

Surface pollen quantification and floristic survey at Shaheed Chandra Shekhar Azad (SCSA) Bird Sanctuary, Central Ganga Plain, India: a pilot study for the palaeoecological implications

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(Received 02 September, 2022; revised version accepted 04 November, 2022)

ABSTRACT

Tripathi S, Srivastava J, Garg A, Khan S, Farooqui A, Quamar MF, Thakur B, Ranhotra PS, Basumatary SK, Trivedi A, Pandey S, Anupama K, Prasad S & Reghu N 2022. Surface pollen quantification and floristic survey at Shaheed Chandra Shekhar Azad (SCSA) Bird Sanctuary, Central Ganga Plain, India: a pilot study for the palaeoecological implications. *Journal of Palaeosciences* 71(2): 159–176.

Accuracy of vegetation reconstruction portraying land cover of the past is based on a careful analysis of pollen production, dispersal and their quantitative deposition. The present attempt to integrate sampling of pollen–vegetation spectrum through Crackles Protocols for vegetation surveys, at three spatial zones with intervals of 0–10 m (A), 10–100 m (B) and 100–1000 m (C) at Shaheed Chandra Shekhar Azaad Bird Sanctuary in Uttar Pradesh with tropical dry deciduous forest, is a maiden approach. In these studies, the standard vegetation survey around the pollen surface sampling sites is prerequisite for quantifying pollen–vegetation relationship in modern analogues of the past. The underlying theory of this approach is based on the fact that the relative pollen productivity (RPP) is constant in space and time within a region or biome. The floristic survey of the sanctuary is integral to this pilot study, Crackles Bequest protocol, and is intrinsic to run the Extended R–Value (ERV) model for obtaining estimates of relative pollen productivities (RPPs) for quantitative palaeoecological interpretations from tropical to subtropical forest covers in northern India. The modern pollen assemblage from surface sediment samples established the dominance of Poaceae pollen, along with those of *Acacia*, *Albizia* and *Mimosa* species. The multivariate principal component analysis (PCA), applied to quantify the data on survey of different vegetation communities revealed that out of the four identified vegetation communities, community D consisted of herbaceous patches including *Ageratum*, *Parthenium*, *Rumex*, *Tephrosia*, *Eclipta alba*, *Oxalis*, *Cannabis* and *Launea*, community B mainly comprised of tree taxa like *Terminalia*, *Barringtonia* and *Pongamia*, whereas the communities A and C represented a mixed vegetation comprising of trees, shrubs and herbs. The present maiden analysis through Crackles Bequest protocol method, served as a preliminary step to establish the quantitative ‘pollen–based’ vegetation reconstruction in the Gangetic Plains of Central India, and is expected to serve as a model for similar studies in other regions.

Key–words—Quantitative Pollen–vegetation relationship, Crackles Protocol, Relative Pollen Productivity, Shaheed Chandra Shekhar Azad (SCSA) Bird Sanctuary, Uttar Pradesh, India.

INTRODUCTION

POLLEN-BASED quantitative plant cover reconstruction is essential for analysing past vegetation, climate and even humans (Li *et al.*, 2018). Pollen accumulation rates (PAR) and percentage occurrence has been widely used as major tool to estimate past vegetation changes from local to global scales using multi-proxy approaches, as other approaches which include biomization, permit correlation of plant functional

types (PFT) and biome of taxa assemblage (Williams *et al.*, 2008; Ni *et al.*, 2014). However, this approach has limitations as it does not provide reconstructions of plant abundance in absolute values in terms of percentage land cover of plants which comprise the individual pollen taxa. A comparison of the satellite-derived and pollen-based estimates of tree cover in northern Asia showed higher pollen-inferred tree cover, mainly due to long-distance transport of pollen (Tarasov *et al.*, 2007). Till date, the most widely accepted

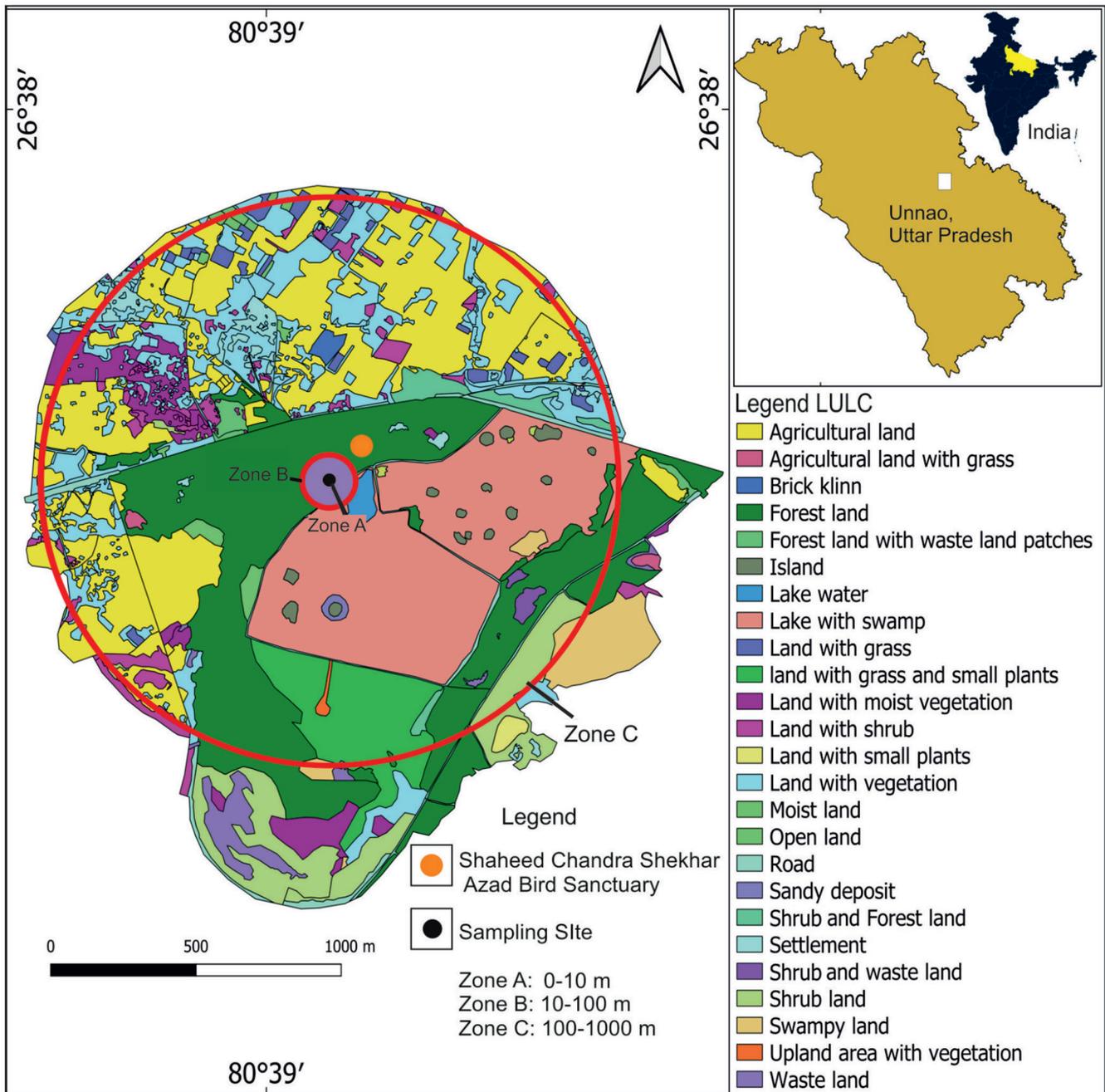


Fig. 1—Location map showing study area in the Shaheed Chandra Shekhar Azad (SCSA) Bird Sanctuary along the Central Ganga Plain, India (prepared through ArcGIS software using Google Earth Pro).

