Supplementary Information

European mushroom assemblages are darker in cold climates
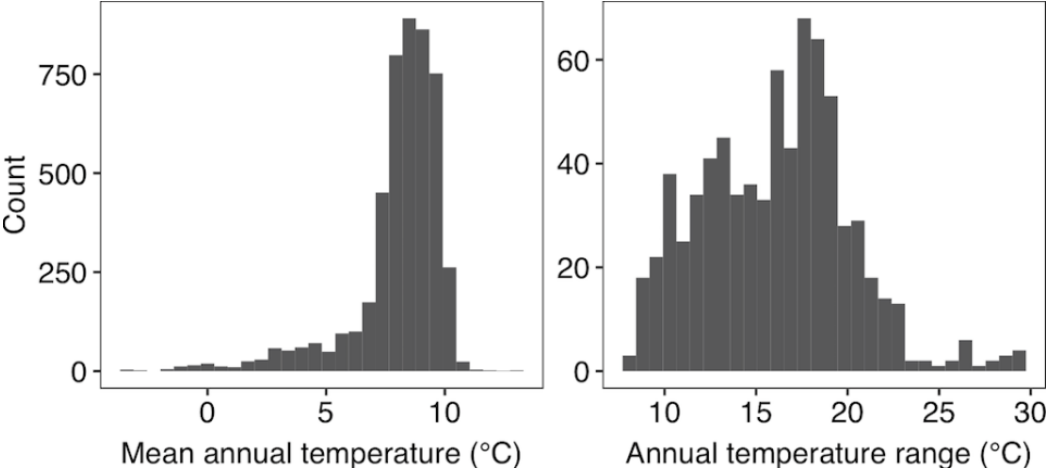
Krah *et al*.

This PDF file includes:

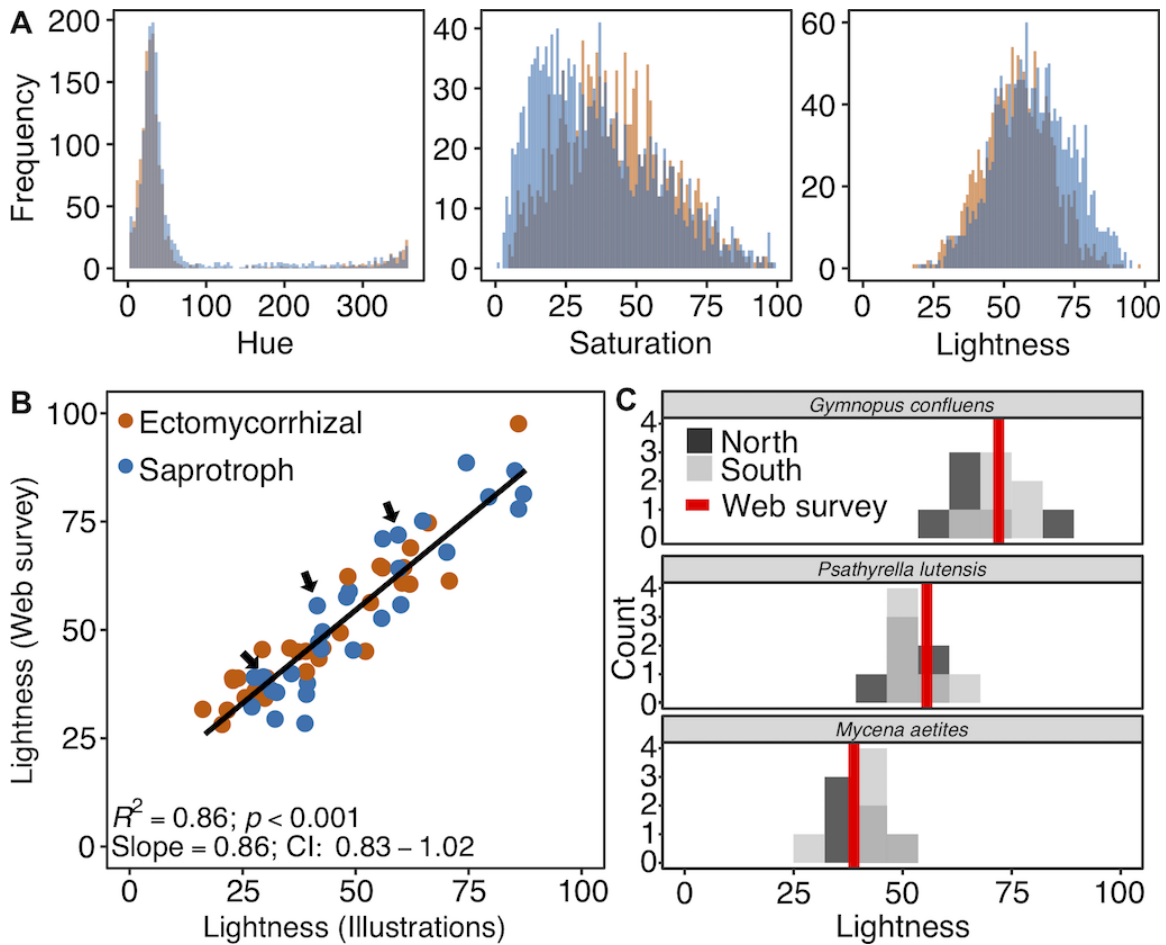Supplementary Figures 1-13

Supplementary Tables 1-7

Supplementary References

Supplementary Figures and Tables

**Supplementary Figure 1. Histograms of temperature of the grids in the European dataset.**

Displayed are mean annual temperature values and the annual temperature ranges (maximum −

minimum monthly temperature) of each grid cell used in this study.

Supplementary Figure 2. Histograms of mushroom colors and data reliability. (**A**) Histograms based on ectomycorrhizal and saprotrophic species (3,054 species). Both, saprotrophs (blue) and ectomycorrhizal fungi (orange), show a hue peak in the interval 1–80 degree, which comprises the colors red, orange, and yellow (note that this includes brown colors). Ectomycorrhizal fungi have on average darker fruit bodies than saprotrophic fungi (Fig. 3A, Supplementary Table 2). Note that overlapping areas are displayed in darker blue. (**B**) Lightness based on web survey (our color sampling approach, see 'Color sampling' in Methods) vs. lightness based on standardized illustrations [1–4]. Illustrations are standardized in terms of geographic and environmental variability of lightness intensity. We randomly selected 10 species of each of three lightness categories (dark, <40; medium, 40-60; and light, >60, see Fig. 2) and nutritional modes (ectomycorrhizal and saprotroph) from our dataset, resulting in a total of 60

