



Grassland fire ecology has roots in the late Miocene

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That fire facilitated the late Miocene C₄ grassland expansion is widely suspected but poorly documented. Fire potentially tied global climate to this profound biosphere transition by serving as a regional-to-local driver of vegetation change. In modern environments, seasonal extremes in moisture amplify the occurrence of fire, disturbing forest ecosystems to create niche space for flammable grasses, which in turn provide fuel for frequent fires. On the Indian subcontinent, C₄ expansion was accompanied by increased seasonal extremes in rainfall (evidenced by $\delta^{18}\text{O}_{\text{carbonate}}$), which set the stage for fuel accumulation and fire-linked clearance during wet-to-dry seasonal transitions. Here, we test the role of fire directly by examining the abundance and distribution patterns of fire-derived polycyclic aromatic hydrocarbons (PAHs) and terrestrial vegetation signatures in *n*-alkane carbon isotopes from paleosol samples of the Siwalik Group (Pakistan). Two million years before the C₄ grassland transition, fire-derived PAH concentrations increased as conifer vegetation declined, as indicated by a decrease in retene. This early increase in molecular fire signatures suggests a transition to more fire-prone vegetation such as a C₃ grassland and/or dry deciduous woodland. Between 8.0 and 6.0 million years ago, fire, precipitation seasonality, and C₄-grass dominance increased simultaneously (within resolution) as marked by sharp increases in fire-derived PAHs, $\delta^{18}\text{O}_{\text{carbonate}}$ and ¹³C enrichment in *n*-alkanes diagnostic of C₄ grasses. The strong association of evidence for fire occurrence, vegetation change, and landscape opening indicates that a dynamic fire–grassland feedback system was both a necessary precondition and a driver for grassland ecology during the first emergence of C₄ grasslands.

C₄ grassland expansion | paleofire | polycyclic aromatic hydrocarbons | leaf wax carbon isotopes | Mio-Pliocene

Fire regime is an emergent property of ecosystems and represents complex feedbacks between climate and vegetation that make it an important component of the earth system and terrestrial carbon cycle (1, 2). Until recently, fire was often treated as a response to, or indication of, global redox state (i.e., atmospheric O₂ concentrations) in the pre-Quaternary paleo record, with feedbacks among fire, climate, and vegetation community often neglected in interpretations of major vegetation transitions in Earth history (3). Fire system insights from modern ecological studies combined with novel tools for deep-time environmental reconstructions allow a more thorough examination of fire in the dynamics of past terrestrial ecosystems (3, 4).

The expansion of C₄-grassland ecosystems in the Miocene, one of the largest ecological shifts of the Cenozoic, transformed carbon cycling of the terrestrial biosphere on a global scale (5, 6). However, transitions on different continents were not synchronous during the interval of 5 to 8 Ma, which implies that regional mechanisms affected these events (7). Underlying drivers for the timing and nature of the expansion at local and global scales remain obscured by limited high-fidelity records of CO₂, drought, and other specific disturbance factors, including fire regime.

Fire is hypothesized to have served as a feedback mechanism linked to changes in Late-Miocene climate (particularly increased rainfall seasonality) that potentially optimized conditions for burning in fuel-limited ecosystems (8). Today, monsoonal conditions foster increased biomass growth during wet seasons, which

dries out and increases fuel loads during dry seasons. Fuel-laden dry seasons increase fire occurrence, which clears forested areas and allows grasses to occupy niche space previously shaded by trees; grasses increase seasonal fuel load with more flammable biomass and self-promote higher fire frequency. This cycle operates on a variety of seasonal to multidecadal timescales and has the potential to convert a woody tropical biome to grassland on continental scales (4). Grassland ecosystems themselves promote increased fire occurrence in modern landscapes and sustain themselves via a series of positive feedbacks (9). Both models and modern observations show that fire and seasonality of rainfall are critical to maintaining the current global distribution of subtropical grasslands (10). This suggests that a monsoon-linked fire feedback was responsible for the initial opening of grassy landscapes in the Miocene and their subsequent maintenance.

