

Lab 7: Energy and Respiration

April 10 & 11, 2013

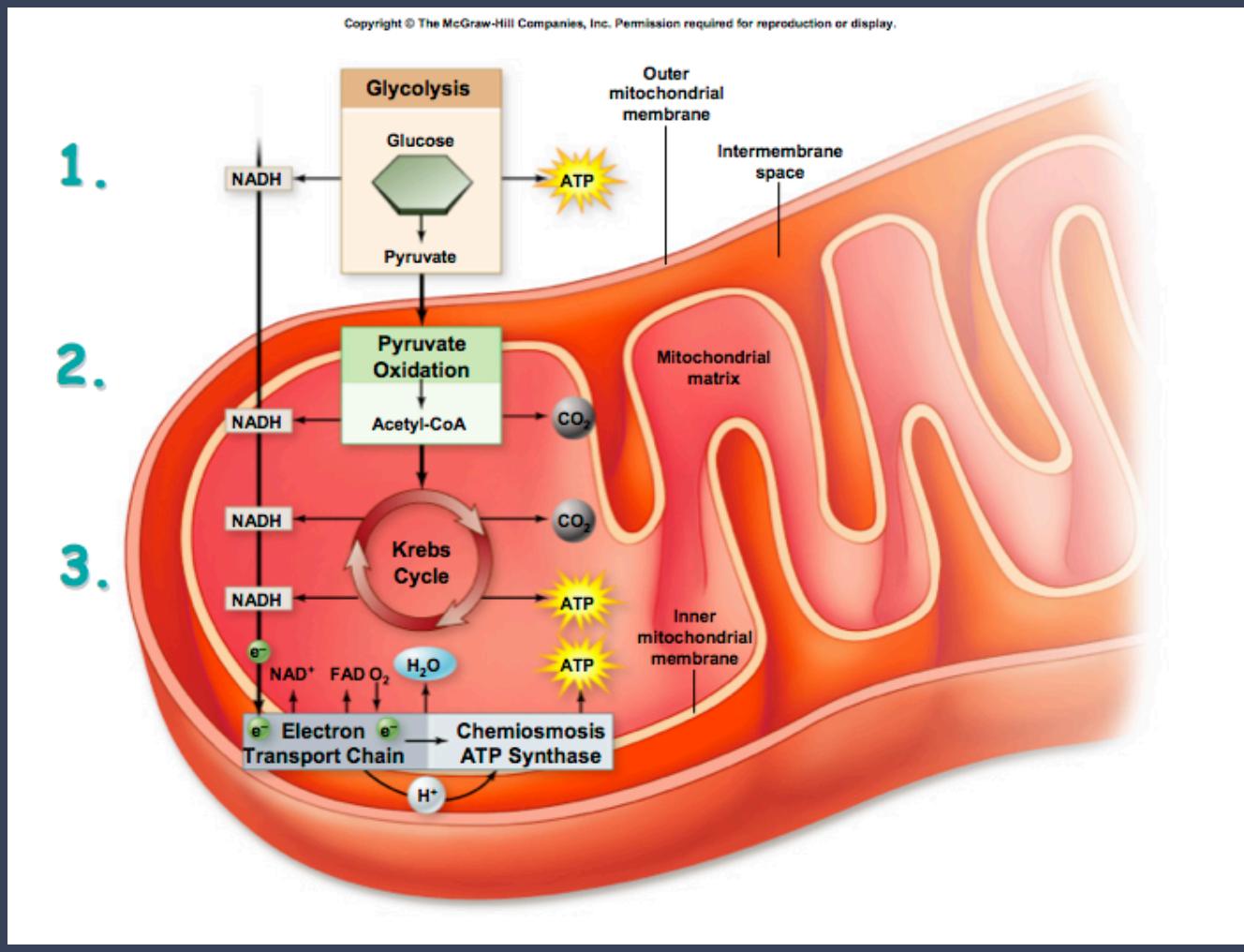
Lab Goals

- * Think quantitatively
 - * Enzyme kinetics
- * Understanding at one level informs understanding at other levels
 - * Scaling up
- * Consider environmental influences
 - * Variation
 - * Global change

Section I. Enzymes



Respiration



Enzymes are proteins that accelerate chemical reactions

A typical enzyme (E) converts a substrate (S) into a product (P):



However the rate at which S can be converted to P has a limit.

