Project Description – Project Proposals

Cristian Estop Aragonés, Münster. Werner Borken, Bayreuth

Thawing Effects on Soil Organic Carbon in Permafrost Peatlands: TESOCPe

Project Description

# Starting Point

State of the art and preliminary work

Permafrost peatlands cover > 1 million km2 in the Arctic and Subarctic and store large amounts of soil organic carbon (SOC) per unit area with an estimated global pool of 185±70 Pg C 1. Due to sub-zero temperatures in permafrost, mineralization of frozen SOC is considered negligible. Amplified warming ~~and increased wildfire frequency~~ at high latitudes are causing widespread permafrost thaw through thermokarst and active layer thickening 2–6 which exposes deep, old SOC stores to microbial activity potentially releasing it to the atmosphere in form of greenhouse gases CO2 and CH4. The release of CO2 and CH4 from previously-frozen SOC represents a net addition into the global C cycle and thus, permafrost thaw due to climate warming may enhance further climate warming. Such ‘permafrost carbon feedback’ is potentially the greatest positive feedback to the climate but there is a large uncertainty in the magnitude of release of CO2 and CH4 from previously-frozen SOC 7. Quantifying rates of mineralization of previously-frozen SOC can be accomplished through radiocarbon measurements of respired gases. A synthesis of >1,800 14C measurements of CO2, CH4, dissolved and particulate organic carbon (DOC and POC) in northern permafrost identified a poor spread of 14C data among regions and, except for one study, none of these 14C measurements occurred in Fennoscandian peatlands 8. Measurements of both previously-frozen SOC losses and recent C gains are needed to assess the effects of thaw on the C balance in peatland ecosystems but the available data in this regard is restricted to few peatland sites with very variable response to thaw.

*Rapidly thawing peatlands in Norway – A timely spatial research gap*

Finnmark is the main area of permafrost peatlands in Norway where these peatland plateaus or palsas cover around 110 km2 and started accumulating following the Last Glacial around 9200-9800 cal. yr BP 9–12. Critically, the extent of peat plateaus is rapidly decreasing in Fennoscandia as indicated by an estimated decrease of 33-71% between 1950 and 2010 in Norway and an accelerating rate in the last decade10. The permafrost temperatures in Finnmark are higher than those in other regions making the study of these sites relevant to inform about the future response of much larger peatland areas such as for example the Western Siberian Lowlands. Additionally, there are noteworthy differences in these Fennoscandian peatlands compared to previous studies that have investigated the mobilization of old SOC release in permafrost peatlands. First, peatlands in this region have remained permafrost-free fens during most of the Holocene 11. This contrasts with work in previous sites investigating the effects of thaw on old SOC release in Canada where the bulk of the organic matter deposit was bog peat 13,14. Given the vegetation origin and organic matter composition differs between fen peat and bog peat 15,16 it is important to understand whether such differences in composition influence the effects of thaw on the C balance in these ecosystems. Second, in contrast to other extensive peatland regions where the process of peat accumulation was terrestrialization (water body filled with sediment/peat), the main process for accumulating peat in Finnmark has been through paludification (dry land converted to peatland) 9,17. Nevertheless, while several studies have documented the coverage, stratigraphy and dating of Fennoscandian peat plateaus 9,10,18,19, no study has yet quantified in situ mineralization rates of previously-frozen SOC or recent SOC gains occurring after thaw in peatlands disturbed by thermokarst or active layer deepening in Finnmark. This knowledge gap is important to fill given i) the large vulnerability to thaw of these relevant carbon stores, ii) the large discrepancy of peat C loss rate and C balance following thaw between the few studies in Canada 13,20 and Alaska 21,22, and iii) the differences in peat origin (fen vs bog) and historical development (paludification vs terrestrialization) of the proposed study sites in comparison to previous work. These differences enlarge the range of peatland site characteristics where the response to thaw has been assessed and thus add value to improve ‘permafrost carbon feedback’ projections in thawing peatlands currently based on few sites and limited spatial coverage 1,23.

