ACE protein as a candidate proxy for microbial functional processes in contrasting Australian cropping soils

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Supplementary Information.

Biology and Fertility of Soils

Table S1. Description of field site locations, aims and treatments applied

Site	Year established	Management Aim	Treatments
Birchip Cropping Group	2018	To determine if herbicide use affects soil and crop health	Control (no herbicide application)
			In-crop Herbicide Regime 1
			In-crop Herbicide Regime 1 + Fallow spray
			In-crop Herbicide Regime 2
			In-crop Herbicide Regime 2 + Fallow spray
Minnipa Agricultural Centre	2021	To lift yields in calcareous soils through addressing soil chemical and biological constraints	Typical practice (Control)
			P + Trace Elements delivered as a fluid
			Fungicide + TEs chelates + fluid P and N
			Banded bespoke biochar + Urea
			Granular N and P to match Biochar + Urea
Central West Farming Systems	2019	To improve soil physical, nutritional and biological activities through increased plant diversity	Control – lime
			Control + lime
			Pulse rotation + lime
			Undersown + lime
			Summer Cover Crop + lime
Wheatbelt NRM	Prior to 2019	To determine differences in soil biological activities related to adoption of regenerative ag. practices	‘Regenerative’ practices – Site 1
			Paired 'Conventional' practices – Site 1
			‘Regenerative’ practices – Site 2
			Paired 'Conventional' practices – Site 2

Table S2. Envfit results for the best set of soil physicochemical variables that explain enzyme activities

Predictor	Dim1	Dim2	R^2	P
pH	0.97325	-0.22977	0.809	< 0.001
TC	0.93316	-0.35947	0.6573	< 0.001
ACE protein	-0.99712	-0.0758	0.6108	< 0.001
ResC	0.65488	-0.75574	0.4999	< 0.001
CN	0.9989	0.04687	0.4867	< 0.001
MinN	-0.96172	-0.27403	0.0805	< 0.001

Table S3. Results of BIOENV analysis identifying the best subsets of environmental variables explaining variation in soil enzyme activities (chitinase, phosphatase, esterase, leucine aminopeptidase, arylsulfatase and glucosidase) measured at standardised and native pH, where model size is the number of predictors in each subset and the Spearman rank correlation between biological and environmental distance matrices is expressed as a percentage

Model predictors	Model size	Correlation (%)
pH	1	59.80
TC, ACE protein	2	70.48
pH, TC, ACE protein	3	70.38
pH, TC, CN, ACE protein	4	70.42
pH, TC, CN, MinN, ACE protein	5	70.72
ResC, pH, TC, CN, MinN, ACE protein	**6**	**71.00**
CharC, pH, TC, TN, CN, MinN, ACE protein	7	70.13
CharC, ResC, pH, TC, TN, CN, MinN, ACE protein	8	69.30
POXC, CharC, ResC, pH, TC, TN, CN, MinN, ACE protein	9	67.99
POXC, TOC, CharC, ResC, pH, TC, TN, CN, MinN, ACE protein	10	66.88
POXC, TOC, CharC, ResC, pH, TC, TN, CN, WSC, MinN, ACE protein	11	65.43
POXC, TOC, CharC, ResC, EC, pH, TC, TN, CN, WSC, MinN, ACE protein	12	63.88
POXC, TOC, CharC, ResC, EC, pH, TC, TN, CN, WSC, HWEOC, MinN, ACE protein	13	61.58
POXC, TOC, POC, CharC, ResC, EC, pH, TC, TN, CN, WSC, HWEOC, MinN, ACE protein	14	59.28
POXC, TOC, POC, CharC, ResC, EC, pH, TC, TN, CN, WSC, HWEOC, MinN, MinNT14, ACE protein	15	57.14
POXC, TOC, POC, CharC, ResC, EC, pH, TC, TN, CN, WSC, HWEOC, MinN, MinNT7, MinNT14, ACE protein	16	53.82

