Bryophytes and lichens are important vegetation components in most tundra vegetation types and they may respond either directly to temperature change or indirectly through interaction with vascular plants. It is, therefore, important to include these groups in ITEX-studies at least in those at community level. Although bryophytes and lichens are quite distinct organisms, in many cases they play a comparable role in plant communities and it is thus appropriate to include both groups under the same protocol. This is particularly true for mat forming mosses and lichens with large cover in the bottom layer. Studies have shown that both mat forming mosses and lichens are extremely efficient in immobilising all atmospheric nutrient input making it unavailable for vascular plants (Crittenden 1989; Lee et al. 1987; Jónsdóttir et al. 1995).

We strongly recommend including bryophytes and lichens in studies at ITEX-sites. This can be done at different levels and we provide some detail on measurements at two of them:

1. Community level measurements.
2. Growth rate of individual shoots.

1. Community level measurements.
In the “point framing” analysis that is recommend in the “Community baseline measurements for ITEX-studies” it is important to record two hits at each point if possible: 1) first hit in the field layer and 2) first hit in the bottom layer (called “ground surface” in the Community baseline-protocol). If you have mat-forming bryophytes or lichens you should try to record the thickness of the mat. This is done by making one additional recording at each point: the distance from the bottom string intersection to the soil surface underneath the mat. It may be difficult to find the surface for two reasons: first, you have to be careful not to disturb the mat and secondly, the interface between the bryophyte/lichen mat and the soil surface is not always obvious. The thickness of the mat is then calculated as the difference between the distance to the soil surface and the distance to the surface of the mat (“ground surface”). In this way valuable information on the responses of mat thickness to the OTCs can be obtained.

If you find it too difficult or impossible to identify species or higher taxa in the bottom layer even a recording of a “functional group” will be informative. We suggest the following groups:

- Crustose lichens
- Foliose lichens
- Fruticose lichens
- Thalloid liverworts
- Leafy liverworts
- Acrocarp mosses (erect, ascending), other than Sphagnum and Polytrichales.
- Pleurocarp mosses (prostrate, for example Hylocomium splendens)
- Semi-prostrate mosses (for example Racomitrium lanuginosum)
- Sphagnum mosses
- Polytrichales

These groups can also be used in later meta-analysis of responses at the next level.
2. Growth measurements.

There are several methods available and different methods are suitable for different functional groups (see above). Clymo (1970) gives a detailed overview of methods used for measuring growth of *Sphagnum* species some of which have also been used for other species. Below we describe some of the methods that have proved most successful or promising in terms of accuracy and labour intensity and we mention one new method (c).

The first three methods (a-c) use reference marks outside the plant, and the two last methods are based on transplantation of plants cut to known length and/or of known mass. Some of the methods (b,d and e) involve removal and replacement of tagged target plants in a moss or lichen mat. In all these methods the target plants may either be placed back directly into the bryophyte/lichen mat or be first placed in a cage. Whether to use cage or not has to be decided from one case to another after evaluating potential disturbances and errors. By using a cage the damage to the "test" plant is minimised.

Especially for lichens, but also for some mosses, pushing a dry plant into, or pulling it out of a lichen/moss mat would do extensive damage. Under method e) there is a description of a cage that can be used for both bryophytes and lichens to decrease risk of such damage. A potential error in using cages is that a physical separation of a moss shoot or lichen thallus from the remainder of the mat can change the micro-climate of the caged plant.

a) Cranked wire.

Suitable for various *Sphagnum* species and other dense, mat forming acrocarp or semi-pleurocarp mosses. A number of ca 15-18 cm long stainless steel wires are cranked twice so there will be two vertical sections and one ca 1-2 cm long horizontal section somewhere in the middle. At time zero \( t_0 \) one end is pushed into the mat until the horizontal section is level with the moss shoots, while the free end projects into the air. It is important that the free end is of exact known length. The difference between this length and the portion still above the moss shoots at a given time \( t_1 \) gives the increase in shoot length during a time interval \( t_1 - t_0 \). By measuring the average dry mass of different length increments in a separate study the increase in shoot length during a time interval can be related to the species specific average mass of plant in unit depth to give an estimate of increase in dry mass. **Advantages of the method**: an easy-to-use and low-disturbance method; non-destructive and so repeated measurements possible; relatively accurate for the right type of mosses provided that many wires are used. **Disadvantages**: difficult to find all wires if not properly marked; risk of disturbance by grazing animals.

b) Tied thread.

Suitable for a range of mosses, but preferably mat forming. A thin thread is tied around the stem at a known distance from the apex of a number of shoots that are then placed back into the carpet at time zero \( t_0 \). The shoots are collected at a given time \( t_1 \) and the length increase measured. If the shoots are to be placed back for another time interval an estimate of dry mass increase can be done as in the previous method. If measurements are not to be continued, the dry mass of the new increment can be measured directly. **Advantages**: Suitable for a range of moss growth forms. **Disadvantages**: time consuming; risk of disturbing the moss shoots too much in repeated measurements; difficult to find all shoots if not properly marked.

c) Fluorescent spray.

