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Supplement of

Soil microbial biomass and function are altered by 12 years of crop rotation

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Cropping Biodiversity Gradient Kellogg Biological Station LTER

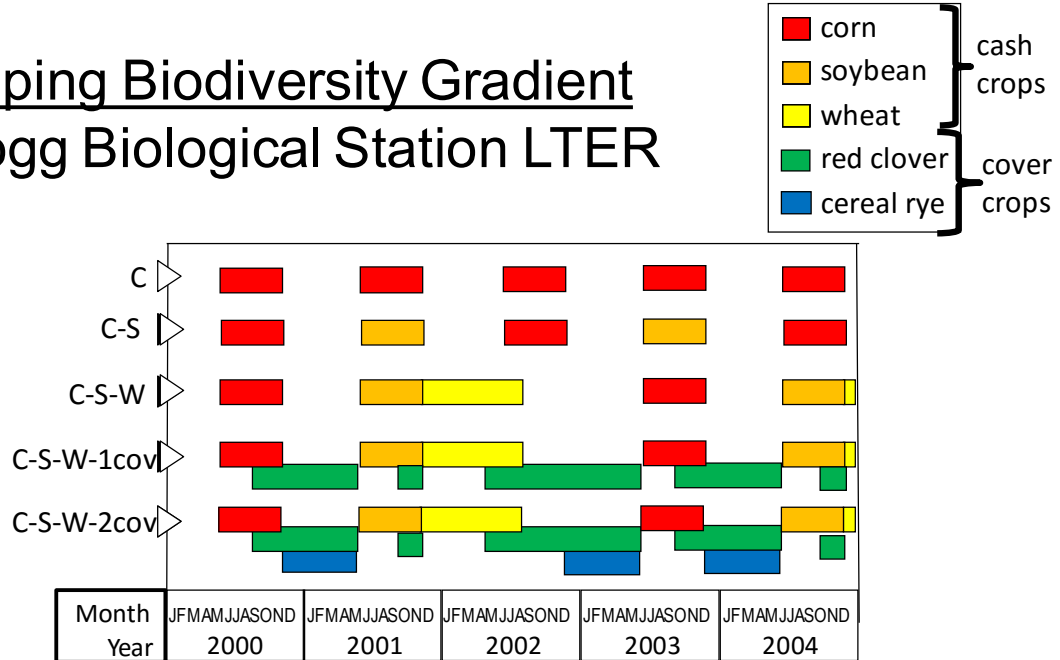


Figure S1. Schematic diagram showing the crop sequences for the five crop rotation treatments sampled in this study. This figure was adapted from Smith et al. (2008), where more details on the cropping systems can be found.