species. We then generated lightness values for the same species based on illustrations [1–4]. Note that standardized illustrations are only available for a limited subset of our species list. Goodness of fit (R^2) and p-value (p) were estimated using linear models. We further tested for proportionality by testing whether the estimated slope differed from a slope of one, using reduced major axis. Arrows indicate species used in the analysis in (**C**). (**C**) Lightness based on web survey in northern latitude (>~58 latitude) vs. southern latitudes (<~50 latitude) based on three saprotrophic species (dark, <40; medium, 40-60; and light, >60) with each 6 individuals from north and south. The red line indicates the lightness value of the web survey (our color sampling approach, see 'Color sampling' in Methods) used in our analysis. Overlapping areas of the northern and southern distribution are shown in an intermediate gray. Source data underlying panel **A** are provided in Supplementary Data 1, 2 and 3. Source data underlying panels **B** and **C** are provided in Supplementary Data 5.
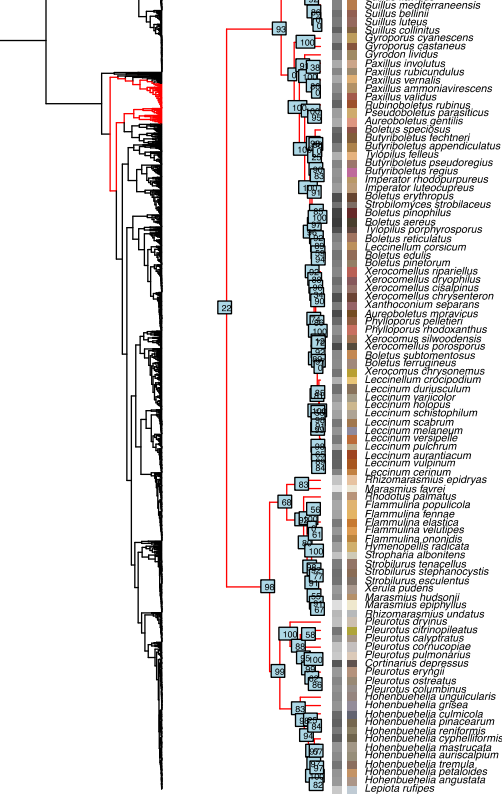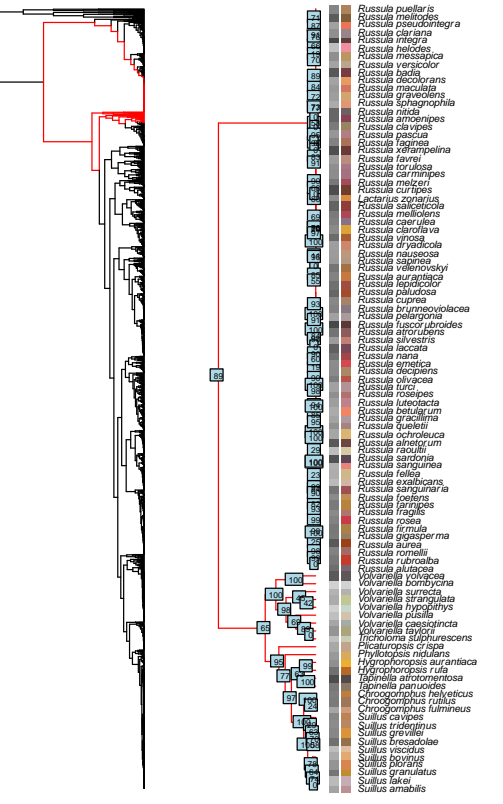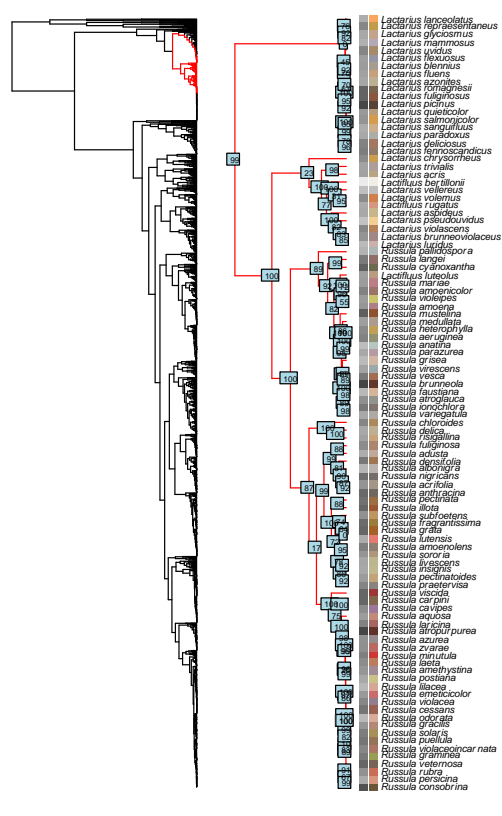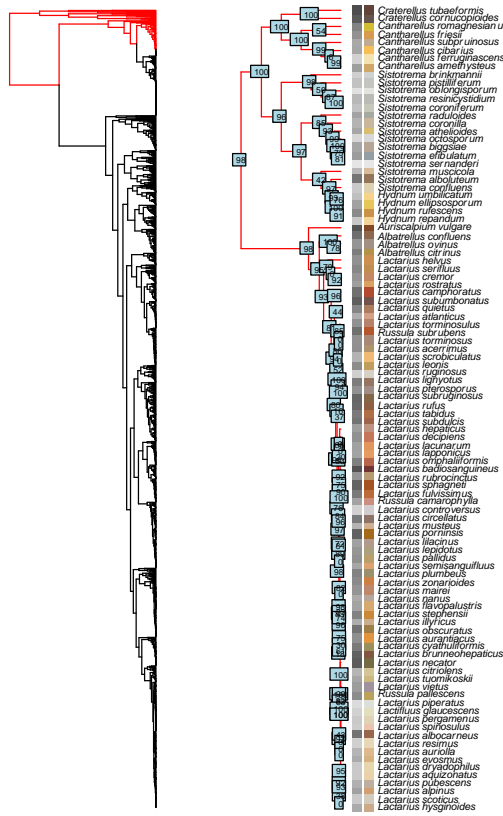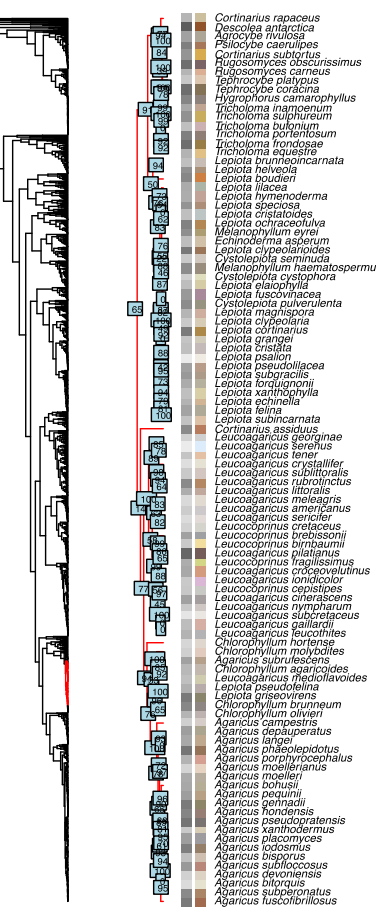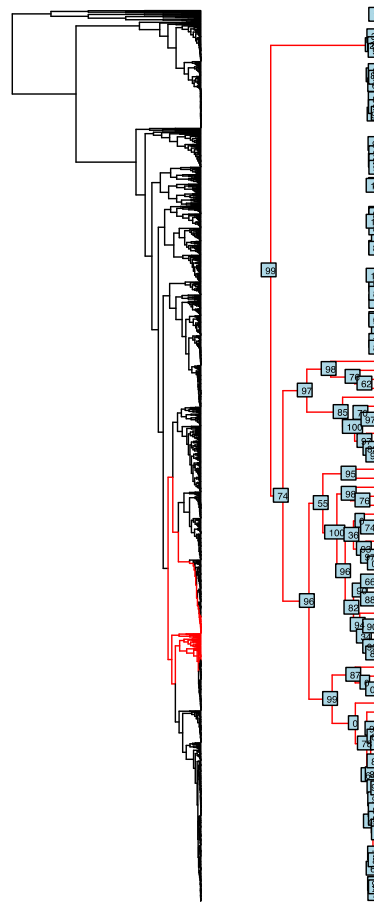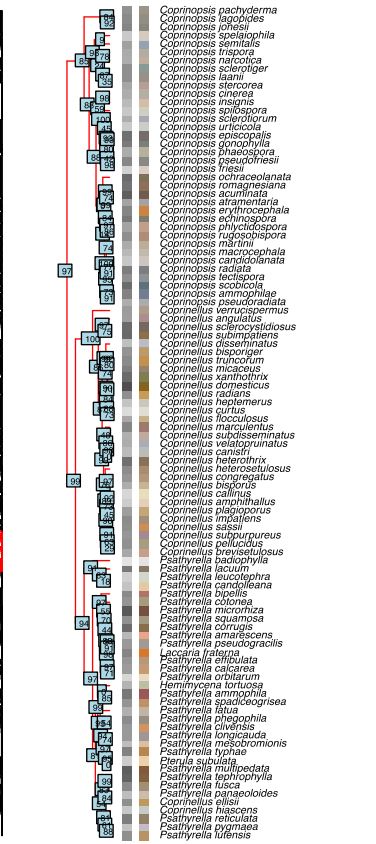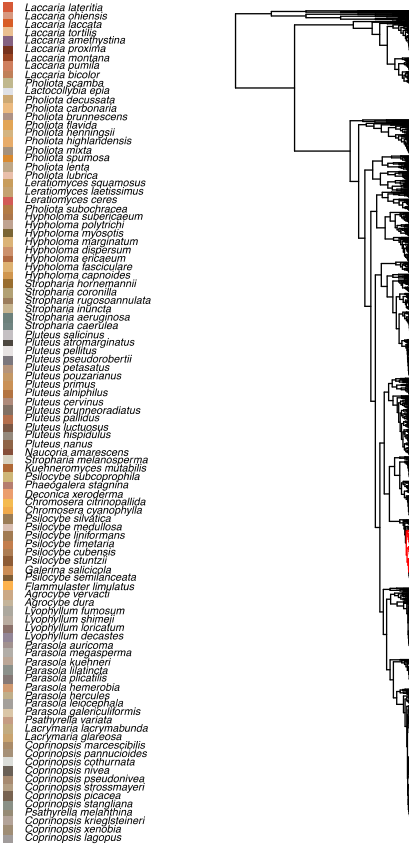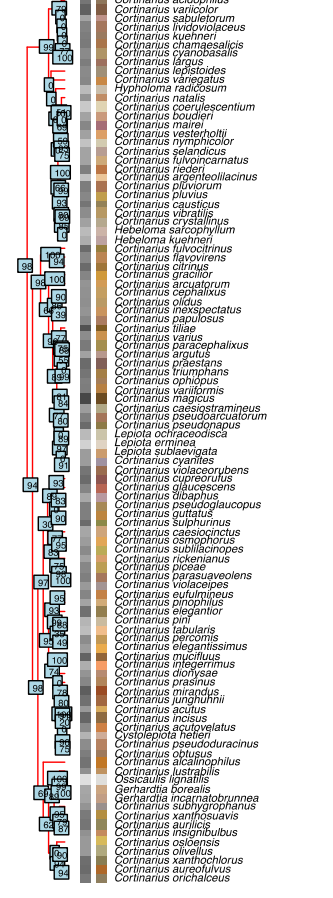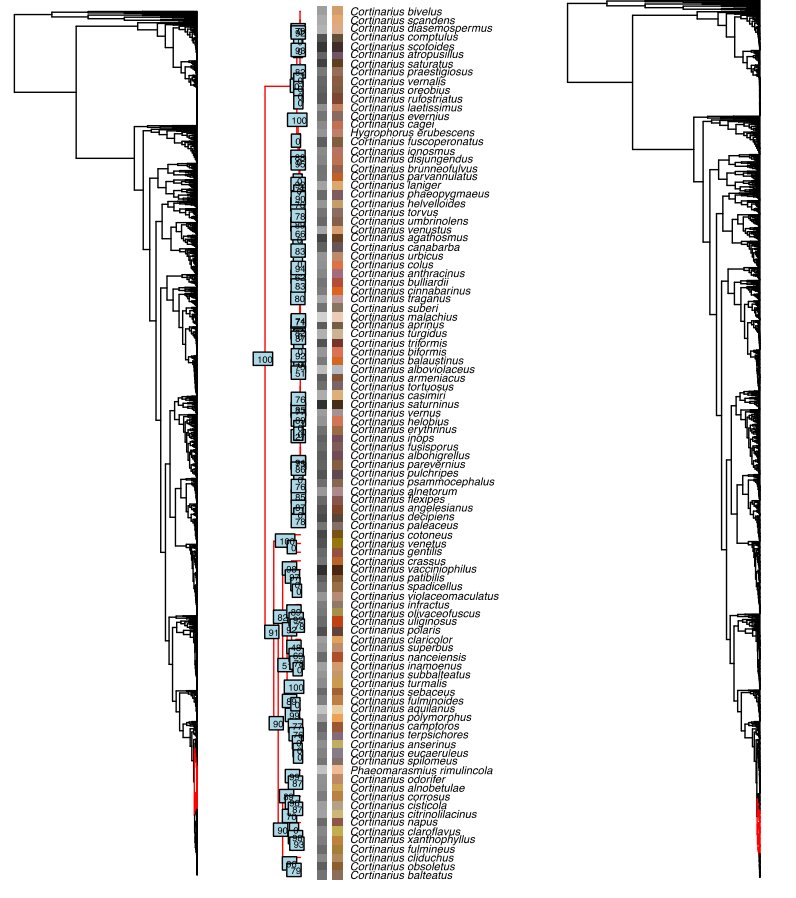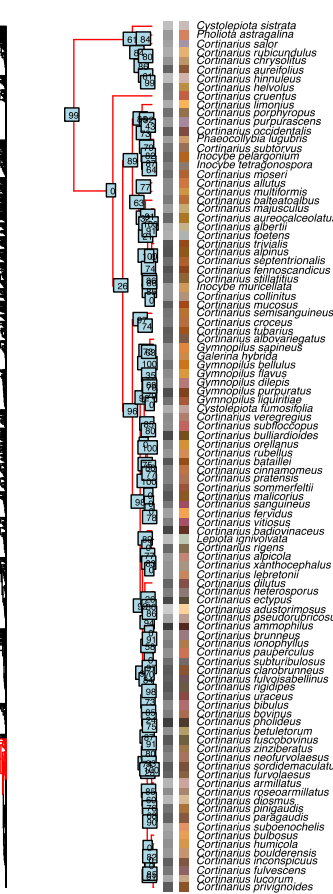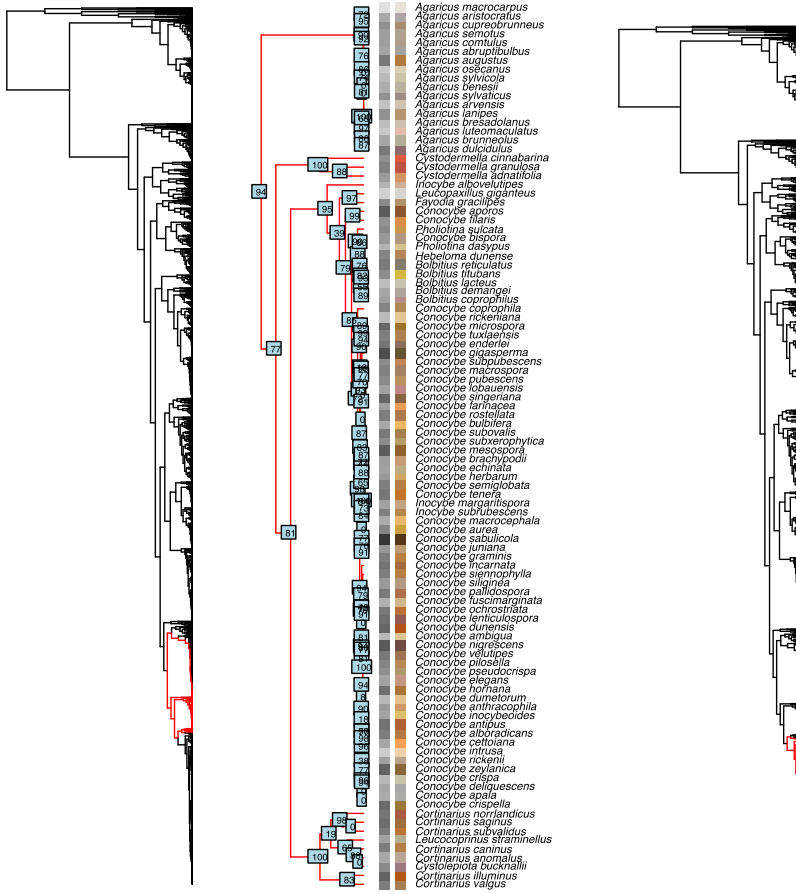
Supplementary Figure 3. Maximum-likelihood phylogeny. Branch support is given as Shimodaira–Hasegawa approximate likelihood ratio tests (SH-aLRT). The phylogeny was produced based on a mega-phylogeny approach (see 'Mega-phylogeny approach' in Methods). Averaged lightness and hue values are provided with the tip labels. Source data are provided in Supplementary Data 2.
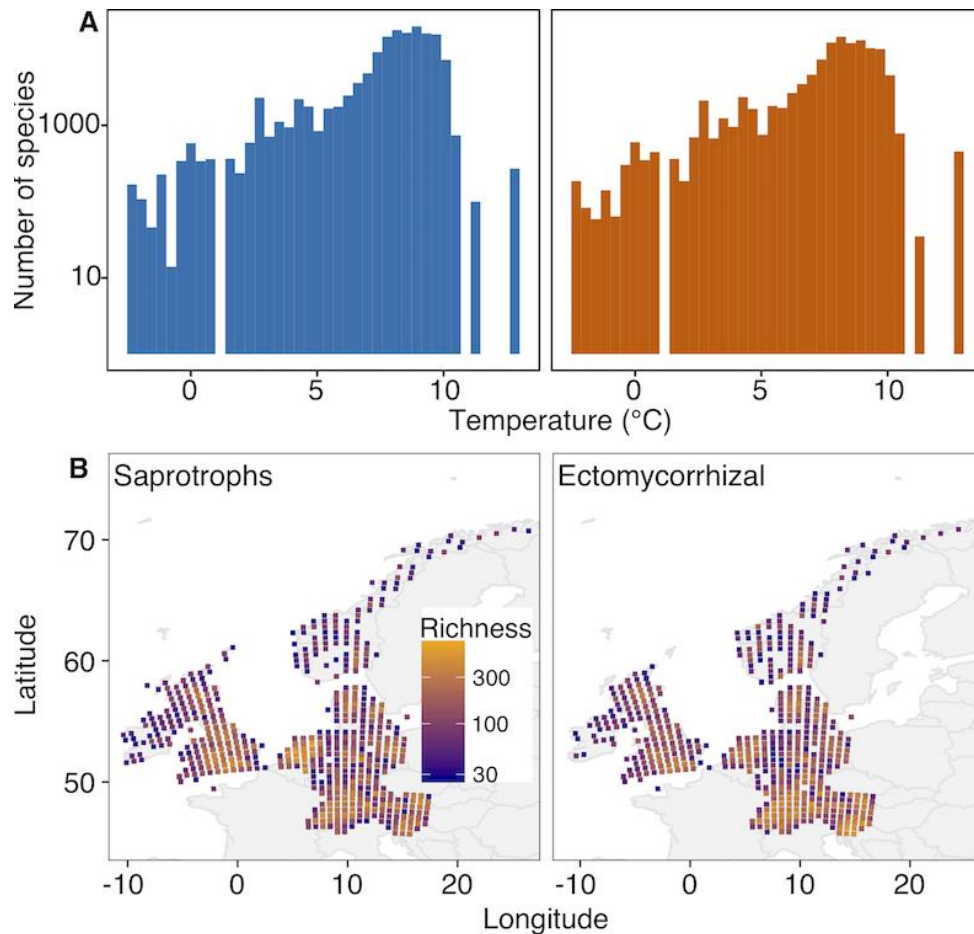
Supplementary Figure 4. Density histogram and map of the number of species. (**A**) Density histogram of the number of species along the mean annual temperature; saprotrophs in blue, ectomycorrhizal fungi in orange. Note $\log_{10}$-transformation of the y-axis and richness. (**B**) Map with occupied grids after data preparation (see Method section) and number of species (richness). Lighter colors indicate higher number of species. Maps were created using the R package ggplot2 with the function *borders*. Source data are provided Supplementary Data 4.
