.	2	.	.	2	.	.	.	1	.	2	.	1	.	.	.	.	.	.	29
Eleocine indica	.	.	1	.	.	2	.	.	.	1	.	.	.	.	.	.	.	.	.	.	.	.	.	.	13
Cymbopogon jawarancosa	1	.	.	.	1	.	.	3	.	3	2	.	.	.	.	.	.	.	1	.	.	.	.	.	25
Phleum paniculatum	.	.	.	2	.	1	1	.	.	3	.	.	.	.	.	.	2	.	.	.	.	.	1	.	25
Saccharum spontaneum	.	.	.	1	.	.	.	2	.	.	.	.	.	.	.	.	.	.	.	.	.	1	.	.	13
Apluda mutica	.	.	1	.	1	.	.	.	.	3	1	1	1	1	.	.	3	.	.	.	.	.	.	.	33
Eragrostis cilianensis	.	.	.	.	.	.	4	2	.	.	.	.	.	.	2	1	.	.	3	.	.	.	.	.	21
Sorghum halepense	.	.	1	1	2	.	2	.	1	.	1	.	.	.	.	.	.	.	.	4	.	.	.	.	29
Oryza sativa	.	.	.	.	.	.	.	.	.	.	2	.	.	.	.	.	.	.	.	.	.	.	.	.	4
Digiteria ciliaris	.	.	1	.	.	.	.	.	.	.	.	2	2	1	.	.	.	.	.	.	.	.	2	.	21
Phalaris minor	.	.	1	.	2	.	.	.	.	.	1	.	.	4	.	.	.	2	.	.	.	.	.	.	21
Heteropogon contortus	.	.	1	.	.	.	.	.	.	.	.	.	.	.	2	.	.	.	.	.	.	1	.	.	13
Avena fatua	.	.	.	1	.	.	.	.	.	.	.	.	.	2	.	3	.	.	.	.	.	.	.	.	13
Dactyloctenium aegyptium	.	.	.	.	2	.	.	.	.	.	.	.	.	1	.	.	.	.	.	.	.	1	.	.	13
Aristida species	.	.	.	1	.	.	1	2	.	.	.	.	.	.	.	.	.	.	1	.	.	.	.	.	17
Paspalidium flavidum	.	.	.	.	1	.	.	.	.	.	.	2	.	.	.	.	.	.	.	1	7	.	.	.	17
Urochloa panicoides	.	.	.	1	.	.	.	.	1	1	.	.	.	.	.	.	1	.	.	.	9	2	.	.	25
Achrachne racemosa	.	.	.	.	.	2	.	.	2	.	.	.	.	.	.	.	2	2	.	.	.	.	.	.	17
Setaria viridis	.	.	.	1	.	1	.	.	.	.	.	2	.	.	.	.	2	.	2	.	.	.	2	.	25

Table 3. Floristic composition of the 24 releve´s in percent cover along the four belts of altitudinal gradient in northern Pakistan **continues……**

Pennisetum orientale	.	.	.	2	.	.	.	.	1	.	.	2	.	.	.	.	.	2	.	1	.	.	.	.	21
Bromus catharticus	.	.	1	.	1	.	.	.	.	.	.	.	.	.	.	.	.	.	.	3	.	.	.	.	13
Imperata cylindrica	.	.	.	.	.	2	1	.	.	2	.	.	.	.	.	.	.	.	2	.	.	.	.	.	17
Isodon rogosus	.	.	.	.	1	.	1	1	.	2	.	.	.	.	.	.	.	.	.	.	.	3	.	.	21
Bromus pectinatus	.	.	.	2	.	.	.	.	.	.	.	.	1	1	.	.	.	1	.	.	.	.	.	.	17
Polypogon fugax	.	.	.	1	.	.	.	.	.	.	.	.	2	.	.	.	.	.	.	.	.	.	2	.	13
Cyperaceae																									
Cyperus species	.	1	.	.	2	.	.	.	.	2	.	2	2	1	.	.	3	1	3	2	3	5	4	2	58
Asteraceae																									
Parthenium hysterophorus	5	2	.	.	.	.	1	.	5	.	.	2	.	.	.	.	.	4	.	.	.	.	.	.	25
Conyza aegyptiaca	2	.	4	2	.	2	2	.	.	.	2	.	.	.	.	10	.	3	4	.	.	1	.	.	42
Conyza Canadensis	.	.	.	2	.	.	.	.	.	.	.	2	.	.	.	.	1	.	.	.	.	3	.	.	17
Tagetes minima	.	.	.	.	2	1	.	.	1	3	.	.	.	.	.	.	.	.	3	.	.	.	.	.	21
Tagetes errecta	.	.	.	.	.	.	.	.	.	.	2	2	1	.	.	.	.	.	.	4	.	2	.	.	21
Echinops species	.	.	.	.	.	.	1	.	.	6	2	.	.	.	.	.	1	.	1	.	.	.	.	.	21
Sonchus asper	.	2	1	.	.	3	.	.	.	.	2	.	.	.	2	.	.	2	.	.	2	.	.	.	29
Artemesia japonica	.	.	.	.	2	.	.	.	.	.	.	1	.	.	.	.	.	.	.	.	.	.	.	.	8
Artemisia dubia	.	.	.	.	.	1	.	.	.	.	.	.	.	3	.	.	2	.	.	.	.	3	3	.	21
Xanthium strumarium	.	.	1	.	.	.	.	.	.	.	2	.	.	3	.	3	.	.	2	.	.	.	.	.	21
Tussilago farfara	.	.	.	.	.	.	.	.	.	.	.	2	.	.	.	2	.	3	1	.	.	.	.	.	17
Bidens species	.	.	.	.	.	.	.	.	.	.	.	.	.	1	.	.	1	.	.	.	.	2	.	.	13
Anaphalis species	.	.	.	.	.	.	.	.	.	2	.	.	.	.	.	.	.	.	1	.	.	.	4	.	13
Taraxicum officinale	.	.	1	.	.	.	1	.	.	.	.	.	.	.	1	.	.	.	2	3	.	.	.	.	21
Calandula arvensis	.	.	1	.	.	.	.	.	.	.	.	.	.	1	.	.	1	1	.	.	.	.	.	.	17
Silybum marianum	1	1	.	.	3	1	.	1	.	.	.	.	.	.	.	.	1	1	1	.	.	.	.	.	33
Senecio chrysanthemoides	.	.	.	.	2	.	.	2	.	.	.	.	.	.	.	.	1	.	1	3	.	.	.	.	21
Fabaceae																									
Alhagi maurorum	2	9	.	.	1	.	.	2	.	2	1	.	.	.	.	1	.	2	.	.	.	.	.	.	33
Medicago denticulata	1	.	1	.	1	.	.	.	.	1	.	2	.	.	.	1	.	5	.	.	.	.	.	.	29
Polygonaceae																									
Rumex hastatus	5	3	.	.	2	1	.	3	.	.	2	.	.	.	2	.	6	3	.	3	.	.	.	.	42
Polygonum barbatum	2	2	5	.	.	1	1	.	.	.	2	.	.	.	.	4	.	.	.	.	.	.	.	.	29
Polygonum plebjum	.	.	1	.	.	1	.	.	.	.	.	2	.	.	.	6	5	3	3	.	.	.	.	.	29

Table 3. Floristic composition of the 24 releve´s in percent cover along the four belts of altitudinal gradient in northern Pakistan continue……

Polygonum aviculare	.	2	.	1	.	.	.	2	5	.	.	.	.	.	.	.	2	.	.	.	.	.	2	.	25
Lamiaceae																												
Mentha viridis	.	.	.	.	2	.	10	.	.	7	2	.	4	6	.	.	.	3	.	.	.	.	.	.	29
Micromeria biflora	1	.	.	.	.	.	.	4	.	.	1	2	.	.	1	.	.	.	.	.	.	.	.	.	21
Origanum vulgare	.	.	.	2	.	.	.	.	8	.	.	1	.	2	.	.	.	.	.	.	.	.	.	.	17
Stachys species	.	.	.	2	.	.	.	.	.	2	.	.	.	.	.	.	.	.	1	.	.	.	.	.	13
Coleus species	.	.	.	.	.	.	.	.	.	.	.	.	.	2	.	.	.	.	.	2	.	3	.	.	13
Dracocephalum nutans	.	.	.	.	.	.	.	.	.	.	.	.	.	2	.	.	.	2	.	.	.	2	2	.	17
Otostegia limbata	.	.	.	1	.	.	7	.	.	2	.	3	8	3	.	1	.	2	.	2	.	.	.	.	38
Plectranthus rugosus	.	.	1	2	.	.	.	8	.	.	.	2	.	3	.	.	4	3	.	.	.	.	.	.	29
Cannabaceae																												
Cannabis sativa	9	3	4	3	.	10	4	9	8	5	2	.	.	.	.	.	.	.	.	.	.	.	.	.	42
Solanaceae																												
Solanum surratense	.	4	.	.	1	.	.	.	.	.	4	.	6	.	2	.	.	.	.	.	.	.	.	.	21
Solanum nigrum	.	.	.	5	.	.	.	.	.	.	.	2	.	2	3	.	1	.	.	.	.	.	.	.	21
Datura stramonium	.	.	1	.	.	.	.	.	.	.	.	.	4	4	5	.	.	.	.	6	.	.	.	.	21
Datura inoxa	.	.	.	3	.	.	.	.	.	.	.	2	.	.	1	.	.	.	1	.	.	.	.	.	17
Withania somnifera	1	.	.	.	.	2	1	.	.	.	3	.	.	3	.	.	1	.	.	.	.	.	.	.	25
Hyoscyamus niger	.	.	.	1	.	.	.	.	.	.	.	.	3	.	2	.	.	.	.	.	.	.	.	.	13
Brassicaceae																												
Nasturtium officinale	.	.	.	.	.	1	.	.	.	.	.	2	.	.	.	.	.	.	2	.	.	.	.	.	13
Brassica campestris	.	3	1	.	.	5	.	.	.	.	.	.	.	.	.	.	1	1	.	.	.	.	.	.	21
Lepedium sativum	.	.	.	5	.	.	.	.	.	2	.	.	.	.	.	.	1	1	.	.	.	2	.	.	21
Lepedium pinnatifidum	.	.	.	.	.	.	.	.	.	.	.	.	.	3	.	.	.	.	4	.	.	3	.	.	13
Capsella bursa-pastoris	.	.	.	2	1	3	1	3	.	.	.	.	2	1	.	2	.	1	.	.	.	.	.	.	38
Rananculaceae																												
Clematis species	.	.	.	.	.	.	.	2	.	3	.	.	.	.	.	.	.	.	4	5	.	3	.	.	21
Rananculus vulgaris	.	.	.	.	.	.	1	.	.	.	2	.	.	.	.	1	.	.	.	.	.	.	.	.	13
Amaranthaceae																												
Achyranthes aspera	2	4	10	.	.	.	.	3	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	17
Alternanthera species	2	.	.	.	.	.	1	.	.	2	.	.	.	.	.	.	1	.	.	.	.	.	.	.	17
Amaranthis spinosus	.	.	.	.	.	.	.	.	.	.	.	2	.	.	.	.	.	.	1	.	.	.	.	.	8

Amaranthis viridis	2	.	2	.	.	.	.	.	.	.	2	.	.	.	.	1	.	.	.	.	.	.	.	.	.	17
Verbenaceae																										
Verbena species	.	.	1	.	.	.	.	.	.	.	2	7	.	.	.	.	.	.	.	.	.	2	2	.		21
Typhaceae																										
Typha angustifolia	4	2	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.		8
Nyctaginaceae																										
Boerhavia diffusa	2	.	4	.	3	.	2	.	.	.	1	.	.	.	.	2	.	.	.	.	.	.	.	.		25
Commelinaceae																										
Commelina benghalensis	.	.	.	.	.	.	.	.	5	.	.	.	.	.	2	.	.	.	.	4	.	.	.	.		13
Acanthaceae																										
Barleria species	.	.	.	.	.	.	.	.	.	.	.	.	1	.	.	.	.	.	.	.	.	.	1	.		8
Justicia adhathoda	.	.	.	.	.	.	.	.	10	.	.	3	8	1	.	.	2	.	1	.	.	.	.	.		25
Balsaminaceae																										
Impatiens bicolor	.	.	.	.	.	.	.	.	.	.	1	.	.	.	.	4	.	.	.	.	.	.	.	.		8
Balsaminaceae species	.	.	.	.	.	.	.	.	.	.	1	.	.	.	.	.	2	.	.	.	.	.	.	.		8
Impatiens edgeworthii	.	.	.	.	.	.	.	.	.	.	2	.	.	.	.	.	.	.	3	.	.	.	.	.		8
Rubiaceae																										
Rubiaceae species	.	.	.	.	.	.	.	.	.	.	.	2	.	.	.	.	2	.	.	.	1	.	.	.		13
Galium aparine	.	.	.	.	.	.	.	.	.	.	2	.	.	.	.	.	2	1	.	.	.	.	.	.		13
Galium debile	.	.	.	.	.	.	.	.	.	.	.	2	.	.	.	.	.	.	.	.	.	3	.		1	13
Plantaginaceae																										
Plantago lanceolata	.	.	.	.	.	.	.	.	.	2	.	.	.	.	2	.	1	.	.	3	.	.	2	.		21
Gentianaceae																										
Swertia species	.	.	.	.	.	.	.	.	.	1	.	.	.	.	.	.	.	.	.	.	.	.	.	.		4
Oxalidaceae																										
Oxalis carniculata	.	1	.	.	.	.	.	2	.	.	.	.	.	.	.	.	1	3	.	.	.	2	.	.		21
Apiaceae																										
Scandix species	.	.	.	2	.	.	.	.	.	.	.	.	.	2	.	.	.	.	.	.	.	.	.	.		8
Foeniculum vulgare	.	.	.	.	3	.	2	.	.	.	.	2	1	1	.	.	.	3	.	4	.	.	.	.		29
Phrymaceae																										
Mazus species	.	.	.	2	.	.	.	.	.	.	.	.	.	2	.	.	.	.	.	.	.	2	.	.		13
Saxifragaceae																										
Bergenia ciliata	.	.	.	.	.	.	.	.	.	.	.	.	.	1	.	.	.	2	.	.	1	.	.	.		13
Convolvulaceae																										

