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Leaf-economic strategies across the Eocene–Oligocene transition correlate with dry season precipitation and paleoelevation

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PREMISE: The Eocene–Oligocene transition (EOT; 34–33 Ma) was marked by global cooling and increased seasonality and aridity, leading to a shift in North American floras from subtropical forests to deciduous hardwood forests similar to today. This shift is well documented taxonomically and biogeographically, but its ecological nature is less known.

METHODS: Using the relationship between petiole cross-sectional area and leaf mass, we estimated leaf dry mass per area (LMA), a functional trait tied to plant resource investment and expenditure, at 22 western North American sites spanning the EOT to determine how the broad restructuring of vegetation during this time was reflected in leaf economics.

RESULTS: There was no overall shift in LMA between pre-EOT and post-EOT floras; instead, changes in LMA across sites were mostly driven by a negative correlation with dry-season precipitation and a positive correlation with paleoelevation. These patterns held for both whole sites and subsets of sites containing taxa with similar biogeographical histories (taxa that persisted in the highlands across the EOT or that migrated to the lowlands) and are consistent with most observations in extant floras.

CONCLUSIONS: Our data provide a geological context for understanding environmentally paced changes in leaf-economic strategies, particularly linking leaf economic strategies to dry-season precipitation and paleoelevation.

KEY WORDS Bridge Creek; CLAMP; Eocene-Oligocene transition; Florissant; functional traits; leaf economics spectrum; leaf mass per area; LMA; paleobotany; paleoclimate.

During the Eocene–Oligocene transition (EOT; 34–33 Ma), the Earth's climate shifted from the globally equable greenhouse climate of the Eocene to a cooler, more seasonal climate similar to today (Wolfe, 1994; Liu et al., 2009). Most of this shift took place just after the Eocene–Oligocene boundary (33.9 Ma; Gradstein et al., 2012) and is closely linked with the Oi-1 glaciation (33.5 Ma), when deep-ocean temperatures cooled rapidly and large ice sheets formed on Antarctica (Zachos et al., 2001). The timing and magnitude of this event's effects on land are uncertain due to the heterogeneity of terrestrial climates (Retallack et al., 2004; Lielke et al., 2012) and asymmetric hemispheric responses (Liu et al., 2018). Nonetheless, a common response in the mid to high latitudes of the northern hemisphere was a rapid cooling of ~5°C alongside an increase in seasonality and aridity (Retallack et al., 1999; Eldrett et al., 2009; Liu et al., 2009).

In western North America, lush subtropical forests were replaced during the EOT by deciduous hardwood forests, the foundation of today's widespread North American temperate biome (e.g., Chaney, 1947; Wolfe, 1977). This ecological shift was originally attributed to the "Arcto-Tertiary" concept (Chaney, 1947)—that during the warm Paleocene and Eocene, temperate deciduous forests were restricted to the colder high latitudes until they later spread southward under cooler global climate conditions. This concept was reevaluated after (1) the discovery of thermophilic forests in the high latitudes during the Eocene, which highlighted the equability of the Eocene greenhouse (Koch and Undersøgelse, 1963; Wolfe, 1980); and (2) the taxonomic revision of taxa from early Oligocene temperate forests, which revealed their affinity with the preceding subtropical Eocene forests (Wolfe, 1977). From this, Wolfe (1977) hypothesized that the deciduous hardwood forests of the early Oligocene were derived from both migrating temperate taxa from high-latitude and high-elevation forests of the Eocene and persistent subtropical taxa from the Eocene lowlands. Taxonomic analyses of Eocene and Oligocene floras provide strong evidence for both pathways

(Wing, 1987; Meyer and Manchester, 1997; Leopold et al., 2008; DeVore and Pigg, 2010). Critically, the physiological and ecological nature of this floral realignment has only been inferred based on knowledge of the extant relatives of the flora, an approach that by definition assumes no changes in plant function between fossils and their associated extant relatives (DeVore and Pigg, 2010; Leopold and Zaborac-Reed, 2019).

To avoid this limitation and to more directly evaluate plant ecological strategies from late Eocene and early Oligocene floras in western North America, we use here the concept of the leaf economics spectrum (LES) (Reich et al., 1997; Wright et al., 2004). The LES is a continuum of viable leaf strategies, described by trade-offs among a group of leaf functional traits that reflect how plants utilize their resources. On one end of the LES are slow-return individuals. These have low rates of photosynthesis, low concentrations of leaf nitrogen and phosphorous, and low rates of respiration; as a trade-off for this slow revenue stream, slow-return individuals have long leaf lifespans, and—to support their longer lived leaves—a high leaf dry mass per area (LMA), which represents the thickness and density of a leaf. On the other end of the LES are fast-return individuals. These have a low LMA and short leaf lifespan, but in exchange have high photosynthesis rates, respiration rates, and nutrient concentrations. These fundamental trade-offs of the LES are largely free from taxonomic influence (Ackerly and Reich, 1999); plants hew closely to the LES with little exception (Wright et al., 2004). Thus, while the position of individuals, species, and floras along the LES is informed by a mixture of evolutionary history (Reich, 2014) and the influence of physical environments (Wright et al., 2005; Poorter et al., 2009), at its core, LES position reveals the energy trade-offs and strategies inherent to a plant.

In this study, we determined the position of 22 late Eocene and early Oligocene floras along the LES by estimating the LMA of fossil leaves using the mechanical relationship between the cross-sectional area of the petiole and the mass of the leaf (Royer et al., 2007). Not only do these floras capture Wolfe's (1977) historical pathways of persistence and dispersal across the EOT as biogeographical affinities realigned, they also each represent different physical environments, covering a wide range of temperature, rainfall, seasonality, and elevation. These environmental traits can all play a significant role in governing viable leaf-economic strategies. For example, mean annual temperature (MAT) and elevation both positively correlate with LMA in extant environments, especially within deciduous taxa (Wright et al., 2005; Read et al., 2014), while mean annual precipitation (MAP) is negatively correlated, especially within evergreen taxa (Wright et al., 2005). When seasonality of precipitation is considered by isolating dry-season precipitation (total precipitation in the three driest months [3DRY]), an even stronger negative correlation with LMA is observed (Niinemets, 2001; Wright et al., 2004). Fossil studies that address these relationships between the LES and climate are rare but promising. In particular, seasonality of precipitation has been linked to LMA in Central European Paleogene floras (Roth-Nebelsick et al., 2017) and in North American Eocene floras (Royer et al., 2007).

By evaluating the biogeographic shifts in floral composition alongside these climate interactions, we can come to a greater understanding of how plant strategies were affected by the Earth-system upheaval of the EOT. To do so, we investigated five LMA scenarios derived from Wolfe's (1977) two pathways of persistence and dispersal. (1) Global shift: Roth-Nebelsick et al. (2017) observed a decrease in LMA across the EOT in European floras. In a global LMA response scenario, North American floras will exhibit similar decreases in LMA across every dimension of the transition (e.g., in both persisting and dispersing taxa). Such a global shift in LMA expression could potentially be tied to the global decrease in MAT and the drawdown of CO_2 across the EOT, both variables which have been shown to positively correlate with LMA (Poorter et al., 2009). (2) Persistence without functional change: The LMA of persisting taxa will not significantly change across the EOT. (3) Persistence with functional change: The LMA of persisting taxa will shift to reflect a change in ecological strategies. Because variance in LMA within a single assemblage is often greater than between assemblages (Wright et al., 2004), significant overlap in LMA range may still occur. (4) Dispersal without functional change: Taxa that migrated from the highlands to the lowlands will maintain their LMA. (5) Dispersal with functional change: Taxa that migrated will shift their LMA.

MATERIALS AND METHODS

Fossil sites

We reconstructed the LMA of woody dicot taxa from 22 Eocene and Oligocene floras (Table 1, Fig. 1) that encompass a wide range of physical environments (Table 2, Fig. 2). To put them into our five-scenario framework, we divided the floras into four bins: pre-EOT highlands, pre-EOT lowlands, post-EOT highlands, and post-EOT lowlands.

Pre-EOT highlands—*Florissant*—Located in the high-elevation Rocky Mountains of Colorado (Meyer, 2001), Florissant (34.07 Ma; $^{40}Ar/^{39}Ar$ dating; Evanoff et al., 2001) is our best-represented pre-EOT highland flora. The flora contains a mix of subtropical evergreen and temperate deciduous species (MacGinitie, 1953; Leopold et al., 2008; Allen et al., 2020) that lived in a temperate climate, approaching microthermal, with winter temperatures that occasionally dipped below freezing (Wolfe, 1994; Gregory and McIntosh, 1996; Meyer, 2003; Boyle et al., 2008). Rainfall was limited, especially during the pronounced dry season (Gregory-Wodzicki, 2001; Leopold and Clay-Poole, 2001; Table 2). Florissant is a lake deposit (MacGinitie, 1953; O'Brien et al., 2008) and captured species from different microhabitats, including a mesic lakeside habitat and a xeric woodland/scrubland from the hills surrounding the lake (Meyer, 2003). This broad sourcing of leaves resulted in a high preserved diversity of species, with an equally diverse range of post-EOT histories. Many of the taxa that rose to dominance in highland floras after the EOT are related to taxa found among Florissant's diverse assemblage (Leopold and Zaborac-Reed, 2019). At the same time, many early Oligocene lowland floras share taxonomic similarities with Florissant, particularly among deciduous trees and shrubs, which likely migrated downward from the highlands (Meyer and Manchester, 1997; Leopold et al., 2008).

