

INTERSITE MONITORING (ITEM) OF INTERANNUAL VARIATIONS

Matthias Diemer, Esther Lévesque and Gus Shaver

Objectives and concerns

Aside from level I and II measurements of plant growth, phenology and meteorology a need exists for additional standardized measurements to quantify annual variation within and among sites. This should facilitate the determination of similarities and distinctions between sites and years, based on population or ecosystem traits (cf. Shaver et al. 1986). Emphasis is placed on simple, integrative parameters and experiments, which can be made in a) brief, annual visits to a site, b) are not strongly dependent on a narrowly defined time period and c) do not require continuous monitoring. This will allow monitoring of a larger number of sites/communities (even ones without OTCs), improve our understanding of regional variability and our ability to detect and predict change.

We distinguish five topics, namely 1) non-destructive plant measurements, 2) destructive harvests, 3) simple experiments, 4) climatic observations and 5) biotic observations. We understand these measurements as an extension of community measurements (see Manual).

One of the difficulties in relating ITEX data to other published sources on growth or productivity is the lack of a reference area (e.g. cm², m²). This deficiency should be addressed and remedied in topics 1) and 2), making accessible a wide range of data (e.g. IBP, LTERS, MaB).

Preliminary protocol

This is a first draft and we encourage you to try out these approaches in the field. We would appreciate your feedback and suggestions for developing a definitive protocol.

1) Non-destructive plant measurements

Non-destructive measurements should be carried out in designated reference plots (could be identical to plots utilized for community baseline measurements, see manual). Ideally several reference plots should be established per field site or plant community. (Refer to section on community baseline measurements for permanently staking plots). Generally a square meter plot should be sufficient for most non-destructive parameters, however one may want to expand or reduce this size (nested plots) depending upon plant density. Regardless, results should be expressed per square meter area. Ideally all species should be surveyed, however if that is not possible focus on ITEX plant species and dominant herbs and graminoids at the site.

In sites with sparse vegetation cover two possibilities exist: use of large plot sizes, or alternatively collect data from 15-20 plants. Use of the latter involves a loss of the reference (ground) area, but we feel this shortcoming is superior to no data at all.

Population density, structure and turnover processes - phenological development and population structure tend to be rhythmic or episodic (seedling recruitment, flowering) in many arctic and alpine plant species. It is important to document these cycles; these data also serve as a baseline for interpreting the effects of climate warming

Parameters:

- a) Plant density per species and plot: count the number of shoots/plants per plots. This may require use of nested plots, however make sure that the nested plot is representative of entire plot (randomization and visual evaluation). It's better to use several nested plots and to use the subplot mean to extrapolate to plot basis.
- b) Number of flowering shoots/plants per species and plot: tabulate the abundance of flowering individuals per reference area.
- c) Proportion of reproductive to vegetative shoots/plants per species: determine the ratio of flowering to non-flowering shoots on an area basis, by dividing b) by a) minus b). Flowering phenologies in arctic alpine species often tend to be cyclical. These cycles are dependent on the age structure of the population (cf. Carlsson & Callaghan 1990) and/or on climatic factors.
- d) Seedling density: count the number of seedlings per species and area. Notice: in many instances seedling distributions are clumped (Diemer 1992, Spence 1990). Therefore a number of small plots (e.g. 10*10cm) gives a better estimate than one large plot. If seedlings cannot be identified, mark several individuals with colored cocktail stirrers for subsequent identification.
- e) Age structure: estimates of c) and d) provide a good indication of age structure (i.e. reproductive, vegetative shoots and seedlings). In some cases it is possible to distinguish additional age classes, based on size or morphology (Callaghan & Emanuelsson 1985, Carlsson & Callaghan 1990).
- f) Mortality: increased population turnover via enhanced growth may affect mortality. Hence it may be valuable to estimate the density of dead shoots, provided that it is possible to distinguish current-year or overwintering mortality from shoots or plants which died previously. It is possible to use markers (tags, cocktail stirrers) to distinguish shoots which died in different years.

Reproduction and reproductive success - in some years weather conditions or the absence of pollinators may prevent seed set.

Parameters

- a) Pollination visits: count the number of pollinator visits per area and time period. Since pollinator activity is dependent upon weather conditions and daytime, try to make observations under similar conditions. Record these along with rates of pollination visits for major species (number m⁻² h⁻¹). This parameter is in fact strongly dependent upon timing within the growing season, but we felt that these data are vital in the context of seed production.
- b) Proportion of aborted to fertile flowers: at the stage of seed set determine the ratio of aborted and fertile flowers.
- c) Seed production: determine fruit : flower ratio and seed number, as described in Manual (Plant response variables). Knowledge of flowering plant density (see above) permits extrapolation on area basis.

2) Destructive measurements

These should not be carried out in permanent plots, but in undisturbed adjacent areas. They serve to relate non-destructive growth measurements to estimates of biomass via allometric relationships and separate analyses. In some instances it may be desirable to harvest individual shoots or branches from within OTCs or Control plots, however these disturbances should be kept to a minimum.