Table 1—A taxon dictionary file of pollen taxa recovered from the study area which is saved in *.dct format.

Master Directory-NBS

*Acacia-t Acacia auriculiformis Acacia nilotica	*Myrt-t=Eucalyp/Callistem type Eucalyptus Callistemon lanceolatus
*Albizia-t Albizia lebbbeck	*Euph=Euphorbiacea Phyllanthus emblica
*Ast-t=Asteraceae type Ageratum conyzoides Parthinium aspera Nymphalium Eclipta alba Launea grandis	*chrz-t=Chrozophora type Chrozophora *Lecy-t=Lecythidaceae Barringtonia acutangula
*Mela.Comb=Melastomataceae/Combretaceae Terminalia arjuna Terminalia chebula	*Lyth-t=Lythraceae Lagerstroemia parviflora
*Fab-t= Cassia fistula Pongamia pinnata Tephrosia Prosopis juliflora	*Can=Cannabaceae Cannabis sativa *Pol=Polygonaceae Rumex type *Poa=Poaceae Poaceae

Note: Plant/Pollen taxa are not written in italic format as per the requirement of the *.dct file for uploading in the vegetation software dictionary.

method for estimating plant cover at taxa level is the multi-step Landscape Reconstruction Algorithm (LRA) of Sugita (2007a, b) and the Multiple Scenario Approach (MSA) of Bunting & Middleton (2009). The LRA comprises two models (REVEALS, LOVE) which corrects the long existing biases regarding the pollen representation of vegetation due to interspecific variability in pollen productivity and dispersal, vegetation heterogeneity and atmospheric conditions influencing pollen–vegetation relationships (Sugita, 2007b) which, along with their nonlinearity, remains an important issue. Study of these relationships therefore, is a major prerequisite to the interpretation of past pollen records (e.g. Gaillard *et al.*, 1992, 1994; Hicks & Birks, 1996; Hjelle, 1997, 1998; Gerasimidis *et al.*, 2006; Mazier *et al.*, 2006, 2008). The present investigations are based on single site which quantified a detailed pollen dictionary (Fig. 1, Table 1) in support of the ERV model runs, as a first step to linearize pollen–vegetation relationship and subsequently, to apply in LRA for understanding the past landcover. Such dictionaries and studied models were never attempted earlier in the tropical to sub-tropical forest of north India.

The main parameter for using the LRA and MSA is the estimation of pollen productivity of the significant taxa and the most common method for estimating relative pollen productivity (RPP) is the Extended R-value (ERV) model (Prentice & Parsons, 1983) on modern data sets of pollen assemblages and related vegetation data. A relatively large number of RPP estimates were found in Europe (Mazier *et al.*, 2012; Bunting *et al.*, 2013) and Northern America (Commerford *et al.*, 2013) through this method which has augmented the understanding of past land cover and climate interactions. However, as per the Intergovernmental Panel on Climate Change (IPCC) the quantitative proxy-based climate reconstructions of tropical regions are scanty, mainly due to wide variety of pollen production and dispersal mechanisms which makes it difficult to quantify (Gaillard *et al.*, 2021) and thus, support the regional climate change assessments (IPCC: climate change, 2021). Further, the pollen-based quantification studies are important for climate modelling (Gaillard *et al.*, 2010) and also essential for evaluation of Dynamic Vegetation Model simulations (Lu *et al.*, 2018) and Anthropogenic Land Cover Change scenarios (Li *et al.* 2020; Gaillard *et al.*, 2021). In concurrence with these studies, Gillson & Duffin (2007)

used process-based model to translate tree pollen percentages into past woody plant cover in savannah habitat in southern Africa to inform the identification of thresholds of concern for conservation monitoring in the region. Recently, some studies on quantitative pollen-based Holocene reconstruction have estimated Relative Pollen Productivity (RPP) of key taxa from tropical and subtropical regions of the world (Jiang *et al.*, 2020; Wan *et al.*, 2020), including South Africa (Hill *et al.*, 2021), Cameroon highlands (Gaillard *et al.*, 2021) and Namibia (Tabares *et al.*, 2021).

Although studies on qualitative pollen-vegetation relationship within different vegetation types have also been carried out in India (Bonnefille *et al.*, 1999; Anupama *et al.*, 2000; Bera, 2000; Dixit & Bera, 2011, 2013; Basumatary *et al.*, 2013; Ranhotra & Bhattacharyya, 2013; Quamar & Bera, 2014; Basumatary *et al.*, 2014, 2017, 2018; Tripathi *et al.*, 2016; Pandey & Holt, 2017; Bajpai & Kar, 2018; Pandey & Minckley, 2019; Srivastava *et al.*, 2019; Quamar & Kar, 2020), but quantitative relationship studies are scanty (Barboni & Bonnefille, 2001; Navya *et al.*, 2017). Further, there are several palynological works on sedimentary soil sequences in northern India (Gupta, 1978; Chauhan *et al.*, 2004; Saxena *et al.*, 2006; Chauhan & Chatterjee, 2008; Chauhan *et al.*, 2009; Ranhotra & Bhattacharyya, 2010; Farooqui & Sekhar, 2011; Trivedi *et al.*, 2011, 2013), but studies on qualitative surface pollen samples are scanty (Sharma *et al.*, 2006; Trivedi & Chauhan, 2011; Tripathi *et al.*, 2016). Dixit and Bera (2012) suggested that comparing multiple points of pollen data, along with variations among taxa and sites would help us to understand the vegetation structure more vividly.

The Crackles Bequest field protocol (Bunting *et al.*, 2013) has been validated for temperate European and Chinese forest covers, along with North American vegetation, by estimating Relative Pollen Productivity (RPP) and fall speeds of the representative pollen taxa. Further, the reliability of vegetation habitats was tested on temperate plant taxa to quantify Holocene pollen estimates and infer palaeoenvironmental reconstruction using different elements such as meadows, heaths and woodlands (Bunting *et al.*, 2013).

In order to obtain the RPP estimation in tropical plant taxa, a sufficient number of sites need to be surveyed for recording the vegetation data for the ERV model. Hence, there is a pertinent need for estimation of RPP for key

plant taxa in the Indian Subcontinent to support the proxy based quantitative reconstruction studies for more accurate evaluation of land cover change over time and its impact on climate. The present pilot study was therefore made to test the Crackles Vegetation Survey protocol in a single site in the Central Gangetic Plains which can later be extended for similar studies in the tropical and subtropical forest ecosystem in north India and clustering of prominent plant taxa within the different vegetation communities were investigated through principal component analysis (PCA). These studies provide the first step in developing the RPP dataset for the ERV model and pollen based quantification reconstruction of past climate and vegetation cover and aims to provide a dictionary for mapping pollen types/pollen taxa to their respective parent plants, through pollen counts and vegetation survey data combining different (local) vegetation zones (A–B) and remote sensing based vegetation zone (C) for the ERV model and contributing an important tool towards pollen based quantification of the landcover in the tropical mixed deciduous forests (Champion & Seth, 1968) of the Central Ganga plain (Fig. 2; Pl. 1)

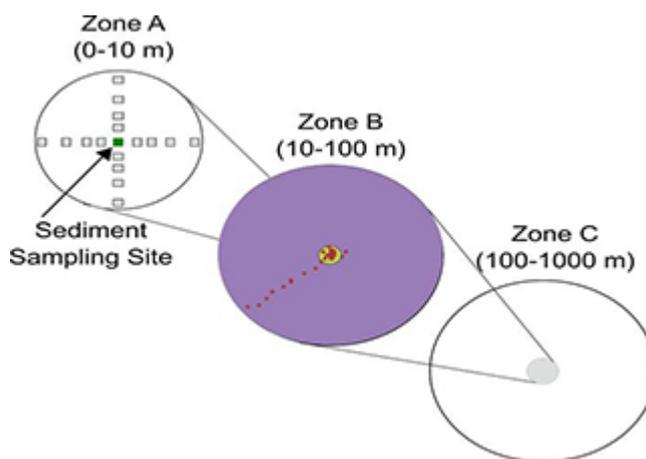


Fig. 2—A sketch diagram showing the detailed vegetation survey in Zone A (0–10 m) using a standard array of 21 quadrates of 1 x 1 m centred on the surface sediment sample collected, zone B (10–100 m) and zone C (100–1000 m).

