The International Drought Experiment:
a distributed approach to assess terrestrial ecosystem responses to extreme drought

PROTOCOL: DROUGHT EXPERIMENTS IN TALL STATURE (FOREST AND SHRUBLAND) ECOSYSTEMS

The goal of the International Drought Experiment (IDE) is to determine how and why terrestrial ecosystems differ in their sensitivity to extreme drought. To accomplish this goal, a coordinated, distributed experiment imposing an extreme drought over a four-year period will be established in a range of ecosystem types across the globe. While ecosystem responses to drought depend on a combination of soil, tree and atmospheric factors, precipitation manipulations represent the most cost effective method for modifying plant available soil water and are the basis for the IDE consortium. Below is a description of the IDE experimental design, measurements, and sampling protocols, specifically designed for tall stature (forest and shrubland) ecosystems – including both unmanaged and managed production systems (e.g., plantations, timber harvesting and/or non-timber forest products). This protocol is intended to strike a balance between providing a sufficient level of methods standardization (such that the results will be robust and comparable), while not being too resource-intensive or technologically complex (such that participation would be severely restricted). Additional details about IDE are available at the Drought-Net website (www.drought-net.org).

I. Experimental Design

A. Site Selection

- Sites should be selected that can accommodate relatively large plot sizes. To the extent possible, the forest matrix selected for establishment of the experimental plots should be relatively homogeneous across the manipulation area with respect to soil properties, topography, and plant species composition.
- Selecting sites on convex topography and with plot edges located along the ridge is preferable to concave topography or areas with a large upslope area above the plots, which would contribute subsurface flows into plots. Plots sited at the top of ridges are desirable, as this will maximize the likelihood that plots are hydrologically isolated (e.g., buffered from subsurface water flows) and minimize the need for trenching (see section d below for more details).

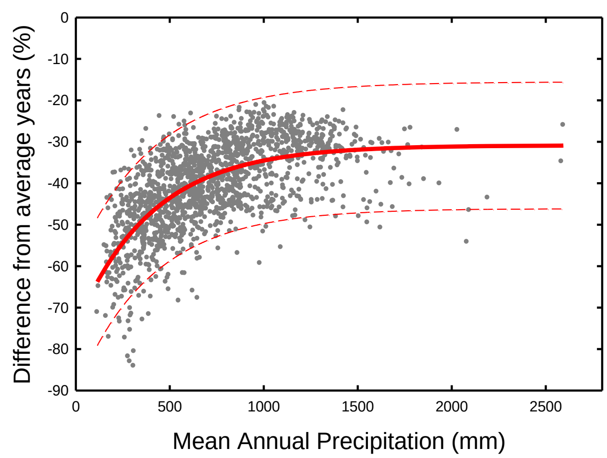

Fig. 1. Relationship between the difference (%) in precipitation amounts between normal years (amounts between the 45-55th percentile in a 100 year record) and extremely dry years (precipitation amounts in the lowest 10 years for a 100 yr record) and mean annual precipitation. Data (grey dots) are from 1614 climate stations arrayed across the globe, representing 12 ecoregions. Shown is the predicted relationship (solid red line) with upper and lower bounds of the 95% confidence interval (dashed red lines). Smith *et al*. in prep.

- Sites that have experienced a major above- or below-ground disturbance event within the past decade should be avoided. Management interventions (e.g., logging, grazing) should be avoided during the course of experiment (unless this is planned as part of the experiment and adequate unmanaged plots are also established).

B. Treatments

Fig.2. Photos of three types of throughfall removal systems: flat panel frames at Caxiunanã National Forest Experiment in Pará, Brazil (top; Meir et al. 2015), fixed troughs at the Hubbard Brook Experimental Forest Experiment in New Hampshire, USA (middle; photo: K. Jennings), and half cut pipes from the Golfo Dulce Forest Reserve Experiment in Costa Rica (bottom; Cleveland et al. 2010).

