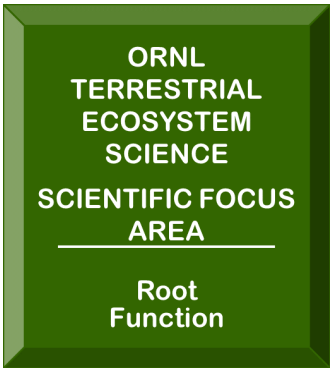
Physiological Responses of *Populus trichocarpa* to Warming



Summary

This data set contains empirical physiological, morphological, and chemical data collected over time on Western Black Cottonwood (*Populs trichocarpa* Torr. & A.Gray ex Hook., Salicaceae) clones, between July and December 2019 at Oak Ridge National Lab. The project was designed to experimentally warm *P. trichocarpa* clones and assess their physiological acclimation of leaves versus roots. Branch cuttings of the Nisqually-1 genotype were obtained from the US Department of Energy Joint Genome Institute (JGI) in Stanford, California, and propagated in leach tubes. Ninety genetically identical clones were planted into specially constructed mesocosm growth boxes and grown for an initial six weeks in the greenhouse, and then grown at three temperature treatments for ten weeks. The daytime air temperatures of treatments were approximately 25°C, 29°C, and 33°C. Measurements on plant physiology and growth were conducted at various intervals throughout the experiment.

Nine individual datasets are included in this data product. Data include measurements of light-saturated net photosynthesis and chlorophyll fluorescence, belowground CO_2 efflux, photosynthetic CO_2 response curves, light-response curves of photosynthesis, leaf photosynthesis temperature-response curves, leaf dark respiration-temperature response curves, root respiration-temperature response curves, data on leaf morphology (specific leaf area) and nitrogen content (on both mass and area basis), data on root nitrogen content (mass-based), and data on plant growth and biomass production. Leaf photosynthesis and respiration were measured with the Li6800 portable photosynthesis system (Li-COR, Lincoln, NE, USA). Root respiration measurements were made using the Li-6800 and the Walz 3010-GWK1 gas exchange chamber (Heinz Walz GmbH, Effeltrich, Germany).

There are nine comma-separated ASCII data files provided with the dataset along with this companion file.

User Note – Data File Updates July 7, 2020:
Two data files have been updated to correct the reporting of leaf mass area (LMA) and two derived variables -- leaf mass nitrogen per cm^2 (Leaf_Narea) and mass nitrogen corrected leaf CO_2 assimilation rate (A_N). The error was that the values reported for LMA, were actually specific leaf area (SLA).

Updates: The reported LMA (actually SLA) values were retained, but the variable name and documentation were changed to reflect the reporting of **SLA** ($cm^2\ g^{-1}$) and use in deriving Leaf_Narea and A_N. All mentions of LMA were removed. No data values were changed.
Leaf_Narea was recalculated, all values replaced, and documentation updated.
A_N values were recalculated, and all values replaced.

Notes: Only two of the nine data files were updated. No changes to the other data files. Several editorial changes were made to the User's Guide, including changing all mentions of leaf mass per area (LMA) to specific leaf area (SLA).

Dataset Change Log

Release Date	File Name	Status	User Action
May 5, 2020	photosynthesis_temp_response_ curve_data.csv	Initial release. Now superseded by 20200707 release.	
July 7, 2020	photosynthesis_temp_response_ curve_data_20200707.csv	First update	Download this new file with SLA updates.
May 5, 2020	respiration _temp_response_ curve_data.csv	Initial release. Now superseded by 20200707 release.	
July 7, 2020	respiration_temp_response_ curve_data_20200707.csv	First update	Download this new file with SLA updates.

Related Publication:

The measurements and results of this study have been described in the following publication:

Hogan, JA, Baraloto, C, Ficken, C, Clark, MD, Weston, D & Warren, J. Limited physiological acclimation and growth response of Poplar to warming. *Plant Physiology*

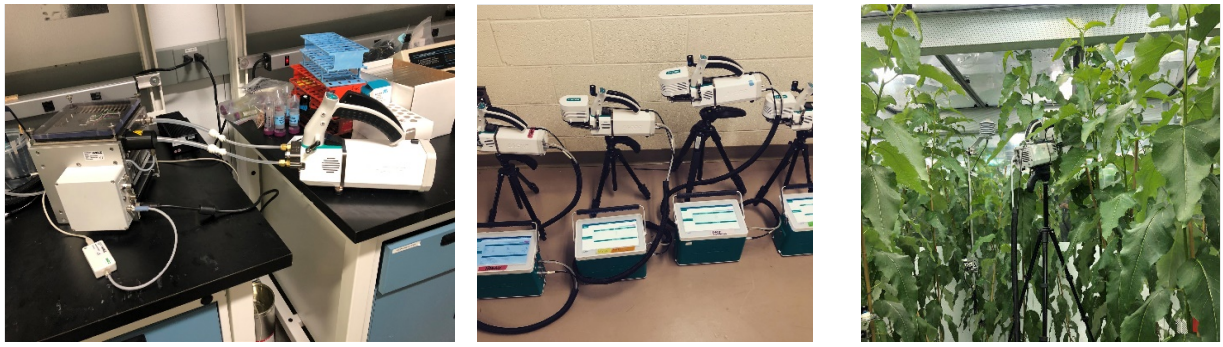


Figure 1. Leaf and root gas exchange measurements were conducted using the Li-6800 portable photosynthesis system. *Left:* the Li-6800 attached to the Walz respiration chamber, which was used to measure root tissue respiration and its response to temperature. *Middle:* fleet of Li-6800s ready to collected coupled leaf gas exchange and chlorophyll fluorescence measurements. *Right:* two Li-6800's collecting data in a growth chamber at Oak Ridge National Lab in Building 1506.

