

Supplementary Information for

Seasonal shifts in bacterial and fungal microbiomes of leaves and associated leaf mining larvae reveal persistence of core taxa regardless of diet

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This file includes:

Figures S1 to S6

Tables S1, S2, S5, and S6

Other supplementary materials for this manuscript are uploaded separately, and include the following:

Figure S7 (hierarchical taxonomic composition of the leaf and larval bacterial microbiota throughout the season)

Figure S8 (hierarchical taxonomic composition of the leaf and larval fungal microbiota throughout the season)

Table S3 (contaminant bacterial and fungal ASVs)

Table S4 (bacterial and fungal taxa, the number of reads, and the variables entering the analyses)

Fig. S1. Map of **a)** Czechia showing the sampling locality at Central Moravia (Střeň in PLA Litovelské Pomoraví); **b)** sampling plots with individual trees. Map backgrounds were taken from the website FreeWorldMaps.net (<https://www.freeworldmaps.net/europe/czechia/czechia-physical-map.jpg>) and Mapy.cz (<https://mapy.cz/zakladni>).

a)



b)

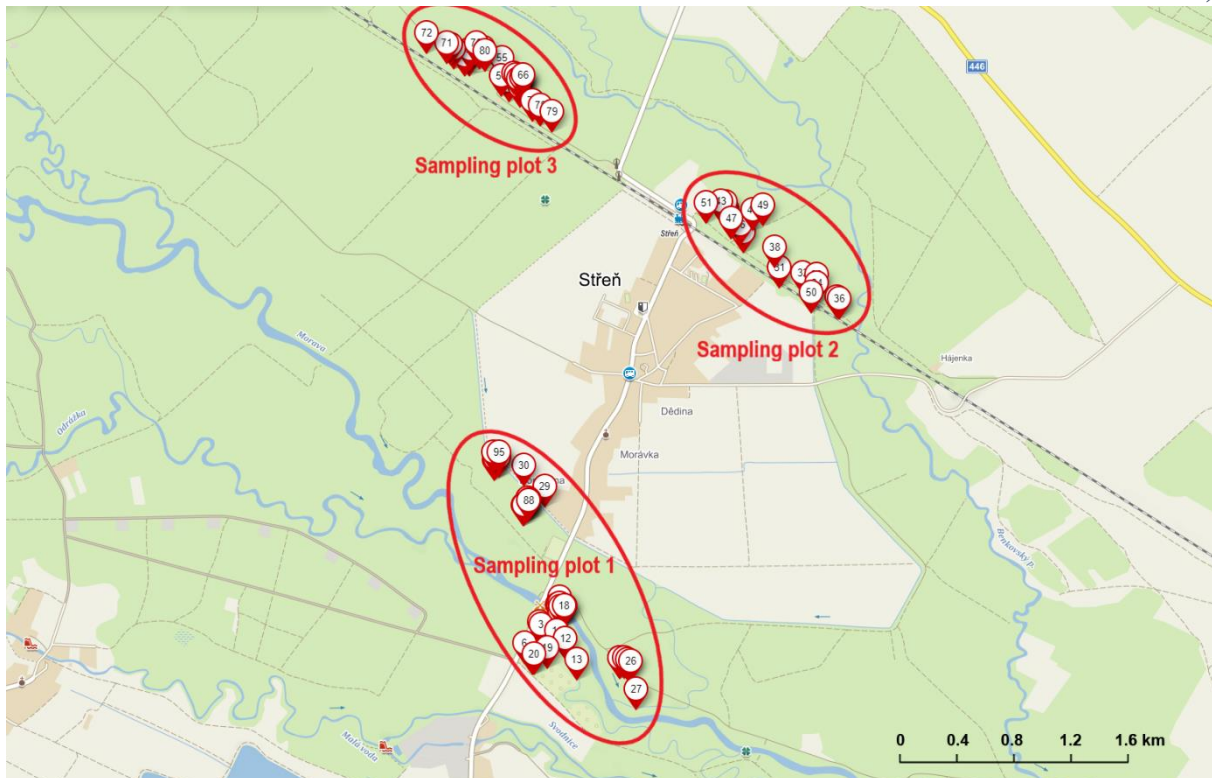


Table S1. Number of leaf-miners and host tree species sampled on individual sampling events.

