

Article

Linking Tree Health, Rhizosphere Physicochemical Properties, and Microbiome in Acute Oak Decline

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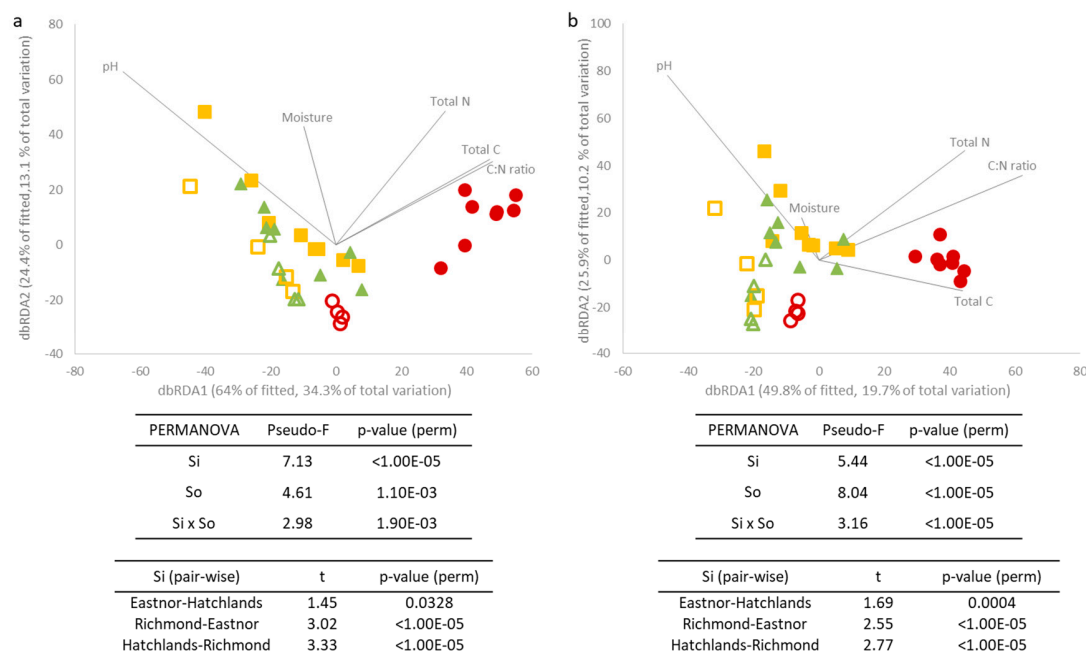
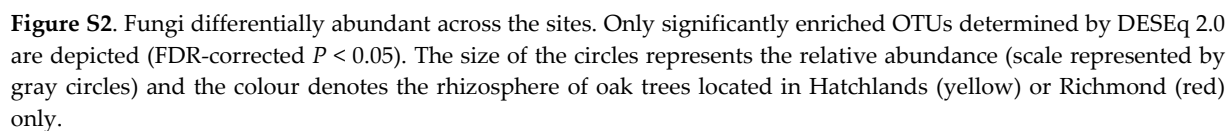


Figure S1 Distance-based redundancy analysis of the (a) bacterial and (b) fungal communities and soil properties of the rhizosphere and bulk soil across the sites ($n = 36$). The longer the line, the stronger the correlation between microbiome composition and soil chemistry. Eastnor—green triangle; Hatchlands—yellow square; Richmond—red circle; filled symbol—rhizosphere; open symbol—bulk soil. PERMANOVA results are shown in the below tables. Si—site; So—Soil compartment.