*The mode of thaw and its effect on soil C balance of permafrost peatlands*

The mode of thaw influences the soil redox state in permafrost peatlands and palsas thereby influencing greenhouse gas release. On the one hand, thaw by deepening of the active layer (part of the soil profile thawing in summer and refreezing in winter) increases the fraction of SOC being thawed and potentially mineralized. Such active layer deepening is enhanced by gradual climate warming ~~and by wildfire~~ 24–26, and previously-frozen SOC may be rapidly mineralized if soils remain relatively well drained and oxic 14,20,27–29. On the other hand, thermokarst occurs when the entire soil profile that contains large excess ice thaws and the frozen portion of the peatland collapses, which results in ground subsidence and water-logged conditions. In this case, substantial SOC stores with basal peats >9,000 years old are potentially available to microbial decomposition although anoxic conditions likely reduce mineralization rates of previously-frozen SOC 14,20,30,31. Quantifying mineralization rates of previously-frozen SOC in sites affected by active layer deepening vs thermokarst is thus critical to assess the vulnerability of permafrost SOC following thaw.

Landscapes with undisturbed peatlands (intact permafrost) adjacent to thermokarst peatlands (degraded permafrost) allow assessing the effects of thaw on C cycling through the space-for-time chronosequence approach. Several studies in boreal North America have reported a clear shift in vegetation and rapid peat accumulation at the surface following thermokarst 20,22,32,33. While these thermokarst peatlands may accumulate organic matter rapidly at the surface due to fast-growing hydrophilic vegetation, previously-frozen SOC at depth may also be mineralized through anaerobic processes and be released as CO2 and CH4. Quantifying both near-surface gains of peat following thaw and losses of deep SOC becomes thus necessary to determine the net effect of thaw on the C balance in thermokarst peatlands. The goal of this project is to determine the net effects of thaw on the C balance in permafrost peatlands affected by thermokarst as well as the effects on soil respiration in peatlands disturbed by active layer deepening in Finnmark. There is so far no study reporting such results in the region.

*Uncertain fate of thawed soil organic matter following thermokarst in peatlands*

The few available data regarding the fate of thawed SOC in thermokarst peatlands come from Alaska and Canada, and these studies show contrasting results. Chronosequence and modelling results in Alaskan thermokarst peatlands suggest that a substantial part (30-50%) of the original SOC stock is lost within decades after thaw, which represents a flux of previously-frozen C >3000 g C m-2 yr-1 21,22. Such results were pioneer and are indeed being used to globally upscale the response of peatland SOC to thermokarst 1,23. However, there is no direct evidence at those same sites supporting such potential large SOC loss in form of released greenhouse gases, which show a loss at least an order of magnitude lower 34. In contrast, studies in other thermokarst peatlands in Canada and Alaska directly quantifying the release of “old” SOC with radiocarbon analysis of CO2 and CH4 showed a maximum flux of previously-frozen C of as little as 1.5 g C m-2 during the growing season 14,20,35,36. Other results comparing SOC stocks 13 and incubations 37 along chronosequences also suggest low mineralization of peat accumulated before thaw but the lack of data in other regions and the use of diverse approaches makes projections of future C losses to be very uncertain 23.

*Approaches to determine the fate of thawed carbon in thermokarst peatlands*

The fate of thawed SOC in peatlands can be studied using different approaches that will be combined in this proposal to provide independent lines of evidence regarding the effects of thermokarst on permafrost SOC mineralization. First, the SOC stocks can be quantified and compared along peatland chronosequences to determine if there are differences in the amount of pre-thaw SOC between undisturbed and thermokarst locations.