Type	Order	Family	Species	Host tree	Sampling event				Total
					April	June	August	October	
M	Lepidoptera	G	<i>Phyllonorycter</i> sp.*			1	6	1	8
M	Lepidoptera	G	<i>Phyllonorycter coryli</i> (Nicelli, 1851)	<i>Corylus avellana</i>		10	9	8	27
M	Lepidoptera	G	<i>Phyllonorycter esperella</i> (Goeze, 1783)	<i>Carpinus betulus</i>		10	12	9	31
M	Lepidoptera	G	<i>Phyllonorycter heegeriella</i> (Zeller, 1846)	<i>Quercus robur</i> , <i>Q. petraea</i>		1		22	23
M	Lepidoptera	G	<i>Phyllonorycter klemannella</i> (Fabricius, 1781)	<i>Alnus glutionsa</i>			10	5	15
M	Lepidoptera	G	<i>Phyllonorycter nicellii</i> (Stainton, 1851)	<i>Corylus avellana</i>		9	9	9	27
M	Lepidoptera	G	<i>Phyllonorycter quercifoliella</i> (Zeller, 1839)	<i>Quercus robur</i> , <i>Q. petraea</i>		27	8	12	47
M	Lepidoptera	G	<i>Phyllonorycter rajella</i> (Linnaeus, 1758)	<i>Alnus glutionsa</i>		9	6	13	28
M	Lepidoptera	G	<i>Phyllonorycter roboris</i> (Zeller, 1839)	<i>Quercus robur</i> , <i>Q. petraea</i>		5	13		18
M	Lepidoptera	G	<i>Phyllonorycter stettinensis</i> (Nicelli, 1852)	<i>Alnus glutionsa</i>		9			9
M	Lepidoptera	G	<i>Phyllonorycter tenerella</i> (Joannis, 1915)	<i>Carpinus betulus</i>		8	6	9	23
M	Lepidoptera	Ti	<i>Tischeria ekebladella</i> (Bjerkander, 1795)	<i>Quercus robur</i> , <i>Q. petraea</i>		18	18	19	55
T	Fagales	B	<i>Alnus glutinosa</i> (L.) Gaertn.		18	19	19	19	75
T	Fagales	B	<i>Carpinus betulus</i> L.		18	18	18	18	72
T	Fagales	B	<i>Corylus avellana</i> L.		18	20	18	18	74
T	Fagales	F	<i>Quercus petraea</i> (Matt.) Liebl.		18	18	18	18	72
T	Fagales	F	<i>Quercus robur</i> L.		19	19	19	17	74
Total					91	201	189	197	678

Type: M – leaf-miner, T – tree, Family: G – Gracillariidae, Ti – Tischeriidae, B – Betulaceae, F – Fagaceae

*data for the individuals from *Quercus robur* sampled in low numbers (including *P. harrisella* and *P. muelleriella*) were merged and analyzed at the genus level

Table S2. List of taxonomic resources used for identification of leaf miners.

1. Laštůvka, Z. & Liška, J. (2010) Checklist of Lepidoptera of the Czech Republic (Insecta: Lepidoptera). URL <http://lepidoptera.wz.cz>
2. Patočka, J. (1980) Die Raupen und Puppen der Eichenschmetterlinge Mitteleuropas. Monographien zur angewandten Entomologie.
3. Ratnasingham, S. & Hebert, P. D. N. (2007) BOLD: The Barcode of Life Data System. *Molecular Ecology Notes* 7, 355–364. URL <http://barcodinglife.org>
4. De Prins, J. & De Prins, W. (2012) Global Taxonomic Database of Gracillariidae (Lepidoptera). URL <http://www.gracillariidae.net>
5. Ellis, W. N. (2007) Leafminers and plant galls of Europe, in, Zoölogisch Museum Amsterdam. URL <https://bladmineerders.nl>
6. Hering, E. M. (1957) Bestimmungstabellen der Blattminen von Europa einschliesslich des Mittelmeerbeckens und der Kanarischen Inseln., Uitgeverij Dr. W. Junk, s'Gravenhage.

Table S5. Explanatory variables (and their interactions) with significant effect on the composition and richness of leaf and larval microbiota. n.s. = the AIC values of their models were higher than of the null model. For leaf microbiomes, we tested the following explanatory variables: tree species, DBH, sampling height, irradiation of crown, sampling plot, and time point. For larval microbiomes, we tested all variables mentioned above plus larval species and family.

