frontiers

Supplementary Material

A poisoned apple: First insights into community assembly and networks of the fungal pathobiome of healthy-looking senescing leaves of temperate trees in mixed forest ecosystem.

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Physiochemical analyses

To obtain water-leachable components, senescing leaf and needle samples were shaken in 30 mL milliQ water for 1 h in falcon tubes, centrifuged for 5 min at 3500 rpm, decanted, and filtered. The remaining leaf/needle material was dried for two weeks at 40 °C to determine dry weight, which was used as reference for all subsequent qualifications. Leachate pH was determined using pH paper with a scale precision of 0.2 units. TN was analyzed using a sum parameter analyzer with high temperature combustion and chemiluminescence detection (Mitsubishi TN-100; a1 environsciences, Düsseldorf, Germany). All samples were measured as triplicates. N_{org} was calculated as the difference: $N_{org} = TN – N_{Min}$. For N_{Min} quantification, a flow injection analyzer (Quikchem QC85S5; Lachat Instruments, Hach Company, Loveland CO, USA) was used with corresponding manifolds to measure ammonium nitrogen $N_{NH_4^+}$, nitrite nitrogen $N_{NO_2^-}$, and nitrate- plus nitrite nitrogen $N_{NO_3^- + NO_2^-}$ content. $N_{NH_4^+}$ was determined by the gas diffusion method. $N_{NO_3^-}$ was reduced to nitrite using a cadmium column in the manifold prior to the chemical reaction to form an azo dye. The nitrate reduced by cadmium and the nitrite originally present in the sample were analyzed using the Griess reaction by diazotization with sulfanilamide and coupling with N-(1-naphthyl) ethylenediamine dihydrochloride. The deep pink color of the resulting dye was measured at $\lambda = 520$ nm. $N_{NO_2^-}$ alone was determined after the same reaction, without using a cadmium column. DOC was quantified as non-purgeable organic carbon (NPOC) with a sum parameter analyzer using high-temperature combustion and infrared detection (vario TOC cube, Elementar Analysensysteme GmbH, Langenselbold, Germany). Each sample was measured as triplicate. A sample volume of 200 µL each was automatically injected into the ash finger of the combustion tube which contains platinum as catalyst. The samples were combusted at 850°C in synthetic air, a hydrocarbon-free mixture of nitrogen and oxygen. After removing moisture from the

combustion gas, NPOC was quantified by IR detection of CO_2 formed from the organic carbon compounds in the sample.

The determination of nutrient content, Ca, Fe, K, Mg, and P of leaves and needles followed two processes. First, the sample digestion, in which 100 mg of sample material were submitted to a microwave-assisted high-pressure digestion (Multiwave 3000, Anton Paar, Graz, Austria) at a maximum microwave power of 1200 W and a maximum pressure of 60 bar after addition of 3 – 5 mL 65% HNO_3, supra-pur, (Merck, Darmstadt, Germany). A rotor 8SXF100 with reaction vessels made of TFM (tetrafluor-modified polytetrafluoroethylene) was used. Overall digestion time was 50 min, including 20 min of cooling at zero microwave power. A blank, consisting of nitric acid only was run to check for possible contamination of reagents and vessels. After accomplishment of digestion, the solutions were filtered and transferred to 50 mL PE vessels which were filled to the mark with ultrapure water (Millipore, Eschborn, Germany). Secondly, the sample solution analyses were carried out using inductively coupled plasma–optical emission spectrometry (ICP-OES) "Arcos" (Spectro, Kleve, Germany) equipped with a 27.12 MHz free-running LDMOS generator and ORCA optical system. A three-point-calibration based on single-element standards issued by Merck, Darmstadt, Germany, was carried out at the following concentration levels: 10, 50, 100 mg/L for Ca, K, Mg, P and 0.5, 2.5 and 5 mg/L for Fe, respectively.

