Supporting Information for

Sediment-encased pressure–temperature maturation experiments elucidate the impact of diagenesis on melanin-based fossil color and its paleobiological implications.

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Supplementary tables

Table S1. Specimen data and experimental maturation treatments. Specimen data includes taxonomic information, and where applicable, museum accession codes, tissue types, fossil localities, and geological ages. PCA No. refers to the data point numbers assigned in the PCA score plots **(Figs. 2, S2, S3)**.

Capsule matured enzymatically extracted melanin from extant feathers **(Colleary et al. 2015)**

PCA No.	Common Name	Scientific Name	T (°C)	P (bars)	Feather Colour	Melanin Type
1	Chicken	*Gallus gallus*	200	250	Brown	Phaeomelanin
2	Carrion crow	*Corvus corone*	200	250	Glossy Black	Eumelanin
3	Chicken	*Gallus gallus*	200	250	Black	Eumelanin
4	Dark-eyed junco	*Junco hyemalis*	200	250	Grey	Eumelanin, Phaeomelanin
5	Mallard	*Anas platyrynchos*	200	250	Grey	Eumelanin, Phaeomelanin
6	Grey catbird	*Dumetella carolinensis*	200	250	Brown	Phaeomelanin
7	Rock dove	*Columba livia*	200	250	Grey	Eumelanin, Phaeomelanin
8	Turkey	*Meliagris gallopavo*	200	250	Iridescent	Eumelanin
9	Chicken	*Gallus gallus*	250	250	Brown	Phaeomelanin
10	Carrion crow	*Corvus corone*	250	250	Glossy Black	Eumelanin
11	House wren	*Troglodytes aedon*	250	250	Brown	Phaeomelanin
12	Chicken	*Gallus gallus*	250	250	Black	Eumelanin, Phaeomelanin
13	Dark-eyed junco	*Junco hyemalis*	250	250	Grey	Eumelanin, Phaeomelanin
14	Grey catbird	*Dumetella carolinensis*	250	250	Brown	Phaeomelanin
15	Rock dove	*Columba livia*	250	250	Grey	Eumelanin, Phaeomelanin
16	Turkey	*Meliagris gallopavo*	250	250	Iridescent	Eumelanin

Melanin-bearing fossil specimens **(Colleary et al. 2015, Xu et al. 2015)**

PCA No.	Taxon	Accession No.	Clade	Tissue	Fossil Locality	Geological Age
17	Pipidae	MU 41-3	Anura	Skin	Mush Valley Ethiopia	Ypresian-Lutetian
18	Pipidae	MU 32-2A/B	Anura	Skin	Mush Valley Ethiopia	Ypresian-Lutetian
19	Indeterminate	FUM-N 2275	Aves	Feather	Fur, Denmark	Ypresian-Lutetian
20	Indeterminate	SMF-ME 3850	Aves	Feather	Messel, Germany	Ypresian-Lutetian
21	*Hassianycteris*	SMF-ME 11407b	Mammalia	Hair	Messel, Germany	Ypresian-Lutetian
22	*Palaeochiropteryx*	SMF-ME 11406a	Mammalia	Hair	Messel, Germany	Ypresian-Lutetian
23	*Pelobates*	PW2005-5034-LS-GDKE	Anura	Skin	Enspel, Germany	Chattian
24	*Keuppia*	NHMUK PI CC578	Cephalopoda	Ink Sac	Hakel/Hjoula, Lebanon	Cenomanian
25	*Glyphiteuthis*	BRSUG 29387	Cephalopoda	Ink Sac	Hakel/Hjoula, Lebanon	Cenomanian
26	*Indeterminate*	-	Cephalopoda	Ink Sac	Lyme Regis, UK	Sinemurian
27	*Messelornis*	SMF-ME 11402a	Aves	Feather	Messel, Germany	Ypresian-Lutetian
28	*Palaeobatrachus*	SMF-ME 11390a	Anura	Eye	Messel, Germany	Ypresian-Lutetian
29	*Palaeobatrachus*	SMF-ME 11390a	Anura	Skin	Messel, Germany	Ypresian-Lutetian
30	*Indeterminate*	-	Cyclostomata	Eye	Mazon Creek	Carboniferous
31	*Sapeornis*	STM 15-18	Avialae	Feather	Yixian, China	Aptian-Albian
32	*Sapeornis*	STM 15-18	Avialae	Feather	Yixian, China	Aptian-Albian
33	*Yi qi*	STM 31-2	Pennaraptora	Feather	Tiaojishan, China	Oxfordian
34	*Yi qi*	STM 31-2	Pennaraptora	Feather	Tiaojishan, China	Oxfordian
35	*Sapeornis*	STM 15-18	Avialae	Feather	Yixian, China	Aptian-Albian