	Bacterial composition		Fungal composition	
	% of variability	statistics	% of variability	statistics
Leaves				
<i>Tree species</i>	13.80	df = 361, F = 17.84, P = 0.001***	29.39	df = 362, F = 47.81, P = 0.001***
<i>Time point</i>	13.33	df = 365, F = 68.92, P = 0.001***	5.13	df = 361, F = 33.38, P = 0.001***
<i>Irradiation</i>	0.78	df = 360, F = 4.03, P = 0.001***		n.s.
<i>Sampling plot</i>		n.s.	2.99	df = 359, F = 9.73, P = 0.001***
<i>Tree species*t. point</i>	3.26	df = 356, F = 4.22, P = 0.001***	3.90	df = 355, F = 6.34, P = 0.001***
<i>Tree species*s. plot</i>		n.s.	5.26	df = 347, F = 4.28, P = 0.001***
Larvae				
<i>Tree species</i>		n.s.	3.88	df = 305, F = 3.47, P = 0.001***
<i>Time point</i>	4.66	df = 298, F = 18.79, P = 0.001***	6.82	df = 309, F = 24.39, P = 0.001***
<i>Larval species</i>	17.64	df = 299, F = 6.46, P = 0.001***		n.s.
<i>Larval family</i>		n.s.	0.55	df = 304, F = 1.97, P = 0.008**
<i>Larval species*t. point</i>	6.23	df = 288, F = 2.51, P = 0.001***		n.s.
<i>Tree species*t. point</i>		n.s.	4.79	df = 300, F = 4.29, P = 0.001***
Bacterial richness				
Leaves				
<i>Tree species</i>		df = 362, F = 5.69, P < 0.001***		df = 362, F = 22.65, P < 0.001***
<i>Time point</i>		n.s.		df = 360, F = 22.75, P < 0.001***
<i>Sampling plot</i>		n.s.		df = 358, F = 3.62, P = 0.028*
<i>Tree species*t. point</i>		n.s.		df = 350, F = 3.23, P = 0.001**
Larvae				
<i>Time point</i>		df = 297, F = 17.76, P < 0.001***		df = 308, F = 140.18, P < 0.001***
<i>Larval species</i>		df = 299, F = 29.07, P < 0.001***		df = 297, F = 3.91, P < 0.001***
<i>Tree species</i>		n.s.		df = 295, F = 3.89, P = 0.021*

Fig. S2. PCoA plot based on p-RDA of **a)** bacterial (df = 360, F = 32.30, P = 0.001), and **b)** fungal community composition (df = 360, F = 19.50, P = 0.001) of leaves showing significant shifts as the season progressed. Leaves were sampled at four time points; April, June, August, and October, which corresponds to young, mature, senescent, and old leaves, respectively