Pre-thaw SOC refers to organic matter accumulated before the time of thaw and that may be still frozen (undisturbed site) or thawed (thermokarst site). If existing, differences in SOC stock can be attributed to differences in mineralization since the time of thaw. The comparison of SOC stocks along chronosequences is typically limited to a single core per location. Plant macrofossil analysis is then used to verify that both the undisturbed and thermokarst locations along the chronosequence share a common developmental history 13,38. The analysis of the plant macrofossils allows to differentiate between stages such as marsh, fen, bog or permafrost palsa in a given site and ensure that such stages are similar in both sites (peatland plateau and thermokarst) so that the comparison in SOC stocks between both sites is appropriate 13. Increasing the number of cores would add robustness to the results once an initial characterization of the site developmental history has been done through plant macrofossils.

Second, direct evidence regarding the mineralization of thawed SOC is provided by radiocarbon analysis of the respired CO2 (14CO2). Our focus for old SOC losses will be on CO2, which constitutes the largest C flux component in these systems and there is increasing evidence of very limited mineralization of previously-frozen SOC sources as CH4 35,36. Given that deep SOC in thermokarst peatland is depleted in radiocarbon and may be several millennia old (up to around 9,000 years), measuring the radiocarbon content of the respired CO2 can be used to quantify the loss rate of previously-frozen SOC. Samples for 14CO2 analysis can be collected from chamber measurement fluxes that include and exclude deep soil respiration (“old” SOC) in order to quantify the contribution of “old” SOC sources to the flux and estimate the rates of “old” SOC release. The age of the “old” SOC source can be defined through radiocarbon analysis of soils at the base of the active layer (or top of permafrost) in the undisturbed location. Preparation of samples prior for 14C analysis (graphitization step) strongly reduce the overall analysis cost and this is possible following established methodologies at the laboratory of the PI Prof. Dr. Werner Borken (U. Bayreuth).

Third, a suite of additional analysis and laboratory experiments can be done to study how organic matter quality, mineralization rates and microbial biomass and carbon use efficiency of thawed SOC in the thermokarst locations compare with SOC of similar age in the undisturbed locations. If deep peat in the thermokarst site has undergone substantial in situ decomposition, the potential for mineralization of organic matter should be lower compared to that of the undisturbed site. Current methodologies at the University of Münster allow characterization of solid phase organic matter properties and its humification degree through proxies obtained from Fourier Transformed Infrared (FTIR) spectroscopy (humification index 1630 cm-1/1090 cm-1), X-ray fluorescence spectroscopy (XRF) and C and N elemental analysis (C/N/P ratios). This information can be used to evaluate whether there are differences in soil organic matter properties between undisturbed and disturbed (thermokarst) sites 13,39 and relate these differences to differences in the potential mineralization between sites 40. Incubations of peat and leached dissolved organic matter can be used to quantify potential CO2 and CH4 production rates and determining mineralization decay constants for SOC as well as for DOC. The potential for DOC mineralization may be limited in peatlands given that organic matter has been exposed to decomposition for long time periods yielding matter rich in aromatic structures in both the solid 13,39 and the dissolved phase 41. Differences in dissolved organic matter properties (SUVA254, C/N/P) provide further information to determine differences in lability between thawed and undisturbed peat as well as their relation with the abovementioned solid phase organic matter properties and mineralization potentials. Changes is substrate quality after permafrost thaw are also associated with changes in microbial metabolism. In particular, the carbon use efficiency (CUE) of microorganisms provides insight into the relative utilization of SOC for microbial respiration and growth of microbial biomass. High CUE indicates intense microbial growth and SOC stabilization, while low CUE favors respiration and relatively high C loss to the atmosphere (Manzoni et al. 2012 #40). Although, this concept has not been applied to thawing permafrost peatlands, CUE could add information on SOC stability in permafrost at different thawing modes. Overall, these approaches provide independent lines of evidence to the same research question and render confidence in the overall findings.