Convolvulus arvensis	.	.	.	.	.	.	.	.	.	.	.	.	2	.	.	.	.	.	.	.	.	.	.	.	.	.	4
Araceae																											
Arisaema species	.	.	.	.	.	.	.	.	.	.	.	2	1	.	.	.	1	1	.	.	.	.	.	.	.	.	17
Zygophylaceae																											
Tribulus terresteris	.	.	1	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	4
Araliaceae																											
Hedera nepalensis	.	.	.	.	.	.	.	.	.	.	.	1	.	1	.	.	.	.	.	.	2	.	.	.	.	.	13
Euphorbiaceae																											
Euphorbia prostrata	10	4	7	2	.	3	2	1	.	.	.	.	.	.	.	.	4	.	.	.	4	.	.	.	.	.	38
Euphorbia helioscopia	.	.	.	.	.	.	.	.	5	.	1	.	.	1	.	.	1	.	.	.	.	.	.	.	.	.	17
Boraginaceae																											
Heliotropium europaeum	4	1	.	.	.	.	.	.	.	4	.	.	.	.	.	.	.	.	.	2	.	.	.	.	.	.	17
Cynoglossum species	.	.	.	.	.	.	.	.	.	.	.	.	.	1	.	.	.	.	.	.	.	.	.	6	.	.	8
Pinaceae																											
Pinus walichiana	.	.	.	.	.	.	4	9	.	9	.	4	8	.	.	9	.	.	.	.	.	.	.	5	5	.	33
Pinus roxburgii	.	.	.	.	.	.	5	5	20	.	10	4	.	1	9	9	.	5	5	5	5	20	15	20	.	.	63
Cedrus species	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	10	15	.	.	8
Abies Pinaceae	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	5	10	20	.	.	13
Picea smithiana	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	4	10	10	.	.	13
Myrtaceae																											
Eucalyptus camaldulensis	10	9	7	5	.	5	9	10	5	9	4	.	4	.	4	.	6	.	.	.	.	.	.	.	.	.	54
Fabaceae																											
Acacia modesta	2	.	4	.	.	15	.	.	.	.	.	.	.	3	.	.	2	.	3	.	.	.	.	.	.	.	25
Dalbergia sissoo	2	.	4	.	.	.	.	.	.	.	.	.	4	.	4	3	6	6	.	.	.	.	.	.	.	.	29
Indigofera heterantha	.	.	7	.	5	.	.	.	.	.	.	2	3	.	.	.	2	.	.	3	.	.	2	.	.	.	29
Salicaceae																											
Salix species	.	6	7	.	.	.	.	.	.	.	.	1	.	1	4	.	.	.	5	5	.	.	.	.	.	.	29
Populus ciliata	3	4	4	5	.	10	4	.	.	5	.	3	.	.	.	.	3	.	.	.	.	.	.	.	.	.	38
Moraceae																											
Broussonetia papyrifera	4	9	5	.	.	.	.	.	.	.	3	.	.	1	.	.	.	.	5	.	2	.	.	.	.	.	29
Morus alba	5	4	.	.	5	.	.	.	.	.	.	.	.	.	1	.	2	.	.	2	.	.	.	.	.	.	25
Ficus palmate	.	.	.	3	.	.	.	.	.	.	.	.	.	2	.	.	.	.	.	.	.	3	.	.	.	.	13
Ficus carica	.	.	.	.	5	.	.	2	.	.	3	.	.	3	1	.	2	.	.	.	.	3	.	.	.	.	29
Meliaceae																											

Melia azedarach	.	1	.	5	.	5	4	.	.	.	.	.	.	.	1	.	.	.	.	.	.	.	.	.	.	21
Celastraceae																												
Maytenus royleana	.	.	.	.	5	.	.	.	.	.	.	2	.	3	.	.	.	.	.	.	.	.	.	.	.	13
Fagaceae																												
Quercus incana	.	.	.	.	.	.	.	.	.	.	.	.	4	.	.	.	.	3	8	.	16	.	.	13	21
Quercus dilitata	.	.	.	.	.	.	.	.	.	.	.	.	.	.	4	.	.	3	.	.	.	.	.	.	8
Simaroubaceae																												
Ailanthus altissima	.	.	.	5	5	.	.	.	.	.	.	.	3	.	2	2	.	.	.	.	.	.	.	.	.	21
Ebenaceae																												
Diospyros kaki	.	1	2	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	8
Platanaceae																												
Platanus orientalis	.	.	.	.	.	.	.	.	.	.	.	2	.	.	.	5	.	.	.	.	.	.	.	.	.	8
Sapindaceae																												
Dodonaea viscosa	.	.	.	.	.	.	12	8	10	5	10	5	8	9	13	5	2	.	.	.	2	.	.	.	50
Asclepediaceae																												
Calotropis procera	.	1	.	5	.	.	4	4	5	.	2	.	.	.	.	5	2	.	.	4	.	.	.	.	38
Rosaceae																												
Duchesnea indica	.	.	.	.	.	.	.	.	.	.	.	6	4	5	2	.	.	.	3	.	.	.	.	.	21
Spirea species	.	.	.	.	3	1	.	.	.	.	5	6	.	3	4	2	2	2	3	.	.	.	.	.	42
Rubus elepticus	.	.	.	.	.	.	.	.	.	.	.	1	.	.	.	.	.	.	.	.	.	.	.	.	4
Prunus species	.	.	.	.	.	.	.	.	.	.	.	.	.	4	2	3	4	1	5	.	.	.	.	.	25
Celastraceae																												
Gymnosporia royleana	.	.	.	2	.	.	.	.	.	.	.	2	.	1	4	1	1	.	.	.	.	.	.	.	25
Berberidaceae																												
Berberis lycium	.	.	.	.	1	.	.	.	3	6	2	.	.	.	2	.	7	3	.	.	9	.	.	.	33
Scrophulariaceae																												
Buddleja lindleyana	.	.	.	.	.	.	.	.	.	.	1	.	.	.	.	.	.	.	.	.	2	.	.	.	8
Ericaceae																												
Thomsonia species	.	.	.	.	.	.	.	.	.	.	.	.	.	2	.	2	.	.	.	2	.	.	.	.	13
Capprifoliaceae																												
Viburnum nervosum	1	.	.	3	.	.	.	.	.	.	.	.	4	.	1	.	.	2	.	1	.	.	.	.	25
Sarraceniaceae																												
Darlingtonia californica	.	.	.	.	.	.	.	.	.	.	.	.	2	.	.	.	1	.	.	.	1	3	.	.	17
Rhamnaceae																												

Ziziphus nummularia	.	.	.	.	.	3	.	.	.	.	.	.	2	.	.	1	.	.	.	1	.	.	.	.	17
Aspleniaceae																											
Asplenium species	.	.	.	.	.	.	.	.	.	.	.	.	.	.	2	2	.	.	1	.	.	3	.	1	21
Davalliaceae																											
Davallia denticulate	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	2	.	5	5	1	1	2	25
Athyriaceae																											
Athyrium spicatum	.	.	.	.	.	.	.	.	.	.	.	.	.	.	1	1	.	1	2	.	.	3	1	2	29
Pteridaceae																											
Adiantum species	.	.	.	.	.	.	.	.	.	.	1	2	.	.	.	2	.	.	2	2	5	1	2	2	38
Adiantum incisum	.	.	.	.	.	3	.	.	.	.	.	.	.	.	1	1	.	2	.	3	10	2	3	2	38
Pteris species	.	.	.	.	.	.	.	.	.	.	2	.	.	.	1	.	.	2	2	5	5	2	3	1	38
Dryopteridaceae																											
Dryopteris	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	1	.	3	4	5	5	3	2	3	33

CHAPTER 4

Vegetation and climate dynamics in Khyber Pakhtunkhwa, north-western Pakistan, inferred from the Kabal Swat pollen record during the last 3300 years

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Abstract

We present a pollen based palaeoenvironmental reconstruction of the past 3300 years in Kabal Swat valley of the Hindu Kush mountains, in the northwestern Khyber Pakhtunkhwa Province of Pakistan. In total, 38 samples taken from a 150 cm long radiocarbon dated sediment core were analyzed by pollen for studying the vegetation history of the area. Only the upper 76 cm of the core with 20 samples recording the last 3300 years had sufficiently preserved pollen. Conifers like *Pinus, Picea, Abies, Cedrus, Taxus* and herbs belonging to Poaceae, Cyperaceae and Amaranthaceae were found consistently throughout the period with varying abundances. Vegetation reconstruction revealed that subtropical Cyperaceae and Poaceae dominated over the conifers from 3300 to 300 cal yr BP. The decrease in herbaceous vegetation from 2400 to 1500 cal yr BP and again its increase from 1500 to 1200 cal yr BP, points to the respective shrinkage and spread of grassland boundaries in Kabal Swat valley, and also suggest that the area went through respective wet-cool and dry-warm periods. Herbs were mostly abundant in most samples from 900-300 cal yr BP. This change can be attributed to a more pronounced impact of widespread deforestation, agricultural activities and to a drier summer climate. Evergreen trees and shrubs such as Oleaceae, Myrtaceae, Moraceae species, *Juglans* and *Dodonaea* dominated and were consistently present from 2400 cal yr BP to the present. Conifers like *Pinus*, *Taxus*, *Picea*, *Abies* and *Cedrus* were frequent in the study area from 300 cal yr BP to the present. These conifers mostly occur in the mixed coniferous forests at higher elevation in the alpine area today.

Key words: Holocene vegetation, climate dynamics, pollen record, Kabal Swat, Khyber Pakhtunkhwa Pakistan, Hindu Kush Himalaya, Indian monsoon

Introduction

Little information is available about the past vegetation and climate changes of Pakistan. Pakistan is susceptible to the Indian monsoon system and is subject to the changing vegetation cover, ongoing desertification and climate change (Claussen et al. 2003). Important progress in the investigation of the Holocene climate in the monsoon regions has been made during the last few decades, based on evidence from cave deposits, speleothems, lake sediments, peat deposits and loess–palaeosoil sequences (An et al. 2000; Hong et al. 2005; 2010; Wang et al. 2010; Chen et al. 2014). Despite the availability of information received from numerous geological studies conducted in Pakistan (Waagen, 1882;1885; Noetling, 1901; Balme, 1970; Pakistani–Japanese Research Group, 1985; Wardlaw and Pogue, 1995; Mertmann, 2003; Sultan, 2004; Alam, 2008; etc.), yet only a few palaeoecological studies have been conducted so far e.g. in Karachi coast near the Arabian Sea (von Rad et al., 1995; Schulz et al., 1996; Ivory and Lézine, 2009) and in the salt range of Pakistan (Schneebeli-Hermann et al., 2014). To infer vegetation history and palaeoclimate during the last glacial–interglacial cycle from records in onshore areas, a study was conducted in southern Pakistan near the coast of the Arabian Sea by the cooperative German–Pakistan project PAKOMIN (Pakistan Oxygen Minimum, von Rad et al., 1995; Schulz et al., 1996). The aim was to analyze the pollen and spore abundance and floral diversity changes in a continuous, high resolution, undisturbed gravity core derived from the present-day oxygen minimum zone (OMZ) of the Indus continental slope (Ansari and Vink, 2006). This joint project provided the first, uninterrupted 30,000 year long record of vegetation and climate of the southern Pakistan hinterland near the Arabian Sea. The analysis of the core and interpretation revealed the recognition of (1) greater glacial aridity than during the following postglacial period up to present-day (2) a downslope retreat of the Himalayan subalpine and

deciduous tree zone during the Last Glacial Maximum (LGM) to the Younger Dryas cold interval (3) a marked change to early Holocene humid conditions.

Beside these few studies, that have mostly been conducted on the extreme southern side of Pakistan near /on the shore of the Arabian Sea (0 m a.s.l.), the first study conducted on northern Pakistan focused primarily on the Neogene mammal faunas that include a family of hominoids, the Ramapithecidae (Badgley and Behrensmeyer, 1980). In the second study, an attempt was made to trace the early human impact in the upper forest ecotone of Hindu Kush-Himalaya (36°23' N/73°07' E) at the Shukan site (Miehe et al., 2009). The present work is the third overall palaeoecological study of the area. Our research area lies in South Asia whose pollen database is yet to appear while the sampling sites covered in the East Asian pollen database (EAPD) include China, Mongolia, the Russian Far East, Vietnam, Cambodia and Thailand (Zheng et al., 2014). To our knowledge there is no published work on the vegetation history of the northwestern side of Pakistan, lying in one of the world's most important mountain system, the Hindu Kush mountain range. This study is the first record on the northwestern side of the lower Hindu Kush mountains for reconstructing the vegetation history of the Kabal Swat area (750 m a.s.l.) in the Khyber Pakhtunkhwa Province of Pakistan.

In the Swat district and surrounding areas, several studies have contributed to the knowledge of woodland types from low elevated subtropics to the high mountains (Beg and Khan, 1984; Hussain et al., 1992; Hussain et al., 1995; Ahmed et al., 2010; Rashid et al., 2011; Sher and Al Yemeni, 2011; Ilyas et al., 2012; Khan, 2012; Akhtar and Bergmeier, 2015). With its varying range of different climate and vegetation zones, with mountain forest and alpine vegetation above the tree line, Swat valley is the most suitable area for reconstructing the

vegetation dynamics of the past and studying the influence of climate change on vegetation is of particular interest.