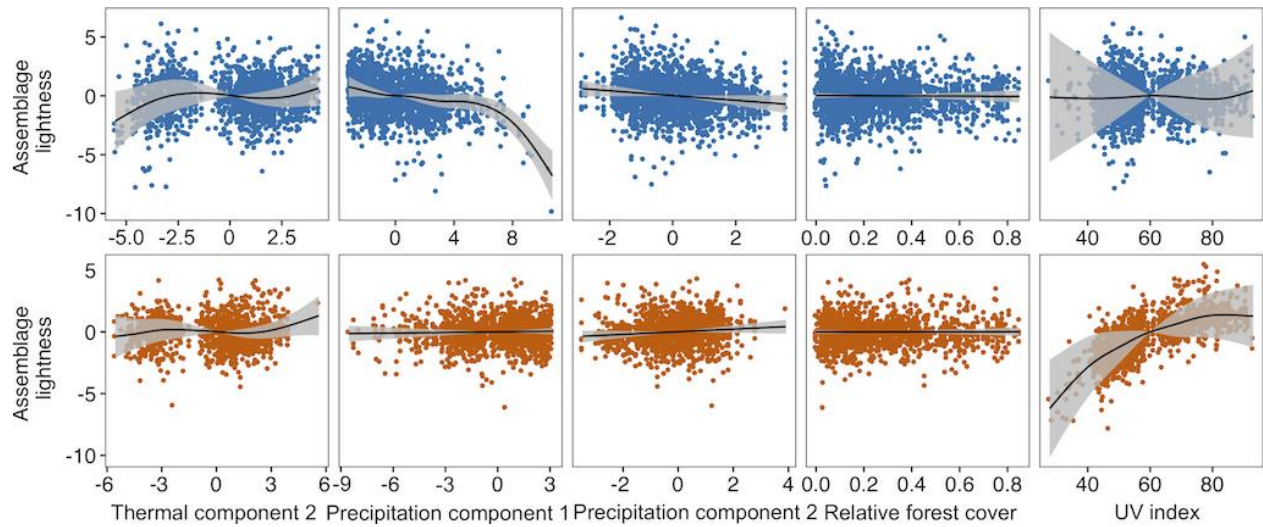
Supplementary Figure 5. Partial effect of covariates on mushroom color lightness. The top row displays effects for saprotrophs (blue), the bottom row for ectomycorrhizal fungi (orange). Plots are based on fits of the generalized additive models (GAM, Table 1). The gap within the thermal component 2 corresponds to the climatological difference between the British Isles and the European mainland, which differ in seasonality variation due to the degree of continentality. Source data are provided in Supplementary Data 4.
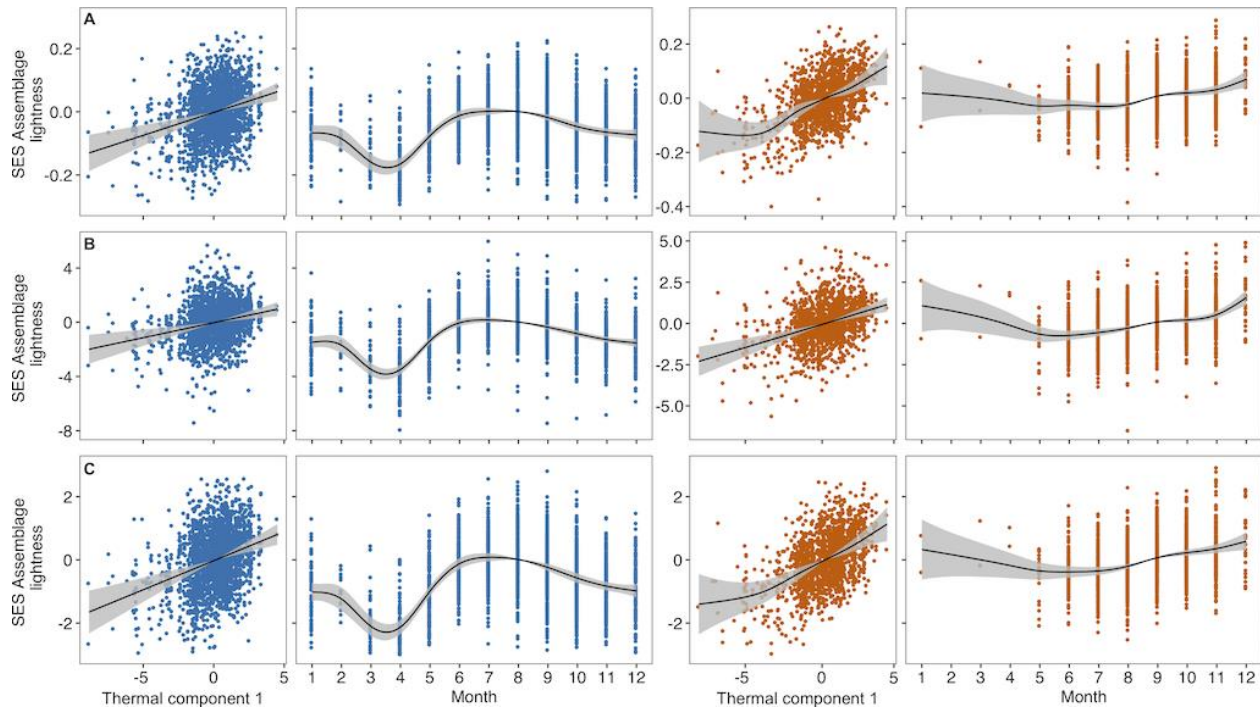
Supplementary Figure 6. Partial effects of temperature and seasonality on standardized effect sizes (SES). Standardized effect sizes of mushroom color lightness were calculated based on 100 randomizations of the community matrix (3,054 species) using the three null models: (**A**) independent swap (**B**), richness, and (**C**) frequency. Saprotroph responses are shown in the two columns on the left (blue); ectomycorrhizal responses in the two columns on the right (orange). For statistics see Table 1. Source data are provided in Supplementary Data 4.
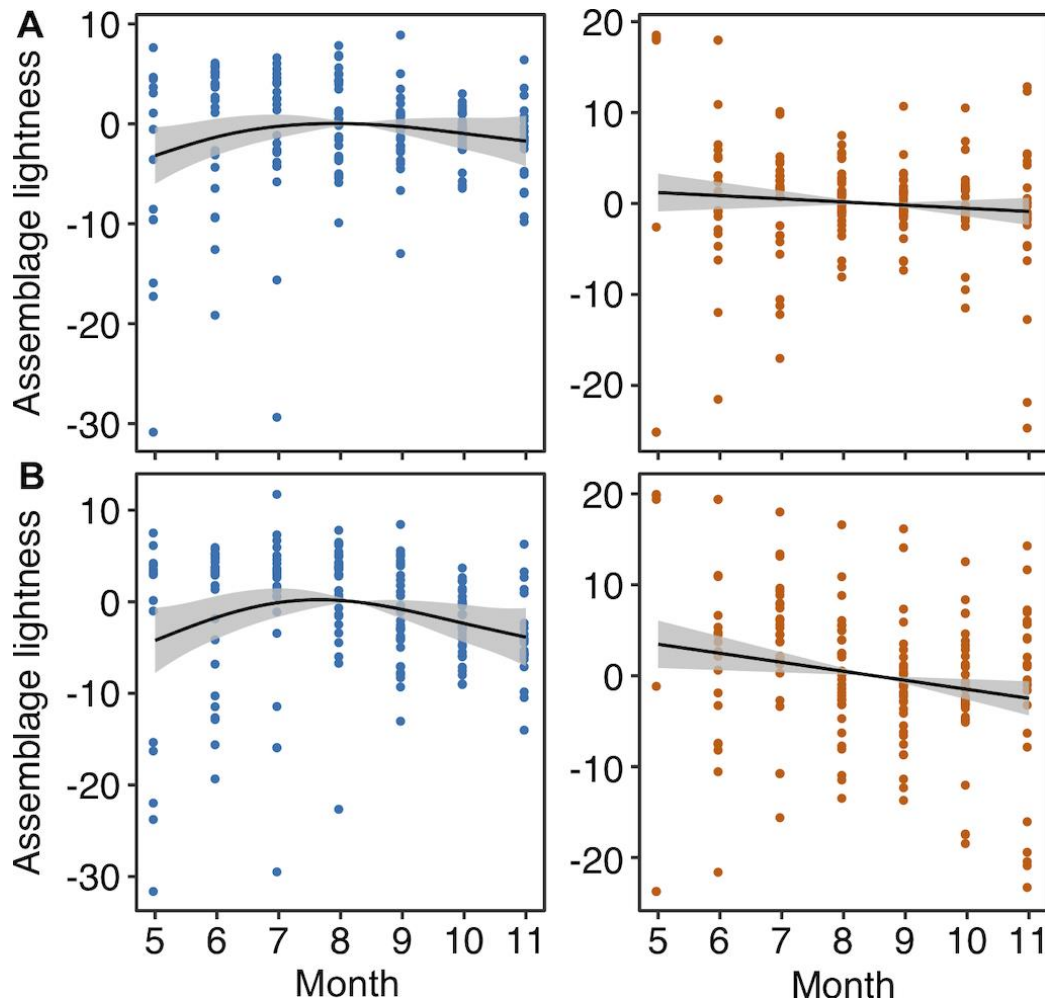
Supplementary Figure 7. Partial effects of seasonality on mushroom color lightness for a local dataset. Effect of months on the mushroom color lightness of saprotrophs (left, blue) and ectomycorrhizal fungi (right, orange). Mushroom color lightness was calculated based on (**A**) presence/absence data (generalized additive models: saprotroph: F-value=1.65, p-value=0.146; ectomycorrhizal: F-value=1.30, p-value=0.255), and (**B**) abundance data (generalized additive models: saprotroph: F-value=2.96, p-value=0.031; ectomycorrhizal: F-value=6.75, p-value=0.01, Supplementary Table 4). Slopes are estimates from generalized additive models (GAMs) with standard deviations. Source data are provided in Supplementary Data 6.
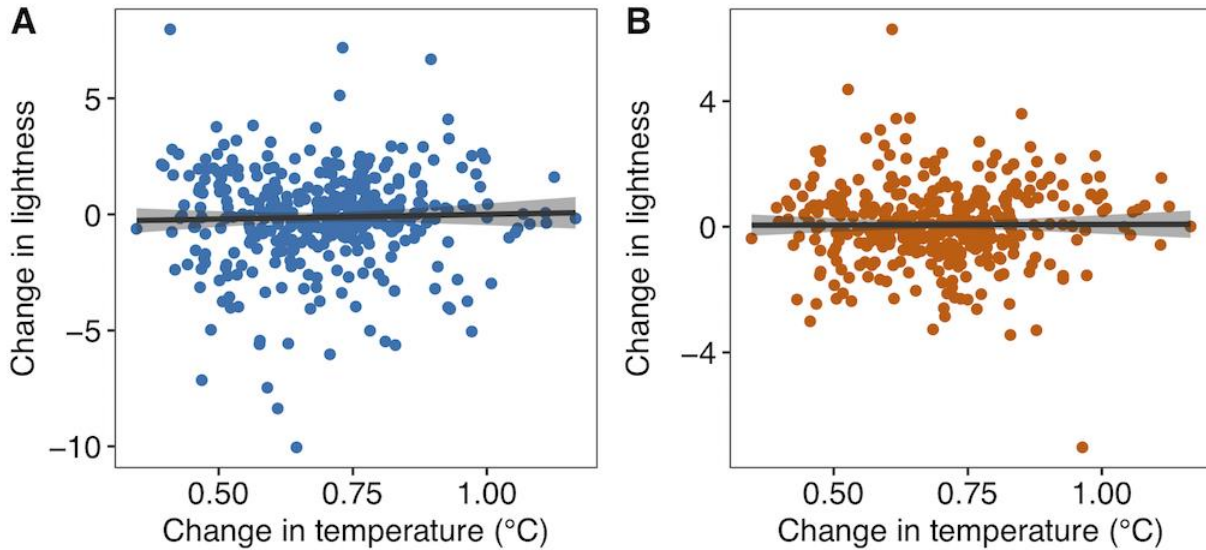
