Inferring ecological disturbance in the fossil record: A case study from the late Oligocene of Ethiopia

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Abstract
Environmental disturbances profoundly impact the structure, composition, and diversity of modern forest communities. A review of modern studies demonstrates that important characteristics used to describe fossil angiosperm assemblages, including leaf margin type, plant form, plant diversity, insect herbivore diversity and specialization, and variation in herbivory among plant species, differ between early and late successional forests. Therefore, sequences of fossil floras that include a mix of early and later successional communities may not be appropriate to study long-term temporal trends or biotic effects of climate, latitude, or other variables.

Keywords: Succession Paleobotany Africa Chilga Oligocene Plant–insect interactions

1. Introduction
Disturbances like fires, hurricanes, floods, and volcanic eruptions play an integral role in structuring forest communities and may even be essential to maintaining that structure (e.g. Connell, 1978; Denslow, 1987; Hubbell et al., 1999). The process of community recovery following a disturbance is called succession (Harper, 1977), and forest communities often go through a series of compositional and structural changes following disturbances, recognized as successional stages, each with different plant and insect herbivore diversity, composition, and life habits. The fundamental structural and taxonomic differences among stages of forest recovery emphasize the importance of determining the successional stage (or stages) represented by a fossil leaf assemblage. Complete sequences of forest succession are rarely preserved in the plant macrofossil record because of its limited temporal and spatial resolution (Behrensmeyer and Hook, 1992; Burnham et al., 1992). Instead, the macrofossil record preserves either isolated leaf floras represented a single successional stage or time-averaged floras that mix early and later successional stages, although there are exceptions (García Massini et al., 2010). Sampling a mix of early and later successional communities in a stratigraphic sequence may mask true long-term temporal trends, create spurious trends, or obscure correlations with climate (Cross and Taggart, 1982; Burnham, 1994), latitude, or other variables. Furthermore, extracting the deep–time record of alpha and beta diversity accurately, especially at tropical paleolatitudes, is critical for identifying drivers of biodiversity change through time.
Spatial heterogeneity in plant composition and insect herbivory within a forest can be assessed in the paleobotanical record through lateral sampling (e.g., Burnham et al., 1992; Wing et al., 1993; Burnham, 1994; Davies-Vollum and Wing, 1998; Dimichele et al., 2007; Currano, 2009). Several paleobotanical studies have attributed abrupt taxonomic changes across a landscape or through time to post-disturbance succession (e.g., Cross and Taggart, 1982; Taggart and Cross, 1990; Frank and Bend, 2004; Samant and Mohabey, 2009; Bashforth et al., 2010; Garcia Massini et al., 2010; Bashforth et al., 2011). For example, Bashforth et al. (2010, 2011) demonstrated succession in Carboniferous macrofossil deposits by linking sedimentological data to plant taxa. Cross and Taggart’s (1982) work on the Miocene Succor Creek palynofloras used analogies to nearest living relatives to conclude that progression through distinct post-disturbance successional stages caused abrupt taxonomic shifts.

Here, we focus on non-taxonomic approaches that can be used to recognize post-disturbance succession in angiosperm-rich fossil assemblages. We first review the ecological literature on plant and insect herbivore responses to disturbance in order to determine what (and whether) structural and functional attributes characterize early vs. late successional angiosperm-dominated tropical forests (Table 1). We then apply these characters to the Oligocene fossil record of the Chilga Basin, northwest Ethiopia. We focus on two stratigraphically equivalent palaeofloras that are 1.5 km apart but have no plant species in common. We hypothesize that sedimentological, taxonomic, and palaeoecological differences between the two palaeofloras will be consistent with communities in different, earlier and later, forest successional stages. This is one of the few paleobotanical studies to attribute physiognomical and paleoecological variations in contemporaneous macrofossil assemblages to succession, and the first to apply insect herbivore damage data to issues involving disturbance and succession.

### 2. Non-taxonomic approaches to recognizing disturbance in modern settings

#### 2.1. Plant physiology and morphology

The first plants to occupy an area following a disturbance generally have rapid resource acquisition, high growth rates, and a life strategy that maximizes seed production (Grime, 1974). Their leaves often have low leaf mass per area, short leaf lifespan, high nutrient concentrations, and high rates of photosynthesis and respiration (Wright et al., 2004), all of which make them highly palatable to insect herbivores (Coley et al., 1985; Wright et al., 2004). Late successional species display a much wider range of life strategies and leaf traits. Some species invest most of their energy in growth and little in anti-herbivore defenses, making them vulnerable to both generalist and specialist herbivores. Others are adapted to maximize nutrient retention by having a long leaf lifespan, low nutrient concentrations in the leaves, and low photosynthetic and respiration rates (Wright et al., 2004). These species, which are more likely to invest in chemical defenses, should be less palatable to generalist herbivores and display a high percentage of specialized damage.