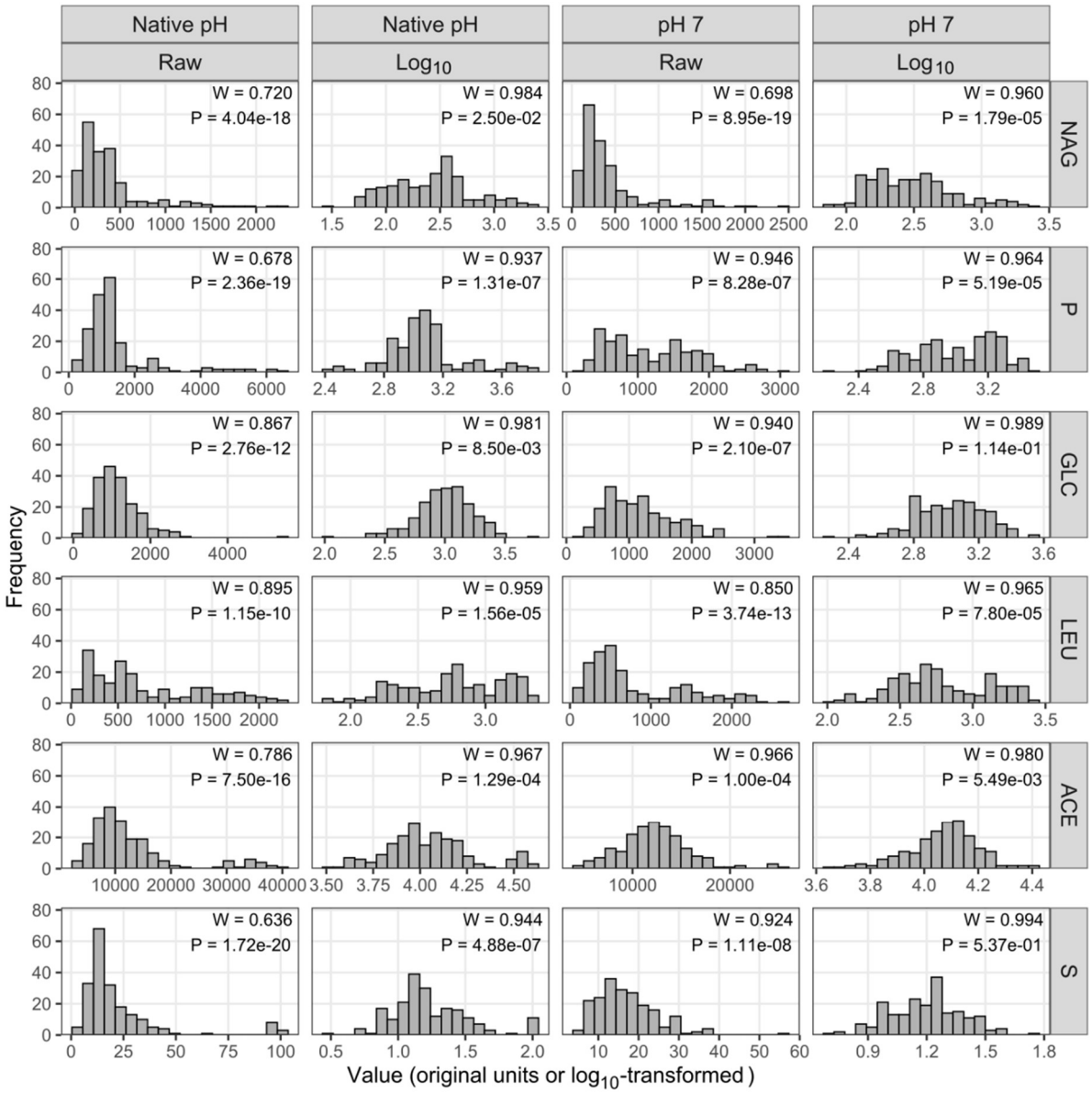


Fig. S1 Distributions of raw and log_{10}-transformed chitinase (NAG), phosphatase (P) and β-glucosidase (GLC), leucine aminopeptidase (LEU), esterase (ACE) and arylsulfatase (S) enzyme measures at pH 7 versus native soil pH (suffix ‘spH) alongside Shapiro-Wilk results