Supplementary Figure 8. Effects of climate warming on change in mushroom color lightness. To test for effects of climate warming on mushroom color lightness, we split our 40-year European dataset into two time intervals: 1970–1990 and 1991–2010. The number of species analyzed were as follows; ECM (1970-1990): 1167, ECM (1991-2010): 1371; SAP (1970-1990): 1497, SAP (1991-2010): 1621. The border between these two time intervals marks the approximate border of accelerated climate warming [5]. We calculated the change in temperature as the mean annual temperature of the first time-interval subtracted from the mean annual temperature of the second time-interval; the change in lightness is the mushroom color lightness of the first time-interval subtracted from the mushroom color lightness of the second time-interval. Effect of a change in temperature on a change in mushroom color lightness of (**A**) saprotrophic fungi (blue) and (**B**) ectomycorrhizal (orange) between the two time-intervals. Lines are linear model regressions with error bars displaying 95% confidence interval. Source data are provided in Supplementary Data 7.
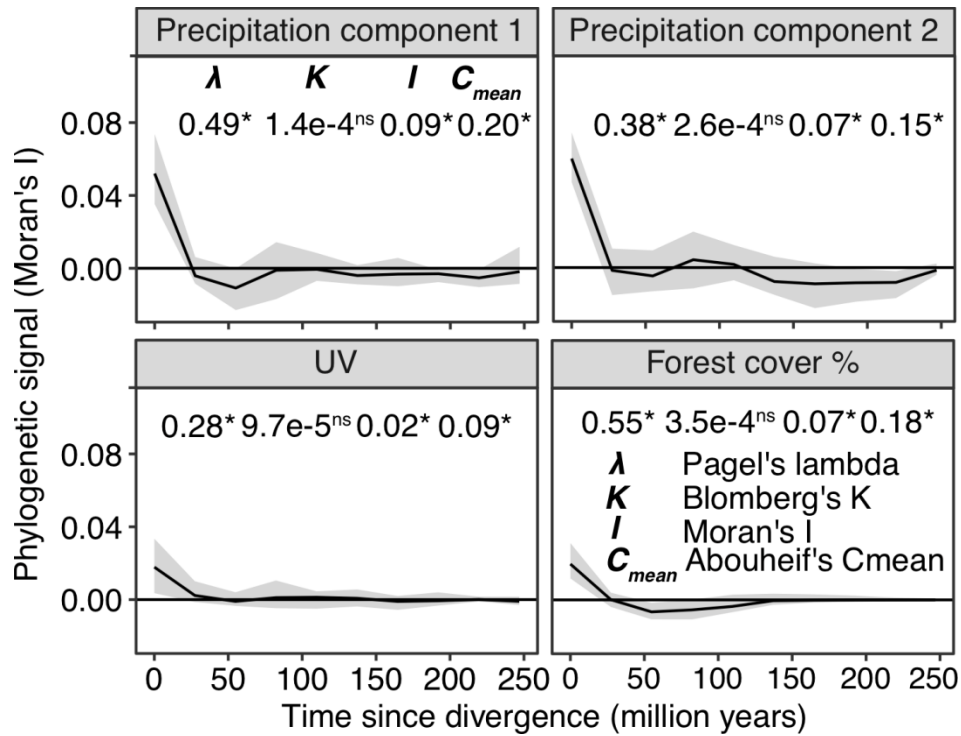
Supplementary Figure 9. Phylogenetic signal of species environmental variables (niche position). The phylogenetic signal was calculated as four different measures, and graphically displayed based on Moran's *I* (phylogenetic correlogram). Species which occupied at least 10 grid cells were used. Confidence interval (CI) based on 99 bootstraps. Significant phylogenetic signal was detectable only on very short phylogenetic distances, i.e., between closely related taxa (CI above null line). To estimate overall phylogenetic signal, we used (from left to right): Pagel's lambda (*λ*), Blomberg's *K*, Moran's *I* and Abouheif's C_{mean}. Source data are provided in Supplementary Data 1, 2 and 3.
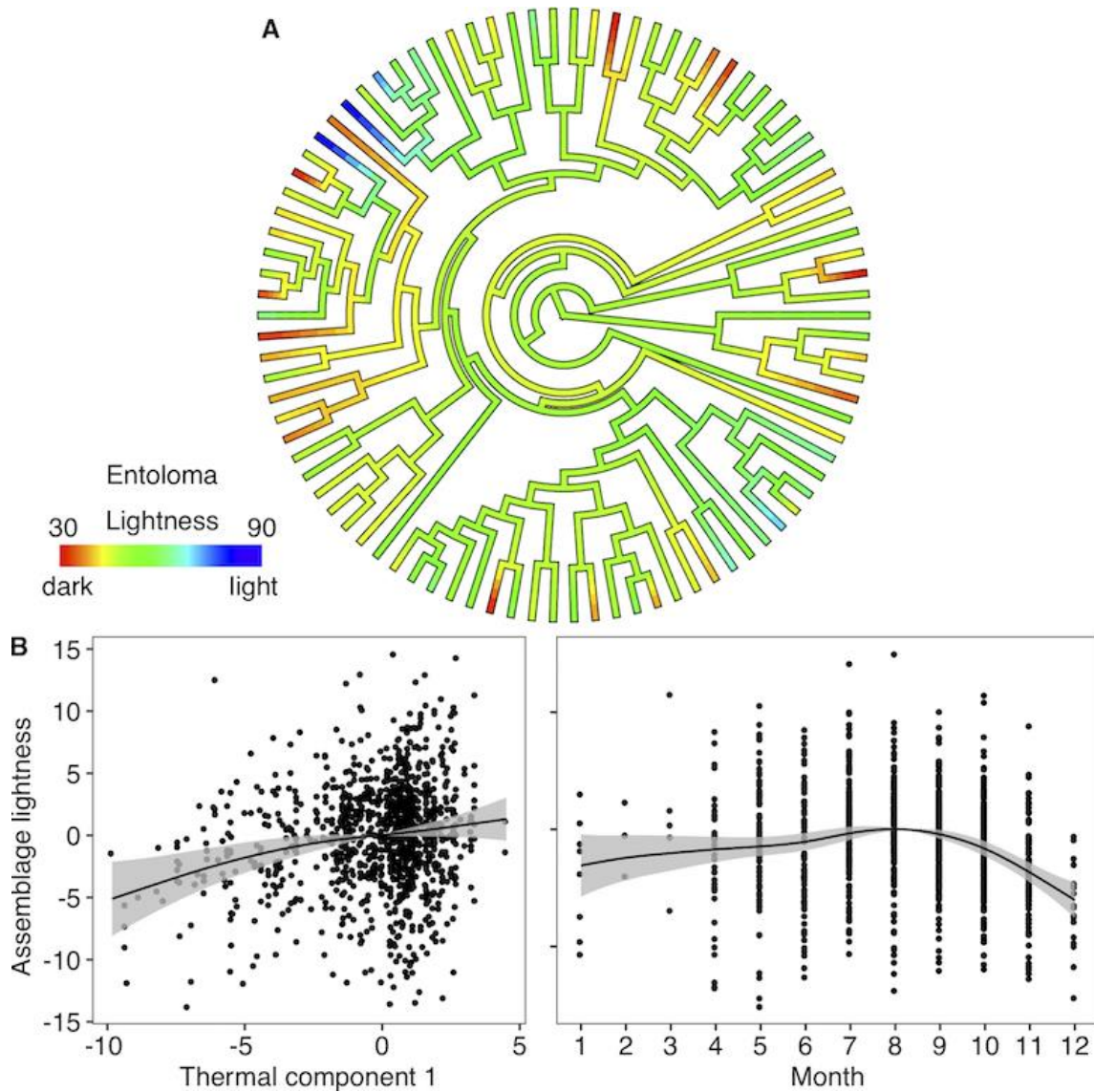
Supplementary Figure 10. Partial effects of temperature and seasonality on the widespread genus *Entoloma*. (**A**) Ancestral state estimation (function *contMap*, R package phytools) of the continuous lightness trait showing single-species or small clade divergences resulting in low phylogenetic signal (Fig. 5). (**B**) Partial effects of temperature (generalized additive models: *F*-value=4.26, *p*-value<0.007) and month (generalized additive model: *F*-value=15.43, *p*-value<0.001) on mushroom color lightness within the genus *Entoloma*, based on a generalized additive model (*R*²=0.22). Model is based on 491 grids and 90 species. We additionally

