

Consistent predictors of microbial community composition across spatial scales in grasslands reveal low context-dependency

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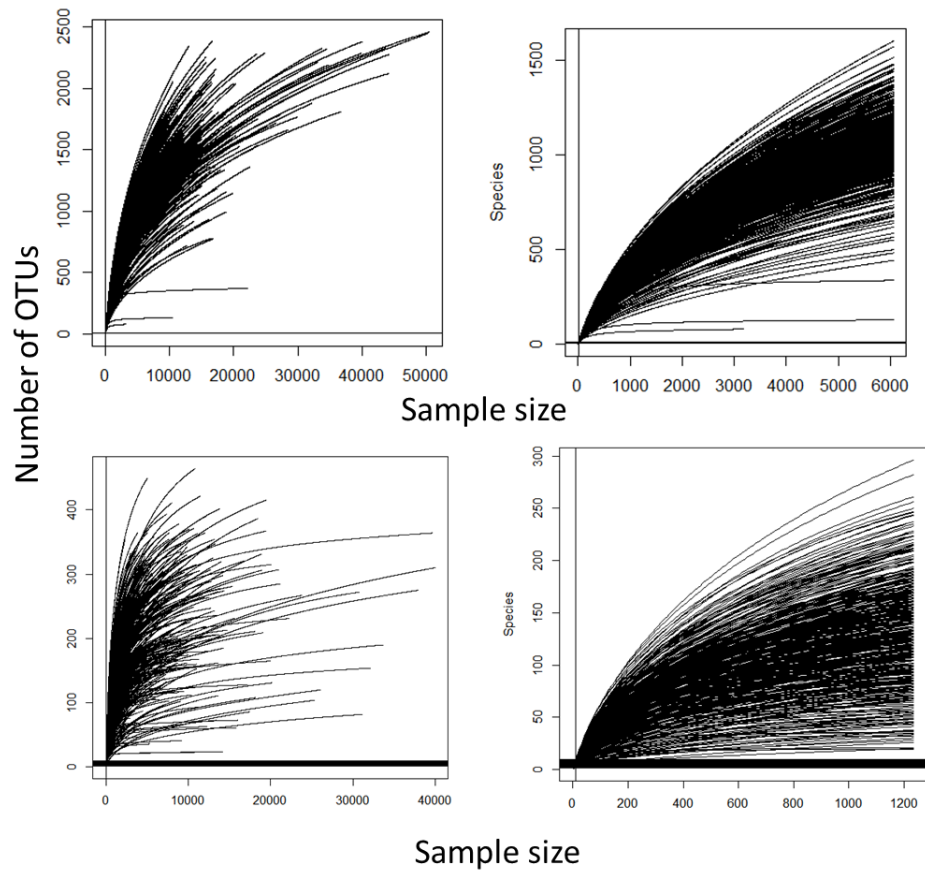


Figure S1 Rarefaction curves for bacteria (top) and fungi (bottom). The graphs on the left show the curves for non-rarefied data, while the graphs on the right show the curves after rarefactions at 6046 and 1231 reads for bacteria and fungi, respectively.