Factors Affecting Enzymatic Reactions



Factors Affecting Enzymatic Reactions

- * Concentration of substrate(s)
- * Concentration of enzyme
- * pH
- * Temperature
- * Presence and/or concentration of inhibitor(s)
- * Concentration of cofactors(s)

Enzyme Kinetics



Leonor Michaelis
1875-1949



Maud Menten
1879-1960

Michaelis – Menten equation:

$$v = \frac{V_{\max}S}{K_m + S}$$

v = velocity of reaction

V_{\max} = maximal velocity of a reaction

S = substrate concentration

K_m = substrate concentration @ $\frac{1}{2}$ V_{\max}

Experiment 1a: Soybeans

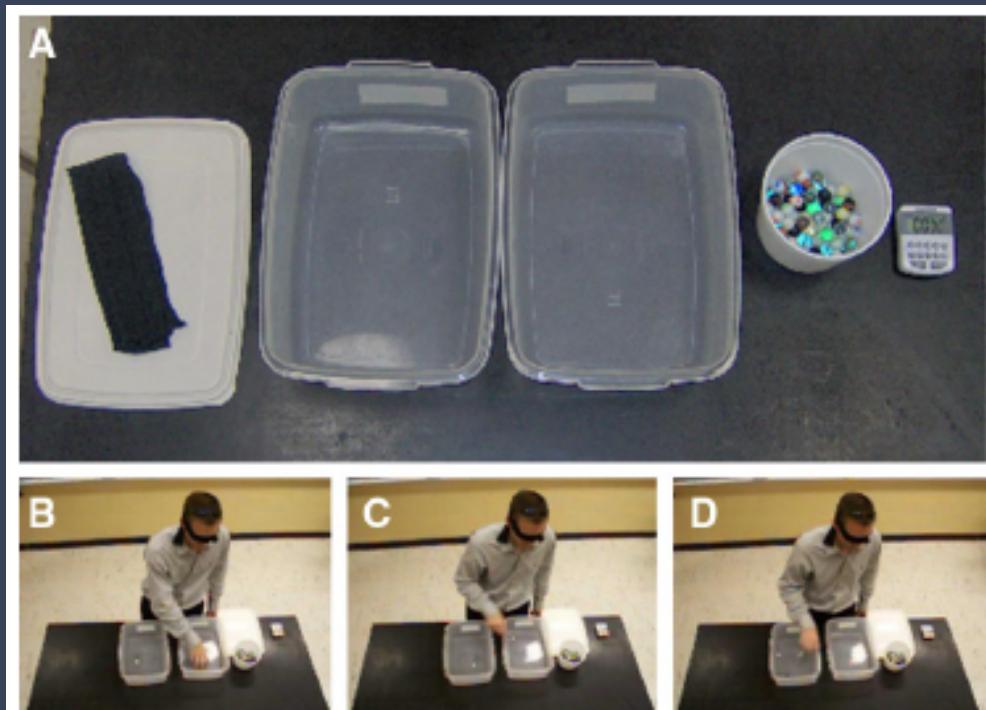


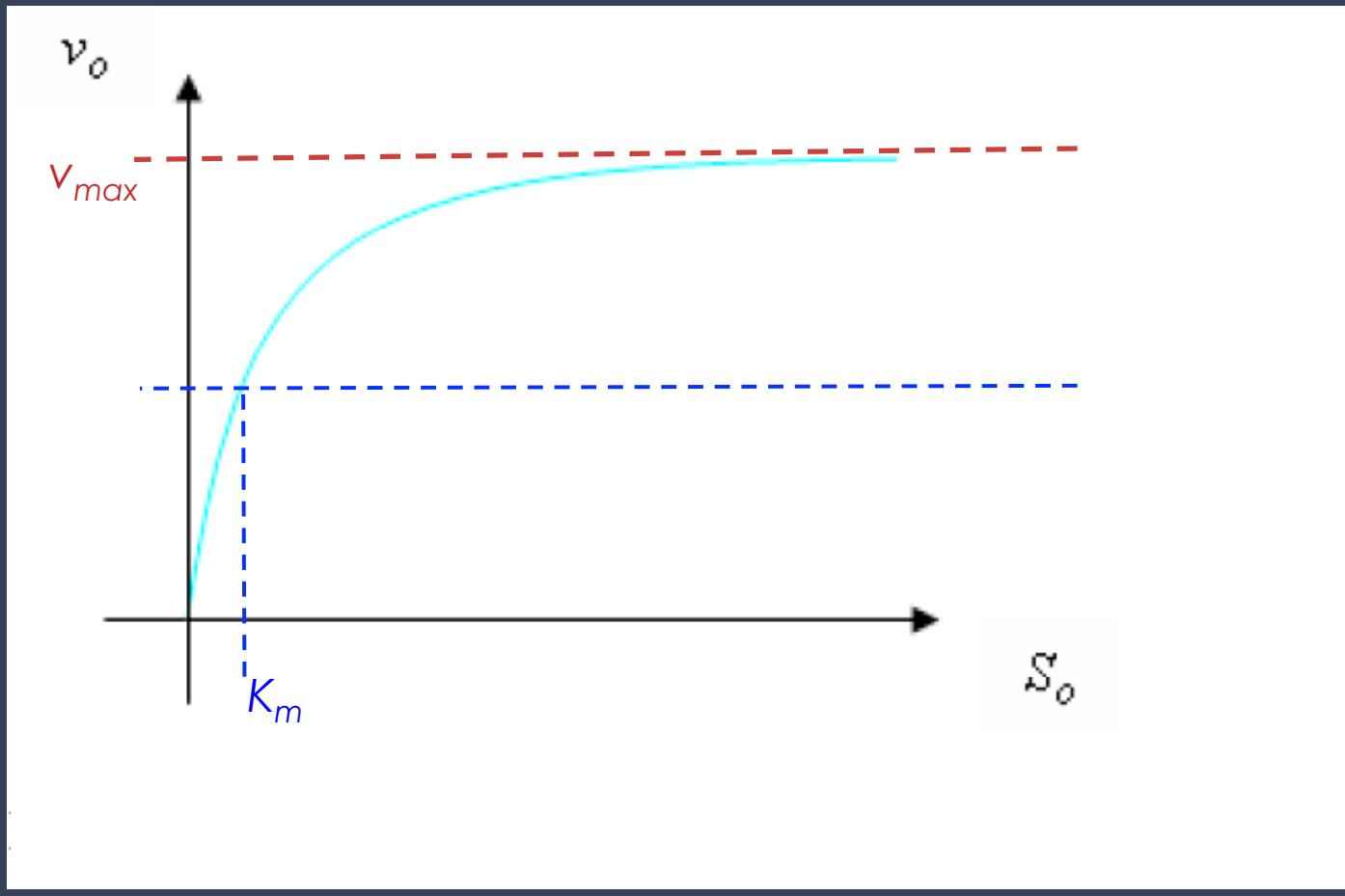
Figure 1. The experimental system. (A) Materials needed include a timer, marbles, two plastic containers, and a blindfold. (B–D) Photographs depicting a student performing one 10-s “run” of the exercise. (B) Finding a marble in the “substrate” container. (C) Transferring a marble to the “product” container. (D) Releasing the marble into the “product” container. The 13-s video of the student performing the exercise is available (see Supplemental Material).