PLATE 1

Sampling and vegetation survey sites within SCSA forest area. →

1. Sample collection and vegetation recording in a standard array of 21 quadrats of 1 x 1 m centred within Zone A (0–10 m).
2. Vegetation communities were measured by using relevés within Zone B (10–100 m) in which each data point was located at a distance of 1.2, 2.9, 3.85, 4.6, 5.2 and 5.75 m.
- 3–5. Vegetation survey and recording as per Crackles protocol.
6. One of the authors (KA) was providing training on vegetation survey, recording and modern pollen quantification at SCSA Bird Sanctuary.



PLATE 1

STUDY AREA

Geographical Setting

The study site, Shaheed Chandra Shekhar Azad Bird Sanctuary (earlier known as the Nawabgunj Bird Sanctuary), located at 26°37'08" N and 80°39'10" E constitutes a part of the Central Gangetic Plains in the Unnao District of Uttar Pradesh, India (Fig. 1). It is situated midway along the busy Lucknow–Kanpur National Highway, at c. 45 km from Lucknow and same distance from Kanpur. The sanctuary covers 224.60 hectares area. It was declared as 'Bird Sanctuary' by the Government of Uttar Pradesh, Lucknow vide Gazette No. 2332/, 14–3–48–83, on 7th August, 1984, under the Wildlife Protection Act 1972 for protection and conservation of resident and migratory bird species in wild (Garg & Joshi, 2015). In the year 2015, the sanctuary was renamed as Shaheed Chandra Shekhar Azad Bird Sanctuary. Its lakes and ponds were formed due to disruption of fluvial channels around 9–8 ka in response to tectonic activity and base–level changes (Singh, 1996, 2002) and fall within the Ganga Plains and the abandoned channel segments are characterized by alkaline soils. The vegetation comprises of dry deciduous to moist deciduous forest (Champion & Seth, 1968) (Pl. 1). The sanctuary also houses a deer park, watchtowers and children's park with cafeteria.

The SCSA Bird Sanctuary is a major hub of resident and migratory birds from far and wide continents which reside here for overwintering and rearing. More than 250 migratory birds flock here from northern higher latitudes during November to March, the best season to visit this sanctuary (Garg, 2016; Garg & Singh, 2018). The main area of the sanctuary is around a shallow lake, which serves as a temporary rest area for migratory and water birds (Garg, 2019).

Climate and Soil

The climate of the region has a high seasonality as it is influenced by the southwest monsoon with extreme winters and summers. The temperature ranges between ~7°C to ~45 °C. Annual rainfall in the state ranges from 1000–2000 mm in the east to 600–1000 mm in the west. About 90% of the rainfall occurs during the peak of the Southwest monsoon, lasting from June to September that is followed by the winter monsoon for a short duration. With most of the rainfall concentrated during these four months, floods are a recurring problem and can cause fatalities and heavy damage to crops and property, particularly in the eastern part of the state. Periodic failure of monsoons results in drought conditions. The relative humidity ranges from 60 to 97%. Much of the area of Uttar Pradesh is covered by a deep layer of alluvium spread by the slow–moving rivers of the Ganges system. These extremely fertile alluvial soils range from sandy to clayey loam. The soils in the southern part of the state are generally

mixed red and black or red to yellow in colour; however, it is alluvium in and around the study area (Tripathi *et al.*, 2016).

Vegetation Type

Anderson (1859) was the first to explore the vegetation of tropical deciduous forests of Uttar Pradesh and document indigenous and cultivated plants. Thereafter, Kapoor (1962) and Patil (1963) studied the floristic of the region from an ecological and phytogeographical view point.

The Unnao District has a tropical mixed to dry deciduous vegetation. The SCSA Bird Sanctuary is divided into six zones as follows: (1) core zone, (2) buffer zone, (3) tourism zone, (4) eco–development zone, (5) biodiversity and eco–restoration zone and (6) restricted zone (Garg, 2019). The study area mainly contains trees such as *Acacia auriculiformis* (Fabaceae), *Acacia nilotica* subsp. *indica* (Fabaceae), *Albizia lebbek* (Fabaceae), *Azadirachta indica* (Meliaceae), *Barringtonia acutangula*, *Bauhinia variegata* (Fabaceae), *Prosopis juliflora* (Fabaceae), *Cassia occidentalis* (Fabaceae), *Cordia dichotoma* (Boraginaceae), *Dalbergia sissoo* (Fabaceae), *Ficus religiosa* (Moraceae), *Holoptelea integrifolia* (Ulmaceae), *Madhuca longifolia* (Sapotaceae), *Mitragyna parviflora* (Rubiaceae), *Pithecellobium dulce* (Fabaceae), *Pongamia pinnata* (Fabaceae) and *Bombax ceiba* (Malvaceae), in addition to planted *Eucalyptus* (Myrtaceae) and *Syzygium* (Myrtaceae) and *Terminalia arjuna* (Combretaceae), etc. The shrubby elements, namely *Calotropis gigantea* (Asclepiadaceae), *Clerodendrum viscosum* (Verbenaceae), *Datura innoxia* (Solanaceae), *Jatropha glandulifera* (Euphorbiaceae), *Lantana camara* (Verbenaceae), *Morinda pubescens* (Rubiaceae), and *Ziziphis nummularia* (Rhamnaceae) grow in a scattered fashion in the region. The list of major trees, shrubs and herbaceous taxa with their family, habit, flowering period and specimen number along with the designated herbarium where deposited are provided in Table 2.

MATERIAL & METHODS

As a prelude to pollen data analysis, the present survey included critical floristic documentation of plants prevalent in the study zones of the sanctuary, followed by their systematic taxonomic identification. For this, healthy and complete plant specimens were collected along with their field data on habit, habitat, flowering fruiting time, etc. dried, preserved and mounted following conventional herbarium methods (Jain & Rao, 1977; Bridson & Forman, 1999), accessioned and deposited in the herbarium of Central Regional Centre of the Botanical Survey of India (BSA). These were identified following standard taxonomic methods including dissections and floristic key consultation (Bor, 1960; Kanjilal, 1966; Hooker, 1872–1897; Duthie, 1960; Chaudhary *et al.*, 2016; Saini *et al.*, 2010) where needed, and matching with the

Table 2—The list of major trees and herbaceous taxa in the SCSA Bird Sanctuary.