considered space (geographical latitude and longitude) as a covariate (generalized additive model: F-value=3.19, p-value<0.001) and grid as random effect.
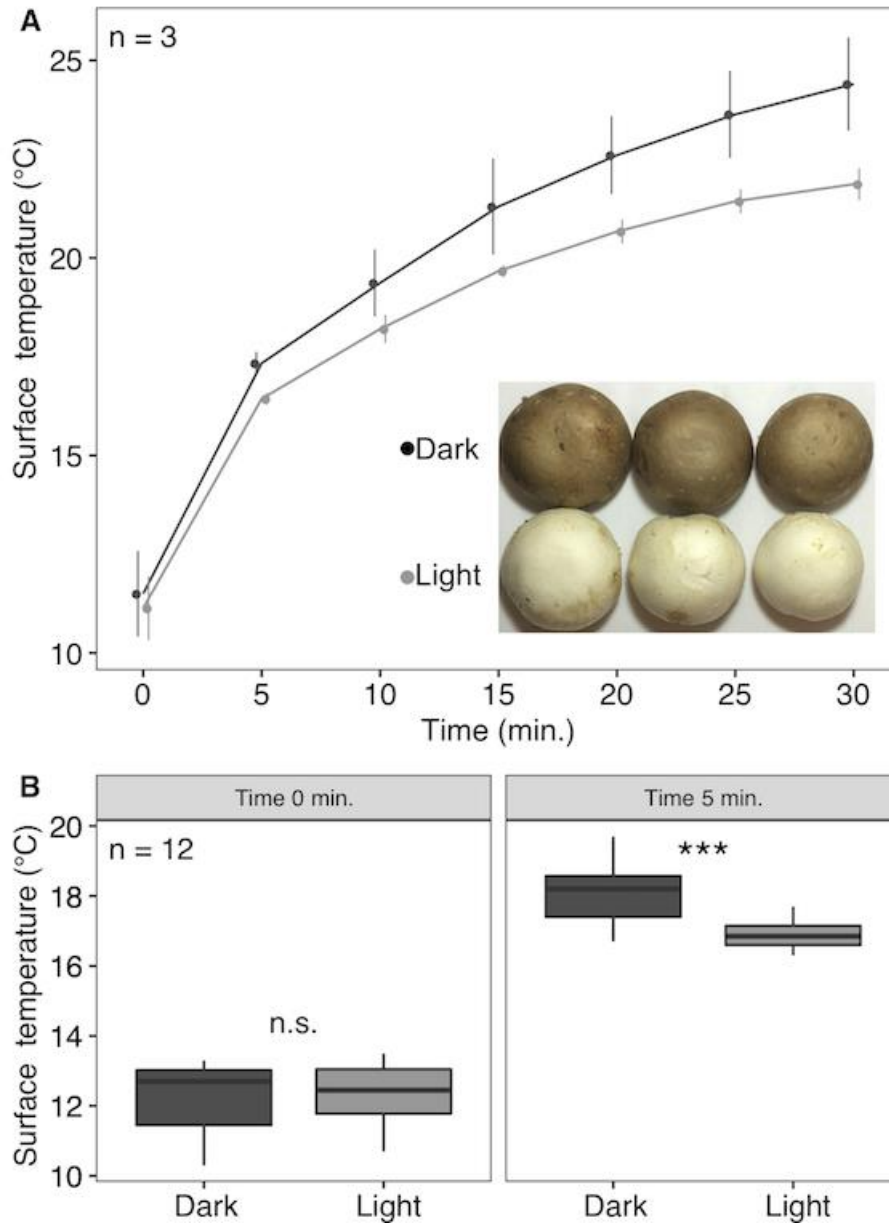
Supplementary Figure 11. Experimental effect of artificial radiation on mushroom cap surface temperature. (**A**) We first tested the temporal effect on mushroom cap surface temperature using 3 dark-colored and 3 light-colored caps of *Agaricus bisporus* individuals. We found that dark-colored mushrooms were warmer than light-colored mushrooms and that this difference increased over time. Error bars denote the upper and lower range of the standard deviation. (**B**) We then tested the warming difference based on 12 dark-and light-colored mushrooms and found significantly warmer mushroom caps of the dark variety after 5 minutes