R scripts for statistical analyses

Specialist/generalist classification

```
setwd("C:\\Initial_leaves_fungi")

install.packages("vegan")

install.packages("spaa")
```

```
install.packages("devtools")

devtools::install_github("GuillemSalazar/EcolUtils")

library(EcolUtils)

library(RCurl)

comm.tab<-
read.table("Pathogen_spe_gen.txt",text=x,sep="\t",row.names=1,header=TRUE,comment.char="@")

comm.tab<-t(comm.tab[,1:60])

comm.tab<-comm.tab[,which(colSums(comm.tab)>0)]

res<-spec.gen(comm.tab,n=100)

comm.tab.bin<-ceiling(comm.tab/max(comm.tab))

colors <- c("dodgerblue4", "gray47", "orangered3")

plot(colSums(comm.tab),colSums(comm.tab.bin)/dim(comm.tab.bin)[1], family = "ArialMT" ,col=
colors[res$sign], pch=19,log="x",xlab="Abundance",ylab="Occurrence", cex.lab = 1.5, cex.axis = 1,
cex = 1)

legend("topleft",levels(res$sign), col=colors,pch=19,inset=0.01,cex=1.5)

install.packages("xlsx")

library("xlsx")

write.xlsx(res, file = "Pathogen_spec_gen.xlsx", sheetName = "Specialist_Generalist",
```

col.names = TRUE, row.names = TRUE, append = FALSE)

Goodness-of-fit statistic and variance partitioning analyses

```
setwd("C:\\Initial_leaves_fungi")

library(vegan)

FUNA=read.csv("PP_presence_absence_factors_C.csv", header=T)

fix(FUNA)

row.names(FUNA)=FUNA$Plant_sample

speciesf=  FUNA[,21 :420]

environmentf=FUNA[,2:20]

fix(speciesf)

fix(environmentf)

FUNA.nmds<-metaMDS( speciesf ,distance="jaccard", k=2, autotransform=FALSE,trace = FALSE)

FUNA.nmds

plot(FUNA.nmds)

fit_FUNA<-envfit(FUNA.nmds, environmentf, perm=999,na.rm = TRUE)

fit_FUNA

modall <- varpart(speciesf, ~ TreeID, ~ TOC + Norg + K + Mg + P , ~ pH + Water_content, ~Lat +
Long,  data=environmentf)

modall

plot(modall)
```

Table S1. Average relative abundance of fungal plant pathogens detected in senescing leaves and needles of 12 temperate tree species (please see in a separate excel file).

Table S2. Comparisons between fungal plant pathogenic community compositions associated with 12 temperate tree species using analysis of similarities (ANOSIM) and non-parametric multivariate analysis of variance (NPMANOVA) based on (a) relative abundance data and the Bray-Curtis distance measure and (b) presence/absence data and the Jaccard distance measure.

(a) ANOSIM and NPMANOVA based on relative abundance data and the Bray-Curtis distance measure.

Comparison	NPMANOVA	ANOSIM
A. pseudoplatanus vs. F. sylvatica	4.49**	0.54**
A. pseudoplatanus vs. P. menziesii	4.63**	0.99**
A. pseudoplatanus vs. Q. robur	4.42**	0.94**
A. pseudoplatanus vs. F. excelsior	3.31*	0.74*
A. pseudoplatanus vs. P. abies	5.50**	1.00**
A. pseudoplatanus vs. C. betulus	5.81**	0.59**
A. pseudoplatanus vs. P. avium	1.78*	0.28*
A. pseudoplatanus vs. P. sylvestris	7.75**	1.00**
A. pseudoplatanus vs. L. decidua	9.43**	0.98**
A. pseudoplatanus vs. T. cordata	4.11**	0.65**
A. pseudoplatanus vs. P. Hybrid	9.29**	1.00**
F. sylvatica vs. P. menziesii	10.49**	1.00**
F. sylvatica vs. Q. robur	8.89**	0.85**
F. sylvatica vs. F. excelsior	8.77**	0.88**
F. sylvatica vs. P. abies	12.93**	1.00**
F. sylvatica vs. C. betulus	16.63**	0.99**
F. sylvatica vs. P. avium	5.07**	0.60**
F. sylvatica vs. P. sylvestris	20.97**	1.00**
F. sylvatica vs. L. decidua	34.76**	1.00**
F. sylvatica vs. T. cordata	9.77**	0.82**
F. sylvatica vs. P. Hybrid	29.93**	1.00*
P. menziesii vs. Q. robur	5.32**	1.00**
P. menziesii vs. F. excelsior	4.84**	1.00**
P. menziesii vs. P. abies	4.36**	0.94**
P. menziesii vs. C. betulus	11.10**	1.00**

