Soil Phenol Oxidase Activity in an Agroecosystem

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Introduction

Soil represents a large reservoir for storage of carbon. Much effort has been devoted to studying the cycling of carbon in soils. Enzymes involved in the carbon cycle, such as phenol oxidase, are useful indicators in the understanding of the storage of carbon. Phenol oxidase is an indicator of lignin storage[1]. One unanswered question regarding phenol-oxidase is the role of fertilizer (nitrogen) additions in undisturbed environments such as no-tillage systems. The objective of this study is to investigate phenol oxidase activity as a function of nitrogen fertilizer rate in a long term, no-till ecosystem.

Methods

Soil (Maury silt loam, Typic Paleudalf) was sampled after corn harvest from the long-term no-tillage agroecosystem located at the University of Kentucky's Spindletop farm (Fig 1). The site has received continuous long-term fertilizer nitrogen additions (0, 84, 168, and 336 kg N/ha as NH_4NO_3) since 1970 and has been managed under no-tillage. Soil was characterized for standard soil properties, including extractable nitrogen species.

Phenol oxidase activity measurements were performed on fresh soil using L-DOPA (L-3,4-dihydroxyphenylalanine, 5mM) as a substrate with a constant temperature water bath (23^0 C) using a pH-buffer (pH 6, MES) (Fig 1). The red product, DOPAchrome, was quantified by absorbance measurements at 475 nm using a UV-VIS spectrophotometer. Activity measurements will be normalized with respect to oven-dried weight of soil and total organic carbon.
We performed statistical analyses of the data to evaluate whether changes in enzyme activity are significantly related to soil properties.

Fig. 1. No-till site at the time of sampling (left) and experimental setup in the lab for measuring soil phenol oxidase activity (right).

Results

- After 50 y of no-tillage, soil org C and N increased with N rate, in agreement with past work (Table 1). Cation exchange capacity also increased, due in part to elevated soil C

Table 1. Characteristics of the no-till soil. Different letters indicate significant differences (P<0.1) according to the least significant difference (LSD) test.

N rate	Soil C	Soil N	Sand	Silt	Clay	CEC	pH	KCl-NH_4^+	KCl-NO_3^-
kg N/ha			g/kg			$cmol_{(+)}$/kg		µmol/g	µmol/g
0	18.9 c	1.83 c	104 b	773.2 a	122.9 b	7.78 b	7.03 a	<0.006	0.67 c
84	21.9 b	2.05 bc	107.7 b	761.7 a	130.6 ab	9.0 b	6.5 ab	<0.006	1.14 c
168	23.7 b	2.23 b	115.1 ab	739.7 b	145.3 a	8.74 b	6.25 b	<0.006	2.87 b
336	26.4 a	2.58 a	128.5 a	735.8 b	135.8 ab	16.3 a	6.07 b	<0.006	5.74 a

- There was a negligible difference in phenol oxidase activity between the control (0 kg N/ha) and lowest N rate (84 kg N/ha) (Fig 2), agreeing with other no-till systems[2]
- Phenol oxidase activity decreased by 13% and 32% as N rate increased from 0 to 168 and 336 kg N/ha (Fig 2)

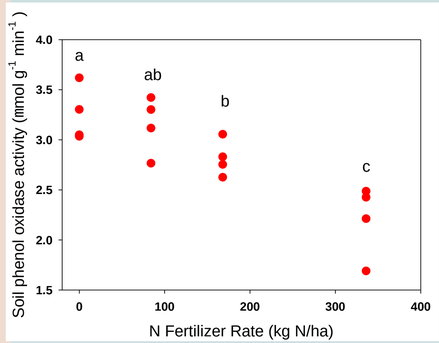

Fig. 2. Soil phenol oxidase activity as a function of N fertilizer rate. Treatments with different letters are significantly different (P<0.1) according to the least significant difference test.

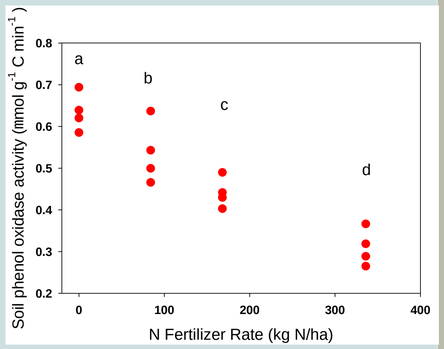

Fig. 3. Soil phenol oxidase activity normalized to organic C as a function of N fertilizer rate. Treatments with different letters are significantly different (P<0.1) according to the least significant difference test.

- Phenol oxidase activity values normalized to organic carbon revealed greater differences between N rates. For example, there was a 30% and 50% decrease in phenol oxidase as N rate increased from 0 to 168 and 336 kg N/ha (Fig 3).

Discussion

- Behavior of phenol oxidase in response to N fertilizer agrees with past results[1,3] and might be due in part to available NO_3^- based on a negative relationship between these properties (r=-0.63, p=0.008) (Table 2). This relationship was more significant when using OC-normalized values (r=-0.72, p=0.001)
- Cation exchange capacity was also negatively related to phenol oxidase (r=-0.76, p=0.006)

Table 2. Correlation coefficients (n=16) describing the relationship between phenol oxidase and soil properties.

Phenol oxidase	Soil C	Soil N	Sand	Silt	Clay	CEC	pH	KCl-NH_4^+	KCl-NO_3^-	
1	-0.632	-0.586	-0.442	0.44	-0.177	-0.76	0.375	-	-0.635	Phenol oxidase
p-value	0.0086	0.0169	0.0864	0.08	0.51	0.0006	0.15	-	0.0081	
	1	0.973	0.81	-0.78	0.28	0.77	-0.57	-	0.68	Soil C
	p-value	0.0001	0.0001	0.0004	0.28	0.0005	0.02	-	0.0033	
		1	0.85	-0.82	0.29	0.81	-0.58	-	0.73	Soil N
		p-value	0.0001	0.0001	0.27	0.0002	0.017	-	0.0012	
			1	-0.78	0.06	0.66	-0.55	-	0.43	Sand
			p-value	0.0003	0.82	0.0048	0.028	-	0.09	
				1	-0.66	-0.57	0.5	-	-0.63	Silt
				p-value	0.0047	0.02	0.046	-	0.008	
					1	0.12	-0.15	-	0.49	Clay
					p-value	0.66	0.57	-	0.05	
						1	-0.39	-	0.75	CEC
							0.13	-	0.0008	
							1	-	-0.51	pH
								-	0.04	
								1	-	KCl-NH_4^+
									-	
									1	KCl-NO_3^-

- The exact mechanism underlying the suppression in phenol oxidase with N is not known, however, it could be related to shifts in microbial communities[3,4] and interactions with soil mineralogy, all of which await further work.
- The anticipated impact of this study is to gain a better understanding of how phenol oxidase is suppressed in the presence of nitrogen and how this might alter lignin storage in no-tillage systems.

References
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