FigShare Dataset Name: **Dataset: 1999 CO2xCommunity Experiment Belowground Biomass**

The dataset is composed of 11 files.
1. **Read Me (2021-08-19).docx**: This document explaining dataset contents (same as files 2 & 3)
2. **Read Me (2021-08-19).pdf**: This document explaining dataset contents (same as files 1 & 3)
3. **Read Me (2021-08-19).htm**: This document explaining dataset contents (same as files (1 & 2)

4. **1999_GCReW_CO2xCommunity_Experiment_Root_Biomass_Assembled.csv**: Main dataset containing dry masses disaggregated by belowground component, depth increment, core replicate, plot, plant community and experimental treatment.

5. **CO2_community_belowground_derivative_data_1999.csv**: Derived dataset containing estimated total belowground biomass and rooting depth disaggregated by core replicate, plot, plant community, and experimental treatment.

6. **GCREW 1999 Below Ground Derivative Traits.jpg**: A figure visualizing the dataset.

7. **GCREW_Root_depth_derived.zip:** A zipped folder containing replicable work flow for calculating derived traits from disaggregated data.

- o   8. **GCREW_root_depth_derived.Rproj**: An R project file which will manage the directories for the workflow.
- o   9. **packages.bib**: A latex file which contains citations documented in the workflow
- o   10. **Root Depth Derived Workflow.Rmd**: An R markdown folder which documents and performs the analysis.
- o   11. **Root-Depth-Derived-Workflow.html**: html report which documents the analysis.

This Read Me file has three tables that describe the methods in detail (Table 1), variables in the disaggregated data file (Table 2), and variables in the derivative data file (Table 3):

Table 1. Description of the datasets and detailed methods.	
1. Name	Pat Megonigal
2. Dataset File Names	1999 CO2xComm Root Biomass Data Assembled.csv, CO2_community_belowground_derivative_data_1999.csv
3. Dataset Version	Version 2
4. Lead Investigators	Megonigal, Patrick; Drake, Bert
5. Other Investigators	Kristin Fitzgerald; Gary Peresta
6. Contact	Megonigal, Patrick. Email: megonigalp@si.edu, Tel: 443-482-2346
7. Start Date	1999
8. End Date	1999
9. Location	38.874214°N, -76.549571°W; Smithsonian Global Change Research Wetland, Smithsonian Environmental Research Center, Edgewater, MD 21037. Known traditionally as Kirkpatrick Marsh. Adjacent to the Rhode River.

