



Supplement of

Soil fungal network complexity and functional roles differ between black truffle plantations and forests

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Table S1 Physical-chemical properties from soil samples of black truffle-productive plantations and forests, modified from Barou et al. (2024). Values are mean $\pm$ standard error (SE). GSM = gravimetric soil moisture, EC = electric conductivity, OM = organic matter, actCA = active carbonate, carb = CaCO₃.

Soil variable (units)	Plantations		Forests	
	Mean	SE	Mean	SE
pH	8.25	0.02	8.09	0.03
GSM (%)	11.99	0.3	11.27	0.57
EC ($\mu\text{s cm}^{-1}$)	272.07	10.76	276.34	13.21
sand (%)	48.6	1.1	52.12	1.41
clay (%)	23.96	0.6	16.72	0.53
silt (%)	27.85	0.71	31.16	1.4
stone (%)	39.53	1.62	38.44	1.94
OM (%)	3.69	0.17	5.6	0.32
actCA (%)	6.57	0.36	4.94	0.33
carb (%)	28.48	1.59	39.2	2.59
N (%)	0.13	0.01	0.18	0.01
P (mg kg^{-1})	6.03	0.81	2.75	0.29
K (mg kg^{-1})	163.65	8.19	126.88	5.54
Ca (mg kg^{-1})	3297.1	78.94	3175.35	100.81
Na (mg kg^{-1})	14.99	1.73	9.71	1.51
Mg (mg kg^{-1})	151.41	9.37	212.16	22.84
Fe (mg kg^{-1})	5.99	0.25	10.71	0.92
Mn (mg kg^{-1})	8.22	0.35	7.9	0.41
Zn (mg kg^{-1})	1.93	0.19	1.79	0.22
Cu (mg kg^{-1})	0.46	0.04	0.25	0.01
B (mg kg^{-1})	0.07	0.00	0.06	0.00