*Rapid peat accumulation following thermokarst may control the peatland C sink-source function*

The collapse of permafrost peatlands through thermokarst results in ground subsidence and water-logged conditions. Shifting from relatively dry and well drained conditions in permafrost peatlands or palsas to water-logged conditions in thermokarst peatlands results in a clear change in vegetation towards hydrophilic species 42 and in the type of peat formed. This results in a sharp change in the peat stratigraphy. Visual inspection of the peat stratigraphy allows identifying the transition depth between peat accumulated before and after thaw, which can be used to determine the time of thaw with 210Pb dating 20,32,43. Age-depth relations using 210Pb dating can be combined with C stock measurements to quantify C accumulation rates since time of thaw during the last 100-150 years 44. This recent C accumulation since time of thaw needs to be put in context with potential C losses from thawed peat to determine the net effect of thaw on the C balance. Several studies have shown that following thermokarst, recent C accumulation rates are high compared to permafrost peatlands and indicated very variable values ranging from as little as 10 to as much as 800 g C m-2 yr-1 20,22,32,33. These large differences in C accumulation rate are important because they may determine whether thermokarst peatlands currently function as C sinks or sources. However, we do not yet understand what explains such large variability in recent C accumulation in thermokarst peatlands. Ground subsidence following thermokarst results in water being temporarily above the ground surface until peat accumulates again to and above the water level. This proposal aims to assess the relation between depth of collapse and recent C gains at the surface by surveying several thermokarst peatlands with waterlogged peat profiles. We refer to depth of collapse as the distance in the waterlogged peat profile from the top to the transition depth between post-thaw and plateau peat (pre-thaw) at a given site. We expect sites where the collapse was deeper (i.e., the ground surface of the site became submerged deeper following thermokarst) to have greater potential for C gain (i.e., greater recent C accumulation rates). Understanding i) how post-thaw C accumulation rates change with time as peat succession occurs and ii) whether post-thaw C accumulation rates are related to the depth of collapse (≈ground ice content) are critical to understand the magnitude and time-scale of the change in the C sink/source function.

*Active layer deepening effects on soil respiration in permafrost peatlands*

In contrast to thermokarst and associated water-logging, thaw through active layer deepening is considered to result in peat remaining well-drained and more likely to be dominated by soil oxic conditions. Permafrost peatlands with deeper active layer offer a great opportunity to quantify mineralization rates of previously-frozen SOC as aerobic decomposition is expected to increase deep soil mineralization. Understanding how the mode of thaw (thermokarst vs active-layer deepening) influences the mineralization of previously-frozen SOC is important to predict future peatland SOC stocks in the permafrost region. Furthermore, the effects of active layer deepening on old SOC respiration are important to be quantified as they may be relatable to wildfire effects. ~~Burnt permafrost peatlands are shown to develop deeper active layers~~ ~~26,45~~~~.~~ ~~Although peat core charcoal analysis suggest wildfire is not a pressing disturbance in Fennoscandian permafrost peatlands~~ ~~46~~, active layer deepening will become a widespread feature in permafrost peatlands in coming years based on current projections in Europe and Western Siberia 47,48 and ~~increased wildfire frequency~~ in northern regions 4,49–51. Nevertheless, the only available data assessing active layer deepening on previously-frozen SOC mineralization in peatlands comes from few sites 14,20,26. Combining flux chamber measurements with 14CO2 analysis allows quantifying mineralization rates of previously-frozen SOC released to the atmosphere. Considering the ongoing and future active layer deepening in peatlands, it is thus critical to quantitatively assess the relation between active layer deepening and mineralization rate of previously-frozen SOC. This relation can be evaluated by performing 14CO2 measurements across an active layer deepening gradient to assess the soil thermal regime and better constrain estimates of previously-frozen SOC mineralization.