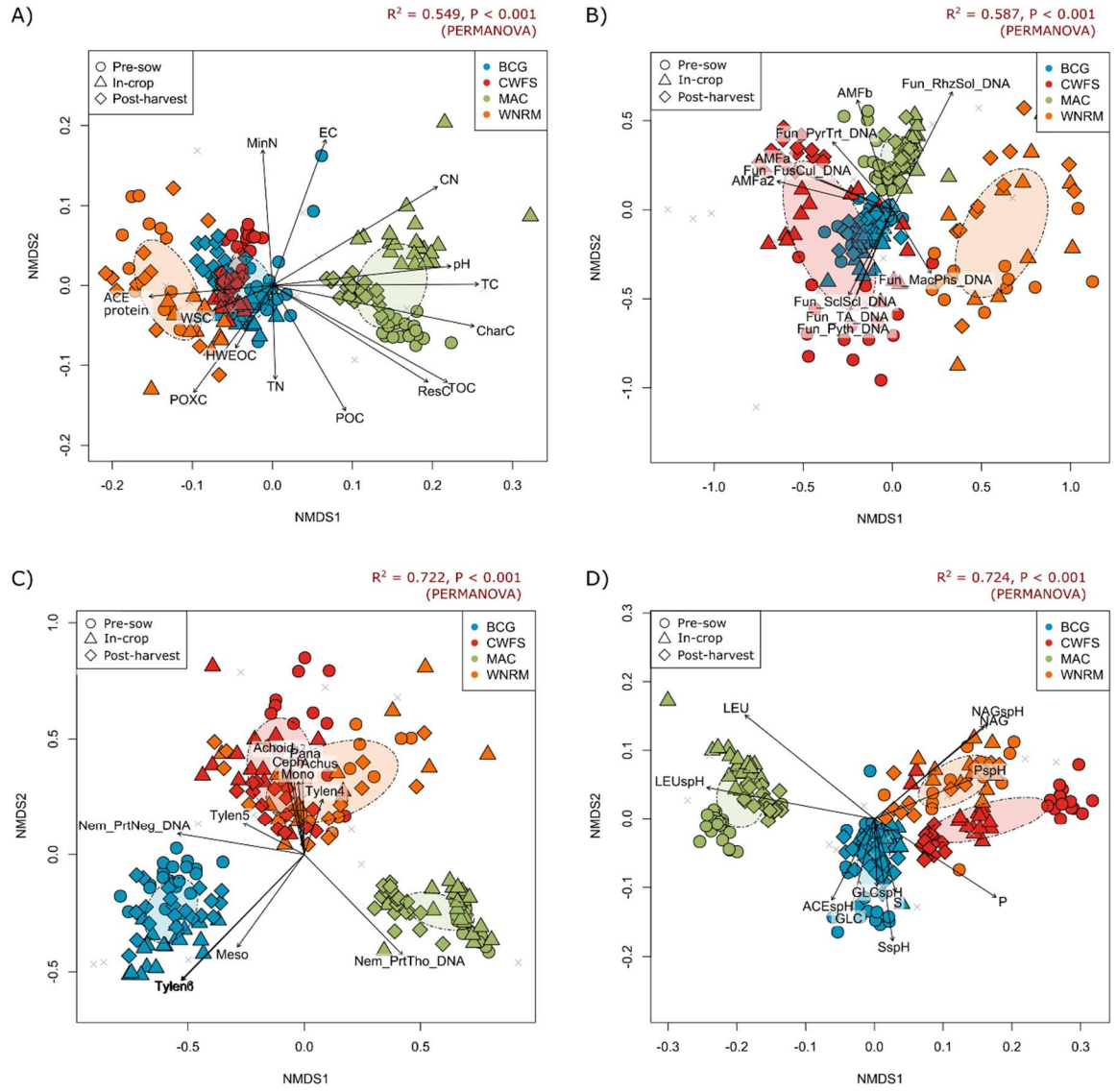


Fig. S2 Non-metric multidimensional scaling (NMDS) ordinations showing distributions of A) physicochemical variables, B) fungal community composition, C) nematode community composition and D) functional enzyme indicators by site and sampling time