Mormon Creek—Mormon Creek, a fluvial flora from the Renova Formation of southwestern Montana (Becker, 1960), is a late Eocene or early Oligocene pre-EOT highlands flora from just before the EOT (Lielke et al., 2012). Unlike Florissant, Mormon Creek did not have a pronounced dry season (Table 2) and experienced warmer

TABLE 1. Floras measured for this study and sources for species identifications.

Study area or flora	No. leaves	No. species	ID source	Photo source
La Porte	38	23	Potbury (1935)	UCMP, UWBM
Badger's Nose	28	10	Myers (2006)	UCMP
Renova Formation (5 floras)	494	80	Becker (1960, 1961, 1966, 1969; 1972, 1973), Lielke et al. (2012)	YPM
Florissant	712	70	MacGinitie (1953), Manchester (2001)	UCMP, YPM, FLFO
Pitch Pinnacle	25	10	Gregory and McIntosh (1996)	CU
Creede	41	9	Knowlton (1923), Axelrod (1987), Wolfe and Schorn (1989), Leopold and Zaborac-Reed (2019)	CU
Goshen	121	38	Chaney and Sanborn (1933), Meyer and Manchester (1997)	UCMP, UO
Bridge Creek (8 assemblages)	661	61	Meyer and Manchester (1997)	UCMP, UO, JODA, Meyer and Manchester (1997)
Rujada	161	17	Lakhanpal (1958), Meyer and Manchester (1997)	UCMP
Willamette	94	12	Myers et al. (2002)	UCMP, UWBM
Gumboot Mountain	23	7	Meyer and Manchester (1997)	UCMP, UWBM

Notes: UCMP, University of California Museum of Paleontology; UWBM, University of Washington Burke Museum; YPM, Yale Peabody Museum; FLFO, Florissant Fossil Beds National Monument; UO, University of Oregon Condon Collection; JODA, John Day Fossil Beds National Monument; CU, Colorado University Museum of Natural History.

temperatures. Most of the taxa at Mormon Creek were subtropical with a secondary component of warm-temperate species (Becker, 1960; Lielke et al., 2012).

Pre-EOT lowlands—*Goshen*—The fluvial Goshen flora of western Oregon's Fisher Formation is our best-represented pre-EOT lowland flora (Chaney and Sanborn, 1933). It is subtropical, with large leaves and abundant "drip tips", indicating a warm and everwet climate (Table 2; Chaney and Sanborn, 1933). The term "Goshen-type" has been used to describe a common type of late Eocene and earliest Oligocene subtropical flora with a combination of broadleaved evergreen and deciduous species that perhaps only existed for a short time prior to the EOT (Wolfe, 1981). Thermophilic remnants found at post-EOT lowland floras may have been survivors from Goshen-type floras (Meyer and Manchester, 1997). Retallack et al. (2004) dated Goshen based on an ^{40}Ar/^{39}Ar date for the underlying Bond Creek Tuff (34.9 Ma) and the observation at Goshen of normal polarity, constraining Goshen to chron 13n (33.7–33.2 Ma), which spans the EOT (Ogg, 2012). Based on the warm and wet climate of Goshen, it seems appropriate to place the flora soon after the beginning of chron 13n—less than a few hundred thousand years before the EOT.

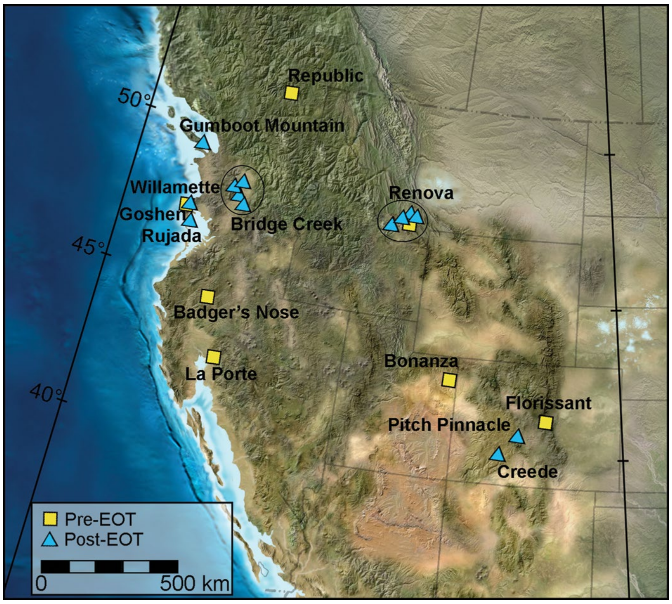

FIGURE 1. Paleogeography of Eocene–Oligocene transition (EOT) sites used in study. Positions of sites were extrapolated from their coordinates today to their estimated paleocoordinates 35 Ma using the Paleolocation Mapping Service (Urban and Hardisty, 2013; www.paleolocation.org). The map layer represents western North America ~35 Ma (©2013 Colorado Plateau Geosystems).

La Porte—The La Porte flora of northern California's Ione Formation is a late Eocene or early Oligocene pre-EOT lowland flora (Meyer and Manchester, 1997; Leopold et al., 2008; DeVore and Pigg, 2010; Prothero et al., 2011) from a fluvial environment in a warm and humid climate (Table 2). La Porte is commonly considered Goshen-type (Potbury, 1935); however, the exact character of La Porte may be intermediary between Goshen and earlier Eocene lowland floras, with a stronger thermophilic character similar to the Chalk Bluff flora found lower in the Ione Formation (Potbury, 1935; Prothero et al., 2011).

Post-EOT highlands—Pitch Pinnacle—Pitch Pinnacle (32.9–29 Ma) is our best-represented post-EOT highland flora. It is a lacustrine deposit from the Colorado Rocky Mountains and is floristically connected to the pre-EOT Florissant, albeit with much lower diversity and with the beginnings of a new shrubby and herbaceous component resembling the shrublands of Colorado today (Leopold and Zaborac-Reed, 2019). Compared to Florissant, a higher percentage of taxa were cool-adapted, mainly due to the loss of broad-leaved deciduous tree species and an increase in small-leaved shrubs (Leopold and Zaborac-Reed, 2019). The temperature at Pitch Pinnacle was similar or possibly colder than that at Florissant, but with slightly more precipitation (Gregory and McIntosh, 1996; Table 2).

Creede—The Creede flora (26.87 Ma) is a post-EOT highland flora that represents a third temporal step in the transition from Florissant to Pitch Pinnacle to the shrub and conifer dominated Rocky Mountain flora of today (Leopold and Zaborac-Reed, 2019).

TABLE 2. Site mean leaf dry mass per area (LMA), climate, paleoelevation, and depositional environment for Eocene and Oligocene floras.