Comparison	NPMANOVA	ANOSIM
P. menziesii vs. P. avium	3.78**	0.93**
P. menziesii vs. P. sylvestris	7.17**	0.98**
P. menziesii vs. L. decidua	10.22**	1.00**
P. menziesii vs. T. cordata	7.64**	1.00**
P. menziesii vs. P. Hybrid	10.28**	1.00**
Q. robur vs. F. excelsior	4.42**	0.95**
Q. robur vs. P. abies	6.31**	1.00**
Q. robur vs. C. betulus	10.70**	0.94**
Q. robur vs. P. avium	3.46**	0.80**
Q. robur vs. P. sylvestris	8.98**	1.00**
Q. robur vs. L. decidua	11.52**	1.00**
Q. robur vs. T. cordata	7.26**	1.00**
Q. robur vs. P. Hybrid	10.78**	1.00**
F. excelsior vs. P. abies	5.58**	1.00**
F. excelsior vs. C. betulus	8.38**	0.83**
F. excelsior vs. P. avium	2.34*	0.52**
F. excelsior vs. P. sylvestris	8.04**	1.00**
F. excelsior vs. L. decidua	9.88**	0.99**
F. excelsior vs. T. cordata	5.08**	0.78**
F. excelsior vs. P. Hybrid	9.36**	1.00**
P. abies vs. C. betulus	13.71**	1.00**
P. abies vs. P. avium	4.26**	0.93**
P. abies vs. P. sylvestris	7.84**	1.00**
P. abies vs. L. decidua	12.03**	1.00**
P. abies vs. T. cordata	9.07**	1.00**
P. abies vs. P. Hybrid	12.00**	1.00**
C. betulus vs. P. avium	5.51**	0.58*
C. betulus vs. P. sylvestris	23.22**	1.00**
C. betulus vs. L. decidua	39.78**	1.00**
C. betulus vs. T. cordata	12.28**	0.92**
C. betulus vs. P. Hybrid	33.98**	1.00**
P. avium vs. P. sylvestris	6.58**	1.00**
P. avium vs. L. decidua	7.90**	0.96**
P. avium vs. T. cordata	3.98**	0.61**
P. avium vs. P. Hybrid	7.87**	1.00**
P. sylvestris vs. L. decidua	18.65**	1.00**
P. sylvestris vs. T. cordata	13.69**	1.00**
P. sylvestris vs. P. Hybrid	18.63**	1.00**
L. decidua vs. T. cordata	19.25*	1.00**
L. decidua vs. P. Hybrid	30.73**	1.00**
T. cordata vs. P. Hybrid	17.11**	1.00**

(b) ANOSIM and NPMANOVA based on presence/absence data and the Jaccard distance measure.