Preliminary work

We have discussed these research gaps and have established collaboration agreements with three main colleagues (section 4.6). To this aim, we have identified research peatland sites with permafrost disturbance in Norway to address these research gaps. Dr. Hanna Lee is based in the Norwegian University of Science and Technology in Trondheim, Norway (NTNU) and has identified and is currently working in suitable research sites in Finnmark, the northernmost county in Norway. Briefly, the challenge of addressing field sampling campaigns in the north will be eased through collaboration with our Norway colleague Hanna Lee (NTNU), which will contribute with data and knowledge of the study region and will facilitate logistical assistance, field equipment, storage space and accommodation. A relevant part of this proposal lies on soil core analyses to i) characterize the historical development of the peatlands and determine succession stages and ii) quantify recent soil C gains in thermokarst peatlands using 210Pb dating. For this, we will collaborate with i) Dr. Mariusz Gałka at the University of Lodz to perform plant macrofossil analysis and ii) Dr. Carolina Olid at the University of Barcelona to analyze and interpret the 210Pb data. All these collaborations represent strong assets for the project and improve the quality of the generated data given the expertise of the collaborators in their respective fields 44,52–54. Additionally, these collaborations reduce the amount of materials and equipment being purchased as well as associated analytical costs. We have planned for logistics that are most cost effective. The arrangements for the logistics of the project between Germany and Norway have been discussed and agreed with our colleague Hanna Lee (NTNU). The equipment and bulky materials for field measurements will be arranged in Münster and transported to Norway by us on road renting a vehicle at Uni Münster. The equipment for soil core sampling will be arranged through Norway colleagues. Given the workload during fieldwork, we plan for PI’s, a PhD student, a MSc student and a student assistant to participate during field campaigns.

Cristian Estop Aragonés has led since 2013 field research campaigns in permafrost sites in the boreal forest, peatlands and lakes in northern Canada (Yukon, Northwest Territories and Alberta). He is experienced in the quantification of loss rates of previously-frozen SOC in thawing ground using 14CO2 and in determining recent C accumulation to determine net effects of thaw on peatland C balance (Figure 1). He assessed in a synthesis paper the use of 14C measurements to quantify rates of permafrost SOC loss following thaw 8, which among others identified limited spatial coverage in some regions and thaw through active layer deepening as major research gaps to constrain estimates of permafrost carbon mobilization.

Werner Borken has more than two decades of experience in the research of C cycling in peatland and forest ecosystems. He uses radiocarbon signatures of soil organic matter and CO2 as powerful tool to partition soil respiration into autotrophic and heterotrophic respiration 55 and to assess the age and turnover time of soil organic carbon 56,57. An extraction vacuum line was established at the University of Bayreuth in 2005 for preparation of graphite from solid, solute and gaseous samples. More than 1000 graphite samples have been processed since 2005, and measured at the KECK Carbon Cycle AMS Laboratory, University of California, Irvine, USA.

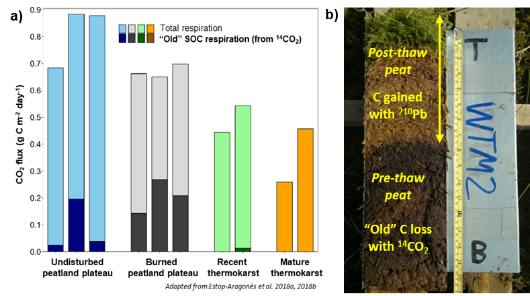


Figure 1 – a) “Old” SOC respiration estimated using 14CO2 measurements in September in northern Alberta peatlands thawing either through active layer deepening (undisturbed and burned) or thermokarst (recent and mature). b) Surface peat core from a thermokarst peatland in Canada showing changes in peat stratigraphy indicating the transition depth (i.e., depth of collapse) between peat accumulated since time of thaw (post-thaw) and before (pre-thaw). The net effect since time of thaw wants to be investigated in peatlands in Finnmark county (Norway) and can be determined by measuring the C gains through 210Pb (panel b) and the C loss through 14CO2 measurements (panel a).