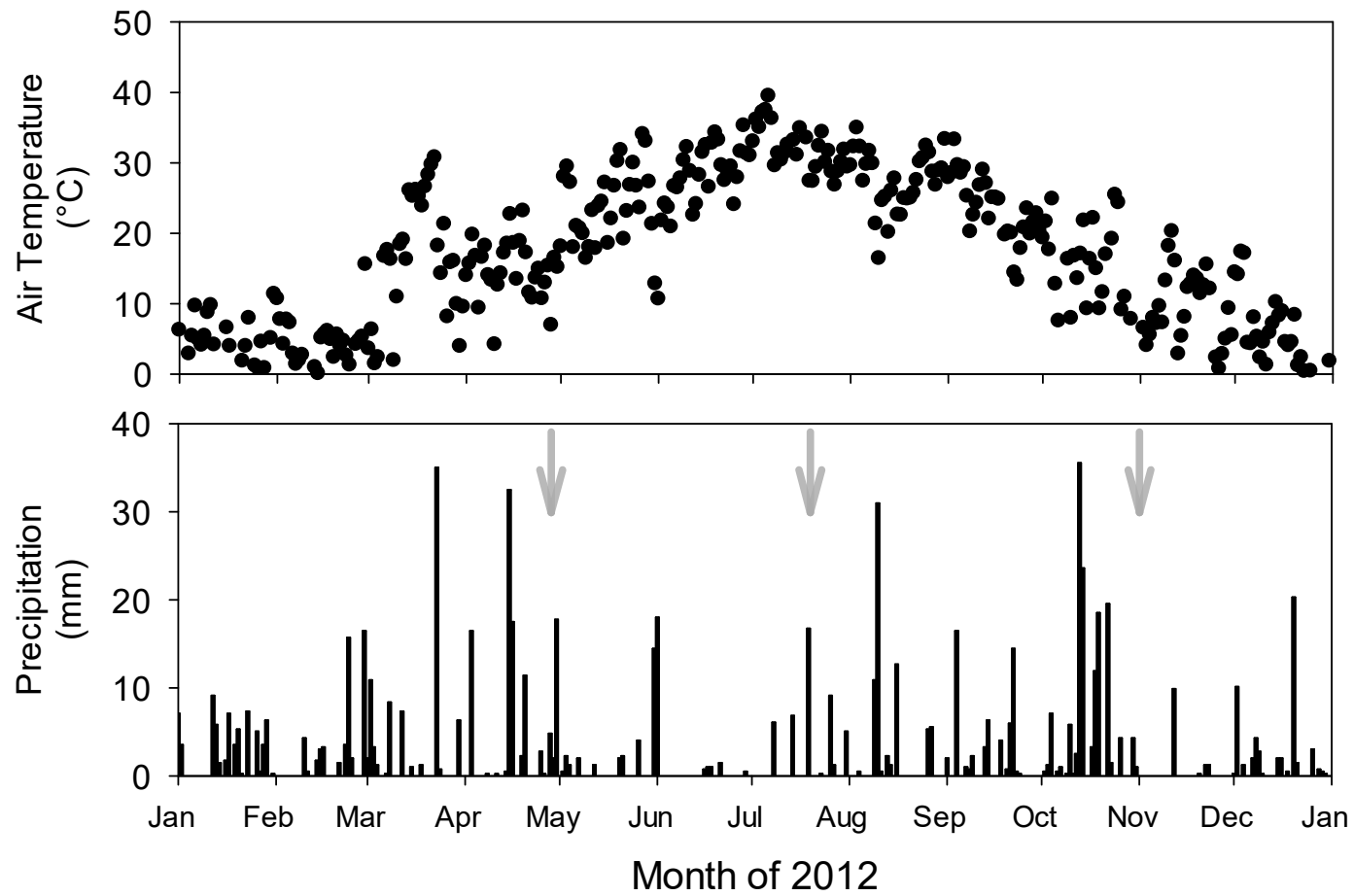


Figure S2. Daily air temperatures (Top Panel) and precipitation (Bottom Panel) from the W.K. Kellogg Biological Station Long-term Ecological Research station (KBS 2013). Dark gray down-arrows indicate when spring, summer, and autumn samples were collected.

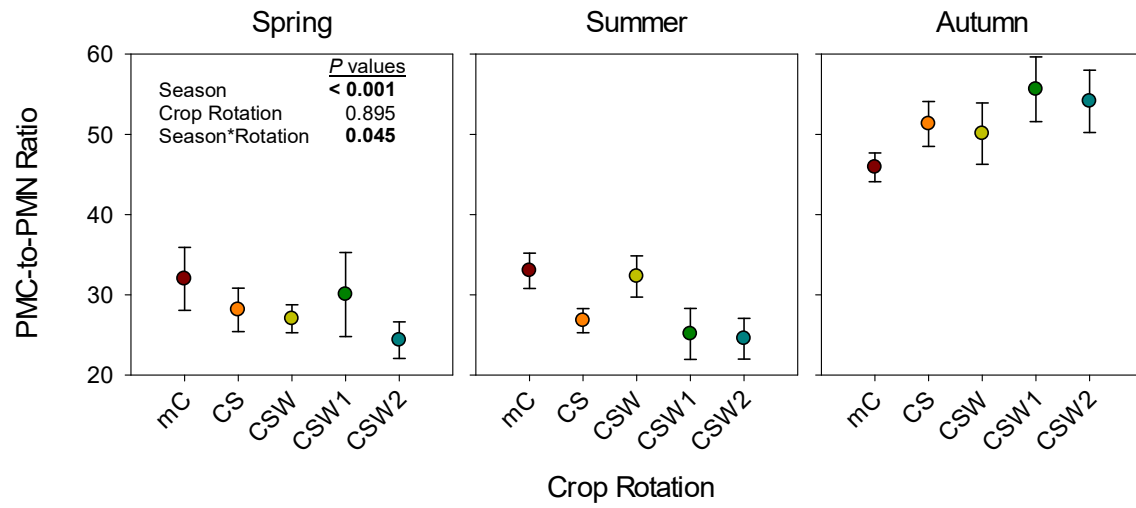


Figure S3. The potentially mineralizable C to potentially mineralizable net N ratio. See Fig. S3 for crop rotation abbreviations. Means are shown and error bars are standard errors (n = 4).