Marine records provide distal evidence supporting a rise of fire during the late Miocene. Charcoal records in marine sediments off the coast of East Asia and Africa dated between 2 and 9 Ma in conjunction with *n*-alkane carbon isotope records link fire and C₄ grasslands on these continents (11, 12). Charcoal in Northern Pacific marine sediment cores potentially suggests that an increase in fire was coincident with the timing of C₄ expansion on the Indian subcontinent (~8 Ma), although the geographic origin of the charcoal is not well defined. Coeval records of both vegetation and fire are needed to directly test the role of fire during grassland expansion in South Asia (13). Further, interactions between fire and vegetation are highly localized, and records that tie these changes together at the landscape scale are needed to untangle climate–vegetation interactions (4).

Significance

Fire is crucial to maintaining modern subtropical grasslands, yet the geologic and ecological history of this association is not well constrained. Here, we test the role of fire during the expansion of C₄ grassland ecosystems in the Mio-Pliocene through innovative molecular proxies from ancient soils in Pakistan. We produce a synoptic terrestrial record of fire and vegetation change in this region, which indicates that increased fire occurrence accompanied two stages of landscape opening. Proxy data confirm that a pronounced fire–grassland feedback was a critical component of grassland ecosystems since their origination and fostered the rise of C₄-dominated grasslands. The approach presented here can be used to examine landscape-scale interactions between paleofire and vegetation for other geographic regions and climatic transitions.

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The Miocene Siwalik Group of Pakistan provides an extended sedimentary record of South Asian terrestrial change in the form of fluvial sediments and paleosols that filled the sub-Himalayan foreland basin between ~ 18 and ~ 1.5 Ma (*SI Appendix, Fig. S1*) (ref. 14 and references therein). Extensive coeval bio- and geochemical records from these deposits have provided evidence to constrain mammalian evolution and vegetation change over the Neogene (15–18).

While there are many records of hydrologic and ecosystem change on the Indian subcontinent from 10 to 6 Ma, direct evidence of fire has remained elusive (19–21). C_4 expansion in this region is best documented in the Pakistan Siwalik sequence through carbon isotopic data from fossil teeth and paleosol carbonates (15, 16, 18, 22). Traditional pollen and charcoal methods for vegetation and fire reconstruction are not possible in this stratigraphic sequence due to the lack of macro- and microscopic plant material preservation (14). However, leaf wax biomarkers are preserved and have been successfully used to characterize vegetation shifts in the Siwalik record despite lack of plant preservation (23, 24). Molecular proxies thus provide an opportunity to resolve the fire history in conjunction with C_4 -grassland expansion for which conventional methods are not viable.

With this study, we present a paleofire reconstruction for the Pakistan Siwalik sequence based on pyrogenic signatures inferred from the concentrations and distributions of polycyclic aromatic hydrocarbons (PAHs) in a succession of Miocene to Pleistocene paleosols. These volatile compounds are produced by the incomplete combustion of organics. In modern environments, these compounds are widely linked to vegetation fires (25), even though they are less widely used as a paleofire proxy in sedimentary records (however, see ref. 26), especially in terrestrial archives. We combine evidence from plant waxes and pyrogenic PAHs extracted from the same samples to provide an integrated terrestrial record that links fire and vegetation change.

Evidence for a Primary Pyrogenic PAH Signal in the Siwaliks

We first examined the distributions of PAHs found in the Siwalik paleosols to ensure that the molecules primarily reflect fire inputs and were not confounded by modern fossil fuel contamination or derived from geologic sources. First, to evaluate potential laboratory contamination, stringent blanks were run during each processing step, and these proved clean. Second, diagnostic ratios of methylated and nonmethylated PAHs widely used in modern environmental chemistry (27) indicated that the PAHs were almost exclusively derived from predominately pyrogenic sources and not from coal or other petrogenic inputs (28). The exception was four samples, all from the oldest Chinji Formation (~ 14 to 11 Ma), which had ratios of methylphenanthrene (MPh) to phenanthrene (Ph) characteristic of petrogenic PAH sources (Fig. 1). As previously reported, n -alkane distributions in the Chinji Formation were degraded and indicated weathered parent-material inputs (23), which suggests that weathered thermally mature parent material was also the source of PAHs with a petrogenic signature. These samples also have some of the lowest concentrations of PAHs in the dataset, and we conclude that petrogenic inputs were rare, low, and do not confound the primary fire signal in this record.