(difference between means: 1.21 °C) (linear model: 0 min: t-value=0.214, p-value=0.833; 5 min: t-value=-4.23, p-value<0.001). Note finally that this result is consistent with studies demonstrating that dark-pigmented insects and yeasts heated up faster and reached higher temperatures than lighter individuals following irradiation [6]. Boxplots denote the median (horizontal line) and interquartile range (colored box); whiskers show three times the interquartile range. Source data are provided in Supplementary Data 8.
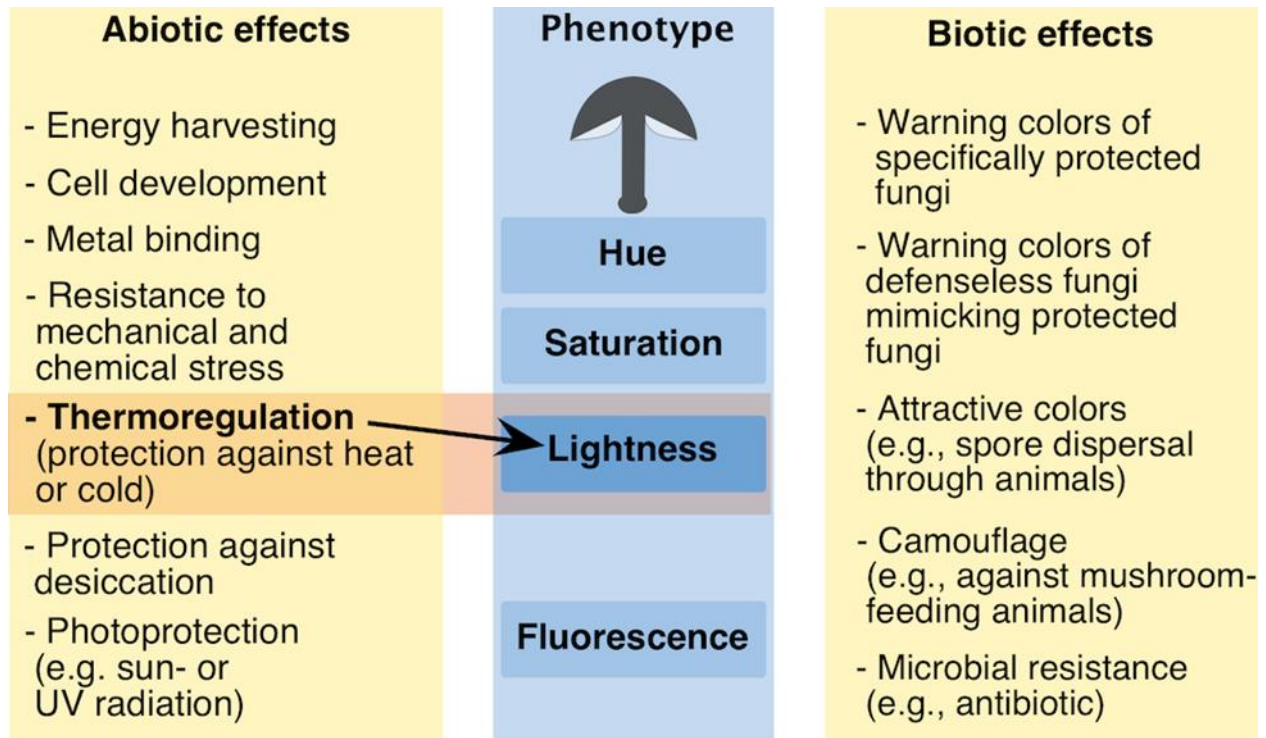
Supplementary Figure 12. Overview of possible abiotic and biotic effects on the mushroom color phenotype. Abiotic effects were adapted from Cordero *et al.* [7]; biotic effects from Caro [8]. The theory of "thermal melanism" is highlighted (see also Fig. 1).
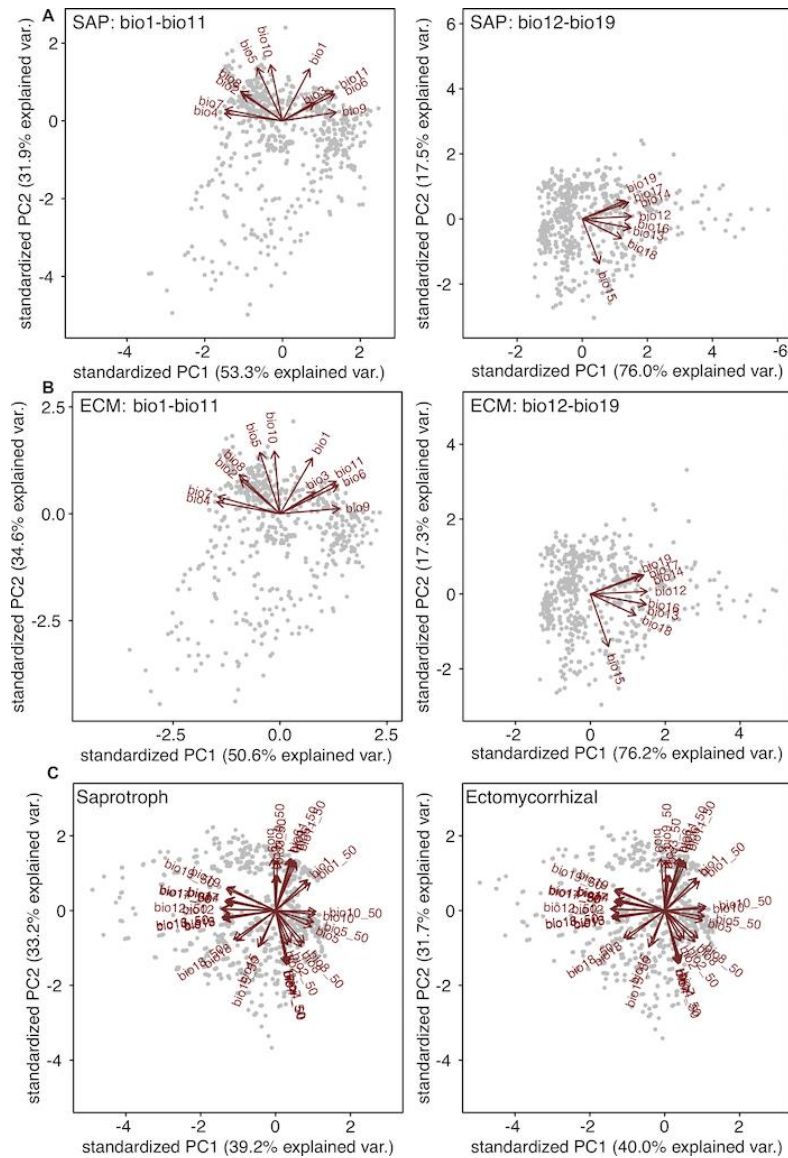
Supplementary Figure 13. Principal component analysis (PCA) for temperature and precipitation. PCAs were based on WorldClim variables for (**A**) saprotrophic (SAP) and (**B**) ectomycorrhizal fungi (ECM). The PCA for the temperature variables of SAP and ECM fungi displays overall mean values on axis 2 (e.g., bio1, mean annual temperature) and temperature variability on axis 1 (e.g., bio4, temperature seasonality). The PCA for the precipitation variables for SAP and ECM displays overall mean values on axis 1 (e.g., bio12, annual precipitation) and variability on axis 2 (e.g., bio 15, precipitation seasonality). (**C**) All WorldClim variables based