The glaciers in the Himalayas are receding faster than in any part of the world and the likelihood of their disappearance by the year 2035 is very high at its current rate (Husnain et al., 2005). This glacier recession will be observed more in the western Himalayas as the contribution of snow to the runoff of major rivers on the western side is about 60 - 70% compared to only 10% on the eastern side (IPCC, 2001). The high elevated mountain region in the Himalayas and Hindu Kush mostly receive winter rains, while the low elevated region mostly receives summer rains (Sheikh and Manzoor, 2004). The winter precipitation is from December to March due to the western disturbances passing along the path between 30 - 60°N, whereas monsoon precipitation in the summer are caused by lows and depressions developing in the Arabian Sea and Bay of Bengal from July to September (Sheikh and Manzoor, 2004). The eastern part of the Hindu Kush becomes more similar to the Himalayas in terms of climate and flora, thus most bio-geographers call it the Hindu Kush-Himalaya. The forests of the Hindu Kush that come under the influence of the monsoon are represented by *Pinus wallichiana*, *Pinus roxburghii*, *Cedrus deodara*, *Picea smithiana* and *Abies pindrow* while important indicator species of the Himalayan range are *Abies pindrow*, *Pinus wallichiana*, *Fragaria nubicola*, *Rhododendron* species, *Viola* and *Clematis* species (Khan, 2014). Floristically, the vegetation of the western and northern Himalayas becomes similar to the Hindu Kush and the monsoon belt of the Karakorum in terms of species composition and richness, perhaps owing to geologic, physiographic and climatic correspondence. Characteristic species of this transitional belt of the western Himalaya, the southern Karakorum and the eastern Hindu Kush are *Cedrus deodara*, *Picea smithiana*, *Ephedra gerardiana*, *Thymus linearis* and *Cotoneaster microphyllus* (Khan, 2014).

In June and July, the equatorial trough of the Inter Tropical Convergence Zone (ITCZ) moves northward. The trade winds swing around as it crosses the equator becoming the south west Monsoon into Asia. Monsoons are the most intense in Asia which has the largest land mass building up an intense low pressure system each summer (Sarfaraz, 2007). The Himalayan Mountains cause the ITCZ to move much further north than anywhere else in the world. As the air flows northward it picks moisture from the Indian Ocean. So the Monsoon brings torrential rain to Asia in July and August. Monsoons in the subtropical Hindu Kush and Himalayan mountains are the result of the seasonally reversing tropical winds bringing dry and wet seasons. Monsoon rains on these mountains resulted in rich flora famous for its unique endemic and threatened biodiversity, forests, wildlife and immense unexplored genetic resources, due to which the western Himalayan moist temperate Ecoregion has been included in the Global-200 priority ecoregion for conservation (Ahmad, 2014).

In this paper we present the first assessment of vegetation and climate history spanning the last 3300 years of the Holocene based on palynological results of Kabal Swat. Our aim is to obtain new insights into the past vegetation patterns of Swat valley and surrounding northern areas and hence derive information on potentially responsible climatic factors.

Setting

Pakistan is located in southwest Asia just above the tropic of cancer, extending northeast to southwest from latitude 37° N to 23° N and longitude 60° E to 75° E (Fig.1a). Khyber Pakhtunkhwa is one of the four provinces of Pakistan, located in the north west of the country (31°49'N, 70°55'E to 35°50'N, 71°47'E) covering an area of 74,521 km^2. In this north western province, the Swat valley is located between the Hindu Kush Mountains of Afghanistan to the west, Indian Himalayas to the east and Karakorum Mountains towards the north separating Pakistan from the Tibetan Plateau of China. The exact point of our coring site is

located in the western part of Swat valley in Kabal village (34°66'N 72°13'E) at an elevation of 750 m above sea level (Fig.1b). Kabal (34°66'N and 72°17'E) is a Tehsil of district Swat

Figure 1a) Map of Pakistan shown in yellow color surrounded by other countries of the region. The coring site is located in Kabal village (shown as red star in the map).

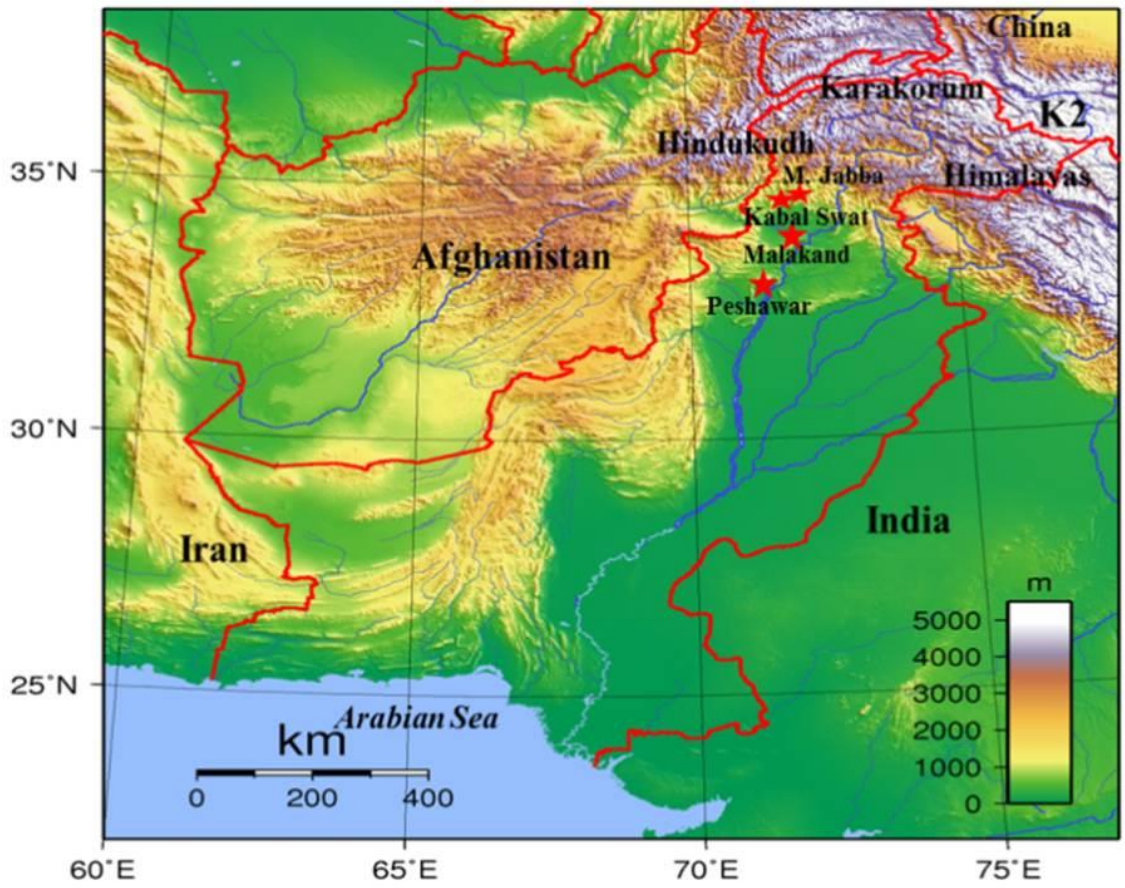

Figure 1b) Topographic map of Pakistan; Vehicle track to the research area shown by red stars (comprising a 200 km long elevational gradient starting from the plain Peshawar valley (275 m) crossing Malakand hills (500 m a.s.l) reaching to Kabal village in Swat valley (750 m). Beyond Kabal, the mountains reach up to Malama Jabba (3000 m). The area is surrounded by China in the north, Afghanistan on the west, Iran on the south west, Arabian sea in the south and India on the east (changed after Sadalmelik, 2007).

lying between the foothills of Hindu Kush and the Himalayas in the northwestern Khyber Pakhtunkhwa Province of Pakistan. Kabal is located 10 km northwest of Mingora (Swat) covering an area of about 400 km^2 between latitude 34°45'N to 35°55'N and longitude 72°08'E to 72°50'E, whose elevation varies from 700 to 2500 m. Being a part of the high altitude Hindu Kush Himalayan region, Swat valley comprises a diverse set of biophysical, ecological and socio-economic characteristics (Qasim et al., 2011).

Pakistan is home to five significant mountain systems lying in different bio-climatic zones. These five mountain systems include the Hindu Kush, the Himalayas, the Karakorum, Kirthar and the Suleiman ranges, all contributing to a high plant biodiversity. Our research area (Kabal Swat valley) is encircled by the Hindu Kush mountain systems. The mountains of the Swat valley and adjoining areas consist of rock units representing the Kohistan Arc sequence, the Tethys oceanic lithosphere and the Indo-Pakistan plate sequence. The Kohistan Arc sequence consists essentially of late Jurassic-Cretaceous and Tertiary plutonic and metamorphic plutonic, volcanic and sedimentary rocks (Arif et al., 2011; Akhtar and Bergmeier, 2015). Swat area consist of Precambrian-Cambrian basement (Arif et al., 2011). The basement rocks in Swat are overlain uncomfortably by Phanerozoic metasedimentary rocks of the Alpurai group (DiPietro, 1991; DiPietro and Lawrence, 1991; DiPietro et al., 1993; Arif et al., 2011). The eastern Hindu Kush forms a triangular ecotone which delimits the Irano-Turanian, the Sino-Himalayan, and the Central Asiatic floristic regions (Walter u. Breckle, 1991; 24).

Climate

Pakistan lies in the northern hemisphere just above the tropic of cancer in the western part of the monsoon climate zone with subtropical climate, experiencing extreme temperature variations. As a result of the climatic impact, elevational gradient and the surrounding topographic conditions, sediments have accumulated at Kabal site since the early Holocene, as revealed from the radiocarbon dating results of our samples. Pakistan has four well marked seasons: 1. Cold (November-February) 2. Hot (March to mid-June) 3. Monsoon (mid-June to mid-September) 4. Post monsoon (mid-September to October). Summer season is extremely

Table 1

Meteorological data of the areas south and north of Kabal Swat in Khyber Pakhtunkhwa Province.

1. Plain zone	**coordinates**	**Elevation m a.s.l**	**ARF(mm)**	**RH %**	**MAT(°C)**
Charsadda	34°80'N 71°43′E	276	> 127	45-50	> 20
Mardan	34°12'N 72°20'E	285	> 127	45-50	> 20
2. LMM zone					
Malakand	34° 33'N71° 55'E	500	> 889	55-60	> 20
Batkhela	34°10′N 71°53′E	650	> 889	55-60	> 20
3. Swat zone					
Kabal village	34°66'N 72°13'E	750	> 1016	60-65	> 15
Fiza gut hill	34°79'N 72°38'E	1150	> 1016	60-65	> 15
4. UMM zone					
Malam Jabba	34°81'N 72°57'E	2600	> 1016	60-65	> 15

ARF: Annual rainfall; RH: Relative humidity; MAT: Mean Annual temperature; LMM: Lower mountain Malakand; UMM: Upper mountain Malam Jabba

hot and the relative humidity ranges from 25 to 50% (Pakistan meteorological department technical report No: PMD-22/2009). The monsoon rainfall is in summer (July-August) and the winter rainfall (December-January) is due to western systems. Except the southern slopes of the Himalayas and sub mountain region, where the annual rainfall ranges from 760 to 2000 mm, most of the country is arid to semi-arid. 75% of the country receives less than 250 mm rainfall, while 20% of the country receives only 125 mm (Pakistan meteorological department technical report No: PMD-22/2009). The temperature varies from the lowest -6 °C in winter (mid-December) in the north, to the highest 50 °C in summer (mid-June) in the center and south of the country. A six-decade study (1931-1960 and 1961-1990) conducted on the climatic normal of Pakistan, revealed that there was cooling over northern and southeastern Pakistan due to increase in the monsoon rainfall and cloudiness (Kruss et al., 1992). An analysis of the reconstructed long term temperature time series from 1876-1993, show the presence of large variability in temperature and warming since the beginning of the last century with a total change of 0.2 °C (Singh and Sontakke, 1996). According to the

Pakistan meteorological department of the federal government, there is a decreasing trend in average annual rainfall (-1.18 mm/decade) all over the country, which may be attributed to the presence of drought period during 1998-2001. Details of the general climate of the area and surrounding high and low elevated areas are provided in Table 1. These details are based on the 30 years' average (1981-2010) of daily maximum-minimum temperatures and rainfall data. The data has been obtained from the regional meteorological center (RMC) Peshawar, recorded at 0300 GMT (Greenwich mean time), measured at 0800 GMT (Meteorological department of Pakistan. ISO 9001: 2008. http://www.pmd.gov.pk/.

Vegetation

Almost 40% of Pakistan's natural forests are located in the north western Khyber Pakhun-khwa Province (Ahmed and Mahmood, 1998; Akhtar and Bergmeier, 2015). The present-day forest areas of Kabal Swat in northwestern Pakistan are located in the Hindu Kush Mountain range, west of Pakistani Himalayas and are under the influence of the monsoon. Representative species are conifers like *Abies pindrow*, *Cedrus deodara*, *Picea smithiana*. *Pinus roxburghii* and *Pinus wallichiana*. The eastern part of the Hind Kush Mountains towards Hazara division is more similar to the Himalayas in terms of climate and flora. The Kabal valley of Swat experience overgrazing, deforestation, logging and clearing of land for terrace cultivation which is the major threats responsible for the overall degradation of forests in this zone (Hussain et al., 1997; Sher et al., 2010; 2011). Natural coniferous forests of *Pinus*, *Abies*, *Cedrus* and *Picea* are also under the heavy social and economic pressure of logging (Siddiqui et al., 1999). Our paper dealing with vegetation and modern pollen rain in Kabal Swat (Jan et al., 2015), revealed that notable taxa of the present day forests are: *Abies pindrow*, *Aesculus indica*, *Ailanthus altissima*, *Amaranthus viridis*, *Artemisia scoparia*,

Cedrus deodara, Chenopodium album, Cynodon dactylon, Dalbergia sissoo, Datura stramonium, Diospyros kaki, Dodonaea viscosa, Euphorbia wallichii, Indigofera heterantha, Juglans regia, Juniperus sp., Otostegia limbata, Picea smithiana, Pinus roxburghii, Pinus wallichiana, Platanus orientalis, Polygonum plebjum, Quercus semecarpifolia and others.

