Kinetics of Soil Enzyme Activities Under Long-Term Cropping Systems

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Background

- Soil extracellular enzymes (EEs) catalyze the rates of biochemical reactions in soils.
- Potential enzyme activity (PEA) is a laboratory measurement that provides insight into carbon and nutrient supply and demand. proxy for mineralization, and general measure of microbial activity.
- The Michaelis-Menten constant (K_m), and the maximum rate of activity (V_{max}) can be readily derived from varying substrate concentrations and measuring EE reaction velocity.
  V_{max} = maximum activity when enzymes are substrate-saturated.
  K_m = substrate concentration at half V_{max} .
- Measuring K_m and V_{max} is important to ensure that the substrate concentration is not limiting PEA for robust comparisons among soil samples.
- While much work has been done on agricultural management and PEA, much less has explored the effects on PEA kinetic parameters

Objective

1. To assess the impacts of cropping systems on enzymes kinetics V_{max} and K_m .
2. Explore the relationship between EE kinetics with other soil properties

Materials and Methods

- **The experiment** was established in 2008 at Comparison of Biofuel Systems near Boone, IA. All plots are tile drained and receive no tillage (Fig. 1)

Fig.1

- **Three experimental treatments**:

Treatments	Description	Fertilizer N rate (kg N ha^{-1} y^{-1})
CS	Corn - soybean rotation	~60
P	Multi-species, perennial restored prairie	0
PF	Fertilized multi-species perennial restored prairie	60-84

- **Soil Properties** (0-15 cm): microbial biomass carbon (MBC) and nitrogen (MBN), organic matter % (OM), clay content (Table. 1).
- **EEs:** β-Glucosidase (BGase), and Leucine aminopeptidase (LAPase).

Fig. 2. Measuring enzyme kinetics (V_{max}) and (K_m) using microplate fluorimetric assay

Results

- The PF treatment increased BGase V_{max} by 83% and 118% compared to the P and CS treatments, respectively. (Table. 2, Fig. 3).
- Across all treatments, there were no significant differences in K_m for either enzyme or LAPase V_{max} (Table.3)
- BGase V_{max} and K_m were positively correlated with MBC and MBN, soil OM, and clay content. (Table.3)
- Only LAPase V_{max} (not K_m) was positively correlated with MBC, MBN, OM, and clay content. (Table.3)
- Both the PF and P treatments had significantly higher MBC and MBN compared to the CS treatment.

Table 1. Background soil data

Treatments	OM (%)	Clay (%)	Microbial Biomass C (mg.kg^{-1})	Microbial biomass N (mg.kg^{-1})
PF	4.10 ± 0.22	30.07 ± 1.62	1023.34 ± 45.38 a (0.019)	47.56 ± 5.72 a
P	3.23 ± 0.34	26.26 ± 2.54	1094.14 ± 89.22 a	37.64 ± 4.18 ab
CS	3.28 ± 0.39	27.23 ± 2.93	724.70 ± 60.90 b	25.48 ± 3.35 b

Values are shown mean ± standard errors (n = 4). CS: Corn-Soybean rotations; PF: Prairie Fertilized; P: Prairie (without fertilization). Different letters (a-c) in a column indicate significant differences at $p < 0.05$. Value in parentheses is the ANOVA p-value.

Table 2. Michaelis constant (K_m) and maximum reaction velocity (V_{max} (nmole.hr^{-1}. g^{-1}Soil))

Soil enzymes	Kinetic parameters	Treatments PF	P	CS
BGase	V_{max}	1576.75 ± 215.08 a **(0.01)**	862.05 ± 140.95 bc	723.55 ± 68.97 c
	K_m (mM)	0.10 ± 0.016 a	0.07 ± 0.003 a	0.07 ± 0.007 a
LAPase	V_{max}	672.93 ± 149.77 a	682.73 ± 95.58 a	346.58 ± 62.95 a
	K_m (mM)	0.17 ± 0.036 a	0.18 ± 0.021 a	0.18 ± 0.015 a

Values are shown mean ± standard errors (n = 4). CS: Corn-Soybean rotations; PF: Prairie Fertilized; P: Prairie (without fertilization). βG: β-Glucosidase; LAP: Leucine aminopeptidase. Different letters (a-c) in a column indicate significant differences at $p < 0.05$. Value in parentheses is the ANOVA p-value.