# Objectives and work programme

## Anticipated total duration of the project

36 months (01.03.2024-28.02.2027)

## Objectives

Study sites

Two main peatland sites have been selected to determine SOC gains and losses following thaw by thermokarst (Iškoras site) and active layer deepening (Áidejávri site) – Figure 2. The sites have peatland plateaus or palsas (peatlands with permafrost) that are elevated relative to thermokarst peatlands where permafrost thaw has resulted in variable ground subsidence (⁓50-100 cm), water-logging and vegetation shift. The peatlands are up to 2 m deep and with an active layer depth between 50 and 100 cm. The Iškoras site has been established since 2016 and maintained by collaborative efforts across several institutes in Norway (i.e. Prof. Dr. Hanna Lee at NTNU, University of Oslo, NIVA-Norwegian Water Research Institute, and NORCE-Norwegian Research Centre). The site is currently well equipped with various continuous and campaign-based monitoring efforts including soil thermal and hydrological regime, greenhouse gas exchange including surface fluxes and eddy covariance fluxes, water chemistry, and monitoring of permafrost degradation with aerial photography and digital elevation models (DEM). However, there is no quantification in this site about i) the rate of release of previously-frozen SOC, which we plan to quantify using 14CO2, ii) C accumulation rates since thaw, which we plan to quantify using 210Pb to capture 100-150 year time scales, and iii) characterization of humification and elemental composition from the peats to compare the thawed and intact areas. Other thermokarst features at the site make it suitable to investigate multiple locations and examine different chronosequences. The Áidejávri site has been more recently identified by Norwegian colleagues (Sebastian Westermann, University of Oslo) and temperature monitoring and DEM have been implemented in the last 3 years. This site has a more extensive peatland plateau and is suitable to select locations of different active layer depth and investigate the effects of active layer deepening on old SOC release. The site can be reached from Iškoras after 200 km by road. The sites can then be reached by car from Karasjok (45 min to Iškoras, 2 h to Áidejávri) and a walk. Car rentals can be arranged if needed at both Alta airport or Lakselv airport for the sampling campaigns and both airports are well connected to Oslo International Airport. The nearest town from the sites is Karasjok, which is used as a basecamp during fieldwork campaigns. By collaborating with the Norwegian colleagues, we will have access to the sites, sampling equipment, as well as sharing of the existing data from the sites since 2016 (weather station data, hydrological data and greenhouse gas exchange). No additional permits will be required since the sites are already permitted for scientific research. A major benefit of working at these peatlands in Finnmark is the relatively easy access and limited logistical difficulties despite the challenge of working in Arctic regions. No study has yet quantified in situ mineralization rates of previously-frozen SOC or recent SOC gains occurring after thaw in peatlands disturbed by thermokarst or active layer deepening in Finnmark (Figure 2a). A field visit to the sites is planned in September 2023 by the PI and Norwegian colleagues to show installed equipment, select potential sampling locations and discuss project details and further collaborations.

Figure 2 – Sketch of research sites. a) Locations (yellow and blue stars) that have investigated thawed C mobilization in northern permafrost using radiocarbon 8. Red star shows the gap we want to fill in Norway where no such data exist. b) Selected sites in Finnmark county (Norway) disturbed by thermokarst and active layer deepening (Iškoras: 69°20’ N 25°18’ E and Áidejávri: 68°45’ N 23°19’ E). c) Aerial image of the Iškoras site showing a mosaic of permafrost peatland plateaus and thermokarst peatlands with variable ground subsidence. d) Ground view at Iškoras site showing an example of a peat plateau collapsing with ⁓70 cm ground subsidence and vegetation shift occurring in the thermokarst peatland.