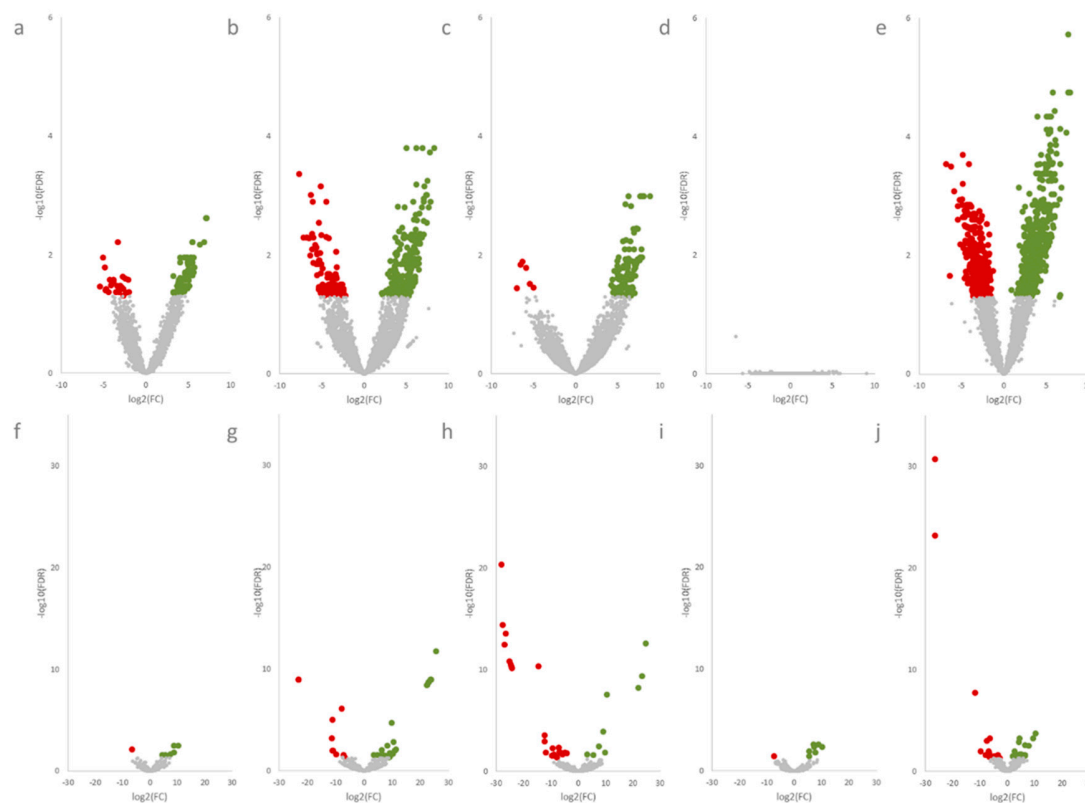


Figure S3. Volcano plots of differential bacterial (a, b, c, d, and e) and fungal (f, g, h, i and j) OTU abundance analysis as calculated by DESeq 2.0 according to tree health condition. Fold change as FDR corrected p-values are plotted for each OTU. Significantly different taxa (FDR-corrected $P < 0.05$) are coloured according to tree health condition: healthy–green (positive \log_2 (FC) axis), AOD–red (negative \log_2 (FC) axis). All sites (a, f) Eastnor (b, g); Hatchlands (c, h); Richmond (d, i); Eastnor and Hatchlands (e, j).