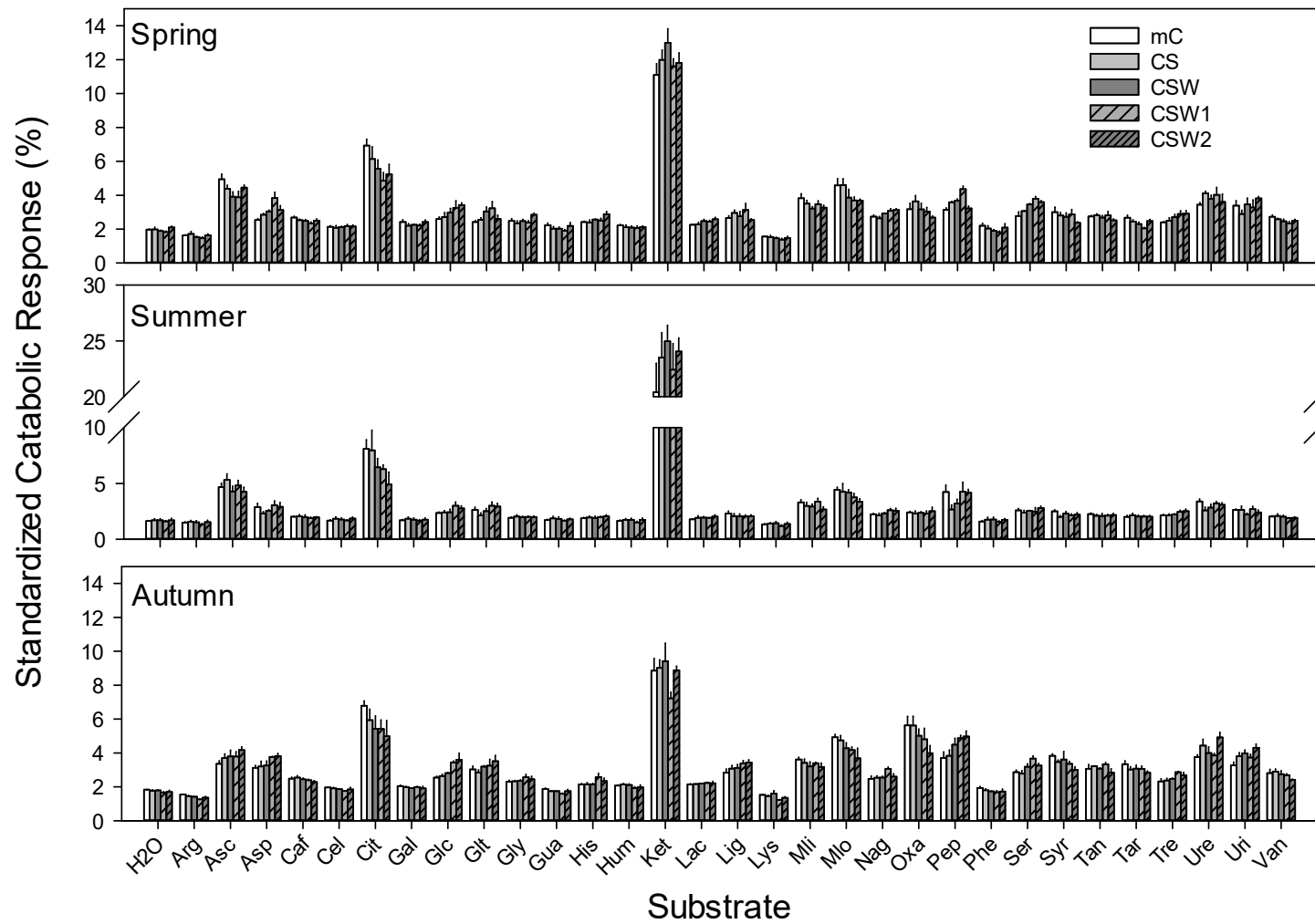


Figure S4. Standardized catabolic response (Respiration rate of Specific Substrate / Respiration rate of All Substrates * 100). Respiration rates were in units of $\mu\text{g CO}_2\text{-C}$ per gram of dry soil. For substrate abbreviations see Table 1 in manuscript.

