ITEX meta-analyses on exp. warming x respiration, methane, microbes, vegetation

Guidelines for 2nd survey round

Thank you for participating in our ITEX meta-analysis!

In this document you will find the main guidelines on how to proceed. This includes

a) a **timeline** with steps to be undertaken by each participant,
b) an **overview of the documents** that you can find in the **OneDrive folder** (where the surveys to be filled in + supporting docs are located),
c) the steps to follow when filling in your **site-specific excel survey**,
d) **protocols** for (potential) additional data collection.

Note: we want to remind each participant on the **main selection criteria** for a study/plots to be included in this meta-analysis is that the only experimental treatment is **warming** (either by OTCs or snow fence or a combination of these), and that **respiration measurements** should be available for each plot (with data available autumn 2020). We do not want data (respiration measurements) from plots with additional.

a) Timeline

	Jun-Jul 2020	Jun-Aug 2020	Oct 2020	Oct-Dec 2020
	2nd survey round (metadata)	Collect additional data/samples	Send datasets + soil samples with completed checklist	Initial data analysis
ACTIONS	▪ Add **missing info** ▪ Add **clarifications** ▪ **Doublecheck** metadata	▪ If **data collection yourself**: add missing data to the survey ▪ If **measurements** to be done by us (additional microbial analysis): store samples until September 2020 ▪ If **no additional data collection** possible: do nothing	▪ Respiration data should be sent as **daily average flux** per replicate for every flux measurement occasion (see example in Onedrive) ▪ For additional soil sampling, see protocol in Onedrive	
DEADLINE	**15 July 2020** (after which a check will be done and changes can still be made until **15 August 2020**)	**End of growing season**	**15 October 2020**	First results expected by **Spring 2021**

b) Overview of OneDrive docs

- **README_Guidelines** [docx]

  Current file, read this before you start completing the survey
- **Site-specific survey files** [xlsx]: *"standardized site name_contact person_survey.xlsx"*

  e.g. AUS_1_Mark_Hovenden_survey.

  Find your own files in the one drive to check. You can look for your own name in the document name, as well as look for your sites's standardized name in the participant + site list. *If you are unsure which files are yours, please contact us.* Please address all items marked with a color coding by changing them in the OneDrive folder (more info below). Please do not make edits to any other file than your site file(s).
- **Walker2005_CAVM [PDF]** (+ Suppinfo1,2,3)

  In the survey we ask you to classify your site in one of these classes. This is a supporting article (+ supporting info files) explaining the vegetation classes derived from the Circumpolar Arctic Vegetation Map.
- **Participant + Site list** [xls]

  The participant + site list that you are asked to doublecheck as part of the survey questions.
- **Itexmanualchapter11_VegComm_Pointframe** [PDF]

  If your site lack vegetation community data, please have a look at this document for instructions for the upcoming 2020 field season. No need to change vegetation sampling protocol though if you have already done vegetation surveys previously.

c) Survey excel procedure

Tab 1 = Your initial survey replies with notes and editing made by us that you are asked to doublecheck/add new information.

Because we want to use as many flux data points as possible, we have **split your survey replies into unique site-flux measurement IDs**. You should see your initial column on the most left in excel, and then between 2 black columns the same information, but copy-pasted for every unique year that you have flux measurement for. You can fill in different info now for the different measurement years (e.g. if different flux measurement methods have been used at different occasions), or you can fill it in for one column and copy paste the info for all years if the methods are always the same (**Just make sure that the unique ID is still correct in row 3 in red**). If there are more flux measurement years available than initially filled in, then copy-paste (a) new column(s) with the info for the additional year(s).

- Text in red = I have changed something → **Please doublecheck** if correct for your site
- Cell highlighted in yellow → Missing, **please add information if available, else explain when available or if it will remain absent**
- Cell highlighted in green → **Please add more detail/clarification**
- Note: sometimes the drop-down menus are not visible anymore in the cells, although they are still there. If it says "Drop-down" in column B, there should be a menu available to choose from. Simply click on "alt + down arrow" when in the cell, and it should reappear.

Tab 2 = Site-specific questions → **Please answer these extra questions** for your site.

Thank you for sending an email to **confirm** you have completed the survey on OneDrive to **sybryn.maes@umu.se** (preferably before 15/7/20).

d) Protocols current meta-analysis

We ask you to collect plant height and a soil sample from each plot, and provide some instructions below. Plant height will be used for biomass estimations, and soil samples for microbial analysis (if not available previously) and potentially measurements of other soil properties. If you visit your plots at several moments during the growing season (eg. repeated vegetation/flux measurements), we prefer the height and soil sample taken at **the timing of the flux measurements** and/or at **peak biomass (mid-season)**, but if this is not possible, we proceed with what you have.

Soil

REQUESTED FROM ALL SITES WITH NO AVAILABLE DNA EXTRACT

Since we want to use the soil potentially for microbial analysis, we ask you to sterilize between collecting samples in each new plot. We describe in the protocols suggested equipment, but if this is not available for you, please use what is available to you and suitable for the data. Our apologies that we cannot send you material to collect the samples with. Thank you for using your own material in **sterile conditions**, i.e. cleaning the tweezers/corer with a clean tissue sprayed with 1% v:v hypochlorite (bleach) then wiping the tweezers/corer twice with a clean tissue sprayed with 70-90% ethanol.