on 50 km × 50 km grids (denoted by the addition: '_50') and 10 km × 10 km subgrids occupied by fungi averaged based on 50 × 50 km grids. Displayed are standardized principal components with explained variances in brackets. Source data are provided in Supplementary Data 4.

Supplementary Table 1. Data overview. The total number of species in the European fungal dataset; the number of mushroom-forming species (mushroom = fruit body with cap and stipe, example: Fig. 2) with color data; and the number of mushroom-forming species with color and sequence data. Percent values refer to the "Mushroom-forming species" dataset. HSL = hue, saturation, and lightness.

	Full dataset	Mushroom-forming species	Mushroom-forming species & HSL data	DNA sequences available
Ectomycorrhizal fungi	1,989	1,770	1,401 (79%)	1,011 (57%)
Saprotrophic fungi	3,481	2,289	1,653 (72%)	1,046 (46%)
Other (parasitic, endophytic, unknown)	261	-	-	-
Total	5,831	4,059	**3,054 (75%)**	**2,057 (51%)**

Supplementary Table 2. Test for differences in color lightness between species of nutritional modes. Phylogenetic linear regressions based on different models of trait evolution based on the maximum likelihood phylogeny (2,057 species). BM, Brownian motion; OU, Ornstein-Uhlenbeck; Lambda, Pagel's lambda. We fitted the Lambda model with 100 alternative trees. Effects are presented as *t*-values (estimates divided by the respective standard error), significant effect sizes are highlighted in bold. Superscripts indicate rank of model selection based on AIC scores. SAP = Saprotroph; ECM = Ectomycorrhizal; ML = maximum likelihood. Source data are provided in Supplementary Data 1, 2 and 3.