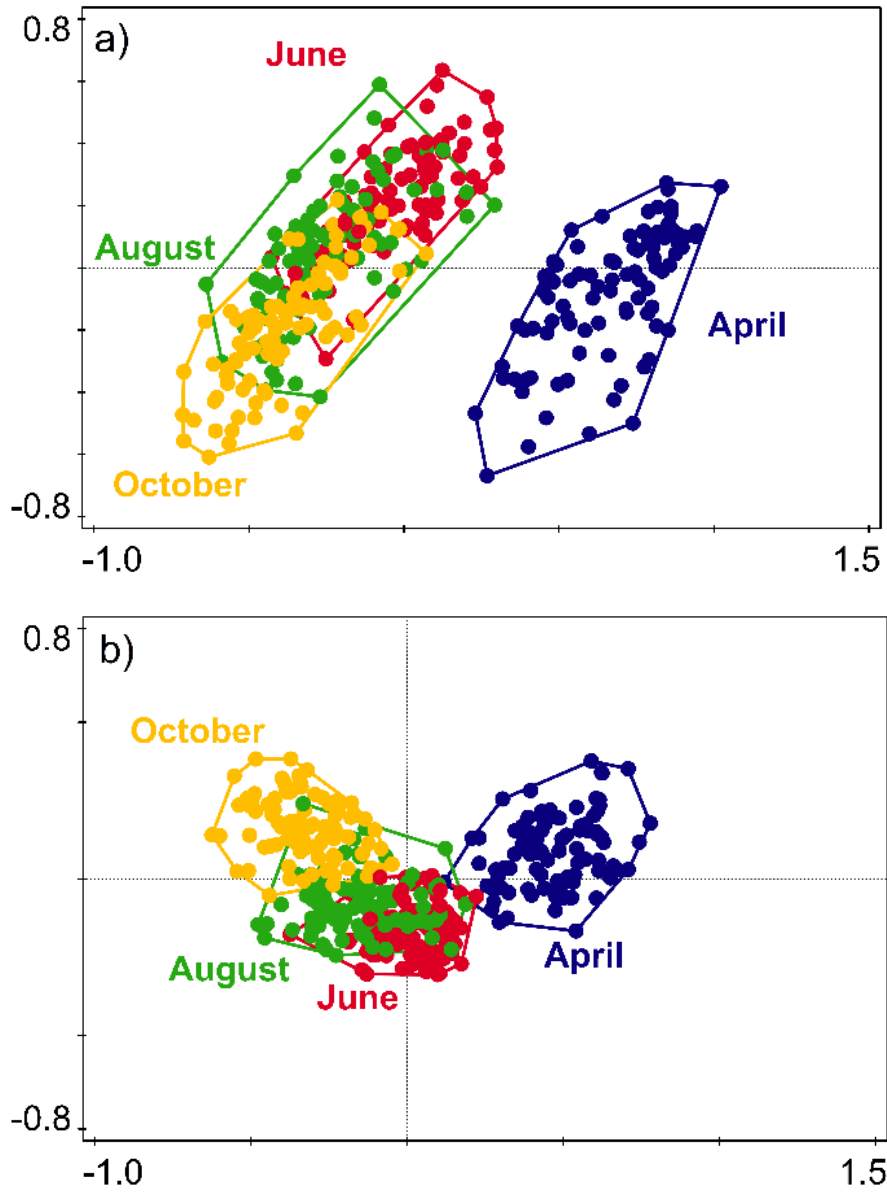


Fig. S3. Rarefied richness of leaf microbiota among tree species for **a)** bacteria (df = 362, F = 5.69, P < 0.001), **b)** fungi (df = 362, F = 22.65, P < 0.001) (mean \pm SE) as fitted by GLMs with Gamma distribution.