Plant Name	Family	Voucher Specimen No. (BSA)	Habit Type	Flowering Period
<i>Acacia auriculiformis</i> Benth.	Fabaceae	Arti Garg 71799	Tree	January-May
<i>Acacia nilotica</i> subsp. <i>indica</i> (Benth.) Brenan	Fabaceae	Arti Garg 71792	Tree	November-April
<i>Albizia lebbbeck</i> (L.) Benth	Fabaceae	Arti Garg 71846	Tree	March-October
<i>Barringtonia acutangula</i> (L.) Gaertn.	Lecythidaceae	Arti Garg 71611	Tree	October-November
<i>Terminalia arjuna</i> (Roxb. ex DC.) Wt. & Arn.	Combretaceae	Arti Garg 71831	Tree	April-August
<i>Azadirachta indica</i> A. Juss.	Meliaceae	Arti Garg 71844	Tree	April-August
<i>Bauhinia variegata</i> L.	Fabaceae	Arti Garg 71818	Tree or Shrub	February-April
<i>Prosopis juliflora</i> (Sw.) DC.	Fabaceae	B.K. Shukla & Arti Garg 71620	Tree	February-December
<i>Cassia fistula</i> L.	Fabaceae	Arti Garg 71845	Tree	April-March
<i>Cordia dichotoma</i> G. Forst.	Boraginaceae	Arti Garg 72346	Tree	February-April
<i>Dalbergia sissoo</i> Roxb. ex DC.	Fabaceae	Arti Garg 71818	Tree	February-June
<i>Eucalyptus umbellata</i> Dum. Cours.	Myrtaceae	Arti Garg 71837	Tree	January-June
<i>Ficus religiosa</i> L.	Moraceae	Arti Garg 71708	Tree	April-September
<i>Holoptelea integrifolia</i> (Roxb.) Planch	Ulmaceae	Arti Garg 71797	Tree	January-April
<i>Mitragyna parviflora</i> (Roxb.) Korth	Rubiaceae	Arti Garg 72152	Tree	March-December
<i>Pithecellobium dulce</i> (Roxb.) Benth.	Fabaceae	Arti Garg 71819	Tree	January-April
<i>Pongamia pinnata</i> (L.) Pierre	Fabaceae	Arti Garg 71801	Tree	May-February
<i>Bombax ceiba</i> L.	Malvaceae	Arti Garg 72524	Tree	February-May
<i>Streblus asper</i> Lour.	Moraceae	Arti Garg 71832	Medium-sized tree	January-March
<i>Syzygium cumini</i> (L.) Skeels	Myrtaceae	Arti Garg 71771	Tree	February-August
<i>Tamarindus indica</i> L.	Fabaceae	Arti Garg 72521	Tree	April-June
<i>Terminalia arjuna</i> (Roxb. ex DC.) Wt. & Arn.	Combretaceae	Arti Garg 71831	Tree	April-August
<i>Calotropis gigantea</i> (L.) Dryand	Asclepiadaceae	Arti Garg 71855	Shrub	March-May
<i>Clerodendrum indicum</i> Kuntze	Lamiaceae	Arti Garg 71890	Shrub	October-January
<i>Datura metel</i> L.	Solanaceae	Arti Garg 71731	Undershrub	Throughout the year
<i>Jatropha curcas</i> L.	Euphorbiaceae	Arti Garg & A.P. Tiwari 77772	Shrub	Throughout the year
<i>Lantana camara</i> L.	Verbenaceae	Arti Garg 71803	Shrub	April-June
<i>Morinda pubescens</i> var. <i>stenophylla</i> (Spreng.)	Rubiaceae	Arti Garg & A.P. Tiwari 77947	Shrub	March-June
<i>Ziziphus mauritiana</i> Lam.	Rhamnaceae	Arti Garg 71783	Shrub	September-February

<i>Parthenium hysterophorus</i> L.	Asteraceae	Arti Garg 71724	Shrub	February- April
<i>Rumex dentatus</i> L.	Polygonaceae	Arti Garg 71729	Herb	May-July
<i>Oxalis corniculata</i> L.	Oxalidaceae	Arti Garg 71725	Herb	June-September
<i>Oxalis debilis</i> Kunth	Oxalidaceae	Arti Garg 71756	Herb	March-April
<i>Launaea procumbens</i> (Roxb.) Amin	Asteraceae	Arti Garg 71826	Herb	November- December
<i>Eclipta prostrata</i> L.	Asteraceae	B.K. Shukla & Arti Garg 71614	Herb	October- December
<i>Cannabis sativa</i> L.	Cannabaceae	Arti Garg 71761	Herb	Autumn season (require light less than 12 hours)
<i>Tephrosia purpurea</i> (L.) Pers.	Fabaceae	Arti Garg & A.P. Tiwari 76963	Herb	April- October
<i>Ziziphis nummularia</i> (Burm. f.) Wt. & Arn.	Rhamnaceae	Arti Garg 71783	Shrub	September- February

available identified Herbarium specimens. In taxonomic treatment, the taxa are listed as per the Bentham and Hooker's system of classification (1862–1883) and in consultation with the Checklist of Flowering plants of India (Mao & Dash, 2020a, b; Dash & Mao, 2020) and complete floral diversity within the sanctuary and dominant floral constituents were analysed.

Vegetation survey—A detailed vegetation survey (Bunting *et al.*, 2013; Navya, 2019) was conducted within Zone A (0–10 m) and recorded using a standard array of 21 quadrates of 1 x 1 m centred on the surface sediment sample collected. (Pl. 1.1). The ground cover of vegetation in each quadrat was recorded as percentages along with the ground flora (Pl. 2). Vegetation communities were mapped in zone B (10–100 m) using quadrat and random relevé (Bunting *et al.*, 2013) (Pl. 1.2, 3) and for the outer zone of the survey system Zone C (100–1000 m) vegetation data was collected from remote sensing GIS (Mazier *et al.*, 2008) (Fig. 1). In Zone, B communities were measured by using relevés in which each data point was located at distances of 1.2, 2.9, 3.85, 4.6, 5.2 and 5.75 m (Pl. 1.2–5). This was followed by software-based estimates to correlate pollen production of vegetation cover. This localised vegetation survey was done to recreate pollen productivity estimates of forest cover for an aerial distribution of 100 m in two concentric zones A and B (Fig. 2). The vegetation data in quadrats and community mapping data was recorded during the field survey in the prescribed reading sheet format (Bunting *et al.*, 2013) (Pl. 1.3, 6). The site map which combines Zone A and B was also drawn during the survey displaying vegetation community segregation and its edges, bare land, water bodies, walking path, obstruction, etc. (Fig. 3). This estimation incorporates contributions of multi-tiered plants covering the highest

tree canopy to lowest, prostrate herbaceous plants. Such a quantitative estimate provides rigorous zone wise spatial and vertical projection of all the component of sparse and dense forest, such as stems, leaves, herbs, shrubs and tree crowns, which were calculated along with the variations and monitored in different plant communities (Pl. 2).

Data obtained from the satellite imagery in Zone C

The landscape/vegetation features were differentiated by their colour in the images and were assigned to different vegetation/ land-use units that had been defined based on the vegetation communities identified in the field survey. Thereafter polygons were drawn to identify each vegetation/ land-use unit and finally a map was created by maximum likelihood classification with ArcGIS 10.3 software to calibrate the modern pollen vegetation with a finer resolution between 100–1000 m (Zone C) (Fig. 1).

Pollen extraction

One surface sample procured from the centre (Lat. 26.6185 N and Long. 80.65219 E) was chemically processed for palynological observation using Erdtman's method (Erdtman, 1943) and the detailed methodology is provided below:

1. About 20 g of material was boiled with 10% potassium hydroxide (KOH) solution for 5 minutes to deflocculate pollen from the soil, mud and moss cushions, and to dissolve the humic acid.
2. Samples were washed 3 times in distilled water to remove alkalis by centrifugation and decantation.