Drought will be imposed using a system of understory troughs that passively remove throughfall by a constant, site-specific percentage (Hanson 2000; Pangle et al. 2012) based on ground area covered and validated with field measurements (see details below). Troughs should be elevated approximately 1 m above the ground to avoid confounding soil effects and allow air movement. The amount of precipitation removed will represent the **1st quantile of annual precipitation** for the site over a hundred-year period (based either on actual data or using the precipitation manipulation tool on the Drought-Net website: http://shiny-smith.biology.colostate.edu/DroughtNet/WebApps/Precip_ShinyApp/). This site-specific approach is necessary given that the reduction amount relative to expected mean annual precipitation will vary greatly depending on the local precipitation regime, which vary widely among locations (Fig. 1; Knapp et al. 2015).

Three types of systems (Fig. 2) have been used effectively: flat-panel frames, fixed-troughs, and half-cut pipes. Flat panel frames work well in environments that do not have significant snowfall or litter removal requirements and where frame placement can avoid trees, or wrap around trees in manner that minimizes stem flow. One significant advantage of this system is that individual flat-paneled frames can be retracted during favorable weather, or removed easily for cleaning, litter collection or repair. The second type, fixed-trough systems, use elevated troughs (usually about 30-60 cm wide) made with flexible reinforced plastic secured to a wooden frame via framing staples. Given its sturdiness, this approach is highly effective at excluding rain and somewhat effective at removing snow (the latter will depend on site-specific conditions related to snow amount, water content, weight, and wind, which must be carefully tested and evaluated). In cases where the trough intersects a tree, the tree can be fitted with a roofing collar that is bonded to the reinforced PVC trough, or alternatively, diverted around the tree or moved slightly, making the gaps somewhat irregular. For both of these approaches, light transmission, strength of the material, and ease of construction are important considerations (e.g., reinforced polyethylene such as "Tough-Scrim" or "Polyscrim" is often used). The third low-cost option, half-cut pipes, takes advantage of transparent plastic pipes cut in half and set into a frame system to create a grid. This design offers considerable flexibility in terms of spacing and allows adequate light penetration to reach the forest understory, but the pipes are

[1] Note: Sealing off the trees will also affect stem flow and nutrient return to the soil (i.e., not just litterfall).

more difficult to keep clean and are vulnerable to damage from branch or tree fall. Thus, this approach is more appropriate for small plot studies with small trees. For all three systems mentioned above, diversion of water away from the plot (i.e., a minimum of 3 m distance from the plot edge) can be facilitated by collecting runoff from the panels, troughs, or pipes into a gutter located along the outer edge of the plot and drained to a downslope location (Fig. 3).

Fig.3. Example of a gutter lining the plot edge to collect and remove runoff at the Thompson Farm Drought Experiment, Durham, NH (photo credit: K. Jennings).

The manipulation will occur year-round where possible. In those instances where snowfall is significant, alternative means of precipitation reduction may be used (e.g., snow removal via shoveling) or, alternatively, the roof can be removed or retracted during the period of heavy snowfall. Snow removal is strongly encouraged for sites where water from snowmelt contributes significantly to plant water uptake (e.g., highly seasonal climates where precipitation is greatest in the winter months). Generally, shoveling is the recommended approach for removing snow, and is ideally conducted just prior to spring snowmelt, as this will reduce the potential for inducing soil freezing as a confounding variable and create a more uniform treatment. Care should be taken not to disturb the soil/litter surface, and to clearly mark equipment that could be damaged by shoveling. If snow is removed in the winter, it is critical that measurements are taken to determine the total amount of precipitation removed with respect to the total annual precipitation, for example, by directly quantifying the snow water equivalent (SWE) of the snowpack with a snow tube before shoveling snow off plots or by measuring SWE in control and treatment plots.

