Changes in Enzyme Activities and Actinomycete Functional Diversity due to Long Term Agricultural Management

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Introduction

Agricultural management can cause long-lasting effects on many aspects of soil function, soil ecology, and soil nutrient status (Acosta-Martínez 2004, 2007). It has been suggested that extracellular enzyme activities could serve as valuable indicators of soil fertility and soil quality (Garcia-Ruizet al., 2008). However, few comprehensive studies have determined not only how extra-cellular enzyme activity relates to soil nutrient status and microbial growth, but also how the growth of specific organisms relates to the production of extracellular enzymes. This type of interdisciplinary effort may offer a deeper understanding of which processes and specific organisms are the best indicators of soil function, and how microbial activity relates to growth conditions.

Actinomycete bacteria are a major group of decomposition enzyme producing microorganisms, with the genus *Streptomycetes* being among the most potent bacterial degraders of cellulose at our site (Ulrich et al., 2008). Streptomycetes are also known to be affected by changes in land management (Hill et al., 2011). In this comprehensive study we used a long-term agricultural field trial to determine how bacterial decomposer abundance and function were altered by long-term management, how the bacterial decomposer community relates to overall soil enzyme activity and microbial growth, and finally how microbial growth and hydrolytic enzyme activity relate to abiotic soil characteristics.

Materials and Methods

- Soil samples were taken at 0-20 cm depth (the plow layer) in June 2010
- The long-term (108 years) field trial consists of 18 fertilization treatments planted with a rotation of sugar beet, spring barley, potatoes, winter wheat, and alfalfa. The following treatments were sampled under alfalfa (n=5 each): **no inputs**, **NPK** (140 N, 60 P, and 230 K kg ha^{-1} yr^{-1}) only, 20 T manure ha^{-1} $2yrs^{-1}$ only (**20 T**), 20 T manure +NPK, (**20 T+NPK**) 30 T manure only (**30 T**), and 30 T manure +NPK (**30 T +NPK**)
- Analysis included:
  - Activity of β-glucosidase (β-gluc), β-xylosidase (β-xylo), N-acetylglucosaminidase (nag), and phosphatase (phos), at pH 7 (German et al., 2011).
  - Microbial biomass (PLFA, using a modified Bligh and Dyer 1959 extraction).
  - *In situ* soil respiration, microbial biomass (SIR, Anderson and Domsch, 1978), and nitrifcation potential (shaken slurry method)
  - Abundance and activity of culturable chitin and cellulose degrading *Streptomycetes*
  - Characterization of soil extractable C and N (hot water extraction and KCl extraction) total C and N (combustion analysis), and pH (in 0.01 M $CaCl_2$)

Figure 1. Response of extra-cellular enzyme activity and specific activity

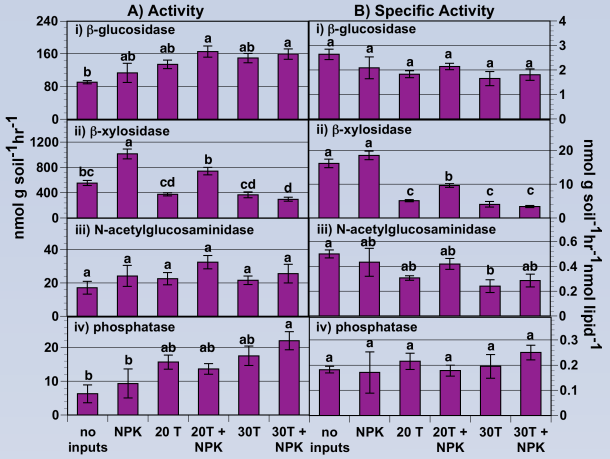

Figure caption: Response of A) extracellular enzyme activity (nmol g $soil^{-1}$ hr^{-1}) and B) specific enzyme activity (nmol g $soil^{-1}$ hr^{-1} nmol $lipid^{-1}$). Different letters indicate statistically different treatments based on Tukey's HSD test at $P<0.05$. All calculations were made per g dry wt. Error bars represent one standard error.