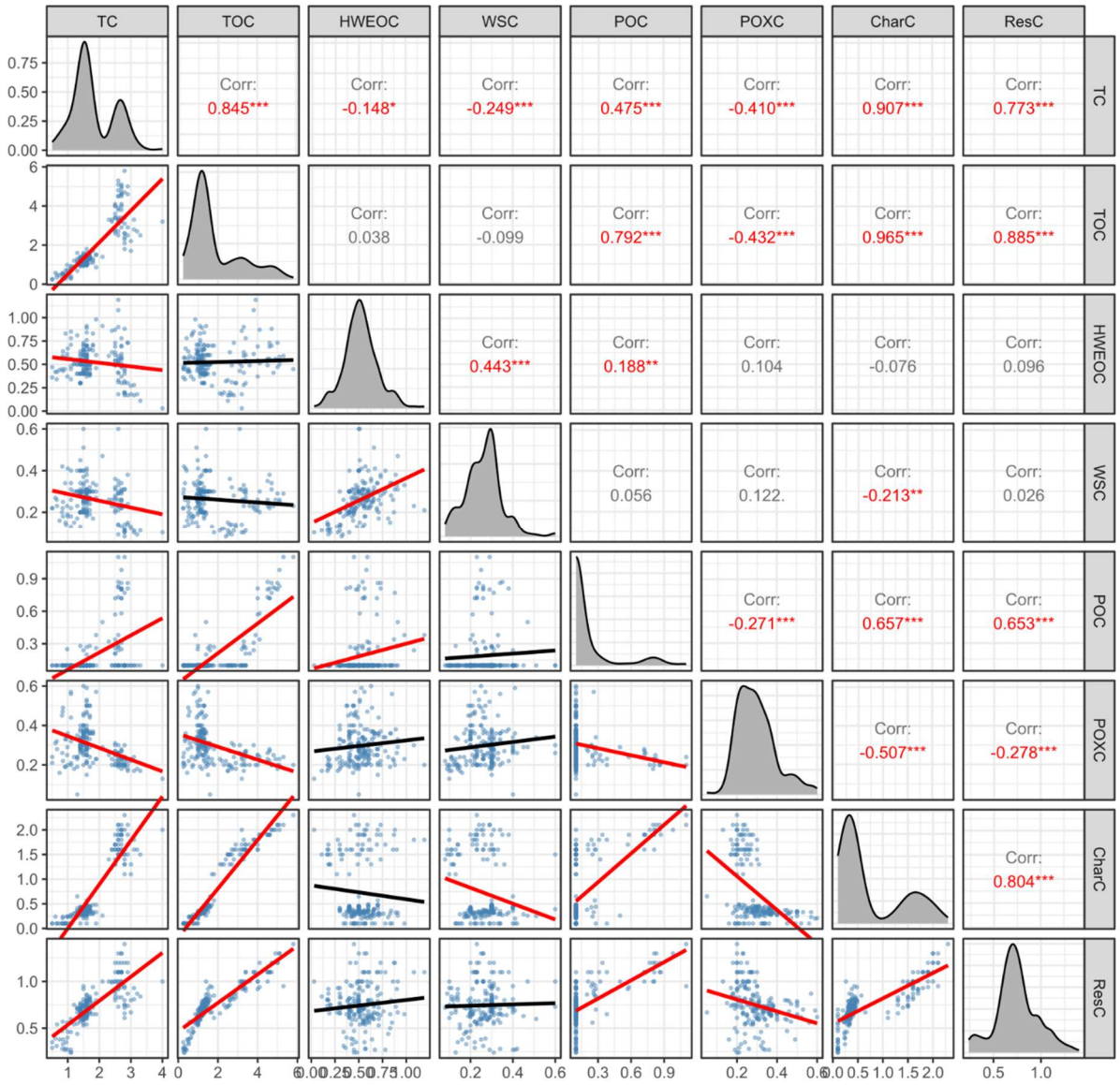


Fig. S3 Relationships between carbon fractions across sites and times, distribution plots of each variable alongside ANOVA effect size and significance notation where * = P < 0.05, ** = P < 0.01 and *** = P < 0.001, red lines and text represent significant (P < 0.05) relationships while black lines and text represent non-significant (P > 0.05) relationships