***Unmatured enzymatically extracted melanin samples* (Colleary et al. 2015)**

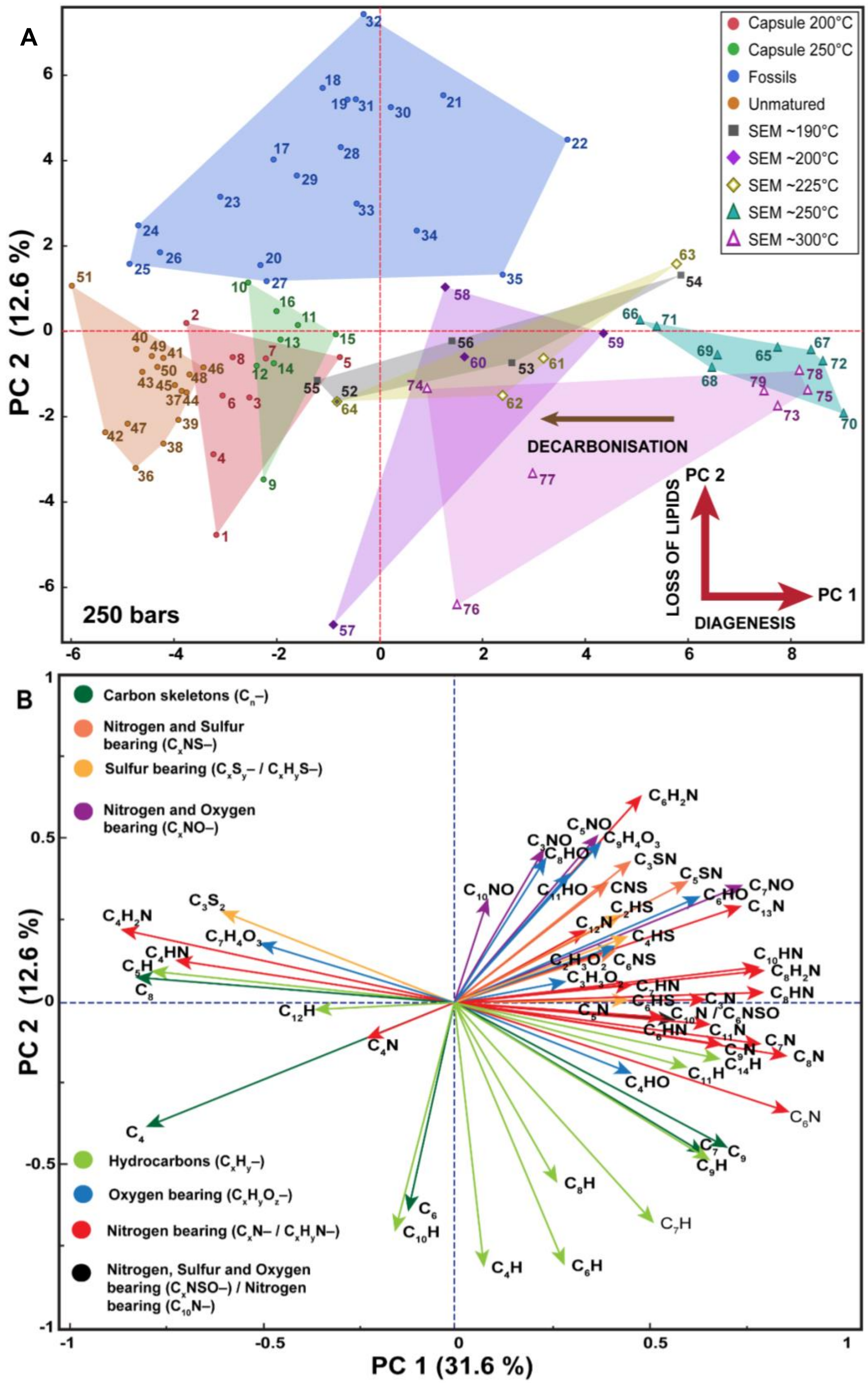
PCA No.	Common Name	Scientific Name	Tissue	Colour	Melanin Type
36	Chicken	*Gallus gallus*	Feather	Brown	Phaeomelanin
37	Carrion crow	*Corvus corone*	Feather	Glossy Black	Eumelanin
38	House wren	*Troglodytes aedon*	Feather	Brown	Phaeomelanin
39	Chicken	*Gallus gallus*	Feather	Black	Eumelanin
40	Dark-eyed junco	*Junco hyemalis*	Feather	Grey	Eumelanin, Phaeomelanin
41	Mallard	*Anas platyrynchos*	Feather	Grey	Eumelanin, Phaeomelanin
42	Dark-eyed junco	*Dumetella carolinensis*	Feather	Brown	Phaeomelanin
43	Rock dove	*Columba livia*	Feather	Grey	Eumelanin, Phaeomelanin
44	Turkey	*Meliagris gallopavo*	Feather	Iridescent	Eumelanin
45	Rock dove	*Columba livia*	Feather	Grey	Eumelanin, Phaeomelanin
46	Rock dove	*Columba livia*	Feather	Black	Eumelanin
47	Edible frog	*Pelophylax kl. esculentus*	Liver	Mixed	Eumelanin, Phaeomelanin
48	Edible frog	*Pelophylax kl. esculentus*	Eye	Mixed	Eumelanin, Phaeomelanin
49	Edible frog	*Pelophylax kl. esculentus*	Eye	Mixed	Eumelanin, Phaeomelanin
50	Eurasian magpie	*Pica pica*	Feather	Iridescent	Eumelanin
51	Purified Sepiamelanin (Sigma -Aldrich)		-	-	Eumelanin standard