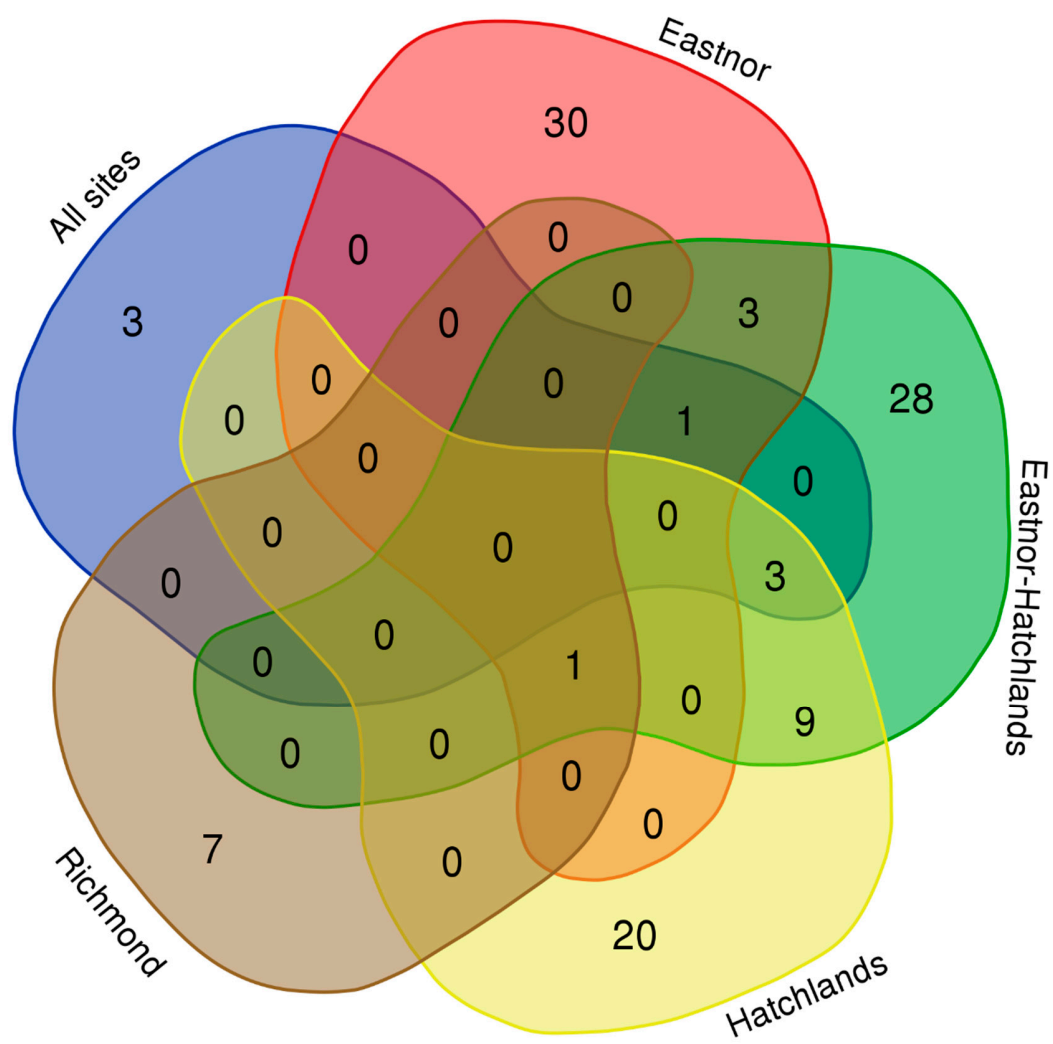


Figure S4. Venn diagram of the intersection of the differentially abundant fungal OTUs between healthy and AOD trees detected across the tree health comparisons.

Table S1 Physicochemical properties according to soil compartment.

Site	AOD stage	Soil Compartment	Moisture (%) *	pH (H ₂ O) ***	Total C (mg/kg) ***	Total N (mg/kg) ***	C:N ratio ***
Eastnor	Low	Rhizosphere	25 ± 6 ab	5.68 ± 0.49 ac	60,740 ± 11,450 a	4,629 ± 651 a	13.04 ± 1.06 ad
		Bulk soil	28 ± 2 b	5.62 ± 0.26 a	34,401 ± 5,065 bd	2,908 ± 376 b	11.81 ± 0.64 bd
Hatchlands	Mid	Rhizosphere	22 ± 3 a	5.92 ± 0.73 a	64,216 ± 9,660 a	4,581 ± 793 a	14.08 ± 0.80 a
		Bulk soil	29 ± 4 b	5.93 ± 0.47 a	42,666 ± 8,150 b	3,860 ± 931 ab	11.17 ± 0.54 b
Richmond	Severe	Rhizosphere	27 ± 6 ab	4.33 ± 0.14 b	225,966 ± 55,586 c	12,921 ± 3,331 c	17.56 ± 0.55 c
		Bulk soil	15 ± 3 c	5.12 ± 0.07 c	27,328 ± 847 d	2,064 ± 102 d	13.25 ± 0.28 ad

Mean values of soil physicochemical parameters ± standard deviation are shown ($n = 36$). Asterisks and letters indicate significant differences as determined by statistical analysis (* for $P < 0.05$; ** for $P < 0.01$; *** for $P < 0.001$).

Table S2 Microbial alpha diversity and total abundance according to soil compartment across the sites.