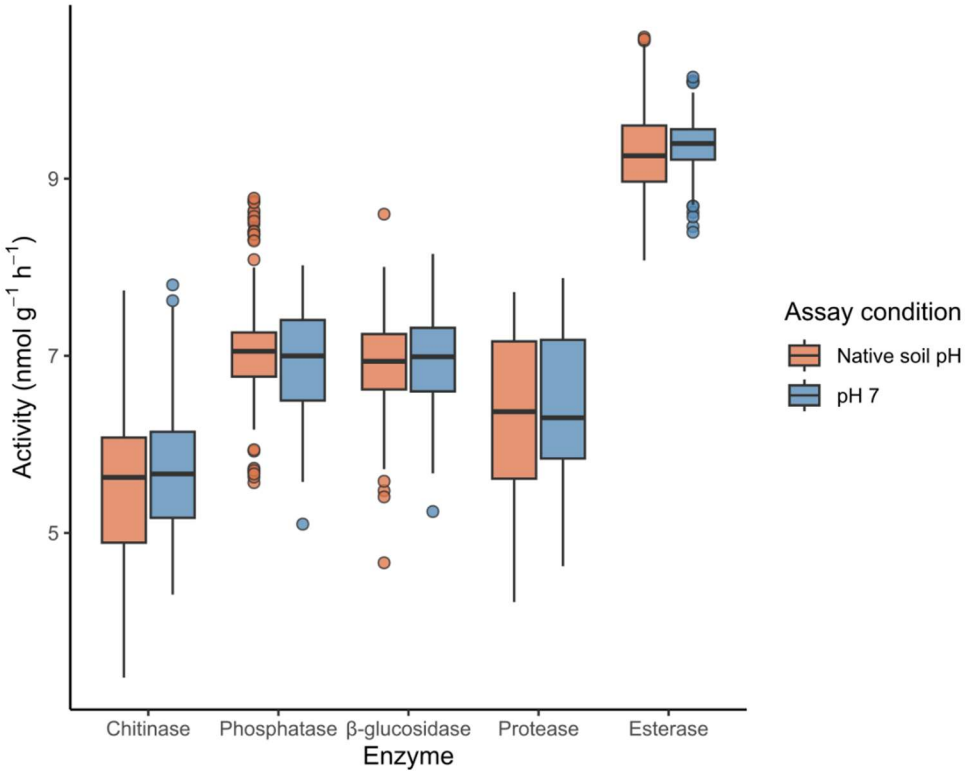


Fig. S4 Boxplot distributions of soil enzyme activities measured under native soil pH and standardised pH 7 assay conditions across all sites, showing strong Pearson correlations for chitinase ($r = 0.946$, $P < 0.001$), phosphatase ($r = 0.696$, $P < 0.001$), glucosidase ($r = 0.716$, $P < 0.001$), leucine aminopeptidase ($r = 0.870$, $P < 0.001$) and moderate for esterase ($r = 0.317$, $P < 0.001$)