Material and Methods

Site selection for coring

Most parts of the north western Khyber Pakhtunkhwa Province in Pakistan have almost no suitable terrestrial sites or even environmental archives for palaeoecological studies probably due to dry and warm climatic conditions. The Kabal Swat site (34°66'N 72°13’E) was selected after a long and tedious survey spanning over the entire month of September 2012.The total depth of this core was 150 cm, taken with a Russian corer in 3 segments of 50 cm length. The cores were covered with splitted PVC tubes and plastic foil after coring, transported to Göttingen Germany and stored in the cold room (4°C) at the Department of Palynology and Climate Dynamics, University of Göttingen.

Radiocarbon dating

For radiocarbon dating, 4 samples were dated in the laboratory of AMS (Accelerator Mass Spectrometry) Radiocarbon dating and Cosmogenic cosmogenic what? in the Department of Geosciences, National Taiwan University (NTUAMS Lab). Three bulk sediment samples taken at a depth of 55, 82 and 148 cm along the core with a fourth wood charcoal sample at 116.5 cm were used for dating. A linear age-depth model (Fig. 2) was fitted with the R software package CLAM (The R Foundation for Statistical Computing 2013), using linear interpolation. Ages were calculated every 1 cm, from 0 cm (top) to 148 cm (bottom),

weighted by the calibrated probabilities (weights =1) and calculations at 95% confidence ranges with 1000 iterations and the goodness-of-fit being 1.93 (-log).

Sample processing and data analysis

For the extraction of pollen and spores, 38 sediment samples (each 0.5 cm^3) were taken from the core every 4 cm, starting from the surface (0 cm) down to 148 cm. For sample processing the standard laboratory method was applied (Faegri and Iversen, 1989). The samples were kept in HF for three days due to large silicate contents. One *Lycopodium clavatum* marker tablet, containing 18583 ± 762 spores, was added to each sample for calculation of the pollen concentration and influx values. The extracted pollen samples were mounted in glycerin for pollen identification and counting. The pollen grain identification was carried out under a light microscopy with 400x and 1000x magnification. Samples were counted to a minimum of 300 pollen grains. In total 18 samples (80-148 cm) had to be excluded from the data analysis, since pollen grains were absent in these samples. Due to very low pollen concentration and insufficient preservation of grains from 64 cm downwards, only a smaller number of pollen grains could be counted. 246 pollen grains could be counted from sample at 64 cm, 198 at 68 cm, 202 at 72 cm and 134 pollen grains at 76 cm.

Pollen identification and illustration

Pollen and spore identification is based on large reference collections at the Depart-ment of Palynology and Climate Dynamics, University of Göttingen. The identification is supported by morphological descriptions provided in different atlases (Fujiki et al., 2005; Tissot et al., 1994; Nayar, 1990) and other relevant publications (Huang, 1972; Seetharam, 1985; Bonnefille, 1999). The pollen percentage diagram was compiled with the program TILIA

(TILIA 1.7.16, Grimm, 2011) illustrating the most abundant and ecologically important taxa. Pollen taxa were assigned to their respective vegetation groups such as conifers, trees/shrubs and herbs. The pollen diagram is based on the pollen sum which includes all pollen taxa excluding the fern, moss, fungal spores, and unidentified taxa. All samples from 76 cm downward without pollen grains were omitted from the pollen diagram. The CONISS (Constrained cluster analysis by sum-of-squares) method was used for the identification of the pollen zones. Samples without pollen were excluded from the CONISS to avoid biases.

Multivariate data analysis

Multivariate data analysis was carried out on pollen percentage data using the program Canonical Community Coordination Package (Canoco) 5 for Windows (ter Braak and Smilauer, 2012). Pollen taxa present in at least 2 samples with a value of >1% were included in the analysis to decrease the effect of rare taxa. To assess the vegetation and climate dynamics of the area during the entire period, less frequent taxa from all age zones had to be ignored in the PCA, to ease assigning of the dominant herbs, shrubs, trees and conifers to the different age zones. The detrended correspondence analysis (DCA) revealed a gradient length of 1.921 and the response data was not compositional, hence a linear model was fitted to the data. We applied a principle component analysis (PCA) as recommended by Leps and Smilauer (2003) for data sets with short environmental gradients. The species data was standardized and log transformed. The ordination diagram was centered by species and the scaling is focused on the distance between groups i.e. conifers, trees/shrubs and herbs.

Results

Stratigraphy and chronology

Because of the topographic and climate conditions sediments have accumulated at Kabal site since the early Holocene as revealed by the radiocarbon dating of samples. From the lowest

150 cm upwards till 100 cm the core is uniform yellowish-grey in color, composed of silty sand having no sharp or gradual boundary with almost no organic matter. The silty material from 100 up to 77 cm is dark blackish grey in color mainly composed of silicate material. The clayey, silty sediment from 77 cm upwards until 55 cm is yellowish black and more compact than the upper part. There are less plant remains and less organic content from 77-55 cm than the lower part of the core from 100-77 cm. The upper 55-0 cm is a uniform stratigraphic structure, having no lateral or vertical adjacent units and no sharp boundary. From 48 cm upwards until 25 cm, the color of the sediment is yellowish grey and from the morphology this part seems to contain a higher content of finely decomposed matter with few plant roots or other remains. The top 25 cm of the profile is mainly black to grayish black and

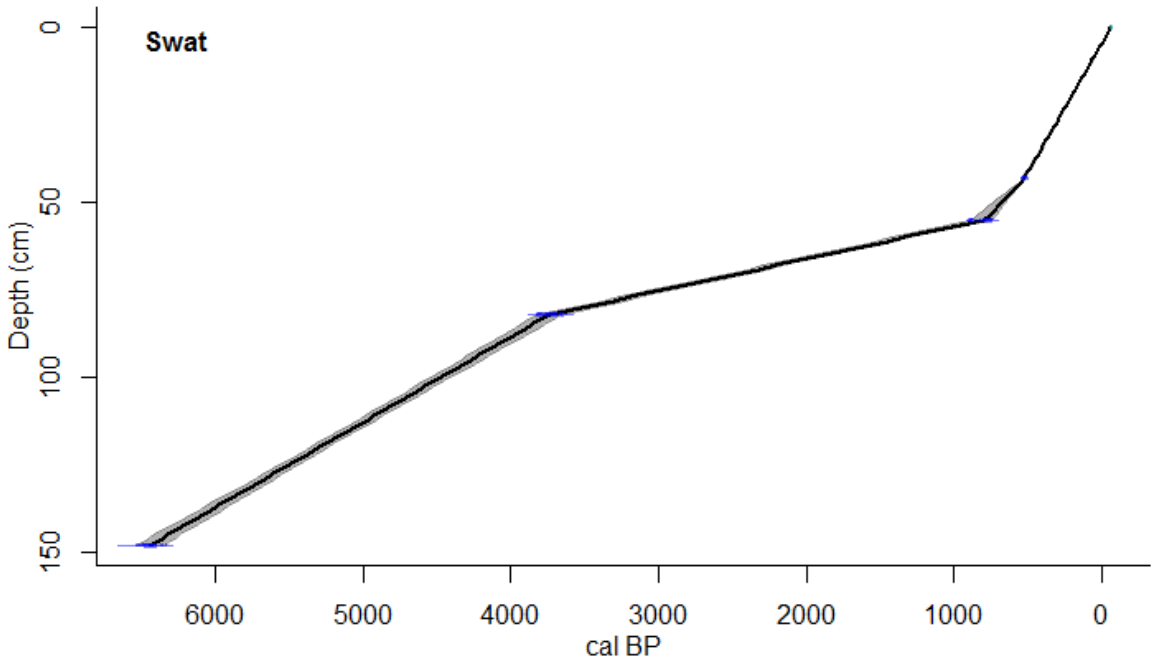

Figure 2: Age-depth relationship (linear interpolation, calibrated years BP/core depth in cm) based on 4 radiocarbon dates and the surface sample (-62 cal yrs BP).

very rich in organic matter with no stratification. Here the sediment texture is composed of granules loosely bounded together, leaving pores for pollen re-location and biological activity and contain abundant root growths. There are occasional charcoal particles and the texture of the sediment is clayey.

Four radiocarbon dates have been used to establish a chronology for the Kabal Swat core (Table 2). The age-depth model (Fig. 2) is not really linear but reveals almost a linear relationship (R^2 = 0.921) between core depth and the calibrated age of 8103 yr. BP until about (~148 cm depth). The surface of Kabal Swat core (0 cm) is of modern age as pollen of present day conifers like those of *Pinus, Picea* and *Abies* are abundantly present in the uppermost sample. The base of the core (148 cm) dates back to the early mid Holocene with an age of 8103 ± 107 cal yr BP.

Table 2. Radiocarbon dates for the Kabal Swat record. OM = organic matter

Sample Id	Lab code	Depth (cm)	Material	C^{13}/C^{12} ratio	^{14}C Age (yr. BP)	Age (cal. yr. BP)
SK- 055	NTUAMS-333	55	Wood	0.0103	781 +- 5	883
SK- 82	NTUAMS-578	82	Bulk OM	0.0101	3461 +- 25	3756
SK- 116	NTUAMS-334	116	Charcoal	0.0101	4932 +- 35	5664
SK- 148	NTUAMS-579	148	Bulk OM	0.009	7266 +- 112	8103

Pollen diagram

The pollen percentage diagram (Fig. 3) shows pollen taxa assigned to the different vegetation groups such as conifers, Trees/Shrubs and herbs. Our published paper on the pollen rain transect study of this area reveals that the source of the pollen is local, originating in the surrounding mountain vegetation with *Pinus, Picea, Cedrus, Abies*, Cyperaceae and Poaceae species (Jan et al., 2015).

Pollen zone description

Zone (Kabal Swat KS -1 76–70cm, 3300–2400 cal yr BP): The predominance of herb pollen grains include Cyperaceae (7-10%), Poaceae (7-9%), Amaranthaceae (4-6%), *Artemisia* (0-4%) and Lamiaceae (0-3%). Less frequent conifer pollen grains include *Picea* (4-6%), *Pinus* (2-6%), *Abies* (1-4%), *Cedrus* (2-3%) and Cupressaceae (0-3%). Other arboreal and shrub pollen types are Bignoniaceae (0-4%), Chloranthaceae (1-3%) and Moraceae (2-3%). The zone is characterized by low pollen concentration values ranging from 8314 to a maximum of 20435 grains/cm^{-3}. The average value of the pollen influx is 187 pollen/cm^{2}/yr. with a sedimentation rate of 0.009 cm/yr (Fig. 4).

Zone KS -2 (70- 55 cm, 2400–900 cal yr BP**).** This period is also dominated by herb pollen grains including Poaceae (8-15%) and Cyperaceae (2-9%) with varying abundances, as well as *Artemisia* (4-6%), Acanthaceae (1-4%) and Amaranthaceae (3-6%). The abundance of Poaceae pollen remains unchanged from 2400 to 1500 cal yr BP but increases notably from 1500 to 900 cal yr BP. Other taxa found are Conifers like *Taxus* (1-6%), *Picea* (2-5%), *Abies* (0-4%) and tree/shrub species include *Quercus* (1-5%), *Juglans* (2-4%), Bignoniaceae (2-4%), Chloranthaceae (3 -5%) and *Myrica* (1-3%). Fungal spores clearly increase in the samples as compared to the previous period zone. This period is also characterized by a low pollen concentration of up to 43185 grains/cm^{-3} and a small average value of the pollen influx (397 pollen/cm^{2}/yr)**.** The sedimentation rate is 0.009 cm/yr.