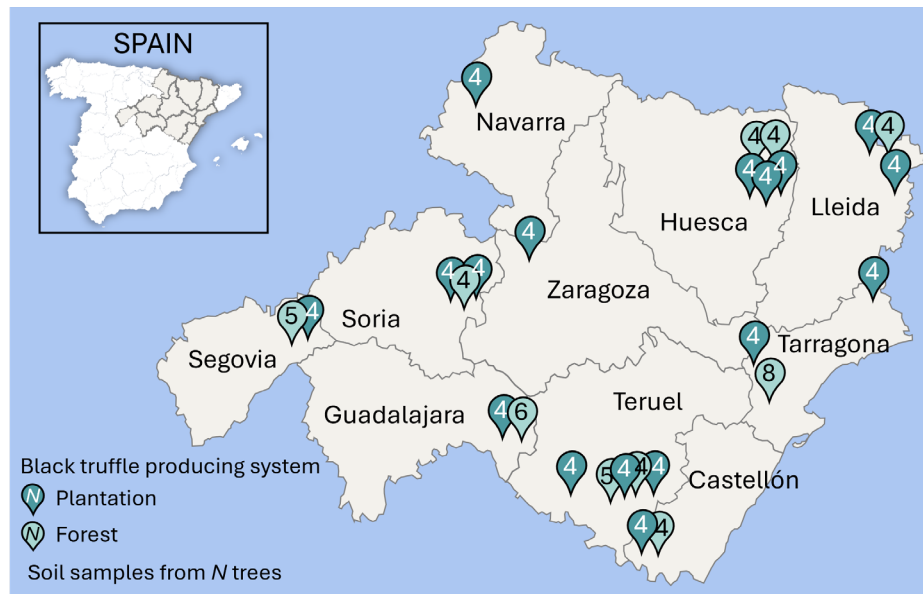
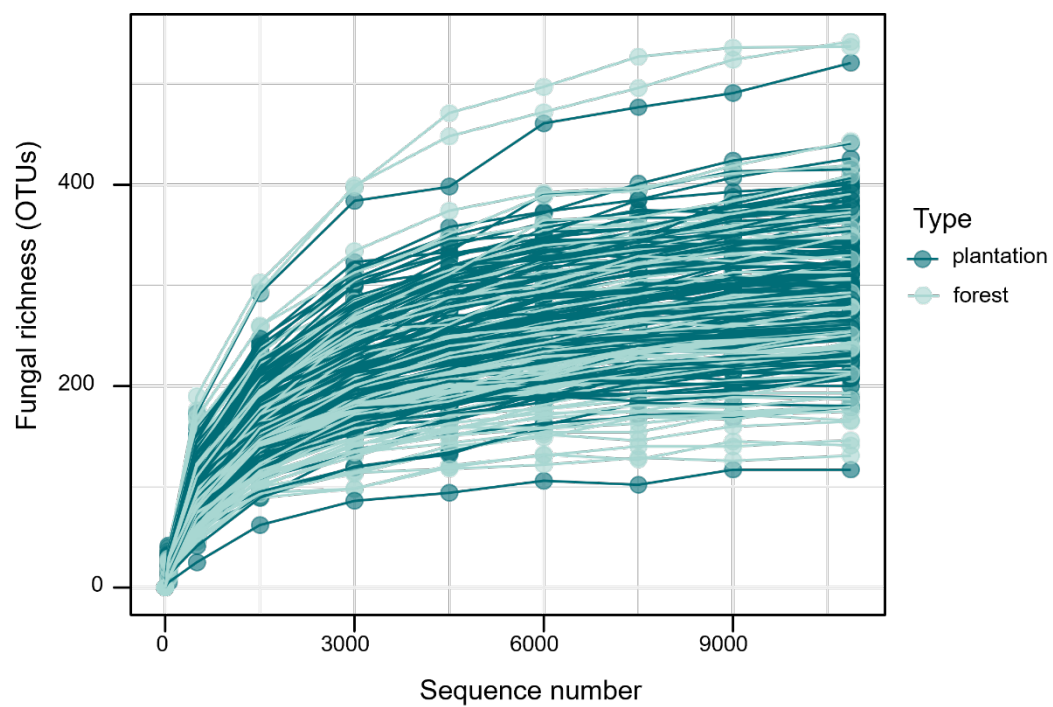


Figure S1 Map with soil samples collected from black truffle plantations and forests across its natural distribution in Spain. The numbers denote the number of holm oaks sampled per site. Map created with flourish.studio (<https://flourish.studio>) and adapted by the authors.