Diagnostic ratios indicated that PAHs were not biologically degraded and give confidence that PAHs are a primary source signal. Modern soil studies show that fluoranthene (Fl) is more readily biodegraded by microbes over its more stable isomer, pyrene (Pyr) (29). $Fl/(Pyr+Fl)$ ratios were >0.5 in all samples, indicating no preferential loss of the less stable form (Fig. 1) (30, 31). $Fl/(Pyr+Fl)$ ratios are also a diagnostic indicator of source, with ratios >0.5 indicating inputs from biomass burning and those <0.5 corresponding to a fossil fuel source (27). Since the MPh/Ph values indicate that the source of these samples was predominantly pyrogenic, it is likely that these $Fl/(Pyr+Fl)$ ratios

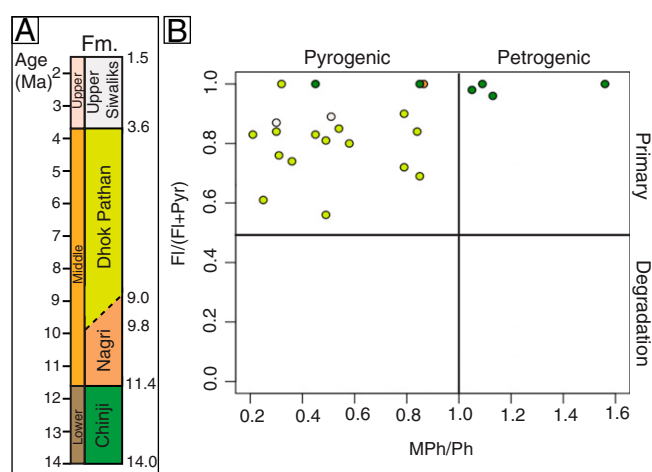


Fig. 1. (A) Generalized stratigraphy of Siwalik Group formations (Fm.) based on Barry et al. (14) and formatted after Patnaik (59). Color coding of formation (Fm.) corresponds to the colors in *SI Appendix, Fig. S1*. (B) Stratigraphically coded PAH diagnostic ratio cross-plot of Fl to Fl+Pyr versus MPh to Ph. Samples are color-coded based on the formations in the stratigraphic framework in A. $Fl/(Fl+Pyr)$ values >0.5 represent samples that have not been degraded, as Pyr is more stable than Fl. MPh/Ph values <1 correspond to samples from a pyrogenic (combustion) source, as petrogenic (thermally mature) PAHs have more methylated forms.

also reflect a primary vegetation burning signal that has not been subject to extensive microbial degradation.

Based on these analyses, we conclude that abundances of the pyrogenic PAHs primarily reflect trends in fire input to the soil during the Mio-Pliocene. Total parent PAH concentrations (nonmethylated) were normalized to C_{31} n -alkane concentration (total PAH/ C_{31}) (Fig. 2C). This normalization of PAH concentrations to a plant-derived biomarker accounts for PAH abundance variations solely due to decreases or increases in plant biomass production (26). Fire-derived PAH concentrations normalized to waxes increased by over an order of magnitude from 13.7 to 1.6 Ma. Notably, the rise in the total PAH/ C_{31} ratio occurred in two distinctive steps: (i) a fivefold increase between 10 and 6 Ma and (ii) an additional 10-fold increase starting at 6 Ma (Fig. 2C).

The PAH distributions in these ancient soil samples were also similar to those in vegetation-derived smoke particulates in modern environments. Samples older than 8 Ma contained retene, phenanthrene, and fluoranthene as major constituents. These three components are dominant in smoke particulates from modern burned conifers (32). In samples younger than 8 Ma, the major constituents were phenanthrene, fluoranthene, and pyrene in distributions that are similar to modern deciduous forest and Gramineae burn products (33). We interpret the decline in retene and the increase in pyrene among the major constituents as reflecting changes in the plant communities that burned.