Comparison	NPMANOVA	ANOSIM
A. pseudoplatanus vs. F. sylvatica	2.90**	0.74**
A. pseudoplatanus vs. P. menziesii	3.87**	1.00**
A. pseudoplatanus vs. Q. robur	2.17**	0.67**
A. pseudoplatanus vs. F. excelsior	2.65**	0.82**
A. pseudoplatanus vs. P. abies	6.08**	1.00**
A. pseudoplatanus vs. C. betulus	3.12**	0.70**
A. pseudoplatanus vs. P. avium	2.13**	0.53**
A. pseudoplatanus vs. P. sylvestris	6.89**	1.00**
A. pseudoplatanus vs. L. decidua	4.20**	1.00**
A. pseudoplatanus vs. T. cordata	2.11**	0.62**
A. pseudoplatanus vs. P. Hybrid	5.30**	1.00**
F. sylvatica vs. P. menziesii	4.95**	1.00**
F. sylvatica vs. Q. robur	3.19**	0.84**
F. sylvatica vs. F. excelsior	4.50**	1.00**
F. sylvatica vs. P. abies	8.32**	1.00**
F. sylvatica vs. C. betulus	3.86**	0.90**
F. sylvatica vs. P. avium	3.40**	0.80**
F. sylvatica vs. P. sylvestris	9.94**	1.00**
F. sylvatica vs. L. decidua	5.93**	1.00**
F. sylvatica vs. T. cordata	2.91**	0.82**
F. sylvatica vs. P. Hybrid	7.73**	1.00**
P. menziesii vs. Q. robur	4.07**	1.00**
P. menziesii vs. F. excelsior	4.66**	1.00**
P. menziesii vs. P. abies	3.92**	0.97**
P. menziesii vs. C. betulus	5.51**	1.00**
P. menziesii vs. P. avium	4.11**	1.00**
P. menziesii vs. P. sylvestris	5.21**	1.00**
P. menziesii vs. L. decidua	3.36**	0.97**
P. menziesii vs. T. cordata	4.36**	1.00**
P. menziesii vs. P. Hybrid	6.14**	1.00**
Q. robur vs. F. excelsior	2.38**	0.72**
Q. robur vs. P. abies	6.16**	1.00**
Q. robur vs. C. betulus	3.03**	0.72**
Q. robur vs. P. avium	1.77**	0.34**
Q. robur vs. P. sylvestris	7.23**	1.00**
Q. robur vs. L. decidua	4.61**	1.00**
Q. robur vs. T. cordata	2.09**	0.61**
Q. robur vs. P. Hybrid	5.47**	1.00**
F. excelsior vs. P. abies	6.75**	1.00**
F. excelsior vs. C. betulus	4.43**	0.91**

Comparison	NPMANOVA	ANOSIM
F. excelsior vs. P. avium	2.39**	0.60**
F. excelsior vs. P. sylvestris	7.87**	1.00**
F. excelsior vs. L. decidua	5.43**	1.00**
F. excelsior vs. T. cordata	2.37**	0.68**
F. excelsior vs. P. Hybrid	5.75**	0.99**
P. abies vs. C. betulus	9.00**	1.00**
P. abies vs. P. avium	6.43**	1.00**
P. abies vs. P. sylvestris	5.93**	1.00**
P. abies vs. L. decidua	5.35**	1.00**
P. abies vs. T. cordata	6.82**	1.00*
P. abies vs. P. Hybrid	8.61**	1.00**
C. betulus vs. P. avium	3.59**	0.78**
C. betulus vs. P. sylvestris	11.05**	1.00**
C. betulus vs. L. decidua	6.47**	1.00**
C. betulus vs. T. cordata	2.87**	0.71**
C. betulus vs. P. Hybrid	8.28**	1.00**
P. avium vs. P. sylvestris	7.26**	1.00**
P. avium vs. L. decidua	4.69**	1.00**
P. avium vs. T. cordata	2.01**	0.44**
P. avium vs. P. Hybrid	5.34**	0.99**
P. sylvestris vs. L. decidua	6.27**	1.00**
P. sylvestris vs. T. cordata	7.98**	1.00**
P. sylvestris vs. P. Hybrid	9.87**	1.00**
L. decidua vs. T. cordata	5.13*	1.00**
L. decidua vs. P. Hybrid	7.09**	1.00**
T. cordata vs. P. Hybrid	5.71**	0.97**

Table S3 Topological characteristics of the ecological networks of the plant pathogen in broadleaved and coniferous tree species.

Network features		Broadleaved tree species	Coniferous tree species
Empirical network	Number of nodes	96	103
	Number of links	286	498
	R^2 of power-law	0.46	0.29
	Number of positive correlations	70 (24.5%)	182 (36.5%)
	Number of negative correlations	216 (75.5%)	316 (63.5%)
	Average degree (avgK)	5.96	9.67
	Average clustering coefficient (avgCC)	0.29	0.22
	Average path distance (GD)	3.23	2.46
	Modularity	0.45	0.33
Random network	avgCC ± SD	0.16 ± 0.02	0.13 ± 0.01
	GD ± SD	2.74 ± 0.05	2.33 ± 0.02
	Modularity ± SD	0.32 ± 0.01	0.24 ± 0.01

Table S4 Latitude and longitude of each tree replicate (please see in a separate excel file).