There are two categories of treatments to consider:

1. Core (required) treatments – The core treatments will consist of 1) an ambient precipitation treatment (unsheltered control) and 2) a drought treatment. The drought treatment will be imposed for at least four consecutive years. The percentage reduction of each rainfall event will mimic an extreme drought, defined as an extreme reduction in precipitation (based on the 1st percentile of the long-term record), which is specific to a particular site (see details above).
2. Optional treatments – Sites are encouraged to implement the optional treatments, but these are not required for participation in the network.
    a. Fixed treatment - Each site will reduce annual precipitation by 50% using passive shelters.
    b. Infrastructure control – To account for shelter effects, (construction disturbance, shading, reduction of wind, introduction of fine-scale heterogeneity in soil moisture, etc.) sites are encouraged to establish infrastructure control plots (in addition to the reference plots), which may consist of inverted troughs or panels that do not empty into a drain system.

C. Plot size and replication

- Sites will need to scale their plot size appropriately to the site-specific size and distribution of trees and/or shrubs. As a general guideline, treatment plots will be large enough to capture the diversity of the target vegetation and their expected lateral rooting distribution (a minimum size of 30 x 30 m is recommended). A common rule of thumb is for the diameter of the plot to be twice the height of the canopy; however, ideally the plot diameter is determined based on site-specific knowledge of root architecture, such that the plots will be large enough to allow for a core area that captures the entire root system of the target vegetation. A buffer zone (usually 3-10 m) will be included to allow for edge effects. Square or rectangular plots will typically make installation and construction simpler and less expensive.

- The level of replication will, in part, be dependent upon costs and the availability of funds. At least two replicate plots per core treatment are recommended (but not required). Plots can be set up randomly or in a blocked design if appropriate.
- The throughfall removal troughs will cover the area of both the core sampling plot and the buffer zone. Troughs will be installed approximately 1.5-2 m above the ground to minimize effects on microclimatic conditions, allow for understory vegetation, and facilitate working in the plots to collect data and maintain equipment (including removing litter from the troughs and performing regular maintenance).

D. Trenching

Trenching along one or more of the borders of each treatment and control plot in order to hydrologically isolate each plot is encouraged but not mandatory. In particular, trenching the upslope plot edge would likely have the maximum benefit in terms of reducing subsurface flow into the plot, while minimizing tree damage and plot disturbance. Depth of trenching is dependent on the rooting architecture of trees and soil and bedrock characteristics, but a minimum of 1 m depth is encouraged for most shrubland and forest sites. Excavated trenches will be lined with an impermeable barrier (e.g., 6 mil (0.15 mm) thick plastic sheeting) and either refilled prior to the initiation of the experiment or left open to further prevent horizontal root growth. Given that trenching is not feasible at all sites, an alternative to trenching is to increase the size of the shelter to accommodate a larger buffer area[2]. Site-specific characteristics that influence the degree of subsurface lateral flow of water into the plot and/or tree access to water outside the plot will be used to determine the need for trenching, including soil texture, slope position and gradient, and root architecture. Additionally, any potential advantages of trenching need to be weighed against possible disadvantages, particularly the severe disruption to the rhizosphere that can confound interpretation of results, and the added costs in establishing and maintaining trenches. If a treatment plot is trenched, the control plot must be similarly trenched.

II. Measurements

Two levels of measurements are included. Level-1 measurements are designed to quantify three key types of response variables that will allow us to test network-level hypotheses, as well as provide important site characteristics. These measurements are required for inclusion in the network, and will be collected both pre- and post-treatment. Level-2 measurements provide additional response and explanatory variables. Level-2 measurements, although optional for inclusion in the network, will be made if possible. Many additional measurements may be of interest to network participants, but the two levels of measurements are meant to capture key responses and explanatory variables to address a range of network-level questions. Detailed methodologies are provided on the Drought-Net website (www.drought-net.org).

[2] Note: The buffer area inside the trench (i.e., where data are not included in analyses) can be increased to allow for root damage negatively affecting the trees immediately inside the trench and other confounding unknown effects. Additionally, trenching with a considerable time lapse before the drought treatment may allow for “root recovery”.

