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Differential temperature sensitivity of intracellular metabolic processes and extracellular soil enzyme activities

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This supplementary material contains:

- **A description of the laboratory procedures used for trial experiment for substrate induced respiration**
- **A description of the laboratory procedures used for trial experiment for extracellular snzyme**
- **Figure S-1: Daily minimum and maximum air temperature measured at the University of Reading Atmospheric Observatory, close to the sampling location, between 1st April 2009 and 30th April 2019**
- **Figure S-2: Soil CO2 measured at various concentration glucose**
- **Figure S-3: β-glucosidase measured at four different reaction time**
- **Figure S-4: Chitinase measure at four different reaction time**
- **Figure S-5: Natural log of β-glucosidase activity, chitinase activity, glucose-induced respiration, and basal respiration plotted against assay temperature.**

Trial experiment for substrate induced respiration

The aim of this trial experiment was to test response of soil CO_2 to five different levels of glucose addition. 15 g of moist soil (equivalent to 13.31 g of dry soil) was weighed into 50 ml incubation vials (Centrifuge tube). Glucose solution (2 ml) was added at five concentrations namely; 0, 0.1, 1, 10, 100 mg/g soil, thus bringing the soil to 58% of its water holding capacity. Three replicates per glucose level (15 samples in total) was used and the soils were incubated at 26°C for 1 hour.

Following soil-substrate mixing, the tube was ventilated by blowing in lab air with a 20ml syringe, ensuring air away from the user was extracted to avoid contamination with human-generated CO_2. The tubes were sealed with septum stoppers and 15ml of lab air was injected. The headspace was flushed by moving the syringe plunger up and down several times before sampling 15ml of head space gas and injecting into a 12ml exetainer vial (T0), creating overpressure, using a tap and needle attached to the syringe. The samples were incubated for one hour at the same five temperatures as for the extracellular enzyme assays, at the end of which the process of injecting air, flushing and sampling was repeated (T1). Headspace gas samples were stored at 20°C prior to analysis by an Agilent 7890B gas chromatograph. The results obtained from the samples were calibrated with CO_2 gas standards (506 ppm, 2542 ppm, 5163 ppm and 19,700 ppm respectively), and the difference in the concentration of CO_2 between T1 and T0 obtained thereafter is presented in Figure S-2 as the actual value for CO_2 flux per hour.

Trial experiment for extracellular enzymes

The purpose of this experiment was to determine the optimal incubation time for the main investigation. 1g of moist soil was weighed into a 50ml centrifuge tube and mixed with 4ml MUB buffer (pH 6) and either 1ml 25mM *p*-nitrophenyl-β-D-glucopyranoside (β-glucosidase) or 10mM *p*-nitrophenyl-N-acetyl-b-D-glucosaminide (chitinase) solution, to assess β-glucosidase and chitinase activity, respectively. Samples were incubated at 45°C for 15, 30, 45 and 60 minutes reaction time respectively, after which 1ml 0.5M $CaCl_2$ and 4ml Tris buffer (pH 12) was added to stop the reaction. Samples were mixed by swirling, then filtered with Whatman No. 2 filter paper.

Additionally, 1 blank (for each reaction time except for 45 minutes which was estimated from others) was created by adding substrate to tubes containing the mixture after the reaction had stopped. Colour intensity of the filtrate - directly proportional to the level of reaction product p-nitrophenol (pNP), and hence level of enzyme activity - was measured using a spectrophotometer at 400nm. Working *p*-nitrophenol standard solutions including 0, 10, 20, 30, 40 and 50 μg *p*-nitrophenol were used and the mass of *p*-nitrophenol in each reaction (0-50 μg) plotted against the OD_{400nm} reading. The level of absorbance was converted to potential enzyme activity by dividing the measured concentration by dry weight equivalent of soil. The relationship between β-glucosidase and chitinase enzyme activity and reaction time is presented in Figure S-3 and Figure S-4, respectively.