Figure S1 NMDS ordinations of plant pathogenic community compositions based on presence/absence data and Jaccard distance similarity.

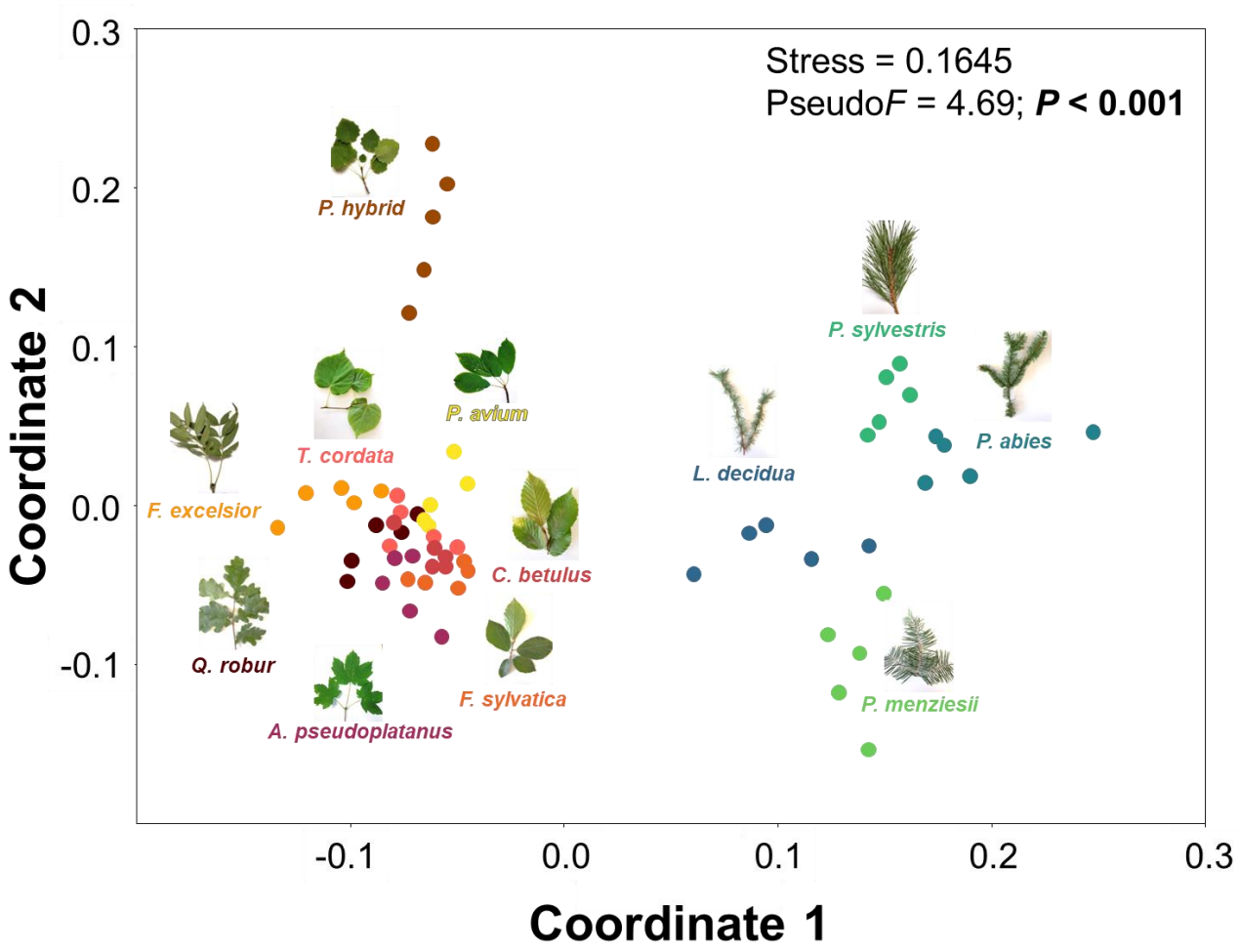

Figure S2 Network between tree species and plant pathogenic fungal ASVs. Large circles indicate each tree species. Tree species name can be found at the outer layer of the network cycle. are Smaller circles in light purple color refer to plant pathogenic fungal ASVs.

