Global Treeline Range Expansion Experiment

Field Protocol – April 2014

The Global Treeline Range Expansion Experiment (G-TREE) is a globally distributed collaborative project aimed at testing the generality of mechanisms driving boundaries of tree distribution at the treeline. Two conditions must be met for plant species' distributions to shift as the climate of marginal environments become more favourable: i) a seed source for colonization; and ii) a suitable substrate for establishment and survival. The goal of G-TREE is to disentangle substrate and seed limitations on range expansion through field experimentation. This initiative will allow for the quantification of edaphic and dispersal factors limiting northern species' distributions, and therefore will permit predictions for future treeline expansion. We aim to produce a manuscript for a high-impact journal following the Year 2 field season, addressing where, and under what conditions, treeline expansion can occur globally, based on our early recruitment data. Subsequent manuscripts will focus on longer term recruitment and survival, and there is potential for more detailed region-specific analyses.

The objective of this protocol is to implement identical seeding trials across the treeline ecotone. Global research networks, in which standardized but modest local data collection efforts are aggregated across many sites, have made unparalleled contributions to understanding complex ecological problems (e.g., NutNet), while being feasible even with a limited budget. Ideally, many sites will be placed in previously established study areas (e.g. PPS Arctic, GLORIA, LTER sites). The benefit of seeding trials in these pre-established sites is that we already have baseline data on stand physical and age structure, tree reproduction, and soil and understory processes and composition. The implementation of these protocols at any treeline ecotone would be beneficial, though, and is encouraged for both new and existing sites. Our goal is to have broad geographic coverage of latitudinal and altitudinal treeline sites.

There are three levels of participation in G-TREE:

Level 1 – Requires the implementation of at least five replicates of the experimental design described below at a site near the treeline ecotone in high elevational or latitudinal tundra. This is the minimum contribution required for participation in G-TREE.

Level 2 – Increased sites, species (see zone descriptions below), and treatments. Level 2 and 3 have the potential to become part of a graduate student research program.

Level 3 – Intensive G-TREE sites. Requires multiple year visits to study sites, multiple treatment combinations (described below), and measurement of environmental variables. Ideally, Level 3 sites will be established as long-term treeline monitoring sites.

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Terminology

For ease of communication, we will refer to three zones (also termed "sites") in the treeline ecotone: forest (F), transition (T), and alpine/arctic tundra (AT; Table 1). We define these ecotone zones based on tree density/ha, where a **tree refers to an individual >2 metres in height**. Study areas can be divided into two treeline forms based on tree structure and density (see Harsch and Bader, 2011, Global Ecology and Biogeography, 20: 582–596 for further descriptions):

1. **Diffuse**: tree height and density decreases gradually across treeline (Fig. 1a); and,

2. **Abrupt**: relatively dense forest abuts alpine/tundra vegetation, without a zone of intermediate height and/or density (Fig. 1b). More frequently present in alpine environments.

Note that our characterization of treeline form specifically refers to the distribution of trees >2m. Site section in zones across the ecotone should be based on the form of the treeline ecotone (diffuse or abrupt) and the criteria outlined in Table 1.

Table 1: Site selection criteria for diffuse and abrupt treeline ecotones based on stand density (trees > 2m height per hectare). Stand density should be verified in a 100m x 100m plot at each site.

	Treeline Form	
Site	**Diffuse**	**Abrupt**
Forest	density >50 trees/ha	density >50 trees/ha
Transition	density of 10 – 50 trees/ha, but not more than 0.5 x forest density	distance of 15 x height of abrupt-edge trees (>2m)*
Alpine/Arctic Tundra	density < 1 tree/ha	density < 1 tree/ha, distance > 30 x height of abrupt-edge trees

*If abrupt edge trees are 2.5m tall, the transition site should be place 15 x 2.5 m = 37.5 m distance from the edge of tree distribution (i.e., upslope). This zone has characteristics and influences from both the forest (e.g., patterns of snow drifting, wind currents) and tundra (e.g., low vegetation, light availability).