Study area	Flora	EOT	High/ Lowland	Age (Ma)	Site mean LMA (g/ m²)	MAT (°C)	MAP (cm)	CMMT (°C)	GSP (cm)	3WET (cm)	3DRY (cm)	Paleo-elevation (m)	Depositional environment
Okanogan Highlands	Republic	Pre	Highland	~49	$77 \pm^{9}_{8}$	8.3	121	−1.8	63	39	24	2500[1]	Lake
Green River	Bonanza	Pre	Highland	47.3	$113 \pm^{14}_{13}$	12.8	88	5.9	127	53	12	2900[1]	Lake
La Porte	La Porte	Pre	Lowland	36.5-33.7	$72 \pm^{8}_{7}$	21.2	201	17.0	241	102	37	0[1]	Stream
Badger's Nose	Badger's Nose	Pre	Highland	35-34	$107 \pm^{15}_{13}$	13.1	100	3.9	121	59	26	1700[5]	Lake
Colorado Highlands	Florissant	Pre	Highland	34.07	$122 \pm^{11}_{10}$	9.0	59	−1.5	92	45	18	3800[1]	Lake
	Pitch Pinnacle	Post	Highland	32.9-29	$118 \pm^{17}_{15}$	10.7	170	1.0	104	53	26	2000[6]	Lake
	Creede	Post	Highland	26.87	$148 \pm^{24}_{20}$	7.7	72	−3.6	69	35	15	2700[1]	Lake
Fisher Formation	Goshen	Pre	Lowland	33.7-33.5	$67 \pm^{8}_{7}$	18.9	260	11.7	276	120	59	0[2]	Stream
	Rujada	Post	Lowland	31.3	$70 \pm^{9}_{8}$	12.5	171	2.4	140	74	48	0[2]	Lake
	Willamette	Post	Lowland	30.1	$76 \pm^{9}_{8}$	13.1	193	3.4	146	75	44	0[2]	Lake
Renova Formation	Mormon Creek	Pre	Highland	>33.5	$84 \pm^{12}_{11}$	14.0	NA	0.6	138	71	49	2300[3]	Stream
	Metzel Ranch	Post	Highland	~33.5	$110 \pm^{15}_{14}$	13.1	NA	0.8	93	52	9	2300[3]	Stream
	Ruby River	Post	Highland	~33.5	$118 \pm^{13}_{12}$	12.2	NA	1.0	128	66	8	2300[3]	Lake
	Beaverhead[a]	Post	Highland	~33.5	$106 \pm^{10}_{10}$	14.6	NA	5.4	85		8	1300[3]	Lake
	York Ranch	Post	Highland	33.5–32.2	$108 \pm^{15}_{13}$	11.8	NA	−1.3	87	51	10	2300[3]	Stream
Bridge Creek	Bridge Creek Total	Post	Lowland	33.6–32.6	$88 \pm^{8}_{7}$	—	—	—	—	—	—	—	Lake
	Iron Mountain	Post	Lowland	33.6	$95 \pm^{10}_{9}$	7.3	150	−5.2	84	53	44	800[4]	Lake
	Painted Hills	Post	Lowland	33.6–32.6	$77 \pm^{9}_{8}$	7.7	148	−4.5	99	59	45	800[4]	Lake
	Crooked River	Post	Lowland	33.6–32.6	$89 \pm^{9}_{8}$	8.1	104	−4.4	86	53	43	800[4]	Lake
	Cove Creek	Post	Lowland	33.6–32.6	$85 \pm^{10}_{9}$	8.9	208	−2.2	75	49	41	800[4]	Lake
	Fossil	Post	Lowland	32.58	$89 \pm^{9}_{8}$	10.2	158	−1.3	116	66	48	800[4]	Lake

Notes: MAP was derived using leaf area analysis (Wilf et al., 1998). All other climate and paleoelevation estimates are based on the Physg3arcAZ_Met3arAZ calibration data set from CLAMP (clamp.ibcas.ac.cn). LMA uncertainties are 95% prediction intervals. NAs are not available. EOT: Eocene–Oligocene transition; MAT: mean annual temperature; MAP: mean annual precipitation; CMMT: coldest month mean temperature; GSP: growing season precipitation; 3WET: precipitation in the three wettest months of the year; 3DRY: precipitation in the three driest months of the year.

[1] Wolfe et al. (1998). [2] Retallack et al. (2004). [3] Lielke et al. (2012). [4] Meyer and Manchester (1997). [5] Myers (2006). [6] Gregory and McIntosh (1996).

[a] Beaverhead Basin climate values are averages of estimates for the three Beaverhead Basin florules (Lielke et al., 2012).

The climate at Creede was colder than at any other site we considered, with winter temperatures far below freezing and with a distinct dry season (Yang et al., 2011). The shift toward cool-adapted taxa seen at the post-EOT Pitch Pinnacle continued at Creede, though many of the remaining taxa were still related to taxa found at Florissant (Leopold and Zaborac-Reed, 2019).

Renova—The Renova Formation of southwestern Montana contains a series of floras that straddled the EOT. These are highland floras from two fossil-bearing basins: Fossil Basin (Becker, 1960, 1961, 1972, 1973) and Beaverhead Basin (Becker, 1969). Fossil Basin contains four floras: Mormon Creek (Becker, 1960), a pre-EOT highland flora described earlier; Metzel Ranch (Becker, 1972), an earliest Oligocene transitional flora that likely dates to during the EOT cooling (Lielke et al., 2012); Ruby River (Becker, 1961, 1966), an earliest Oligocene flora coeval with Metzel Ranch (Lielke et al., 2012); and York Ranch (Becker, 1973), a post-EOT flora somewhat younger than Metzel Ranch and Ruby River (Lielke et al., 2012). The Beaverhead Basin consists of three florules that are roughly coeval with Metzel Ranch and Ruby River (Lielke et al., 2012). Overall, MAT dropped about 2°C across the EOT in Fossil Basin, while 3DRY plummeted (Lielke et al., 2012; Table 2). Metzel Ranch, Ruby River, and Beaverhead Basin all show the replacement of Mormon Creek's subtropical elements by previously subordinate temperate species and the appearance of a new xeric component, similar to the scrub/woodland forests around Florissant (Lielke et al., 2012). York Ranch represents further progression of the temperate deciduous forest, made up almost entirely of extant genera that today live in eastern North America (Lielke et al., 2012).

Post-EOT lowlands—*Bridge Creek*—The Bridge Creek flora (33.6–32.2 Ma) of the Lower John Day Formation in northeastern Oregon is our best-represented post-EOT lowland flora. Bridge Creek was dominated by temperate deciduous species, with a small, persistent broadleaved evergreen component (Meyer and Manchester, 1997). Many of the temperate deciduous genera found at Bridge Creek are thought to have dispersed from pre-EOT highland floras such as Florissant (Meyer and Manchester, 1997; Meyer, 2005; DeVore and Pigg, 2010), while the thermophilic broadleaved evergreen component was a holdover from pre-EOT subtropical lowland floras like Goshen (Meyer and Manchester, 1997). Climatically, Bridge Creek had a higher MAP than at Florissant but was still seasonal (Meyer and Manchester, 1997; Retallack et al., 1999), and a similar MAT, but with a much greater temperature range that likely reached below freezing in the coldest months of the year (Meyer and Manchester, 1997; Table 2). Bridge Creek is often treated as a single unit on the basis of taxonomic similarity, but comprises at least seven different assemblages that are geographically distinct (Meyer and Manchester, 1997). We consider them both separately and in aggregate (Table 2).

Rujada and Willamette—Rujada (31.3 Ma) and Willamette (30.1 Ma) are post-EOT lowland floras from the Fisher Formation of western Oregon. They represent the direct transition from Goshen's pre-EOT subtropical lowland forest to a temperate deciduous forest. They also are comparable to the similar-aged but more inland Bridge Creek flora. In terms of climate, both Willamette and Rujada were significantly colder than Goshen, but warmer than Bridge Creek, as cold month temperatures stayed well above freezing (Wolfe, 1994; Myers et al., 2002; Myers, 2003; Yang et al., 2011; Table 2). At the same time, MAP was lower than at Goshen, with pronounced seasonality similar to Bridge Creek (Myers et al., 2002; Yang et al., 2011). Rujada and Willamette had more thermophilic elements like those found at Goshen than Bridge Creek, likely because frost was uncommon, but on the whole were dominated by a similar temperate deciduous flora (Lakhanpal, 1958; Meyer and Manchester, 1997; Myers et al., 2002).

Gumboot Mountain—The Gumboot Mountain flora from southern Washington State is also a post-EOT flora that was taxonomically and climatically similar to Rujada and Willamette, with a strong temperate deciduous representation and a few broadleaved evergreen taxa (Meyer and Manchester, 1997). Relative to the other floras presented here, its taxonomy and physical setting is less known.

Other sites—Badger's Nose (35–34 Ma) is a pre-EOT flora from the lacustrine siltstones of the Steamboat Formation in northeastern California (Myers, 2006). Badger's Nose has an intermediate paleoelevation (~1700 m a.s.l.) and a climate between the highland Florissant and the lowland Goshen (Myers, 2006; Table 2). Correspondingly, the flora itself is considered a mix between the temperate Florissant and the subtropical floras of the lowlands, with a 60/40% split between deciduous and evergreen dicot taxa (Myers, 2006).

To broaden the range of environments considered when correlating LMA to climate and elevation, we included the Bonanza and Republic floras, both middle Eocene lacustrine assemblages with LMA reported by Royer et al. (2007). Although earlier in the Eocene than any other sites considered here, these floras have strong taxonomic similarities to Florissant (MacGinitie, 1969; Wolfe and Wehr, 1987; DeVore and Pigg, 2010). Bonanza and Republic add combinations of high elevation with either warmer temperatures (Bonanza) or wetter dry seasons (Republic) than Florissant (Table 2), allowing further exploration of environmental gradients.