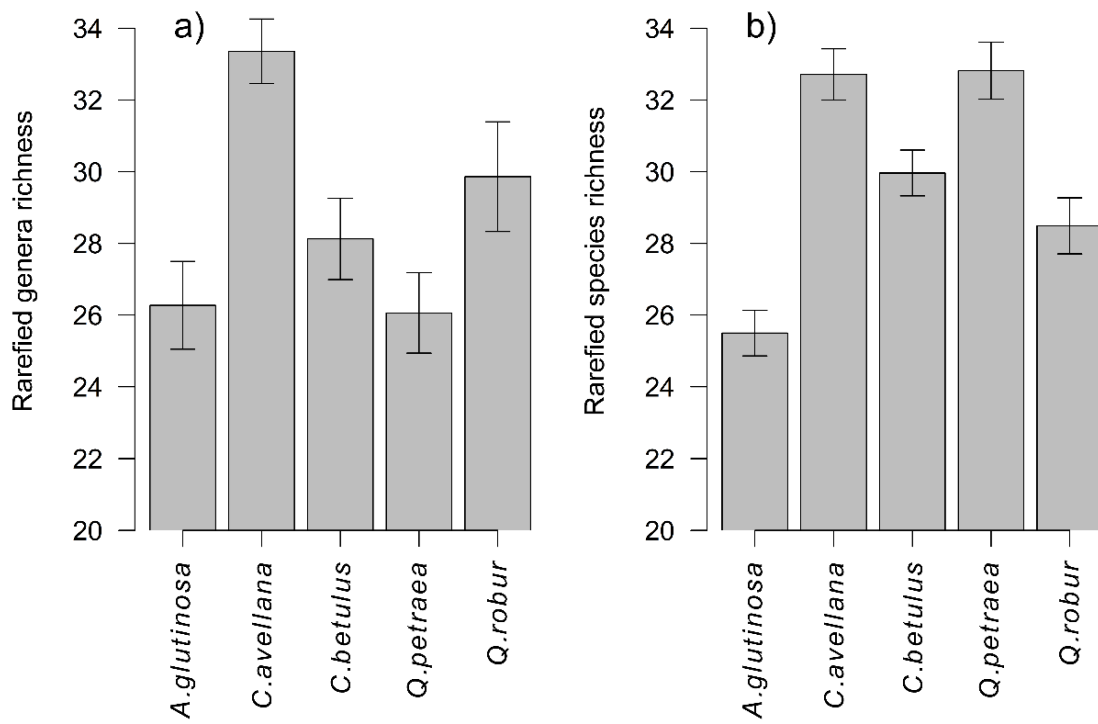


Fig. S4. PCoA showing shifts in composition of larval microbiota due to seasonal progression based on a) p-RDA for bacteria (df = 305, F = 9.90, P = 0.001) and b) p-CCA for fungi (df = 305, F = 20.00, P = 0.001)

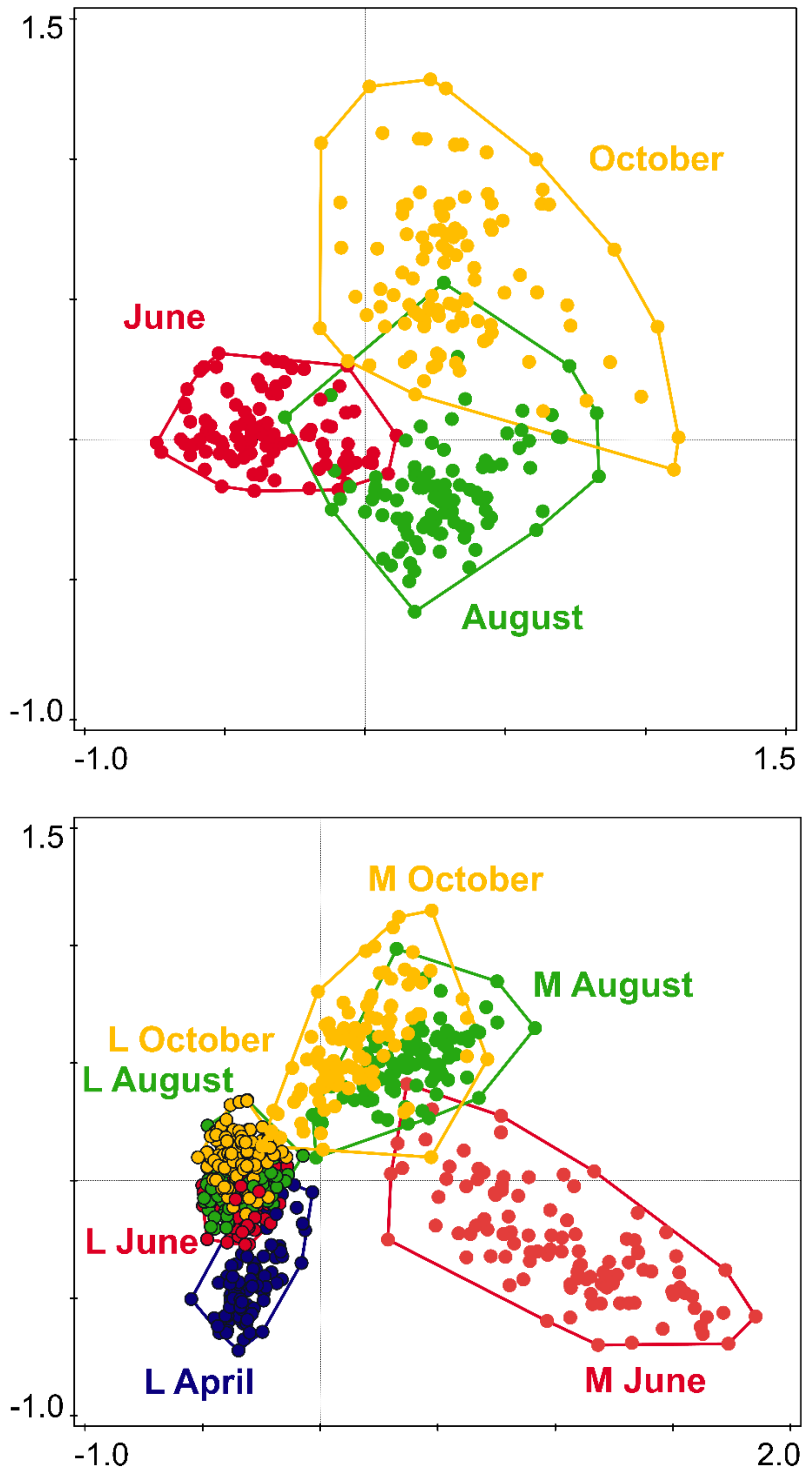


Fig. S5. The p-CCA showing differences in **a)** bacterial (df = 297, F = 2.30, P = 0.001), and **b)** fungal composition of larval microbiota (df = 297, F = 1.60, P = 0.001). Data for *Quercus petraea*, and *Q. robur* were merged for the purposes of this analysis