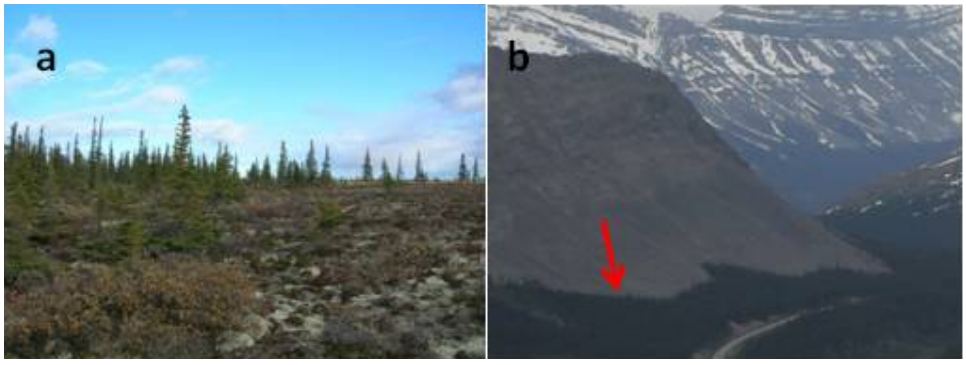


Figure 1: (a) Diffuse treeline at Churchill, Canada; (b) Abrupt treeline indicated by red arrow, near Lake Louise, Canada.

LEVEL 1

Year 1 (2013)

Two seeding (**control**, **seeded**) and two substrate (**control**, **scarified**) treatments will be applied beyond the range edge in **tundra** sites. Seeding treatments identify seed limitations for populations beyond the range edge. Substrate treatments allow for the assessment of current suitability (control), and suitability following disturbance (scarified).

This results in four treatment combinations for Level 1:

1. **full control** (unseeded, unscarified) plots;
2. **seeded** (seeded, not scarified);
3. **scarified** (unseeded, scarified); and
4. **seeded and scarified**.

Using a local seed source is encouraged and preferred. If no seed is available from the local seed or adjacent forest stands, seek out regional seed. In Canada, for example, seed can be obtained from the National Tree Seed Centre, free of charge. Other agencies have similar programs. If you are unsure whether your seed population is appropriate, or have difficulty obtaining seed, please contact us.

Materials:

- transect tape
- random number table or other method of generating random numbers
- corner markers: bamboo skewers or galvanized nails (for long-term plots – can be relocated with an inexpensive metal detector)
- string (note: cotton string will decompose in the field over several years)
- **local seed** for the dominant species at treeline*
- garden hand-cultivator or similar tool (Fig. 2)
- camera
- flagging tape

Figure 2: Example of a hand cultivator for plot scarification.

* It is very important that we know the viability of the seed used; a test of seed viability should be conducted on a sub-sample of the seeds used in the experiment. **Box 1** contains methods for assessing seed viability. The amount of seed required per plot is dependent on species and the viability of your supply. The goal is to have an even coverage of viable seed across each 0.5 m x 0.5 m plot (see below for plot specifications), but to avoid potential overcrowding of emerging seedlings. For reference, black spruce has ~0.0012 g seeds. Applying 250 seeds (~0.3 g) to a plot would be appropriate. However, smaller or larger-bodied species would require more or fewer seeds per plot, respectively. The most important thing is that you record the number of seeds applied to each plot and the viability of those seeds.

Methods:

1. Choose seed plot locations. Level 1 requires that seed plots be located in at least sites beyond the current treeline in **alpine/arctic tundra** sites. Plots may also be positioned in forest and transitional sites. Tundra plots should be placed beyond the current limit of tree occurrence, but within a feasible distance

from the treeline for future treeline expansion; i.e., this distance will likely be measured in metres rather than kilometres (depending on species dispersal distances; this is more relevant for latitudinal treeline sites).

All plots should be located on areas with similar soils, microtopography, drainage, and seedbed to reduce the variability between plots. Measure and record the density of trees ≥2m and <2m (separately) using a fixed-area plot (e.g., 100m x 100m) positioned around the area where the experimental plots will be established.