Data Citation:

Cite this data set as follows:

Hogan, J.A., Baraloto, C., Ficken, C.D., Clark, M.D., Weston, D.M., Warren, J.M. 2020. **Physiological Responses of *Populus trichocarpa* to Warming.** Oak Ridge National Laboratory, TES SFA, U.S. Department of Energy, Oak Ridge, Tennessee, U.S.A. https://doi.org/10.25581/ornlsfa.018/1617459

Acknowledgments:

Data were collected by J. Aaron Hogan (DOE SCGSR fellow from April 2019 to May 2020 at Oak Ridge National Laboratory, Environmental Sciences Division) with the direct supervision of Dr. Jeffrey Warren. Direct correspondence to: jamesaaronhogan@gmail.com

Thanks to Lionel Perez-Collazo, David MacLennan, Keith van Schaick, and Zach Ziegler for help with measurements and plant harvests.

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Data and Documentation Access:

Get Data

For public access to data from the US Department of Energy Terrestrial Ecosystem Science Scientific Focus Area (TES-SFA), please visit: https://tes-sfa.ornl.gov/

Description and Links to Supplemental Information

The Joint Genome Institute: https://jgi.doe.gov/

Table of Contents

1. Data Set Overview:

We grew 90 *Populus trichocarpa* clones and subjected them to warming at two intensities using walk-in growth chambers and had a control treatment. The 90 poplar clones of the Nisqually-1 genotype (wild genotype) were propagated from material obtained from the Joint Genome Institute in October 2018 in leach tubes. Plants were planted into mesocosm growth boxes on July 26, 2019, and experimental warming of two-thirds of the plants began on September 16, 2019. Plants were grown until November 22, 2019

This dataset contains photosynthesis and respiration measurements of leaves, respiration measurements of roots and soil, leaf and root functional traits, and plant growth and biomass data for 90 *Populus trichocarpa* clones grown of 16 weeks and subjected to three temperature treatments. The growth treatment air temperatures were approximately 25°C (ambient T1 treatment), 29°C (moderate warming T2 treatment), and 33°C (severe warming T3 treatment).

2. Data Characteristics:

Temporal Coverage:
Populus trichocarpa Torr. & A. Grey ex Hook. (Salicaceae) clones were propagated in October 2017; however, the experiment began with the planting of propagated clones on July 26, 2019. The research ran for 16 weeks and concluded with the final harvest on November 22, 2019.

Temporal Resolution:
Survey photosynthesis and belowground CO_2 efflux measurements were conducted weekly. Photosynthesis CO_2 response curves, light curves, and temperature response curves of photosynthesis and leaf dark respiration were done every two weeks. Leaf functional traits and nutrient data were measured on leaf areas where response curves were measured (also every two weeks).

Spatial Coverage:
This warming experiment was conducted at Oak Ridge National Lab (ORNL): 1 Bethel Valley Road, Oak Ridge, TN 37830 (35.9311 N, -84.3100 W).

Data File Descriptions:

These data are considered at **Quality Level 1**. Level 1 indicates an internally consistent data product that has been subject to quality checks and data management procedures.

Plant growth rates and biomass are given in separate datasets.

- Data photosynthesis and respiration measurements of leaves taken with the Li-6800 portable synthesis system (Li-COR Inc., Lincoln, NE, USA) are grouped by measurement technique and machine settings (i.e., there is one dataset for CO_2 response curves and a separate dataset for photosynthesis-temperature response curves, etc.).
- Leaf and root functional traits (i.e., specific leaf area and foliar and carbon and nitrogen concentrations) are given in datasets where rates of photosynthesis or respiration were standardized by leaf nitrogen content.

All datasets are provided in comma-separated (*.csv) ASCII files.

Please refer to Section 5, Measurements, for details of the measurement methods for data reported in each data file.

Data File Name	Description
biomass_data.csv	Plants were harvested, dried, and weighted for total and organ-based (i.e., leaf, stem, and root) biomass measurement. Plants were harvested at various intervals, wherein three plants per treatment were harvested. Harvest dates were: 1) 2019-09-17 (just prior to warming), 2) 2019-09-24 (7 days post-warming), 3) 2019-10-01 (14 days post-warming), 4) 2019-10-28 (41 days post-warming), and 5) 2019-11-12 (56 days post-warming). The remainder of the plants were harvested at the end of the experiment on 2019-11-22.
growth_data.csv	We measured basal diameter stem growth at two positions on the stem: 5 centimeters above (+5) and below (-5) the first sprout off of the planted clone main ramet. We also measured plant height growth. Measurements of plant stem diameter and height growth were done at four times over the 16-week experiment: 1) just after planting (2019-7-29), 2) at the start of warming (2019-9-16), 3) when plants were trimmed (2019-10-7), and 4) at the end of the experiment (2019-11-22). This dataset presents stem diameter and height growth rates over the various intervals between the four measurement periods