Table 3. Correlation coefficient (r) between Vmax, Km, and soil properties.

Soil enzymes	Parameters	OM	MBC	MBN	Clay content
BGase	Vmax	**0.709****	**0.547***	**0.842*****	**0.542***
	Km (mM)	**0.706****	0.327	**0.626***	**0.619***
LAPase	Vmax	**0.672****	**0.716****	**0.886*****	**0.637***
	Km (mM)	0.415	0.0354	0.169	0.459

***Correlation is significant $p < 0.0001$; ** $p < 0.01$; * $p < 0.05$.
Organic matter: OM %; Clay (g. g^{-1}); Microbial biomass C (mg. kg^{-1}); Microbial biomass N (mg. kg^{-1}).

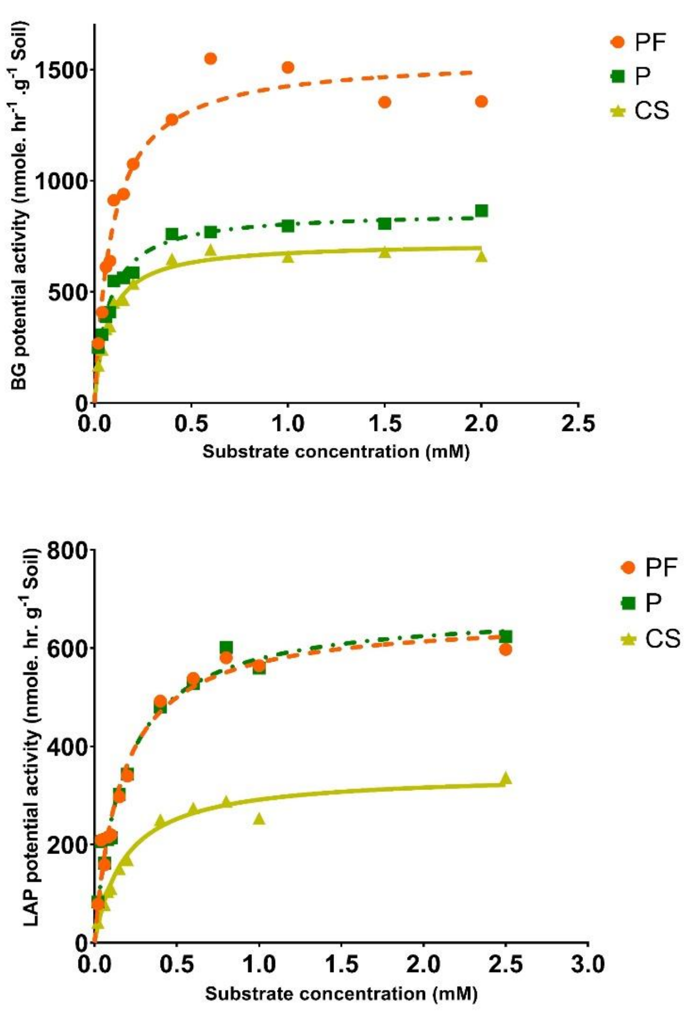

Fig. 3 : Michaelis-Menten kinetics (MM) of BGase (top) and LAPase (bottom). Values are means of four replicates (± SE). Curves present fitting of (MM) kinetics by non-linear regression. The fitted Vmax and Km values are presented in (Table.2). PF increased BGase V_{max} compared to both P and CS.