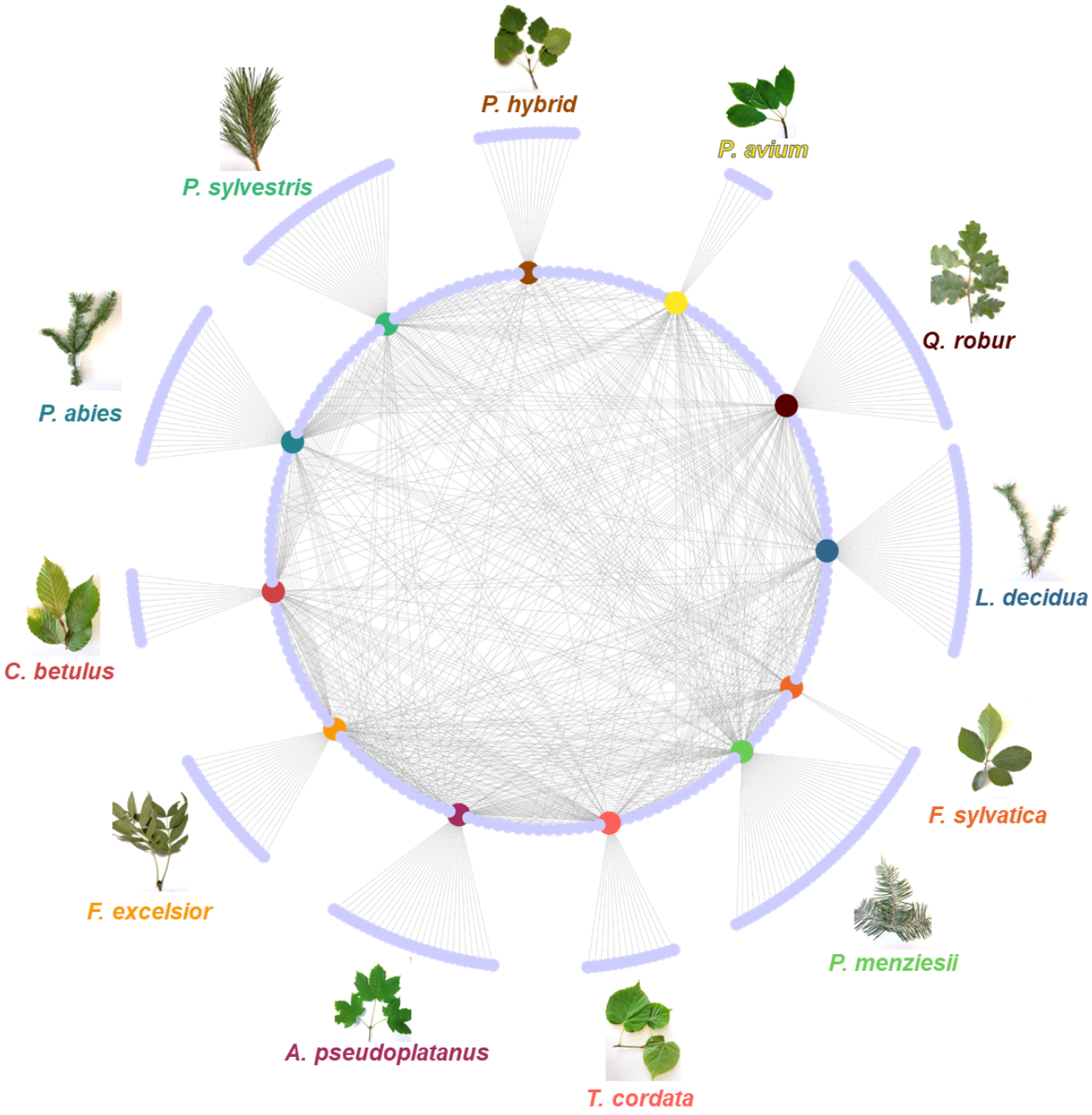

Figure S3. The taxonomic normalized stochasticity ratio (tNST) (A) and ecological stochasticity (B) in broadleaf and conifer trees. *, $P<0.05$