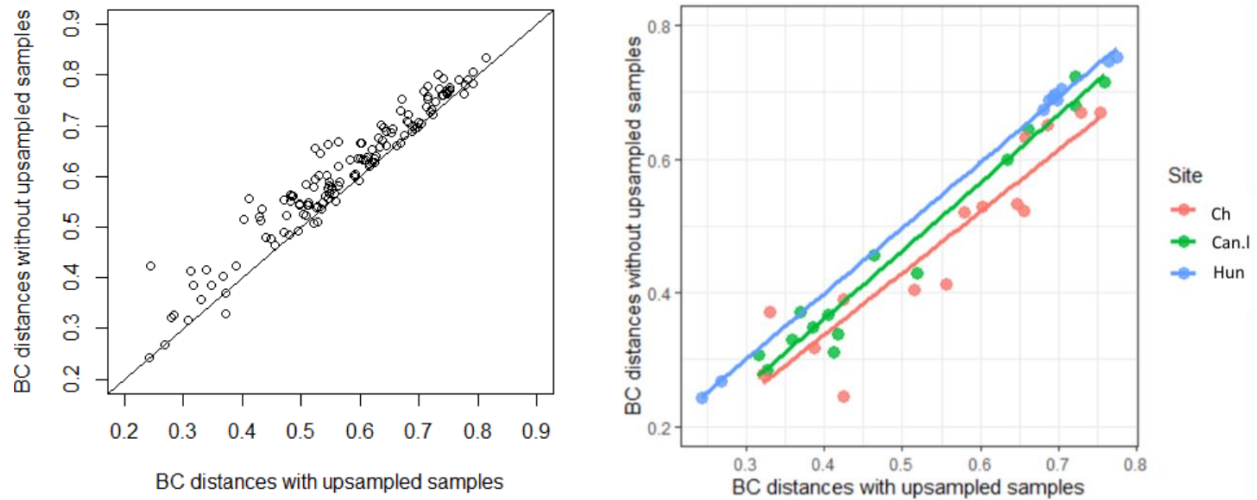


Figure S2 Left: The Bray-Curtis distances in bacterial communities between the upsampled samples aggregated per plot in this study (number of samples per region: China - 19/30, CanadaL - 12/30, Hungary - 8/30 and CanadaE - 7/10) and the samples aggregated per plot where the upsampled samples were excluded. Mantel rho = 0.97. **Right:** The same relationship focusing on the within-site distances for three sites (China, Hungary, CanadaL) that contained more than two plots. These plots demonstrate that the inclusion of usampled samples did not strongly influence the between and within-site bacterial community distances.

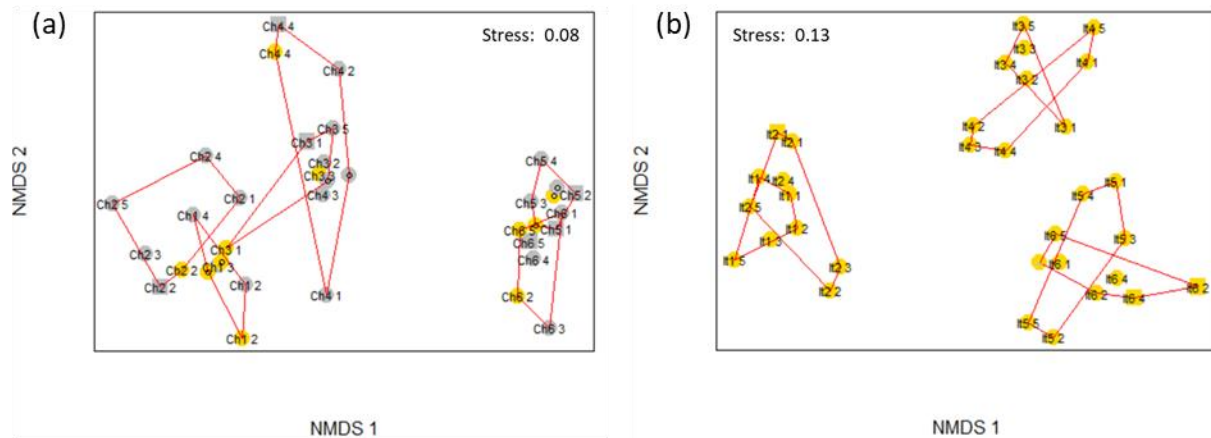


Figure S3 The NMDS ordination of bacterial (a) and fungal (b) community composition in the sites Ch (China) and It (Italy), respectively. The plots provide an example of distances of upsampled samples (for bacteria) and technical replicates (for bacteria and fungi) from other samples within the same plot and region. The yellow colour indicates samples that contained more sequences than the rarefaction threshold while grey represents upsampled samples. Circles represent samples used in the analyses and squares technical replicates. The text indicates the site, plot number and the sample number within the plot. Red lines connect the samples from the same plot. These graph demonstrate i) that upsampled samples cluster closely together with those with higher number of sequences and ii) the close clustering of technical replicates.