A variety of studies suggest that plant species with toothed margins are more abundant in early successional forests than in later successional forests. In upper montane Costa Rican forests, about 60% of plant species in early successional forests had toothed margins, versus ~50% in late successional and ~30% in primary forests (Kappelle and Leal, 1996). Subsequent studies in Ecuador, Australia, and North America have demonstrated that plant species with toothed leaves are more common in riparian habitats than distal floodplain or terra firma sites (Burnham, 1994; Burnham et al., 2001; Kowalski and Dilcher, 2003; Greenwood, 2005; Royer et al., 2009).

The studies referenced above have proposed three main explanations for the abundance of toothed species in riparian and disturbed habitats: increased availability of water (Bailey and Sinnott, 1915; Wolfe, 1993; Kowalski and Dilcher, 2003; Royer et al., 2009), higher diversity of toothed lianas (Burnham et al., 2001), and selection against untoothed species in disturbed environments (Kappelle and Leal, 1996; Burnham et al., 2001). Higher rates of transpiration, respiration, and photosynthesis have been measured in toothed margins than in untoothed margins (Wolfe, 1993; Royer and Wulf, 2006), interpreted as advantageous in places where rapid leaf expansion would be beneficial. Rapid leaf growth could provide toothed pioneer and ruderal species a competitive advantage in disturbed habitats, especially where water availability is high and can support high rates of transpiration and photosynthesis. Although the mechanism driving the pattern may vary based on geography, phylogeny, and the presence/absence of a well-defined successional sequence (Royer et al., 2009; Little et al., 2010), these data overwhelmingly support a higher percentage of toothed angiosperm species in riparian and disturbed habitats.

#### 2.2. Plant ecology

Ecological studies of disturbance and succession in neotropical ecosystems have focused on forest regeneration following timber harvesting, clear-cutting, and hurricanes. Guariguata and Ostertag (2001) review the literature and propose a four stage sequence for tropical terra firma forest recovery. Initial colonization by grasses, herbs, and ferns occurs during the first five years following the disturbance. Years 5–20 are characterized by early forest development, as short-lived pioneers create a closed canopy forest. Lianas, shrubs, and herbs are also an important component of the forest (Guariguata et al., 1997; Pena-Claros and De Boo, 2002; Capers et al., 2005). Species richness steadily increases, and age since disturbance is the best predictor of plant diversity (Aide et al., 1996; Letcher and Chazdon, 2009). Over roughly the next hundred years, long-lived successors replace the short-lived pioneers. Plant species richness can reach pre-disturbance levels in as few as forty years (Aide et al., 1996; Barberena-Arias and Aide, 2003), and richness in late successional forests often exceeds that in primary forests because of the mixing of early successional, late successional, and mature forest tree species (e.g. Vandermeer et al., 2000; Urquhart, 2009). The final stage of recovery is the return to old growth forest composition. The nature and rate of this process depends on seed bank dynamics, soil characteristics, frequency and intensity of disturbance, and distance.

### Table 1

<table>
<thead>
<tr>
<th>Character</th>
<th>Early successional forest</th>
<th>Late successional forest</th>
</tr>
</thead>
<tbody>
<tr>
<td>Leaf margin</td>
<td>Higher percentage of toothed species</td>
<td>Lower percentage of toothed species</td>
</tr>
<tr>
<td>Plant form</td>
<td>Abundant lianas, shrubs, herbs, and ruderal species</td>
<td>Abundant canopy trees and understory vegetation</td>
</tr>
<tr>
<td>Plant diversity</td>
<td>Lower</td>
<td>Higher, many rare species</td>
</tr>
<tr>
<td>Insect herbivore diversity</td>
<td>Lower</td>
<td>Higher, echoing the higher plant taxonomic and structural diversity</td>
</tr>
<tr>
<td>Insect herbivore specialization</td>
<td>Lower percentage of specialized feeders; wider niche breadth</td>
<td>Higher percentage of specialized feeders; narrower niche breadth</td>
</tr>
<tr>
<td>Variation in herbivory among plant species</td>
<td>Low. Most species have a lot of damage.</td>
<td>High. Some species have very little damage, whereas others have many types and occurrences of damage.</td>
</tr>
</tbody>
</table>
to and characteristics of the remnant forest. Although species richness can rebound to old-growth values within a few decades after a disturbance, it often takes hundreds of years for the species composition to return to what it was before the disturbance (Guariguata and Ostertag, 2001; Urquhart, 2009).

