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Differentiating Microbial Contributions to Soil Protease Activity

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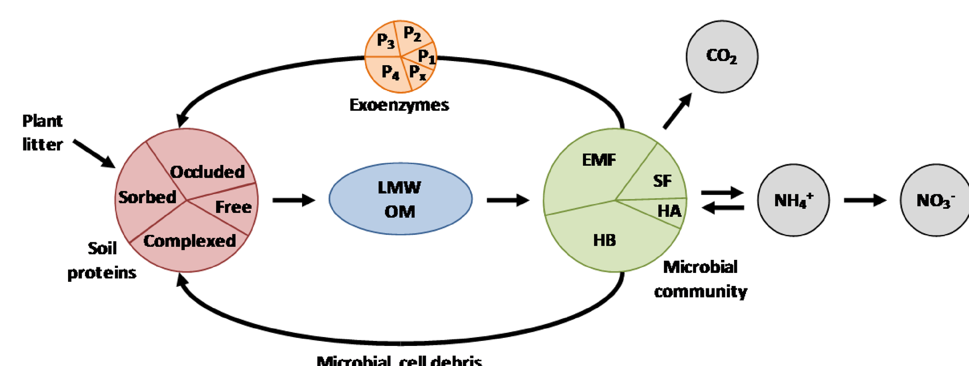
Introduction

The low molecular weight (LMW) organic N pool in soils is small and turns over very rapidly. Therefore, the bottleneck for generating bioavailable N is the breakdown of high molecular weight organic N (predominantly protein-like in nature) into smaller organic fractions. This conversion is accomplished through the activity of protease enzymes that are produced by bacteria and fungi in response to their need for C and/or N.



Conceptual model of the microbial N cycle in an aerobic soil (Schimel & Bennett, 2004; Jan et al, 2009).

Objectives

(1) Characterize soil proteases at two research locations representing gradient of N availability and mineralogy.
(2) Explore soil protease turnover by looking at changes in potential protease activity over time in non-amended and chloroform fumigated samples.
(3) Differentiate microbial contributions to protein turnover in soils by looking at protease activity of bacterial and fungal groups.

Hypotheses

(1) Potential protease activity is inversely related to N availability.
(2) Protease turnover varies among soils.
(3) Contributions of bacteria and fungi vary among soils and are proportional to their biomass.

Experimental Design

Soils

Soil from replicate plots (3) of Douglas-fir and red alder were sampled at two sites in Oregon: Cascade Head and H.J. Andrews Experimental Forest (Table 1).

Chloroform fumigation

Chloroform was used to lyse microbial cells and stop protease production. Soils were incubated under chloroform. Respiration rates and protease activities were measured.

Protease inhibition by antibiotics

Protein synthesis was inhibited in bacteria using streptomycin (1 mg g^{-1} soil) and in fungi using cycloheximide (2 mg g^{-1} soil). Changes in respiration rates and protease activities were captured measured. This experiment used H.J Andrews Douglas-fir soils.

Measurement methods

Measure respiration using Picarro Cavity Ringdown Spectrometer or Carle Analytical Gas Chromatograph.

Measure total protease activity using casein degradation assay and exoenzymatic assay using the leucine amino peptidase (LAP) assay.

Results and Discussion

Table 1: Properties of Douglas-fir and red alder soils at each study site

Characteristics	Cascade Head		H.J Andrews	
Vegetation	Douglas-fir	Red alder	Douglas-fir	Red alder
Total C (g kg^{-1})	128±4	144±18	90±13	82±21
Total N (g kg^{-1})	6.7±0.2	9.2±1.6	2.7±0.1	3.4±0.3
C:N ratio	19.2±1.0	17.1±3.9	33.2±3.5	24.0±4.3
NH_4^+ (gN g^{-1})	4.5±0.6	3.4±0.8	1.0±0.4	2.6±0.8
NO_3^- (gN g^{-1})	4.4±1.7	8.0±2.2	0.3±0.1	3.2±0.7
DON (g g^{-1})	12.7±1.0	17.8±1.5	5.2±0.5	8.3±1.0
pH	4.1±0.1	3.6±0.0	5.0±0.1	5.1±0.1
Microbial biomass (gC g^{-1})	665±62	820±123	468±41	435±4
Leucine amino peptidase (nmolMC g^{-1} h^{-1})	21.2±4.5	20.4±11	9.2±2.8	15.3±2.5
Total Protease (nmolTyrosine g^{-1} h^{-1})	197±32	109±16	321±61	250±39

Data are means and standard errors (n=3). For Leucine amino peptidase and Total protease data, there was a significant effect of site (p=0.01) and marginally significant effect of tree species (p=0.09)

Soil protease activities

Total protease and LAP activities followed different patterns (Table 1); total protease activity decreased with microbial biomass whereas LAP activity increased. When activity was normalized to microbial biomass, there was no correlation between LAP activity and measures of N availability (p=0.50 for C:N, p=0.87 for DON). This suggests that LAP is not regulated by C or N availability. It may be constitutively produced by this microbial community (there was a positive, but not statistically significant, correlation of LAP activity with microbial biomass).