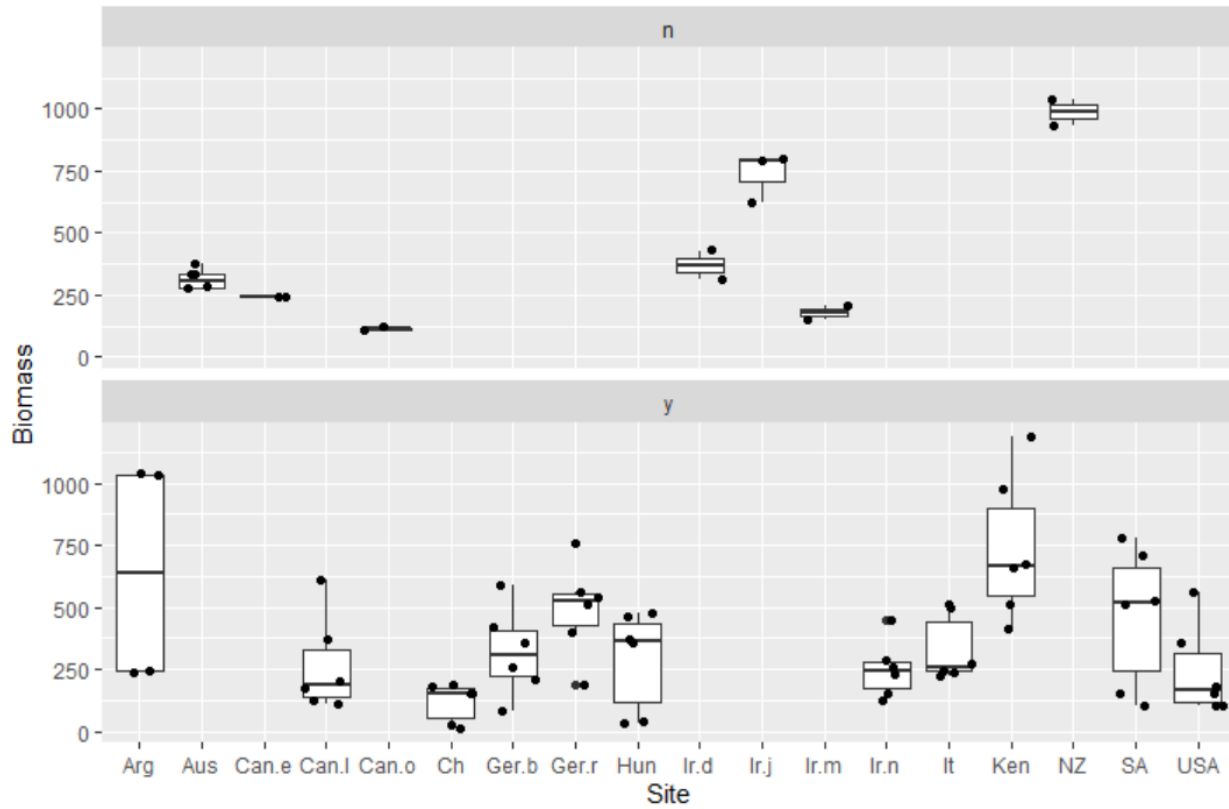


Fig S4 Within-site biomass production [g/m^2] for different sites. Only those sites that had at least 100% difference in biomass production between two pairs of replicate plots (y = includes productivity gradient) were included in the analyses of the relationship between productivity and microbial community composition. All the other sites have little to no difference in the productivity levels between the plots (n = no productivity gradient).

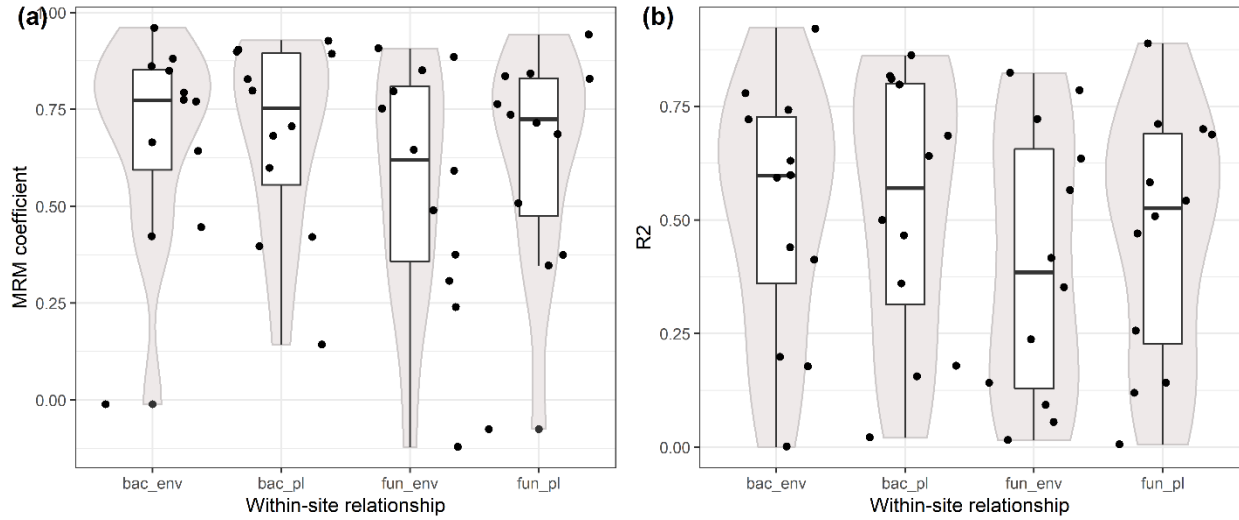


Figure S5 Coefficient and R^2 values of the MRM models of the within-site relationships between bacterial or fungal community composition and environmental and plant community distances.