10. Taxa	C3 sedge *Scirpus olneyi* (A.) Gray; the C4 grass *Spartina patens* (Aiton) Muhl.; and the C4 grass *Distichlis spicata* (L.) Greene. Other species include *Atriplex patula*, *Iva frutescens*, *Kosteletskya virginica*, *Lythrum lineare*, and *Polygonum hydropiper*.
11. Keywords	global change, elevated CO2, open-top chamber, saltmarsh, long-term experiment, *Schoenoplectus*, *Spartina*, *Distichlis*, climate change experiments, roots, rhizomes
12. Abstract	**Abstract** Belowground biomass for 20 of the 45 experiment plots in the "CO2xCommunity" experiment taken in August 1999. Samples were taken after 13 years of continuous treatment with elevated CO_2 at an approximate CO_2 concentration of 750 ppm. Two soil cores were taken from each ambient CO_2 and elevated CO_2 plot in two plant communities, the C3 and C4 communities. A third plant community in the experiment was not sampled. The soil cores were 5.1 cm in diameter and 100 cm in length, extracted with a piston corer in three sections at target intervals of 0-33 cm, 34-66 cm, and 67-100 cm. The soil cores were sub-sectioned horizontally by depth. All belowground biomass was recovered from the top 15 cm of the soil core. At depths greater than 15 cm some soil sub-sections were used to estimate belowground biomass while others were used to estimate bulk density. Belowground biomass was sorted into the morphological categories living roots, rhizomes, and culms, and litter (i.e., dead roots and detritus), and color categories that can be interpreted as coming from C4 grasses or C3 sedges to a rough approximation. The data were gap-filled for depth sub-sections with no belowground biomass data, and modeled to provide estimates of whole-profile root biomass to a depth of 1 m. Total below ground biomass was significantly (p=0.017) and positively affected by the experimental treatment. *Spartina patens* had significantly less below ground biomass than *Schoenoplectus americanus* (p<0.0001). Detailed methods and other metadata are provided in the Read Me file. The dataset is composed of 11 files including the disaggregated data, the derived data for total biomass, and replicable workflow in R for calculating the derived data from disaggregated data. **Methods** *Experimental Design* The experiment from which the cores were taken is a collection of 45 experimental plots across three plant communities (initially sedge-dominated, initially grass dominated, and initially mixed). Each community has 15 plots and three treatments (ambient, elevated and unchambered control). The ambient treatment chambers (n=5) are ventilated with ambient air. The elevated chambers receive enough additional CO2 to raise the concentration to approximately 700 ppm. The third treatment is a plot that is not enclosed by a chamber (i.e. no-chamber control); these which were not sampled in this campaign. *Coring* This dataset contains the data on the standing stock of the "CO2xCommunity" experiment started by Bert Drake in 1987. In August 1999 after 13 years of continuous CO2 treatment, we collected two replicate soil cores from each chamber in the C3 and C4 plant communities (the Mixed community was not sampled). The cores were 5.1 cm in diameter and 100 cm in length. They were extracted with a piston corer in three sections at target intervals of 0-33 cm, 34-66 cm, and 67-100 cm. The cores were stored on ice in the field then at 4 oC until they were processed. The data reported here are roots recovered from these cores. *Sectioning and Sub-sampling*

The soil cores were sectioned horizontally by depth. Consistent depth intervals were used with the exception that the surface most interval (0-2.5 cm) was further sectioned into two intervals (0-1.25 cm and 1.25-2.5 cm) on one of the two replicate cores from each chamber (core 2). All roots were recovered from the top 15 cm of the soil core, while at deeper depths some sections were used to estimate root biomass while other sections were used for bulk density. Subsamples from 0-23 cm depth were nominally 2.5 cm thick while deeper subsamples were nominally 5.0 cm thick. The true thickness of each subsample was estimated by measuring the thickness with a micrometer at four cardinal locations and calculating the average. The volume of the subsample was calculated as the average thickness multiplied by the diameter of the corer (5.1 cm). The nominal center of each section was used as the label.

Washing and Sorting
The samples were wet sieved through a 1.0 mm sieve then sorted into categories defined by size, tissue type, and color. The smallest roots were subsampled by dispersing them in shallow DI water in a glass pan and randomly selecting 4 of 8 quadrants in the pan for sorting into categories that represent function (roots, rhizomes, culms) and species (based on color).

Color is a reliable indicator of species in this ecosystem. Red roots and rhizomes are from C3 sedges (Schoenoplectus americanus) and white rhizomes are from C4 grasses (Spartina patens and Distichlis spicata). White roots can be either C3 sedges or C4 grasses but are dominantly C4 grasses even in C3-dominated plan communities as shown by Saunders et al. 2006 (Saunders, Megonigal, and Reynolds. 2006. Comparison of belowground biomass in C3- and C4-dominated mixed communities in a Chesapeake Bay brackish marsh. Plant Soil 280: 305–322. https://doi.org/10.1007/s11104-005-3275-3). The categories reported here are LRT=light-colored roots; RRT=red roots; DRT=dark roots; WRH=white rhizome; RRH=red rhizome; RHC=rhizomotous culms; NRHC=non-rhizomotous culms; LITT=litter. Litter is detritus that included dead roots identified based on color, rigidity, and the condition of the epidermis.

