**Influence of enzyme activity on decomposition depends on substrate recalcitrance in an ombrotrophic bog**

Kenna Rewcastle

**Introduction**

Northern bog ecosystems are significant terrestrial carbon (C) sinks because they store approximately 30% of global soil organic carbon (SOC) but make up just 3% of land area (Gorham, 1991; Yu *et al*., 2010). The large bog C sink is generated through an imbalance between inputs via net primary production and outputs via decomposition and respiration (Limpens et al. 2008). Bog C stocks increase with inputs of organic matter and global changes, such as increased levels of CO2, are expected to increase the amount of labile C in ecosystems through root exudation of sugars (Hungate *et al.* 1997, Jones *et al.* 1998, Heath et al. 2005, Korner et al. 2005, Phillips et al. 2011). . Clearly, bogs are a hugely important reservoir of C, and we are only beginning to understand what regulates bog carbon dynamics and the resulting feedbacks to climate change.

Is microbial activity linked to the decomposition of labile C substrates? Is the strength of this relationship between microbial decomposers and decomposition different for substrates of varying recalcitrance? We traced we traced isotopically labeled labile C (i.e., 13C-starch) from organic matter into respired CO2 to answer these questions and explore bog decomposition processes. We installed mesocosms in a boreal bog, and after a seven month long *in situ* incubation period, added a small amount of 13C-starch to each mesocosm and captured gas samples to measure 13CO2 respiration and overall soil CO2 respiration to track decomposition. To understand the influence of microbial activity and abiotic environment on organic matter (OM) decomposition, we simultaneously collected samples to assess a suite of peat physical and chemical properties as well as microbial activity and biomass. We predict that the rapid decomposition of labile starch will be directly linked to microbial activity, while the decomposition of more recalcitrant C substrates that results in overall respiration will perhaps be indirectly linked to microbial activity through the influence of the abiotic environment. These analyses ultimately lend insight to the future of microbial-mediated decomposition in a boreal bog in the face of climate change.

**Methods**

*Site description*

We conducted our study in an ombrotrophic bog in the Marcell Experimental Forest (47° 30’ 17” N, 93° 28’ 97’’ E) of Bovey, Minnesota, U.S.A. Ombrotrophic bogs are characterized as having all water inputs from precipitation rather than groundwater (Tfaily *et al.*, 2014). We installed our experimental mesocosms in an 18-m2 area on the southwest side of Boardwalk 4 in the S1 plot at Marcell Experimental Forest. The dominant tree species at our site were black spruce (*Picea mariana*) and tamarack (*Larix laricina*). Three ericaceous shrubs, Labrador tea (*Ledum groenlandicum*), swamp laurel (*Kalmia polifolia*), and wild blueberry (*Vaccinium angustifolium*), dominated the understory vegetation (Malloch and Malloch, 1981; Largent *et al.*, 1979; Dalpé, 1989). A bryophyte layer of *Sphagnum* species covered the bog surface. Mean annual temperature was 3.3 ºC, and mean annual precipitation was 768 mm (Verry *et al.*, 1988). During the sampling period, mean temperatures were 18°C with an average daily high of 27°C and low of 10°C. Soils were characterized as Typic Haplohemist and were overlain by approximately 2.5 m of sphagnum peat (Boelter and Verry, 1977; Nichols, 1998).