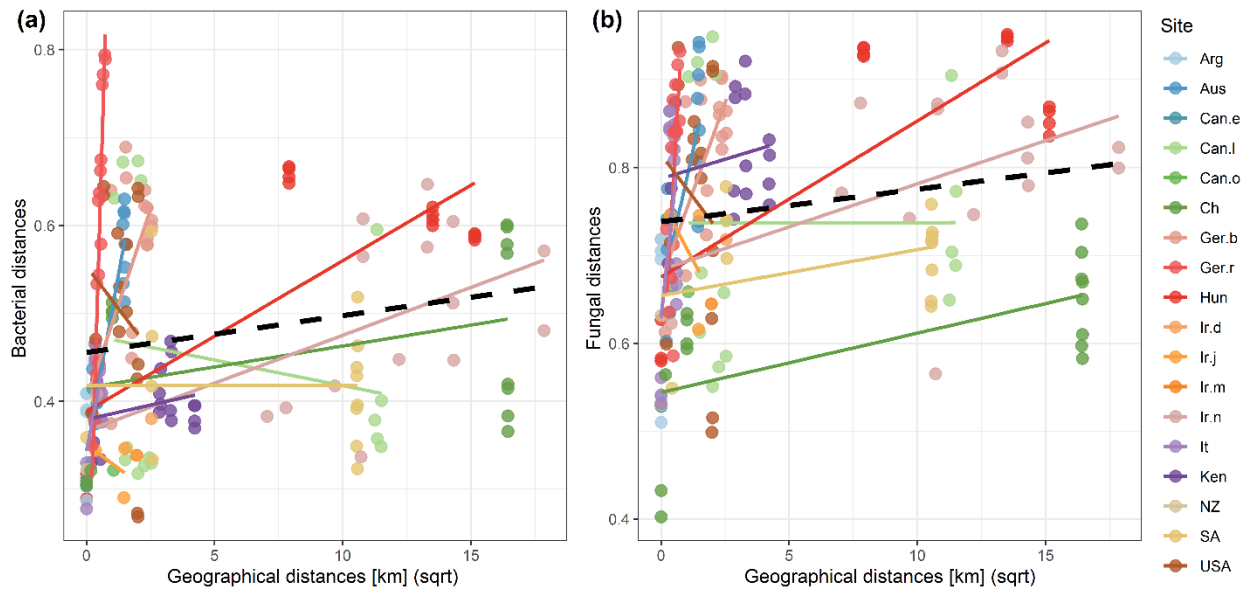


Figure S6 The relationships between within-site geographical distances and within-site BC distances in a) bacterial and b) fungal community composition. Out of the six sites that have larger distances between plots (between 10 and 300 km), the relationship was found to be relatively strong for only one site in the case of bacteria (Ir.n; $R^2 = 51\%$) and two sites for fungi (Ir.n; $R^2 = 47\%$ and Ch; $R^2 = 39\%$). Site references as in Table S1.

Table S1 Information about 18 sites included in the analyses. The sites shaded in grey contained a productivity gradient (i.e. they contained 2 pairs of plots located in the same region and exposed to similar climatic conditions but with a more than 2-fold difference in biomass production).

<i>Site ID</i>	<i>Site name</i>	<i>Country</i>	<i># of plots</i>	<i>Year: biomass sampling</i>	<i>Year: soil sampling</i>	<i>Mean MAP (sd)</i>	<i>Mean MAT (sd)</i>
Aus	Pitztal	Austria	6	2012	2017	796 (50)	4.6 (0.9)
Arg	El Cuadrado, Córdoba	Argentina	4	2012	2017	995 (0)	12.9 (0)
Can.e	Elginfield Observatory	Canada	2	2012	2018	1001 (0)	7.9 (0)
Can.l	Lac du Bois	Canada	6	2012	2018	379 (18)	6.5 (0.5)
Can.o	Onefour, AB	Canada	2	2012	2017	301 (10)	6.7 (0.1)
Ch	Inner Mongolia	China	6	2017	2018	207 (82)	2.1 (0.6)
Ger.b	Bayreuth	Germany	6	2012	2017	721 (38)	8.5 (0.2)
Ger.r	Rostock	Germany	6	2015	2017	668 (0)	8.7 (0)
Hun	Soroksár, Fülöpháza, Battonya	Hungary	6	2012	2018	561 (5)	11 (0.4)
Ir.d	Damavand	Iran	2	2012	2017	425 (16)	7.0 (0.5)
Ir.j	Javaherdeh	Iran	3	2012	2017	1230 (13)	5.3 (1.3)
Ir.m	Masuleh	Iran	2	2012	2017	843 (54)	5.5 (0.1)
Ir.n	North Khorasan, Golestan	Iran	6	2012/2017	2017	398 (65)	8.6 (2.5)
It	Torricchio Nature Reserve	Italy	6	2012	2017	1057 (21)	8.3 (0.1)
Ken	Laikipia	Kenya	6	2012	2018	562 (67)	19.9 (0.3)
NZ	Waitati South Island	New Zealand	2	2012	2018	689 (0)	10.3 (0)
SA	Loskopdam	South Africa	6	2012	2017	699 (96)	16.6 (1)
USA	Fort Keogh	USA	6	2016	2017	333 (16)	8.9 (0.1)

Table S2 Three groups of variables used as predictors of bacterial and fungal community composition. *N and Ca were excluded from the analysed due to high correlation with SOM and Ca, respectively.