Data Reduction
In core 2 of each chamber the two surface-most depth intervals (0-0.125 and 0.125-2.5) were added together to yield a single depth interval of 0-2.5 cm, matching the intervals in core 1. In cases where the roots were subsampled by counting half of the full sample, the mass was multiplied by 2.

Calculation of Whole-Profile Carbon Stocks
The whole-profile carbon stock calculations provided by James Holmquist were done as follows. We post-processed the belowground biomass data in three steps: 1. we gap filled depth increments that were not directly measured; 2. we calculated an estimated total belowground biomass based on the gap-filled biomass; and 3. we estimated the maximum (95% percentile) rooting depth by fitting an exponential function to the shape of the gap filled root mass depth profile. We then performed statistical tests on the post- processed data to determine whether differences between experimental effects or between species nested within experimental effects were significant.

Step 1. Gap Filling
The measurements for each core were not all continuous. Near the top of the core measurements were continuous with depth increments that were nominally 2.5 cm

wide, but deeper down the measurements were discontinuous and nominally 5 cm wide.

We gap-filled the sections of the core that were not measured by assigning representative depth increments to each measured increment, then scaling the measured biomasses by the ratio of representative to measured volume. Representative depth horizons were assigned from top to bottom, as the halfway point between the bottom of one depth increment and the top of the next deeper increment. The representative minimum depth was always 0 for the shallowest sample and was always the deepest measured depth for the deepest section. Near the top of the core where measurements were continuous, representative depth horizons were equivalent to measured depth horizons.

For each depth increment we calculated a scaler by dividing the measured sample volume by the representative depth increment's volume. The representative volume was calculated as the width of the depth increment, multiplied by the area of the 5.1 cm diameter corer (=0.051 m; Area=$\pi[0.051/2]^2$. Note that the variable used for the measured volume was "volume" which was calculated from the measured thickness of each subsample and the known width of the core (i.e. the variable "target_width" was not used).

For each representative depth increment we estimated a scaled biomass by multiplying the scaler by the measured biomass.

Step 2. Estimating Total Biomass

For each core we estimated total biomass by summarizing all scaled biomass values, then converting grams to grams per square meter by dividing summed mass by the area of the core (Area=$\pi[0.051/2]^2$).

3. Estimating 95% maximum rooting depth

We estimated the maximum rooting depth for each core as the depth of 95% of the cumulative belowground biomass by fitting a model of cumulative root biomass as a function of maximum representative depth. The 95% maximum rooting depth is how exponential rooting depths are parameterized in the Marsh Equilibrium Model (Schile et al., 2014).

We calculated cumulative biomass for each representative horizons from top to bottom by summarizing the biomass in the target horizon as well as all of the shallower horizons in the core. Visually plotting the cumulative sum as a function of maximum representative horizon depth indicated that the root mass has an exponentially decaying shape, so we fit the cumulative density function of the exponential distribution.

In order to satisfy the assumptions of the exponential distribution we normalized biomass values by divided the cumulative sum for each depth increment by the total biomass in the core. We then rescaled this normalized biomass to equal 0.999, or 99.9% of the total biomass. The reason for this step is to satisfy an assumption of the exponential distribution, that the cumulative biomass approaches 100% asymptotically with depth but never actually reaches it. We fit a function describing the cumulative biomass proportion (C) as a function of the maximum representative depth (d; C=$1-\exp[-\lambda d]$). We solved for λ for each core using the non-linear solver (nls) function in R. We then extrapolated the depth of