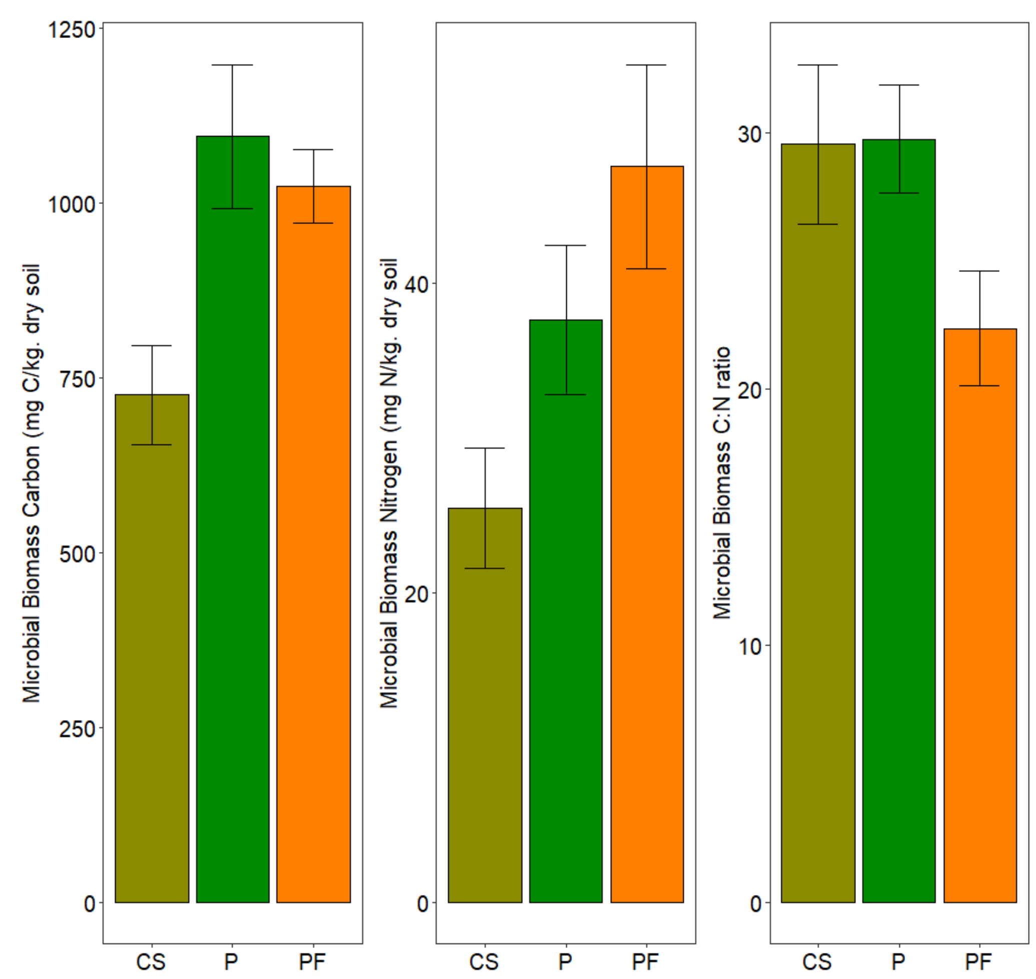

Fig. 4 : Microbial biomass carbon (MBC) and nitrogen (MBN). Values are means of four replicates (± SE). PF and P had significantly higher MBC and MBN compared to CS. (Table.1)

Conclusion

- Soil in both reconstructed prairie treatments (PF, P) had greater OM%, MBC, MBN, and PEA compared to soil in the corn-soy rotation treatment.
- The fertilized prairie (PF) treatment increased BGase V_{max} compared to the unfertilized prairie treatment (P) and compared to the to the corn-soy rotation treatment (CS). However, there were no treatment effects with LAPase.
- Soil properties across management were related to EE kinetics.

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