Sediment encased P/T- maturation samples of extant feathers

PCA No.	Common Name	Scientific Name	T (°C)	P (bars)	Colour	Melanin Type
52	Wrinkled hornbill	*Rhabdotorrhinus corrugatus*	~190	250	Black	Eumelanin
53	Common pheasant	*Phasianus colchicus*	~190	250	Brown	Phaeomelanin
54	Rock dove	*Columba livia*	~190	250	Grey	Eumelanin, Phaeomelanin
55	Wrinkled hornbill	*Rhabdotorrhinus corrugatus*	~190	250	Black	Eumelanin
56	Rock dove	*Columba livia*	~190	250	Iridescent	Eumelanin
57	White-rumped munia	*Lonchura striata*	~200	250	Black	Eumelanin
58	Von Shrenck's bittern	*Ixobrychus eurhythmus*	~200	250	Brown	Phaeomelanin
59	Von Shrenck's bittern	*Ixobrychus eurhythmus*	~200	250	Grey	Eumelanin, Phaeomelanin
60	Rock dove	*Columba livia*	~200	250	Iridescent	Eumelanin
61	Common pheasant	*Phasianus colchicus*	~225	250	Brown	Phaeomelanin
62	Turkey	*Meliagris gallopavo*	~225	250	Iridescent	Eumelanin
63	Wrinkled hornbill	*Rhabdotorrhinus corrugatus*	~225	250	Black	Eumelanin
64	Rock dove	*Columba livia*	~225	250	Grey	Eumelanin, Phaeomelanin
65	Common pheasant	*Phasianus colchicus*	~250	250	Brown	Phaeomelanin
66	Wrinkled hornbill	*Rhabdotorrhinus corrugatus*	~250	250	Black	Eumelanin
67	Rock dove	*Columba livia*	~250	250	Grey	Eumelanin, Phaeomelanin
68	Turkey	*Meliagris gallopavo*	~250	250	Iridescent	Eumelanin
69	Turkey	*Meliagris gallopavo*	~250	250	Iridescent	Eumelanin
70	Common pheasant	*Phasianus colchicus*	~250	250	Brown	Phaeomelanin
71	Wrinkled hornbill	*Rhabdotorrhinus corrugatus*	~250	250	Black	Eumelanin
72	Rock dove	*Columba livia*	~250	250	Grey	Eumelanin, Phaeomelanin
73	Wrinkled hornbill	*Rhabdotorrhinus corrugatus*	~300	250	Black	Eumelanin
74	Common pheasant	*Phasianus colchicus*	~300	250	Brown	Phaeomelanin
75	White-rumped munia	*Lonchura striata*	~300	250	Black	Eumelanin
76	Von Shrenck's bittern	*Ixobrychus eurhythmus*	~300	250	Brown	Phaeomelanin
77	Von Shrenck's bittern	*Ixobrychus eurhythmus*	~300	250	Grey	Eumelanin, Phaeomelanin
78	Rock dove	*Columba livia*	~300	250	Iridescent	Eumelanin
79	Wrinkled hornbill	*Rhabdotorrhinus corrugatus*	~300	250	Black	Eumelanin

Supplementary Figures

Fig. S1. PCA on ToF-SIMS data of fossil and experimental samples. **(A)** Comparison of secondary ion spectra of fresh melanin extracts, capsule-matured melanin extracts, sediment-encased maturation (SEM) of whole feathers, and fossil samples. **(B)** Loading plot indicating the relative contributions of secondary ions on PC1 and PC2 axes (next page).



A
Capsule 200°C
Capsule 250°C
Fossils
Unmatured
SEM ~190°C
SEM ~200°C
SEM ~225°C
SEM ~250°C
SEM ~300°C
DECARBONISATION
LOSS OF LIPIDS
DIAGENESIS
PC 2
PC 1
250 bars
PC 2 (12.6 %)
B
Carbon skeletons (C_n–)
Nitrogen and Sulfur bearing (C_xNS–)
Sulfur bearing (C_xS_y– / C_xH_yS–)
Nitrogen and Oxygen bearing (C_xNO–)
Hydrocarbons (C_xH_y–)
Oxygen bearing ($C_xH_yO_z$–)
Nitrogen bearing (C_xN– / C_xH_yN–)
Nitrogen, Sulfur and Oxygen bearing (C_xNSO–) / Nitrogen bearing ($C_{10}N$–)
PC 2 (12.6 %)
PC 1 (31.6 %)

Fig. S2. Comparison of ToF-SIMS spectra of fresh, experimental, and fossil samples. Aligned time-of-flight secondary ion negative spectra for α-keratin reference (K0253, Sigma-Aldrich) (Schweitzer et al. 2018), white feather β-keratin (Schweitzer et al. 2018), fresh (unmatured) melanin extracted from black and brown feathers (Colleary et al. 2015), (spectra collected at room temperature under the same analytical parameters on the same make and model of equipment as used here and provided by Peter Sjövall, RISE Research Institutes of Sweden, Chemistry and Materials, Borås, Sweden), 200°C capsule-matured melanin extracted from black and brown feathers, 200°C sediment-encased P/T-matured black and brown feathers, and a fossil feather (*Messelornis*, SMF-ME 11402a). Spectra are colour coded as: feather β-keratin reference (red), unmatured melanin extract (purple), capsule-matured melanin extract (pink), sediment-matured feathers (green), and fossil feather (blue). Characteristic melanin fragments based on Colleary et al. (2015) and Lindgren et al. (2014) and Lindgren et al. (2012) are shown as dots above each spectrum. Black dots indicate eumelanin-specific fragments, whereas brown dots indicate phaeomelanin-specific fragments. A large peak present at m/Z 75 and two large peaks m/Z 90.84 and 92.93, as well as the absence of peaks beyond m/Z 120, in the α-keratin spectrum indicate that experimental and fossil samples do not match α-keratin, but instead are dominated by melanin. β-keratin spectrum also largely shows peaks which do not match the unmatured melanin, experimental samples or fossil melanin. We expect melanin to be a more heavily cross-linked polymer in many of these samples than a polypeptide chain, possibly explaining the lack of large mass fragments in the feather β-keratin reference. ~190-200°C experimentally matured samples are shown here because these appear to be most similar to fossil specimens in PCA (next page).