2. Establish plots. A site will have **5 replicates of each treatment combination** (full control, seeded, scarified, seeded and scarified) for a minimum of 20 plots per site. Each plot is 0.5m x 0.5m.Plots should be randomly positioned within a site. Lay down a transect tape across your site, parallel to the existing treeline (Fig. 3; e.g., this may be east-west in latitudinal treeline sites, or perpendicular to the slope in alpine sites). Avoid establishing your transect on gradients occurring along slopes. The goal here is to reduce variability between plots, so use your judgement to place your transect tape in a relatively homogenous area. Use a method of random number generation (e.g., a random number table) to choose the location of the first plot along the transect tape. The random number, and each subsequent, should be two digits (i.e., 10-99) and will represent centimetres. The first two-digit random number will designate the location of the first seeded plot (e.g., random number of 24 means you place the first plot at 24cm along your transect tape. Subsequent random numbers will dictate how many centimetres farther along the tape the next plot will be located.

BOX 1: Assessing seed viability

We recommend using one of the following methods to assess viability of a sub-sample of your seed:

1. Lab germination trials – seeds are allowed to germinate on moist substrate in Petri dishes for four weeks, or one week beyond the last observed germination.

2. Cutting test – seeds are sectioned to determine the presence of an embryo.

3. X-Ray test - seeds are x-rayed to determine the presence of an embryo.

4. Hydrogen peroxide test – seeds with the micropylar end removed are immersed in a hydrogen peroxide solution for one week. Seeds with emerged radicles are considered viable.

Details of these methods can be found in: Leadem, C.L., Gillies, S.L, Yearsley, H.K., Sit, V., Spittlehouse, D.L., and Burton, P.J. 1997. **Field Studies of Seed Biology**. Land Management Handbook 40, Government of British Columbia. **Available free online** from the Government of British Columbia, Canada. Contact us if you need more information on these methods. Also, refer to www.seedtest.org for species-specific requirements.

Example: your first random number is 24. Place the corner of your first plot at 24 cm. Since the plots are 50 cm wide, the first plot ends at 74 cm. Your second random number is 31. Place the second plot 31 cm farther along the transect tape from the edge of the first plot, at 105 cm.

A quick way to delimit each seed plot is to place a 0.5m x 0.5m quadrat on the ground and stake each corner with a bamboo skewer or metal nail. Tie the cotton string to one corner and create the seed plot boundary by wrapping the string around each corner (one full loop around) to make the square (Fig. 3

and 4). **FLAG EACH TRANSECT WELL** for relocation, potentially by other researchers in the future. Take GPS coordinates of each transect end. Record the slope aspect and compass orientation of each transect.

The configuration of your site may require that you use multiple transects within a single site. If so, just repeat the process above for each transect until you have established the required number of plots, keeping the transects in as homogenous an area as possible.