Zone KS-3 (55 to 46 cm, 900-600 cal yr BP**).** Herb pollen of this zone are Poaceae (11-14%), Cyperaceae (5-9%), Amaranthaceae (3-6%), Brassicaceae (2-6%), Rosaceae (1-5%), *Artemisia* (2-5%), Acanthaceae (1-4%), Euphorbiaceae (1-3%) and *Polygonum* (1-3%). Conifers include *Taxus* (5-10%), *Pinus* (2-6%), *Picea* (3-6%), *Cedrus* (3-6%) and *Abies* (3-4%). The different trees and shrub pollen are *Juglans* (4-7%), *Quercus* (5-6%),

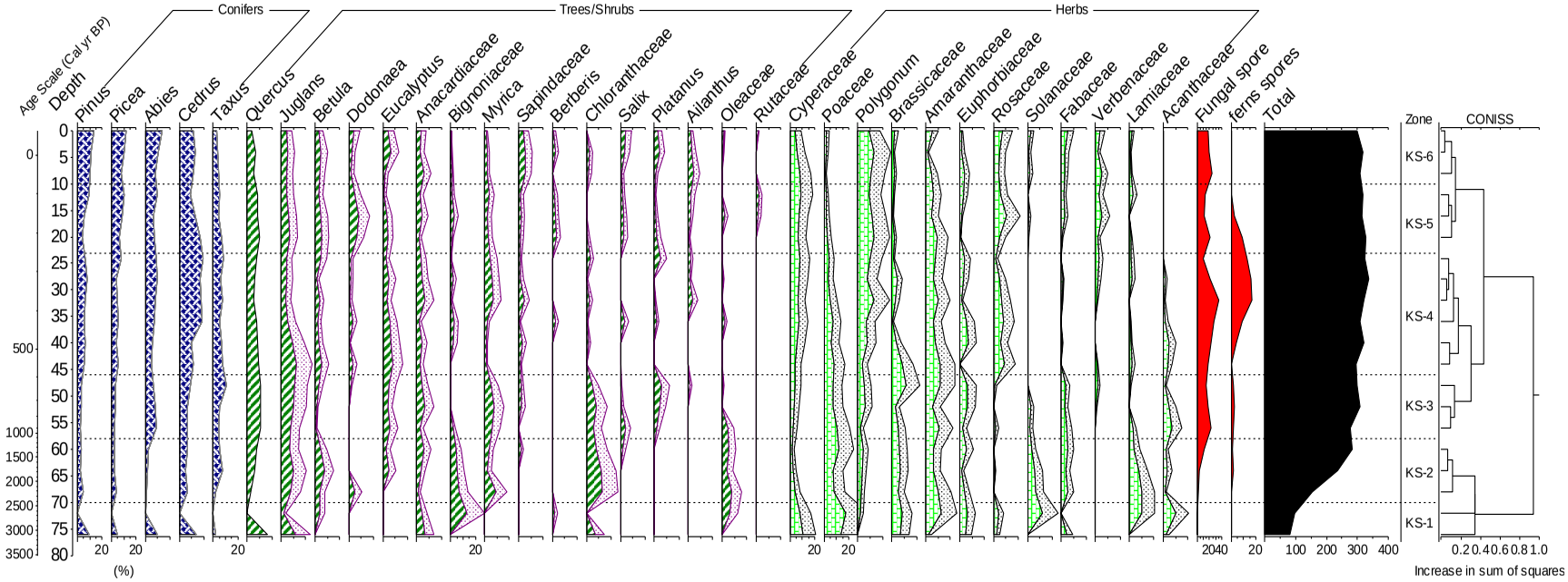

Figure 3: Pollen diagram of core Kabal Swat in the Hindu Kush (Khyber Pakhtunkhwa province north western Pakistan). Cluster analysis based on the percentage data for the most important taxa grouped into Conifers, Trees and shrubs, Herbs and spores. The age scale is based on calibrated years before

Chloranthaceae (0-4%), Anacardiaceae (1-4%), Myrtaceae (2-4%), *Myrica* (1-4%), *Betula* (0-3%), *Platanus orientalis* (0-3%) and Ericaceae (1-3%). Pollen concentration values increased to a maximum of 66713 grains/cm^{-3} during this period and the pollen influx values increased to 2917 pollen/cm^2/yr. The sedimentation rate is 0.004 cm/yr.

Zone KS -4 (46-23cm, 600–300 cal yr BP). This period is still dominated by herb pollen like Poaceae (7-12%) and Cyperaceae (5-11%) but conifers like *Cedrus* (5-10%), *Pinus* (5-9%), *Taxus* (6-8%), *Picea* (4-6%) and *Abies* (3-5%) are also abundant. Other herbs include *Artemisia* (1-4%), Brassicaceae (0-5%), Euphorbiaceae (1-4%), *Polygonum* (2-7%), Amaranthaceae (3-6%) and Rosaceae (3-5%). Among trees and shrubs, pollen grains of *Juglans* (2-7%), *Quercus* (3-5%), Myrtaceae (2-4%), Anacardiaceae (1-4%) and *Myrica* (1-4%) are found. The pollen concentration values in the different samples representing this period vary from 66713 to 83652 grains/cm^{-3} while the average pollen influx value is 6146 pollen/cm^2/yr. The sedimentation rate is 0.007 cm/yr.

Zone KS-5 (23-12 cm, 300–100 cal yr BP). This zone represents a time span of 200 calibrated years. Pollen of the different herbs found in this zone include Cyperaceae (7-14%), Poaceae (2-8%), *Artemisia* (1-3%), Verbenaceae (1-3%), Amaranthaceae (3-5%), *Polygonum* (5-7%) and Rosaceae (1-4%). Conifers found include *Taxus* (5-9%), *Picea* (8-14%), *Abies* (4-11%), *Cedrus* (5-11%) and *Pinus* (7-10%). Tree pollen include *Quercus* (4-6%), *Juglans* (2-4%), *Betula* (2-3%) and *Platanus orientalis* (0-3%). Shrubs include Bignoniaceae (1-3%), *Dodonaea* (1-4%) and *Myrica* (1-3%). This period is characterized by high pollen concentration values in different samples ranging from 77223 to a maximum of 89632 grains cm^{-3} while the average pollen influx value is equal to 6586 pollen/cm^2/yr. The sedimentation rate is 0.007 cm/yr.

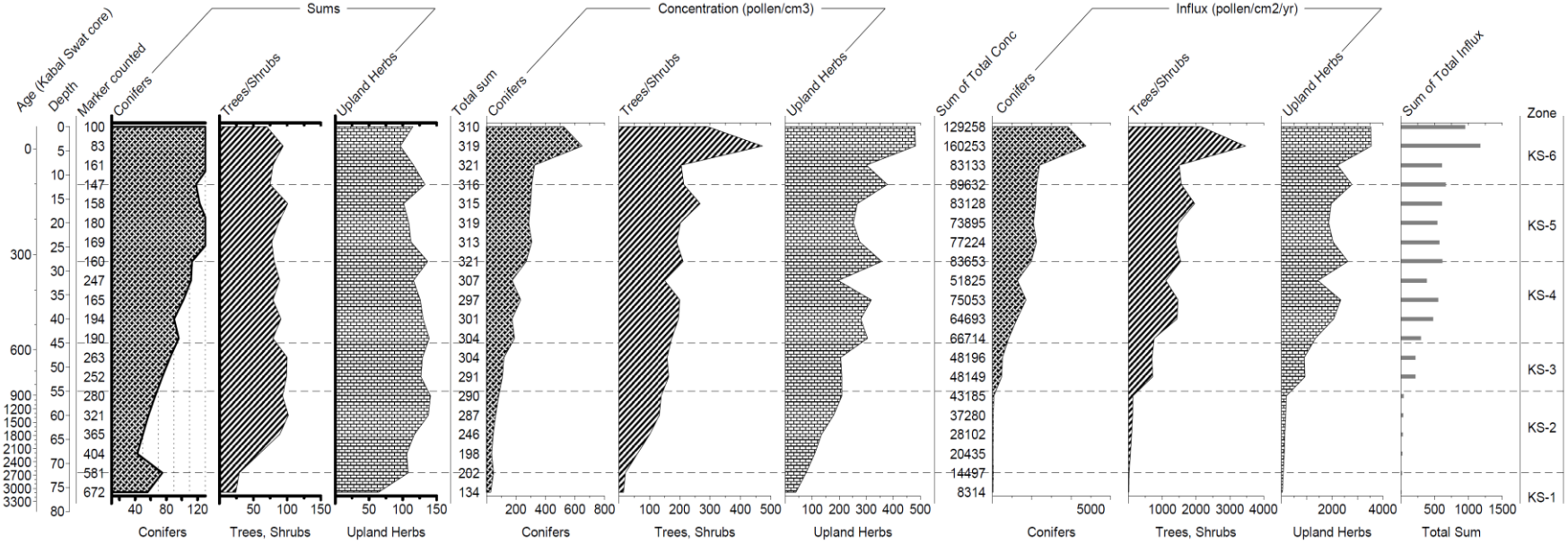

Figure 4: Percentage sums of ecological groups (Conifers, Trees/ shrubs (TRSH and upland herbs-UPHE) records of pollen concentrations (pollen /cm^3) and pollen influx (pollen /cm^2/yr).The pollen sum of the last three samples equals only 198, 202 and 134 pollen respectively.

Zone KS -6 (12-0 cm, 100– -62 cal yr BP**).** This is the top zone representing the most modern pollen spectra of the Kabal Swat area. Conifers constitute the most abundant taxa including *Pinus* (12-19%), *Picea* (8-12%), *Abies* (4-9%), *Cedrus* (6-7%) and *Taxus* (3- 4%). The different tree species include *Quercus* (2-4%), Chloranthaceae (1-4%), *Juglans* (2-3%), Myrtaceae (1-3%), *Betula* (2-3%), Anacardiaceae (2-3%). Shrubs are represented by Sapindaceae (2-3%) and Meliaceae (1-4%). Pollen grains of different herbs found in this zone include Cyperaceae (8-12%), Fabaceae (1-3%), Verbenaceae (1-3%), *Artemisia* (1-4%), Amaranthaceae (1-3%) and Polygonum (5-7%). Maximum pollen concentration is 129258 grains/cm^{-3} and maximum pollen influx is 9497 pollen/cm^{2}/yr. The sedimentation rate is 0.007 cm/yr.

Multivariate data analysis

The PCA ordination diagram (Fig. 5) shows a clear division of trees/shrubs on the lower left and herbs on the lower right quadrants while the conifers are present in the upper left quadrant. Only those 18 taxa are displayed which are present up to at least 5% on average, in each of the respective six age zones. The calibrated ages for all the six age zones from base to the top of the core, are respectively represented by symbols (KS-1 to K-S6, KS for Kabal Swat). The lower right quadrant shows that the herbs belonging to Poaceae, Cyperaceae, Brassicaceae, Fabaceae, Amaranthaceae and Asteraceae (*Artemisia*) are mostly concentrated between 900- here is something missing cal yr BP, represented by their respective age symbols (KS-3 and KS-4). The lower left quadrant shows that shrubs/trees belonging to Oleaceae, Myrtaceae, Bignoniaceae, *Juglans*, *Dodonaea* and *Sapindaceae* are consistently present from 2400 cal yr BP to the present represented by the symbols (KS2-KS6). The upper left quadrant shows that conifers including *Pinus*, *Taxus*, *Picea*, *Abies* and *Cedrus* are predominantly present during the fifth age zone (300-100 cal yr BP represented by KS-5)

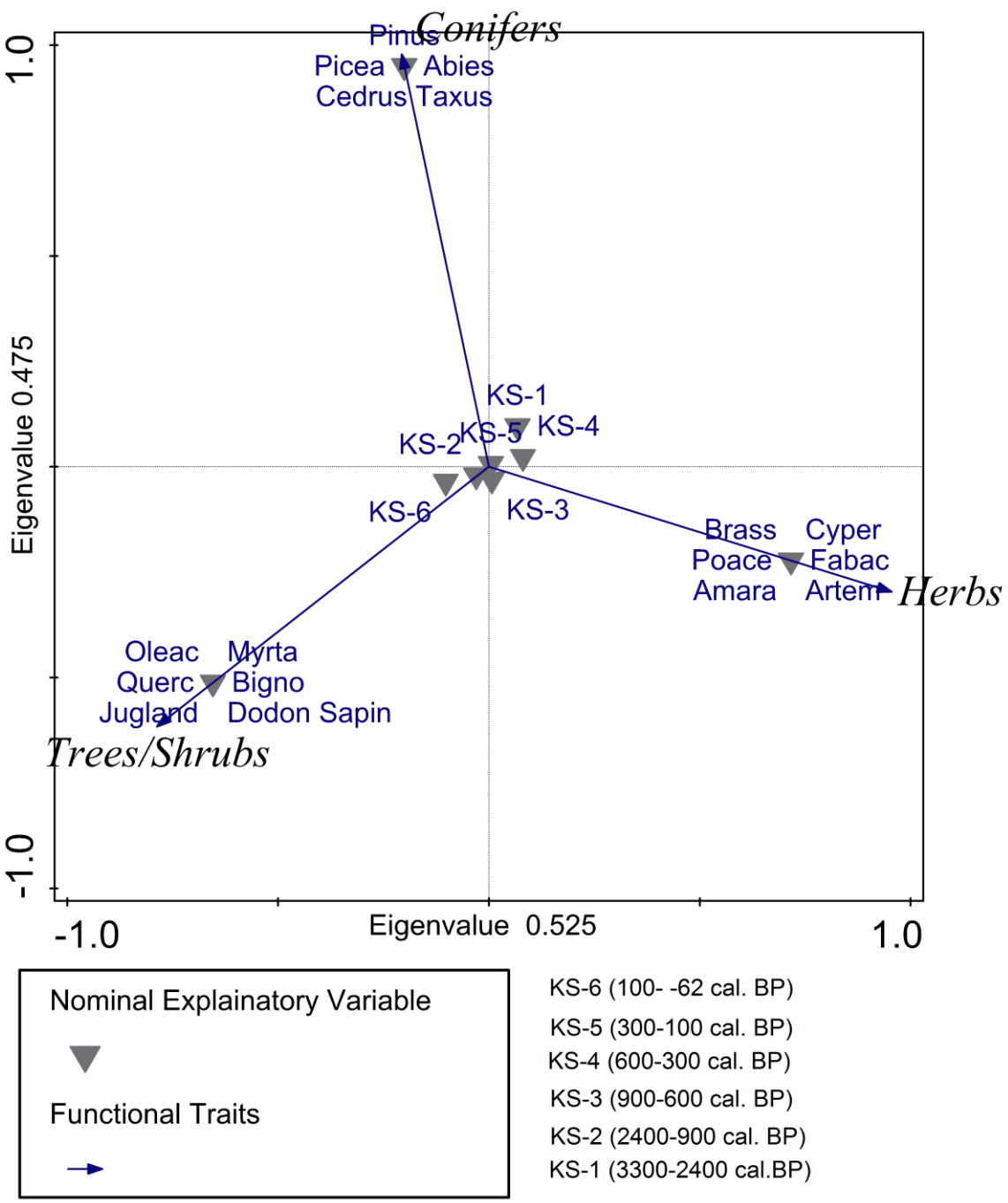

Figure 5: Results of the PCA of square root transformed percent pollen data from the Swat Kabal region in the Khyber Pakhtunkhwa province of Pakistan. KS-1 = represents the oldest age interval of the core (76-70 cm; 3300-2400 cal yr BP.). KS-2 = represents the second age interval (70-55 cm; 2400-900 cal yr BP.). KS-3 = represents the third age interval (55-46 cm; 900-600 cal yr BP.). KS-4 = represents the fourth age interval (46-23 cm; 600 -300 cal yr BP.). KS-5 = represents the fifth age interval (23-12 cm; 300 -100 cal yr BP.). KS-6 = represents the sixth age interval (12-0 cm; 100 --62 cal yr BP.).

followed by their dominance during the second age zone (2400-900 cal yr BP) and thirdly by the most recent age zone (100 cal yr BP - 2012 represented by the age symbol KS-6).