29 **Figure S2** Rarefaction curves of all samples ($N = 231$) in each type of black truffle producing system
 30 (plantation and forest). The graph was performed with “microeco” R package.
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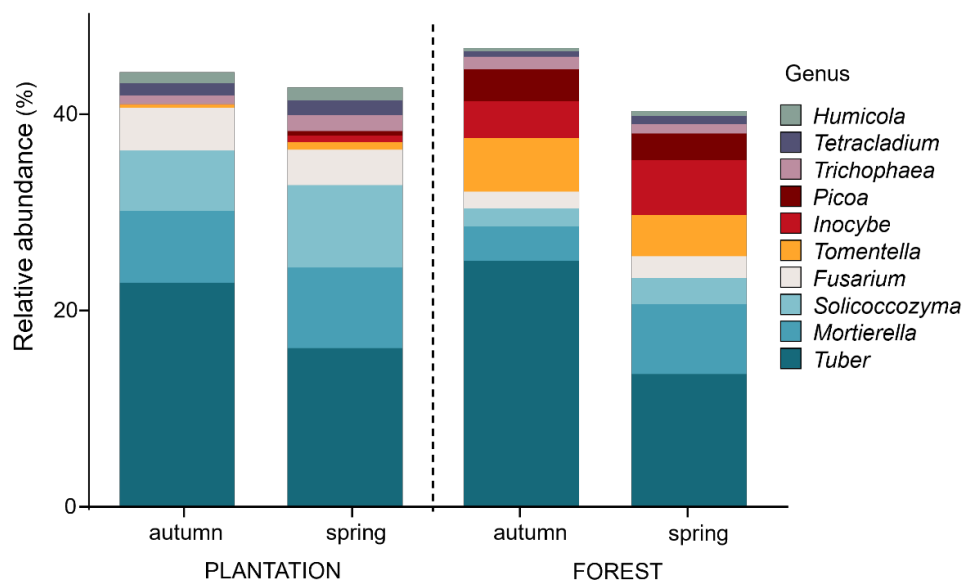
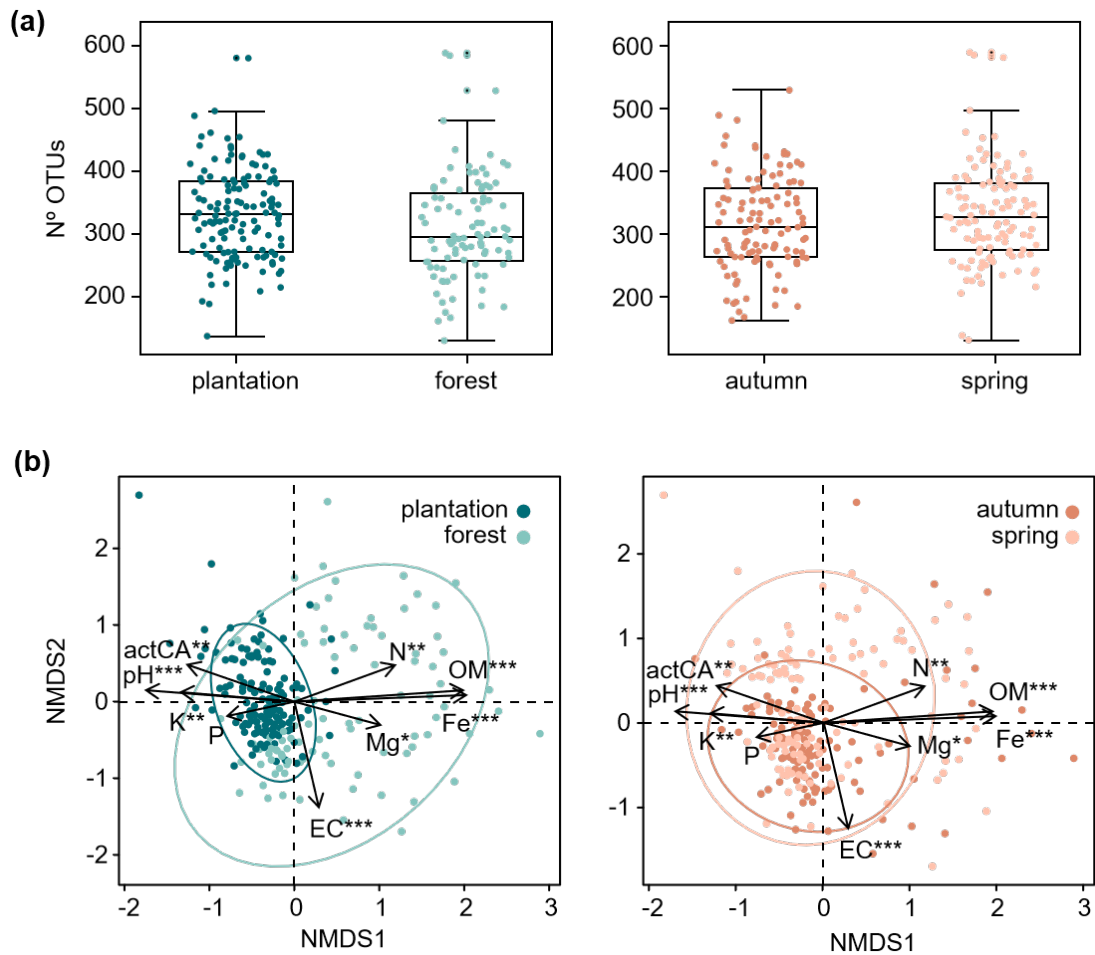


Figure S3 Top-ten most abundant genera across treatments of the studied factors: type of truffle-producing system and seasons of sampling ($N = 231$, OTUs = 8,654).