Data File Name	Description
CO2_response_curve_data.csv	CO_2-response curves were done every two weeks using the Li-6800. Chamber CO_2 concentration was cycled across the following reference IRGA concentrations in µmols per mol: 400,300,200,100,50,375,425,600,800,1000,1200,1500,2000,300 This dataset contains gas exchange measurement data in the simplest form possible, containing all the variables needed to fit the FvCB model for leaf photosynthesis and derive estimates for key photosynthetic parameters (e.g., J_{max}, V_{cmax}). For the paper, this was done using the 'fitaci' function in Remko Duursma's "plantecophys" package (1) in R.
light_response_curve_data.csv	Light-response curves were done every two weeks using the Li-6800. . Light (PPFD) incident on the leaf was varied from high intensity to low in at the following 15 levels (in µmols photon m^2 sec^{-1}): 2000,1800,1500,1200,1000,800,600,400,200,150,100,75,50,25,0. This dataset contains gas exchange measurement data in the simplest form possible, containing all the variables needed to fit the nonlinear regression model of the non-rectangular parabola of Marshall and Biscoe (2). From fits of that model, key photosynthetic parameters related to the light reactions of photosynthesis (e.g., the light compensation point and the apparent quantum yield) can be derived. For the paper, this was done using R code written by Mason Heberling (3).
photosynthesis_temp_response_curve_data_20200707.csv	Photosynthetic-temperature response curves were done every two weeks using the Li-6800. Chamber air temperature was varied at about ± 10°C from growing temperatures from low to high temperature. The sequence of Li-6800 air temperature variation for photosynthetic temperature response curves was as follows (in °C): Ambient & T1 sequence: 8,20,22,24,26,28,30,32,34,36,38,40,42; T2 sequence: 20,22,24,26,28,30,32,34,36,38,40,42,44; T3 sequence: 24,26,28, 30,32,34,36,38,40,42,44,46,48. This dataset contains gas exchange measurement data in the simplest form possible, containing all the variables needed to fit linear quadratic regression models. From quadradic parabola fits key photosynthetic parameters related to the temperature sensitivity of photosynthesis (e.g., T_{opt}, A_{max} at T_{opt}) can be derived. The rightmost four columns contain leaf functional trait values for specific leaf area (SLA), leaf % nitrogen, leaf % carbon, and leaf mass nitrogen per area. Those leaf functional traits were measured on 6 leaf punches taken using an 18.5mm Ø leaf punch (2.69 cm^2 area, about the size of a US Nickle) from the leaf tissue where the temperature response curve was measured. Values for SLA were averaged among all 6 punches, and the punches were homogenized into one leaf tissue sample, which was measured for leaf carbon and nitrogen (using an elemental analyzer).

Data File Name	Description
respiration_temp_response_curve_data_20200707.csv	Leaf dark respiration-temperature response curves were done every two weeks using the Li-6800. Respiration-temperature response curves were run concurrently with photosynthesis-temperature response measurements, in that temperature was increased with the light level in the leaf chamber at 1000 µmols m^{-2} sec^{-1}. Then the light level was decreased to 0, and the temperature was decreased, measuring leaf dark respiration. The sequence of Li-6800 air temperature variation for photosynthetic temperature response curves was as follows (in °C): Ambient & T1 sequence: 42,40,38,36,34,32,30,28,26,24,22,20,18; T2 sequence: 44,42,40,38,36,34,32,30,28,26,24,22,20; T3 sequence: 48,46,44,42,40,38,36,34,32,30,28,26,24. This dataset contains gas exchange measurement data in the simplest form possible, containing all the variables needed to fit linear, exponential models. From the exponential linear model fits key photosynthetic parameters related to the temperature sensitivity of dark respiration (e.g., Q_{10} values) can be derived. The rightmost four columns contain leaf functional trait values for specific leaf area (SLA), leaf % nitrogen, leaf % carbon, and leaf mass nitrogen per area. Those leaf functional traits were measured on six leaf punches 18.5mm Ø leaf punch (2.69 cm^2 area, about the size of a US Nickle) from the leaf tissue where the temperature response curve was measured. Values for SLA were averaged among all 6 punches, and the punches were homogenized into one leaf tissue sample, which was measured for leaf carbon and nitrogen (using an elemental analyzer).
root_respiration_temp_response_curve_data.csv	Root tissue respiration-temperature response curves were done twice during the experiment. Data were collected at the last two plant harvests prior to the conclusion of the experiment, on 2019-10-29 and 2019-11-12 (the experiment concluded with the final plant harvest on 2019-11-22). Measurements were done using the Li-6800 attached to a Walz temperature-controlled respiration chamber 3010-GK01 model (Heinz Walz GmbH, Eiffeltrich, Germany This dataset contains gas exchange measurement data in the simplest form possible, containing all the variables needed to fit non-linear models relating root tissue respiration rates to temperature. We used the following equation from Palta & Noble (3): $R_T = R_0(c - bT)^{T/10}$, , where R is root respiration at temperature T, R_0 is root respiration at 0°C, and b and c are constants which describe the slope and intercept, respectively, of the Q_{10} vs. T relationship.
survey_photosynthesis.csv	Survey measurements of leaf photosynthesis (gas exchange and chlorophyll fluorescence) were done weekly during the experiment using a Li-COR Li-6800 portable photosynthesis system. A pulse-modulated, multi-phase fluorometer induction flash followed by a dark pulse was used to measure chlorophyll fluorescence. This dataset contains only the essential leaf gas exchange and chlorophyll fluorescence parameters.

Data File Name	Description
soil_collar_belowground_CO2efflux_data.csv	Survey measurements of belowground CO_2 efflux were taken weekly throughout the experiment using a Li-6252 Infrared gas analyzer (IRGA) (Li-COR Inc., Lincoln, NE, USA). The data are given in the simplest form, with one row per chamber (i.e., CO_2 efflux collar) per sampling period.

Data Dictionary:

Biomass data: biomass_data.csv

Column Number	Column Name	Units/format	Description
1	Plant	integer	plant number (range:1-90).
2	Treatment	text	four-level factor for plant growth conditions: **GH:** initial six-week greenhouse establishment phase, **T1:** control treatment in the greenhouse (~25°C air temperature), **T2:** moderately warmed (+4°C) treatment using a walk-in growth chamber (~29°C air temperature), **T3:** severely warmed (+8°C) treatment using a walk-in growth chamber (~33°C air temperature).
2	Harvest	integer	Harvest number (range 1-6).
3	Harvest_date	yyyy-mm-dd	The date on which the plant was harvested for biomass measurement.
4	Age_at_harvets	days	Days elapsed from planting (on 2019-07-26) until plant harvest.
5	Leaf_biomass	grams	Total leaf biomass (dry weight).
6	Stem_biomass	grams	Total stem biomass (dry weight).
7	Root_biomass	grams	Total root biomass (dry weight).
8	Total_biomass	grams	Total biomass (dry weight).
9	Plant_height	cm	Maximum plant height reached. This is the total plant height (accounting for when plants were trimmed).
10	Leaf_area	cm^2	Total leaf area at harvest. Measured using Li-3100C Leaf Area Meter (Li-COR Inc., Lincoln, NE USA).
11	Biomass_increment	$g\ day^{-1}$	Total biomass increment of the plant.
12	Leaf_biomass_increment	$g\ day^{-1}$	Leaf biomass increment of the plant.
13	Leaf_area_increment	$cm^{-2}\ day^{-1}$	Leaf area increment of the plant.