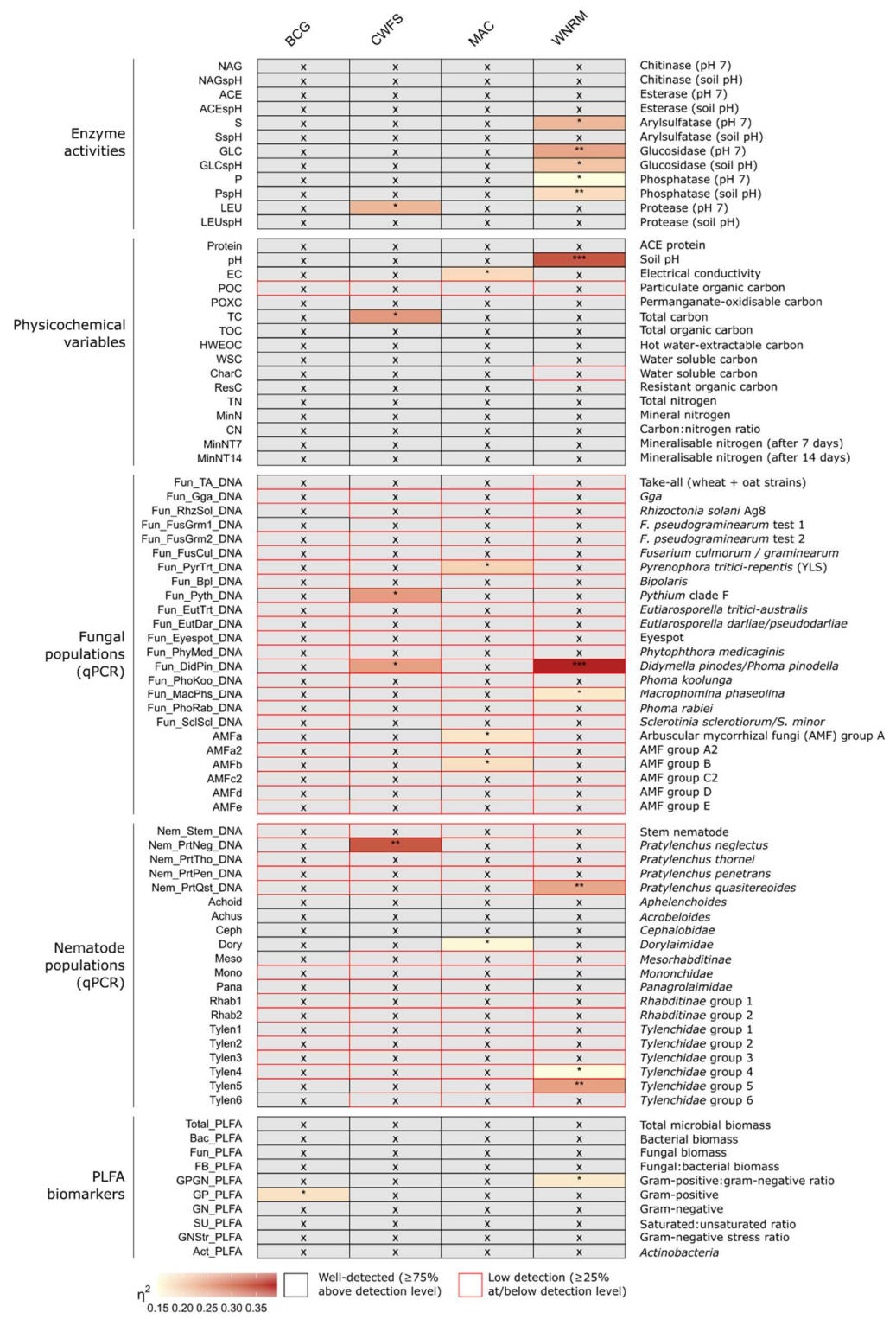


Fig. S5 Treatment effects on soil variables by site where significance notation: *, ** and *** represent significance at P < 0.05, P < 0.01 and P < 0.001, respectively, x represents non-significant (P > 0.05) treatment effects, tile colour represents ANOVA effect size, black borders represent well-detected variables (≥75% samples above detection level) while red borders around tiles represent variables of low detection levels (≥25% samples at/near detection level) where significance should be interpreted with caution