Parameters

- a) Leaf growth: in graminoids it is usually quite simple to establish allometric relationships between leaf length and leaf area or mass. Often 15-20 specimens representing a broad range of leaf lengths are sufficient to obtain high regression coefficients ($R^2 > 0.9$, see also Croy & Dix 1984). Measure leaf length preferably in the field prior to removal. Leaf area measurements can be carried out with commercial leaf area meters (LiCor, CID, ADC), hand scanners or graph paper. For dry weight determination samples should be dried at 80 °C for 24h - try to dry samples as soon as possible after harvesting, particularly if they are used for additional chemical analyses (see d)). Allometric relationships are generally quite robust and can be extended over years. Care should be taken to apply them to OTCs based on material from Control plots, since simulated warming may increase not only change leaf length, but also specific leaf area. For herbaceous plants, allometric relationships can be established using non-destructive measures, such as leaf breadth, breadth * length, or length of longest lamina (compound leaves).
- b) Shoot or branch growth: in woody species annual increments can be determined from bud scars, in some cases retrospectively. Allometric relationships

relating leader length and diameter to biomass can easily be established (cf. Shaver 1981, Shaver 1989).

- c) Litter, standing dead biomass: these analyses can be carried out on a shoot/plant or on an area basis. In the latter case simply clip a 10*10 cm patch and determine dry mass.
- d) Leaf samples for isotopic analysis: J. Welker volunteered to carry out analyses of stable isotopic composition (¹³C, ¹⁵N) of leaf tissue. ¹³C gives an indication of the integrated water and CO₂ supply over a leaf's lifespan, thus permitting an approximation of OTC effects on leaf carbon gain. Leaf samples (2-3 leaves/plot) should be collected toward the end of the growth period, oven dried (80 °C) and sent to Jeff. He has also indicated interest in obtaining soils samples, but should be contacted directly concerning details on sampling and handling.

3) Simple experiments

Here we describe two very simple experiments which can be used to quantify ecosystem-level responses to warming. In addition an experiment aimed at testing the extent of outcrossing is included.

- a) Decomposition: increased soil temperatures increase microbial breakdown and thus decomposition, provided that tissue quality (C:N ratio) is not altered substantially. Two methods are available to quantify decomposition, namely use of litter bags or wooden dowels (tongue depressors). Both methods involve determination of dry mass loss per given time interval. In the case of litter bags, litter samples are weighed and placed in mesh bags (mesh size ca. 1mm) and exposed in the field. Make sure that bags are secured at the soil surface (use inert 'nails') and that replicates can be identified. In the case of wooden dowels replicates can be identified via permanent waterproof markers. Use a minimum of 5 replicates to incorporate microsite variation.
- b) Root growth: an estimate of belowground responses to warming can be obtained via so-called ingrowth cores. These are mesh cylinders (mesh size 1-2mm) filled with root-free soil, which are sunk into holes in the soil (diameter 20-25mm, depth 10-15cm). Mesh bags are removed at intervals from the soils and the dry mass of roots, which grew into the mesh is determined. Make sure to mark the location of the ingrowth cores and cut off roots alongside the outer perimeter of the mesh bag with a knife, prior to removal. Use sifted soils from the core holes to fill ingrowth bags. Although root ingrowth is not a direct measure of belowground productivity (Hansson & Andren 1986), it is an easy, relatively non-invasive means for quantifying responses of warming on root dynamics. Before applying the method within OTCs, we recommend that you try it out elsewhere first, since little data is available from arctic soils.

- c) Emasculation of anthers: experimental removal of anthers can be used to test the extent of outcrossing in plant species. We suggest that seed mass and number of emasculated flowers be compared to non-manipulated controls. Since OTCs tend to serve as a barrier for wind pollination and reduce or even concentrate insect pollinators it is important to determine the extent of outcrossing particularly in ITEX plant species.

4) Climatic observations

In order to link results of 1) to 3) to climatic conditions in the current or previous years an effort should be made to obtain integrated measures of annual climatic variation. In addition to the ITEX climatic data (GDD), date of ice breakup or thawdepth (see Manual), we recommend use of year-round records of standard meteorological data. These data used for interannual intrasite and intersite comparison need not necessarily be local - even regional data from weather service stations could be utilized, particularly in cases where presence at the field site is intermittent. In these cases we recommend calibration of local weather data (i.e. short-term local site data) with those of a nearby permanent weather station. Regressions can be used to estimate local climate, although in some instances monthly means are sufficient. In mountainous terrain care should be taken with these correlations, since altitude, topography and exposition can have profound influences on local climate.

Note: In a number of species flower and leaf primordia are pre-formed, thus current year growth and reproductive status more accurately reflects climatic conditions of the preceding year.

5) Biotic observations

Other climate-related observations of biotic activity (first appearance of birds, hatching dates, insect phenology - see Manual) could be used to augment climatic observations.

Analyses

We recommend that the methodology described above should be incorporated into the standard ITEX protocol. These data will provide a basis for comparisons among and within sites, that extends far beyond the present scope of comparisons (i.e. ITEX species, climate-related data) to include population, community and ecosystem properties. It should for example permit a detailed study of regional and annual patterns of flowering rhythms, biomass accumulation and decomposition, at the same time incorporating the effects of simulated warming.

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