Column Number	Column Name	Units/format	Description
14	Stem_biomass_increment	g day^{-1}	Stem biomass increment of the plant.
15	Root_biomass_increment	g day^{-1}	Root biomass increment of the plant.
16	Root_shoot	ratio	Root to shoot biomass ratio.
17	Root_leaf	ratio	Root to leaf biomass ratio.
18	Above_below	ratio	Above to below-ground biomass ratio.

Growth data: growth_data.csv

Column Number	Column Name	Units/format	Description
1	Plant	integer	plant number (range:1-90).
2	Treatment	text	four-level factor for plant growth conditions: **GH:** initial six-week greenhouse establishment phase, **T1:** control treatment in the greenhouse (~25°C air temperature), **T2:** moderately warmed (+4°C) treatment using a walk-in growth chamber (~29°C air temperature), **T3:** severely warmed (+8°C) treatment using a walk-in growth chamber (~33°C air temperature).
3	Interval	text	Measurements of plant stem diameter and height growth were done at four times over the 16-week experiment: 1) just after planting (2019-7-29), 2) at the start of warming (2019-9-16), 3) when plants were trimmed (2019-10-7), and 4) at the end of the experiment (2019-11-22). Growth rate was calculated over these defined Intervals: • planting_to_warming • planting_to_trim • warming_to_trim • planting_to_end • warming_to_end • trim_to_end
4	Growth_type	factor	Basal diameter stem growth was measured at two positions on the stem and plant height. • diameter+5: 5 centimeters above first sprout off the planted clone main ramet • diameter-5: 5 centimeters below first sprout off the planted clone main ramet • height: plant height growth
5	Growth_rate	cm or mm day^{-1}	The rate of growth, either in cm day^{-1} for height growth or mm day^{-1} for diameter growth.

CO_2 response curve data & light response curve data: CO2_response_curve_data.csv

All the variables in this data file are in common with several of the data files below. This table serves as the reference for the data files below.

Column Name	Units/format	Description
Date	YYYY-mm-dd	the date of measurement.
Week	integer	the week of the experiment (since planting).
Plant	integer	plant number (range:1-90).
Treatment	text	four-level factor for plant growth conditions: **GH:** initial six-week greenhouse establishment phase, **T1:** control treatment in the greenhouse (~25°C air temperature), **T2:** moderately warmed (+4°C) treatment using a walk-in growth chamber (~29°C air temperature), **T3:** severely warmed (+8°C) treatment using a walk-in growth chamber (~33°C air temperature).
Curve	integer	continuous unique identifier for each individual response curve.
Plant_Age	days	the age of the plant at the time of measurement (days since planting).
A	µmols m^{-2} sec^{-1}	leaf CO_2 assimilation rate (corrected for leaks).
Ci	µmols mol^{-1}	concentration of intracellular CO_2, a calculated parameter from the Li-6800 (based on stomatal conductance, and the difference in CO_2 between the reference and sample IRGAs).
Tleaf	°C	leaf temperature measured from the Li-6800 leaf thermocouple from the bottom of the leaf chamber.
Qin	µmols m^{-2} sec^{-1}	the light level (PPFD) incident on the leaf.

Photosynthesis temperature response curve data & respiration temperature response curve data:

photosynthesis_temp_response_curve_data_20200707.csv
respiration_temp_response_curve_data_20200707.csv

Column Number	Column Name	Units/format	Description
1	Date	YYYY-mm-dd	Variable in common.
2	Curve	integer	Variable in common.
3	Plant_Age	days	Variable in common.
4	Plant	integer	Variable in common.
5	Treatment	text	Variable in common.
6	A	µmols m^{-2} sec^{-1}	Variable in common.
7	Ci	µmols mol^{-1}	Variable in common.

Column Number	Column Name	Units/format	Description
8	Tleaf	°C	Variable in common.
9	Qin	µmols m^{-2} sec^{-1}	Variable in common.
10	A_N	µmols gN^{-1} sec^{-1}	the mass nitrogen corrected leaf CO_2 assimilation rate (calculated by taking A and dividing it by Leaf_Narea).
11	SLA	cm^2 g^{-1}	Specific leaf area. The average value of 6 circular leaf punches (Figure 2).
12	Leaf_N	%	leaf percent nitrogen from the homogenized leaf tissue sample (measured with an elemental analyzer).
13	Leaf_C	%	leaf percent carbon from the homogenized leaf tissue sample (measured with an elemental analyzer).
14	Leaf_Narea	g cm^{-2}	leaf mass nitrogen per leaf area (calculated using average leaf punch SLA and leaf %N).

Root Respiration-temperature response curve data:

root_respiration_temp_response_curve_data.csv

Column Number	Column Name	Units/format	Description
1	Date	YYYY-mm-dd	Variable in common.
2	Plant	integer	Variable in common.
3	Treatment	text	Variable in common.
4	Chamber_temp	°C	Walz respiration chamber temperature.
5	E	moles H_2O m^2 sec^{-1}	the rate of water loss from the root tissue.
6	R	µmoles CO_2 m^2 sec^{-1}	root respiration rate.
7	R_g	µmoles CO_2 g sec^{-1}	root mass-normalized respiration rate.
8	R_g_N	µmoles CO_2 g N sec^{-1}	root mass nitrogen-normalized respiration rate.
9	R_g_N_mmoles	mmoles CO_2 g N sec^{-1}	root mass nitrogen-normalized respiration rate (in millimoles).
10	Root_mass	g	dry mass of the amount of root tissue measured in the chamber.
11	Root_C	%	percent carbon in the root tissue measured.
12	Root_N	%	percent nitrogen of the root tissue measured.
13	Root_Nmass	g N	root dry mass nitrogen for the amount of root tissue measured in the chamber.