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39 **Figure S4** Alpha and beta-diversity of soil fungal communities, depending on the factors studied: type
 40 of black truffle producing system (plantation, forest) and season of sampling (autumn, spring). In (a)
 41 medians, min, max and quartiles of α -diversity are represented. In (b) β -diversity is represented by
 42 non-metric multidimensional scaling (NMDS) based on Bray-Curtis dissimilarities for soil fungal
 43 community data: ellipses indicate 95% confidence intervals of fit and vectors represent the strength
 44 and direction of soil variables weight ($k = 2$, stress = 0.22, $R^2 = 0.95$). EC = electric conductivity, OM =
 45 organic matter, actCA = active carbonates. ($N = 231$, OTUs = 8,654).

Table S2 Linear mixed models fitted for structural properties of the co-occurrence fungal network in black truffle brûlés (see **Fig. 2**). The type of truffle producing system and season of sampling were included as fixed factors and site as random factor in models. Significant *p*-values (< 0.05) are shown in bold. *R*²*m* = marginal; variance explained by fixed factors; *R*²*c* = conditional; variance explained by fixed and random factors. Network complexity = ratio of links to OTU richness.

Structural network property	Predictor	Estimate ± SE	F value	<i>p</i> -value	<i>R</i> ² <i>m</i>	<i>R</i> ² <i>c</i>
OTU richness	type	41.431 ± 5.316	60.743	< 0.001	0.23	0.423
	season	3.782 ± 4.287	0.779	0.379		
Fungal links	type	211.054 ± 23.6	79.974	< 0.001	0.286	0.445
	season	20.277 ± 19.193	1.116	0.292		
Network complexity	type	0.496 ± 0.051	96.382	< 0.001	0.321	0.495
	season	0.057 ± 0.041	1.937	0.165		

Table S3 Taxonomic identification from Phylum to Species and fungal guild assignment of OTUs co-occurring with *Tuber melanosporum* Vittad. in truffle producing plantations and forests (see **Fig. 3**). Positive links represent co-occurrence and negative links represent co-exclusion with *T. melanosporum*. ECM = ectomycorrhizal fungi; SAP = saprotrophic; PATH = plant pathogens; PAR=parasites; AMF = arbuscular mycorrhizal fungi; UN = unassigned fungal guild.

PLANTATIONS								
OTU ID	Phylum	Class	Order	Family	Genus	Species	Fungal guild	Type of link
OTU00021	Ascomycota	Sordariomycetes	Hypocreales	Nectriaceae	Ilyonectria	<i>I. macrodidyma</i>	PATH	positive
OTU00050	Ascomycota	Dothideomycetes	Capnodiales	Mycosphaerellaceae	Mycosphaerella	<i>M. tassiana</i>	PATH	negative
OTU00059	--	--	--	--	--	--	UN	negative
OTU00357	Mortierellomycota	Mortierellomycetes	Mortierellales	Mortierellaceae	Mortierella	--	SAP	negative
OTU00419	Ascomycota	Dothideomycetes	Pleosporales	Pleosporaceae	Alternaria	--	PATH	negative
OTU00492	Ascomycota	Sordariomycetes	Hypocreales	Incertae sedis	Trichothecium	<i>T. crotocinigenum</i>	PATH	positive
OTU00569	Ascomycota	Sordariomycetes	Coniochaetales	--	--	--	UN	positive
OTU00636	Basidiomycota	Tremellomycetes	Tremellales	Bulleribasidiaceae	Vishniacozyma	<i>V. victoriae</i>	SAP	negative
OTU00689	--	--	--	--	--	--	UN	negative
OTU01199	Ascomycota	Dothideomycetes	Pleosporales	Amorosiaceae	Angustimassarina	<i>A. acerina</i>	PAR	negative
OTU01202	--	--	--	--	--	--	UN	negative
OTU01203	Basidiomycota	Tremellomycetes	Tremellales	Bulleribasidiaceae	Vishniacozyma	--	SAP	negative
OTU01704	--	--	--	--	--	--	UN	negative
OTU02017	--	--	--	--	--	--	UN	negative
OTU03029	--	--	--	--	--	--	UN	negative
OTU03110	Ascomycota	Sordariomycetes	--	--	--	--	UN	positive
OTU03521	Glomeromycota	Glomeromycetes	Diversisporales	Diversisporaceae	--	--	AMF	positive