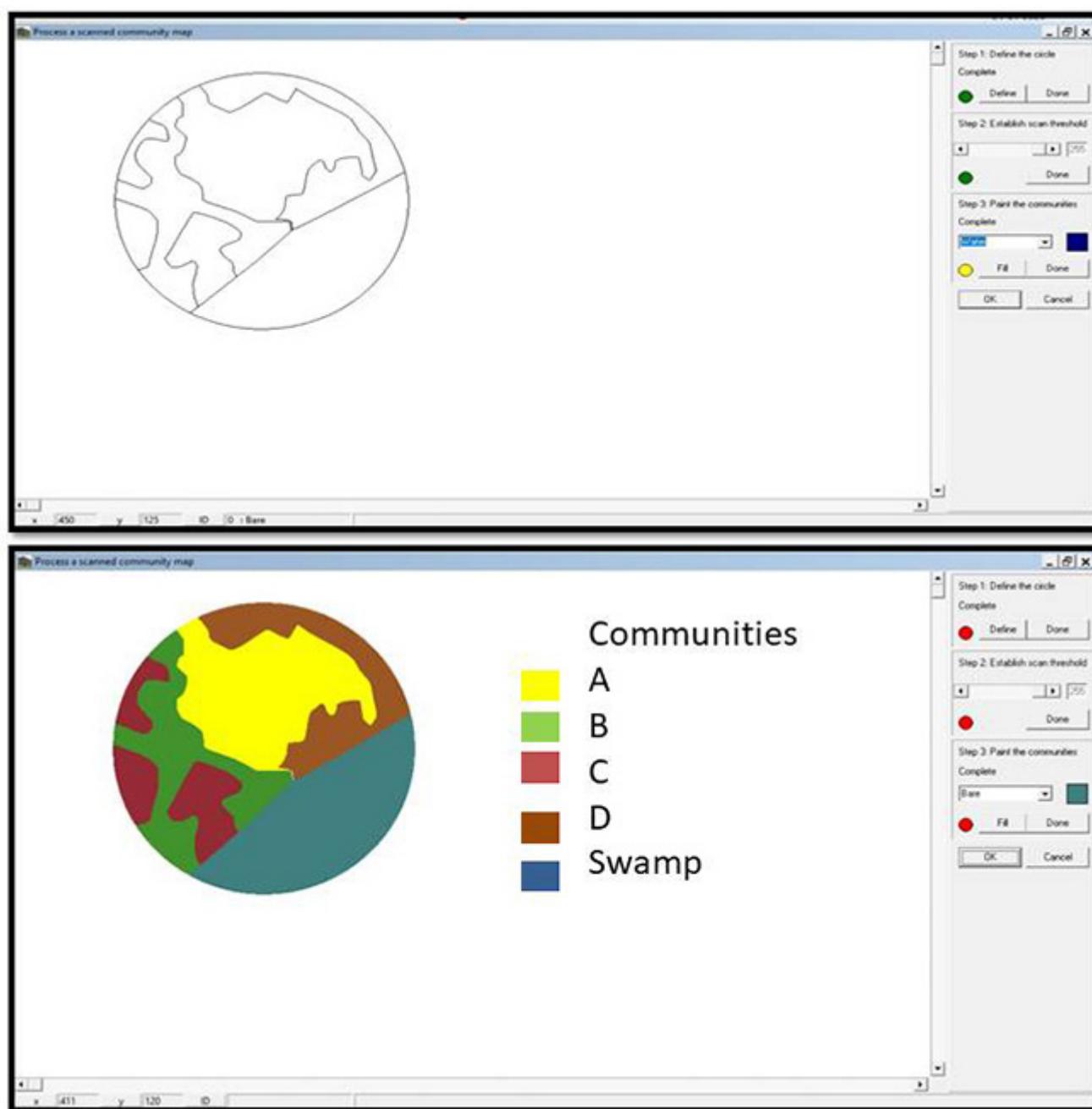


Fig. 3—A scanned sketch map (in .BMP format) of the survey site was loaded under tool option and different colours were assigned for different vegetation communities, water bodies and bare soil.

3. The materials were sieved through a 150- μm mesh size (0.146- μm pore size) sieve to remove the coarse debris.
4. The samples were then treated with 10% hydrochloric acid (HCl) to remove carbonates, if present, and thereafter samples were washed three times with distilled water for the removal of acid contents by means of centrifugation and decantation.
5. The materials were treated with 40% hydrofluoric acid (HF) for 2 to 5 days, to remove the silicates.
6. The samples were again washed with distilled water 2–3 times to render them free from silica and HF.
7. Samples were then treated with glacial acetic acid (GAA) and centrifuged two times.
8. The materials were treated with an acetolysing mixture (9: 1 ratio of acetic anhydride and concentrated sulphuric acid) in a water bath for 5 minutes or until the water started boiling.

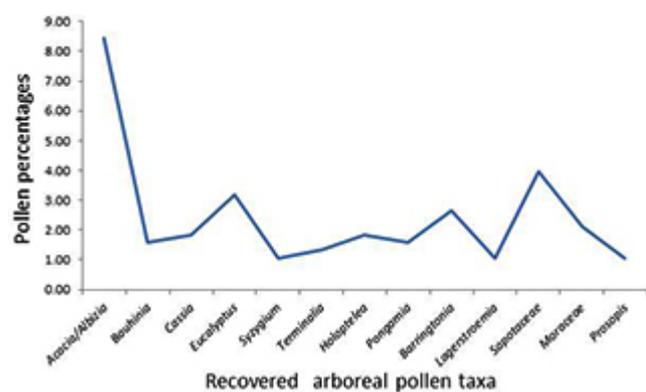


Fig. 4—Line diagram displaying arboreal pollen taxa (trees and shrubs) frequencies procured from surface sediment sample of 0–10 m quadrat.

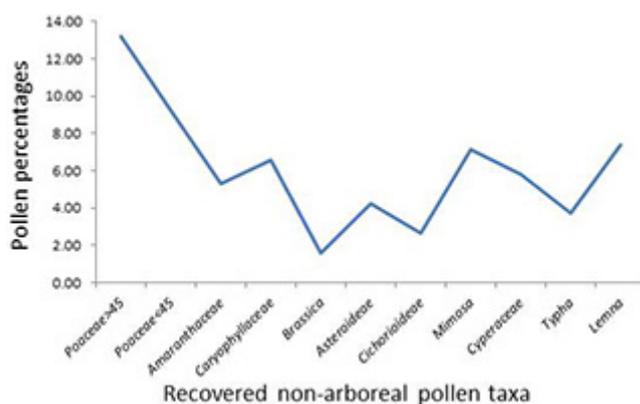


Fig. 5—Line diagram displaying non-arboreal taxa (herbs and aquatics) and high-land pollen taxa frequencies procured from surface sediment sample of 0–10 m quadrat.

9. Samples were then centrifuged and decanted in order to remove the acid.
10. Again, the materials were treated with GAA and washed with distilled water, 3 or 4 times.
11. Thereafter, 50% glycerine was added to the residue, and a few drops of phenol were added to protect the samples from microbial decomposition.
12. Temporary pollen slides were made for identification and counting of pollen under an Olympus BX 61 light microscope. Photography of the same was performed through the attached Olympus DP-25 camera (Pl. 3).

Identification of modern palynomorphs recovered from the surface soil sample was carried out through the consultation of reference slides available in the Herbarium of Birbal Sahni Institute of Palaeosciences (BSIP), Lucknow, and the published literature related to pollen studies (Chauhan & Bera, 1990; Nayar, 1990; Bera *et al.*, 2009). About 1000 pollen grains were counted from the surface soil sample. The percentages of the recovered pollen were calculated in terms of total pollen count (Figs 4–5). Poaceae (grasses) in the text are categorised with pollen < 45 μm (non-cereal) and with pollen > 45 μm (cereal) (Joly *et al.*, 2007).