Survey photosynthesis data: survey_photosynthesis.csv

Column Number	Column Name	Units/format	Description
1	Date	YYYY-mm-dd	Variable in common.
2	Week	integer	Variable in common.
3	Treatment	text	Variable in common.
4	Plant	integer	Variable in common.
5	A	µmols m^{-2} sec^{-1}	Variable in common.
6	E	moles H_2O m^2 sec^{-1}	leaf transpiration rate (corrected for leaks).
7	Ci	µmols mol^{-1}	Variable in common.
8	gsw	moles H_2O m^2 sec^{-1}	stomatal conductance to water vapor.
9	Tleaf	°C	Variable in common.
	wue	µmols CO_2 mol^{-1} H_2O	photosynthetic (or instantaneous) water use efficiency (calculated as the ratio of assimilation to stomatal conductance).
	Fs	arbitrary units	steady-state fluorescence.
	Fm	arbitrary units	Fm' – maximal (light-adapted) fluorescence.
	Fv_Fm	ratio	fluorescence ratio (the ratio of variable fluorescence Fv' to maximal fluorescence Fm').
	PhiPS2	arbitrary units (range: 0-1)	the quantum yield of photosystem II (sometimes called the photochemical efficiency of photosynthesis).
	ETR	µmols m^{-2} sec^{-1}	the electron transport rate of photosystem II.
	PhiCO2	arbitrary units	the quantum yield of CO_2 assimilation corrected for dark respiration.
	NPQ	arbitrary units	the quantum yield of non-photochemical quenching (the approximate proportion of open reactions centers in photosystem II), calculated as (Fm – Fm')/Fm'.

Soil collar belowground CO_2 efflux data: soil_collar_belowground_CO2efflux_data.csv

Column Number	Column Name	Units/format	Description
1	Date	YYYY-mm-dd	Variable in common.
2	Week	integer	Variable in common.
3	Plant	integer	Variable in common.
4	Treatment	text	Variable in common.

Column Number	Column Name	Units/format	Description
5	Chamber	text	two-level factor indicating the mesocosm growth box section where the collar was installed. • "plant + soil" for the section containing the plant, • "soil" for the soil-only control section.
6	T0_CO2_ppm	ppm (parts per million)	The concentration of CO_2 at T0 (i.e., upon initial capping of the respiration collar), this value is calculated from the IRGA integration value of the air sample injected into the Li-6252 using a standard curve (integration value data, standard curves, and calculations are not included).
7	T1_CO2_ppm	ppm (parts per million)	The concentration of CO_2 at T1 (i.e., after incubation), this value is calculated from the IRGA integration value of the air sample injected into the Li-6252 using a standard curve (integration value data, standard curves, and calculations are not included).
8	Incubation_Length_sec	sec	Duration of collar incubation in seconds. The time between when the T0 and T1 samples were injected into the IRGA.
9	Incubation_Length_min	min	Duration of collar incubation in minutes.
10	CO2_flux_ppmv_min	ppm volume^{-1} (196 cm^3) min^{-1}	the change in chamber CO2 concentration over time
11	CO2_flux_uL_m2_min	µL m^{-2} min^{-1}	change in chamber CO_2 concentration in microliters per square meter per minute
12	CO2_flux_mL_m2_min	mL m^{-2} min^{-1}	change in chamber CO_2 concentration in milliliters per square meter per minute
13	CO2_flux_mL_m2_sec	mL m^{-2} sec^{-1}	change in chamber CO_2 concentration in milliliters per square meter per second
14	CO2_flux_g_m2_min	g m^{-2} sec^{-1}	change in chamber CO_2 concentration in grams per square meter per minute
15	CO2_flux_umols_m2_min	µmols m^{-2} min^{-1}	change in chamber CO_2 concentration in micromoles per m^2 per minute
16	CO2 flux_umols_m2_sec	µmols m^{-2} sec^{-1}	change in chamber CO_2 concentration in micromoles per m^2 per second.

3. Applications and Derivation:

Data can be used to assess the effect of warming on net photosynthesis (carbon uptake) and dark respiration (carbon loss) for the model tree species, *P. trichocarpa*. The dataset also allows the user to comprehensively evaluate the acclimation of photosynthesis and leaf dark respiration to increased temperature over time for individual plants. Measurements on any changes in leaf nutrients, morphology, and physiological characteristics, for example, leaf nitrogen content, specific leaf area, and stomata conductance as a response to the warming treatments and their effects on the leaf carbon fluxes can be evaluated. Photosynthesis CO_2 response curves can be used to study underlying photosynthetic biochemical mechanisms (V_{cmax}: maximum carboxylation capacity and J_{max}: maximum rates of electron transport). We directly measured photosynthetic and leaf dark respiration responses to temperature as well as evaluate treatments effects on their temperature sensitivity. We measured the temperature sensitivity of root respiration on two occasions near the end of the experiment. Additionally, plant growth and biomass responses to temperature can be investigated.

4. Quality Assessment

These data are considered at **Quality Level 1**. Level 1 indicates an internally consistent data product that has been subjected to quality checks and data management procedures. Established calibration procedures were followed.

5. Data Acquisition Materials and Methods:

Study Species Description:
Western black cottonwood, *Populus trichocarpa* Torr. & A.Gray ex Hook. (Salicaceae), is a model plant species, native to the northwestern coastal areas of North America. It is a fast-growing species with low wood density, long narrow leaves, and an extensive, aggressive root system. The species can reproduce clonally, like many other members of the willow family, and is cultivated by the North American timber industry for plywood, pulpwood, paper, and biofuel production. The US Department of Energy considers *Populus* species to be the choice genus of woody biomass production for bioenergy applications because if its fast growth, adaptability, and ease of propagation (5). In the wild, *P. trichocarpa* can hybridize with other poplar species, and many hybrid varieties have been developed for agriculture.