Evidence for Changes in Pakistan Plant Communities

Conifer presence on the landscape decreased in the late Miocene, as indicated by declining abundances of retene, a product of conifer resin and softwood combustion (34). The proportion of retene to three-ring PAHs was greatest in the oldest samples (0.79) and decreased in samples younger than 10 Ma. After 6.5 Ma, ratios fell below 0.2, and in some cases, no retene was detected (Fig. 2D). The inferred decline in conifers suggests that the vegetation community changed several million years before C_4 plants appeared in the Pakistan Siwalik record.

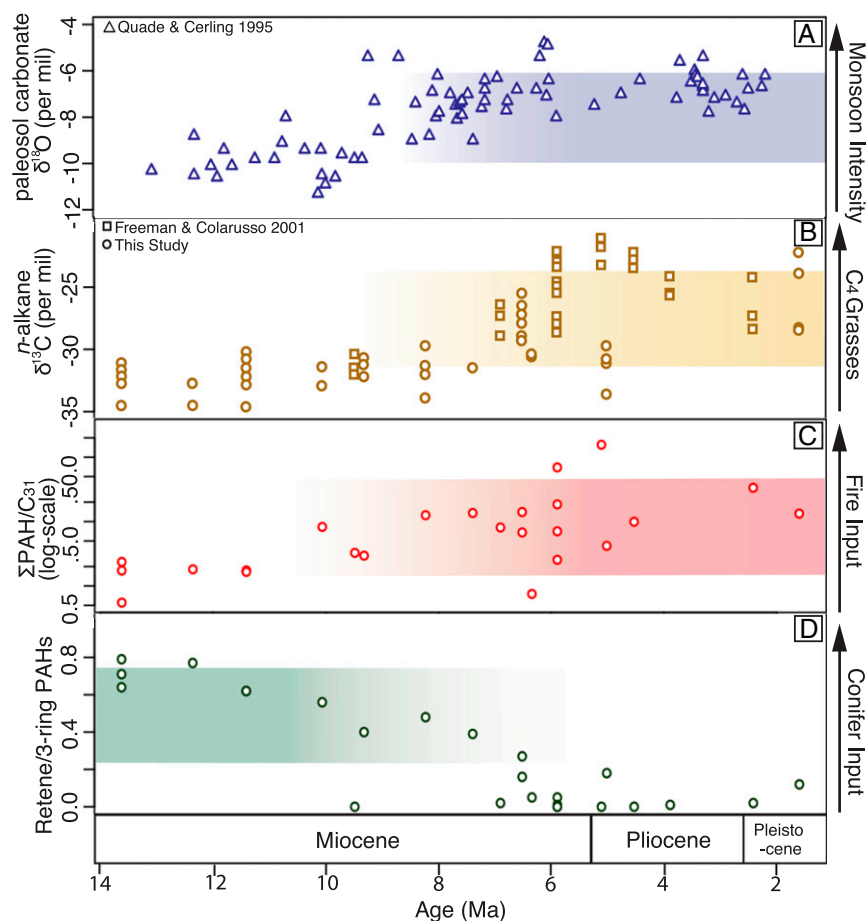


Fig. 2. Proxy evidence for monsoon, fire, and vegetation change in the Siwaliks from the late Miocene to the Early Pleistocene. Shading indicates transitions as shown by each proxy record. (A) $\delta^{18}\text{O}$ data from Siwalik paleosol carbonates replotted from ref. 15. (B) $\delta^{13}\text{C}$ values from C_{29} , C_{31} , C_{33} , and C_{35} *n*-alkanes from Siwalik paleosols. Squares are data from ref. 23, which correspond to PAH paleosol samples in this study. Circles are new data from this study. (C) PAH concentrations normalized to C_{31} *n*-alkane ($\Sigma\text{PAH}/\text{C}_{31}$) plotted on a logarithmic scale from Siwalik paleosols. (D) Retene normalized to retene plus other three-ring PAHs from Siwalik paleosols.