Laboratory method for vegetation survey

A taxon dictionary file was created which is a set of all plant species found in the study area assigned to their pollen types and is saved in *.dct format (Table 1). This helped to correlate pollen taxa with their corresponding plants (group

of a plant), each specified pollen grain is assigned with the identifier “ex. *Ast-t=Asteraceae type” to feed into the ERV model. All these identifiers were also assigned with different plant taxa (e.g. *Ageratum conyzoides*) which have pollen grains of similar morphology. The list of these plants was used to demarcate identified plants in Zone A and B with the help of Quadrats and Community mapping, respectively (Pl. 1). Zone A and B vegetation data were inputted using Vegetation System Survey v2.0.1 (June 2012) which allows the user to enter their vegetation data into the 21 defined quadrats of Zone A (Pl. 2), and upload their field sketch map as a scanned image and attach the vegetation data to it (Fig. 3). The dictionary and the scanned map (in bitmap format) were saved in the same folder file where the software SURVEY was running. After the loading of Zone A vegetation data, different community data for Zone B was loaded. Later, a scanned sketch map (in .BMP format) of the survey site was loaded under the tool option and different colours were assigned for different vegetation communities, water bodies and bare soil (Fig. 3).

Statistical analysis

The statistical significance of the quantified data of relevés of different vegetation communities obtained from the SCSA forest area was determined by Canoco-5 software with constrained and unconstrained values and was taken to indicate statistical significance (Smilauer & Leps, 2014). The resulting data were imported into the Corel draw-12 Software package for further modification (Fig. 6).

PLATE 2

Vegetation System Survey v2.0.1 (June 2012) with the input of Zone A vegetation data into the 21 defined quadrats. 

Vegetation Survey System v2.0.1 : June 2012

File Communities Shortcuts Tools

N

S

Site name

Surveyor Date

Centre E Centre N

Crackles Project Survey Storage System v1.1 Richard Middleton 2011
data access date Thursday 9 January 2020

VERSION=2.0
 DICTIONARYTITLE=Master Directory-NBS
 SITENAME=not known
 SURVEYDATE=not known
 SURVEYORNAME=not known
 CONTACT=none
 NOTES=

RINGS=
 0 to 0.5 = qC
 0.5 to 1.5 = qN1*0.25+qE1*0.25+qS1*0.25+qW1*0.25
 1.5 to 3 = qN2*0.25+qE2*0.25+qS2*0.25+qW2*0.25
 3 to 6 = qN3*0.25+qE3*0.25+qS3*0.25+qW3*0.25
 6 to 10 = qN4*0.125+qE4*0.125+qS4*0.125+qW4*0.125+qNE*0.125+qSE*0.125+qSW*0.125+qNW*0.125
 10 to 100 = every 10

EASTINGS=0
 NORTHINGS=0
 SCANSOURCE=undefined

IMWIDTH=0
 IMHEIGHT=0
 CENTREX=0
 CENTREY=0
 RADIUS=0

----- The following are the quadrat data -----

SURVEYCOUNT=24

0 0 S \$FF Bare

0 0 S \$800000 Water

0 0 X \$DFDFC Corners

1 5 F \$FCFCFB qC
 sample

Eucalyptus	60.00
Cassia fistula	10.00
Acacia auriculiformis	40.00

<

Taxon dictionary Master Directory-NBS

PLATE 2

RESULTS

Pollen assemblage in sediment

The dominance of Poaceae pollen, including both cereal and non-cereal (22.42%) taxa, were observed in the surface sediment, followed by the high frequencies of polyads like *Acacia*, *Albizia* and *Mimosa* (7–9%). Pollen of *Lemna* was also recorded in good frequency (7.59%), while pollen incidence of other plants pertaining to families Sapotaceae, Amaranthaceae, Caryophyllaceae, Asteraceae, Cyperaceae and Typhaceae were in lesser frequencies (4–7%). Arboreal pollen taxa like *Bauhinia*, *Cassia*, *Eucalyptus*, *Syzygium*, *Terminalia*, *Pongamia*, *Barringtonia*, *Holoptelea*, *Lagerstroemia*, *Prosopis* and *Brassica* were also encountered frequently in the pollen assemblage. Among high-land taxa, *Pinus* pollen were best represented (1.06%) along with the scanty occurrence of *Corylus* (Figs 4–5).

PCA results of vegetation community mapping

The PCA score plot illustrated the Eigen values of 0.7643 (Axis 1) and 0.1977 (Axis 2). Simultaneously the total cumulative explained variation for the PCA score plot is 53.51% (Fig. 6). This means that the total variance of 53.51% could be explained by the plant taxa in the four different vegetation communities of SCSA forest. By examining the PCA score plot, it was observed that the plant taxa responsible for the difference between community A and community C were *Phyllanthus*, *Acacia*, *Albizia* and *Prosopis* which were the key taxa for major cluster differentiation. The taxa like *Acacia*, *Albizia* and *Eucalyptus* are found in extremely high frequencies in community A, whereas plant taxa like *Phyllanthus* is abundant in community C. Tree taxa like *Barringtonia*, *Pongamia* and *Terminalia* are placed at the high range of the PCA quadrant near community B. Community D is represented by the herbaceous patch including *Ageratum*, *Parthenium*, *Rumex*, *Tephrosia*, *Eclipta alba*, *Oxalis*, *Cannabis*, *Launea*, etc.

DISCUSSION

Step towards the modern pollen quantification

Conventional modern pollen surface samples with a checklist of plants in the immediate vicinity of the sites has been used as the basis for interpreting the past records extensively in the Indian subcontinent (Anupama *et al.*, 2000; Dixit & Bera, 2011, 2013; Quamar & Stivrins, 2021; Quamar, 2022). Quantitative estimates of the plants in the immediate vicinity and upto 1 km around the site combining field surveys and remote sensing take this one step further in providing distance-weighted plant abundances and are important for understanding the pollen dispersal and deposition characteristics of particular taxa in an environment. It is necessary to know how the pollen is spatially dispersed and deposited at the site of preservation, with reference to its source taxon. Once the spatial frameworks are attached to the pollen analysis, investigation of a range of important issues particularly, environmental and climate change would be possible with improved reliability and confidence which further facilitate the use of palaeoecological data to address issues in ecology, climatology, palaeontology and archaeology. The formulation of a quantitative relationship between pollen and vegetation follows a vegetation survey method that has been used in past studies to collect distance-based vegetation data (Broström *et al.*, 2004; Räsänen *et al.*, 2007; Mazier *et al.*, 2008). Bunting and Hjelle (2010) surveyed the area around a set of moss polsters repeatedly using different methods during a single field week and estimated the relative pollen productivity of *Potentilla*-type relative to *C. vulgaris* was around 0.5 using the rooted frequency method and around 2.5 using the visual estimate of the cover method. Therefore, a more precise vegetation survey protocol was adopted to avoid this confusion (Bunting *et al.*, 2013). This is the first attempt in the northern Indian plains to follow the Crackles Protocol, after its successful application in south-eastern India by Navya (2019) for the successful estimation of RPP of prominent plant taxa of a tropical deciduous forest at the SCSA Bird Sanctuary using vegetation survey software and utilizing the generated data in the ERV model.

PLATE 3

Palynomorphs recovered from the surface sediment samples of 0–10 m quadrat. 