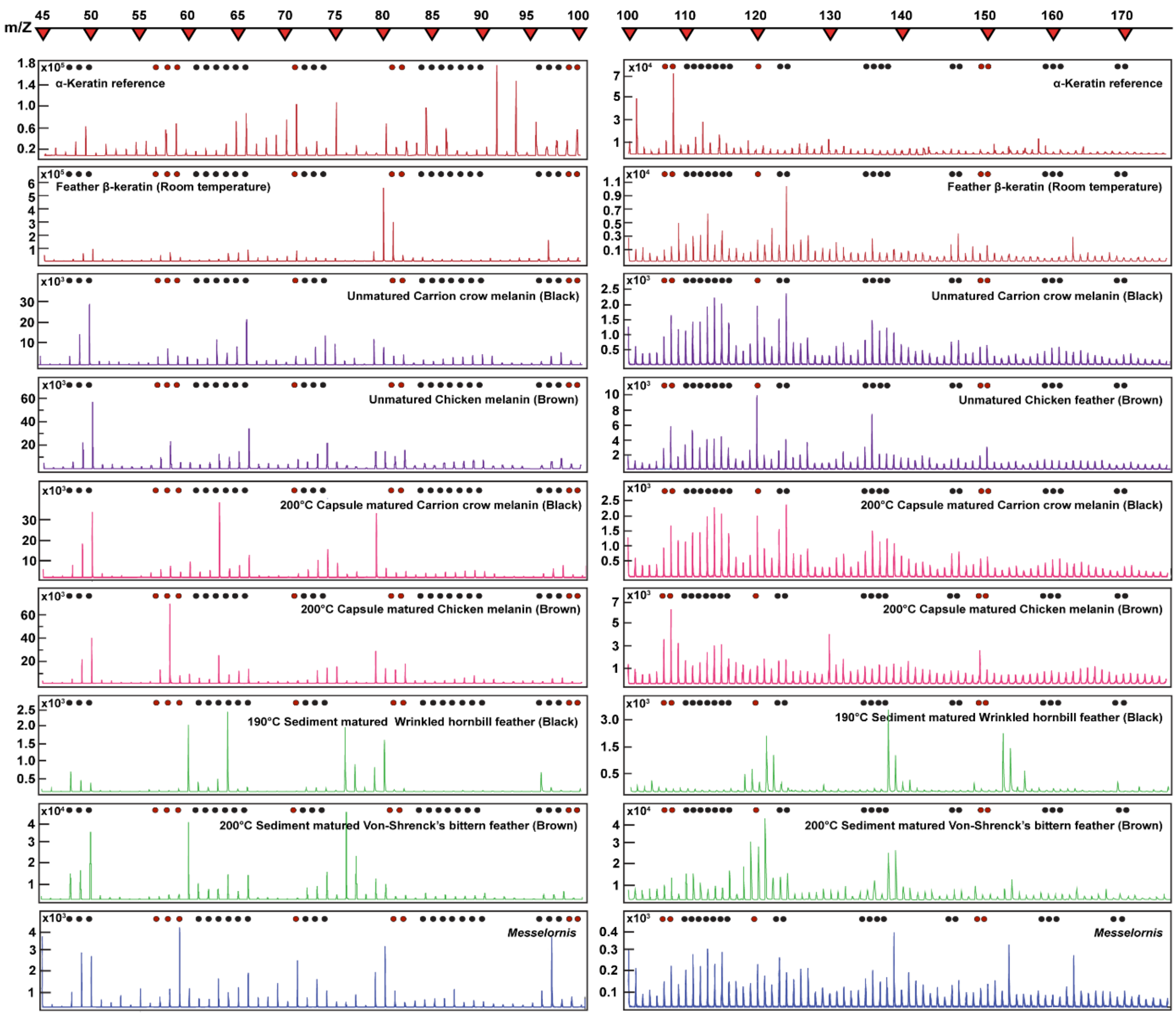


Fig. S3. Loadings of ToF-SIMS secondary ions on PC1–PC4 of the same PCA as shown in Fig. 2 (i.e., all 55 fragments included). Different chemical groups are colour coded. The black bar indicates C_6NSO- (m/Z 134.00) but the fragment size is also very similar to that of $C_{10}N-$ (m/Z 133.97) thus two chemical groups cannot be unambiguously identified (next page).