	95% of the root mass (D) for each core using a probability value (p) of 0.95 and λ fit using the quantile function of the exponential distribution (D = log[1-p]/λ).
	4. Statistics on Post-Processed Data
	We tested for significant differences between experimental treatments and species. We fit linear models with the experimental treatment as the independent variable and total belowground biomass or max rooting depth as the dependent variable. We treated plant community as a random effect. We used the lme4 package (Bates et al. 2015) to fit hierarchical linear models, and the lmerTest package (Kuznetsova, Brockhoff, and Christensen 2017) to test the significance of random effects. We assumed normality for response variables based on my visual inspection of their density plots.
	Bates, Douglas, Martin Mächler, Ben Bolker, and Steve Walker. 2015. "Fitting Linear Mixed-Effects Models Using lme4." *Journal of Statistical Software* 67 (1): 1–48. doi:10.18637/jss.v067.i01.
	Kuznetsova, Alexandra, Per B. Brockhoff, and Rune H. B. Christensen. 2017. "lmerTest Package: Tests in Linear Mixed Effects Models." *Journal of Statistical Software* 82 (13): 1–26. doi:10.18637/jss.v082.i13.
	R Core Team. 2019. *R: A Language and Environment for Statistical Computing*. Vienna, Austria: R Foundation for Statistical Computing. https://www.R-project.org/.
	Schile, Lisa M, John C Callaway, James T Morris, Diana Stralberg, V Thomas Parker, and Maggi Kelly. 2014. "Modeling Tidal Marsh Distribution with Sea-Level Rise: Evaluating the Role of Vegetation, Sediment, and Upland Habitat in Marsh Resiliency." *PloS One* 9 (2). Public Library of Science: e88760.
	Wickham, Hadley. 2017. *Tidyverse: Easily Install and Load the 'Tidyverse'*. https://CRAN.R-project.org/package=tidyverse.
13. Related Materials	None
14. Related Links	http://serc.si.edu/GCREW
15. Related Datasets	http://serc.si.edu/GCREW
16. Research Topic	Belowground biomass response of three tidal marsh plant communities to elevated carbon dioxide.
17. Study Type	Net Primary Production

Table 2. Description of the variables: 1999 CO2xComm Root Biomass Data Assembled.csv
Note that "no data" values = -99

Variable Name	Variable Description	Units	Codes
community	plant community where each of three elevated CO2 experiments was established	none	SC=initially pure *Schoenoplectus americanus* (C3); SP=initially pure *Spartina patens* (C4);

plot	chamber number assigned to each plot in a community	none	none
treatment	carbon dioxide treatment	none	A=ambient CO2, E=elevated CO2
core	replicate core 1 or replicate core 2	none	1=replicate 1 and 2=replicate 2 from a single chamber
depth_center	depth below soil surface at the center of the sectioned subsample	centimeters	none
target_width	nominal width of the sectioned subsample, as opposed to the true or measured width	centimeters	none
volume	Volume of the sectioned subsample calculated as the product of the fixed core diameter and the true (measured) width	cubic centimeters	none
washer	name of person who washed the cookie	none	none
plucker	name of person who plucked the roots	none	none
category	type of root material plucked	none	LRT=light-colored roots; RRT=red roots; DRT=dark roots; WRH=white rhizome; RRH=red rhizome; RHC=rhizomotous culms; NRHC=non-rhizomotous culms; LITT=litter
mass	dry mass of recovered roots, rhizomes and culms	grams	none
seeds	number of Scirpus seeds in subsample	none	number; -99 indicates seed data are missing because we did not attempt to recover seeds from these samples.

Table 3. Description of the variables: CO2_community_belowground_derivative_data_1999.csv
Note that "no data" values = NA

Variable Name	Variable Description	Units	Codes
community	plant community where each of three elevated CO2 experiments was established	none	*Schoenoplectus americanus* = initially pure *Schoenoplectus americanus* (C3); *Spartina patens* = initially pure *Spartina patens* (C4);
plot	chamber number assigned to each plot in	none	none

	a community		
treatment	carbon dioxide treatment	none	Ambient=ambient CO2, Elevated=elevated CO2
core	replicate core 1 or replicate core 2	none	1=replicate 1 and 2=replicate 2 from a single chamber
belowground_biomass_grams_per_square_meter	Estimated total biomass by dry weight.	grams per square meter	none
maximum_rooting_depth_centimeters	Estimated depth below the surface of 95% of root mass.	centimeters	none