Taxonomy

Bridge Creek (Meyer and Manchester, 1997), Badger's Nose (Myers, 2006), Pitch Pinnacle (Gregory and McIntosh, 1996), and Creede (Knowlton, 1923; updated by Axelrod, 1987; Wolfe and Schorn, 1989; Leopold and Zaborac-Reed, 2019) have been fully described under a modern view of fossil plant systematics (Table 1). In contrast, other floras considered here have not been comprehensively classified since the 1960s (or earlier) and their associated taxonomies are now largely considered outdated. Subsequent updates have been made for certain taxa from many of these sites, making them somewhat taxonomically reliable. Florissant was last monographed in full by MacGinitie (1953), but a partial revision by Manchester (2001) presents modern generic assignments for some species and indicates which of the earlier classifications are not supported by the fossil record. Similarly, the Renova Formation floras were described by Becker (1960, 1961, 1966, 1969, 1972, 1973) and updated by Lielke et al. (2012), who preliminarily reviewed the accuracy of Becker's generic assignments. Systematics for the Willamette flora have never been published, but Myers et al. (2002) presented a preliminary species list. Rujada was last described by Lakhanpal (1958), but Meyer and Manchester (1997) listed the taxa that Rujada had in common with the Bridge Creek flora, and thus this subset of assignments can be considered robust. Gumboot Mountain has never been described, but Meyer and Manchester (1997) again listed taxa in common with Bridge Creek. Neither La Porte (Potbury, 1935) nor Goshen (Chaney and Sanborn, 1933) have been comprehensively revised; Wolfe (1977) and Doyle et al. (1988) reevaluated select taxa from Goshen and Meyer and Manchester (1997) listed taxa in common with Bridge Creek, but these account for only a small percentage of the species found at these two sites. For Goshen and La Porte, we consider outdated classifications as morphospecies—species that are distinct from other morphospecies, but that carry no further taxonomic information.

Where modern taxonomy is available, we grouped genera by their trans-EOT histories. At Florissant, Pitch Pinnacle, and Creede, we distinguished between genera that persisted in the Colorado Highlands through the EOT, genera that went extinct, and genera that appeared post-EOT based on the observations of Leopold and Zaborac-Reed (2019) of these sites and another post-EOT Oligocene Colorado highland flora, Antero. At Bridge Creek, we determined which taxa may have come from Eocene lowland survivors based on genera listed in common with the pre-EOT lowland Clarno Nut Beds (Manchester, 1994), Clarno Formation, La Porte, and Goshen floras (Meyer and Manchester, 1997). Genera that survived, went extinct, or newly appeared in the Oligocene of the Renova Formation were determined by presence/absence within the Renova Formation floras based on Lielke et al. (2012). Genera that may have dispersed from the highlands (i.e., Florissant) to the lowlands (i.e., Bridge Creek) were determined based on shared Florissant and Bridge Creek genera listed in Manchester (2001) and Meyer and Manchester (1997), respectively. In general, we considered dispersers as taxa from genera known at both Florissant and Bridge Creek but not known in any pre-EOT lowland floras. At Badger's Nose, lowland taxa that survived the EOT were noted by Myers (2006).

LMA measurements

To estimate LMA of fossil leaves, we followed the protocol of Royer et al. (2007), which relies on a biomechanical link between petiole width and leaf mass. We measured leaf area and petiole width from digital images of fossils in Adobe (San Jose, CA, USA) Photoshop. Leaf area was obtained using the polygonal lasso tool to trace the margin of the leaf, while making appropriate reconstructions of damaged segments of the margin. Petiole width was measured using the ruler tool perpendicular to the direction of the midvein at the basalmost point of petiole insertion into the leaf lamina. Species LMA was then calculated following Royer et al. (2007), using the mean petiole width^2/leaf area for each species:

$$\log_{10}\mathrm{LMA} = 3.070 + 0.382\ \log_{10}(\text{petiole width}^2/\text{leaf area}).$$

Estimates for site LMA were calculated following Royer et al. (2007), using the mean petiole width^2/leaf area of the means for each species represented at the site:

$$\log_{10}\mathrm{LMA} = 3.214 + 0.429\ \log_{10}(\text{petiole width}^2/\text{leaf area}).$$

We calculated 95% prediction intervals for both species and site means following Sokal and Rohlf (2012) and as described by Royer et al. (2007); the necessary coefficients from the calibration data are provided by Royer et al. (2007, their table 2). For each equation (species and site means), the size of the 95% prediction interval scales with sample size; for example, for species represented by three leaves the 95% prediction interval is +60%/−38% of the mean LMA, while for sites represented by 10 species it is +16%/−14% (Royer et al., 2007). All specimen-level (Appendix S1) and species-level (Appendix S2) estimates are presented in the Supplementary Data.

Paleoenvironmental estimates

Most of our paleoenvironmental estimates (Table 2) were generated using the Climate Leaf Analysis Multivariate Program (CLAMP). CLAMP relies on the strong relationship between climate and leaf physiognomy to derive paleoclimate and paleoelevation estimates (Wolfe, 1993; Yang et al., 2011). CLAMP is based on leaf physiognomic character states from extant angiosperm communities across a wide range of climate combinations and geographies. For all CLAMP analyses here, we used the Physg3arcAZ_Met3arAZ calibration data set, which correlates 173 extant sites, mostly from the northern hemisphere, to climate data drawn from nearby meteorological stations (Spicer et al., 2009). Physiognomic scoresheets for Republic, Bonanza, La Porte, Florissant, Goshen, Willamette, Rujada, and Creede were available from CLAMP online (http://clamp.ibcas.ac.cn/), while a scoresheet for Badger's Nose was provided by Jeff Myers. Scores for the Pitch Pinnacle flora were published in Gregory and McIntosh (1996). The Iron Mountain, Painted Hills, Crooked River, Cove Creek, and Fossil assemblages from the Bridge Creek flora were all newly scored for this study using a combination of the Bridge Creek monograph (Meyer and Manchester, 1997) and photographs taken for LMA analysis (scores in Appendix S3). Fossil flora scores were then analyzed via CLAMP online, which generated predictions for climate parameters including MAT, cold month mean temperature (CMMT), warm month mean temperature (WMMT), length of growing season (LGS), growing season precipitation (GSP), 3DRY, precipitation in the three wettest months (3WET), and enthalpy.

Climate predictions for the Renova Formation floras come from Lielke et al. (2012), who generated multiple linear regressions for MAT, CMMT, GSP, 3DRY, and enthalpy based on the CLAMP Physg3arcAZ_Met3arAZ calibration data set. These climate estimates generally correspond with previous estimates of MAT and GSP made with older iterations of CLAMP, which use the same calibration and characters but slightly different weightings of the characters (Lielke et al., 2012). CLAMP scores were not reported, however, so we were unable to apply CLAMP to the Renova Formation floras.

MAP is not directly estimated by CLAMP online, which reports GSP as a replacement (Wiemann et al., 1998). Instead, we used the regression MAP = 6.18(% mesophyll) + 47.5, which uses the percentage of species with large (mesophyll) leaves, a character state scored in CLAMP, to estimate MAP based on calibrations from the CLAMP data set (Wilf et al., 1998). Although this regression likely overestimates MAP (Wilf et al., 1998), more recent univariate regressions rely on Raunkiaer–Webb leaf size classes that cannot be extracted from CLAMP scores.

Paleoelevation estimates also mostly come from CLAMP (Wolfe et al., 1998; Lielke et al., 2012). Forest et al. (1995) developed a method for estimating paleoelevation based on differences in enthalpy between a coastal site (elevation = 0 m), and an inland site at the same latitude. Paleoelevation estimates for Bridge Creek and Badger's Nose were based on assumptions about terrestrial lapse rates and knowledge of local geologic history (Meyer and Manchester, 1997; Myers, 2006).

Uncertainties for these leaf-physiognomic climate and elevation predictions can be high. For example, according to CLAMP online, 3DRY has a 2σ range of 26.4 cm, which is more than 50% of the estimated 3DRY at Goshen, the site with the highest 3DRY estimate. Moreover, the true uncertainties of all CLAMP estimates may be larger than the stated uncertainties, owing to human error in the scoring process and the use of categorical character states for quantitative characters (e.g., Wiemann et al., 1998; Wilf et al., 1998; Peppe et al., 2010, 2011; Royer, 2012). Because we applied CLAMP in a uniform way, the relative changes in climate and elevation between sites are probably more robust than the absolute values. To confirm the CLAMP results, we also present an alternative analysis of MAT using leaf-margin analysis (Wilf, 1997), a univariate method based on the presence/absence of leaf teeth.