Microbial Group	Site	AOD stage	Soil Compartment	Chao 1	Observed Species	Shannon	Evenness	Simpson	Total abundance
Bacteria	Eastnor	Low	Rhizosphere	7,840 ± 804 a	4,234 ± 492 a	9.92 ± 0.37 a	0.82 ± 0.02 a	0.996 ± 0.001 ab	11.02 ± 0.11 a
			Bulk soil	7,506 ± 412 ad	4,143 ± 392 a	9.97 ± 0.31 a	0.83 ± 0.02 a	0.997 ± 0.001 a	10.92 ± 0.10 a
	Hatchlands	Mid	Rhizosphere	7,721 ± 682 a	4,326 ± 426 a	10.06 ± 0.31 a	0.83 ± 0.02 a	0.997 ± 0.001 a	11.08 ± 0.05 a
			Bulk soil	6,396 ± 229 b	3,519 ± 212 ab	9.51 ± 0.24 ab	0.81 ± 0.01 ab	0.995 ± 0.001 b	10.94 ± 0.14 a
	Richmond	Severe	Rhizosphere	5,478 ± 746 c	2,996 ± 441 b	8.77 ± 0.42 c	0.76 ± 0.02 c	0.988 ± 0.004 c	11.09 ± 0.05 a
			Bulk soil	6,618 ± 338 bd	3,598 ± 226 ab	9.17 ± 0.20 bc	0.78 ± 0.01 bc	0.990 ± 0.004 c	11.00 ± 0.05 a
Fungi	Eastnor	Low	Rhizosphere	1,187 ± 190 a	875 ± 152 a	6.77 ± 0.43 ab	0.69 ± 0.03 ab	0.974 ± 0.008 ab	9.08 ± 0.20 bc
			Bulk soil	1,062 ± 141 ac	807 ± 107 ab	6.46 ± 0.20 ab	0.67 ± 0.01 ab	0.966 ± 0.008 ab	8.87 ± 0.06 ac
	Hatchlands	Mid	Rhizosphere	1,125 ± 97 a	883 ± 70 a	6.94 ± 0.32 a	0.71 ± 0.03 a	0.976 ± 0.006 a	8.92 ± 0.26 abc
			Bulk soil	853 ± 130 b	710 ± 90 ab	6.44 ± 0.40 ab	0.68 ± 0.03 ab	0.959 ± 0.016 ab	8.64 ± 0.16 a
	Richmond	Severe	Rhizosphere	1,140 ± 102 a	767 ± 43 ab	6.27 ± 0.25 b	0.65 ± 0.02 b	0.961 ± 0.018 b	9.46 ± 0.15 d
			Bulk soil	896 ± 74 bc	674 ± 60 b	6.52 ± 0.29 ab	0.69 ± 0.02 ab	0.970 ± 0.011 ab	9.00 ± 0.07 b

Mean values ± standard deviation are shown ($n = 36$). Chao1 and Observed species (expressed as number of OTUs) indicate species richness. Pielou's Evenness, Shannon, and Simpson indexes indicate species diversity. Total abundance (qPCR) is expressed as log copy number of 16S or ITS genetic markers/g of soil. Significant differences ($P < 0.05$) are indicated by letters. The statistical analyses were performed for bacterial and fungal communities separately.

Table S5 Microbial alpha diversity and total abundance of the oak rhizosphere soils across the sites and tree health conditions.