A. Level 1 measurements

1. **Site data**

   Each site must provide the following information:
   - Latitude, longitude (precision: 5 decimal places)
   - Elevation
   - Slope, aspect
   - Dominant overstory and understory vegetation (including a list of species)
   - Long-term air temperature and precipitation data (preferably 50-100 yr record, ideally on a daily time scale)
     - These data can be from a nearby representative weather station or based on interpolated data (e.g., PRISM, CRU-TS)
   - Soil Taxonomy (US or FAO system preferred)
   - Other site characteristics (if known)
     - Disturbance history
     - Depth of known soil impediment (shallow bedrock, caliche layer, etc.)
     - Average water table depth
     - Other unusual site characteristics (saline, serpentine, etc.)

   In addition, each site is required to make the following measurements during the study period:
   - Annual precipitation (based on daily precipitation if possible) for each year of the study, or more frequent data (i.e., weekly, monthly) if appropriate.
   - Annual average air temperature of each year of the study based on daily measurements, or less frequently if appropriate.

2. **Plot data**

   The core measurements required for participation in the network are focused on primary productivity, soil moisture, soil carbon (C) and nitrogen (N), and plant community composition.
   1. **Aboveground productivity and standing biomass will be measured annually** using methods appropriate for a particular ecosystem (refer to Fahey & Knapp 2007). These can include both destructive and/or non-destructive measurements. Estimates of biomass will be separated into live and dead biomass. Live biomass will be further separated by growth form (seedling, sapling, tree). Dead biomass will be separated into current and previous year's when appropriate. Annual stand productivity will be determined from measurements of diameter increment growth (dbh measurements, dendrometer bands, or tree rings) or height growth (for younger forests) and litterfall (litter baskets; elevated above the structure between the troughs, if possible). Woody biomass production will ideally be estimated using allometric equations and expressed in units of total C storage and productivity.

   2. **Soil moisture content will be measured for the drought and control treatments at a depth of at least 0.5 m.** These measurements will be made as frequently as possible (e.g., continuous, bi-weekly, monthly). Ideally, continuous soil moisture measurements using sensors placed in at least two depths (0-15 cm and as deep as possible, depending on the rooting distribution at the site) are recommended; alternatively, gravimetric samples can be taken at critical time periods during the experiment[3]. A horizontally stratified design

[3] Note: Relatively cheap sensors that integrate over 30 cm are available (e.g., Campbell Scientific CS616); site-specific calibrations using gravimetric measurements (during at least one wet and one dry period) should be conducted to

can also be used to detect edge effects. Many soil moisture probes also provide concurrent measurements of soil moisture and soil temperature and are highly recommended. Additionally, preliminary assessments of rooting depth and/or groundwater wells will be conducted to assess the soil volume likely to be available to the treated vegetation.

3. **Soil C and N concentration and content will be measured twice, once during the pre-treatment data collection year and in year 4 of the drought.** For each plot, two to three soil samples will be collected to a depth of 0-15 cm and composited. Samples will be sent to a central lab (tbd) for analysis and archived. Bulk density will also be measured to assess changes in C and N pools. Standard protocols will be used for the analysis (including correcting for inorganic C if necessary, Robertson *et al*. 2007).

4. Species composition of mature trees in forest ecosystems is not expected to change during the four-year duration of this experiment; however, **tree dieback and mortality should be monitored, as well as any changes in species dominance** across different forest strata (e.g., dominant, co-dominant, intermediate, suppressed canopy classes).

B. Level 2 measurements

Level 2 measurements are not required for participation in the network, but are strongly encouraged; if collected, including both pre- and post-treatment data is strongly recommended. These measurements include quantifying infrastructure effects and performance, precipitation inputs and changes in soil moisture, belowground productivity, decomposition rates, and plant traits. Of these, quantifying soil moisture content is the <u>*highest priority*</u> and most valuable for understanding drought impacts.

1. Soil characteristics: soil texture, bulk density, chemical characterization (pH, C, total N, P, % OM), hydraulic conductivity, description of soil horizons present and the parent material.

2. Infrastructure effects and performance
   - The effects of “striping”, the creation of heterogenous wet and dry strips located underneath and between the troughs, can be quantified by measuring soil moisture beneath and in the gaps between the troughs.
   - Infrastructure performance can be quantified by measuring the amount of throughfall reaching each treatment plot relative to the amount of throughfall removed from the plot and the total amount of rainfall.