Runge et al. 2006, CBE Life Science Education

V_{max} Describes how high the line goes

K_m Describes how curved the line is

$$v = \frac{V_{max} S}{K_m + S}$$



Steps

$$v = \frac{V_{\max}S}{K_m + S}$$

1. Hypothesize about how changing K_m and V_{\max} influence the rate of a reaction (v)
2. Test your hypothesis graphing the equation in Excel.

Experiment 1b: Factors controlling reaction rates

We will consider how the hexokinase reaction rate changes after changing substrate levels and V_{max} .

Blood glucose levels in humans = 5 mM (before chocolate) to 12 mM (after chocolate)

Basal glucose transporters can uptake 1 mM glucose (K_m)

Let's look at how the reaction rate changes if:

a. $K_m = 1 \text{ mM (Glucose)}$; $V_{max} = 15 \text{ reactions s}^{-1}$

Experiment 1b: Factors controlling reaction rates

Let's make our predictions:

- * How will the reaction rate be affected by increasing/decreasing K_m and V_{max} ?

In Excel, calculate and observe how the reaction rate changes if:

b. $K_m = 0.5, 1,$ and 2 mM ; $V_{max} = 15 \text{ reactions s}^{-1}$

c. $K_m = 1 \text{ mM}$; $V_{max} = 7.5, 15,$ and $30 \text{ reactions s}^{-1}$

Section II. Forest Tree Respiration and Climate Change



Forest tree respiration



Respiration is enzyme mediated, sugar consuming and energy producing.

It is strongly controlled by temperature but the temperature response can vary between species.

Black Rock Forest



www.blackrockforest.org/



LAMONT DOHERTY
EARTH OBSERVATORY
THE EARTH INSTITUTE AT COLUMBIA UNIVERSITY

<http://blackrockforest.org/>

Respiration can be measured instantaneously on a small scale.

How can we scale this up in space and time?



Steps

1. Use the respiration app. to evaluate the response of respiration of oaks and maples to temperature change:
<http://blackrock.ccnmtl.columbia.edu/> **USE FIREFOX!**
2. Use the **leaf-level** tab to evaluate the effect of changes in input variables on tree respiration (**Experiment 2a**)
3. Use the **canopy-level** tab to evaluate changes in seasonal respiration in the forest canopy (**Experiment 2b**)
4. State and test hypotheses on the relationship between input variables and respiration (**Experiment 3**)

Respiration Module

Leaf Level Respiration



In Cooperation with
CNML

Tree Respiration

Leaf Level Canopy Level Teaching Resources

Instructions: Plant respiration is often measured as CO₂ flux from the surface of a leaf, stem, soil or canopy. Highly regulated by temperature, the absolute rate of respiration and the temperature response varies among plant species. Respiration can be modeled by a modified version of the Arrhenius Function, described by Lloyd and Taylor (1994), which incorporates simple temperature coefficients to consider the impact of climate on this physiological process.

Enter values for the parameters of the Arrhenius function to graph the respiration rate for a given tree species. Use the red vertical indicator bar on the graph to view the calculated respiration rate (R) value(s) for all given tree species for your selected ambient temperature value (T_a).