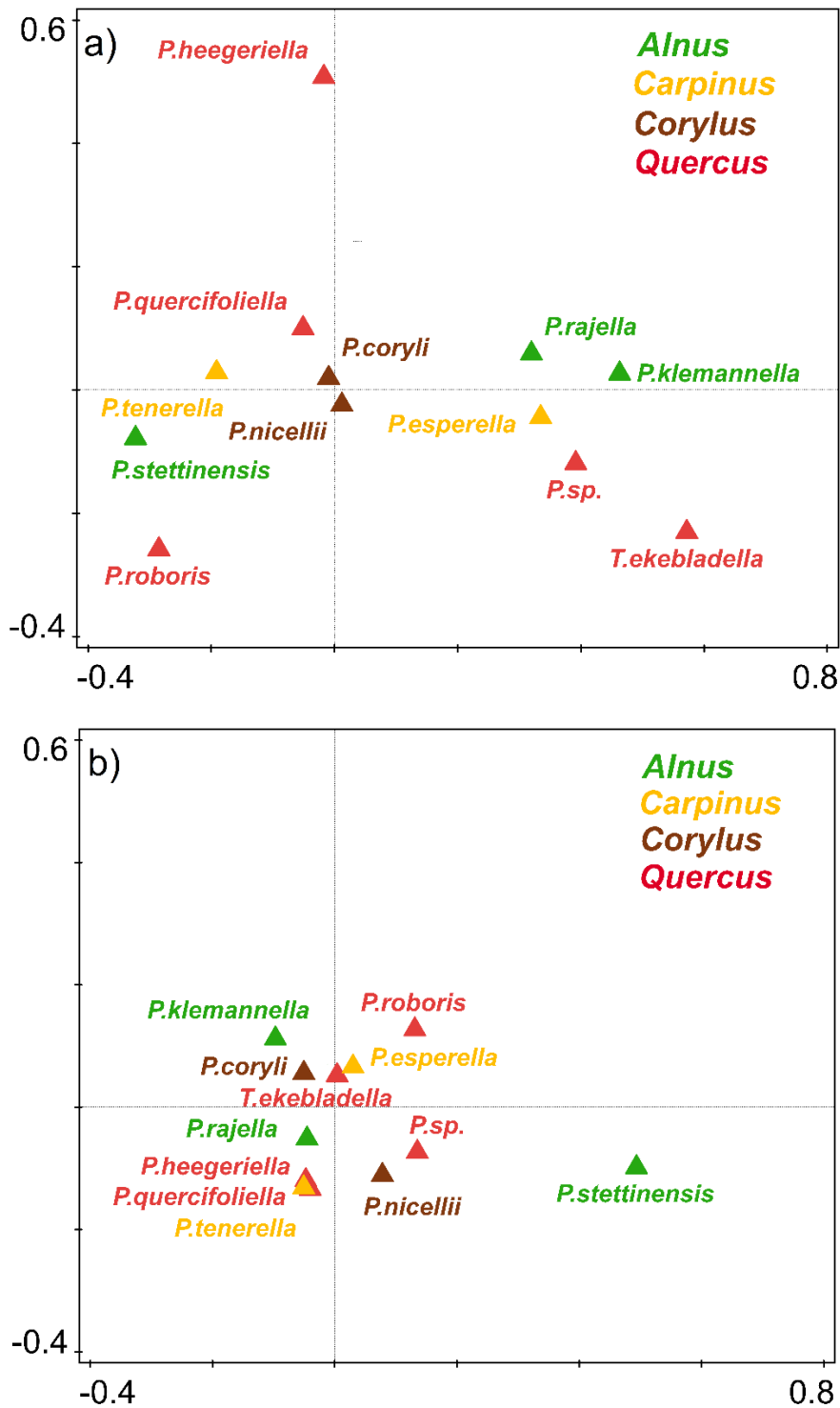


Fig. S6. Rarefied richness among larval species for **a)** bacteria (df = 299, F = 29.07, P < 0.001), **b)** fungi (df = 297, F = 3.91, P < 0.001) as fitted by GLM with Gamma distribution and LM, respectively