Table 1. Treatment affects on soil nutrient status, microbial biomass, and measures of microbial activity

treatment	pH	N inorg	TOC	HWC	HWN	soil resp	nitrification potential	microbial biomass	SIR biomass
no input	5.26 d	1.62 c	1.61 c	399.6 c	30.05 d	2.40 a	0.154 d	34.53 c	241.7 b
NPK	5.08 d	3.11 bc	1.93 b	504.4 bc	40.18 cd	4.29 a	0.303 c	55.00 bc	356.0 b
20 T	5.92 bc	6.24 ab	2.15 b	540.8 abc	47.82 abc	5.93 a	0.398 bc	73.49 ab	537.1 a
20T +NPK	5.62 c	7.29 a	2.49 a	647.1 ab	60.44 a	4.84 a	0.553 a	76.93 ab	617.0 a
30T	6.04 b	8.79 a	2.54 a	677.8 a	58.40 ab	5.43 a	0.423 abc	98.50 a	633.8 a
30T +NPK	6.38 a	9.23 a	2.60 a	512.4 bc	43.35 bcd	5.19 a	0.529 ab	87.61 a	652.3 a

Table caption: Different letters indicate statistically different treatments based on Tukey's HSD test at $P<0.05$. Abbreviations: pH = pH in 0,.01 M $CaCl_2$; Ninorg = sum of NH_4 and NO_3 (mg kg^{-1}); TOC = total organic carbon (%); HWC and HWN = hot water extractabl C and N resp. (mg kg^{-1}); soil resp = soil respiration (mmol CO_2 m^{-2} s^{-1});nitrification potential = (μg g $soil^{-1}$ hr^{-1}); microbial biomass = (nmol lipid g $soil^{-1}$); SIR biomass = substrate induced respiration (μg CO_2-C g $soil^{-1}$ hr^{-1}).

Figure 2. Response of *Streptomyces*

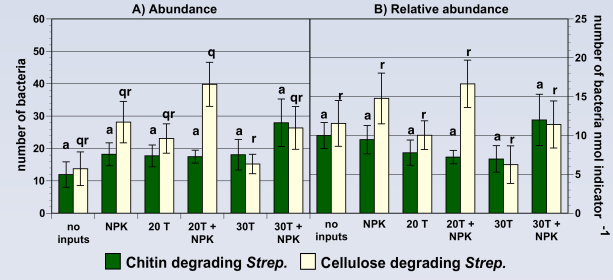

Figure caption:Response of culturable chitin and cellulose degrading *Streptomycetes* (number of bacteria) and number relative to Actinomycete indicator biomass (number of bacteria nmol 18:0 10 me $^{-1}$ g $soil^{-1}$). Different letters indicate statistically different treatments based on Tukey's HSD test at $P<0.05$. All calculations were made per g dry wt. Error bars represent one standard error.

Results and Discussion

Extracellular enzyme activities responded to agricultural management according to microbial biomass

We found that β-gluc, phos, and nag (an insignificant trend) activities were higher with increasing manure and mineral fertilizer, but when taken in terms of microbial growth (specific activity), activities were the same or lower with fertilizer amendment. Thus, the microbial community was similarly or less nutrient limited with more fertilization and produced similar or less extra-cellular enzymes per capita. We also saw that in addition to increasing the nutrient levels directly, long-term manure amendments may change soil characteristics such as pH, to allow for more microbial growth and activity. Further, enzyme activity alone may not predict or indicate the full response of the microbial community in terms of growth conditions or nutrient availability.

The response of chitin and cellulose degrading Streptomycetes was similar to that of total extracellular enzyme activity

Culturable abundances of chitin and cellulose degrading *Streptomycetes* followed similar, but not the same, patterns as total enzyme activities. This suggests that these organisms are important indicators for decomposition in agro-ecosystems and that other enzyme producing organisms are important as well. In conclusion, it is important to assess microbial activity in terms of growth of the organisms for a more complete view of microbial processes under human management.

References

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