1. Broad-scale predictors - vary strongly at the large scale	2. Regionally-varying predictors- vary at the large and the regional scale	3. Community-related variable - varies at the large and the regional scale
Mean annual precipitation (MAP)	Soil organic matter (SOM)	Plant community composition
Mean annual temperature (MAT)	Cation exchange capacity (CEC)	
Nitrogen (N) deposition	pH	
Geographical distances	Base saturation (BS)	
	Carbon to nitrogen ratio (C:N)	
	Total nitrogen (N)*	
	Total phosphorus (P)	
	Available phosphorus (P Olsen)	
	Sand	
	Extractable calcium (Ca)*	
	Extractable magnesium (Mg)	
	Extractable potassium (K)	
	Plant biomass	

Table S3 Large-scale environmental predictors of bacterial and fungal community distances. Environmental variables (atmospheric and geographic as those varying primarily across sites and soil and productivity as those varying both across and within sites) that had a significant effect in the MRM model are shown (n = 83). R² values of the individual relationships between the variables and bacterial/fungal community composition are shown together with the coefficients and P values for each of the variables in the MRM model. The coefficients of variables shaded in grey were used to weigh these variables before summing them to obtain a single representative composite environmental variable in Fig. 3.

		Bacterial community composition			Fungal community composition		
		Individual R ²	Coefficient (model)	P-value (model)	Individual R ²	Coefficient (model)	P-value (model)
Broad-scale variables	Geo. dist.	-	-	-	0.05	0.099	0.009
	MAT	-	-	-	0.18	0.330	0.001
	MAP	0.02	0.060	0.010	-	-	-
	N deposition	0.18	0.295	0.001	0.11	0.216	0.001
Ecosystem fertility-related variables	Plant biomass	0.02	0.07	0.009	0.05	0.124	0.003
	pH	0.25	0.21	0.001	0.08	0.234	0.001
	CEC	0.14	0.14	0.001	0.05	0.092	0.008
	N total	0.07	0.15	0.001	0.06	0.123	0.002
	BS	0.36	0.41	0.001	-	-	-
	Sand	0.03	0.08	0.008	-	-	-
	Full model R²	0.60			0.38		
	F-test	629.29			296		

Table S4 The individual and compound effect of three groups of variables: broad-scale, ecosystem fertility-related variables and plant community composition on broad-scale bacterial and fungal community composition. The results were produced using MRM models and were used to create variance partitioning between the three groups of variables.

	Bacterial community composition	Fungal community composition
	Individual R²	Individual R²
1. Broad-scale variables	0.19	0.26
2. Ecosystem fertility-related variables	0.50	0.18
3. Plant community distances	0.21	0.42
Full model R²	0.65	0.52
F-test	701.62	457.22

Table S5 PERMANOVA results on the differences in bacterial and fungal community composition at low vs high productivity levels (n = 44).

Bacteria	Df	SumsOfSqs	MeanSqs	F.Model	R2	Pr(>F)
prod_level	1	0.5596	0.55959	2.2042	0.04986	0.001
Residuals	42	10.6628	0.25388	0.95014		
Total	43	11.2224	1			
Fungi	Df	SumsOfSqs	MeanSqs	F.Model	R2	Pr(>F)
prod_level	1	0.5325	0.53247	1.2026	0.02784	0.001
Residuals	42	18.5956	0.44275	0.97216		
Total	43	19.1281	1			

Table S6 The explained variance of the best environmental predictor and plant community composition of bacterial and fungal community composition at high (n =22) and low productivity grasslands (n = 22).

	Bacteria		Fungi	
		R ²		R ²
High productivity				
Best env. predictor	Base sat.	0.33	MAT	0.31
Plant community		0.21		0.41
Low productivity				
Best env. predictor	Base sat.	0.65	MAT	0.14
Plant community		0.23		0.30

Table S7 The results of the mixed-effect model (n = 44) showing the difference in bacterial and fungal abundance (and their ratio) as well as the relative abundance of three fungal functional groups (symbiotrophs, saprotrophs and pathotrophs) at high vs low productivity levels.

	Value	Std.Error	DF	t-value	p-value
Bacterial abundance					
(Intercept)	10328.66	3549.525	31	2.910	0.0066
prod_level	7190.45	2736.761	31	2.627	0.0133
Fungal abundance					
(Intercept)	1664.657	337.215	32	4.936	0
prod_level	687.9539	222.837	32	3.087	0.0042
Fungi:bacteria					
(Intercept)	0.211939	0.052	31	4.040	0.0003
prod_level	0.041453	0.054	31	0.770	0.4469
	Value	Std.Error	DF	t-value	p-value
Symbiotroh					
(Intercept)	9.883575	1.099	32	8.996	0
prod_level	1.377614	0.940	32	1.465	0.1526
Saprotroph					
(Intercept)	18.99543	1.195	32	15.898	0
prod_level	0.303075	1.106	32	0.274	0.7859
Patotroph					
(Intercept)	18.20772	1.085	32	16.789	0
prod_level	0.664656	1.225	32	0.543	0.5911

Table S8 Taxonomic assignment (phylum, family, genus) of the significant bacterial indicators of high or low productivity levels.