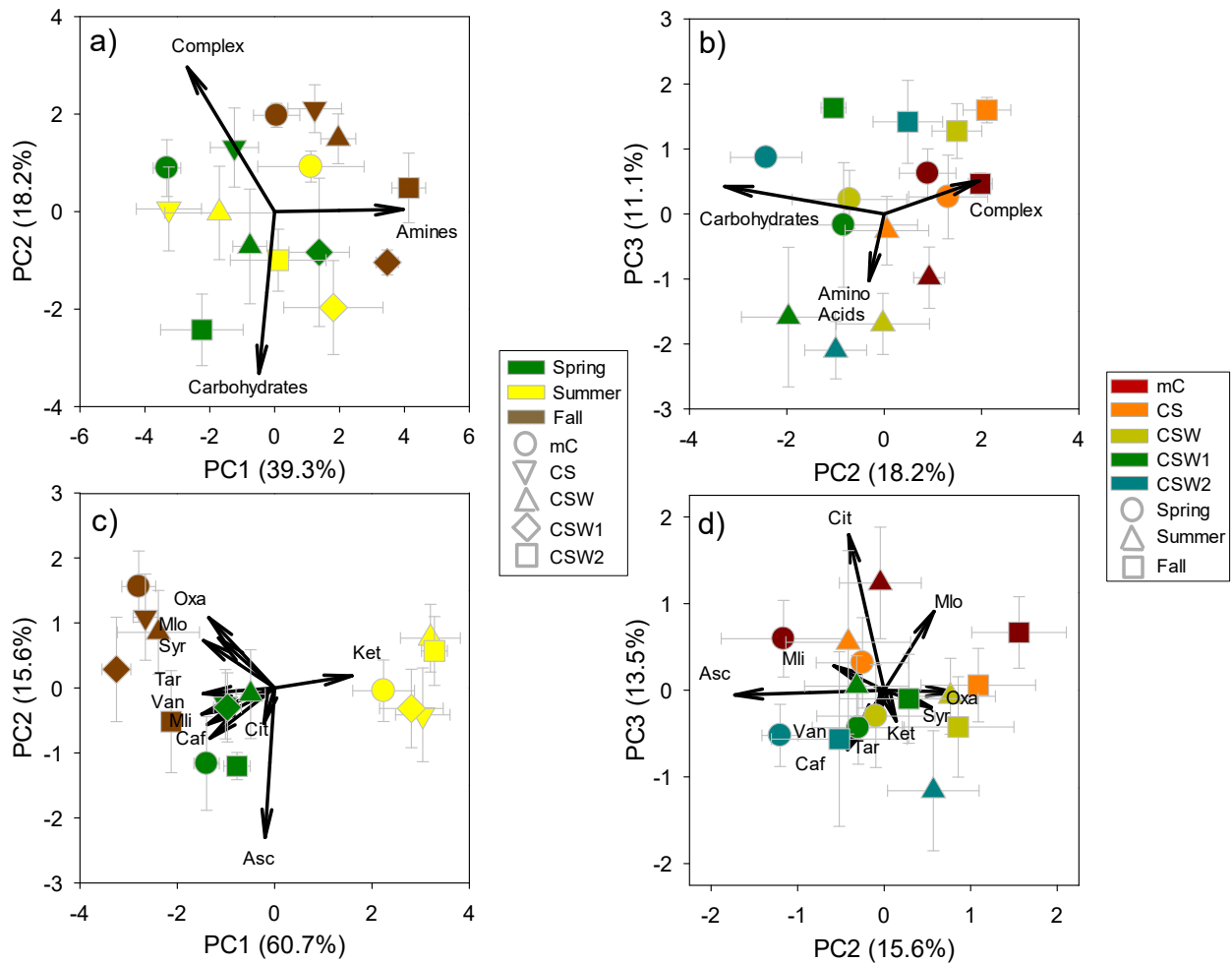


Figure S5. Principal components analysis (PCA) on all substrates except for carboxylic acids [a and b)] and only carboxylic acids [c) and d)]. Principal components 1 and 2 [a) and c)], where Season is dominant discriminating factor ($P < 0.001$) and Principal components 2 and 3 [b) and d)] where Rotation is highlighted as a dominant discriminating factor. See also Table 5 for PCA and ANOVA results. Crop rotation abbreviations are: monoculture corn (mC), corn-soy (CS), corn-soy-wheat (CSW), corn-soy-wheat with red clover cover crop (CSW1), and corn-soy-wheat with red clover + rye cover crops (CSW2). The carboxylic acid abbreviations are: ascorbic acid (Asc), caffeic acid (Caf), citric acid (Cit), ketobutyric acid (Ket), malic acid (Mli), malonic acid (Mlo), oxalic acid (Oxa), syringic acid (Syr), tartaric acid (Tar), and vanillic acid (Van). Data are means ($n = 4$) with standard errors of PC loading values.