Arrhenius Function

$$R = R_0 e^{\frac{E_0}{R_g} \left(\frac{1}{T_0} - \frac{1}{T_a} \right)}$$

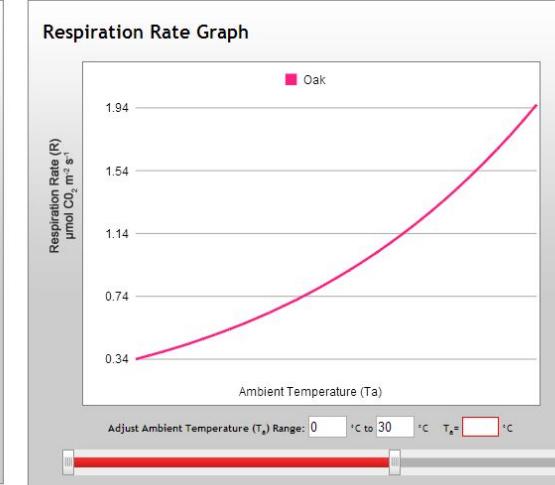
Set Respiration Rate Variables

Base Temperature: T₀ = 15 °C = 288.15 K

Species List

| | |
|---|--|
| Oak | R ₀ = <input type="text"/> |
| Respiration Rate @ base temperature | R ₀ = 86 umol m ⁻² s ⁻¹ |
| Energy of Activation @ base temperature | E ₀ = 40000 J mol ⁻¹ |

[Print](#)



Arrhenius function

$$R = R_0 e^{\frac{E_0}{R_g} \left(\frac{1}{T_0} - \frac{1}{T_a} \right)}$$

- R = Respiration
- R_g = *Ideal gas constant*
- R_0 = Base respiration, measured at the temperature T_0
- E_0 = Energy of activation for respiration, measured at the temperature T_0
- T_0 = Base temperature
- T_a = Ambient temperature → WE ASSUME THIS IS LEAF TEMPERATURE

To scale from leaf to canopy level, what do we need to consider?



Leaf Area Index (LAI)



Ratio of leaf area to ground area

Expressed as $\text{m}^2 \text{ leaf}$
 $\text{m}^{-2} \text{ ground}$

Ranges from 0 (bare leaves) to 15 (dense canopy) – BRF has an LAI of around 2.5

LAI plays important role in controlling interactions between terrestrial and atmospheric environmental variables

Respiration Module

Canopy Level Respiration

The Virtual Forest Initiative blackrock.ccnmtl.columbia.edu/respiration/forest

In Cooperation with 

Tree Respiration

Leaf Level Canopy Level Teaching Resources

Instructions: Explore how climate fluctuations and changes in species composition affect the respiration output of a forest using actual temperature data from Black Rock Forest. Enter the following scenario parameters into the fields below. Click on the Graph button to determine and view the total amount (moles) of carbon respired from a m² of forest for each of your scenarios.

Advanced options include a forest leaf area index that takes into consideration the canopy layers of the forest as well as a delta function that modifies the temperature data points across the board.

Arrhenius Function

$$R = R_0 e^{\frac{E_0}{R_g} \left(\frac{1}{T_0} - \frac{1}{T_a} \right)}$$

Set Scenario Variables

Print Graph >

Scenario List: 100% Oak Forest

Base Temperature T_0 = 15 °C = 288.15 K

Species List: Oak SC = 100 %

$R_0 = 0.86 \text{ umol m}^{-2} \text{s}^{-1}$

$E_0 = 40000 \text{ J mol}^{-1}$

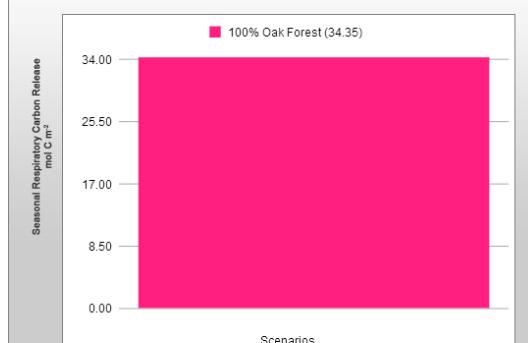
Advanced Options: Forest Leaf Area Index = 2.5, Field Station: Open Lowland, Year: 2005, Season Start (mm/dd): 5/15, Season End (mm/dd): 9/15, ΔT_a = 0 °C

[Export temperature data to CSV](#)