Statistical methods

The Mann–Whitney *U* test (Sokal and Rohlf, 2012) was used to assess differences in median between LMA distributions of different assemblages, where the site medians are calculated from

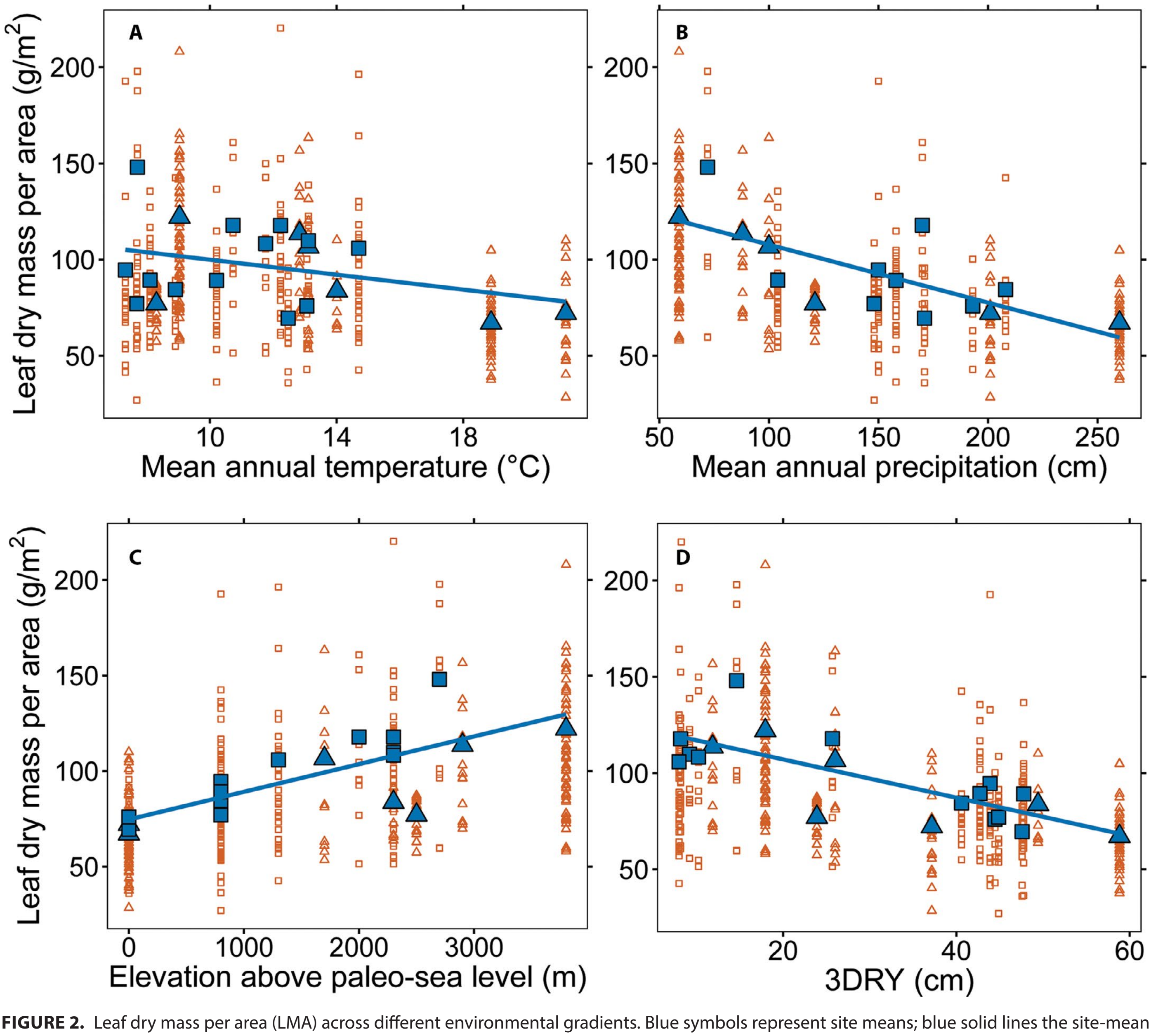

FIGURE 2. Leaf dry mass per area (LMA) across different environmental gradients. Blue symbols represent site means; blue solid lines the site-mean regressions. Orange symbols represent species means. Triangles of all colors are from sites pre-dating the Eocene–Oligocene transition (EOT); squares are from post-EOT sites. (A) LMA and mean annual temperature (MAT); Site LMA = 119.35 – 1.94.MAT ($r = -0.33$; $P = 0.15$); (B) LMA and mean annual precipitation (MAP); Site LMA = 137.91 – 0.30.MAP ($r = -0.73$; $P < 0.01$); (C) LMA and paleoelevation; Site LMA = 74.74 + 0.01 × Elevation ($r = 0.76$; $P < 0.001$); (D) LMA and three driest month precipitation (3DRY); Site LMA = 126.99 – 0.99.3DRY ($r = -0.78$; $P < 0.001$).

species means. Similarly, the Kolmogorov–Smirnov test was used to assess differences in the shape of the distributions (e.g., dispersion and skewness; Sokal and Rohlf, 2012).

RESULTS

Response of LMA to the EOT and environmental gradients

The median site LMA post-EOT (13 sites) did not significantly differ from the pre-EOT (7 sites; $P = 0.49$). Restricting sites to within ±3.5 Myr of the EOT (17 total sites) showed a similar lack in difference ($P = 0.57$; Appendix S4). Instead, LMA was strongly inversely correlated with the precipitation variables MAP and 3DRY, and positively correlated with paleoelevation (Table 3; Fig. 2B–D). These correlations are all in keeping with extant relationships (see introduction). In contrast, we found no significant correlation between site LMA and MAT (Fig. 2A), a correlation that is commonly observed in present day-plants (Wright et al., 2004; Wright et al., 2005; Poorter et al., 2009). This lack of correlation persists when using leaf-margin analysis instead of CLAMP to estimate MAT (Appendix S5). Critically, none of these environmental variables changed significantly across the EOT (comparing all post-EOT sites to pre-EOT sites), in part because of the wide range of baseline climates and paleoelevations at our sites (Table 2).

Using multiple linear regression, we looked for combinations of these environmental variables in which each individual variable was significant and where there was no multicollinearity (variance inflation factor < 10; Sokal and Rohlf, 2012). The combination 3DRY + paleoelevation was the only one to meet these criteria (Fig. 3), highlighting two independent axes of variation with LMA expression.

Differences in depositional environments may affect both leaf physiognomic climate estimates and LMA estimates (e.g., Greenwood, 1992; Blonder et al., 2014). Five of our sites come from fluvial environments, while the other 16 come from lacustrine environments (Table 2). We reevaluated the correlations between LMA and climate with fluvial sites omitted and found them to be similar to those of the full data set: an inverse correlation with MAP and 3DRY and a positive correlation with paleoelevation (Appendix S6). This consistency of result suggests that any effects of depositional environment on LMA or climate estimates do not overwrite the strong correlations between them.

Distribution of species-mean LMA within sites

The pre-EOT highland Florissant had a site LMA of 122 g/m^2 (Table 2), with species-mean LMA ranging between 58–208 g/m^2 (Fig. 4A). Species were relatively evenly distributed across this range, suggesting that no one leaf-economic strategy was strongly favored at Florissant. These results are consistent with independent LMA estimates of fossils from Florissant excavation site 9 (Allen et al., 2020), a quarry in the same middle shale layer as most of the fossils in our study, which includes 152 leaves from excavation site 9 itself. Compared to Florissant's two taxonomic ancestors, its higher site LMA and wide range of species-mean LMA most closely resemble the warm and dry Bonanza flora (113 g/m^2; 70–157 g/m^2) rather than the cold and wet Republic flora (77 g/m^2; 57–87 g/m^2; Table 2).

In the lowlands, at the pre-EOT Goshen, the site LMA was lower (67 g/m^2; Table 2), with a narrower species-level range (38–105 g/m^2) that was dominated by species in the 40–80 g/m^2 range (Fig. 4B). Indeed, the median LMA at Goshen was significantly lower than at Florissant ($P < 0.001$), and the distribution of species in LMA differed significantly as well ($P < 0.001$). This contrast between highland and lowland pre-EOT sites was further supported by the lowland La Porte flora, which had a low site LMA (72 g/m^2) and narrow range (29–110 g/m^2) dominated by low LMA taxa (Fig. 4B).

Mormon Creek and Badger's Nose are our other two pre-EOT floras. Mormon Creek, a highland flora, had a much lower site LMA (84 g/m^2) than Florissant along with a narrower range (64–110 g/m^2; Fig. 5A). Each of the measured taxa at Mormon Creek came from its subdominant temperate component (vs. the dominant subtropical component), suggesting that this LMA distribution may not be fully representative. Still, the high 3DRY (48 cm) at Mormon Creek is in keeping with lower LMA values relative to Florissant. At Badger's Nose, the site LMA (107 g/m^2) and range (54–163 g/m^2) were between the highland Florissant and lowland Goshen/La Porte; this is consistent with Badger's Nose's intermediate climate, elevation, and taxonomic composition (Table 2; Fig. 4D).

In the post-EOT highlands, both Pitch Pinnacle and Creede had high site LMA (118 and 148 g/m^2, respectively; Table 2). Both also had wide distributions in LMA similar to Florissant (Fig. 4A). The median LMA and overall LMA distribution at both Pitch Pinnacle ($P = 0.96$; $P = 0.95$) and Creede ($P = 0.40$; $P = 0.11$) were indistinguishable from Florissant. The four post-EOT floras from the highland Renova Formation were also similar to Florissant, once again exhibiting wide and relatively evenly distributed LMA ranges (Fig. 5). Each of these sites had a higher site LMA (Table 2) than their pre-EOT highland counterpart, Mormon Creek. Except for the lower elevation Beaverhead Basin flora ($P = 0.03$), the wide LMA distributions of these post-EOT Oligocene floras (Fig. 5) were indistinguishable from Florissant's (all $P > 0.28$). Tellingly, the climate at the post-EOT Renova sites and the pre-EOT Florissant were quite similar (Table 2).