*Experimental design and implementation*

To assess decomposition *in situ*, we installed 60 PVC mesocosms (15 cm long × 5 cm diameter) in the bog on November 7, 2014. We cut 10.5 cm × 8 cm rectangular openings into two sides of the mesocosms and covered them with stainless steel mesh (1.45 mm, 55 µm, or 5 µm opening size) to control root and mycorrhizae access to the mesocosms (for mesocosm description, see Moore et al. 2015). The mesocosms were covered with three different mesh sizes as part of an on-going research project at this site. Mesh size was not an important factor as it did not alter root biomass (there was no root ingrowth into any of the mesocosms), microbial biomass (p = 0.100), or peat water saturation (p = 0.094), allowing us to combine mesocosms from all mesh sizes into one pool of 60 replicates. To standardize the organic matter inputs of the peat substrate, we filled each mesocosm with 300 cm3 of dry, commercially available *Sphagnum* peat (Sun Gro Horticulture, Agawam, MA, U.S.A.). We installed the mesocosms in bog hummocks (to control for microtopography) by gently moving aside the bryophyte layer and inserting the mesocosm until the openings were below the bog surface and air gaps between the mesocosm and peat were minimized.

On June 16, 2015, we injected half of the mesocosms (n = 30) with 5 mg of 99 atom% 13C-starch suspended in 30 mL DI water using a syringe (Cambridge Isotope Laboratories, Tewksbury, MA, U.S.A.) (Moore *et al.* 2015, Zak and Kling, 2006). The remaining 30 mesocosms received injections of 30 mL DI water to control for water addition and disturbances.

*Sample collection and analysis*

From June 17 to June 21, 2015, we collected ambient gas samples from the mesocosms to detect metabolism of the 13C-starch. To collect gas samples, we capped each mesocosm with a 5 cm PVC cap containing a rubber septum. After waiting 20 minutes to allow for gas to build up within the mesocosm, we collected a 15 mL gas sample using a syringe and injected the sample into a 12 mL Exetainer vacuum vial (Labco Limited, Lampeter, Ceredigion, U.K.). To assess background atmospheric 13C-CO2 concentrations, we collected two ambient air samples on each day. All gas samples (60 mesocosms + 2 ambient) × 5 days = 310 samples) were analyzed for 13C-CO2 (UC Davis Stable Isotope Facility, Davis, CA) using a ThermoScientific Precon-GasBench system coupled with a ThermoScientific Delta V Plus isotope ratio mass spectrometer (ThermoScientific, Bremen, DE). On four of the five sampling days, we also measured soil respiration in each mesocosm using a LI-6400XT with a soil chamber attachment (LiCOR Instruments, Lincoln, NE). We report soil respiration in μmol CO2 m-2 s-1. Soil respiration was not measured on June 20 due to inclement weather. On June 21, after collecting final gas samples, we removed the mesocosms from the bog, transported them back to the laboratory on ice, and stored them at 4°C.

All laboratory measurements were conducted on sieved peat (2 mm) from within each mesocosm. We measured the percent water saturation of each sample by determining gravimetric water content (GWC) of the sample relative to GWC of saturated peat. Sample GWC was determined by weighing field-moist samples, then oven-drying peat at 105 ºC for 48 h, and reweighing dried samples (Robertson *et al.*, 1999). The average GWC of saturated peat was determined by soaking 12 replicates of 100 g of commercially-available *Sphagnum* peat (Sun Gro Horticulture, Agawam, MA, U.S.A.) in DI water for 8 h and repeating the same GWC measurement as stated above. Water saturation for each sample was expressed as a percentage relative to average GWC of fully saturated peat.

Microbes contribute to the decomposition of organic matter by producing extracellular enzymes (Nanniperi *et al*., 2002). Microbes target C in cellulose substrates by producing cellulase enzymes (e.g., β-glucosidase, α-glucosidase, cellobiohydrolase) and C in proteins with proteases (e.g., N-acetylglucosamindase) (Sinsabaugh 2008). Nutrients are necessary for microbial growth and enzyme production, thus microbial acquisition of N using proteases and P using phosphatase affects decomposition rates as microbes continue to mine organic matter for nutrients. Thus, we analyzed microbial activity within each mesocosm by measuring potential activity of cellulose-degrading (cellobiohydrolase (CBH), β-glucosidase (BG), α-glucosidase (AG)) nutrient-acquiring enzymes (acid-phosphotase (PHOS), N-acetylglucosaminidase (NAG), and leucine aminopeptidase (LAP)) following the protocol by Bell *et al.* (2013). CBH, BG, AG, NAG, and PHOS are linked to the substrate methylumbelliferyl (MUB) and LAP is linked to the substrate methylcoumarin (MUC), which fluoresce when depolymerized by enzymes. Fluorescence was measured using an excitation wavelength of 365 nm and an emission wavelength of 450 nm. Potential enzyme activity was expressed in units of nmol h-1 g-1 dry peat.