Following earlier work, the emergence of C_4 vegetation was inferred from carbon isotopes of leaf waxes to represent C_4 -vegetation change in the Siwalik paleosol record (23). Carbon preference index (CPI) is a common measure often used to verify the source of *n*-alkanes (see *SI Appendix*, Fig. S3 for equation). CPI values for samples ranged between 1.1 and 5, with an average of 2.8 ($n = 25$) (*SI Appendix*, Fig. S3A). CPI values >1 indicate an odd-over-even dominance of longer *n*-alkane chain lengths and indicate higher plant inputs to sediments and soils (35). CPI values in this study are comparable to previous studies of plant-derived *n*-alkanes in the Siwaliks, as well as in both modern soils and paleosols in other regions (23, 24, 36, 37). Previous researchers hypothesized that the generally low CPI values in the Siwaliks reflected oxidation and/or microbial alteration of plant waxes (23). Authors of modern-soil studies attribute low CPI values to spatial and temporal averaging of plant inputs to soils and consider them still indicative of terrestrial plant sources (37). Despite attenuated CPI values in the Siwaliks, carbon isotopic enrichment in organic (both bulk and *n*-alkanes) and inorganic ^{13}C are similar in both magnitude and timing in the Pakistan Siwaliks (15, 23) (*SI Appendix*, Fig. S5). This agreement suggests that, despite organic degradation processes, $\delta^{13}\text{C}$ values of leaf waxes represent vegetation changes in this record.

Leaf wax $\delta^{13}\text{C}$ values mark a significant vegetation transition in the late Miocene Siwalik record. Over the course of the entire

interval, from the Middle Miocene (14 Ma) to the Early Pleistocene (1.6 Ma), $\delta^{13}\text{C}$ values of C_{29} , C_{31} , and C_{33} *n*-alkanes all displayed a robust 10‰ increase (Fig. 2B). Strong ^{13}C enrichment (2 to 4‰) first emerged between 8 and 6.5 Ma, at the onset of the transition to a C_4 -grassland state, consistent with carbonate $\delta^{13}\text{C}$ data from previous studies in this region (*SI Appendix*, Fig. S5) (15).

The Relationship Between Vegetation Turnover and Active Fire Feedbacks

Both *n*-alkane $\delta^{13}\text{C}$ values and PAH abundance records exhibit considerable variability (*SI Appendix*, *Statistical Analyses*). High variance is not unexpected, given the inherent heterogeneity of fire processes on nonuniform natural landscapes (17). To determine whether this scatter confounded larger patterns in the data, we calculated correlation coefficients and their significance between C_{29} , C_{31} , and C_{33} *n*-alkane $\delta^{13}\text{C}$ values and log-transformed total PAH/C_{31} (*SI Appendix*, *Statistical Analyses*). Correlations of normalized PAHs to $\delta^{13}\text{C}_{29}$, $\delta^{13}\text{C}_{31}$, and $\delta^{13}\text{C}_{33}$ had r values of 0.70, 0.61, and 0.79, respectively ($P < 0.01$), and indicate a robust and significant correlation between fire occurrence and C_4 -grassland states. Thus, in the following discussion, we use trends in the molecular records to infer relationships between proxy for fire occurrence and vegetation-community change through time (Fig. 3).

The biomarker data suggest three stages, punctuated by two fire-linked transitions between fire increase and vegetation shifts in time and space, on the Indian subcontinent (Figs. 2 and 3). First, from 10 to 8 Ma, biomarkers indicate that fire increased as conifer inputs declined (Figs. 2 C and D and 3). Second, between 8 and 6.5 Ma, C₄-grass dominance increased as conifers further declined and, ultimately, were no longer important contributors to the regional ecosystem (Figs. 2 B and D and 3). Third, after 6.5 Ma, an order of magnitude increase in fire signatures coincided with the rapid onset of a fully C₄-grassland state (Figs. 2 B and C and 3).

The initial increase in fire at 10 Ma suggests that vegetation transitioned toward increasingly fire-prone communities before C₄-grassland expansion in the Potwar Plateau record. Vegetation type can modify fire occurrence by increasing flammable fuel, independent of changes in climate that promote fire (26, 38). In the Nepal section of the Siwaliks, vegetation-community shifts indicated by pollen and plant macrofossils that preceded isotopic evidence for C₄-grassland expansion suggest that mixed conifer and angiosperm evergreen communities were replaced by deciduous angiosperms before the expansion of C₄ grasslands (39, 40). Additionally, pollen records indicate that grasses were a potentially significant part of the Nepal landscape 2 My before C₄ plants expanded (40, 41). We suggest that similar changes took place in Pakistan, as indicated by the observed decline in relative retene abundance 2 My before the C₄ grassland transition (Fig. 2 B and D). Mixed conifer communities were replaced by frequent fire-promoting vegetation, such as a C₃ grassland or a dry angiosperm forest.