- | | |
|-------------------------------|---------------------------|
| 1–2. <i>Acacia</i> type. | 15–16. Poaceae > 45 µm. |
| 3–4. <i>Albizia</i> type. | 17. Amaranthaceae. |
| 5. Sapotaceae. | 18. <i>Brassica</i> type. |
| 6–7. <i>Eucalyptus</i> type. | 19–20. Cyperaceae. |
| 8–9. <i>Pongamia</i> type. | 21. <i>Mimosa</i> type. |
| 10. <i>Barringtonia</i> type. | 22. <i>Lemna</i> type. |
| 11–12. <i>Bauhinia</i> type. | 23. <i>Glomus</i> type. |
| 13. <i>Pinus</i> type. | 24. Microthyriaceae. |
| 14. <i>Corylus</i> type. | |

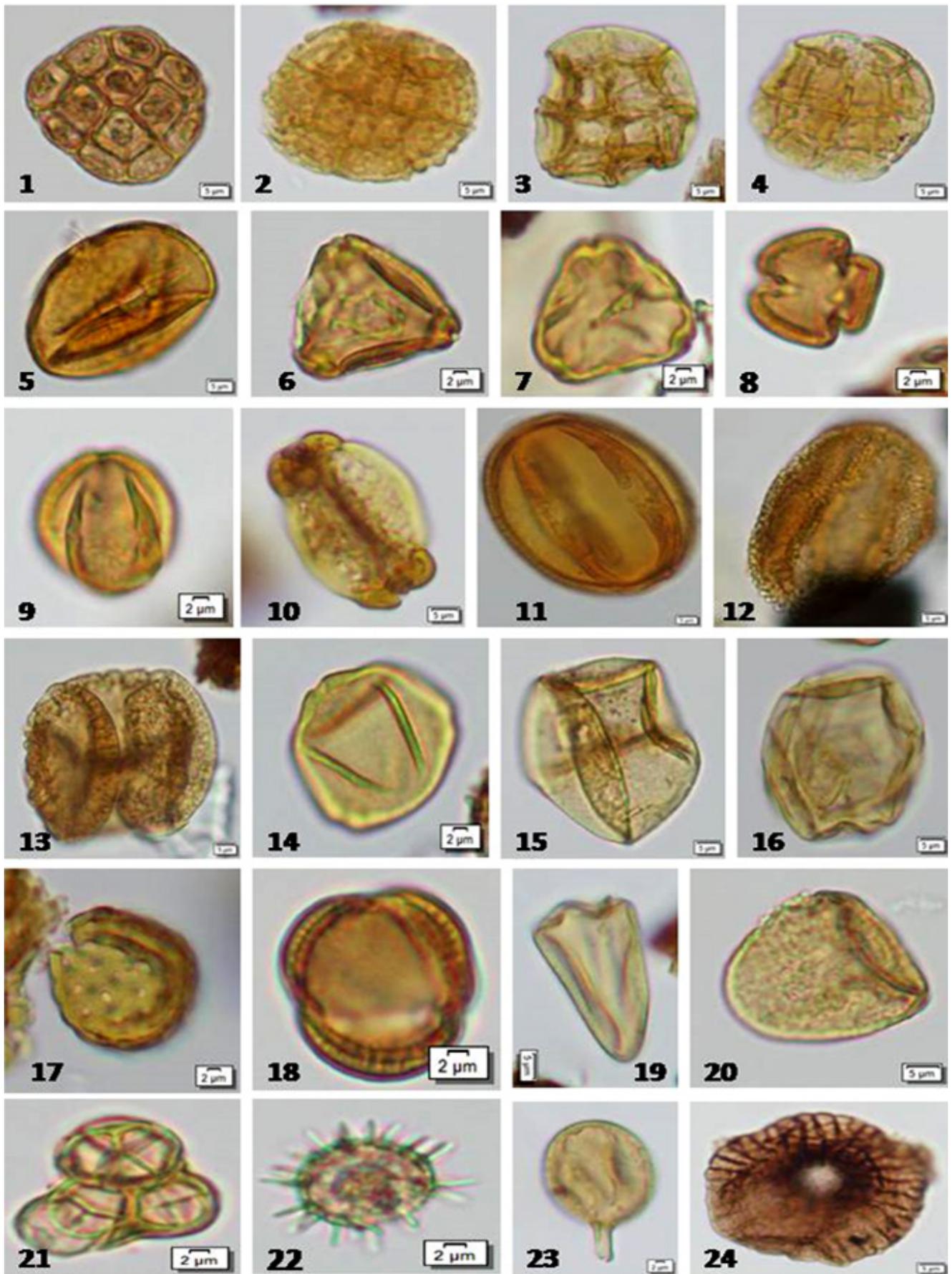


PLATE 3

The major plant taxa encountered during the survey were represented by *Acacia*, *Albizia*, *Terminalia*, *Dalbergia*, *Phyllanthus*, *Barringtonia*, *Callistemon*, *Tephrosia*, *Prosopis*, Poaceae, etc., signifying the occurrence of tropical dry deciduous forest. A high abundance of aquatic taxa was also luxuriantly growing around the perennial lake near the forest area. The recovered pollen from the centre of 0–10 m radius more or less correlates with the extant tropical dry deciduous vegetation represented by *Acacia*, *Albizia*, *Eucalyptus*, *Syzygium*, *Barringtonia*, Sapotaceae, etc. The presence of polyad pollen like *Acacia*, *Albizia* and *Mimosa* in high frequencies (7–9%) reflects the occurrence of local taxa in surface pollen assemblage. The high frequencies of aquatic taxa like *Lemna* and *Typha* indicates the occurrence of perennial water system which is found in real towards the vicinity of the SCSA forest. The herbaceous pollen taxa like Amaranthaceae, Caryophyllaceae, *Brassica*, Asteroideae, Cichorioideae indicates the presence of open-land in and around the forest area. The dominant occurrence of cereal (> 45 µm) and non-cereal (< 45 µm) pollen in the palynoassemblage indicates the high anthropogenic activities in this region in the form of agricultural practices. However, about three decades before palynologists used to distinguish non-cereal and cereal pollen on the basis of 60 and 80 µm size difference, respectively (Connell, 1987), which requires further examination.

Interpretation for PCA analysis

A variance of 53.51% could be explained by the first two principal components. The PCA score plot clearly showed four cluster separations between different vegetation communities in which vegetation community C falls in between community A and community B and thus shows closer resemblance with community A and B (Fig. 6). While the vegetation community D falls apart in opposite quadrant. According to PCA score plot, vegetation community D is independent and consist of herbaceous patch while community A consist of tree taxa like *Acacia*, *Albizia*, *Callistemon*, *Lagerstroemia* and planted *Eucalyptus* which shows that the forest was not consolidated and dense which reflect a mixed forest and open-land vegetation. According to score plot, the relatively high occurrence of Poaceae (cereal and non-cereal pollen) and other crop pollen taxa like *Lycopersicon* near the community A is the direct indicator of agricultural and anthropogenic activity in the nearby region. The presence of non-cereal pollen (Poaceae) could perhaps reflect human activity, which could indicate changes in mineral content of the sediment, increased fire frequency or a replacement of pollen types of mesic habitats with those of xeric conditions (Behling & Negrelle, 2001). The occurrence of *Pongamia*, *Terminalia* and *Barringtonia* along with marshy and aquatic taxa indicates the close vicinity of community B to the lake.

Limitations in the vegetation survey methodology

One concern in using a team with multiple individuals is the increase of error between the multiple recorders. Hence, the possibility of recorder bias is the main limitation in vegetation survey which can be avoided by giving common initial training to all the vegetation recorders. During the field trips, the recorders can analyse a few quadrats together and compare their results so that any individual errors in vegetation survey can be checked. In smaller teams, swapping of the team members will help in standardizing the datasets. The number of species that are recordable varies across the year (Losvik, 1991), suggesting that the best time for vegetation survey in a given habitat will often be much earlier in the field season than the preferred time for pollen sample collection. Many of the taxa of interest are relatively persistent in the vegetation (e.g. trees and shrubs, their coverage remains fairly constant even though their identification may vary) but not all (e.g. grasses and herbs), suggesting that ideally vegetation surveys should be carried out at the same phenological point for each habitat. However, this is often not possible due to the costs or logistical challenges of revisiting locations. Empirical investigations of the extent to which this can alter the estimates of RPP are underway as part of the modification.