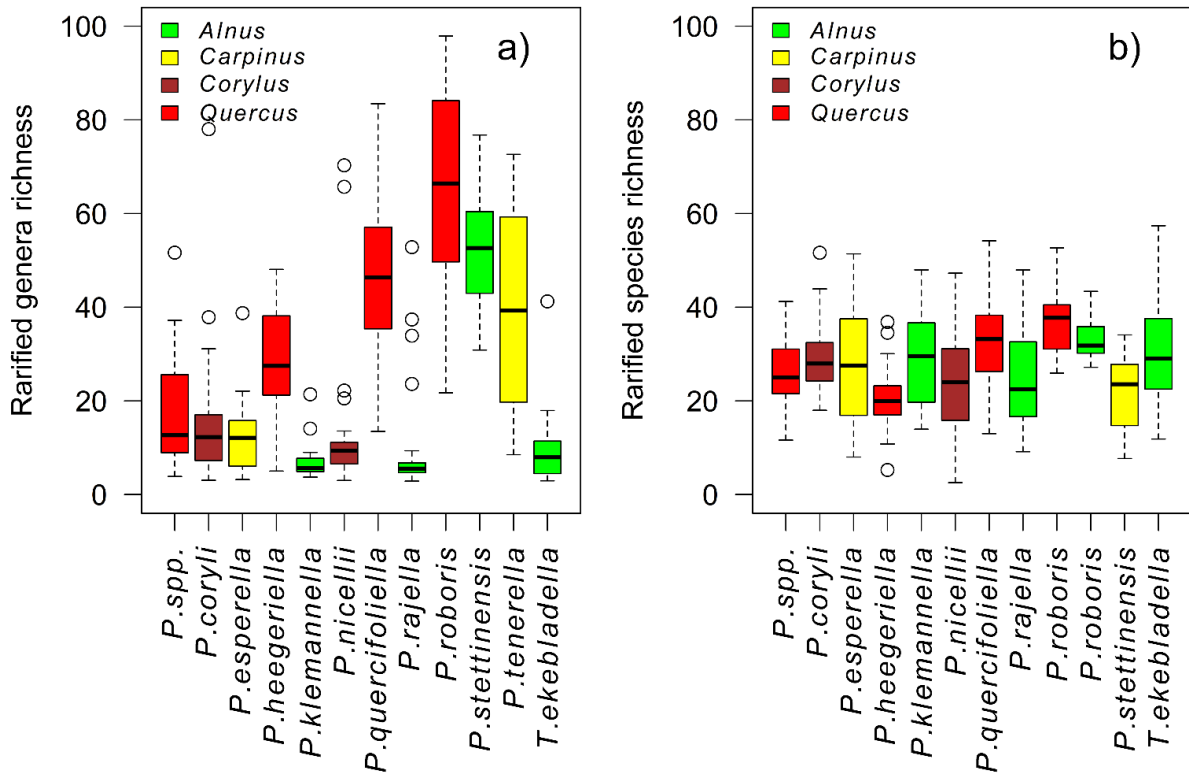


Table S6. Top ten bacterial genera and fungal species with identified seasonal trends. AIC_{Δ} = difference in AIC of model based simply on differences between leaf and larval samples, and model with added seasonal trend and its interaction with sample type, Sample Type P_{adj} = adjusted p-value from analysis of deviance of the model for differences between leaf and larval samples, Sample Time P_{adj} = adjusted p-value for simple seasonal trend, Interaction P_{adj} = adjusted p-value of interaction between those explanatory variables, Frequency in dataset = Number of samples with presence of given bacteria/fungi, only samples with frequency ≥ 100 were used in the analysis. Taxa occurring in larvae more frequently than predicted by null models are highlighted.