*Statistical analyses*

A Bayesian approach was used to compute parameter estimates for the following linear model:

Yi | β, σ2 ∼ Normal(β0 + X1iβ1 + X2iβ2 + X3iβ3, σ2)

This model was fit separately for both total soil respiration (n = 60) and ∂13CO2 respiration (n = 30), where *Y* is the response variable (either total soil respiration or ∂13CO2 respiration), β*0* is the is the model intercept, β*1* is the effect of carbon-degrading enzyme activity, β*2* is the effect of nutrient grading enzyme activity, β*3* is the effect of peat water saturation, *X1* is total carbon degrading enzyme activity (the sum of BG, AG and CBH activity) in nmol h-1 g-1 dry peat, *X2* is total nutrient degrading enzyme activity (the sum of PHOS, NAG, and LAP activity) in nmol h-1 g-1 dry peat, and *X3* is percent peat water saturation.

The JAGS package was used with RStudio (JAGS version 4.3.0, R version 3.4.2 “Short Summer”) to fit the model to both data sets, and to retrieve the resulting parameter estimates with their corresponding 95% confidence intervals. Priors used were as follows for both models:

β 0 ∼ Normal(0, 1)

β 1 ∼ Normal(0, 1)

β 2 ∼ Normal(0, 1)

β 3 ∼ Normal(0, 1)

σ2∼InvGamma(0.01, 0.01)

The posterior for each parameter was sampled 100,000 times using 4 simultaneous chains. Parameter estimates for which zero was not included in the 95% confidence interval were deemed ‘significant’ predictors of total respiration or ∂13CO2 respiration. The corresponding R code used to fit these models is appended to this manuscript, and in addition, parameter estimates were compared to the coefficients producing using least square means linear regression in R.

**Results**

After estimating all predictive parameters for total respiration, it is clear that percent peat saturation has a significant positive influence on total respiration, and carbon degrading enzyme activity has a marginal negative effect on total respiration. Table 1 shows the result of the JAGS model fit and the confidence intervals surrounding each parameter estimate.

|  |  |  |  |
| --- | --- | --- | --- |
| Parameter | Empirical Mean | Standard Deviation | 95% Confidence Interval |
| β 0 | -0.286 | 0.496 | [-1.258 : 0.689] |
| β 1 | -0.003 | 0.001 | [-0.006 : -0.001] |
| β 2 | 0.002 | 0.003 | [-0.003 : 0.007] |
| β 3 | 0.090 | 0.017 | [0.058 : 0.123] |

**Table 1:** Model fit results for total respiration. Parameter β3 (peat water saturation) and, to a marginal degree, β1 (carbon degrading enzyme activity), were statistically significant predictors of total respiration, with greater peat water saturation leading to higher total respiration.

Parameter estimates were essentially identical to the coefficients produced using least square means (from lm output: β0 = -0.388, β1 = -0.003, β2 = 0.002, β3 = 0.092).