2.3. Insect herbivory

Ecological disturbances should have both direct and indirect effects on insect herbivore communities. Frequent disturbances keep insect herbivore diversity low, and most insect species have a short generation time, high migratory ability, high reproductive potential, and low niche specialization (Brown, 1985). Insect communities also feel the bottom-up effects of changes in the plant community, and specialized herbivores must be most susceptible because of their dependence on one or a few closely related host plants. Several studies have demonstrated an increase in herbivore specialization with plant successional stage. Brown’s (1985) landmark study of Heteroptera populations showed a decline in the proportion of generalist insect species and an increase in the proportion of specialists along a gradient of early to late successional plant communities. Similarly, Brown and Southwood (1983), using a numerical scale to express niche breadth of sap sucking insects, documented a decline in breadth as succession proceeded. More recently, a study of restoration plots in the Brazilian Amazon found that forest age is the most important factor determining the richness of galling insects (Fernandes et al., 2010), considered among the most specialized of insects.

Published theories regarding the effects of plant succession on insect herbivore diversity are all founded on the idea that a more complex resource base can support more species than a simple resource base. Most insect species display some degree of specialization (Bernays and Chapman, 1994; Termonia et al., 2001; Farrell and Sequeira, 2004), and increasing the complexity of the resource base creates more niches for insect herbivores. Resource complexity can be defined taxonomically or structurally. According to the taxonomic diversity hypothesis, the more taxonomically diverse a plant community is, the more insect species it can support (Siemann et al., 1998; Bröse, 2003), and a variety of studies have demonstrated a positive correlation between plant diversity and insect diversity (Wright and Samways, 1998; Hawkins and Porter, 2003; e.g. Haddad et al., 2009).

The structural heterogeneity hypothesis, on the other hand, states that heterogeneity in plant architecture and structure increase as a forest matures, driving higher insect diversity in later successional stages (Southwood et al., 1979; Bröse, 2003; Jeffries et al., 2006). Architectural diversity refers to the distribution of different types of plant structure and tissues, and structural diversity to the distribution of plant parts spatially above ground level. In all likelihood, both plant taxonomic and structural diversity influence insect species diversity, and higher herbivore diversity is therefore expected in a late successional forest than an early successional forest.

3. Methods

3.1. Geologic overview

The study area is located 60 km west of Gondar in Chilga Woreda, Amhara Region, northwestern Ethiopia. Massive flood basaltus up to 2000 m thick were extruded about 30 Ma (Hofmann et al., 1997), followed by faulting in the middle to late Oligocene to create a basin no less than 325 and probably no greater than 1000 km². Fossiliferous clastic and volcanoclastic sediments interbedded with volcanic deposits filled the basin (Feseha, 2002; Jacobs et al., 2005). A total of 150 m of stratigraphic section containing fossil plants and animals is exposed; radiometric dates and paleomagnetic reversals place these strata in the late Oligocene Chattian stage, between 27 and 28 Ma (Kappelman et al., 2003; Jacobs et al., 2005).

The most productive and well-preserved fossil floras in the Chilga Basin occur in autochthonous overbank and pond deposits. These floras, which consist of leaf, fruit, seed, and flower compressions, provide a unique view of wet, tropical vegetation from the interior of the Afro-Arabian continent. They predate both the major mammalian turnover event that occurred near the Paleogene–Neogene boundary (Maglio, 1978; Bernor et al., 1987; Gagnon, 1997; Kappelman et al., 2003; Stevens et al., 2008; Rasmussen and Gutierrez, 2009; Ducrocq and Boisserie, 2010) and Miocene rainforest. The plant species found at Chilga are characteristic of lowland or submontane tropical moist forests and represent a historical biogeographic link between the currently disjunct Central and West African forests and the East African coastal and Eastern Arc forests (Jacobs et al., 2005; Pan et al., 2006; Pan, 2007; Pan and Jacobs, 2009; Pan, 2010). Similar to modern African tropical forests, legumes (Fabaceae) dominate the Chilga floras. However, the occurrence and diversity of fossil palms at Chilga contrasts strongly with extant African tropical forests, which are notably depauperate in palms (Pan et al., 2006).