Western black cottonwood was the first woody tree species to have its genome sequenced and mapped (6), with the work mostly having been done at the Joint Genome Institute. Its genome is of modest size for plants, containing about 485 million bases and 45,000 genes (7). The genome that was sequenced is that of "Nisqually-1", a single female specimen that was collected in eastern Washington State, near the Nisqually River.

The native range for the western black cottonwood is the humid coastal forests of the Pacific Northwest from northern California to southern Alaska along the coast, with larger ranges in inland from Oregon to British Columbia, Canada. Within that geographical range, precipitation varies from about 250 mm to >3050 mm, and the growing season ranges from about 70 days in the northern, more inland areas to > 260 days in the southern part. The species can tolerate cold

temperatures by going physiologically dormant during the winter months. Minimum and maximum temperatures for the habitat range of *P.trichocarpa* are 0 to -47°C, and 16 to 47°C, respectively (8).

Materials and Methods:

Mesocosm growth boxes and P.trichocarpa clones

Western black cottonwood (*Populus trichocarpa* Torr. & A.Gray ex Hook., Salicaceae) ramets of the Nisqually-1 genotype were planted into leach tubes containing growth medium and allowed to establish for ten months, beginning in October 2018. The growth medium used throughout the experiment was a well-draining potting mix (pH 5.5 - 6.5), consisting of peat moss, vermiculite, perlite and processed pine bark (Farfad 52 mix, SunGro Horticulture, Agawam, MA), mixed with time-release Osmocote Plus Fertilizer (0.7 g kg-1 15-9-12, NPK). Ninety established clones with an average height of 14.4 cm (range: 8.1–24.8 cm) and average basal stem diameter of 4.5 mm (range:2.9–6.5 mm), were transplanted from leach tubes into mesocosm growth boxes on July 29, 2019. Prior to transplanting, in order to standardize clone size, stems were trimmed at the 7th leaf node and any roots extending beyond the bottom of the leach tube were cut off.

Mesocosm growth boxes (38 × 23.5 × 18 cm L× W × H, 15.14 L capacity) were constructed from AkroGrid boxes (model 33168, Akro-Mils, Akron, OH), using a modified methodology of Ficken & Warren (9). Three small holes were drilled on either side of the boxes, near the bottom to allow for water drainage. Each box was filled with 3.5 kg. of potting mix and had a 1-micron mesh partition installed to separate one-third of the box volume. The mesh barrier was designed to exclude plant roots but permit the movement of microbes and water between mesocosm portions, effectively creating a soil control for each mesocosm. *P. trichocarpa* clones were positioned in the middle of the larger compartment, and each compartment was equipped with a PVC soil CO_2-efflux collar. Collars were made from schedule 40 PVC (5 cm diameter and 10 cm in length), with twelve 3.7 cm-diameter holes drilled into the portion of the collar that sat below the soil surface. Mesocosm boxes were encased in CoolShield thermal bubble wrap (ULINE, Pleasant Prairie, WI) to mimic natural temperature differences between the soil and air.



Figure 2. *Populus trichocarpa* growing in mesocosm growth boxes. The PVC soil CO_2-efflux collars are evident.

Growth conditions & experimental warming

The duration of the experiment was 16 weeks, wherein plants were grown at experimental temperatures for ten weeks. Throughout the experiment, axillary sprouts were pruned to constrict plant growth to the main stem. All ninety of the plants were grown together for six weeks to allow them to establish root biomass in mesocosms. During this initial establishment period, the plants were drip irrigated, receiving about 1 liter of water day^{-1}, and they received two doses of soluble fertilizer (about 1.4 g $dose^{-1}$, Southern Ag Nitrate Special 20-10-20, NPK) roughly two weeks apart. When the plants were 44 days old, sixty of the plants were transferred to two walk-in growth chambers (30 per chamber, CONVIRON, Ontario, Canada).

Growth chambers were set to 70% relative humidity and at 29°C and 33°C, respectively, each with a 4°C degree nighttime temperature drop. Growth chamber temperatures were chosen based on the environmental conditions of the greenhouse during the initial establishment period, where temperatures maximized at 25°C with at most a 4°C nighttime decrease. A climate station, with two air thermometers, two relative humidity sensors, two quantum sensors, and 12 soil temperature sensors, was used throughout the experiment and rotated among the three treatments at 2-week intervals to monitor their environmental conditions.

The plants grew vigorously, on average >2 cm day^{-1}, were 1.5 to 2 m in height when transferred to the growth chambers, and reached the ceiling in the growth chambers about three weeks into warming. Therefore, at week 10 (day 71) of the experiment, all plants were pruned to 1.5 m height. Additionally, at week 10 (day 71), the experimental treatments were swapped between growth chambers, to spread any potential latent effects of each specific growth chamber over both treatments.



Figure 3. Some methodological considerations of the Poplar Phys project. *Left:* Specific leaf area (SLA) and leaf nitrogen content were assessed using the leaf punch method, where six leaf discs were punched out from the leaf tissue area where the Li-6800 was clamped to measure photosynthetic responses (to CO_2, light, and temperature). SLA was averaged across all six discs, and they were homogenized into one sample for elemental analysis. *Middle:* A mass of *P. trichocarpa* roots and soil at harvest (view from bottom). *Right:* the Li-6252 IRGA (Li-COR Inc., Lincoln, NE, USA) was used for measuring soil CO_2 efflux. Soil respiration collars installed into mesocosm growth boxes were capped, and a gas sample was taken from the

chamber headspace using a syringe and analyzed using the Li-6252 adapted for low CO_2 concentration gas analysis. pictured: David MacLennan (in plants) and J. Aaron Hogan (at the computer) take measurements inside a growth chamber at ORNL.

Measurements:

Biomass

Plants were harvested, dried, and weighted for total and organ-based (i.e., leaf, stem, and root) biomass measurement. Plants were harvested at various intervals, wherein three plants per treatment were harvested.