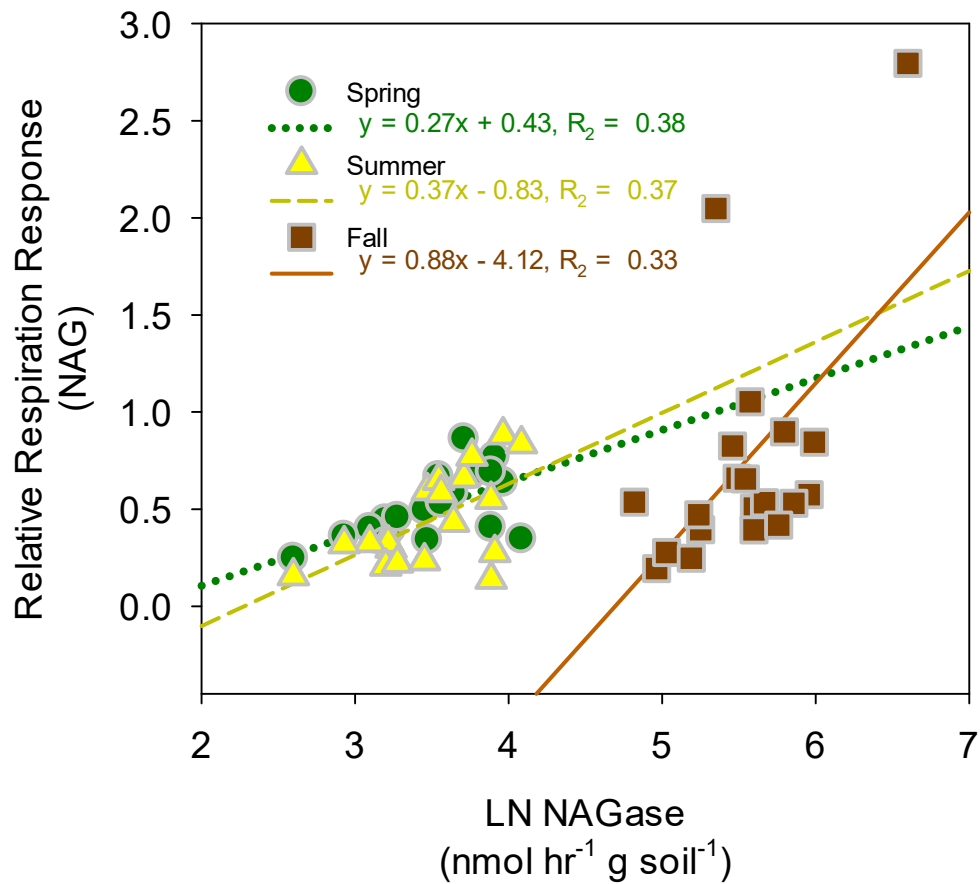


Figure S6. Regression between NAGase enzyme activity and respiration response to adding NAG. Log normal (LN) N-acetyl glucosaminidase extracellular enzyme activity, on the x-axis, and the N-acetyl glucosamine Relative Respiration Response $[(R_{\text{sub}} - R_{\text{H}_2\text{O}}) / R_{\text{H}_2\text{O}}]$ from the community-level physiological profile data, on the y-axis. Lines are linear regressions through the data (all P values on fits were less than 0.008).