In contrast, total protease activity, on either a soil or microbial biomass basis, increased with soil C:N ratio ($p<0.1$) and significantly decreased with DON ($p<0.01$). These relationships suggest that exo- and endoprotease production were regulated by N availability, with greater protease in more N-limited soils.

Soil protease turnover

Chloroform fumigation has been using as a effective soil sterilizing method (Blankinship et al., 2014). In this experiment, continuous chloroform exposure during incubation disrupted cellular activities, preventing microbial re-growth, and inhibiting CO_2 respiration compared to control samples (Fig. 1).

Accordingly, chloroform stopped LAP and total protease production. Total protease activity sometimes increased initially, possibly from the release of intracellular proteases due to cell lysis, but thereafter declined in a regular pattern (Fig. 1). The decline in protease activity is an indication of protease turnover and could be fitted with a double-exponential curve, suggesting that there are two pools of proteases: one that turns over rapidly (half-life <1 day) and one that turns over more slowly (half-life of ~1 month). The fast pool may represent free protease enzymes and the slow pool proteases associated with the soil matrix. Besides microbial regulation of protease production, matrix factors may also contribute to the level of protease activity in soils.

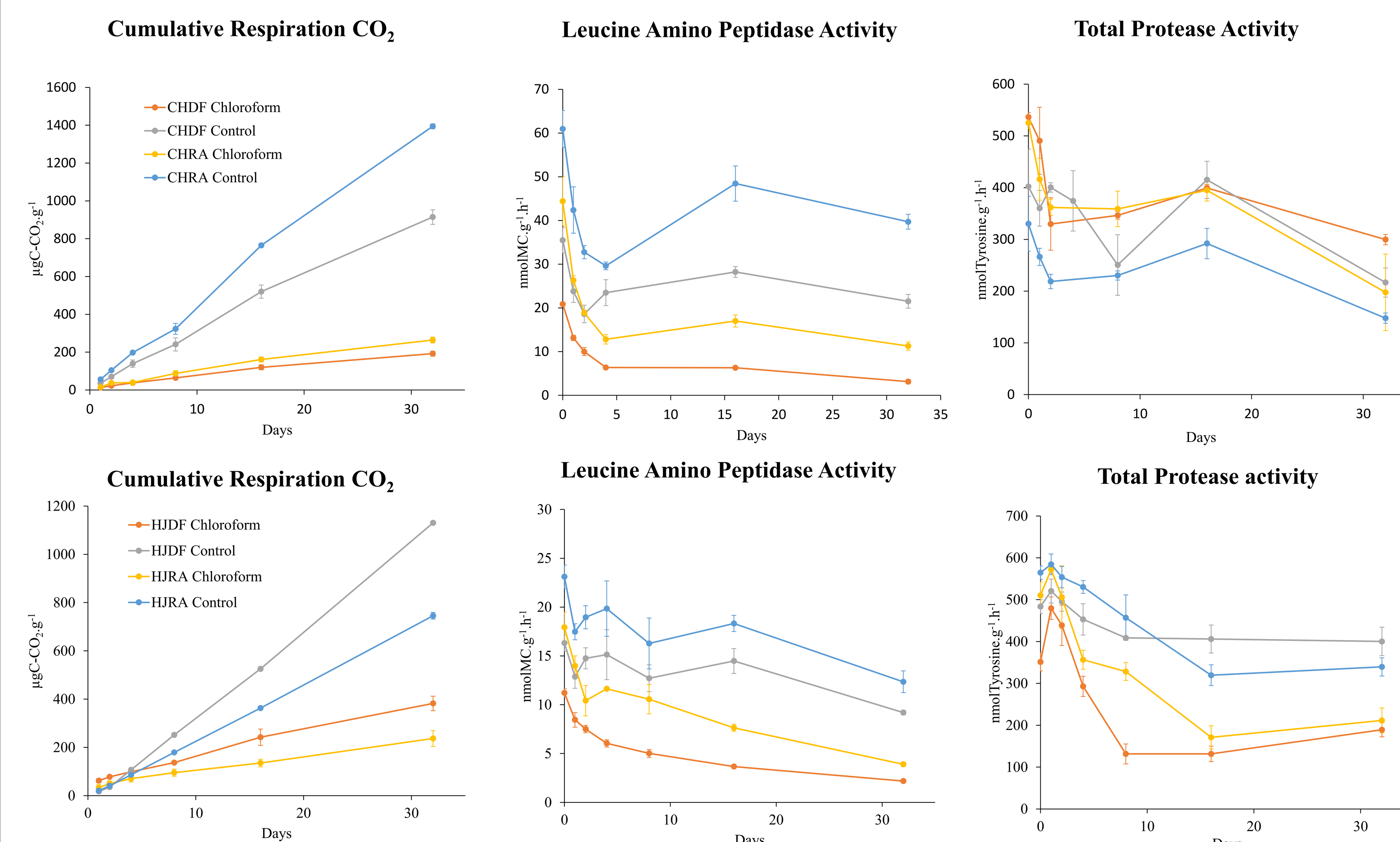


Figure 1: *Chloroform fumigation inhibits microbial activity and soil protease activities HJ=H.J. Andrews, CH=Cascade Head, DF=Douglas-fir, RA=red alder Data are means and standard errors*