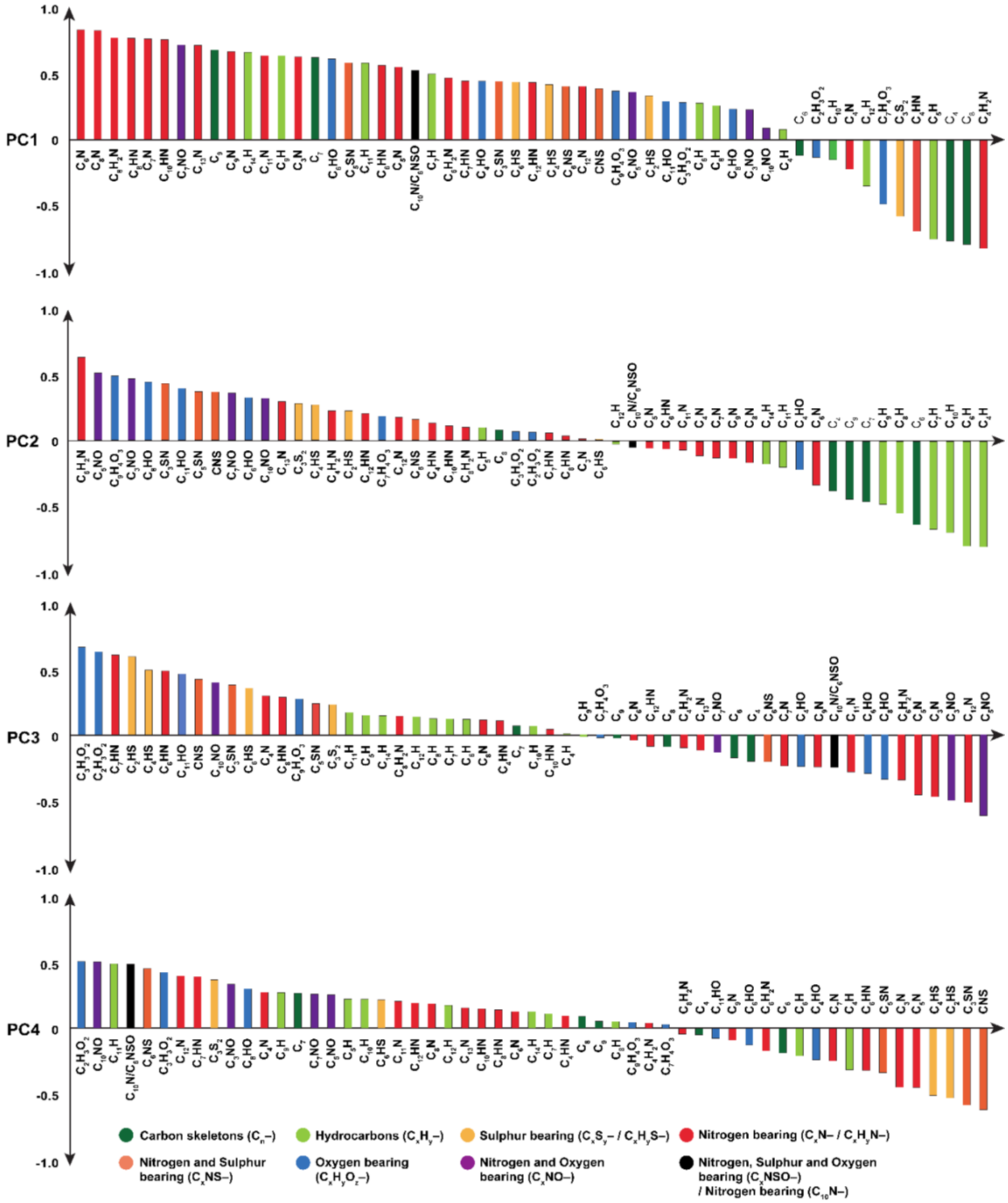
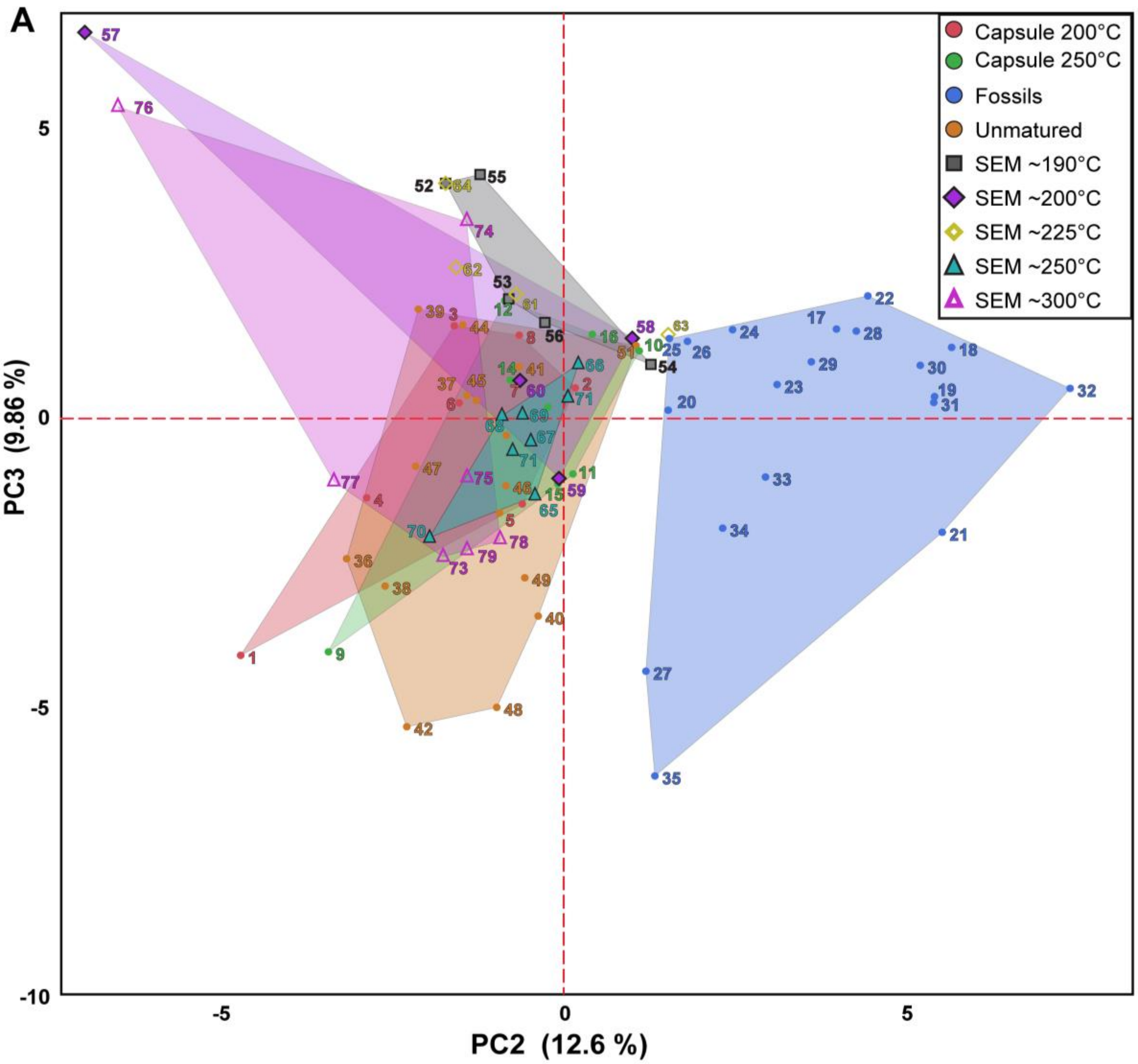