FORESTS

OTU ID	Phylum	Class	Order	Family	Genus	Species	Fungal guild	Type of link
OTU00004	Mortierellomycota	Mortierellomycetes	Mortierellales	Mortierellaceae	Mortierella	<i>M. alpina</i>	SAP	positive
OTU00028	Basidiomycota	Agaricomycetes	Russulales	Russulaceae	Russula	<i>R. ilicis</i>	ECM	negative
OTU00070	Ascomycota	Dothideomycetes	Pleosporales	Sporormiaceae	Preussia	--	SAP	positive
OTU00184	Ascomycota	Dothideomycetes	Pleosporales	Didymellaceae	Epicoccum	<i>E. dendrobii</i>	PATH	negative
OTU00274	Ascomycota	--	--	--	--	--	UN	positive
OTU00492	Ascomycota	Sordariomycetes	Hypocreales	Incertae sedis	Trichothecium	<i>T. crotocinigenum</i>	PATH	positive
OTU00508	Ascomycota	Eurotiomycetes	Chaetothyriales	Herpotrichiellaceae	Rhinoclaadiella	--	SAP	negative
OTU00690	--	--	--	--	--	--	UN	positive
OTU00717	Ascomycota	Dothideomycetes	Pleosporales	Phaeosphaeriaceae	Wojnowiciella	<i>W. dactylidis</i>	SAP	negative
OTU00718	Ascomycota	Sordariomycetes	Hypocreales	Clavicipitaceae	Metarhizium	<i>M. marquandii</i>	PAR	negative
OTU00798	Basidiomycota	Agaricomycetes	Thelephorales	Thelephoraceae	Pseudotomentella	--	ECM	positive
OTU01198	Chytridiomycota	Rhizophydiomycetes	Rhizophydiales	Rhizophydiaceae	Rhizophydium	--	PAR	positive
OTU01717	Ascomycota	Eurotiomycetes	Chaetothyriales	Herpotrichiellaceae	Cladophialophora	--	SAP	negative
OTU02555	--	--	--	--	--	--	UN	negative
OTU02586	Ascomycota	Leotiomyces	Thelebolales	Pseudeurotiaceae	Connersia	--	SAP	positive
OTU02750	Chytridiomycota	Spizellomycetes	Spizellomycetales	Powellomycetaceae	--	--	SAP	positive
OTU03788	--	--	--	--	--	--	UN	negative
OTU04160	Ascomycota	Eurotiomycetes	Eurotiales	Aspergillaceae	Penicillium	<i>P. megasporum</i>	SAP	positive

61 **References**

- 62 Barou, V., Zabal-Aguirre, M., Parladé, J., Rincón, A. (2025). *Tuber melanosporum* Vittad. abundance and
63 specific soil parameters predict soil enzymatic activity in wild and managed truffle producing
64 systems. *Applied Soil Ecology*, 206, 105872. <https://doi.org/10.1016/j.apsoil.2025.105872>