Bridge Creek was the best-sampled post-EOT lowland site. The individual sites at Bridge Creek had similar LMA distributions to the combined flora (Fig. 6). Compared to its Oregonian pre-EOT predecessor at Goshen, Bridge Creek had a significantly higher median LMA ($P < 0.001$) with a distribution weighted toward higher LMA values ($P < 0.001$) even though the range of LMA was similar (Fig. 4B). Compared to its pre-EOT highland predecessor at Florissant, Bridge Creek had a significantly lower median LMA

TABLE 3. Site level correlations between site-mean leaf dry mass per area (LMA) and paleoenvironment (climate and paleoelevation).

Statistic	MAT	MAP	WMMT	CMMT	LGS	GSP	3WET	3DRY	Elevation
r	–0.33	–0.73	0.010	–0.31	–0.41	–0.49	–0.62	–0.78	0.76
P	0.15	0.002	0.97	0.19	0.13	0.035	0.005	<0.001	<0.001
N	20	15	20	20	15	19	19	20	20

Notes: MAT, mean annual temperature; MAP, mean annual precipitation; WMMT, warmest month mean temperature; CMMT, coldest month mean temperature; LGS, length of growing season; GSP, growing season precipitation; 3WET, precipitation in the three wettest months of the year; 3DRY, precipitation in the three driest months of the year.

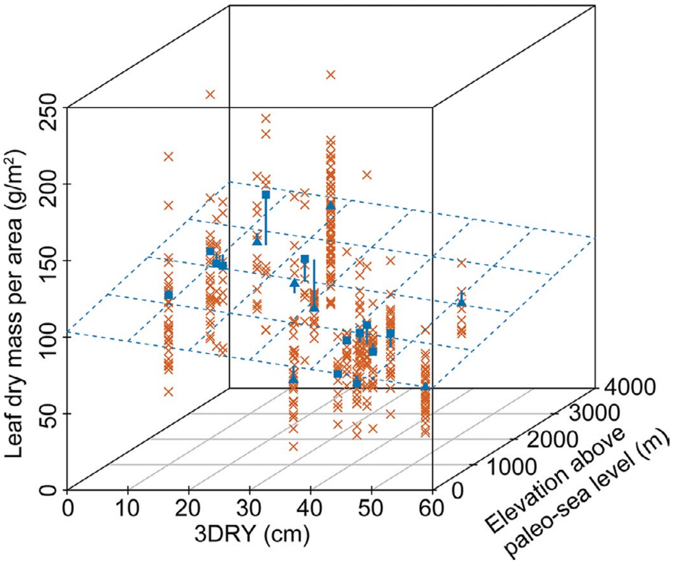

FIGURE 3. Multiple linear regression with leaf dry mass per area (LMA) as a function of rainfall during the three driest months of the year (3DRY) and paleoelevation. Blue triangles represent means from sites pre-dating the Eocene–Oligocene transition (EOT); blue squares represent post-EOT site means; orange x's represent species means. For site LMA, the best-fit plane (blue grid) is: LMA = 103.36 + 0.008.Elevation – 0.609.3DRY ($r = 0.82$; $P_{3DRY} = 0.03$; $P_{elevation} = 0.06$).

($P < 0.001$) as well as a distribution significantly weighted toward lower LMA ($P < 0.001$) due to a complete lack of a slow-return element (120–180 g/m^2; Fig. 4C). Bridge Creek was also significantly different from its highland contemporaries at Pitch Pinnacle and Creede by median LMA and LMA distribution (all $P < 0.001$).

The LMA of the post-EOT lowland Rujada and Willamette sites had lower medians than Bridge Creek ($P = 0.001$; $P = 0.03$) even though they shared many taxa. Their median LMA and LMA distribution instead more closely resembled that of Goshen, which had a similarly high 3DRY, MAP, and low elevation (all $P > 0.27$; Fig. 4D). Gumboot Mountain could not be differentiated by median or distribution (all $P > 0.11$) from either Goshen or Bridge Creek, although its site LMA (89 g/m^2) suggests that it had more similarities with Bridge Creek.

Persistence and dispersal

The pre-EOT Florissant, post-EOT Pitch Pinnacle, and post-EOT Creede floras make up a suite of geographically close (Fig. 1) highland sites with many species that persisted in the highlands across the EOT. We compared the LMA of these taxa to determine whether there was a shift in LMA tied to persistence. The median LMA of persisting taxa at Florissant (106 g/m^2), Pitch Pinnacle (96 g/m^2), and Creede (126 g/m^2) was high at all three sites (Fig. 4A), and there was no significant difference between any combination of medians or distributions (all $P > 0.25$), indicating that the LMA of persisting taxa in the highlands did not change across the EOT. Due to a lack of a reliable taxonomy at our pre-EOT lowland sites, we were unable to do similar analysis for lowland persisting taxa. Nonetheless, the median LMA of persisting taxa at Bridge Creek (84 g/m^2) was significantly higher than the median LMA at Goshen (67 g/m^2; $P = 0.003$), and indeed higher than 89% of individual Goshen species (Fig. 4B), suggesting a shift in leaf economic strategy among persisting taxa across the EOT in the lowlands.

To evaluate dispersal, we compared the LMA of dispersed taxa from the pre-EOT highland Florissant and the post-EOT lowland Bridge Creek (Fig. 4C). We found a distinct shift toward lower LMA from Florissant to Bridge Creek (103 vs. 72 g/m^2; $P < 0.001$), suggesting that dispersers may have undergone significant ecological changes during the EOT.

DISCUSSION

Environmental gradients

The LMA distributions within our sites fall along a spectrum that can largely be tied to paleoelevation and 3DRY. One end is defined by an even distribution centered around a high median LMA and is associated with high paleoelevation and low 3DRY. Florissant exemplifies this distribution (Fig. 4A), but lesser-sampled sites such as Pitch Pinnacle and Creede follow the same pattern; these sites contain a mix of temperate deciduous trees/shrubs with low LMA and hardy slow-return trees/shrubs with high LMA. The other end of the spectrum is defined by a narrow distribution dominated by low LMA species and is associated with low paleoelevation and high 3DRY. Goshen and Rujada showcase this pattern (Fig. 4B, D). These sites contain a mix of high rainfall-adapted deciduous trees and broad-leaved evergreens. Between these end points are sites with more intermediate environments and LMA distributions such as Bridge Creek and the post-EOT Renova Formation floras (Figs. 4–6).

Temperature is commonly proposed as the underlying cause for the paleoelevation correlation (Körner, 2007; Read et al., 2014), but we found no evidence for a correlation between LMA and MAT ($P = 0.15$). Further, because climates generally cooled across the EOT, we also found no correlation across all sites between paleoelevation and MAT ($P = 0.14$); this decoupling thus allows us the opportunity to independently test the roles of paleoelevation and MAT. Together, our observed patterns strongly suggest that in our data set temperature does not drive the correlation between LMA and paleoelevation.

Crucially, although the EOT resulted in large changes in regional climates, among our sites, no singular response links environmental variables to the EOT. On the other hand, our measured correlations reveal consistent floral responses to regional variations in environment, indicating that the 3DRY and paleoelevation gradients have far more power than a simple binary categorization of the EOT (pre- vs. post-EOT).

Functional responses to the EOT

Did LMA shift synchronously with the EOT (scenario 1 from the introduction)? At two sites in the Czech Republic, Roth-Nebelsick et al. (2017) found that site LMA dropped by approximately 40 g/m^2 across the EOT. In contrast, at our sites, we found a mixed response. From the pre-EOT Goshen to any of the nearby post-EOT lowland floras, site LMA either remained about the same (e.g., 67 vs. 69 g/m^2 at Rujada) or significantly increased (e.g., 67 vs. 89 g/m^2 at Fossil, Bridge Creek). In the highlands, we saw LMA slightly decrease from Florissant to Pitch Pinnacle (122 vs. 118 g/m^2), slightly increase from Florissant to Creede (122 vs 148 g/m^2), or significantly increase from Mormon Creek to Ruby River (84 vs. 118 g/m^2). Because the