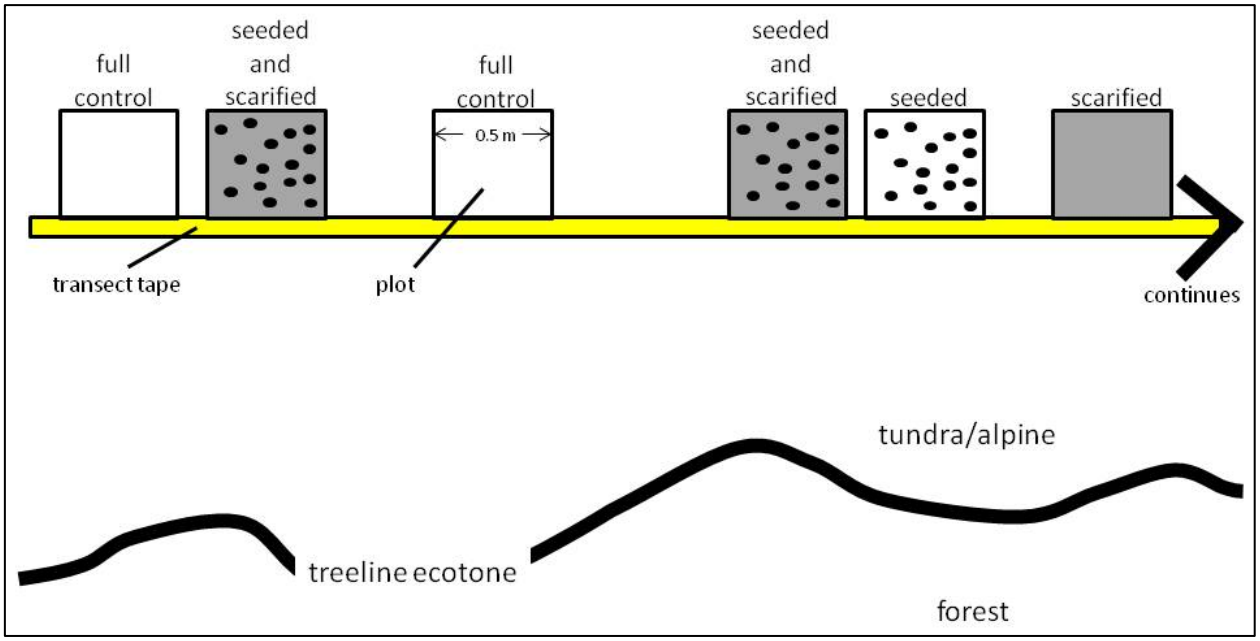


Figure 3: Arrangement of plots randomly along a transect. Each site consists of five replicates of each treatment combination, a portion of which is shown here. Transect should be oriented parallel to forest limit. Figure is not drawn to scale.

3. Randomly assign treatments. This experiment follows a completely random design for the distribution of plots within sites. Label four markers/replicate with the treatment combinations. Mix them up in your hand/bag/hat and place a marker in each plot.
4. Record baseline data on your plots using the attached template datasheet, including an estimate of substrate cover (e.g., litter, lichens, moss, rock), depth of surface organics (distance from the forest floor surface to mineral soil surface), and presence of any natural seedlings within the plots. Take several photos for documentation:
    a. Surface cover. Take one photo aimed straight down of each plot within the block (Fig. 4). For scarified plots, a photo should be taken before and after scarification.
    b. Horizon. Take one photo at each site (not necessary for each plot) with the landscape horizon visible, with a small blackboard or sheet indicating the site information included in the photo. Using a blackboard avoids overexposure, allowing the label to be easily read.

Figure 4: Example of a 0.5m x 0.5m seeded plot, constructed with bamboo skewers and cotton string. Each corner is flagged for ease of future location. This plot was located on a burned surface.

5. Scarify 1/2 of the plots. In the plots that were randomly assigned "scarified" and "seeded and scarified" treatments, drag the hand-cultivator across the seedbed, first in one direction and then in the perpendicular direction. This should remove surface plant litter and live surface cover of mosses and lichens, while leaving vascular plants rooted in the plot. Sub-surface layers (organic horizons, dead moss layers) can be left intact if the organic layer is thick (>5 cm), but ideally this treatment would expose mineral soil. This treatment works best if you can scarify a little bit (~10 cm) beyond the edges of the plot, but avoid scarifying adjacent plots. The described method of scarification will not be effective in some types of seedbed, such as dense alpine meadows. With these seedbeds, break apart and remove the sod with a spade to expose the soil. **See Box 2** for an optional intermediate scarification treatment in dense alpine meadows.

Box 2: *Optional* Intermediate Scarification Treatment

Biotic and abiotic interactions are complex in dense alpine meadows (e.g., facilitation vs. competition by grasses), thus we propose an ***optional*** intermediate scarification treatment in these sites.

When to use it: Where there is dense herbaceous cover.

Why to use it: To provide scarified seedbeds that also have vegetation cover within the plot.

- Create a grid of exposed mineral soil across the plot by cutting through the grass biomass with the tip of a spade or soil knife. The diameter of each opening should be ~1/3 the height of the surrounding vegetation (e.g., 3 cm diameter openings within 10 cm tall grasses) and openings should be spaced a distance of twice the diameter apart (e.g., 6 cm apart in our example).
- Seed should only be applied to scarified areas of the plot, following the seeding instructions below. Depending on seed size, several should be applied per opening.
- Adding this intermediate (partial) scarification treatment will require an increase in the total number of plots at each site. Instead of four treatment combinations (full control, seeded, scarified, seeded and scarified), there will six treatment combinations (the standard four plus partially scarified and seeded and partially scarified) to be replicated five times.