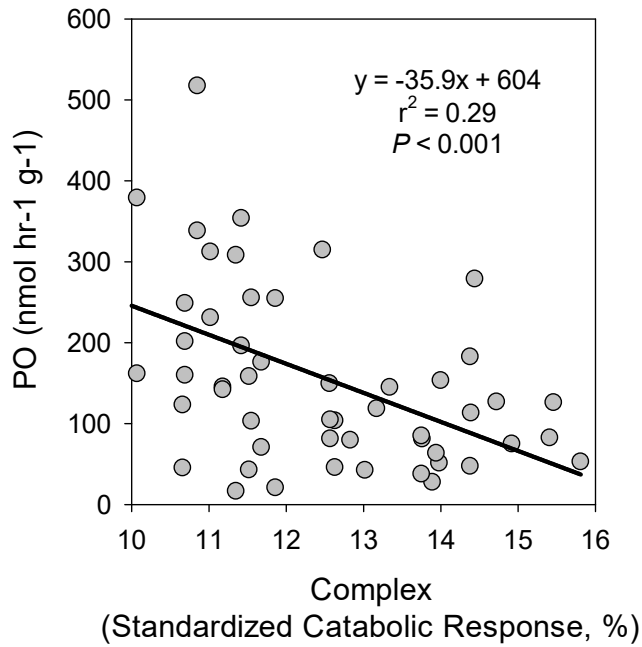
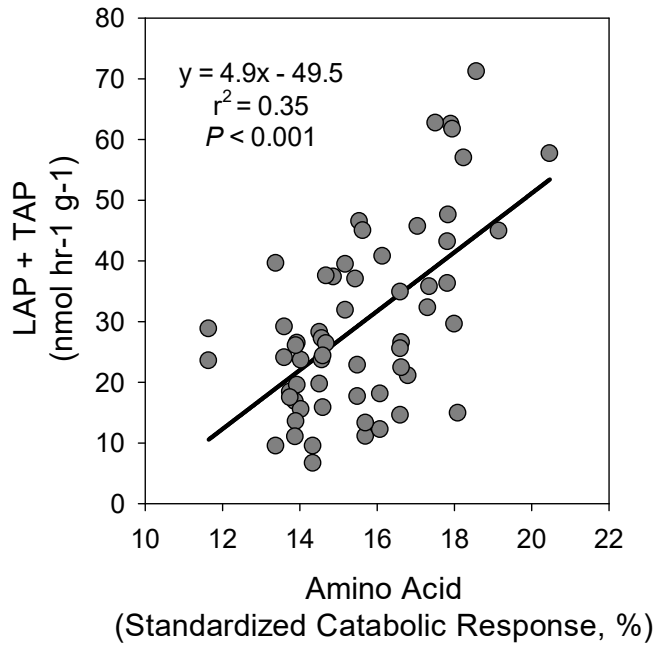


Figure S7. Relationships between substrate guild (Complex or Amino Acid) catabolic response and extracellular enzyme activities (EEA). EEA abbreviations are: Tyrosine aminopeptidase (TAP), Leucine aminopeptidase (LAP), and phenol oxidase (PO).

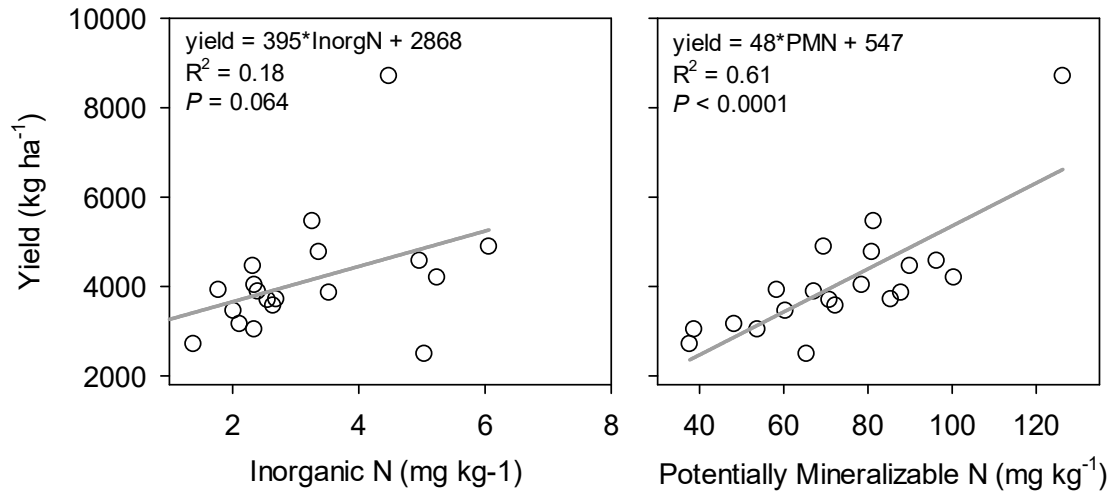


Figure S8. Relationships between spring soil nitrogen and 2012 yield in corn. *Note:* Inorganic N = NO₃⁻-N + NH₄-N.