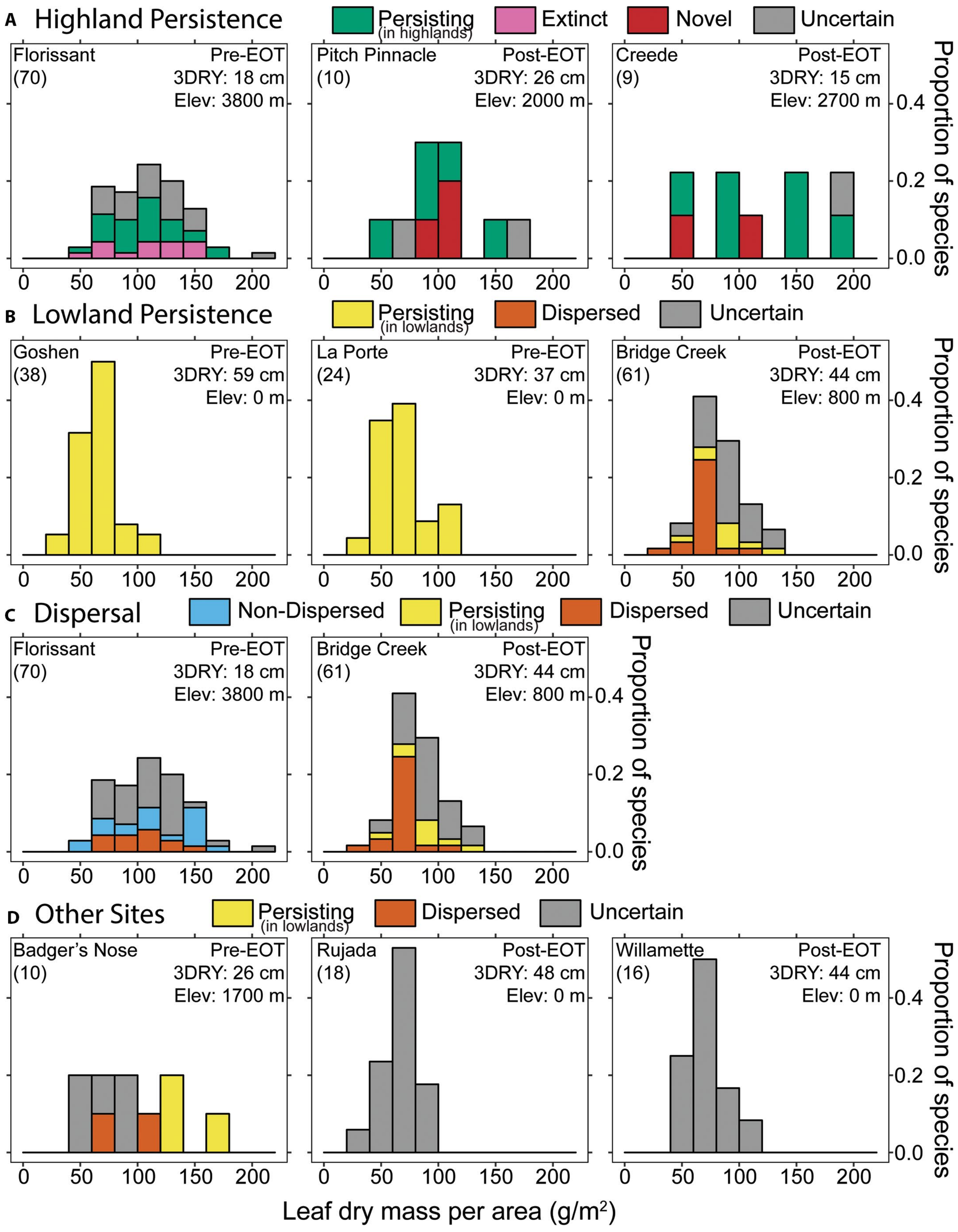
A Highland Persistence
Persisting (in highlands)
Extinct
Novel
Uncertain
Florissant (70)
Pre-EOT
3DRY: 18 cm
Elev: 3800 m
Pitch Pinnacle (10)
Post-EOT
3DRY: 26 cm
Elev: 2000 m
Creede (9)
Post-EOT
3DRY: 15 cm
Elev: 2700 m
B Lowland Persistence
Persisting (in lowlands)
Dispersed
Uncertain
Goshen (38)
Pre-EOT
3DRY: 59 cm
Elev: 0 m
La Porte (24)
Pre-EOT
3DRY: 37 cm
Elev: 0 m
Bridge Creek (61)
Post-EOT
3DRY: 44 cm
Elev: 800 m
C Dispersal
Non-Dispersed
Persisting (in lowlands)
Dispersed
Uncertain
Florissant (70)
Pre-EOT
3DRY: 18 cm
Elev: 3800 m
Bridge Creek (61)
Post-EOT
3DRY: 44 cm
Elev: 800 m
D Other Sites
Persisting (in lowlands)
Dispersed
Uncertain
Badger's Nose (10)
Pre-EOT
3DRY: 26 cm
Elev: 1700 m
Rujada (18)
Post-EOT
3DRY: 48 cm
Elev: 0 m
Willamette (16)
Post-EOT
3DRY: 44 cm
Elev: 0 m
0
50
100
150
200
0.0
0.2
0.4
Proportion of species
Leaf dry mass per area (g/m²)

FIGURE 4. Leaf dry mass per area (LMA) distributions for key floras across the Eocene–Oligocene transition (EOT). Fill colors represent different trans-EOT outcomes for individual species: persisting at either high or low elevation, dispersing from high to low elevation, going extinct before the EOT, or originating after the EOT. Numbers in parentheses are the number of species measured from the flora. The bin size for leaf dry mass per area is 20 g/m^2. Abbreviations: 3DRY, precipitation in the three driest months of the year; Elev, elevation above paleo sea level. (A) High elevation sites. (B) Low elevation sites. 3DRY for Bridge Creek is an average of estimated 3DRY of the five Bridge Creek assemblages (see Fig. 6 and Table 2). (C) Dispersal from the highlands to the lowlands across the EOT. (D) Three sites with adequate sampling that represent different combinations of environmental and biogeographic factors. Badger's Nose (pre-EOT, intermediate elevation, intermediate 3DRY) and Rujada and Willamette (post-EOT, lowlands, high 3DRY).

paleoelevation does not change substantially in these examples, 3DRY is the likely master variable, inversely covarying with LMA in each case. And even at the Czech Republic sites, 3DRY increased across the EOT as LMA decreased (Roth-Nebelsick et al., 2017). Thus, there was no simple shift in LMA across the EOT. Instead, we hypothesize that changes in 3DRY—that were likely a response to global changes associated with the EOT—drove predictable changes in LMA.

The LMA of persisting taxa covaried with 3DRY and paleoelevation across the EOT, further corroborating our model. For instance, support for scenario 2 (persistence without a shift in LMA) is present in the highlands: the LMA of persisting taxa from our three Colorado highland floras (Florissant, Pitch Pinnacle, and Creede) did not significantly differ (Fig. 4A); but neither did paleoelevation (all >2000 m) nor 3DRY (18 cm, 26 cm, and 15 cm, respectively; Table 2). We find support for scenario 3 (persistence with a shift in LMA) in the lowlands, where environmental conditions did shift. At the post-EOT Bridge Creek, the LMA of lowland survivor taxa was higher than most taxa found at the pre-EOT Goshen and La Porte, despite many of these comparisons being between deciduous and evergreen leaves (Fig. 4B). The paleoelevation of Bridge Creek (~800 m) was higher than the sea level Goshen and La Porte, while 3DRY at Bridge Creek (~45 cm) was lower than at Goshen (59 cm) but higher than at La Porte (37 cm). Thus, we conjecture that the changes in LMA in persisting taxa were largely driven by environmental gradients, with both paleoelevation and 3DRY playing important roles.

Our observations also support the interpretation that changes in LMA of taxa that dispersed (i.e., migrated from highlands to lowlands) can be explained in the context of 3DRY and paleoelevation (scenarios 4 and 5). From the pre-EOT highland Florissant to the post-EOT lowland Bridge Creek, the LMA of dispersing taxa dropped significantly (103 vs. 72 g/m^2; scenario 5; Fig. 4C) as 3DRY increased (18 vs. ~45 cm) and paleoelevation dropped (3800 vs. 800 m).