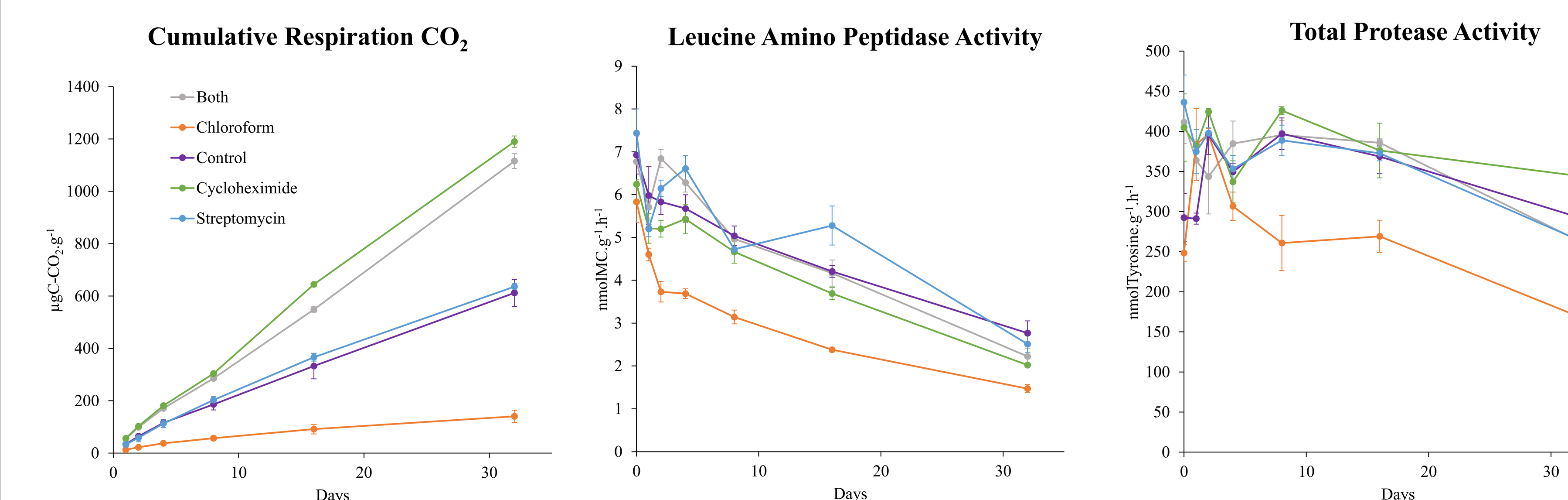


Figure 2: *Differentiation of bacterial and fungal contributions to protease activity of soils H.J. Andrews, Douglas-fir, Plot 2 soil. Data are with means and standard errors*

Protease biosynthetic inhibition

According to cumulative respiration (Fig. 2), chloroform successfully inactivated microbial respiration. The bacteria inhibitor had little effect, possibly because this soil is dominated by fungi. The fungal inhibitor unexpectedly increased respiration, perhaps because the fungi respired C that could not be used for protein synthesis (overflow metabolism) or because bacteria utilized C that was not used by fungi or additional C from fungal death.

Chloroform treated samples worked well as negative controls for protease activity, however, antibiotic-treated samples did not work as we expected: protease activity was not inhibited (Fig. 2). Although the reasons for this behavior are unclear, one possibility is that the conditions in this soil were not conducive to protease production.

Conclusions

Protease activity varied among sampling locations, depending on existing microbial community as well as environmental conditions. Total protease activity was higher in N-limited soils, suggesting a microbial response to N limitation. In contrast, LAP may be produced constitutively.

Chloroform fumigation enables the measurement of protease turnover in soils. Future studies will evaluate the effect of soil mineral and organic matter composition as matrix regulators of protease turnover.

Streptomycin and cycloheximide did not work as we expected to differentiate microbial contributions to protease synthesis. Further tests under conditions conducive to protease production will be done.

References

1. Jan, M.T., Roberts, P., Tonheim, S.K., Jones, D.L., 2009
2. Schimel, J.P., Bennett, J., 2004
3. Blankinship, J.C., Becerra C.A., Schaeffer S.M., Schimel J.P., 2014

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