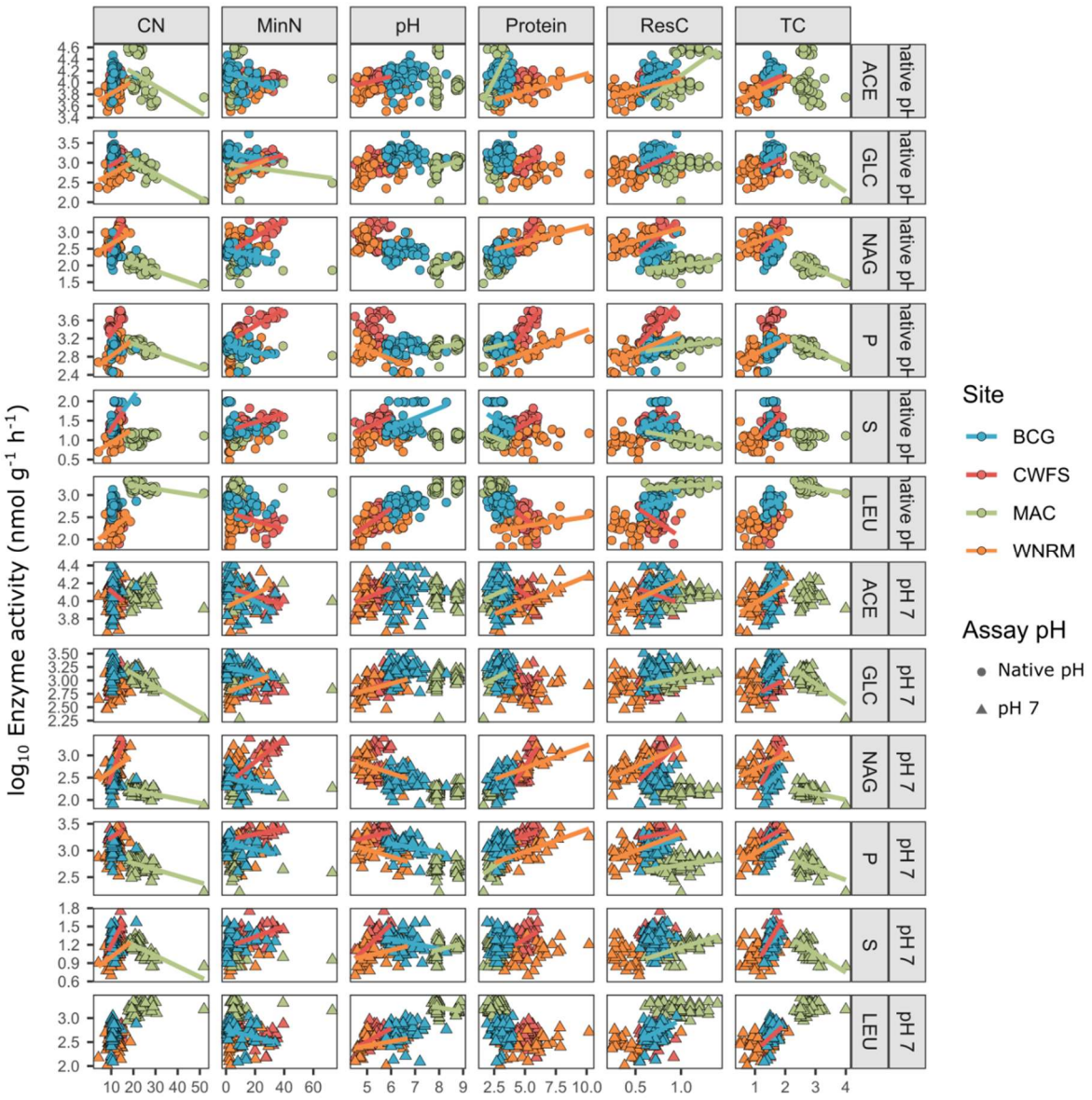


Fig. S6 Relationships between environmental predictor variables and enzymes (log_{10}-transformed nmol g $soil^{-1}$ hr^{-1}) measured at pH 7 and native soil pH by study site where the presence of a linear trend line indicates a significant relationship ($P < 0.05$, ANOVA)