Carbon degrading enzyme activity was on the only significant predictor of 13CO2 respiration according to this model. Table 2 shows the results of the JAGS model fit for the ∂13CO2 model including 95% confidence intervals surrounding the estimates of each model parameter. In contrast to the model fit for total respiration, parameter estimates in the ∂13CO2 model using JAGS did not fit the coefficients producing using least square means (from lm output: β0 = -3.165, β1 = -0.268, β2 = -0.402, β3 = 1.539).

|  |  |  |  |
| --- | --- | --- | --- |
| Parameter | Empirical Mean | Standard Deviation | 95% Confidence Interval |
| β 0 | 0.008 | 1.000 | [-1.960 : 1.970] |
| β 1 | 0.270 | 0.120 | [0.035 : 0.505] |
| β 2 | -0.323 | 0.209 | [-0.728 : 0.090] |
| β 3 | 0.651 | 0.749 | [-0.841 : 2.102] |

**Table 2:** Model fit results for ∂13CO2. Parameter β1 (carbon degrading enzyme activity) was the only statistically significant predictor of ∂13CO2 respiration, with greater carbon degrading enzyme activity contributing to higher levels of 13CO2 in the CO2 respired from each mesocosm, indicating greater decomposition of the 13C-labeled starch substrate.

There was an especially large discrepancy between the estimates of the intercept (β0), but it should be noted that the standard error associated with the least square means estimate of the intercept was associated with an extremely large standard error (47.491). Inconsistent model fit most likely results from the limitation of sampling from the posterior of a model derived from a small dataset, as the ∂13CO2 respiration experiment (n = 30) was a subset of the larger total respiration experiment (n = 60).

**Discussion**

The original hypothesis, that decomposition of labile carbon substrates such as the labeled 13C-starch would be more closely linked to microbial activity than the decomposition of more recalcitrant substrates, was supported by the results of this study. This work corroborates studies in other systems that have highlighted the tight relationship between microbial production of extracellular enzymes and the decomposition of labile carbon substrates that serve as sources of energy and carbon for biomass incorporation (Sinsabaugh 2008). Given the importance of root exudates as sources of labile carbon substrates (Phillips et al. 2011), this model should be expanded to include mesocosm proximity to vascular plants to determine if the presence of nearby roots perhaps primes the microbial community to not only take advantage of the labeled-starch addition, but also degrade the more recalcitrant SOM stores that have accumulated in the bog once the more labile substrates have been consumed.

Our results showed that peat water saturation was the strongest predictor of total respiration. Many studies have shown that the combination of warming and woody plant encroachment into bogs will cause a draw-down of the water table in the future (McLatchey and Reddy, 1998; Gunnarsson *et al.*, 2000; Blodau *et al.*, 2004; Davidson and Janssens, 2006; Delarue *et al.*, 2011; Heijmans *et al.* 2013; Briones *et al.*, 2014; Dieleman *et al.*, 2015; Pinsonneault *et al.*, 2016), making these results particularly relevant to an assessment of bog carbon stocks with climate change. Our results agree with those from a study of water draw-down in an ombrotrophic bog in North Wales, UK (Ellis *et al.* 2009). In that study, draw-down combined with elevated CO2 reduced decomposition by 16% compared to a control. Our results also showed that decomposition (i.e. total respiration) was lowest where water saturation was also lowest. We hypothesize that oxygenation of the bog via water table draw-down and tree encroachment changes the microbial community to one that utilizes carbon less efficiently.

The link between respiration, microbial activity, carbon substrate recalcitrance, and other abiotic and biotic factors involved in decomposition established in this study has implications for understanding the fate of C stored in boreal peatlands. When predicting how C cycle dynamics may change in high C ecosystems like peat bogs, it is important to understand what abiotic and biotic drivers are most important in influencing microbial-mediated decomposition and, ultimately, the release of CO2 during respiration. Our study shows that the interactions between abiotic and biotic drivers are especially important when predicting soil respiration, and that a more biologically-dominated suite of drivers predicts decomposition of labile C substrates. By analyzing drivers of soil respiration and the decomposition of labile starch substrates, these results differentiate between factors that will be important in long-term considerations of C storage in bog systems and factors that have short-term implications for the cycling of labile C additions to the bog through root exudation. We highlight that the indirect control of abiotic drivers of C dynamics are likely important predictors of C cycling in bogs through their influence on microbial-mediated decomposition processes.

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