Discussion

The present-day vegetation and climate of Kabal and surrounding areas of Qalagai hills in Swat is of temperate mountain type (Champion et al., 1965; Beg, 1975; Ilyas et al., 2012). Because of the marked differences in edaphic, physiographic and local climatic conditions in different slopes at different elevations, they support different plant associations (Ahmad, 1986; Ilyas et al., 2012). The Kabal area in Swat is surrounded by mountains and majority of the pollen and spores are most likely to have originated from vegetation growing on the adjacent mountain system. Hence the pollen assemblage in Kabal area of Swat valley is likely to originate from plants having a broad spectrum of altitudes. Consequently, changes in past climate must have left a notable effect on the altitudinal occurrence of taxa and these changes are likely to be represented in the pollen record. As a result, the natural vegetation around Kabal area in Swat valley should be a reasonably sensitive indicator of the climatic conditions on the surrounding mountains at any period of time. Our results based on the pollen diagram and multivariate data analysis reveal changes in the vegetation and environmental conditions of Kabal area in Swat during the last 3300 cal yr BP. Two bulk samples (116 cm and 148 cm depths) recorded time up to 5660 and 8100 cal yr BP, respectively. But since no pollen or negligible pollen grains were found from 76 cm downwards, probably due to local effects in the sediment archive or erosion in the sediment archive or may be due to drier climatic conditions, therefore we relied on the samples till 76 cm which recorded the vegetation history up to 3300 cal yr BP.

From 3300 to 2400 cal yr BP, the presence of Poaceae shows that the Kabal area represented fairly open landscape during the early late Holocene. The dominance of Cyperaceae

represents wet swampy vegetation during this period. The pollen record shows that alpine coniferous vegetation mainly *Picea*, *Pinus*, *Abies* and *Cedrus* or mixed forests are present in the area since the last 8000 years. The vegetation is characterized by coniferous forests on the surrounding mountains while herbs like Amaranthaceae and *Artemisia* were present in the surrounding lower plains of Kabal area. The presence of both *Pinus* and *Picea* by up to 6% in the samples shows that *Picea* was growing within a few hundred meters of the sampling site as compared to the more abundant pollen producer, *Pinus*. The characterization of this period by very low pollen concentration values (up to 20400 grains/$cm^{-3)}$) and a very low value of the pollen influx (190 pollen/cm^2/yr.) shows a very weak vegetation cover during this period. The lower value of sedimentation rate (0.009 cm/yr. shows that Kabal Swat experienced lower erosion rates probably due to less rain fall on the surrounding Hindu Kush mountains.

From 2400 to 900 cal yr BP, Poaceous vegetation became more abundant than Cyperaceae, indicating that the climatic conditions became drier and the landscape remained open during this phase. Increase in fungal spores as compared to the earlier period (3300-2400 cal yr BP) also reveals a dry period. The decrease in Poaceae from 2400 to 1500 cal yr BP and again its increase from 1500 to 1200 cal yr BP show that the area went through respective wet-cooler and dry-warmer periods. Climatic change has often been cited as a determining factor in cultural changes in the context of the Harappa Civilization in the Hindu Kush-Himalayas of north-western South Asia, 2500 to 1900 cal yr BP (Madellaa and Fuller, 2006). Similar to our findings of this period, It has been reported that increase in the percentage of *Artemisia* reflects the weakening of the grazing pressure in Hindu Kush mountains (Miehe et al., 2009) and the abundance of *Artemisia* and Amaranthaceae/Chenopodiaceae increase under either cold and moist or temperate and arid conditions in northern China (Xiangjum et al., 1996). Among the conifer forest species, *Taxus* and *Abies* increased while *Picea* and *Pinus*

decreased. Our findings are also in line with Schickhoff (1995) and Miehe et al. (2009), who reported that during this period forest ecotone of southern High Asia of Hindu Kush-Himalaya forests in the south of Gilgit, were represented by *Cedrus deodara* (2400-2900 m), *Picea smithiana* (2800-3500 m), *Pinus wallichiana* (2500- 3500 m) and *Abies pindrow* (3000 to 3600 m)**.** This period is also characterized by a very low pollen concentration (up to 43200 grains cm^{-3}) and a small value of the pollen influx (400 pollen/cm^2/ yr.) showing sparse vegetation cover**.** This period also experienced low sedimentation rate (0.009 cm/yr, which again shows that Kabal Swat experienced lower erosion rates probably due to less rain falls on the surrounding Hindu Kush mountains.

From 900 to 600 cal yr BP, the decreasing vegetation of Cyperaceae coupled with the increasing Poaceous vegetation, Amaranthaceae and Brassicaceae reflects the opening of the landscape with *Taxus* and *Juglans* growing on the surrounding mountains. This change can be attributed to the drier climate and increased agricultural activities. Our findings are in line with Miehe et al. (2009), who reported that Poaceae pollen doubled and open shrub land over represented mainly by *Artemisia* and *Euphorbia* in the upper Shukran valley of Gilgat during this phase. They further reported the absence of *Pinus* and a remarkable decrease in other conifers from the upper Gilgat area during this period. However, the presence of *Pinus*, *Picea*, *Cedrus* and *Abies* in the vegetation indicates that the lower Hindu Kush Mountains were covered by coniferous forests beside the mixed forests with species of *Juglans*, *Quercus*, Chloranthaceae, Anacardiaceae, Myrtaceae, *Myrica*, *Betula*, *Platanus orientalis*. Pollen concentration values increased to a maximum of 66700 grains cm^{-3} during this period. But again the lower value of the pollen influx value (2900 pollen/cm^2/yr) and the lower sedimentation rate (0.004 cm/yr) show the stability of land cover during this period.

The period from 600-300 cal yr BP shows a wet and cooler trend in which Cyperaceae show an increase and Poaceae decreased towards the end of the period. Conifers including *Cedrus*, *Taxus*, *Picea* and *Pinus* increase which indicates the spread of coniferous forests on the surrounding Hindu Kush mountains in which ferns spread abundantly on the forest floor as it is similar to the modern vegetation of the area. The present day Hindu Kush mountains have similar vegetation to that of the western Himalayas (Wazir et al., 2008; Noroozi et al., 2008; Ali and Qaiser, 2009; Khan et al., 2012). The representation of this period by western Himalayan type herbs and tree/shrub species like *Artemisia*, Brassicaceae, Euphorbiaceae, *Polygonum*, Amaranthaceae, *Juglans*, *Quercus*, Myrtaceae, Anacardiaceae and *Myrica* show that the climate of the Kabal Swat during this period was warmer and drier compared to the upper northern Karakorum mountains, but colder and wetter as compared to the lower southern plains of Peshawar valley. During this period the pollen concentration values (66700 to 83650 grains/cm^{-3}), the pollen influx value (6150 pollen/cm^{2}/yr) and the increased sedimentation rate of 0.007 cm/yr reveals that the abundant pollen producing pollen conifers spread on the surrounding mountains but the vegetation cover weakened which resulted in increased erosion from the upper mountains down to the valley.

From 300 to 100 cal yr BP, the abundant increase of Cyperaceae and conifers like *Abies*, *Picea* and *Pinus* on one hand and the simultaneous decrease of Poaceae show that the climate showed a wet cooler change during this phase. The drastic decrease in the herbaceous vegetation show that the openness of the area reduced and mixed forests expanded in the area including tree/shrub species like *Quercus*, *Juglans*, *Betula*, *Platanus orientalis*, Bignoniaceae, *Dodonaea* and *Myrica*, *Artemisia*, Verbenaceae, Amaranthaceae, *Polygonum* and Rosaceae. *Berberis lyceum* is a shrub found mostly in the foothills of the present day Hindu Kush mountains and the introduction of *Berberis* to Kabal area occurred during this period. The

increase in the spread of trees and shrubs on the surrounding mountains resulted in high pollen concentration values (77220 to 89630 grains/cm^{-3}) and increased pollen influx value (6590 pollen/cm^2/yr). But simultaneously agricultural activities and grazing pressure also increased which resulted in higher sedimentation rate (0.007 cm/yr) during this period.

The top three samples (12- 0 cm) of the core provide sufficient details regarding the climate and vegetation changes of the last 100 years. During this period coniferous forests consistently dominated and spread in the area represented by *Pinus*, *Picea*, *Abies*, *Cedrus* and *Taxus* species. An increase in forest cover of the district Swat has been reported from 1996 to 2000 (Government of Khyber Pakhtunkhwa, 2008). Despite the Government claims, a significant deforestation has occurred due to large scale exploitation of forest resources, partly illegal agricultural expansion and fuel wood collection in deprived rural areas of Swat (Ali, Shabaz, and Suleri, 2006; Shabaz and Ali, 2006; Qasim et al., 2011). The higher occurrence of Cyperaceae shows that the climate predominantly remained wet and cold Mixed forests on the surrounding Hindu Kush forests were also represented by *Polygonum*, *Quercus*, Meliaceae, Chloranthaceae, *Artemisia*, Amaranthaceae, Fabaceae, Verbenaceae, Myrtaceae, Anacardiaceae, Sapindaceae, *Juglans* and *Betula* species. The uppermost zone, based on 3 samples representing the pollen spectra of the past 100 years, is characterized by maximum pollen concentration values (83130 -129260 grains cm^{-3}) and maximum pollen influx value (9500 pollen/cm^2/yr). The sedimentation rate of 0.007 cm/yr reveals that the erosion of the land cover has increased pointing to increased agricultural activities and ongoing deforestation. The pollen record of the past 100 years reflects an overall vegetation spectrum almost similar to the present day vegetation, revealing that the annual average values of different climatic variables have remained the same in Kabal Swat area of the Hindu Kush mountains.

The PCA ordination diagram (Fig. 5) separates conifers, trees/shrubs s and herbs found in all the six age zones (KS1-KS-6) of the upper 76 cm part of the Kabal Swat core during the last 3300 years. Herbs belonging to Poaceae, Cyperaceae, Brassicaceae, Fabaceae, Amaranthaceae and Asteraceae (*Artemisia*) were mostly abundant from 900-300 cal yr BP (KS-3 and KS-4). The occurrence of abundant herbs during this period might be attributed to a more pronounced impact of widespread deforestation, agricultural activities and to the drier summer climate (Pignatti, 1997). At the warmer end of the gradient (the lower part of the diagram), evergreen trees and shrubs dominate the pollen assemblages notably Oleaceae, Myrtaceae, Bignoniaceae, *Juglans*, *Dodonaea* and Sapindaceae which were consistently present from 2400 cal yr BP to the present (KS-2 to KS-6). At the cooler end of the gradient (the upper left quadrant of the PCA diagram), the overall distribution of conifer taxa mainly reflects the major biomes of the study area. Conifers including *Pinus*, *Taxus*, *Picea*, *Abie*s and *Cedrus* are predominantly present from 300 -100 cal yr BP, represented by KS-5 i.e. the fifth age zone) followed by their dominance during the second age zone (2400-900 cal yr BP) and thirdly by the most recent age zone (100 cal yr BP to - 62, KS-6). These conifers occur at higher elevation in the alpine area today. A pollen dispersal bias, however, may be responsible for high values of the wind-pollinated *Pinus wallichiana*, *Pinus roxburghii*-type pollen (Prentice, 1985) in Kabal Swat above the forest line.

Conclusions

The study site of Kabal Swat is located at the transition between three of the world's important mountains systems i.e. the Hindu Kush of Afghanistan to the west, Indian Himalayas to the east and the Karakorum mountains below the Tibetan Plateau of China to the north. Our pollen record from this area unravels new and interesting aspects providing a history of the vegetation and climate of the Hindu Kush mountains in Pakistan since the last

3300 years of the late Holocene. In our study we reveal shifts in the upper vegetation zones of Kabal Swat during the late Holocene period. From 3300 to 2400 cal yr BP the subtropical semiarid herbaceous vegetation represented by Cyperaceae and Poaceae species was dominant in the valley which was replaced by mixed coniferous forests of *Taxus, Pinus, Juglans*, Poaceae and Cyperaceae from 2400 to 900 cal yr BP, suggesting a comparatively moderate climatic variability during the late Holocene. The decrease in Poaceae from 2400 to 1500 cal yr BP and again an increases from (1500 cal yr BP to 1200 cal yr BP) show that the Kabal Swat went through respective wet- cooler and dry-warmer periods. Herbs belonging to Poaceae, Cyperaceae, Brassicaceae, Fabaceae, Amaranthaceae and Asteraceae (*Artemisia*) were abundant from 900 to 300 cal yr BP. This can be attributed to a more pronounced impact of widespread deforestation, agricultural activities and to the drier summer climate. Evergreen trees and shrubs dominated the pollen assemblages notably Oleaceae, Myrtaceae, Moraceae species, *Juglans* and *Dodonaea*, which were consistently present from 2400 cal yr BP to the present. Conifers like *Pinus*, *Taxus*, *Picea*, *Abies* and *Cedrus* are concentrated in the upper two zones of the core spanning from 300 cal yr BP to the present reflecting the major biomes of the study area. These conifers mostly occur in the mixed coniferous forests at higher elevation in the alpine area today. Further high resolution Holocene pollen records of the Hindu Kush are needed, to allow a more elaborate comparison with other south and central Asian palaeo-archives, providing more detailed and applicable knowledge for management and conservation issues.