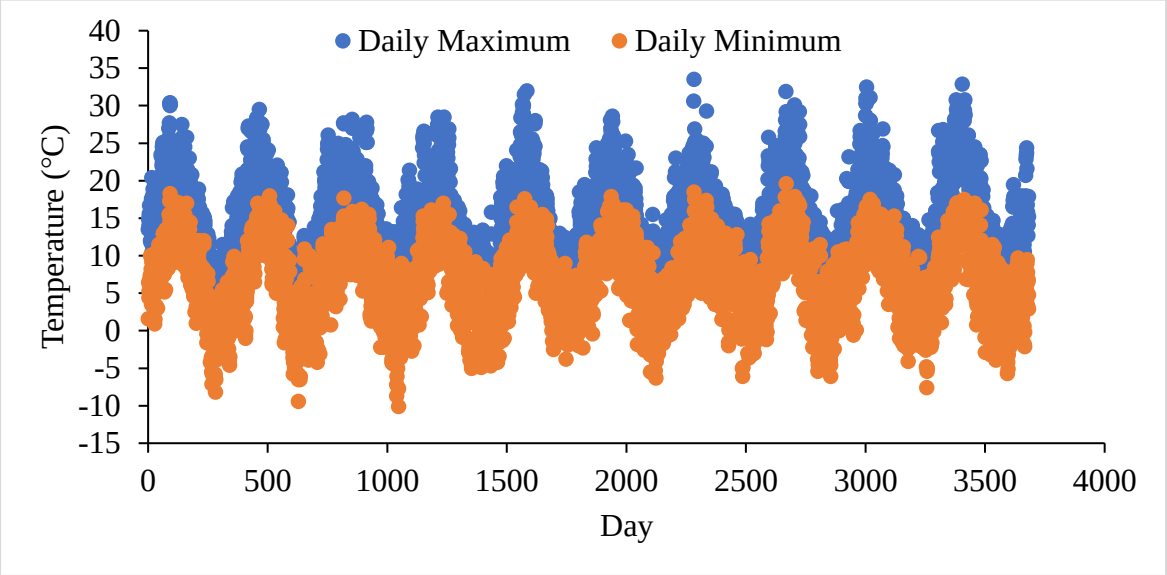

Figure S-1: Daily minimum and maximum air temperature measured at the University of Reading Atmospheric Observatory, close to the sampling location, between 1st April 2009 and 30th April 2019

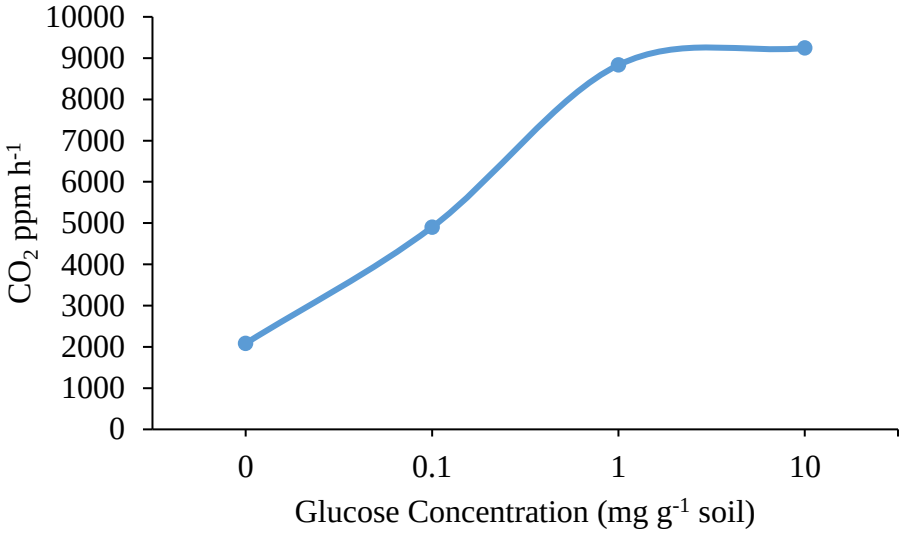

Figure S-2: Soil CO_2 measured at various glucose concentrations. Each data point represents the mean of 3 replicates.

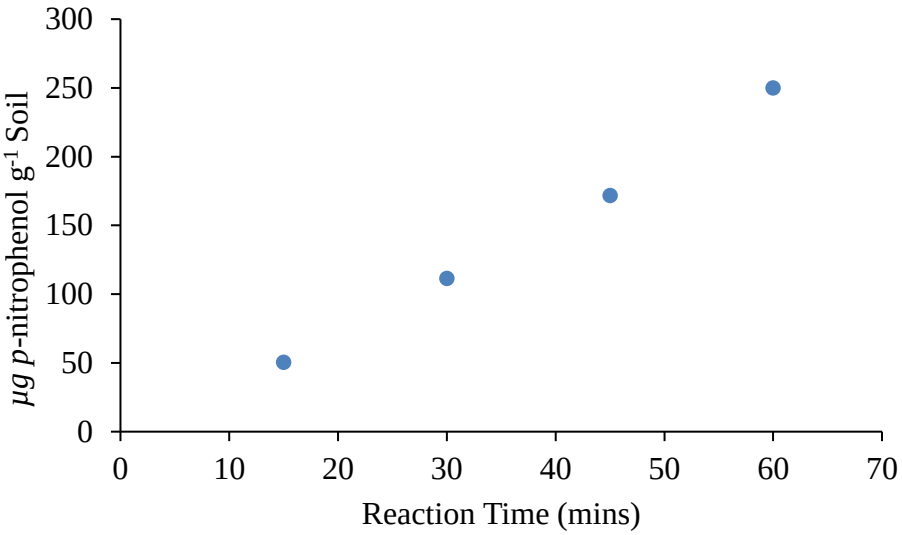

Figure S-3: β-glucosidase activity at four different reaction times. Each data point represents the mean of 3 replicates.