3.2. Fieldwork and paleobotanical analyses

In this study, we compare two fossil floras that occur in the same facies and are stratigraphically and temporally equivalent. The Guang River plant fossil horizon is exposed laterally for 60 m along the Guang River, and the Bull’s Bellow locality is 1.5 km to the south, along the Margargaria River (Fig. 1, Table 2). Detailed lithological descriptions were made at four different sublocalities at Bull’s Bellow and at a single locality (CH40) along the Guang River (Fig. 2). Sections were dug back minimally 300 mm in order to avoid modern weathering features. Descriptions include lithology, sedimentary structure, color, acid-reaction class, bed thickness and characteristics of boundaries between sedimentary rock layers. Sediment and paleosol profile colors were determined by comparison of dry samples against Munsell® Color Chips (Munsell Color, 1975).

Because the two fossil horizons are laterally extensive, we excavated and collected fossil plants from four sublocalities at Guang and seven sublocalities at Bull’s Bellow to capture spatial heterogeneity within each locality. Sublocality sites were selected based on accessibility, rock cohesiveness, and preservation quality of plant remains. At each sublocality, the fossil-bearing strata were treated with a dilute bleach solution to further isolate the cuticle, washed with distilled water, and finally immersing them in 48% HF until the cuticle was completely matrix-free (2–24 h). The specimens were then treated with a dilute bleach solution to further isolate the cuticle, which was then mounted on slides in glycerine jelly for light microscopy. Fossil specimens were compared with extant plant collections in herbaria at the Royal Botanic Gardens, Kew, Missouri Botanical Garden, and Botanical Research Institute of Texas.

Every identifiable leaf (or leaflet) with at least half of the blade intact was scored for insect herbivore damage using the Labandeira et al. (2007) damage type (DT) system. Insect folivory was distinguished from detritivory or taphonomic alteration by the presence of plant response tissue; consistency in the pattern, position, or type of damage; or specific micromorphological features, such as papal chambers, particular mandible bite marks, or exit slits in mines and galls (Labandeira, 1998). Damage types can be divided into generalized...
DTs, made by insects whose diet typically includes many taxonomically unrelated plants, and specialized DTs, made by insects that typically consume only one or a few closely related host plants (Wilf and Labandeira, 1999; Labandeira et al., 2002; Labandeira et al., 2007). Specialization is recognized by similarity to extant specialized feeders, by morphologically stereotyped damage patterns, and by restricted occurrences confined to particular host-plant species or tissue types in either fossil or extant host taxa (Labandeira, 2002; Labandeira et al., 2007). Here, we consider DTs to be specialized if they have a host specialization value of 2 or 3 on the 1–3 scale in the Labandeira et al. (2007) system.

3.3. Quantitative analyses

All analyses were performed in R version 2.9.2 (R Development Core Team, Vienna, Austria). We analyzed plant species richness, evenness, and heterogeneity for each flora. Floral richness, or the number of plant species standardized for sample size, was measured using analytical rarefaction, and errors were estimated using Heck et al.’s (1975) standard error. Probability of interspecific encounter (PIE) was used to compare evenness among sublocalities and between Guang and Bull’s Bellow. Floral heterogeneity within a locality was quantified by calculating the Bray–Curtis distance between all pairs of sublocalities. These distances were calculated from a matrix of plant species’ relative abundances. We averaged the results within each locality to obtain a single value for heterogeneity.

Insect folivory was examined using three damage metrics: diversity, frequency, and composition. Damage diversity, or the number of DTs present at a site or on a host species, was normalized for the number of leaves sampled as in previous studies (Wilf and Labandeira, 1999; Currano et al., 2008). Damage diversity on a per-leaf basis is a core measure of how many ecological feeding types are present on a certain amount of foliage resource. Although damage diversity is not completely analogous to insect diversity, preliminary data from Panama show a good correspondence between DT diversity and actual insect diversity (Ramírez-Carvalho et al., 2009). Damage frequency is simply the percent of leaves in a sample that are damaged. Because a leaf either has damage or does not, error bars for damage frequency are calculated using a binomial sampling distribution. Damage composition refers to the relative abundance of the DTs in different samples. Only DTs that occurred on two or more leaves were included in these analyses. The data were first arcsine square-root transformed to improve normality (Sokal and Rohlf, 1995). The “metaMDS” function in R was then used to perform nonmetric multidimensional scaling (NMS) ordinations.