This is a relatively new method that we have not tried, but should be rather accurate for a range of both bryophyte and lichen species forming dense mats (Russel 1988). A layer of fluorescent chemicals is sprayed on the mat at \( t_0 \). Shoots are collected at \( t_1 \) and the length increment above the fluorescent layer and its dry mass is measured. **Advantages**: low-disturbance method; spraying of
the mats can be repeated at intervals without disturbing the mats and at the final harvest the growth could be analysed retrospectively using the different fluorescent layers as markers. 

Disadvantages: there may be a problem finding the right chemicals that will stay in the plant tissue without harming them.

d) Plants of known length and known mass.

This method can be used for a range of mat forming mosses, but is probably not suitable for Polytrichales because of the primitive vascular tissue they possess. A number of moss shoots are cut to constant length (ca 5 cm) and weighed (fresh mass). The shoots are divided into two groups, one is tagged and placed back into the moss mat at \( t_0 \), while the dry mass is measured in the other half. The ratio average dry mass : fresh mass is used to calculate the dry mass of the transplanted shoots at \( t_0 \). The shoots are collected at \( t_1 \), their length and weight measured. This method is very similar to the next method which is mainly designed for lichens. While the shoot length of most mosses is easily measured and can be related to dry mass, this is usually impossible to do in most lichen species. The details of the weighing procedure and calculations given under method e) for the lichens can also be followed for mosses. The greatest advantage of both these methods is that they are relatively accurate. Disadvantages are that there is risk of disturbance and it may be difficult to find transplanted shoots again.

e) Plants of known mass

**General principle**

The dry mass of a lichen is measured at time zero \( (t_0) \), the lichen is secured in the field to grow and then dry mass measured again at the end of the growth period \( (t_1) \).

**Experimental**

Test thalli should be selected, trimmed in size if necessary, cleaned of extraneous debris and placed in the field until they dry naturally during a rain-free period. The air dry thalli are then transferred to a laboratory and allowed to equilibrate with laboratory air for c.12 h and weighed \((t_0)\) using a 4 decimal place analytical balance. The weighed thalli are tagged for identification purposes and then returned to the field to grow. At the end of the growth period the lichens are recovered, air dried under laboratory conditions, weighed \((t_1)\), oven dried \( (80 \, ^\circ \text{C} \, \text{for 12h}) \) and weighed again.

On both occasions \( (t_0 \) and \( t_1 \)) procedures similar to those above are carried out on a duplicate set of "dummy" thalli (but these are not tagged) for which both air dry mass and oven dry mass are determined. The air dry:oven dry mass ratio for dummy thalli is then used to estimate the oven dry mass of the test thalli. Oven dry mass of test thalli at \( t_1 \) can then be measured both directly (by drying the test thalli) and indirectly (by using data for dummy thalli): this provides a check on the accuracy of the "dummy" lichen approach.

The suggestion above is that lichen thalli are selected, cleaned etc. in the wet state. This will reduce damage since dry lichens are brittle. It is probably desirable to deviate from natural conditions as little as possible and, thus, to avoid artificially rewetting lichens, especially during periods with strong evaporative forces (e.g. when exposed to strong direct solar radiation). Of course, occasions may arise when there is no other option and spraying with deionized water or simulated rainwater becomes necessary in order to select intact lichen thalli. To avoid errors it is essential that (i) after weighing at \( t_0 \) lichen thalli are handled with the greatest of care (e.g. watchmakers forceps) and (ii) thalli are wet when they are recovered from the field at \( t_1 \): if thalli in the field are dry on this occasion then they should be artificially re-wetted to minimise the likelihood of mechanical damage.

Tags can be made from small pieces of acetate or polyester sheet (e.g. 4 x 3mm) attached to the lichen with polyester thread. I have found it useful to photocopy numbers onto acetate overlays.
for this purpose. Some lichens (e.g. foliose species) do not lend themselves readily to having things tied to them, instead tags might be tied to their securing mesh (see below).

Securing lichens in the field
Useful materials for this purpose are fine woven stainless steel (s.s.) wire mesh (or fine nylon mesh) and s.s. wire strong enough to be pushed into the soil. Lichens should be oriented in the field in a manner comparable with undisturbed specimens of the same species. A suggestion for mat-forming species that grow vertically upwards (e.g. species of Cladonia, Stereocaulon, Cetraria) is to construct small cylinders from s.s. mesh which are then inserted into otherwise undisturbed lichen mats thus creating a recess or well. These cylinders should be wide enough to allow air dry thalli to be gently "dropped" into place. It may be desirable to add some material to the bottom of the cylinder to raise the apex of the test thallus to those of the surrounding lichen (suitable material might be dead basal parts of lichen thalli or inert black plastic beads). Foliose lichens (e.g. Peltigera, Nephroma) can be placed under fine mesh which is pinned to the soil with s.s. wire. Thalli of Thamnolia vermicularis might be held in position using s.s. wire bent like a shepherd's crook.

Relative growth rate
Mean RGR = (log_eM1 - log_eM0) / (t1 - t0) (unit mass per unit mass per unit time)

References