Fig. S4. PC2 and PC3 of the same PCA on TOF-SIMS data of fossil and modern/experimental samples as in Fig. 2 (i.e., all 55 fragments included).**(A)** Comparison of secondary ion spectra of modern melanin extracts, capsule-matured melanin extracts, sediment-encased maturation (SEM) of whole feathers, and fossil samples along PC2 and PC3. **(B)** Loading plot indicating the relative contributions of secondary ions on PC2 and PC3. The black arrow indicates C_6NSO^- (m/Z 134.00) but the fragment size is also very similar to that of $C_{10}N^-$ (m/Z 133.97) thus two cannot be unambiguously identified. **(C)** Box plot of PC3 values according to sample category.



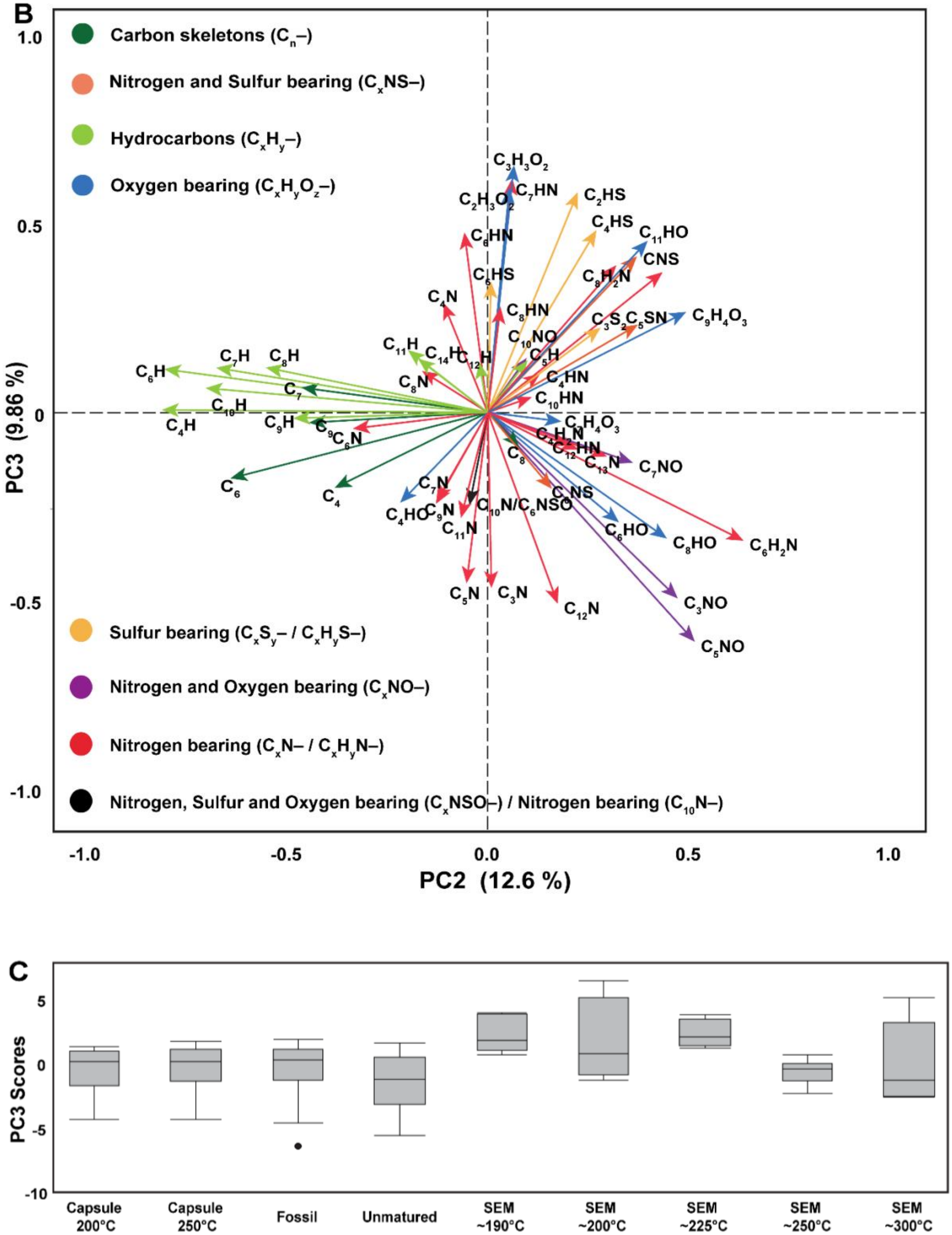
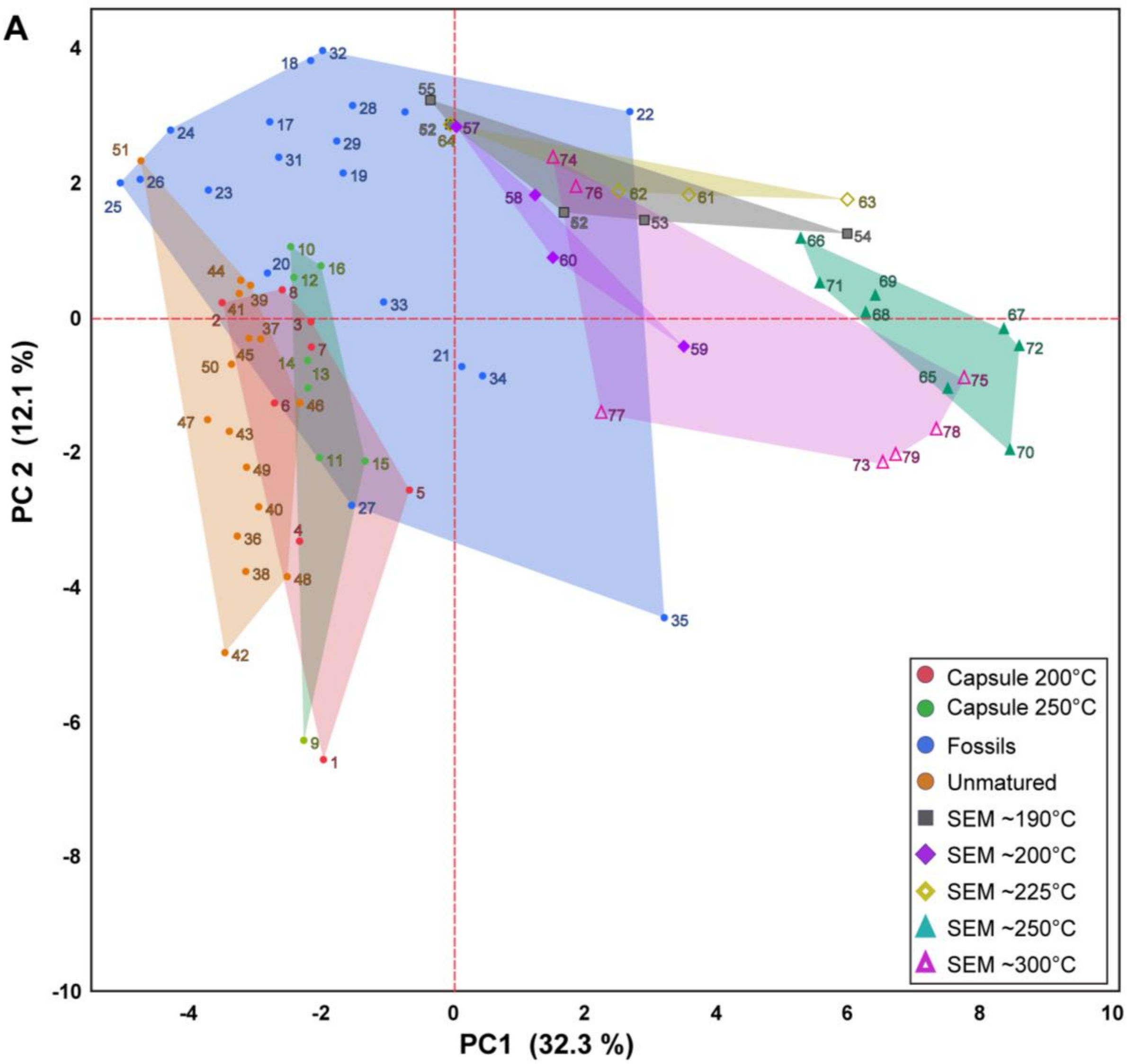
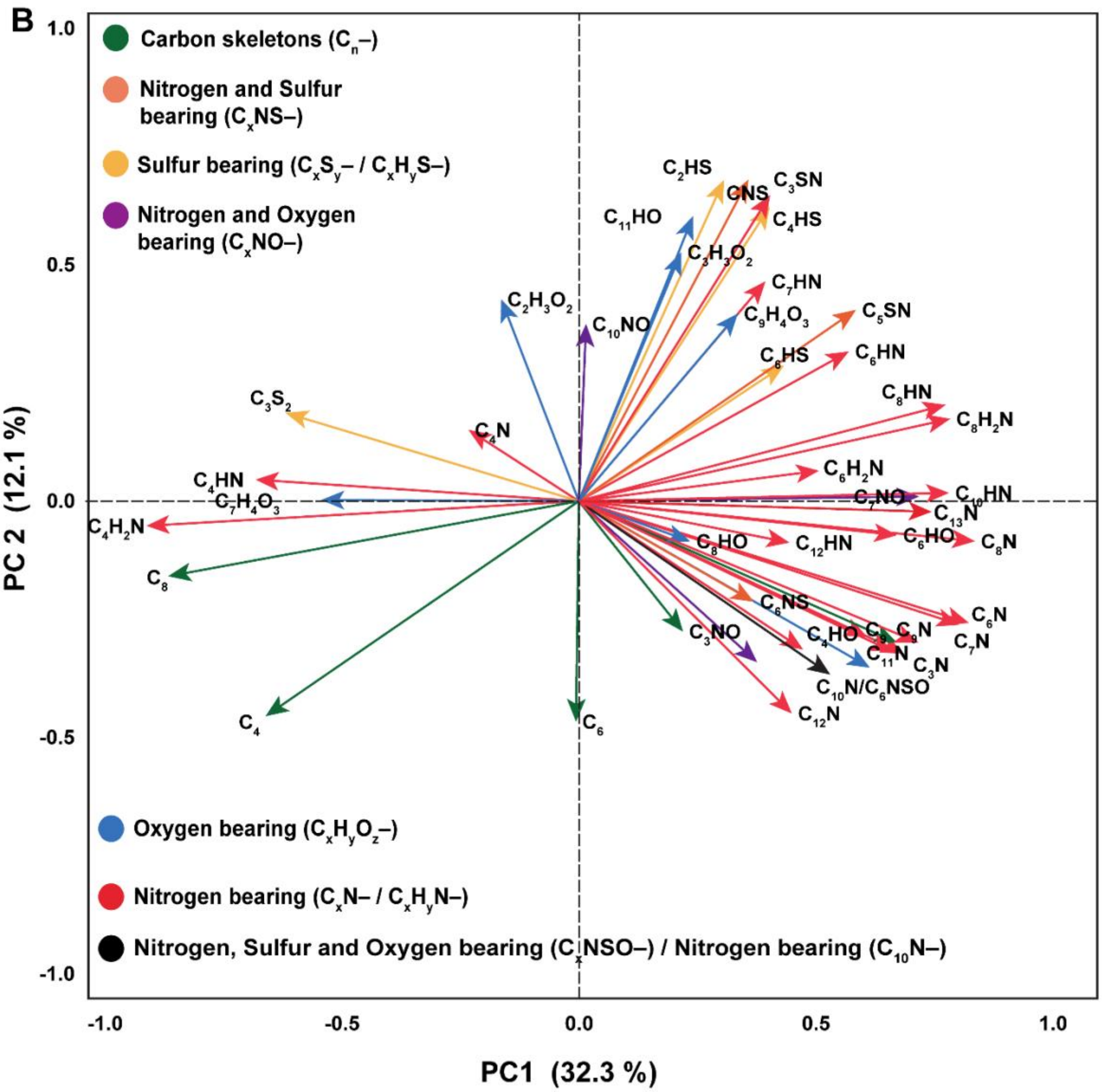