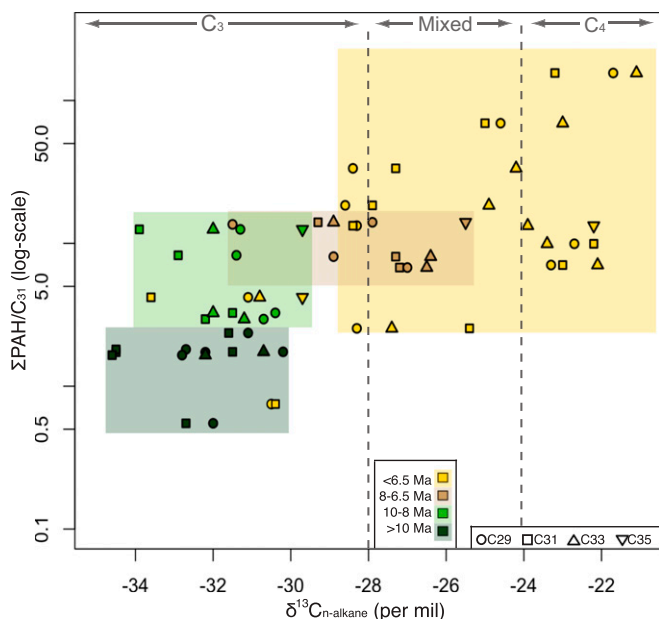


Fig. 3. The relationship between fire and vegetation change in the Siwaliks through time. PAH concentrations normalized to C₃₁ n-alkane ($\Sigma\text{PAH}/\text{C}_{31}$) plotted on a logarithmic scale against $\delta^{13}\text{C}$ values from C₂₉, C₃₁, C₃₃, and C₃₅ n-alkanes. Shaded boxes correspond to time slices in the record; symbols correspond to each chain length. Dotted lines represent approximate limits of C₃ and C₄ values (23). Respective correlation coefficients and significance levels for log-transformed PAH values vs. n-alkane $\delta^{13}\text{C}$ are C₂₉, $r = 0.70$ and $P < 0.01$; C₃₁, $r = 0.61$ and $P < 0.01$; C₃₃, $r = 0.79$ and $P < 0.01$; and C₃₅, $r = 0.57$ and $P = \text{nonsignificant}$. Two samples (R17-B, CF5-4 and R29-1, Psol 2) have both relatively depleted $\delta^{13}\text{C}$ values and low PAH concentrations relative to other samples of their time slice and fall outside the annotated boxes. However, these samples fit with the statistical relationship between $\delta^{13}\text{C}$ values and PAH concentrations. We interpret these as C₃ patches on the landscape that were subject to less burning.

A transition to a more open landscape at 10 Ma is supported by multiple lines of faunal evidence in Pakistan. Consistent with loss of closed woodland habitats before C₄ dominance, frugivorous and browsing mammals in the Potwar faunal record declined between 10.0 and 8.0 Ma (18). Further, $\delta^{13}\text{C}$ and $\delta^{18}\text{O}$ isotopic records of equid enamel indicate that grassland habitats started to expand before 9.4 Ma (16, 18, 22). Microwear analysis of multiple taxa indicates the presence of C₃ grass between 10 and 8 Ma, well before C₄ vegetation became dominant (16, 42). Eventually, mixed feeders and grazers shifted toward more C₄ diets between 8.5 and 6.0 Ma, accompanying the rising dominance of C₄ vegetation (16, 18, 22).

Today, fire regimes with low frequency (i.e., rare and intermediate return intervals) tend to dominate in tropical conifer (89%), temperate conifer (97%), and mixed conifer (97%) biomes, while frequent fire regimes (with short return intervals) are almost exclusively characteristic of grassland biomes (43). Lower-frequency fires tend to be litter fueled, whereas high-frequency fires are fueled by the more flammable biomass characteristic of grass biomes (43). We suggest that the appearance of a more open vegetation state increased fire frequency in the paleofire regime on the Siwalik floodplains and adjacent uplands. An increase in the flammability of biomass is indicated by a fivefold increase in PAHs that preceded isotopic evidence for C₄-grassland expansion and a sharp uptick in seasonal precipitation (15).