Objectives

The overall aim is to quantify thaw effects on SOC loss and gain in permafrost peatlands and contrast thaw by active layer deepening and thermokarst in relation to the abovementioned research gaps. We define four objectives (O) and hypotheses (H), which will be implemented in four work packages (WP):

O1:To quantify and compare SOC stocks and solid phase organic matter properties in peat profiles from undisturbed and thermokarst peatlands using plant macrofossils (WP 1)

H1: S*OC stocks accumulated before thaw are smaller in thermokarst peatlands than in undisturbed adjacent peatland due to mineralization of previously-frozen peat following thaw*. Thermokarst peat (add layer) shows (Increased) higher mineralization, reduced SOC stock and higher humification than undisturbed peatlands (Figure 3a).

O2: To quantify the mineralization rate of previously-frozen SOC using 14CO2 in intact peatlands, thermokarst peatlands and peatlands with active layer deepening (WP 2)

H2: *Previously-frozen peat (“old” SOC) is more vulnerable to mineralization when thaw occurs through active layer deepening (well-drained, oxic) than when it occurs through thermokarst (water-logged, anoxic).* (Figure 3a).

O3: To assess post-thaw SOC accumulation in thermokarst peatlands of different collapse depths/intensities using PbXX records from intact peat cores (WP 3)

H3:Sites with greater “depth of collapse” result in greater subsidence and deeper waterlogging leading to higher post-thaw SOC accumulation rates compared to sites with shallow or medium collapse. A deeper collapse creates a thicker water column and requires longer for the peat to “fill the gap”, thus extending the C sink function over decades (Figure 3b).

O4: To quantify potential SOC and DOC mineralization rates, microbial biomass, turnover and carbon use efficiency in undisturbed and thermokarst peatlands (which - shallow, medium, deep?) and explore their relation with organic matter properties (WP 4)

H4: *The potential for SOC and DOC mineralization of deep peat is lower in thermokarst peatlands than in undisturbed peatlands reflecting increased humification following thaw.* Previously-frozen peat in the thermokarst site has undergone substantial decomposition following thaw compared to peat of similar age in the peatland plateau, resulting in lower SOC and DOC mineralization (CO2 and CH4 production and decay constants) and altered SOC and DOC composition (C/N, humification index, aromaticity). Further, we hypothesize lower microbial biomass, microbial turnover and carbon use efficiency in the stronger humified thermokarst peat than in undisturbed peat.

The activities related to each work package are described below and the planned timeline and associated deliverables are shown in Table 1.