#	Phylum	Family	Genus	Indic.	P-sidak
1	Nitrospirae	0319-6A21	uncultured	high	0.008
2	Actinobacteria	0319-6M6	uncultured	high	0.032
3	Actinobacteria	Acidimicrobiaceae	uncultured	high	0.047
4	Actinobacteria	Acidimicrobiaceae	llumatobacter	high	0.036
5	Chloroflexi	Anaerolineaceae	uncultured	high	0.044
6	Firmicutes	Bacillaceae	Bacillus	high	0.047
7	Firmicutes	Bacillaceae	Bacillus	high	0.038
8	Firmicutes	Bacillaceae	Bacillus	high	0.026
9	Proteobacteria	Blrii41	unknown	high	0.004
10	Bacteroidetes	Chitinophagaceae	uncultured	high	0.028
11	Firmicutes	Clostridiaceae	unknown	high	0.018
12	Firmicutes	Clostridiaceae	unknown	high	0.014
13	Firmicutes	Clostridiaceae	unknown	high	0.022
14	Firmicutes	Clostridiaceae	unknown	high	0.006
15	Proteobacteria	Desulfurellaceae	H16	high	0.004
16	Actinobacteria	Elev-16S-1332	unknown	high	0.032
17	Actinobacteria	Elev-16S-1332	uncultured	high	0.047
18	Actinobacteria	Elev-16S-1332	uncultured	high	0.036
19	Bacteroidetes	env.OPS	unknown	high	0.006
20	Firmicutes	Erysipelotrichaceae	Turicibacter	high	0.020
21	Firmicutes	Family	unknown	high	0.028
22	Actinobacteria	Gaiellaceae	Gaiella	high	0.016
23	Gemmatimonadetes	Gemmatimonadaceae	uncultured	high	0.002
24	Proteobacteria	Haliangiaceae	Haliangium	high	0.022
25	Proteobacteria	Haliangiaceae	Haliangium	high	0.026
26	Proteobacteria	Haliangiaceae	Haliangium	high	0.024
27	Actinobacteria	Intrasporangiaceae	unknown	high	0.010
28	Bacteroidetes	KD3-93	uncultured	high	0.002
29	Actinobacteria	Mycobacteriaceae	Mycobacterium	high	0.004
30	Proteobacteria	Nitrosomonadaceae	uncultured	high	0.034
31	Proteobacteria	Nitrosomonadaceae	uncultured	high	0.038
32	Actinobacteria	Nocardiodaceae	Nocardioides	high	0.036
33	Actinobacteria	Nocardiodaceae	Nocardioides	high	0.012
34	Firmicutes	Peptostreptococcaceae	Intestinibacter	high	0.012
35	Firmicutes	Planococcaceae	unknown	high	0.030
36	Proteobacteria	Rhodobiaceae	uncultured	high	0.034
37	Proteobacteria	Rhodospirillales	unknown	high	0.049
38	Acidobacteria	Solibacteraceae	unknown	high	0.042
39	Acidobacteria	unknown	unknown	high	0.004

40	Actinobacteria	uncultured	uncultured	high	0.028
41	Actinobacteria	uncultured	uncultured	high	0.044
42	Actinobacteria	uncultured	unknown	high	0.026
43	Proteobacteria	uncultured	unknown	high	0.010
44	Proteobacteria	uncultured	uncultured	high	0.036
45	Proteobacteria	uncultured	uncultured	high	0.002
46	Acidobacteria	unknown	unknown	high	0.012
47	Acidobacteria	unknown	unknown	high	0.047
48	Acidobacteria	unknown	unknown	high	0.028
49	Acidobacteria	unknown	unknown	high	0.006
50	Elusimicrobia	unknown	unknown	high	0.014
51	Chloroflexi	unknown	unknown	high	0.028
52	Chloroflexi	unknown	unknown	high	0.008
53	Verrucomicrobia	unknown	unknown	high	0.004
54	Verrucomicrobia	unknown	unknown	high	0.008
55	Acidobacteria	unknown	unknown	high	0.002
56	Proteobacteria	Xanthobacteraceae	Pseudolabrys	high	0.038
57	Proteobacteria	Xanthobacteraceae	Pseudolabrys	high	0.047
58	Proteobacteria	Xanthobacteraceae	unknown	high	0.016
59	Proteobacteria	Xanthomonadales	unknown	high	0.006
60	Proteobacteria	unknown	unknown	high	0.002
61	Elusimicrobia	unknown	unknown	high	0.016
62	Acidobacteria	unknown	unknown	high	0.010
63	Thaumarchaeota	unknown	unknown	low	0.004
64	Thaumarchaeota	unknown	unknown	low	0.032
65	Thaumarchaeota	unknown	unknown	low	0.002
66	Thaumarchaeota	unknown	unknown	low	0.002
67	Actinobacteria	0319-6M6	uncultured	low	0.032
68	Actinobacteria	0319-6M6	uncultured	low	0.020
69	Actinobacteria	0319-6M6	uncultured	low	0.010
70	Actinobacteria	0319-6M6	uncultured	low	0.002
71	Actinobacteria	0319-6M6	uncultured	low	0.002
72	Actinobacteria	0319-6M6	uncultured	low	0.042
73	Actinobacteria	0319-6M6	unknown	low	0.004
74	Actinobacteria	0319-6M6	uncultured	low	0.014
75	Actinobacteria	0319-6M6	uncultured	low	0.012
76	Actinobacteria	288-2	uncultured	low	0.020
77	Proteobacteria	Acetobacteraceae	uncultured	low	0.002
78	Acidobacteria	Blastocatellaceae	unknown	low	0.012
79	Acidobacteria	Blastocatellaceae	unknown	low	0.040
80	Acidobacteria	Blastocatellaceae	unknown	low	0.012
81	Acidobacteria	Blastocatellaceae	unknown	low	0.002
82	Acidobacteria	Blastocatellaceae	unknown	low	0.032