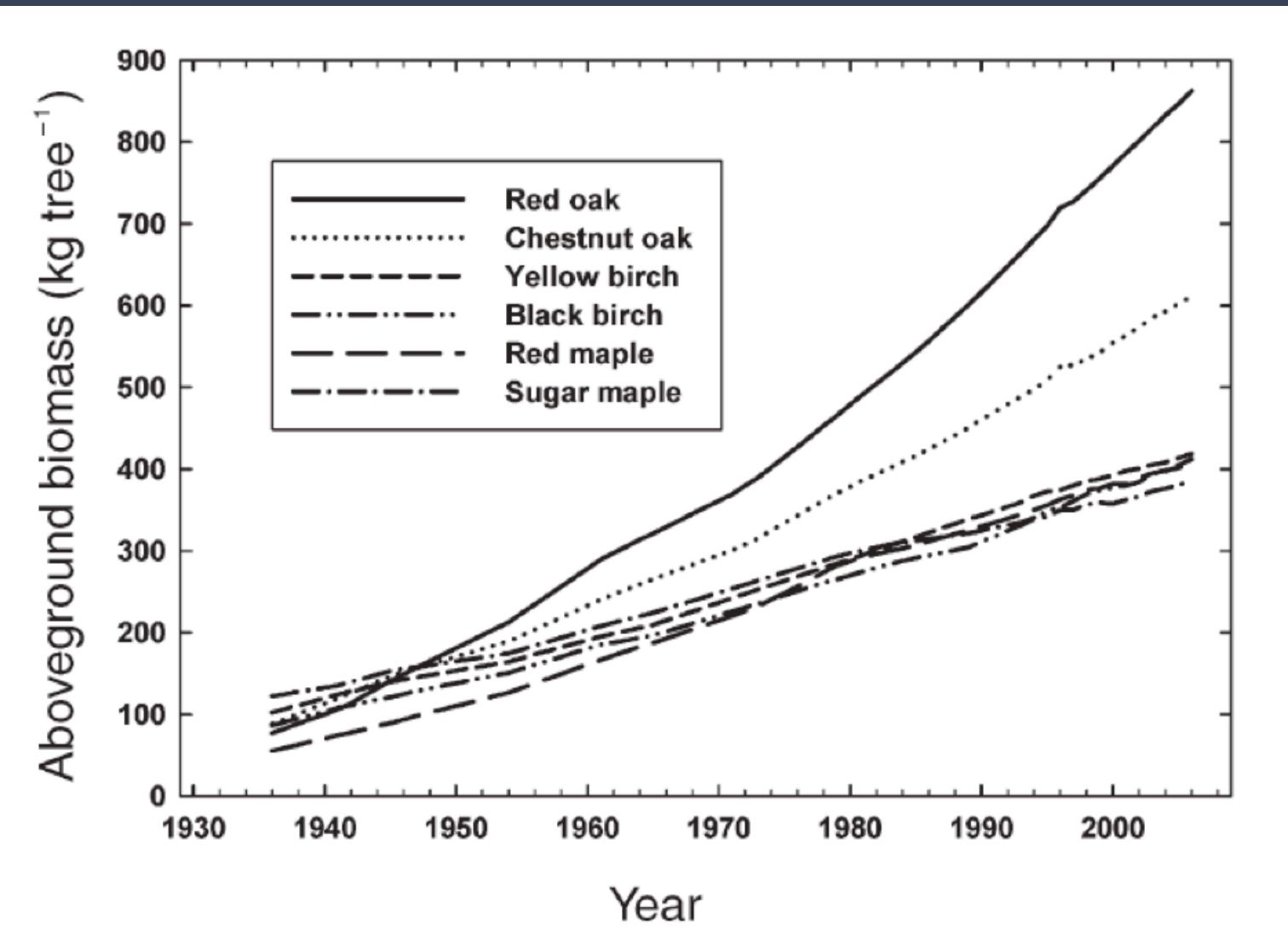
Scenario Graph

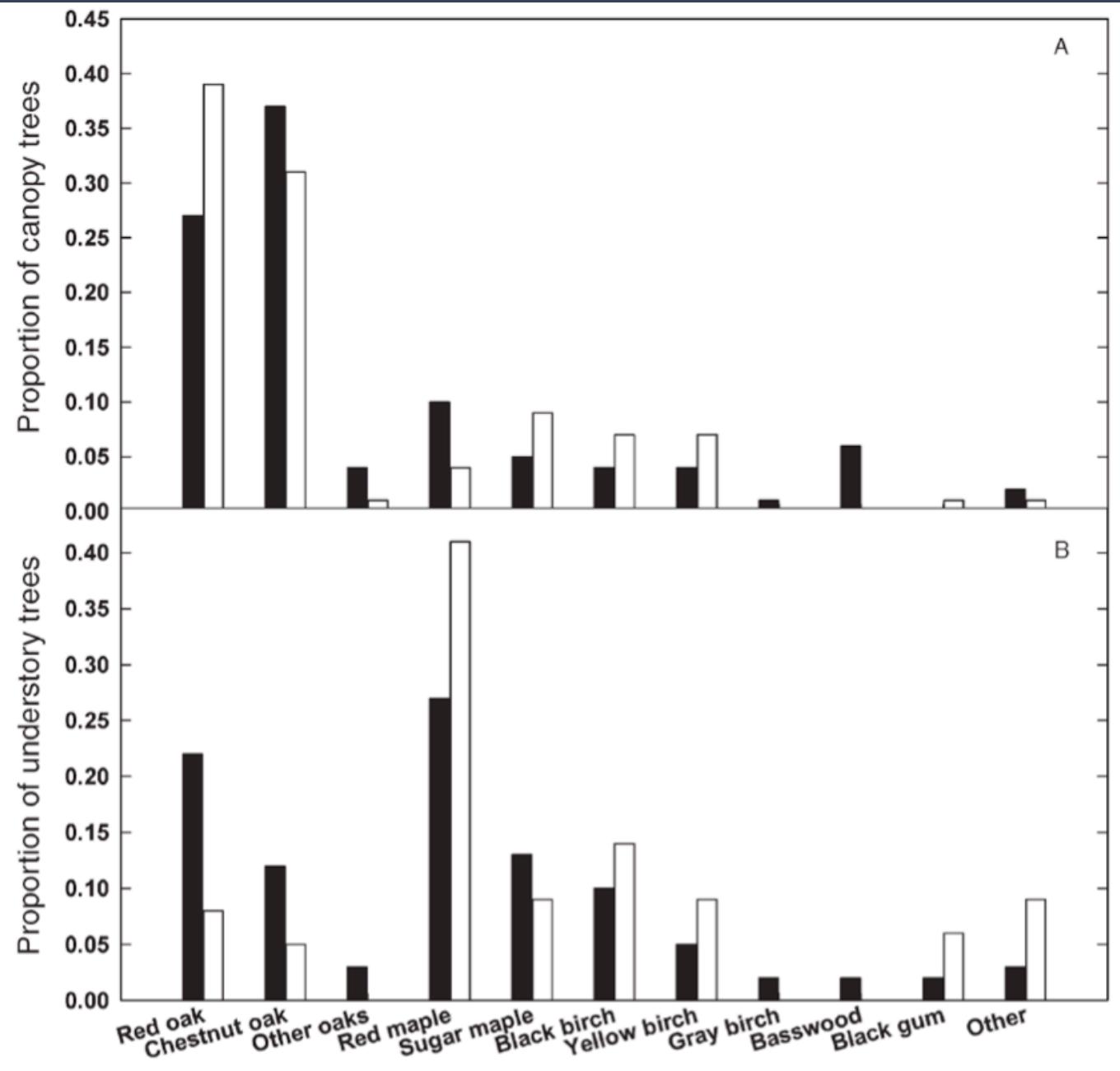
Seasonal Respiratory Carbon Release (moles C/m²)

Scenarios: 100% Oak Forest (34.35)



How might different tree species respond to climate change?





BRF Climate 2005