B
Carbon skeletons (C_n–)
Nitrogen and Sulfur bearing (C_xNS–)
Hydrocarbons (C_xH_y–)
Oxygen bearing ($C_xH_yO_z$–)
Sulfur bearing (C_xS_y– / C_xH_yS–)
Nitrogen and Oxygen bearing (C_xNO–)
Nitrogen bearing (C_xN– / C_xH_yN–)
Nitrogen, Sulfur and Oxygen bearing (C_xNSO–) / Nitrogen bearing ($C_{10}N$–)
$C_3H_3O_2$
C_7HN
C_2HS
C_4HS
$C_{11}HO$
CNS
C_8H_2N
$C_9H_4O_3$
C_6HN
C_6HS
C_4N
C_8HN
$C_{10}NO$
C_5H
C_4HN
$C_{10}HN$
C_6H
C_7H
C_8H
$C_{11}H$
$C_{14}H$
$C_{12}H$
C_8N
C_7
$C_{10}H$
C_4H
C_9H
C_9
C_6N
C_6
C_4
$C_3H_4O_3$
$C_{12}HN$
$C_{13}N$
C_7NO
C_8
C_7N
C_9N
$C_{11}N$
C_4HO
$C_{10}N/C_6NSO$
C_6HO
C_8HO
C_6H_2N
C_5N
C_3N
$C_{12}N$
C_3NO
C_5NO
PC3 (9.86 %)
PC2 (12.6 %)
1.0
0.5
0
-0.5
-1.0
-1.0
-0.5
0.0
0.5
1.0
C
PC3 Scores
5
0
-5
-10
Capsule 200°C
Capsule 250°C
Fossil
Unmatured
SEM ~190°C
SEM ~200°C
SEM ~225°C
SEM ~250°C
SEM ~300°C

Fig. S5. A separate PCA on negative secondary ions in which hydrocarbon (C_xH^-) fragments are excluded (i.e., the remaining 45 fragments) **(A)** Excluding hydrocarbon fragments lead to an increase in overlap between experimental categories (Capsule 200-250°C; 195-225°C) and fossils suggesting that fossils are depleted in hydrocarbon fragments due to early microbial decay or late oxidative weathering. PC1 scores of low temperature sediment matured samples (~190°C, ~200°C, ~225°C) become more comparable with fossil samples when this hydrocarbon discrepancy is accounted for. There is some heterogeneity in fossil samples probably due to differences in age, locality, and taphonomy. Fossils overlap with a few unmatured melanin extracts further supporting the idea that melanin, rather than protein, produces the stains of fossils. **(B)** Loading plot indicating the relative contributions of secondary ions on PC1 and PC2. The black arrow indicates C_6NSO^- (*m/z* 134.00) but the fragment size is also very similar to that of $C_{10}N^-$ (*m/z* 133.97) thus two chemical groups cannot be unambiguously identified (next page).





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