83	Acidobacteria	Blastocatellaceae	unknown	low	0.042
84	Acidobacteria	Blastocatellaceae	unknown	low	0.008
85	Acidobacteria	Blastocatellaceae	unknown	low	0.018
86	Bacteroidetes	Chitinophagaceae	Flavisolibacter	low	0.030
87	Bacteroidetes	Chitinophagaceae	Flavisolibacter	low	0.047
88	Bacteroidetes	Chitinophagaceae	Ferruginibacter	low	0.042
89	Bacteroidetes	Chitinophagaceae	Niastella	low	0.032
90	Bacteroidetes	Chitinophagaceae	Ferruginibacter	low	0.022
91	Bacteroidetes	Chitinophagaceae	Flavisolibacter	low	0.022
92	Verrucomicrobia	DA101	unknown	low	0.016
93	Verrucomicrobia	DA101	unknown	low	0.004
94	Verrucomicrobia	DA101	unknown	low	0.002
95	Proteobacteria	Desulfurellaceae	G55	low	0.002
96	Actinobacteria	Elev-16S-1332	uncultured	low	0.030
97	Actinobacteria	FFCH11085	uncultured	low	0.006
98	Actinobacteria	Frankiaceae	Jatrophihabitans	low	0.040
99	Actinobacteria	Frankiaceae	Jatrophihabitans	low	0.034
100	Actinobacteria	Frankiaceae	Jatrophihabitans	low	0.020
101	Gemmatimonadetes	Gemmatimonadaceae	uncultured	low	0.006
102	Gemmatimonadetes	Gemmatimonadaceae	uncultured	low	0.008
103	Actinobacteria	Geodermatophilaceae	Geodermatophilus	low	0.030
104	Proteobacteria	KCM-B-15	uncultured	low	0.006
105	Proteobacteria	Methylobacteriaceae	Microvirga	low	0.008
106	Proteobacteria	Methylobacteriaceae	unknown	low	0.002
107	Proteobacteria	Methylobacteriaceae	Microvirga	low	0.008
108	Proteobacteria	Methylobacteriaceae	Microvirga	low	0.002
109	Proteobacteria	Methylobacteriaceae	uncultured	low	0.004
110	Proteobacteria	Methylobacteriaceae	Psychroglacielcola	low	0.010
111	Actinobacteria	Micromonosporaceae	Phytohabitans	low	0.002
112	Actinobacteria	Micromonosporaceae	Actinoplanes	low	0.008
113	Actinobacteria	Mycobacteriaceae	Mycobacterium	low	0.042
114	Actinobacteria	Mycobacteriaceae	Mycobacterium	low	0.045
115	Actinobacteria	Nakamurellaceae	Nakamurella	low	0.034
116	Proteobacteria	Oxalobacteraceae	Massilia	low	0.040
117	Proteobacteria	P-102	uncultured	low	0.016
118	Planctomycetes	Planctomycetaceae	uncultured	low	0.006
119	Planctomycetes	Planctomycetaceae	Singulisphaera	low	0.002
120	Planctomycetes	Planctomycetaceae	Gemmata	low	0.028
121	Planctomycetes	Planctomycetaceae	uncultured	low	0.004
122	Planctomycetes	Planctomycetaceae	uncultured	low	0.010
123	Actinobacteria	Propionibacteriaceae	uncultured	low	0.014
124	Actinobacteria	Propionibacteriaceae	Friedmanniella	low	0.047
125	Actinobacteria	Propionibacteriaceae	uncultured	low	0.044