Increased fire occurrence during the C₃-dominated and fire-prone intermediate state likely promoted its own eventual replacement by C₄ grasslands. If the intermediate ecosystem was a dry angiosperm forest, an additional increase in flammability would have intensified landscape clearing. The 10 \times fire marker increase starting at 6 Ma provides evidence for a disturbance trigger that further cleared away woody vegetation and allowed C₄ grasslands to dominate (8). Alternatively, a set of linked fire-seasonality-C₄ positive feedbacks could have fostered the transition from a C₃-grassland intermediate state to C₄ grassland. Dynamic global vegetation models invoke C₄ characteristics based on the quantum yield model for modern C₃/C₄ plants (44, 45). When used to simulate Miocene C₄-grassland expansion, these models showed that fire favored an intermediate C₃-grassland state when a condition promoting the C₄ pathway over the C₃ pathway was lacking (i.e., a drop in CO₂, summer growing season, or low mean annual precipitation) (44). However, when fire was present and one of these climatic or biological conditions was also met, C₄ grasslands rapidly replaced C₃ grasslands.

The quantum yield model indicates that a wet summer growing season favors the C₄ pathway (46). A seasonality switch in the late Miocene favored summer precipitation over winter precipitation, indicated by the Siwalik oxygen isotope record (Fig. 2A) (17), promoting C₄ grasses over C₃ grasses. Additionally, C₄ grasslands are commonly more fire-prone than C₃ grasslands due to the evolution of fire-promoting traits (44, 47). This advantage, combined with hydrologic conditions that promoted increased fires, is consistent with the large jump in fire compounds observed with the advent of C₄ grasslands (Fig. 2A and C) (15).

The rapid and simultaneous rise in indicators for fire, C₄ grasslands, and seasonal drying provides strong evidence of a fire feedback system that fostered C₄-grassland expansion in South Asia (8). This does not preclude other factors that could have affected C₄ expansion (e.g., a drop in CO₂ levels or herbivory), but our results show that fire was important to the development of C₄ ecosystems on the Indian subcontinent.

Implications of a Late Miocene Link Between Fire and Grassland Expansion

Large-scale patterns of fire frequency and intensity are linked to both vegetation and climate conditions in the present (43). The interaction of these factors with fire, as represented in ecological

threshold models, characterizes rapid, nonlinear transitions between forest and grassland vegetation states (48, 49). We suggest that two distinctive fire-regime states accompanied the vegetation changes in the late Miocene. The first transition (~10 Ma), with a modest increase in fire signatures, reflected a mostly linear system response to fire (Fig. 3). The second transition—the expansion of C₄ grasses and the accompanying exponential jump in fire signatures (~6 Ma)—has characteristics of a system crossing a critical ecologic threshold and transitioning to a new stable vegetation state (Fig. 3) (43). It is possible that through positive feedbacks, favorable fire conditions were a necessary precondition that enabled the proliferation of grasslands in the late Miocene, and the two-staged ecosystem shift culminated in the establishment of frequent fire regimes unique to modern seasonal grassland ecosystems (43).

An early association between a fire regime with frequent burning and C₄ grasslands has important implications for both the development of subtropical ecosystems and the evolution of grassland fauna. In South Asia, the expansion of grasslands prompted the extinction of some browser and frugivore taxa, along with a transition to dominance of grazers and mixed feeders (16, 18, 50). Additionally, open habitats are linked to the development of traits such as hypsodonty and cursorial traits (51, 52). Animals in modern grassland ecosystems are adapted to open landscapes and, therefore, to environments characterized by frequent fire. In modern habitats, synergistic relationships between fire and herbivory are vital to the maintenance of open and productive grassland habitat (51, 53). Our results indicate that changing fire dynamics and the establishment of frequent fire regimes characterized the initial expansion of grassland floras and suggest that plant–fire–animal functional and evolutionary relationships have been in operation ever since.