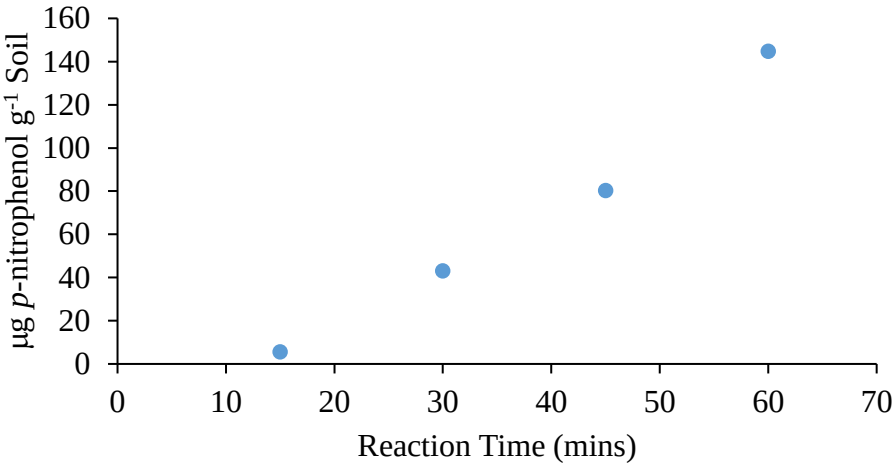

Figure S-4: Chitinase activity at four different reaction time. Each data point represents the mean of 3 replicates.

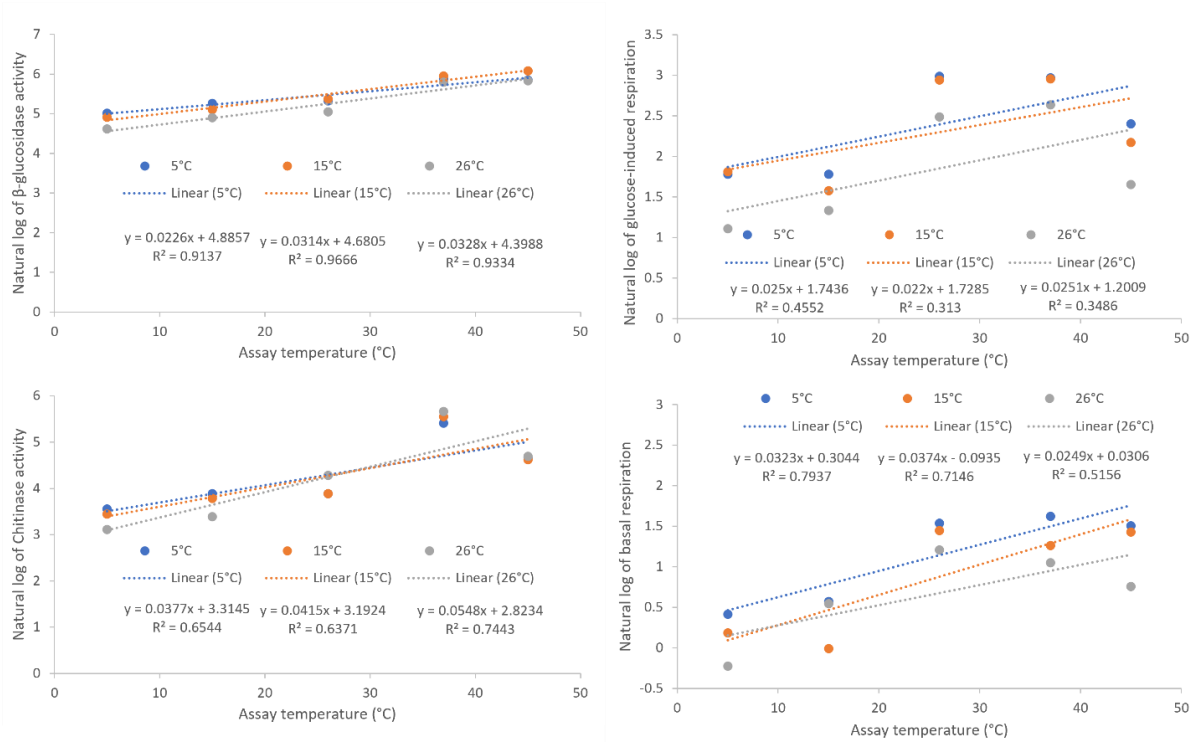

Figure S-5: Natural log of β-glucosidase activity, chitinase activity, glucose-induced respiration, and basal respiration plotted against assay temperature.