Author Biographies

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Farooq Jan was awarded a B.Sc. in biology (1998) and M.Sc. in Botany (2001) from Peshawar University Pakistan, M.Phil. in plant taxonomy (2010) from Quaid-i-Azam University, Islamabad, Pakistan and Ph.D. in Biodiversity and Ecology (2015) in the department of palynology and climate dynamics at Georg-August-University of Göttingen, Germany. He served as lecturer in Botany at the government postgraduate college Mardan Pakistan from 2003 to 2009. He pursued his PhD under the supervision of Professor Dr. Hermann Behling in the Department of Palynology and Climate Dynamics at the Georg-August-University of Göttingen Germany, on the palaeoecology of Khyber Pakhtunkhwa Province in the northwestern Pakistan. Presently he is serving as Assistant Professor of Botany at Abdul Wali Khan University Mardan in Pakistan and teaches plant ecology, biodiversity & conservation, plant taxonomy, research methodology and Biostatistics at the undergraduate and graduate level. His research interests include terrestrial palynology, palaeoecology, vegetation studies, biodiversity and climate dynamics, palaeoclimatology, floods, disaster management and landscape restoration in the context of global warming and climate change, human settlement/cultural history focusing on the Holocene palaeoenvironmental studies in the subtropical Hindu Kush Himalayan region between Afghanistan, Pakistan and India.

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Lisa Schüler did her BSc (2007), MSc (2009) and PhD (2012) in biodiversity and ecology in the department of Palynology and Climate Dynamics at Göttingen University, Germany. From 2013-2015, she worked as postdoctoral researcher/lecturer in the Department of Plant Systematics, Bayreuth University, Germany. Since 2015 she has a postdoc position in the department of Palynology and Climate Dynamics of University Göttingen, Germany Her main research interest is the transformation of modern-pollen rain and vegetation communities to fossil assemblages, using ecological monitoring, pollen traps and vegetation study data to improve and refine palaeoenvironmental inferences. Her scientific focus lies on the development and exploration of pollen environmental data sets using multivariate approaches. She is interested in the effects of selection and transformation of plant taxa composition on the accuracy and reliability of pollen-based transfer functions. She is especially interested in a long-term perspective on the development and response of terrestrial environments to both natural and anthropogenic impacts on ecosystems.

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Hermann Behling is a professor of Botany at the University of Göttingen in Germany and head of the Department of Palynology and Climate Dynamics since October 2005. He has about 20 years of teaching / research experience and has published about 200 peer-reviewed research papers. He graduated in biology (Diplom) and continued in the subject to attain his PhD in biology at the University of Göttingen. During his PhD work, at the Federal University of Rio de Janeiro in Brazil, he was funded by the Deutscher Akademischer Austauschdienst (DAAD). He gathered postdoctoral experience at the Smithsonian Tropical Research Institute (STRI) in Panama (2 years), the University of Amsterdam, The Netherlands (3 years), and the Centre for Marine Tropical Ecology (ZMT) in the Department of Geosciences at the University of Bremen, where he had a Deutsche Forschungsgemeinschaft (DFG)-Habilitation Scholarship (2 years) and worked on late Quaternary Neotropical ecosystems. His major research interests are terrestrial and marine palynology, palaeoecology, biodiversity dynamics, palaeoclimatology, fire history and human settlement history. His research focuses on late Quaternary palaeoenvironmental studies in tropical and subtropical South America, Asia and Africa, land ocean interactions, and palaeoecology in different ecosystems such as rainforests, savannas, mangroves and mountain vegetation. He is a member of the editorial boards of Palaeogeography, Palaeoclimatology, Palaeoecology and Review of Palaeobotany and Palynology.

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CHAPTER 5

Synthesis

The chapters 2, 3, and 4 included in this thesis lay the foundation for further palynological and palaeoecological research in the unexplored subtropical Hindu Kush Himalayan region of Pakistan and Afghanistan - the center of Gandhara civilization (1500-500 BC). The results of modern and palaeoenvironmental investigation in Khyber Pakhtunkhwa provide important insights into the subtropical grassland and mountain ecosystems and their response to the impacts of past climate change. Biodiversity changes along elevational gradient are also investigated.

The three studies each presented in a separate chapter, provide the knowledge from three perspectives about the late Holocene palaeoenvironmental conditions in the north western Pakistan. The analyses of pollen grain size variation within Poaceae along with a detailed study of modern pollen rain –vegetation relationships along a 200 km elevational gradient, together with the study of the last 3300 years vegetation and climate history, provide a detailed overview of the broader patterns of Holocene ecosystem dynamics in the north western Khyber Pakhtunkhwa Province. Moreover the approaches used in this research contribute to a better understanding of how the subtropical grassland and mountain ecosystems and their climate respond to changes in environmental forcing.

Discussion of the research outcomes

1. Trends of pollen grain size variation in C3 and C4 Poaceae species using pollen morphology for future assessment of grassland ecosystem dynamics

C4 grasses are mostly dominant in regions with warm seasonal precipitation, while C3 grasses prefer cool seasonal precipitation (Epstein et al. 1997; Sage 2004). Moreover C3 and C4 Poaceae species in different grasslands can be interpreted as climate proxies for past temperature, atmospheric CO2 concentration and precipitation variation (Schüler & Behling 2011). The first study in chapter 2, entitled above, is aimed at looking for pollen grain size variation in Poaceae

on continental scale, beyond the 200 km elevational gradient in the Hindu Kush Himalayan region of Khyber Pakhtunkhwa. Previous studies on Poaceae in Pakistan are mostly of taxonomic or anatomic nature (Cope, 1982; Moinuddin et al. 1994; Ahmad et al.2011; Ullah et al. 2011; Perveen & Qaiser, 2012). The present study addressing the palynological, ecological and climate dynamics aspects of Poaceae, is different from previous studies conducted in this region. This pilot project (though based on a limited dataset of 160 species only i.e. 1.45% of all Poaceae species) is the first ever ecological attempt on Poaceae on continental scale.

Results of this study reveals that C3 and C4 (diploid and polyploid species) are not clearly separable based on the measurement results of their pollen grain length, pollen grain width, pore diameter and annulus width. However, the measurement results and statistical analysis performed on these specific Poaceae species reveal clear trends showing that C3 polyploids have larger grain sizes than C3 diploid species while C4 polyploids have larger grain size than C4 diploid species. Pollen grain size is larger in C4 species as compared to C3 species. This study suggests that grasslands dominated by C3 and C4 Poaceae species from different regions and habitats can be separated by studying the pattern of trends in their pollen grain sizes. The method used in this study can be applied on Poaceae pollen grains deposited in environmental archives to reconstruct past climate and assess the dynamics of past grassland ecosystems. The average difference in the pollen grain sizes counted from a set of samples along a core may indicate shifts between C3 and C4 Poaceae composition of grasslands during the past. This approach will not only help the ongoing palaeo-ecological studies in unravelling aspects such as changes in vegetation composition and shifts in biomes of past grasslands ecosystems but also provide useful insights for future predictions.

2. Vegetation and pollen along a 200 km transect in Khyber Pakhtunkhwa Province, northwestern Pakistan

Chapter 3 is based on the relationship between vegetation and modern pollen rain along a 200 km elevational gradient (275-2600 m a.s.l.), which is the first study in Pakistan. Previous studies conducted in Pakistan mostly deals with ethnobotany; taxonomy or phytosociological work (Hussain et al. 1989; 1997; Iqbal & Hamayun, 2005; Hussain et al. 2005; Sher et al 2010; 2011; Shaheen et al. 2012; Khan et al. 2013; Akhtar et al. 2013; Khan et al. 2014).

This study provides insights as how the modern vegetation is reflected in the pollen rain along the 200 km-long elevational gradient in Khyber Pakhtunkhwa. This part of the study recommends the use of higher-ranked systematic levels such as genera or families instead of species for unexplored remote areas like north western Pakistan. Taxonomic surrogacy at family level seems to be the right choice for a comparison of pollen and plant taxa in the tropics (Odgaard 2001; Jantz et al. 2014). Comparisons at family level have previously been proven to be reliable for explaining the patterns of biodiversity (La Torre-Cuadros et al. 2007; Leal et al. 2010).

Results of this study suggest that it is possible to analyze the pollen rain on plant family level in order to derive altitudinal zones or belts of the surrounding vegetation which will help us to assess the role of involved climatic conditions. Four distinct elevational zones are defined by dominating plant families which are reflected in the pollen assemblages by different proportions, indicating a significant correlation between pollen rain and vegetation in families like Poaceae, Asteraceae, Cyperaceae, Verbenaceae, Acanthaceae and Euphorbiaceae. Pollen assemblages also vary considerably from the associated vegetation composition and major discrepancies are caused by large differences in pollen and vegetation proportions in Boraginaceae, Saxifragaceae, Apiaceae, Balsaminaceae and Rubiaceae families.

Results based on the comparison of vegetation and the pollen spectra at family level and their transfer factors (TF) revealed that large sized Poaceae cereal pollen grains >45 µm, Myrtaceae, Polygonaceae, Brassicaceae, Asteraceae, Chenopodiaceae/Amaranthaceae and Cannabaceae reflect the proximity of cultivated land and human habitation in the lower three zones including Peshawar valley, lower montane Malakand hills and Swat valley. All these species reflect secondary vegetation due to the strong human impact. Similarly Pinaceae, Pteridaceae, Dryopteridaceae and other spores reflect natural vegetation in the upper montane Malam Jabba zone.

As past shifts in the vegetation types and their distributions are mostly reconstructed from fossil pollen assemblages therefore the exact relationship between the existing vegetation and modern pollen compositions is crucial for the calibration of the fossil pollen records. The establishment of a modern pollen rain – vegetation relationship, at least at the family level, is necessary for calibration and interpretation of the fossil pollen record from such sites. The differences in the representation of plant families along an elevational gradient recorded from the vegetation

survey and the pollen signal are measurable as transfer factors. Such transfer factors can be applied to calibrate palaeorecords in future studies enabling us to quantitatively reconstruct the past climate and vegetation of this remote part of north western Pakistan in the Hindu Kush Himalayan region of South Asia.

3. Holocene vegetation and climate dynamics in Khyber Pakhtunkhwa, northwestern Pakistan, inferred from the Kabal Swat pollen record during the last 3300 years

Chapter 4 of this thesis deals with the Holocene vegetation and climate dynamics in Khyber Pakhtunkhwa, northwestern Pakistan, inferred from Kabal Swat pollen record. This part of the thesis is the first assessment of vegetation and climate history spanning over the last 3300 years of the Holocene for northwestern Pakistan. Only a few palaeoecological studies have been conducted in Pakistan mostly in southern parts of the country on/ near the Arabian Sea (von Rad et al. 1995; Schulz et al. 1996; Ivory & Lézine, 2009), and in the salt range of Pakistan (Schneebeli-Hermann et al. 2014). This study in the north western terrestrial mountainous region of Swat, attempts to achieve a comprehensive understanding of the past vegetation changes and identification of environmental conditions as well as their impact on local and regional ecosystem dynamics. The aim of the study is to obtain details on past environmental changes in Swat and surrounding northern areas to get new insights into the biodiversity and vegetation patterns and hence derive information on potentially responsible climatic factors.

From 3300 to 2400 cal yrs BP the subtropical semiarid herbaceous vegetation represented by Cyperaceae and Poaceae species was dominant in the valley. This was replaced by mixed coniferous forests of *Taxus*, *Pinus*, *Juglans*, Poaceae and Cyperaceae, from 2400 to 900 cal yrs BP, suggesting a comparatively moderate climatic variability during the late Holocene. The decrease in Poaceae from 2400 to 1500 cal yrs BP and again an increase from 1500 to 1200 cal yrs BP show that Kabal Swat went through respective wet-cooler and dry-warmer periods. Conifers like *Pinus*, *Taxus*, *Picea*, *Abies* and *Cedrus* are concentrated in the upper two zones of the core spanning from 300 cal yrs BP to the present, reflecting the major biomes of the study area. These conifers mostly occur in the mixed coniferous forests at higher elevation in the Alpine area today.

Phytogeographically, the study site of Kabal Swat is located at the transition between three of the world's important mountains systems i.e. the Hindu Kush of Afghanistan to the west, Indian Himalayas to the east and the world´s second highest peak (K2 – 8611 m in Karakorum Mountains of Pakistan) below the Tibetan Plateau of China to the north. Monsoon rains on these mountain systems resulted in rich flora famous for its unique endemic and threatened biodiversity, forests, wildlife and immense unexplored genetic resources, due to which the western Himalayan moist temperate Ecoregion has been included in the Global-200 priority ecoregion for conservation (Ahmad, 2014).

Data of this study establishes a base for a detailed comparison with the other study sites in South Asia. Moreover the present study provides a basis for formulating/hypothesizing broader scientific questions relating to different aspects of ecosystem research in Khyber Pakhtunkhwa and subsequently relating them to the ongoing archaeological and geological studies in the region. Further high resolution Holocene pollen records of the Hindu Kush Himalayan region are needed, to allow a more elaborate comparison with other South and Central Asian palaeo-archives, providing more detailed and applicable knowledge for management and conservation issues.