Harvest dates were:
1) 2019-09-17 (just prior to warming),
2) 2019-09-24 (7 days post-warming),
3) 2019-10-01 (14 days post-warming),
4) 2019-10-28 (41 days post-warming), and
5) 2019-11-12 (56 days post-warming).

The remainder of the plants were harvested at the end of the experiment on 2019-11-22.

Growth

We measured basal diameter stem growth at two positions on the stem: 5 centimeters above (+5) and below (-5) the first sprout off of the planted clone main ramet. We also measured plant height growth.

Measurements of plant stem diameter and height growth were done at four times over the 16-week experiment:
1) just after planting (2019-7-29),
2) at the start of warming (2019-9-16),
3) when plants were trimmed (2019-10-7), and
4) at the end of the experiment (2019-11-22).

This dataset presents stem diameter and height growth rates over the various intervals between the four measurement periods

CO_2-response Curves

CO_2-response curves were done every two weeks using the Li-6800. The light level was set to a non-limiting 1000 µmols mol^{-1}. Leaf VPD was maintained at 1.25 KPa, and the chamber air temperature was set to the ambient conditions of the growth environment, which varied over time and by the experimental treatment. Chamber CO_2 concentration was cycled across the following reference IRGA concentrations in µmols per mol:
400,300,200,100,50,375,425,600,800,1000,1200,1500,2000,300. (note: the last data point was typically left out when fitting the Farquhar-von Cammerer-Berry (FvCB) model of

photosynthesis, it was included in the cycle to get the machine back close to 400 ppm chamber [CO_2] for the next leaf).

Light-response Curves

Light-response curves were done every two weeks using the Li-6800. Chamber incoming CO_2 concentration was held constant at 400 µmols mol^{-1}, leaf VPD was maintained at 1.25 KPa, and the chamber air temperature was set to the ambient conditions of the growth environment, which varied over time and by the experimental treatment. Light (PPFD) incident on the leaf was varied from high intensity to low in at the following 15 levels (in µmols photon m^2 sec^{-1}): 2000,1800,1500,1200,1000,800,600,400,200,150,100,75,50,25,0.

This dataset contains gas exchange measurement data in the simplest form possible, containing all the variables needed to fit the nonlinear regression model of the non-rectangular parabola of Marshall and Biscoe (2). From fits of that model, key photosynthetic parameters related to the light reactions of photosynthesis (e.g., the light compensation point and the apparent quantum yield) can be derived. For the paper, this was done using R code written by Mason Heberling (3).

Photosynthetic-temperature Response Curves

Photosynthetic-temperature response curves were done every two weeks using the Li-6800. Chamber incoming CO_2 concentration was held constant at 400 µmols mol^{-1}, leaf VPD was maintained at 1.25 KPa, and the light level in the leaf chamber was set to a non-limiting 1000 µmols photon per square meter per second. The chamber air temperature was varied, and leaf photosynthesis was allowed to stabilize before logging the data point. Chamber air temperature was varied at about ± 10°C from growing temperatures from low to high temperature. The sequence of Li-6800 air temperature variation for photosynthetic temperature response curves was as follows (in °C): Ambient & T1 sequence: 8,20,22,24,26,28,30,32,34,36,38,40,42; T2 sequence: 20,22,24,26,28,30,32,34,36,38,40,42,44; T3 sequence: 24,26,28, 30,32,34,36,38,40,42,44,46,48.

This dataset contains gas exchange measurement data in the simplest form possible, containing all the variables needed to fit linear quadratic regression models. From quadradic parabola fits key photosynthetic parameters related to the temperature sensitivity of photosynthesis (e.g., T_{opt}, A_{max} at T_{opt}) can be derived.

The rightmost four columns contain leaf functional trait values for specific leaf area (SLA, leaf % nitrogen, leaf % carbon, and leaf mass nitrogen per area. Those leaf functional traits were measured on 6 leaf punches taken using an 18.5mm Ø leaf punch (2.69 cm^2 area, about the size of a US Nickle) from the leaf tissue where the temperature response curve was measured. Values for SLA were averaged among all 6 punches, and the punches were homogenized into one leaf tissue sample, which was measured for leaf carbon and nitrogen (using an elemental analyzer).

Leaf Dark Respiration-temperature Response Curves

Leaf dark respiration-temperature response curves were done every two weeks using the Li-6800. Respiration-temperature response curves were run concurrently with photosynthesis-temperature response measurements, in that temperature was increased with the light level in the leaf chamber at 1000 µmols m^{-2} sec^{-1}. Then the light level was decreased to 0, and the temperature was decreased, measuring leaf dark respiration. Chamber incoming CO_2 concentration was held constant at 400 µmols mol^{-1}, leaf VPD was maintained at 1.25 KPa, and the light level in the leaf chamber was off. The chamber air temperature was varied, and leaf respiration was allowed to stabilize before logging the data point. Chamber air temperature was varied at about ± 10°C from growing temperatures in a decreasing fashion. The sequence of Li-6800 air temperature variation for photosynthetic temperature response curves was as follows (in °C): Ambient & T1 sequence: 42,40,38,36,34,32,30,28,26,24,22,20,18; T2 sequence: 44,42,40,38,36,34,32,30,28,26,24,22,20; T3 sequence: 48,46,44,42,40,38,36,34,32,30,28,26,24.

This dataset contains gas exchange measurement data in the simplest form possible, containing all the variables needed to fit linear, exponential models. From the exponential linear model fits key photosynthetic parameters related to the temperature sensitivity of dark respiration (e.g., Q_{10} values) can be derived.

The rightmost four columns contain leaf functional trait values for specific leaf area (SLA), leaf % nitrogen, leaf % carbon, and leaf mass nitrogen per area. Those leaf functional traits were measured on six leaf punches 18.5mm Ø leaf punch (2.69 cm^2 area, about the size of a US Nickle) from the leaf tissue where the temperature response curve was measured. Values for SLA were averaged among all 6 punches, and the punches were homogenized into one leaf tissue sample, which was measured for leaf carbon and nitrogen (using an elemental analyzer).