3. Root production and biomass
   - Annual root production can be estimated with root-ingrowth cores (recommended minimum of n = 10 per plot, with more cores needed for larger plots). The diameter and depth of in-growth cores may vary by soil depth and/or vegetation type. In-growth cores will be installed at the end of each growing season and removed a year later. The depth of the ingrowth

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help remove biases in the data. Deeper groundwater wells and stable isotope analysis of plant water and source waters can also be used to more precisely determine whether trees are accessing deep water sources.

cores/screens will depend on site-specific conditions (a common depth used in forest ecosystems is 20 cm).

- Standing root biomass can be estimated from the cores extracted for root-in-growth measurements.
- Root density, length, and depth can be measured from soil pits.

4. Understory species composition. Due to the effects of infrastructure and data collection activities on the belowground environment (e.g., plant growth, seed reproduction, etc.), assessing changes in understory plant community composition is problematic for forest drought experiments. However, if the understory response is of particular interest, the shelter and/or sampling design could potentially be modified to better accommodate such measurements. Alternatively, the forest understory response could be investigated by establishing rain shelters on smaller plots (see the protocol for short-stature ecosystems for details).

5. Litter Decomposition. Decomposition can be measured using native litter from the site and/or a standardized material, such as tea bags (Keuskamp *et al*. 2013) and wooden dowels/tongue depressors (Robertson *et al*. 2007), ideally with enough samples to remove as a time series over multiple years.

6. For the most dominant tree species (those collectively comprising 90% of basal area in plots), each site is encouraged to provide qualitative and/or quantitative trait data (for many sites, this information may be readily available from the literature). Examples of these traits include: specific leaf area, wood density, stomatal size and density, wood density and anatomy (e.g., ring-porous versus diffuse porous wood), growth form (shrub, tree), symbiotic associations (rhizobia, mycorrhizae), hydraulic strategies (isohydric-anisohydric) and root architecture. Additionally, physiological and ecosystem indicators of drought response may be measured, including (but not limited to) the following:
    - Pre-dawn and mid-day plant water potential
    - Leaf relative water content
    - Foliar chemistry (N, P) and biochemistry (chlorophyll, pigments content)
    - Non-structural carbohydrates
    - Hydraulic conductivity
    - Leaf gas exchange (stomatal conductance, photosynthesis)
    - Water use efficiency (calculated from instantaneous gas exchange and/or stable C isotope data)
    - Sap flux
    - N mineralization, nitrification (beneath and between the troughs)
    - Soil respiration (beneath and between the troughs)
    - Stem water content
    - Phenology

Literature Cited

Cleveland, C.C., W.R. Wieder, S.C. Reed, A.R. Townsend. 2010. Experimental drought in a tropical rain forest increases soil carbon dioxide losses to the atmosphere. Ecology. 91(8):2313-2323.

Fahey, T.J. and A.K. Knapp (eds). 2007. Principles and Standards for Measuring Primary Production. Oxford University Press.

Hanson PJ (2000) Large-scale Water Manipulations. Chapter 23 in Sala OE, Jackson RB, Mooney HA, Howarth RW (Eds.) *Methods in Ecosystem Science*, Springer-Verlag, New York. pp. 341-352.

Meir P, Wood TE, Galbraith DR, et al. 2015. Threshold responses to soil moisture deficit by trees and soil in tropical rain forests: insights from field experiments. *Bioscience*. 65, 9 Pages: 882-892.

Robertson, G.P., D.C. Coleman, C.S. Bledsoe, and P. Sollins (eds). 1999. Standard Soil Methods for Long-term Ecological Research. Oxford University Press.

Pangle, R.E., J.P. Hill, J.A. Plat, E.A. Yepez, J.R. Elliot, N. Gehres, N.G. McDowell, W.T. Pockman. 2012. Methodology and performance of a rainfall manipulation experiment in a piñon-juniper woodland. Ecosphere. 3(4):28. http//dx.doi.org/10.1890/ES11-00369.1