### Table 2
Sampling summary, Guang River and Bull’s Bellow.

<table>
<thead>
<tr>
<th>Sublocality</th>
<th>Site</th>
<th>GPS coordinates</th>
<th>No. identifiable leaves</th>
<th>No. leaf species or morphotypes</th>
<th>No. insect herbivore DTs</th>
</tr>
</thead>
<tbody>
<tr>
<td>CH40</td>
<td>Guang</td>
<td>N 12.5134 E 37.1240</td>
<td>209</td>
<td>14</td>
<td>21</td>
</tr>
<tr>
<td>CH41</td>
<td>Guang</td>
<td>N 12.5129 E 37.1240</td>
<td>94</td>
<td>25</td>
<td>14</td>
</tr>
<tr>
<td>CH52</td>
<td>Guang</td>
<td>N 12.5134 E 37.1240</td>
<td>74</td>
<td>15</td>
<td>12</td>
</tr>
<tr>
<td>CH54</td>
<td>Guang</td>
<td>N 12.5129 E 37.1239</td>
<td>56</td>
<td>18</td>
<td>13</td>
</tr>
<tr>
<td>Guang total</td>
<td></td>
<td></td>
<td>433</td>
<td>40</td>
<td>32</td>
</tr>
<tr>
<td>CH72</td>
<td>Bull’s Bellow</td>
<td>N 12.50890 E 037.11300</td>
<td>195</td>
<td>22</td>
<td>18</td>
</tr>
<tr>
<td>CH90</td>
<td>Bull’s Bellow</td>
<td>N 12.50888 E 037.11288</td>
<td>126</td>
<td>15</td>
<td>9</td>
</tr>
<tr>
<td>CH91</td>
<td>Bull’s Bellow</td>
<td>N 12.50886 E 037.11277</td>
<td>34</td>
<td>10</td>
<td>2</td>
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<tr>
<td>CH92</td>
<td>Bull’s Bellow</td>
<td>N 12.50873 E 037.11270</td>
<td>155</td>
<td>14</td>
<td>17</td>
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<tr>
<td>CH95</td>
<td>Bull’s Bellow</td>
<td>N 12.50937 E 037.11417</td>
<td>75</td>
<td>14</td>
<td>6</td>
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<tr>
<td>CH101</td>
<td>Bull’s Bellow</td>
<td>N 12.50529 E 037.11397</td>
<td>5</td>
<td>4</td>
<td>1</td>
</tr>
<tr>
<td>CH103</td>
<td>Bull’s Bellow</td>
<td>N 12.50936 E 037.11423</td>
<td>55</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>Bull’s Bellow total</td>
<td></td>
<td></td>
<td>645</td>
<td>35</td>
<td>27</td>
</tr>
</tbody>
</table>

* Damage types.
‡ CH40 and CH52 are less than 5 m apart and therefore have identical GPS coordinates at this resolution.
4. Results

4.1. Sedimentology and stratigraphy

The fossil flora stratigraphic unit considered herein was collected from a single layer ranging in thickness from 9 to 40 cm (Fig. 2) composed of drab-colored, wavy laminated to thinly bedded, claystone to mudstone. This single bed is correlatable among all study sites based upon the authors' ability to walk this bed, and verify its continuity, from the Guang River to Bull's Bellow collection sites.

The stratigraphy of the Bull's Bellow and Guang River sites is dominated by drab-colored and fine-grained lithologies (Fig. 2). The lower half to two-thirds of each measured and described sublocality section is dominated by massive to angular blocky claystone to mudstone with common, vertical to horizontal, branching organic matter compressions as well as common (2–5% by volume) to abundant (5–15% by volume) mm-sized, orange to brown, sphaerosiderite concretions (Jacobs et al., 2005). The upper half to one-third of the measured sections is dominated by claystones, silty claystones and mudstones and only rarely preserves carbonized organic matter compressions or concretionary sphaerosiderite. At Bull's Bellow, this upper part of the stratigraphy preserves sedimentary transport structures including plane-to-wavy laminations, ripple cross-lamination, small-scale (10–20 cm deep) trough cross-bedding and clear examples of a scour-and-fill deposit (CH90, 160–180 cm) and mud drapes (CH90, 245–260 cm). In addition, fallen and burnt tree trunks occur among three of the five measured sublocality sections, and only in the beds within the fossil flora unit.