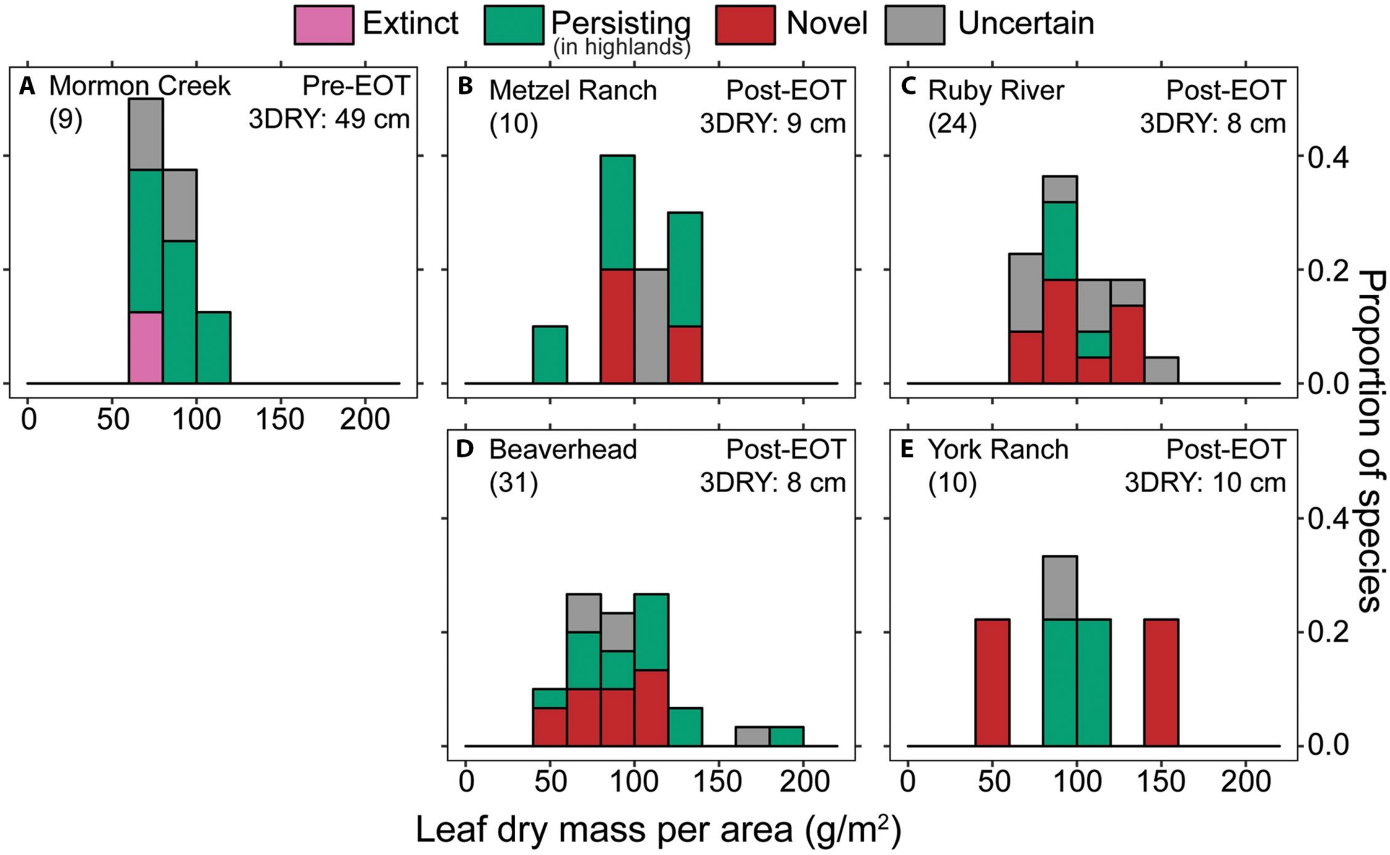

FIGURE 5. Leaf dry mass per area (LMA) distributions for the five Renova Formation highland floras across the Eocene–Oligocene transition (EOT). Numbers in parentheses are the number of species measured. Fill colors represent different trans-EOT outcomes for individual species based on their presence or absence in the Renova Formation. 3DRY: Precipitation in the three driest months of the year. The LMA bin size is 20 g/m^2.

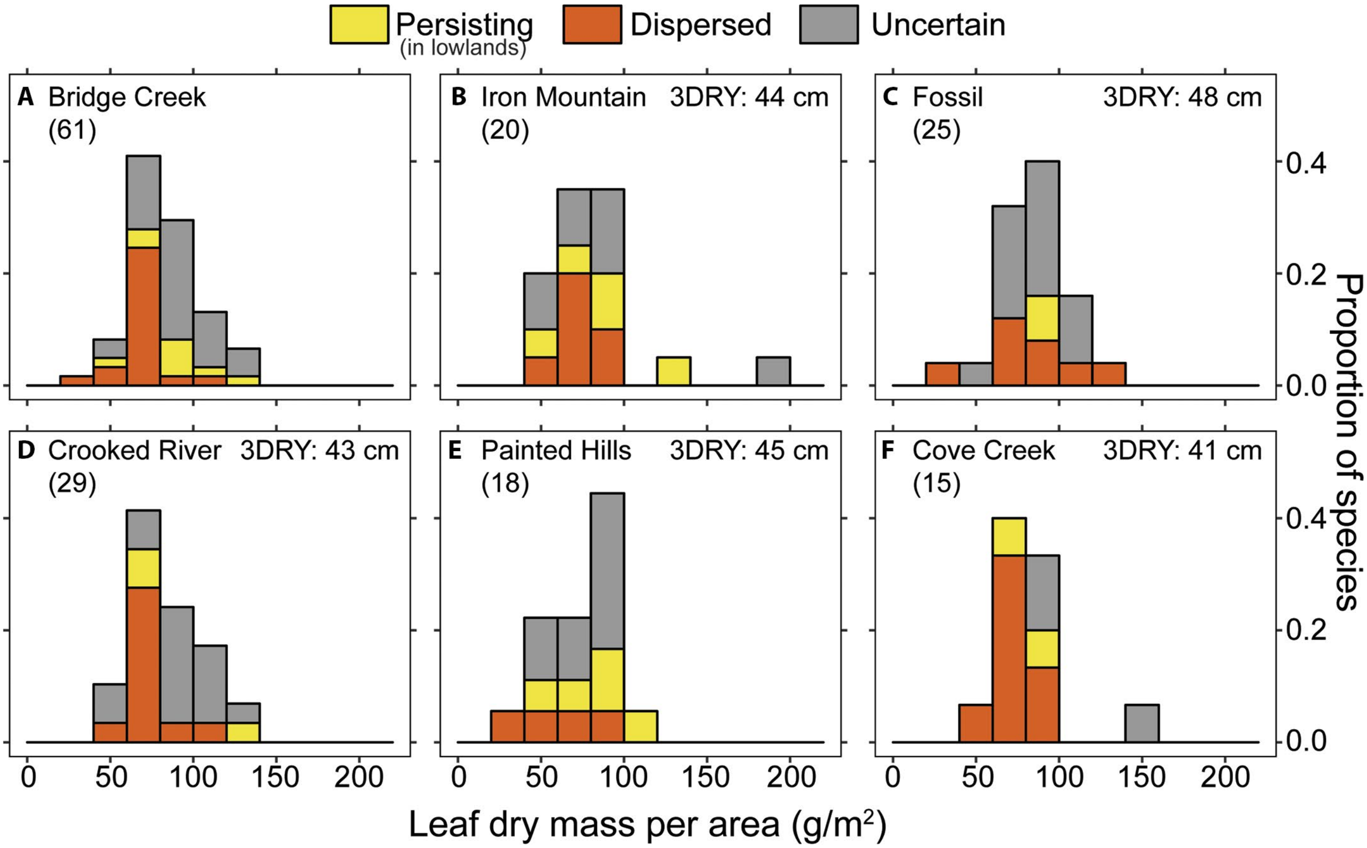

FIGURE 6. Leaf dry mass per area (LMA) distributions for five individual lowland post-Eocene–Oligocene transition Bridge Creek assemblages (panels B–F) along with the combined Bridge Creek flora (panel A). Numbers in parentheses are the number of species measured. Fill colors represent different trans-EOT outcomes for individual species. Panel A is identical to the Bridge Creek distribution in Fig. 4B and Fig. 4C; it combines the five assemblages shown here plus the Butler Basin (8 species), Lost Creek (7 species), and Twickenham (4 species) assemblages. 3DRY: precipitation in the three driest months of the year. The LMA bin size is 20 g/m^2.

CONCLUSIONS

Together, we find no evidence for a global shift in LMA across the EOT; instead, changes in LMA were tied to site-specific environmental factors, particularly dry-season precipitation (3DRY) and paleoelevation. At high elevation and low 3DRY, plants tended to have high LMA and follow slow-return leaf-economic strategies, while at low elevation and high 3DRY, plants tended to have low LMA and follow fast-return strategies. The link between LMA and these environmental conditions was apparent at multiple scales, including whole sites and groups of species at sites sharing similar biogeographical histories. This linkage persisted even in the midst of a period of global climate disruption and ecological turnover associated with the EOT. Our observed patterns are broadly consistent with extant patterns and provide a geological context for understanding how leaf-economic strategies respond to environmental change.

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AUTHOR CONTRIBUTIONS

M.J.B. and D.L.R. contributed to the design and implementation of the research, to the analysis of the results. and to the writing of the manuscript.

SUPPORTING INFORMATION

Additional Supporting Information may be found online in the supporting information tab for this article.

APPENDIX S1. Measurements and reconstructions of leaf dry mass per area of individual fossils.

APPENDIX S2. Mean estimated leaf dry mass per area for species/morphotypes at each site.

APPENDIX S3. Character scores used to generate climate predictions.

APPENDIX S4. Site mean leaf dry mass per area (LMA) of sites close to the EOT.

APPENDIX S5. Comparisons between CLAMP and leaf-margin analysis estimates of mean annual temperature (MAT).

APPENDIX S6. Leaf dry mass per area (LMA) across different environmental gradients with depositional environment controlled for.

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