6. Seed the plots **timed in synchrony with natural seed dispersal for your species**. Spread the seed evenly across the surface of the "seeded" and "seeded and scarified" plots by hand. Be wary of windy or wet conditions, ensuring that all seed remains in the designated plot. Seedlings of coniferous and deciduous species are easily distinguished; if both types are being seeded at your site, they can be sown in the same plot. For fall-seeding species, seed should be sown as late as possible in the growing season (prior to the first snow) or, if necessary, within a week or two of snowmelt, depending on the species-specific stratification requirements. **Record the approximate number of seeds applied per plot and the percent viability of those seeds**, as determined using laboratory trials (Box 1).

   NOTE ON SEEDING: Two points related to seed application: 1) Number of viable seeds to apply to each 0.5m x 0.5 m plot. The optimal situation would be ~100 viable seeds applied to each plot. However, because our emphasis is on using local seed sources, some groups may not have sufficient viable seed to apply that quantity. The most important thing is that you know how much seed was applied to a plot (# of seeds) and what the viability of that seed is, so we can calculate number of viable seeds applied. 2)

Density effects. If you are seeding two species in one plot (e.g., a coniferous and deciduous species whose seedlings are easy to distinguish), we may have issues with density effects as the seedlings develop. Our recommendation is to cut the number of seeds of each species applied in half if you are double-seeding plots.

Year 2 (2014)

1. Count emerged seedlings one year after seeding (Fig. 5). Mark each seedling with a piece of aluminum wire (20-24 gauge; ~10cm inserted on an angle to avoid frost thrust) or similar marker. If possible, return the following year (2 years after seeding) to do a second census and assess over-winter survival.

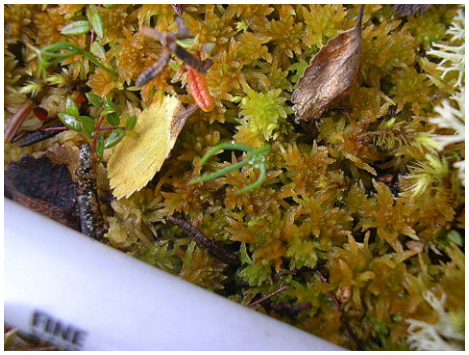


Figure 5: First year *Picea* sp. seedling on sphagnum moss seedbed.

LEVEL 2

Add additional treatments to the field experiment. Level 2 **MUST include** (but is not limited to):

- seeded plots in both transition and alpine/arctic tundra sites (T, AT)
- multiple years of seeding, applied to new experimental replicates, to assess impacts of annual variation in weather or other factors
- multiple species used in the seeding treatment, where more than one tree species occurs at or near treeline
- estimate of mean monthly air temperatures (annual and summer; recorded at the site or estimated from a nearby weather station)

NOTE ON PHENOLOGY: Snowmelt will occur at low elevation/latitude sites before high elevation/latitude sites. For those setting up sites across the gradient (Level 2 or 3) and are using spring-seeded species, plot set up and seeding should follow the natural phenology of snow melt along your gradient; i.e., low elevation/latitude sites should be seeded first, and high elevation/latitude sites later, once the snow has melted.

LEVEL 3

Level 3 **MUST include** (but is not limited to):

- seeded plots in all three sites across the treeline ecotone (F, T, AT)
- multiple years of seeding, applied to new experimental replicates, to assess impacts of annual variation in weather or other factors
- split-plot mesh cages to exclude small herbivores from half the seeded area (note: this may not be feasible on sites prone to frost heave and snow loading). A mesh size of 1cm is sufficient for excluding rodents.
- multiple provenances of regional seed, including at least one provenance from near the treeline (species range edge) and another provenance from a fully forested area (species range interior)
- multiple species used in the seeding treatment, where more than one tree species occurs at or near treeline

- direct measurements of soil temperature (10 cm depth below the soil surface) and snow depth (iButton snow stakes)
- some estimate of mean air temperatures (annual and summer; recorded at the site or estimated from a nearby weather station)

To reduce the total number of plots at each site for Levels 2 and 3 (Table 2), see Box 2 for an alternative plot configuration to reduce the space and effort required.

Table 2: Number of plots (or sub-plots, if following alternative configuration in Box 3) required for each level.