Bacterial taxon	AIC_{Δ}	Sample Type P_{adj}	Sample Time P_{adj}	Interaction P_{adj}	Frequency in dataset	Prevalence environment	Potential function	Reference(s)*
<i>Bacillus</i>	250.54	<0.001	0.200	<0.001	391	leaf/larva	plants: antifungal activity, promotion of plant growth and health; larvae: proteolytic activity counteracting plant protease inhibitors	Bao et al. 2020, Nair et al. 2002, Perissol et al. 1993
<i>Staphylococcus</i>	173.60	<0.001	<0.001	<0.001	354	larva	plants: alleviation of biotic stresses; larvae: protease activity against plant secondary metabolites	Araya et al. 2020, Visôto et al. 2009
<i>Cutibacterium</i>	155.71	<0.001	<0.001	0.280	417	larva	common core member of arthropod guts, digestive processes in vertebrate guts (carbohydrate fermentation)	Kumar et al. 2020, Koskinioti et al. 2019, Papadopoulos et al. 2020, Perry & Lambert 2006, Wang et al. 2011
<i>Erwinia</i>	134.20	<0.001	<0.001	<0.001	155	leaf	common colonist of leaves	Rastogi et al. 2012
<i>Deinococcus</i>	128.07	<0.001	<0.001	<0.001	246	leaf	promoting plant resistance to environmental stressors (desiccation, pollution, UV radiation, etc.)	Bauermeister et al. 2011, Waight et al. 2007, Díaz-Riaño et al. 2019
P3OB-42	125.85	<0.001	<0.001	<0.001	367	leaf	suppression of phytopathogens	Dobrovol'skaya et al. 2016
<i>Acinetobacter</i>	114.08	<0.001	<0.001	0.228	356	larva	plants: denitrification and organic pollutant degradation; larvae: metabolizing of toxic phenolic glycosides	Bao et al. 2020, Mason et al. 2016
<i>Methylobacterium/Methylorubrum</i>	99.39	<0.001	0.317	<0.001	501	leaf/larva	plants: plant-growth promotion, pathogen resistance; larvae: synthesis of amino acids, production of digestive enzymes and vitamins	Senthilkumar et al. 2015, Ardanov et al. 2012, Jayashree et al. 2011, Martins et al. 2019
<i>Curtobacterium</i>	88.61	<0.001	<0.001	0.044	250	leaf	plant pathogen	Hsieh et al. 2005
<i>Streptococcus</i>	88.50	<0.001	<0.001	0.001	223	larva	degradation of plant secondary metabolites	Osawa et al. 1990, Visôto et al. 2009
Fungal taxon	AIC_{Δ}	Sample Type P_{adj}	Sample Time P_{adj}	Interaction P_{adj}	Frequency in dataset	Prevalence environment	Potential function	Reference (s)*
<i>Neosascochyta europaea</i>	226.31	<0.001	<0.001	<0.001	154	larva	synthesis of amino acids, oxidative processes	Heisel et al. 2017
<i>Ampelomyces quisqualis</i>	132.18	<0.001	<0.001	0.001	283	larva	suppression of phytopathogens	Falk et al. 1995

<i>Curvibasidium cygneicollum</i>	115.27	<0.001	<0.001	<0.001	185	leaf/larva	plant pathogen	Robert et al. 2019
<i>Botrytis cinerea</i>	106.06	0.999	<0.001	1.000	133	leaf/larva	plant pathogen, regulation of lepidopteran life cycle via sterol synthesis	Elad et al. 2016, Rizvi & Raman 2015
<i>Debaryomyces prosopidis</i>	101.68	<0.001	<0.001	0.685	123	larva	nutrition (carbohydrate assimilation)	Wrent et al. 2014
<i>Ceramothyrium carniolicum</i>	80.49	<0.001	<0.001	<0.001	270	larva	plant pathogen	Nguyen et al. 2019
<i>Mycosphaerelloides madeirae</i>	78.91	<0.001	<0.001	<0.001	102	larva	plant pathogen	Crous et al. 2004
<i>Exobasidium arescens</i>	69.12	<0.001	<0.001	<0.001	104	leaf	plant pathogen	Bergerow et al. 2002
<i>Seimatosporium quercinum</i>	57.32	<0.001	<0.001	<0.001	109	leaf	common colonist of oak twigs	Goonasekara et al. 2016
<i>Candida sake</i>	56.51	<0.001	<0.001	1.000	229	larva	pathogen suppression, nutrition	Malassigné et al. 2021, Usall et al. 2000, Morales et al. 2008

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