Please collect min. 10g soil with tweezers and/or apple corer (2cm Diameter) and stored in zipped plastic bags. Please take pooled/homogenized soil samples from 5 points spread evenly across each plot to reach at least 10g soil. Sampling depth should be **0-10 cm**, mixing both mineral and organic soil, but please write down the **organic layer depth**. For an estimate of the organic layer depth, measure the total depth of the hole (soil surface to bottom) where you cored, as well as the length of mineral layer and organic layers of the actual core (the length of the organic layer in the actual core will likely be somewhat shorter than that on the ground). Measuring these 2 variables achieves the best estimate of the depth of the organic layer which is often disturbed (compacted) during coring.

It is very important to keep the sampling "clean": **sterilize** tweezers used to collect soil between the different plots with bleach and ethanol as explained above. Please, immediately **freeze** the samples and store them frozen (at minimum -20°C) until we request you to send it to us (likely mid-October).

- Collect 10 g **in sterile conditions**
- Collect 0-10 cm depth, mixing mineral and organic layer
- Write down organic layer depth: a) total depth of cored hole, b) length of mineral layer of core, and c) length of organic layer of core
- Ship in frozen state

REQUESTED FROM ALL SITES WITH AVAILABLE DNA EXTRACT (FROM PAST MICROBIAL ANALYSES)

Please collect min. 10g soil with tweezers and/or apple corer (2cm Diameter) and stored in zipped plastic bags. Please take pooled/homogenized soil samples spread evenly across each plot to reach 10g soil. Sampling depth should be **0-10 cm**, mixing both mineral and organic soil, but please write down the **organic layer depth**. Please, immediately **freeze** the samples and store them frozen (at minimum -20°C) until we request you to send it to us (likely mid-October).

- Collect 10 g
- Collect 0-10 cm depth, mixing mineral and organic layer
- Write down organic layer depth
- Ship in frozen state

DNA samples

REQUESTED FROM ALL SITES WITH AVAILABLE DNA EXTRACT (FROM PAST MICROBIAL ANALYSES)

If the **DNA extract samples** from past microbial analysis are still available, please do not throw them away, and keep them in **the freezer at minimum -20°C**. We will ask you to send them to us in a later stage, to do standardized microbial analyses across all sites. In this case, please send us a diluted subsample, of at least 20 µL totaling at least 50 ng - as quantified using Qubit or similar fluorescence method (if using Nanodrop-type absorbance ratios, please increase total DNA content to at least 100 ng) - and have it shipped in a frozen/freeze dried state.

- Keep frozen at min. -20°C
- Send in later stage when asked for
- Send diluted subsample
- Ship in frozen/freeze dried state

Plant height measurements

REQUESTED FROM ALL SITES

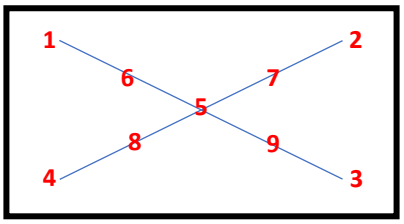
To obtain a uniform trait measurement for community height and to derive biomass/productivity estimates in each plot, we ask you to collect **9 plant height measurements evenly distributed across each plot.** Please take the measurements within the **(permanently marked) subplot** that was/will be used for vegetation community measurements. If you use the **pinpoint** method, measure the height of the **first hit** at each sample location. If you use **another method**, measure plant **height without extending** the plant at each sample location.

The sample locations are fixed as follows:

= subplot used for vegetation community measurements

= 2 diagonals of the subplot

1 = 9 locations for sampling plant height



Plant height should be measured to 1 decimal accurately with a ruler. Focus on **vascular plants** only; if your system is dominated by non-vascular plants (e.g. >80% mosses), still measure plant height where vascular plants are present.

- Collect 9 plant height measurements spread evenly across each plot
- Measure plant height from the fixed 9 sample locations (see figure above)
- Measure the height of the first hit if you use the pinpoint method
- Measure the height without extending the plant if you use another method
- Focus on vascular plants only

Point frame measurements

REQUESTED FROM SITES WITH NO PREVIOUS VEGETATION COMMUNITY DATA:

Please follow the point-frame protocol from the ITEX manual.

Supporting environmental info

If you are collecting samples to provide missing data for the Supporting environmental info (for which thank you!), please collect as though you would do it in your own way. There are **no specific protocols** to follow here. The following supporting environmental info are requested, although we will work with whatever you have or can provide us with. For the requested data based on actual

samples, we prefer to have data that were **taken as close in time to the flux measurements** as possible.

Requested plot-level data based on actual samples (preferable from the year of respiration data, but if not available please state in which year it was measured):

- Air Temperature, Soil Temperature (°C)
- Soil moisture/Water table
- Soil C characteristics
- Soil pH
- Permafrost (continuous/discontinuous/no)
- Permafrost active layer depth (cm)
- Organic layer depth (cm)
- Soil organic matter (%)
- Bulk density (g/cm^3)

Requested data not based on additional samples:

- Air Temperature (°C) (growing season and annual average T)
- Soil moisture class (dry/mesic/wet)
- Parent material (sand/silt/clay/bedrock/unknown/other)
- Ca-content of bedrock
- Snow melt date (approximate date due to yearly fluctuations)
- Photosynthetically active radiation (W/m^2)
- Ambient snow depth (m) (approximate date due to yearly fluctuations)
- Annual Precipitation (mm)