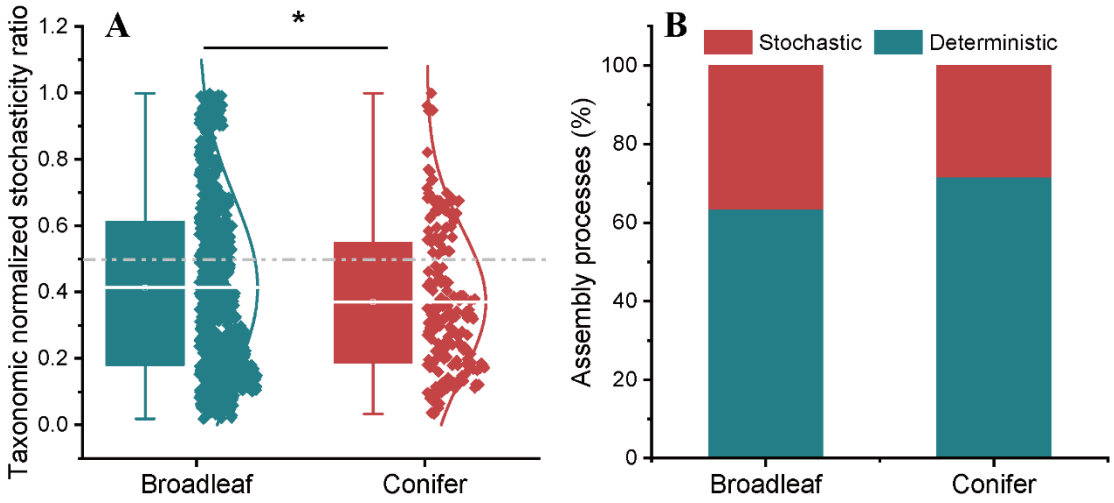

Figure S4 Goodness-of-fit statistics (R^2) of environmental variables fitted to NMDS ordination of fungal plant pathogenic community based on presence/absence data and Jaccard distance measure (a), Venn diagrams showing the contributions of the factors shaping fungal plant pathogenic community (b–c). The locations (latitude and longitude) of each tree replicate are provided in Supplementary Table S4. Nutrient evaluated in the analysis of broadleaf trees was NH_4^+–N. Nutrients evaluated in the analysis of coniferous trees were DOC, N_{org}, K, Mg, and P. The number and percentage in the parentheses in the Venn diagram indicate the explained variance and its percentage in the total explainable variance.

a

Presence/absence	Broadleaf		Conifer	
	R^2	*P*	R^2	*P*
Tree species	**0.83**	**0.001**	**0.91**	**0.001**
Water content	0.01	0.776	0.45	**0.008**
pH	0.45	**0.001**	0.60	**0.002**
DOC	0.11	0.123	**0.71**	**0.001**
NH_4^+ – N	0.35	**0.001**	0.07	0.548
NO_2^- – N	0.03	0.570	0.26	0.051
NO_3^- – N	0.00	0.997	ND	ND
N_{Min}	0.12	0.070	0.07	0.530
N_{Org}	0.10	0.139	0.41	**0.004**
Ca	0.12	0.109	0.20	0.146
Fe	0.09	0.154	0.10	0.414
K	0.12	0.115	0.38	**0.015**
Mg	0.04	0.480	0.51	**0.001**
P	0.06	0.303	0.37	**0.024**
Latitude	0.08	0.223	**0.82**	**0.001**
Longitude	0.05	0.325	**0.91**	**0.001**

*Bold letter indicates statistical significances
**ND = Not detected