	Lambda[1]						OUrandomRoot[2]		BM[3]	
	t-value			*p*-value			*t*-value	*p*-value	*t*-value	*p*-value
	ML	lower	upper	ML	lower	upper				
Intercept	**7.35**	**4.73**	**6.74**	**<0.001**	**<0.001**	**<0.001**	**132.55**	**<0.001**	0.13	0.894
SAP vs. ECM	**4.06**	**3.07**	**4.25**	**<0.001**	**<0.001**	**0.002**	**10.63**	**<0.001**	1.32	0.187

Supplementary Table 3. Test for differences in mushroom color lightness between nutritional modes. Linear mixed effects model to test for differences in assemblage-based mushroom color lightness between saprotrophic and ectomycorrhizal fungi. A random effect of grid cell was incorporated due to repeated temporal sampling of the nutritional modes within grid cells.

	t-value	p-value
Intercept	**822.04**	**<0.001**
Saprotroph vs Ectomycorrhizal	**126.39**	**<0.001**

Supplementary Table 4. Seasonality and mushroom color lightness on local scale. Here we use generalized additive models (GAMs) based on a standardized local scale data set (La Chanéaz). Effects of mushroom color lightness in response to month are given for saprotrophic and ectomycorrhizal fungi. Effects are presented as F-values (F) and significant effects are emboldened (p=p-value). The assemblage-based calculation was based on an abundance community matrix of mushroom-forming fungi (194 ectomycorrhizal and 121 saprotrophic species). Results (standardized effect sizes) based on three null models are shown.

		Mushroom color lightness (non-linear)		Standardized effect sizes Null models					
				Independent swap		Richness		Frequency	
		F	p	F	p	F	p	F	p
Saprotroph	Month	**2.97**	**0.031**	**2.94**	**0.033**	**2.84**	**0.037**	**3.31**	**0.018**
	Adj. R^2	0.08		0.07		0.07		0.07	
Ecto-mycorrhizal	Month	**6.75**	**0.010**	**6.56**	**<0.001**	**6.66**	**0.011**	**3.76**	**0.011**
	Adj. R^2	0.03		0.11		0.03		0.06	

Supplementary Table 5. Phylogenetic regression of the effect of thermal components on species lightness. Thermal components were averaged for each species occurring at least on 10 grid cells (793 ectomycorrhizal and 869 saprotrophic species). Besides testing the overall effect of the thermal components on species lightness, we furthermore tested the interaction of the species' nutritional mode (see 'Statistical analyses' in the Methods for details). Note that the thermal components are significantly positive correlated with species lightness and that there is no difference in the response of this relationship between the nutritional modes. BM, Brownian motion; OU, Ornstein-Uhlenbeck; Lambda, Pagel's lambda. We fitted the Lambda model based on 100 alternative trees. Effects are presented as *t*-values (estimates divided by the respective standard error), and significant effects are emboldened. Superscripts indicate rank of model selection based on AIC scores.

| | Lambda[1] | | | | | | OUrandomRoot[2] | | BM[3] | |
| | *t*-value | | | *p*-value | | | | | | |
	ML	lower	upper	ML	lower	upper	*t*-value	*p*-value	*t*-value	*p*-value
Intercept	**7.28**	**6.43**	**9.03**	**<0.001**	**<0.001**	**<0.001**	32.31	<0.001	0.14	0.892
Thermal component 1 (TC 1)	**2.60**	**2.25**	**2.58**	**0.009**	**0.010**	**0.025**	4.83	<0.001	4.01	<0.001
Thermal component 2 (TC 2)	**2.16**	**1.98**	**2.49**	**0.031**	**0.013**	**0.048**	1.74	0.081	4.21	<0.001
Nutritional mode: TC 1	-0.42	-0.46	-0.13	0.672	0.648	0.899	-0.30	0.767	4.23	<0.001
Nutritional mode: TC 2	0.85	0.81	1.18	0.397	0.236	0.420	1.87	0.061	0.50	0.619

Supplementary Table 6. Linear mixed effects model to test of differences in species lightness between saprotroph and ectomycorrhizal fungi and the effect of the thermal components. Phylogenetic regressions might provide incorrect degrees of freedom if clades share the same trait owing to common descent. According to Grafen [9], each radiation (e.g., radiation of ectomycorrhizal fungal clade) should be treated as an independent data point with one degree of freedom ('radiation principle'). Thus, in addition to the phylogenetic regressions (Supplementary Table 5), we also used a linear mixed model (LMM) that included "genus" clades as a random effect in the function *lmer* from the R package lmerTest ("+ (1|genus)"). We used the genus because we found increased phylogenetic signal mainly within genera (Fig. 5). (**A**) LMM to test for lightness differences between nutritional modes. (**B**) LMM to test for the effects of the thermal components (niche positions) on mushroom lightness. Degrees of freedom (DF) were calculated based on type III analysis of variance table with Satterthwaite's method (typically applied in mixed models [10]; here using function *anova*).

	DF	*t*-value	*p*-value
A)			
Intercept	92	**36.10**	**<0.001**
Nutritional mode – SAP vs. ECM		**3.75**	**<0.001**
B)			
Intercept	1049	**32.38**	**<0.001**
Thermal component 1 (TC 1)	1659	**1.98**	**0.048**
Thermal component 2 (TC 2)	1636	**2.00**	**0.045**
Nutritional mode: TC 1	1653	-0.45	0.652
Nutritional mode: TC 2	1642	0.81	0.417

Supplementary Table 7. Pairwise correlation coefficients (*r*) among environmental variables. Upper triangle (orange) for ectomycorrhizal fungi and lower triangle (blue) for saprotrophic fungi. (**A**) Correlation coefficients for the assemblage-based approach. Source data are provided in Supplementary Data 4. (**B**) Correlation coefficients for the cross-species approach (see 'Statistical analyses' in the Methods for details). Source data are provided in Supplementary Data 1, 2, and 3. Correlation coefficients above 0.6 or below −0.6 are emboldened.

	TC 1	TC 2	PC 1	PC 2	UV	RFC	Month
A)							
Thermal component 1 (TC 1)	-	0.22	–0.39	0.14	0.25	0.05	–0.01
Thermal component 2 (TC 2)	0.18	-	–0.04	**0.66**	0.55	0.50	–0.12
Precipitation component 1 (PC 1)	–0.40	–0.06	-	–0.05	0.43	-0.002	–0.04
Precipitation component 2 (PC 2)	0.10	**0.69**	–0.06	-	0.31	0.24	–0.06
UV	0.15	0.58	0.45	0.36	-	0.28	–0.10
Relative forest cover (RFC)	0.02	0.52	–0.02	0.29	0.30	-	–0.08
Month	–0.07	–0.07	0.05	–0.01	–0.10	–0.05	-
B)							
Thermal component 1 (TC 1)	-	0.25	**–0.62**	**–0.78**	0.06	–0.17	
Thermal component 2 (TC 2)	0.05	-	0.08	0.10	**0.73**	**0.62**	
Precipitation component 1 (PC 1)	**–0.63**	–0.02	-	0.51	0.52	0.48	
Precipitation component 2 (PC 2)	**–0.74**	0.27	0.36	-	0.04	0.23	
UV	0.02	**0.78**	0.38	0.10	-	**0.75**	
Relative forest cover (RFC)	–0.46	0.56	0.46	0.41	**0.60**	-	

Supplementary References

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