4.2. Plant taxonomy

The Bull's Bellow flora includes numerous taxa typically associated with early successional stages of tropical forest regrowth (Fig. 3). These consist of abundant fruit compressions representing the tribe Naucleeae sensu lato (Rubiaceae; Fig. 3A, B) and fossil vegetative
material of heliophytic vine and liana taxa including *Cardiospermum* (Sapindaceae; Fig. 3C), an African rattan (Arecaceae: Lepidocaryeae; Fig. 3D), and a morphotype that likely represents either Aristolochiaceae or Menispermaceae. In addition, numerous toothed-leaves of Euphorbiaceae (*Macaranga* or a closely related euphorbiaceous taxon; Fig. 3E) and Salicaceae sensu lato (Fig. 3F) are present and represent ruderal elements (George et al., 1993; Slik et al., 2003; LaFrankie et al., 2006).

The Guang River flora differs from Bull’s Bellow in the co-occurrence of forest taxa that are typical of early secondary forests and those found in later successional stages of forest regrowth. Guang River fossil taxa representing groups commonly associated with forest gaps, forest edges, and recolonized areas include *Eremospatha* (Arecaceae: Lepidocaryeae), *Clausena* (Rutaceae), *Dioscorea* section *Lasiophyton* (Dioscoreaceae), and *Strychnos* (Loganiaceae; Fig. 3G; Pan et al., 2006; Pan, 2007; Sunderland, 2007; Pan, 2010), whereas genera with living relatives commonly occurring as understorey, shade tolerant trees in African moist forests include *Cola* (Malvaceae sensu lato: Sterculioidae), *Ocotea* (Lauraceae), and *Eugenia* (Myrtaceae; (Pan, 2007; Pan and Jacobs, 2009). The Guang River flora also consists of fossils that likely represent slow growing, canopy or emergent trees including *Cynometra*, *Afzelia* (Fabaceae: Detarieae sensu lato), and two Sapotaceae morphospecies (*Sapotaceae* sp. and *Chrysophyllum* sp.; (Pan, 2007; Pan et al., 2010)). While the presence of disturbance indicator taxa is consistent with a secondary forest community, the abundance of understorey and mature canopy forest groups in the Guang River flora suggest a later stage of forest regrowth capable of recruiting and sustaining such elements.

4.3. Plant morphology

Two out of 34 dicot species at Guang have toothed leaves, compared to seven out of 35 at Bull’s Bellow. Because margin type is a binomial character, a binomial distribution was used to compute errors on the percent of untoothed species as described by Wilf (1997). The Guang River flora, with 5.9±4.0% toothed species, has significantly fewer toothed species than the Bull’s Bellow flora, with 20±6.8% toothed species.

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**Fig. 3.** Bull’s Bellow and Guang River disturbance plant taxa. A, B: Naucleeae (Rubiaceae) reproductive structures, scale bar — 10 mm, (A) inflorescence or immature infructescence, (B) infructescence; C–G: Leaf compressions, scale bar — 20 mm, (C) *Cardiospermum* sp., (D) African rattan (Arecaceae: Lepidocaryeae) leaflet fragment, (E) Euphorbiaceae, (F) Salicaceae sensu lato.
4.4. Plant richness and heterogeneity

In order to examine plant richness standardized for sample size, rarefaction curves were plotted for the Guang River and Bull’s Bellow sublocalities (Fig. 4). Although there is a range in rarefied species richness at sublocalities within a site, the Guang localities generally have more species than those at Bull’s Bellow (Table 3). When the sublocalities at each site are lumped together, Guang has significantly higher floral richness than Bull’s Bellow. Furthermore, the lumped Bull’s Bellow curve and most Bull’s Bellow sublocality curves are beginning to level off, indicating that we have sampled much of the diversity at this site. In contrast, additional collecting is needed to fully sample diversity at Guang, as illustrated by the steadily increasing rarefaction curves.

Both sites are strongly dominated by a single plant species and over half of the plant species are represented by less than five leaves (Fig. 5). Thus, there is very little difference in evenness between Guang and Bull’s Bellow at both sublocality and locality levels (Table 3). The most abundant species at each site are legumes. *Cynometra chaka* makes up 44% of the leaves at Guang, an *Albizia*-like leaf (mimosoid sp. 1) is 34% of the total at Bull’s Bellow, and *Swartzzieae* (cf. *Mildbraediadendron*) composes another 13% of the leaves at Bull’s Bellow. To examine lateral plant heterogeneity within each locality, we calculated Bray–Curtis distances between sublocalities (Tables 4 and 5), which measure differences based upon species compositions and frequencies. The mean Bray–Curtis distance between sublocalities is 0.65 at Guang and 0.51 at Bull’s Bellow. This difference is significant ($t = -2.78$, $p = 0.02$, $df = 7$), indicating that the Guang flora is more heterogeneous.