Microbial Group	Site	AOD stage	Tree health condition	Chao 1	Observed Species	Shannon	Evenness	Simpson	Total abundance
Bacteria	Eastnor	Low	Healthy	8,358 ± 804 a	4,566 ± 477 a	10.14 ± 0.39 a	0.83 ± 0.02 a	0.997 ± 0.001 ab	11.11 ± 0.05 ab
			AOD	7,323 ± 334 ac	3,903 ± 191 ab	9.70 ± 0.16 a	0.81 ± 0.01 a	0.996 ± 0.001 b	10.93 ± 0.07 c
	Hatchlands	Mid	Healthy	7,974 ± 466 a	4,570 ± 319 a	10.30 ± 0.25 a	0.85 ± 0.01 a	0.997 ± 0.001 a	11.11 ± 0.05 a
			AOD	7,469 ± 766 a	4,082 ± 377 a	9.82 ± 0.12 a	0.82 ± 0.00 a	0.996 ± 0.000 b	10.93 ± 0.07 b
	Richmond	Severe	Healthy	5,292 ± 765 b	2,950 ± 371 b	8.73 ± 0.23 b	0.76 ± 0.01 b	0.988 ± 0.002 c	11.04 ± 0.03 b
			AOD	5,664 ± 677 bc	3,041 ± 498 b	8.81 ± 0.54 b	0.76 ± 0.03 b	0.988 ± 0.005 c	11.13 ± 0.03 a
	Eastnor	Low	Healthy	1,181 ± 178 a	905 ± 128 a	6.84 ± 0.44 ab	0.70 ± 0.03 ab	0.974 ± 0.010 a	9.03 ± 0.14 ab
			AOD	1,193 ± 201 a	844 ± 167 a	6.69 ± 0.40 abc	0.69 ± 0.02 ab	0.975 ± 0.010 a	9.13 ± 0.24 abc
Fungi	Hatchlands	Mid	Healthy	1,101 ± 77 a	915 ± 66 a	7.15 ± 0.24 a	0.73 ± 0.02 a	0.980 ± 0.004 a	8.71 ± 0.13 a
			AOD	1,149 ± 109 a	851 ± 57 a	6.72 ± 0.23 abc	0.69 ± 0.02 ab	0.973 ± 0.006 a	9.13 ± 0.16 abc
	Richmond	Severe	Healthy	1,173 ± 78 a	777 ± 40 a	6.20 ± 0.33 bc	0.65 ± 0.03 b	0.954 ± 0.022 a	9.49 ± 0.20 c
			AOD	1,108 ± 113 a	757 ± 44 a	6.34 ± 0.07 c	0.66 ± 0.01 b	0.969 ± 0.002 a	9.42 ± 0.06 bc

Mean values ± standard deviation are shown ($n = 24$). Chao1 and Observed species (expressed as number of OTUs) indicate species richness. Pielou's Evenness, Shannon, and Simpson indexes indicate species diversity. Total abundance (qPCR) is expressed as log copy number of 16S or ITS genetic markers/g of soil. Significant differences ($P < 0.05$) are indicated by letters. The statistical analyses were performed for bacterial and fungal communities separately.

Table S6 PERMANOVA and pairwise comparison results of the comparison of the bacterial and fungal community according to site and tree health condition ($n = 24$).

Microbial Group	Factor	Pseudo-F	P-value (perm)
Bacteria	Si	10.53	$< 1 \times 10^{-5}$
	Tr	3.37	0.0181
	Si \times Tr	1.51	0.1416
Fungi	Si	6.43	$< 1 \times 10^{-5}$
	Tr	1.90	0.0426
	Si \times Tr	1.29	0.1522
Microbial Group	Pairwise	<i>t</i>	P-value (perm)
Bacteria	Eastnor-Hatchlands	1.33	0.0791
	Eastnor-Richmond	3.77	$< 2 \times 10^{-5}$
	Hatchlands-Richmond	4.01	$< 2 \times 10^{-5}$
	Healthy-AOD (E)	2.85	0.0288
	Healthy-AOD (H)	2.60	0.0357
	Healthy-AOD (R)	0.81	0.9715
Fungi	Eastnor-Hatchlands	1.62	0.0003
	Eastnor-Richmond	2.85	$< 1 \times 10^{-5}$
	Hatchlands-Richmond	3.06	$< 3 \times 10^{-5}$
	Healthy-AOD (E)	1.80	0.0081
	Healthy-AOD (H)	1.64	0.0421
	Healthy-AOD (R)	1.07	0.3151

Si–Site; Tr–Tree health condition; E–Eastnor; H–Hatchlands; R–Richmond

Table S7 Combinations of rhizosphere soil explanatory variables that best explain the variance in the rhizosphere microbiome ($n = 24$).

Microbial group	Environmental variable(s) (no. of variables)	Spearman's coefficient (rs)*
Bacteria	pH, C, C:N (3)	0.896
	pH (1)	0.895
	pH, C (2)	0.892
	pH, C:N (2)	0.892
	pH, N, C:N (3)	0.889
Fungi	pH, C:N (2)	0.848
	pH, C, C:N (3)	0.827
	pH (1)	0.825
	pH, N, C:N (3)	0.817
	pH, C (2)	0.799

* Significant differences ($P < 0.01$) were obtained in all the combinations