126	Actinobacteria	Pseudonocardiaceae	Crossiella	low	0.008
127	Actinobacteria	Pseudonocardiaceae	Actinomycetospora	low	0.020
128	Actinobacteria	Pseudonocardiaceae	Pseudonocardia	low	0.004
129	Actinobacteria	Pseudonocardiaceae	Pseudonocardia	low	0.034
130	Chloroflexi	Roseiflexaceae	Roseiflexus	low	0.049
131	Actinobacteria	Rubrobacteriaceae	Rubrobacter	low	0.002
132	Actinobacteria	Rubrobacteriaceae	Rubrobacter	low	0.016
133	Actinobacteria	Rubrobacteriaceae	Rubrobacter	low	0.002
134	Actinobacteria	Rubrobacteriaceae	Rubrobacter	low	0.006
135	Actinobacteria	Rubrobacteriaceae	Rubrobacter	low	0.004
136	Actinobacteria	Rubrobacteriaceae	Rubrobacter	low	0.004
137	Actinobacteria	Rubrobacteriaceae	Rubrobacter	low	0.002
138	Acidobacteria	Solibacteraceae	unknown	low	0.038
139	Acidobacteria	Solibacteraceae	unknown	low	0.036
140	Actinobacteria	Solirubrobacteraceae	Solirubrobacter	low	0.030
141	Actinobacteria	Solirubrobacteraceae	Solirubrobacter	low	0.024
142	Actinobacteria	Streptomycetaceae	Streptomyces	low	0.008
143	Actinobacteria	Streptomycetaceae	Streptomyces	low	0.004
144	Planctomycetes	Tepidisphaeraceae	uncultured	low	0.016
145	Planctomycetes	Tepidisphaeraceae	uncultured	low	0.028
146	Planctomycetes	Tepidisphaeraceae	uncultured	low	0.004
147	Actinobacteria	Thermomonosporaceae	unknown	low	0.034
148	Actinobacteria	uncultured	uncultured	low	0.026
149	Actinobacteria	uncultured	uncultured	low	0.004
150	Actinobacteria	uncultured	uncultured	low	0.040
151	Actinobacteria	uncultured	uncultured	low	0.028
152	Actinobacteria	uncultured	uncultured	low	0.006
153	Proteobacteria	uncultured	uncultured	low	0.004
154	Actinobacteria	uncultured	unknown	low	0.002
155	Actinobacteria	uncultured	uncultured	low	0.044
156	Actinobacteria	uncultured	uncultured	low	0.008
157	Actinobacteria	uncultured	uncultured	low	0.008
158	Actinobacteria	uncultured	uncultured	low	0.012
159	Proteobacteria	uncultured	unknown	low	0.014
160	Acidobacteria	unknown	unknown	low	0.016
161	Actinobacteria	unknown	unknown	low	0.002
162	Chloroflexi	uncultured	unknown	low	0.022
163	Chloroflexi	unknown	unknown	low	0.008
164	Chloroflexi	unknown	unknown	low	0.004
165	Chloroflexi	unknown	unknown	low	0.028
166	Armatimonadetes	unknown	unknown	low	0.020
167	Armatimonadetes	unknown	unknown	low	0.004
168	Armatimonadetes	unknown	unknown	low	0.034

169	Tectomicrobia	unknown	unknown	low	0.047
170	FBP	unknown	unknown	low	0.018
171	Proteobacteria	unknown	unknown	low	0.032
172	Proteobacteria	unknown	unknown	low	0.047
173	Proteobacteria	unknown	unknown	low	0.002
174	Chloroflexi	unknown	unknown	low	0.030

Table S9 Taxonomic assignment (phylum, family, genus) and lifestyle of the significant fungal indicators of high or low productivity levels.

#	Phylum	Family	Genus	Lifestyle	Indic.	P-sidak
1	Ascomycota	Cucurbitariaceae	Pyrenochaetopsis	Pathotroph	high	0.020
2	Ascomycota	Nectriaceae	Ilyonectria	Pathotroph	high	0.000
3	Ascomycota	Nectriaceae	Ilyonectria	Pathotroph	high	0.035
4	Ascomycota	Phaeosphaeriaceae	Neosetophoma	Pathotroph	high	0.001
5	Ascomycota	Clavicipitaceae	Metarhizium	Pathotroph	high	0.010
6	Basidiomycota	Ustilaginaceae	Ustilago	Pathotroph	high	0.009
7	Mortierellomycota	Mortierellaceae	Mortierella	Saprotroph	high	0.012
8	Olpidiomycota	Olpidiaceae	Olpidium	Pathotroph	high	0.042
9	Ascomycota	Teratosphaeriaceae	Capnobotryella	Pathotroph	low	0.021
10	Ascomycota	Hypocreaceae	Trichoderma	Symbiotroph	low	0.044
11	Glomeromycota	Glomeraceae	Glomus	Symbiotroph	low	0.020