Conclusions, uncertainties and open questions

The first pilot study dealing with a small dataset of 160 Poaceae species is certainly limited and the results are specific to our dataset. Hence, the number of species needs to be substantially increased for replicating the study in future. Moreover, pollen grain studies of paired C3/C4 Poaceae taxa would allow a more robust comparison in future studies. Due to the swelling effect of glycerine on the grain size, we recommend, as did (Schüler & Behling 2011), to prepare only a limited number of samples that can be measured on the same day or shortly after sample preparation Phenotypic plasticity with in Poaceae may pose a problem when this method is applied to fossil samples, e.g. some C3 species of Pooideae are found in very dry habitats indicating the correlation of grain size variation more to their characteristic ecological envelope than to the photosynthetic pathway of species in the community. In such cases, the composition and dynamics of existing grasslands based on the grain size analysis of fresh Poaceae species can be studied but interpretation of the palaeo-samples for detecting the composition of past grasslands becomes complicated. Hence, the interpretation of signals from fossil samples for

detecting the composition of past grasslands should be managed with caution. It also cannot be ruled out that pollen size may also be a product of phylogenetic relatedness (Little et al. 2010). Members of the monophyletic PACMAD clade (Panicoideae, Arundinoideae, Chloridoideae, Micrairoideae, Aristidoideae and Danthonioideae), where C4 photosynthesis evolved 22–24 times from their C3 ancestors, might have larger pollen grains than pooids, can potentially provide taxonomic information about past grasslands, i.e. which clades were present, but not about the photosynthetic pathway as all PACMADs are not C4. However, phytolith assemblages can indicate the proportion of C3 and C4 grasses in the flora, if supported by evidence of isotopic studies (Wang et al. 1994).

In the second study based on the pollen rain vegetation relation along the 200 km gradient in the area, the size of plots needs to be increased from 10x10 to at least 20x20 m for broadening the documentation scope of the herbaceous vegetation, in the much disturbed lower two elevations zones of Peshawar valley and lower Malakand. For the upper mountain Malam Jabba zone, the density and frequency of species in the mixed forests can best be documented by extending the plot size to 40x40 m, because of the presence of large Conifers. Phytosociology of the whole transect has been attempted to be recorded in 24 plots. The plot number should also be substantially increased in future studies. At our study site it is possible to analyze the pollen rain - vegetation relationship on plant family level, enabling us to draw boundaries in the surrounding vegetation along the gradient (275-2600 m a.s.l.). Schüler et al. 2014 also found this relationship at family level between vegetation and modern pollen-rain along an elevational gradient on Kilimanjaro, Tanzania using Non Metric Multidimensional Scaling. It is therefore recommended for future studies to focus our scientific questions as how to establish such relationships at the genus and even at species level. The uncertainties/limitations with the ongoing modern day aerial imagery and remote sensing techniques applied to answer the questions of vegetation ecology, climate change biology, forestry, agriculture and related fields of Botany can surely be reduced to a greater extent, if palynologists come up with statistically sound approaches as how to establish such vegetation-pollen relationships at the genus and species level across varying gradients.

To carry out further work regarding the reconstruction of the past vegetation history of the area, in connection with the third part of the study, the very first limiting factor is to find proper sites for taking cores with common Russian corer or other affordable instruments. Unlike Southeast Asia, Europe or other regions where the soil texture / structure is quite soft and loose, there are negligible number of sites in the Khyber Pakhtunkhwa where routine coring may be easy. The soil is extremely hard, compact and dry throughout with almost no forest hollows or swamps either in the plains or mountains. Comparing past and present vegetation with meteorological data and investigating the role of Monsoon rains and wind patterns including the effects of Inter Tropical Convergence Zone (ITCZ) in research area are open questions.

South Asian region is home to over one fifth of the world's population and is known to be the most disaster prone region in the world due to its increasing urbanization, climate change and global warming (UNEP, United Nations Environment Program 2003). Considering the immense potential and importance of this area regarding biodiversity, extreme vulnerability to climate change in the form of ever increasing floods, deforestation and natural disasters, this area should receive special attention in terms of conservation. The impact level of climate change and global warming on the modern day vegetation in the region are questions that need serious attention. Compared to previous annual floods which normally starts from the extreme north of the country, in 2010 there was a record flood in Pakistan which was the worse during the past 60 years causing US $ 10 billion losses, leaving 2000 people dead, 17,553 villages destroyed, 160,000 km^2 area affected (Federal flood commission report 2010, Government of Pakistan). Keeping this scenario in mind paleoecologists, vegetation ecologists, geologists, meteorologists, foresters, agriculturists, conservation biologists, wild life managers and ecosystem researchers working in the area, need to formulate relevant questions for future studies.

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Appendix

Common pollen and spores of Poaceae, dicots, fungi and ferns found along the 200 km long elevational transect in the north western Khyber Pakhtunkhwa province of Pakistan

Appendix A1: Table of identified pollen and spore types

Appendix A2: Plates of identified pollen and spore types

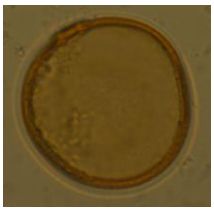
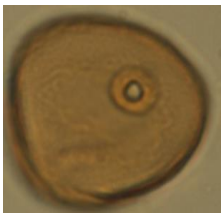
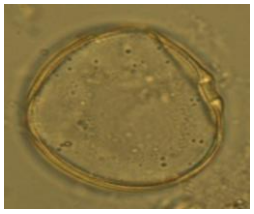
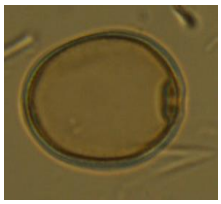
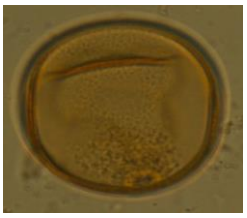
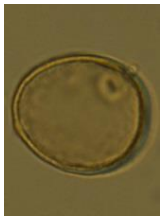
Note: Pollen and spores obtained either from fresh plants or retrieved from surface samples or from Kabal Swat core.

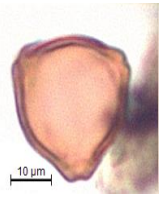
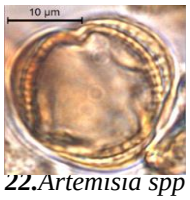
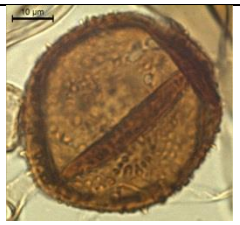
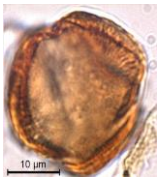
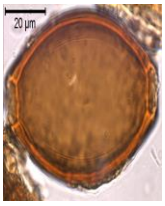
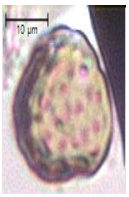
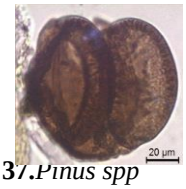
Appendix A: Table of identified pollen and spore types

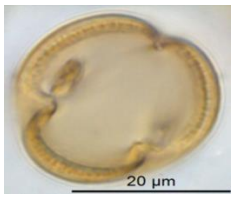
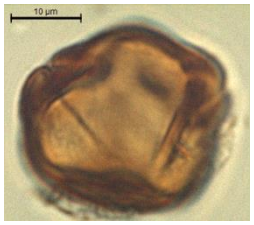
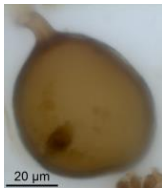
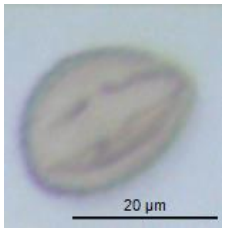
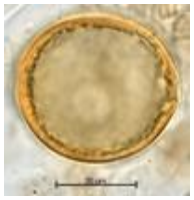
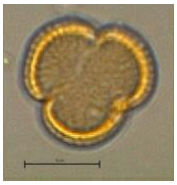
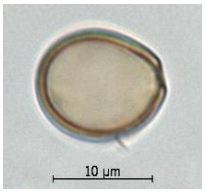
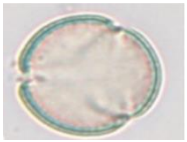
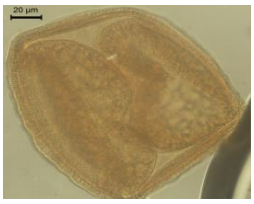
S.No	Taxa of Pollen or spore	Source
1	*Pennisetum typhoidum* Poaceae	Fresh Poaceae specimen
2	*Setaria pumila* Poaceae	Fresh Poaceae specimen
3	*Triticum aestivum* Poaceae	Fresh Poaceae specimen
4	*Helectrotrichon virescense* Poaceae	Fresh Poaceae specimen
5	*Sporobulus* spp Poaceae	Fresh Poaceae specimen
6	*Digitiria ciliaris* Poaceae	Fresh Poaceae specimen
7	*Heteropogon contortus* Poaceae	Fresh Poaceae specimen
8	*Eragrostis cilianensis* Poaceae	Fresh Poaceae specimen
9	*Sporobulus spp* Poaceae	Fresh Poaceae specimen
10	*Dactyloctenium aegyptium* Poaceae	Fresh Poaceae specimen
11	*Polypogon fugax* Poaceae	Fresh Poaceae specimen
12	*Phararis minor* Poaceae	Fresh Poaceae specimen
13	*Phalaris paradoxa* Poaceae	Fresh Poaceae specimen
14	*Phalaris spp* Poaceae	Fresh Poaceae specimen
15	*Urochloa panicoides* Poaceae	Fresh Poaceae specimen
16	*Acrachne racemosa* Poaceae	Fresh Poaceae specimen
17	*Bromus pectinatus* Poaceae	Fresh Poaceae specimen
18	*Bromus unioloides* Poaceae	Fresh Poaceae specimen
19	*Dichanthium annulatum* Poaceae	Fresh Poaceae specimen
20	*Digitaria ciliaris*	Fresh Poaceae specimen
21	*Myrica spp* Myricaceae	Surface sample
22	*Artemisia spp* Asteraceae	Surface sample
23	Malphigiaceae spp	Surface sample
24	Poaceae	Surface sample
25	*Eucapyptus spp* Myrtaceae	Surface sample
26	*Plantago lanceolata* Plantaginaceae	Surface sample
27	*Osmunda* fern Osmundaceae	Surface sample
28	*Pinus spp* Pinaceae	Surface sample
29	Urticaceae spp	Surface sample
30	Verbenaceae	Surface sample
31	Artemisia	Surface sample
32	Fungal spore	Surface sample
33	Fungal spore	Surface sample
34	Chenopodiaceae	Surface sample
35	*Cheno/Amaranthaceae*	Surface sample
36	Poaceae	Surface sample
37	*Pinus spp Pinaceae*	Surface sample
38	*Picea spp*	Surface sample
39	Trilet spore	Surface sample

40	*Dodonaea viscosa* Sapindaceae	Surface sample
41	*Ilex spp.* Aquifoliaceae	Kabal Swat core
42	*Ailanthus altissima* Simaroubacaceae	Kabal Swat core
43	*Pinus wallichiana*	Kabal Swat core
44	*Picea smithiana*	Kabal Swat core
45	*Pinus spp*	Kabal Swat core
46	*Dodonaea spp* Sapindaceae	Kabal Swat core
47	Acanthaceae	Kabal Swat core
48	Fabaceae	Kabal Swat core
49	Oleaceae spp	Kabal Swat core
50	Fungal spore	Kabal Swat core
51	*Quercus* Oak. Fagaceae	Kabal Swat core
52	Anacardiaceae	Kabal Swat core
53	Cyperaceae	Kabal Swat core
54	*Nerium oleander* Apocynaceae	Kabal Swat core
55	Brassicaceae	Kabal Swat core
56	*Ficus spp* Moraceae	Kabal Swat core
57	*Oxalis spp* Oxalidaceae	Kabal Swat core
58	Rananculaceae	Kabal Swat core
59	Caryophylaceae	Kabal Swat core
60	*Abies spp.* Pinaceae	Kabal Swat core

Appendix B: Plates of identified pollen and spore types

 ***1.****Pennisetum typhoidum*	 ***2.*** *Setaria pumila*	 ***3.****Triticum aestivum*	 ***4.*** *Helectrotrichon virescense*
 ***5.*** *Sporobulus* spp	 ***6.****Digitiria ciliaris*	 ***7.****Heteropogon contortus*	 ***8.****Eragrostis cilianensis*
 **9.** Sporobulus spp	 *10. Dactyloctenium aegyptium*	 ***11.****Polypogon fugax*	 ***12.****Phararis minor*
 ***13.****Phalaris paradoxa*	 ***14.****Phalaris spp*	 ***15.****Urochloa panicoides*	 ***16.****Acrachne racemosa*
 ***17.****Bromus pectinatus*	 *18.Bromus unioloides*	 ***19.****Dichanthium annulatum*	 **20.***Digitaria ciliaris*

 **21.***Myrica spp*	 ***22.****Artemisia spp*	 ***23.****Malpighiaceae*	 ***24.*** *Poaceae*
 ***25****.Eucapyptus spp*	 ***26****.Plantago lanceolata*	 **27.***Osmunda* fern	 ***28.*** *Pinus spp*
 **29.**Urticaceae	 ***30.***Verbenaceae	 ***31.****Artemisia spp*	 ***32.****Fungal spore*
 **33.**Fungal spore	 ***34.****Chenopodiaceae*	 ***35.****Cheno/Amaranthaceae*	 ***36.*** *Poaceae*
 **37.***Pinus spp*	 ***38.*** *Picea*	 ***39.****Trilet spore*	 ***40.****Dodonaea viscosa*

 **41**.Aquifoliaceae (Ilex spp)	 ***42.****Ailanthus altissima* *Simaroubacaceae*	 **43**.*Pinus wallichiana?*	 ***44.****Picea smithiana*
 ***45.*** *Pinus spp*	 ***46.****Dodonaea spp*	 ***47.****Acanthaceae*	 ***48.****Fabaceae*
 **49.** Oleaceae spp	 **50.**Fungal spore	 ***51.****Quercus spp*	 ***52****.Anacardiaceae spp*
 ***53.****Cyperaceae*	 **54.** *Nerium oleander* Apocynaceae	 ***55.****Brassicaceae*	 **56.** *Ficus spp* Moraceae
 **57.***Oxalis spp*	 *58.Rananculaceae*	 59. Caryophylaceae	 ***60.****Abies spp*

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Statement

Herewith, I confirm that this thesis is my own work and that I have documented all sources used.

Göttingen, April 14th, 2015