### Table 3

<table>
<thead>
<tr>
<th>Sublocality</th>
<th>Shannon</th>
<th>Probability of interspecific encounter</th>
<th>Rarefied diversity at 50 leaves</th>
</tr>
</thead>
<tbody>
<tr>
<td>CH40 (G)</td>
<td>1.57</td>
<td>0.66</td>
<td>9.33</td>
</tr>
<tr>
<td>CH41 (G)</td>
<td>2.58</td>
<td>0.88</td>
<td>17.87</td>
</tr>
<tr>
<td>CH52 (G)</td>
<td>1.53</td>
<td>0.58</td>
<td>12.32</td>
</tr>
<tr>
<td>CH54 (G)</td>
<td>2.53</td>
<td>0.91</td>
<td>17.11</td>
</tr>
<tr>
<td>Average of Guang River sublocalities</td>
<td>2.05 ± 0.58</td>
<td>0.76 ± 0.17</td>
<td>14.16 ± 4.05</td>
</tr>
<tr>
<td>Lumped Guang River</td>
<td>2.32</td>
<td>0.78</td>
<td></td>
</tr>
</tbody>
</table>

### Table 4

<table>
<thead>
<tr>
<th>Sublocality</th>
<th>CH54</th>
<th>CH41</th>
<th>CH40</th>
<th>CH52</th>
</tr>
</thead>
<tbody>
<tr>
<td>CH54</td>
<td>-</td>
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<td></td>
</tr>
<tr>
<td>CH41</td>
<td>0.64</td>
<td>-</td>
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<td></td>
</tr>
<tr>
<td>CH40</td>
<td>0.75</td>
<td>0.64</td>
<td>-</td>
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### Table 5

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4.5. Insect herbivory

We analyzed insect damage diversity, frequency, and composition at each locality for both individual host plants and the bulk flora. Total, specialized, and mine damage diversity on the bulk flora are all significantly higher at Guang than at Bull’s Bellow (Fig. 6). However, when analyzing herbivory on individual host species at the two sites, only total damage diversity is higher at Guang (Table 6). Therefore, the higher damage diversities on the Guang bulk flora are most likely due to elevated plant richness at Guang.

Damage frequency is significantly higher at Guang than at Bull’s Bellow (31.6 vs. 23.1% of leaves damaged, Fig. 7). The percent of leaves with specialized damage is nearly double, and the percent of leaves with generalized damage is also slightly higher. Elevated specialization at Guang is also apparent when percent of damage occurrences rather than percent of leaves damaged are analyzed, and it appears to be primarily driven by an increase in galling at Guang.

An NMS ordination of percent of leaves with each DT at each sublocality confirms that there are important differences in damage composition between Guang and Bull’s Bellow (Fig. 8A). The Guang River sublocalities plot close to each other and are separated from most of the Bull’s Bellow sublocalities. The Bull’s Bellow sublocalities show more scatter, indicating greater variation in damage composition, and Bull’s Bellow sublocality CH92 plots closer to the Guang sublocalities than to other Bull’s Bellow sublocalities. Fig. 8B considers damage composition on individual plant hosts at the two sites. The Guang and Bull’s Bellow hosts do not form distinct groups, and show a similar amount of variation in damage composition within locality. Thus, both localities contain a mix of little damaged and highly damaged plant taxa.

5. Discussion

5.1. Sedimentology and depositional environments

We consider the massive to angular blocky claystones to mudstones, which may preserve concretionary sphaerosiderites, to be paleosol profiles. Furthermore, we consider the branching
With the exception of CH92, all of the sublocalities inspected preserve a paleosol profile directly beneath the unit containing the fossil flora. Note that scour and erosion of this paleosol profile can be quite extensive (e.g., scour of paleosol in Bull’s Bellow CH90), and therefore it is possible that a correlative soil profile at CH92 was present, but completely eroded, prior to deposition of the overlying unit containing the fossil flora. Nevertheless, the regional sedim-
tology directly beneath this fossiliferous stratigraphic unit repre-
sents a generally stable yet swampy landscape across this part of the basin.