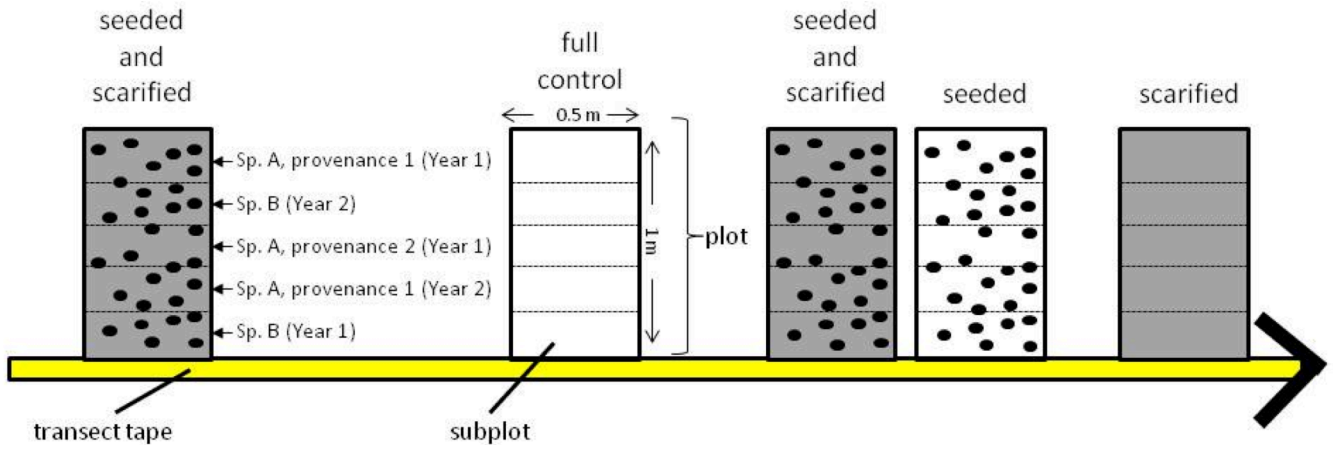
	Level 1	**Level 2**	**Level 3**
Basic seed x substrate treatment	4 treatment combinations	4	4
Replicates	5	5	5
Treeline sites (F, T, AT)	1 (AT)	2 (T, AT)	3 (F, T, AT)
Species (e.g., 2)		2	2
Years of seeding (e.g., 2)		2	2
Provenances (e.g., 2)			2
Herbivore exclosures			split-plot
Total number of (sub)plots	20	160*	480**

*80 per site (transition and alpine/tundra), or 20 plots per site each containing 4 subplots.

**160 per site (forest, transition, and alpine/tundra), or 40 plots per site each containing 4 subplots.

BOX 3: Alternative plot configuration for Level 3

Levels 2 and 3 of the protocol can result in a large number of plots being established at each site. To reduce the total number of plots, multiple species, provenances, or seeding years may be applied to subplots within larger treatment plots. The overall treatment should be assigned and applied to the entire plot in the same manner as Level 1. Seed sources should be randomly applied to subplots within each plot. Add as many subplots to each plot that are needed for your treatment combinations. This may result in the plot being longer than is shown in the figure, below. In the example below, the experimental design included two species (A and B) and two years of seeding (1 and 2). Seed from two provenances (e.g., low and high elevation populations) of species A were available for seeding in year 1; however only provenance 1 was seeded in year 2. NOTE: Level 3 suggests a split-plot design for the exclosures. If you using the alternative plot configuration, this would mean that each exclosure would be 20x25cm. As it is easier to build cages that are square, the subplot size can be increased to allow for 25cm x 25cm cages.



Additional treatments may be added to levels 2 or 3, but please ensure that the minimum requirements for each level are met. Examples:

- multiple substrate treatments (e.g., burned treatment, varying severities of scarification, vegetation removal)
- additional species used in the seeding treatment
- additional provenances of seed (populations of origin)
- seedlings may be followed for multiple years; survival and growth (height and basal diameter) of individual seedlings may be tracked; functional traits of individuals across the gradient may be measured
- seedlings may be outplanted as transplants to monitor growth even in areas where germination and early survivorship are zero.

Please **contact us** with any questions or comments through **treelineresearch.com** or:

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