Working with a greater variety of sites (locations, variety of basin sizes) helps further an investigation of the effect of vegetation heterogeneity pattern and basin size on the estimates of the relevant source area of pollen (RSAP). Thus heterogeneity will later persist in interpretation of vegetation type for vast tropical flora, more localised Crackles based survey will document flora and pollen grains, and principal component scores will define floral assemblage to improve the ambiguity. Sites for the collection of pollen and vegetation data should be of sufficient number and randomly distributed to meet the requirements of the ERV model. A sufficient number of sites are defined as, a minimum, double the number of plant taxa for which relative pollen productivity will be estimated, e.g. 14 sites for 7 taxa (Li *et al.*, 2018). However, the exact number of taxa for which RPP will be generated is not known in advance. In several studies from Europe, the strategy applied was to assume that a maximum of 15 to 20 taxa will be appropriate for ERV-model analysis and thus, 30 to 40 sites were used for pollen and vegetation data collection (Brostrom *et al.*, 2008; Mazier *et al.*, 2012). As the Crackles protocol was investigated globally for temperate regions, which has revealed estimation of Relative Pollen Productivity (RPP) and fall speeds of the representative pollen taxa. For the RPP estimation of the plant taxa, sufficient number of sites is needed for recording the vegetation data for the ERV model. Besides, using a small dataset seriously compromises PPEs when outliers are removed. This paper also documents multivariate analysis (Fig. 6) of pollen grains and their modern nearest living relatives to pre-requisites taxonomical affinity of plant taxa which is defined into arboreal and non-arboreal

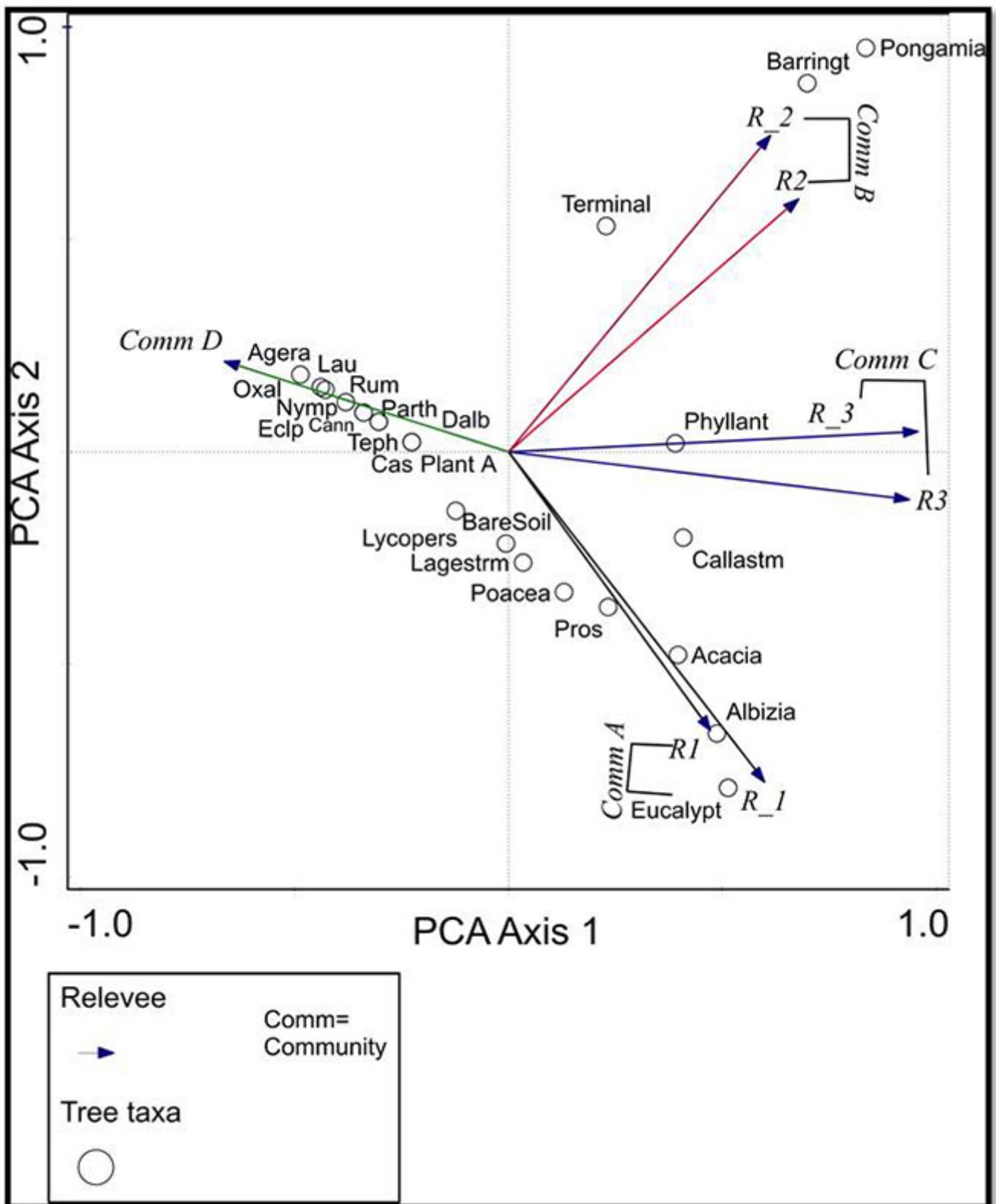


Fig. 6—The PCA analysis of the plant taxa surveyed in releves of different vegetation communities (within 10–100 m quadrat) in SCSA forest area. Tree taxa abbreviations with their extensions: Agera–*Ageratum*; Oxal–*Oxalis*; Lau–*Launaea*; Nymp–*Nymphalium*; Rum–*Rumex*; Eclp–*Eclipta*; Cann–*Cannabis*; Parth–*Parthenium*; Teph–*Tephrosia*; Dalb–*Dalbergia*; Cas–*Cassia*; Lycopers–*Lycopersicum*; Lagestrm–*Lagerstroemia*; Pros–*Prosopis*; Terminal–*Terminalia*; Barringt–*Barringtonia*; Phyllant–*Phyllanthus*; Callastm–*Callistemon*; Eucalypt–*Eucalyptus*.

dominance (Figs 4–5), vegetation type distribution, and identification of remote sensing based land cover patches (Fig. 1) with special reference to tropical region in north India.

CONCLUSIONS

The adopted Crackles Bequest field protocol for estimation of relative pollen productivity is pragmatic, designed to be replicable by different research groups, usable in a wide range of habitats, and requires minimum effort to collect adequate data for ERV model. Although the focus of current work and the papers cited here is in the temperate deciduous forest biome region of the Northern Hemisphere, the principles discussed are sufficiently general thus the method should be appropriate for the tropical forest biome also.

Vegetation communities documented from Zone A to B (10–100 m) are mapped in the field and their composition is recorded using standard methods for open, semi-open and tree dominated community stands and for linear features such as ditches or herbaceous patches. Pollen grains assemblage documented from the sediment, which were sampled from the centre of Zone A combined with modern flora surveyed in both zones (A–B) concised a pollen dictionary, which can be later extended as per the variation in assemblage. However, such protocols and dictionary can be followed further to investigate major pollen type and their RPP. This extensive vegetation sampling, even with the sparse quadrat array and recording the whole area in rings produced statistically genuine results, supporting the argument that the present vegetation survey is a sufficient strategy for ERV analysis data collection.

Acknowledgements—The authors extend their thanks to Dr. Vandana Prasad, Director, BSIP, Lucknow for facilities, infrastructure and permission to publish the paper (permission no. BSIP/RDCC/Publication No. 51/2020–21) and Dr. A.A. Mao, Director, Botanical Survey of India (BSI) for grant of project on floristic studies of the SCSA Bird Sanctuary and providing herbarium (BSA) facilities and data access. The authors also acknowledge the funding received by the BSIP for the training programme at SCSA Bird Sanctuary. Thanks are due to the forest officials of SCSA Bird Sanctuary, Unnao for their permission and support to explore the area. Dr. Ratan Kar, Dr. Santosh Kumar Shah and Dr. Vartika Singh are indebted for their cooperation during the field survey.

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