Root Tissue Respiration-temperature Response Curves

Root tissue respiration-temperature response curves were done twice during the experiment. Data were collected at the last two plant harvests prior to the conclusion of the experiment, on 2019-10-29 and 2019-11-12 (the experiment concluded with the final plant harvest on 2019-11-22). Measurements were done using the Li-6800 attached to a Walz temperature-controlled respiration chamber 3010-GK01 model (Heinz Walz GmbH, Eiffeltrich, Germany). Whole plant root systems were washed, and approximately 100 grams of fresh root biomass from the most distal end of the root system was cut off and placed in the Walz chamber. Incoming CO_2 concentration and relative humidity were held constant at 400 µmols mol^{-1} and 70%, respectively. The chamber was covered with thick paper to prevent light from entering. The temperature in the Walz chamber was precisely controlled using the GFS-Win software, where it was increased from 15 to 50°C in 2.5°C increments. Li-6800 IRGA readings were allowed to stabilize before data point logging; Stability criteria (assessed on the sample IRGA) were *ΔH2O.meas2* slope and standard deviation both < 0.1 mmol per mol (water vapor) & *ΔCO2.meas2* slope < 0.25 and standard deviation < 0.1 µmols per mol CO_2, over a period of 15 seconds.

This dataset contains gas exchange measurement data in the simplest form possible, containing all the variables needed to fit non-linear models relating root tissue respiration rates to temperature. We used the following equation from Palta & Noble (3): $R_T = R_0(c - bT)^{T/10}$, where R is root respiration at temperature T, R_0 is root respiration at 0°C, and b and c are constants which describe the slope and intercept, respectively, of the Q_{10} vs. T relationship.

Survey Measurements of Leaf Photosynthesis (gas exchange and chlorophyll fluorescence)

Survey measurements of leaf photosynthesis (gas exchange and chlorophyll fluorescence) were done weekly during the Poplar Phys experiment using a Li-COR Li-6800 portable photosynthesis system. Chamber incoming CO_2 concentration was held constant at 400 µmols mol^{-1}, leaf VPD was maintained at 1.25 KPa, and the light level in the leaf chamber was set to a non-limiting 1000 µmols m^2 sec^{-1}. The flow rate was 600 µmols mol^{-1}, and the chamber air temperature was set to match the environmental conditions at the time of measurement.

A pulse-modulated, multi-phase fluorometer induction flash followed by a dark pulse was used to measure chlorophyll fluorescence, using the following parameters: measuring beam - dark mod rate = 5 KHz, light mod rate = 50 KHz, flash mod rate = 250 KHz, averaging interval = 15 seconds; multiphase flash - red target = 10000 µmols m^2 sec^{-1}, phase 1,2 & 3 = 300 ms, ramp = 40%, output rate = 500 Hz, margin = 5, dark pulse - far-red target = 25 µmols m^2 sec^{-1}, duration = 5s, before & after = 1s, margin = 5.

Four stability criteria were used over a 15-second window: 1) *ΔH2O.meas2* (mmols mol^{-1}) – measures the difference in [H_2O] between the reference and sample IRGA, slope < 0.5. 2) *ΔCO2.meas2* (µmols mol^{-1}) – measures the difference in [CO_2] between the reference and sample IRGA, slope < 1 & standard deviation < 0.75. 3) g_{sw} (mols m^2 sec^{-1}) – the calculated rate of leaf stomatal conductance to water vapor, slope < 0.1. 4) A (µmols m^2 sec^{-1}) – calculated rate of leaf CO_2 assimilation, slope < 0.5 standard deviation < 0.5.

This dataset contains only the essential leaf gas exchange and chlorophyll fluorescence parameters.

Survey Measurements of Belowground CO_2 Efflux

Survey measurements of belowground CO_2 efflux were taken weekly throughout the Poplar Phys experiment. The experimental set involved mesocosm growth boxes that were partitioned into two sections using a 1-micron mesh, which prevented root growth (but permitted the flow of water, microbes and fungi) from one-third of the growth box. The poplar clone was grown in the remaining two-thirds of the mesocosm growth box volume. In each section of the mesocosm, a 5 cm diameter PVC soil CO_2 efflux collar was installed into the soil during clone planting. Collars were 10 cm long and had twelve large (3.7 cm diameter) holes drilled into the portion of the collar that sat below the soil level. This design allowed us to repeatedly measure the "plant + soil" and "soil" CO_2 efflux.

Measurements were done weekly using a Li-6252 Infrared gas analyzer (IRGA) (Li-COR Inc., Lincoln, NE, USA). The IRGA was connected to an N_2 carrier gas stream which was regulated to flow through the IRGA at a rate of 0.1 liters per minute. PVC collars were capped (using a 5cm diameter PVC cap equipped 20mm butyl septums) with 1, and the headspace air was mixed by using a 50mL syringe (3x pumping). Then a 1 mL air sample was collected from the headspace and injected into the IRGA via the N_2 carrier gas, and the total integration value was recorded (this is the T0 measurement). Capped collars were incubated (i.e., accumulated belowground CO_2 efflux) for at least an hour, and a second measurement (T1) was taken using the same method (headspace mixing followed by analyzing a 1 mL gas sample). The incubation length was closely monitored and recorded, and (accounting for any variation in incubation time) the difference in the T1 and T0 measurements were used to calculate soil CO_2 efflux rates. Li-6252 integration values were converted to CO_2 concentrations using the standard curves, which were done at the beginning and end of each measurement cycle.

The data are given in the simplest form, with one row per chamber (i.e., CO_2 efflux collar) per sampling period.

6. References

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7. Data access

For public access to ORNL TES SFA data please visit the TES SFA Web Site: https://tes-sfa.ornl.gov/home

Contact for Data Access Information: https://mnspruce.ornl.gov/contact