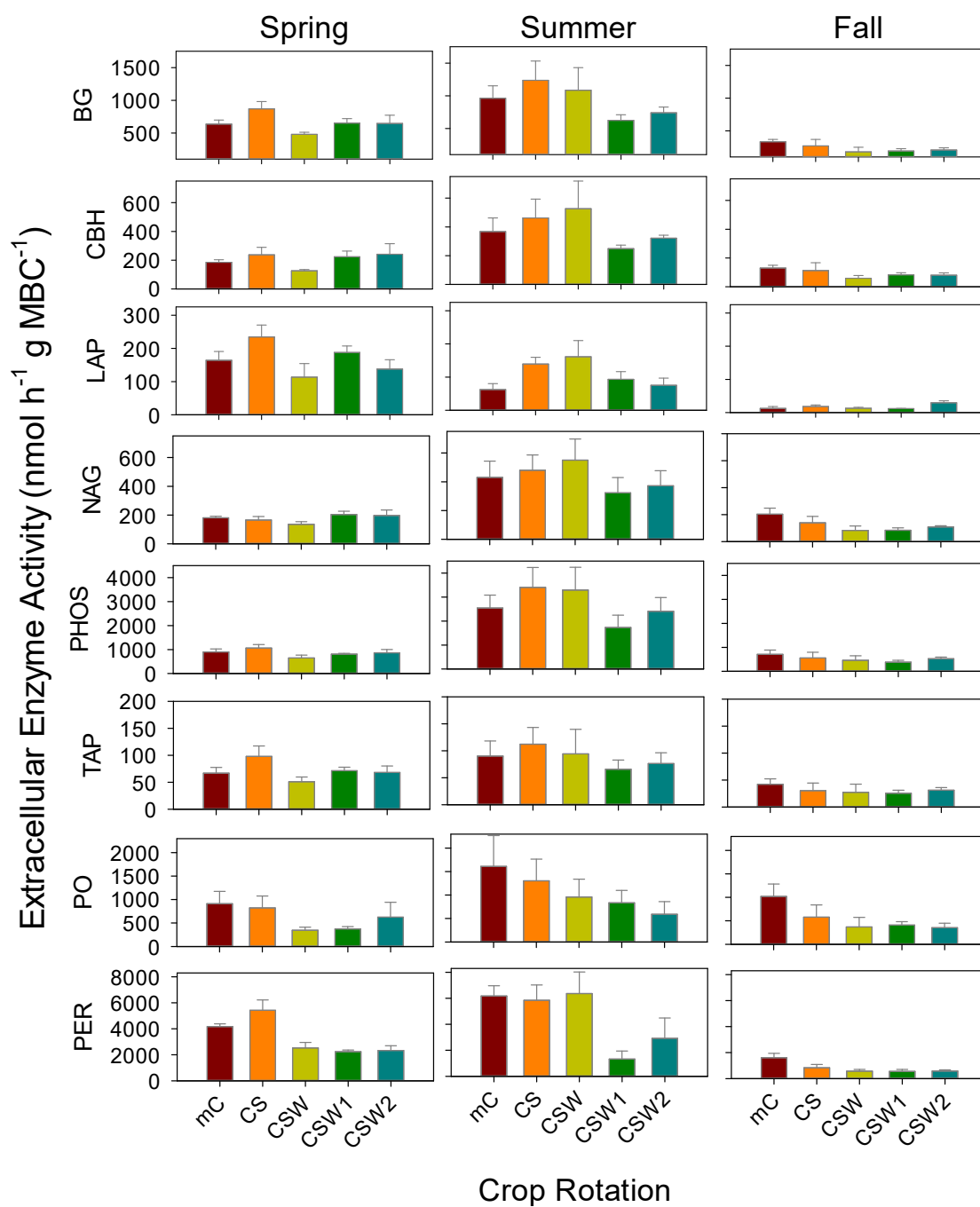


Figure S9. Soil extracellular enzyme activity expressed per unit of soil microbial biomass carbon (MBC). See Fig. S3 for crop rotation abbreviations. Means are shown and error bars are standard errors (n = 4).

Table S1. List of substrates used for the community-level physiological profile (CLPP).