The common feature of burnt, fallen and transported fossil tree trunks at two of the four Bull’s Bellow sublocalities and at Guang River sublocality CH40 suggests an important ecological shift that resulted in destruction, transport, deposition, and preservation of large-statured plants at least in this part of the basin. It is impossible to determine from our data the mechanism responsible for this ecological shift, but it nevertheless included fire as part of the disturbance process. However, the fossiliferous units of the Bull’s Bellow sublocalities are composed of numerous beds and laminations, suggesting multiple episodes of deposition and a generally more “disturbed” environment than the fossiliferous unit at the Guang River sublocality, which is composed of a single, featureless mudstone that may represent a single depositional event subsequently overprinted by pedogenesis (Fig. 2). These differences in sedimentary structure suggest that the Guang River locality represents a more stable, relatively more quiescent and low-energy depositional system than the Bull’s Bellow locality during deposition of the fossiliferous unit.

5.2. Resolving fossil and depositional records

Basin-wide studies of the sedimentology, stratigraphy, and paleosols at Chilga indicate a heterogeneous landscape (Jacobs et al., 2005). A mixture of poorly-drained and well-drained paleosol types occur within the basin, indicating that landscape heterogeneity was likely caused by differences in water table height across space and with time. Sedimentological analyses of stream channel and overbank deposits suggest that rivers had relatively shallow gradients and wandered across the landscape, changing course and affecting plant communities via periodic flooding events (Jacobs et al., 2005). Additionally, charred tree trunks at three of five measured sections provide evidence for fires altering the landscape. In this study, we consider the consequences of this disturbance regime across space at a single time.

Because the Guang River and Bull’s Bellow floras are stratigraphically equivalent and only 1.5 km apart, it is surprising that the two have no plant species in common. However, our stratigraphic and sedimentological data provide evidence that the Bull’s Bellow site was a less stable, higher energy environment than the Guang River site, and a detailed analysis of the taxonomy, plant physiognomy, and paleoecology at each site confirms that the two floras represent different stages of tropical forest recovery. Bull’s Bellow has a higher percentage of plant species with toothed margins, and the nearest living relatives of many of the plant species are associated with early successional forests. Both plant and insect herbivore diversity are relatively low, and there is very little specialized damage. In contrast, the Guang River flora has a very low percentage of plant species with toothed margins, and the plants are a mix of early and late suc-
cessional species. Plant and insect damage diversity is significantly higher than at Bull’s Bellow, and the frequency of specialized damage is nearly twice as high. A review of the ecological literature indicates that these physiological and ecological attributes are consistent with a more mature forest community (Table 1).

Consequently, multiple biotic and abiotic proxies indicate that disturbance, succession, and other landscape-level ecological processes explain variations in plant physiognomy, plant and insect
herbivore composition, and paleoecology among angiosperm-dominated fossil leaf assemblages within a single basin. In particular, insect damage is strongly influenced by disturbance and forest succession. Because insect herbivore specialists often make distinct feeding marks that are preserved on fossil leaves, it is possible to determine the frequency and diversity of specialist feeders, which today show a positive relationship with forest age (Brown, 1985; Fernandes et al., 2010). Our data show that insect damage diversity is higher in a later successional tropical forest, which had greater floral richness, and support the hypothesis that insect diversity tracks plant diversity across a landscape and through time (Wright and Samways, 1998; Hawkins and Porter, 2003; e.g. Haddad et al., 2009).

The many differences between the Guango River and Bull’s Bellow floras illustrate the importance of disturbances in structuring forest communities and the necessity of collecting at multiple localities of the same age within a basin and of sampling from the same paleoecological setting if comparing floras and herbivory from two time intervals. Accurate estimates of diversity and feeding specialization in late Oligocene tropical Africa are essential to understanding the origin and persistence of high species diversity in the tropics generally, and the relatively low level of diversity in the African tropics compared with other regions today. For example, if only Bull’s Bellow were considered, the late Oligocene of tropical Ethiopia would look considerably less diverse and show far less herbivore specialization than actually existed in the Chilga depositional basin. In contrast, the rarefaction curves for both plant and insect damage at Guango indicate that considerably more sampling would be needed to capture the real pattern of richness and diversity at that site. As there is considerable interest in correlating plant and insect damage diversity with global climate change in the fossil record, our work in the Chilga Basin documents the need to understand whether successional stage has introduced bias to the leaf assemblages being studied.

Of broad significance is the issue of assessing plant biodiversity through time. If we sum the mutually exclusive taxa from Bull’s Bellow and Guango River, species richness nearly doubles, indicating beta diversity was high for the Chilga region in the Late Oligocene. As both alpha and beta scales are important for assessing overall biodiversity, our study points to the importance of lateral sampling of paleobotanical sites if biodiversity comparisons among floras from different time intervals are to be made.

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