The results of this study emphasize the need to reconstruct paleofire in the geologic record alongside vegetation and climate to fully examine the development of grassy biomes in Earth history. Feedbacks proposed by modeling studies (53) can now be explicitly tested using proxy data from the geologic record. The recognition of two distinct increases in fire input in the Siwaliks revealed unexpected contingencies between fire-regime changes and intermediate vegetation states that previously were not considered when examining this region's C₄ expansion and vegetation change. We have demonstrated that pyrogenic PAHs are preserved in paleosols and can be used to characterize paleofire inputs in conjunction with the development of grasslands in terrestrial contexts. The combination of this proxy approach with the interpretative paradigm of fire as an emergent property with feedbacks into the earth system is a powerful lens through which to decipher the role of paleofire in paleoecological transitions (3, 4). In modern grasslands, the fire–vegetation–climate system responds differently in distinct continental contexts, and likely did so in the past as well (54). We hope further use of this approach will address how regional differences in fire dynamics contributed to the globally asynchronous late Miocene grassland expansion, as well as other terrestrial ecosystem questions in Earth history.

Conclusion

Here, we provide a fire reconstruction in the Pakistan Siwaliks tied to C₄-grassland expansion by using PAHs preserved in paleosols as

a molecular proxy for pyrogenic input. There is a strong correlation between PAH concentrations and ¹³C enrichment in *n*-alkanes associated with C₄-grassland expansion in South Asia, indicating C₄ grasslands were linked in both time and space to increased fire occurrence. Increased fire signatures at 10 Ma, accompanied by a decrease in conifer input, suggest that a fire-prone transition state preceded C₄ grasslands by 2 My. Fully developed seasonal C₄ ecosystems, as indicated by δ¹⁸O_{carbonate} evidence for increased seasonality as well as increased δ¹³C_{*n*-alkane} values, coincided with highly elevated PAH abundances. The 10-fold greater PAH abundance clearly indicates that increased fire occurrence accompanied vegetation changes and landscape opening and provides robust evidence for a pronounced fire–grassland feedback in operation during the late Miocene grassland expansion. Our paleosol-based molecular fire reconstruction offers an approach that can reveal how fire affected the evolution of grassland ecosystems in other continental terrestrial archives.

Samples and Methods

Siwalik Paleosols. The Siwalik Group consists of fluvial channel and overbank deposits with floodplain paleosols deposited by the proto-Indus and Ganges rivers in the foreland basin of the Himalayas. We analyzed organics extracted from 25 paleosol samples collected by A.K.B., Thure Cerling, and Jay Quade from the Chinji, Nagri, and Dhok Pathan formations of the Potwar Plateau in Pakistan. Chronostratigraphy in this area is based on paleomagnetism and is constrained with ⁴⁰Ar/³⁹Ar dating from rare ash deposits (15, 17, 55). Age assignments were recalibrated based on the timescale of Gradstein et al. (56).

Analytical Methods. Ten of these samples had already been analyzed for *n*-alkane carbon isotopes in a previous study (23), and new analyses were conducted to investigate PAHs in all samples. Paleosol sediments were cleaned, freeze-dried, and powdered. Lipids were extracted from sediments using Soxhlet extraction and separated via accelerated solvent extraction as per Magill et al. (57). PAHs and *n*-alkanes were quantified and identified via GC-MS (Hewlett-Packard 6890 GC and Hewlett-Packard 5973 MS). Carbon isotopes of *n*-alkanes were measured using an irm-GCMS (Varian 3400 GC connected to Thermo MAT 252), and standard mean error for each measurement was 0.3‰ or less (1σ) (58). Carbon isotope values are reported in per mil (‰) delta notation normalized to the international standard Vienna Pee Dee Belemnite (VPDB) using certified reference materials (Schimmelmann A6). Delta notation is defined as δ¹³C = [(¹³C/¹²C)_{sample} / (¹³C/¹²C)_{VPDB} - 1] × 1000. To evaluate potential laboratory contamination, blanks were run for each processing step, and molecules of interest in this study were below detection. Detailed methods are available in *SI Appendix, Extended Methods*.

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