Substrate Name	Abbreviation	Molecular weight	C:N	Guild
L-arginine	Arg	216.238	1.5	amino acid
L-ascorbic acid	Asc	176.124		carboxylic acid
L-asparagine	Asp	189.169	2	amino acid
caffeic acid	Caf	180.175		carboxylic acid
cellulose	Cel	342.296		complex
citric acid	Cit	192.124		carboxylic acid
D-(+)-galactosamine	Gal	215.623	6	amine
D-(+)-glucose	Glc	180.16		carbohydrate
L-glutamine	Glt	146.145	2.5	amino acid
glycine	Gly	75.067	2	amino acid
guaiacol	Gua	94.112		complex
L-histidine	His	169.181	2	amino acid
humic acid	Hum	???		complex
2-ketobutyric acid	Ket	102.089		carboxylic acid
lactose	Lac	342.296		carbohydrate
lignin	Lig	534.508		complex
L-(+)-lysine	Lys	146.188	3	amino acid
malic acid	Mli	134.087		carboxylic acid
malonic acid	Mlo	104.061		carboxylic acid
N-acetyl glucosamine	Nag	221.208	8	amine
oxalic acid	Oxa	90.044		carboxylic acid
pepsin	Pep	672	5.6	amine
phenol	Phe	94.112		complex
L-serine	Ser	105.93	3	amino acid
syringic acid	Syr	198.173		carboxylic acid
tannic acid	Tan	1701.2		complex
L-(+)-tartaric acid	Tar	150.087		carboxylic acid
D-(+)-trehalose	Tre	378.27		carbohydrate
urea	Ure	60.055	0.5	amine
uric acid	Uri	170.126	1.25	amine
vanillic acid	Van	168.147		carboxylic acid

Table S2. Total MANOVA results for overall community-level physiological profile (and subsets of substrate additions).

MANOVA [§]			
	Factors	F	<i>P</i> value
All Substrates			
	Season	33.28	< 0.001
	Rotation	2.19	0.003
	Season*Rot.	0.65	0.949
No Carboxylic Acids			
	Season	17.98	< 0.001
	Rotation	1.96	0.012
	Season*Rot.	1.33	0.105
Carboxylic Acids			
	Season	21.37	< 0.001
	Rotation	1.72	0.035
	Season*Rot.	0.599	0.972
Carboxylic Acids (no Ket [‡])			
	Season	11.93	< 0.001
	Rotation	1.27	0.207
	Season*Rot.	0.69	0.917

§ Degrees of freedom: Season = 2, Rotation = 4, Season*Rotation = 8.

‡ Ket = ketobutyric acid

Table S3. ANOVA and means comparisons of individual principal components.

ANOVA [§] run	Parameter	PC1		PC2		PC3		PC4		PC5		Total
All Substrates												
	Proportion of variance	38.7		17.7		14.5		9		3.8		83.7
	ANOVA Factor	F	<i>P</i> value	F	<i>P</i> value	F	<i>P</i> value	F	<i>P</i> value	F	<i>P</i> value	
	Season	64.02	< 0.001	22.57	< 0.001	5.4	0.008	0.68	0.510	10.33	< 0.001	
	Rotation	0.69	0.605	3.03	0.028	12.82	< 0.001	0.36	0.834	1.81	0.146	
	Season*Rot.	0.16	0.995	1.22	0.311	0.55	0.81	0.88	0.544	0.27	0.973	
	Significant comparisons	1=3≠2		1=2≠3, CS ≠ CSW1		1=2≠3, mC=CS≠CSW=CSW 2				1≠2=3,		
No Carboxylic Acids												
	Proportion of variance	39.3		18.2		11.1		6.8		4.4		79.8
	ANOVA Factor	F	<i>P</i> value	F	<i>P</i> value	F	<i>P</i> value	F	<i>P</i> value	F	<i>P</i> value	
	Season	11.04	< 0.001	3.83	0.03	24.6	< 0.001	0.68	0.510	10.33	< 0.001	
	Rotation	4.06	0.007	5.6	0.001	0.58	0.678	0.36	0.834	1.81	0.146	
	Season*Rot.	1.97	0.075	0.7	0.691	1.05	0.419	0.88	0.544	0.27	0.973	
	Significant comparisons	1=2≠3, mC=CS≠CSW1		2≠3, mC=CS≠CSW1=C SW2		1≠2=3				1≠2=3		
Carboxylic Acids												
	Proportion of variance	60.7		15.6		13.5		4		2.3		96.1
	ANOVA Factor	F	<i>P</i> value	F	<i>P</i> value	F	<i>P</i> value	F	<i>P</i> value	F	<i>P</i> value	
	Season	200.2	< 0.001	5.19	0.01	0.18	0.84	12.33	< 0.001	0.36	0.699	
	Rotation	1.8	0.148	0.8	0.532	0.03	0.026	0.99	0.421	2.98	0.031	
	Season*Rot.	0.26	0.976	1.16	0.346	0.24	0.98	0.51	0.84	0.48	0.864	
	Significant comparisons	1≠2≠3		1≠3		mC≠CSW2		1≠2=3		CS≠CSW1		