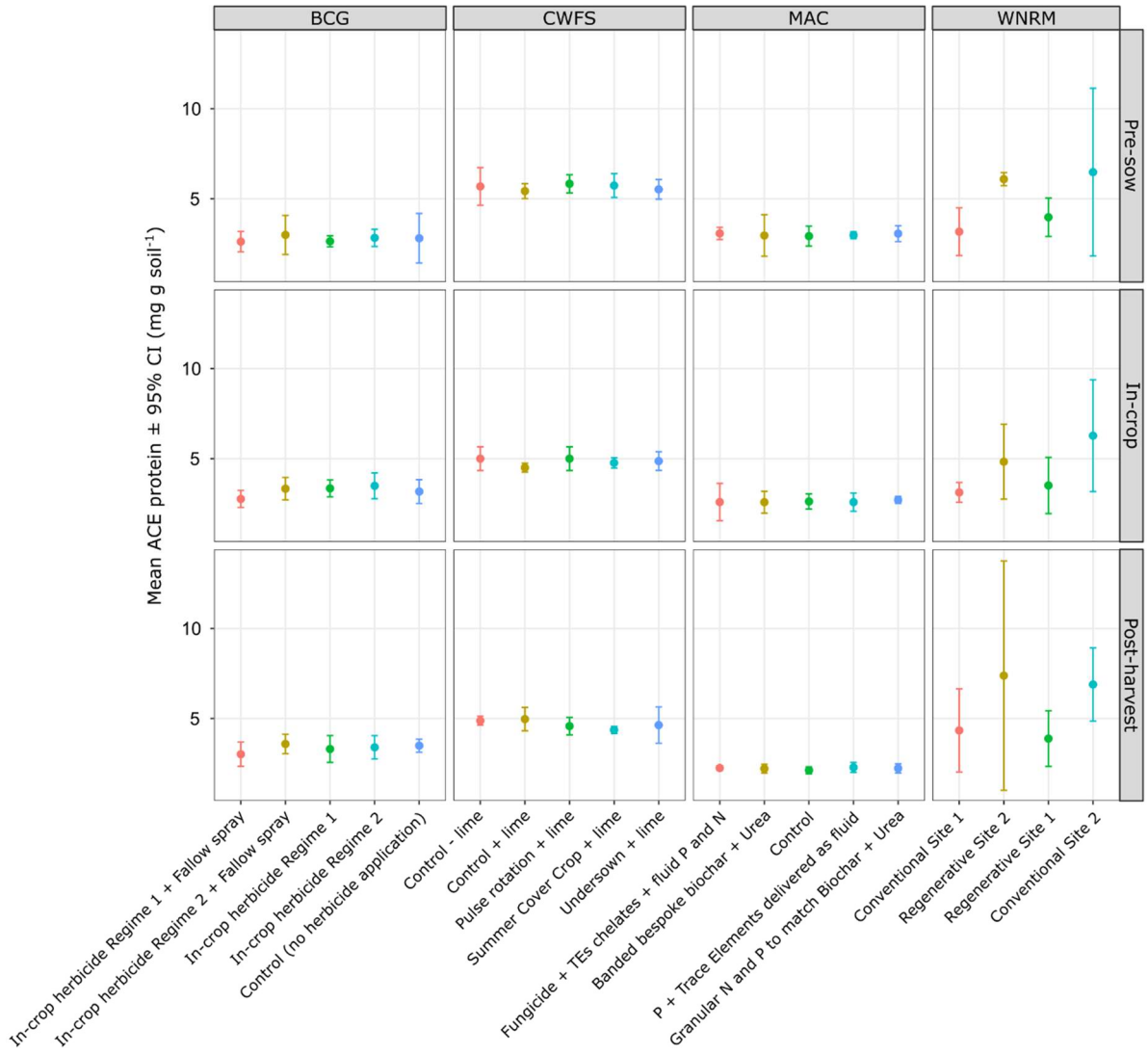


Fig. S7 Treatment effects on mean soil ACE protein content (mg g soil^{-1}) ± 95% confidence interval by treatment by site and sampling time. Error bars represent the 95% CI of the mean