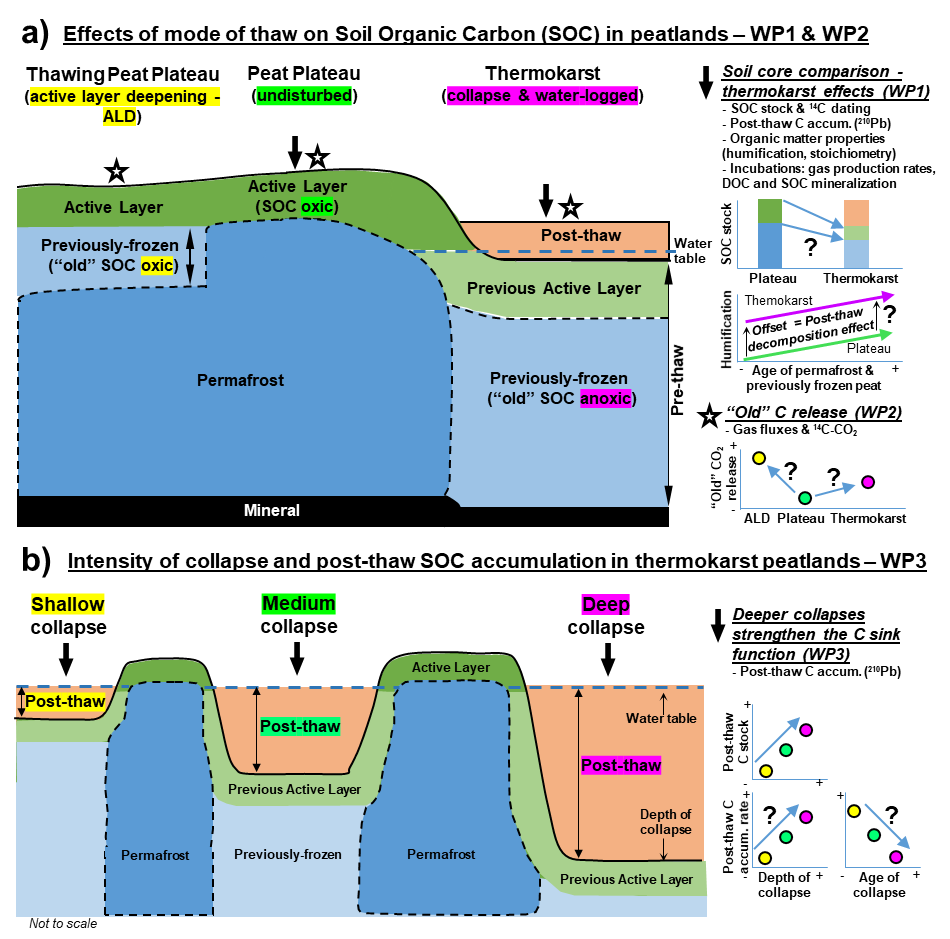


Figure 3 – Different modes of thawing in peat plateau and thermokarst (a) and different intensities of post-thaw in thermokarst (b). Expected changes in SOC stocks, humification and CO2 release and SOC accumulation are addressed in WP1, WP2 and WP3.

## Work programme including proposed research methods

WP1: Effect of thaw on SOC stocks following thermokarst (C Estop Aragonés, U Münster & W Borken, U Bayreuth)

Sampling and soil cores: We will take four soil cores in the peatland plateaus and four soil cores in adjacent thermokarst peatlands down to the organic-mineral transition (total 8) at the Iškoras site. This level of replication adds value regarding site spatial variability in comparison to previous studies that usually investigate one or two cores. In the thermokarst peatland, soil cores will be sampled with a serrated knife near the surface and with a Russian corer (50 cm depth intervals) at deeper depths. Two boreholes will be used for each soil core to ensure stratigraphic continuity and avoid peat compaction. In the peatland plateau, soil core sampling in frozen ground will be done with drilling equipment arranged in situ with Norwegian colleagues. Stratigraphy will be described in situ and samples will be double-bagged, secured in half-cut PVC tubes for transport and stored frozen. Additional surface cores from the same location will be taken to ensure enough material for both 210Pb and macrofossil analysis. Samples will be sectioned at 1 cm resolution as needed and processed for further analysis in Münster. Subsamples will be used to determine bulk density, loss on ignition (LOI 550°C) and C and N content (and δ13C and δ15N) to calculate soil carbon storage. Plant macrofossil abundance will be quantified using the Quadrat and Leaf Count macrofossil analysis technique 58; a starting point may be the identification of Sphagnum, brown moss, sedge, ligneous matter, and ericaceous rootlets. Specific depths based on changes in color/structure of peat stratigraphy (for example basal peat, fen to bog, bog to peat plateau, pre-thaw to post-thaw peat) will be selected for plant macrofossil sampling and sent for 14C analysis. Graphitization will be carried out at the University of Bayreuth (PI Borken) and the 14C analysis at the Keck Carbon Cycle AMS facility at UC Irvine. The upper part of the core will be dried, milled and sent for 210Pb analysis (Dr. Olid). Two cores in the plateau and two in the thermokarst will be used for plant macrofossil analysis and 14C analysis to verify synchronicity of peat development before thaw and be able to compare SOC stocks between sites. The other two cores of each site will be used to compare SOC stocks based on the information of the dated cores assuming a similar stratigraphy. We aim to analyze 10 samples for 14C analysis per core (4 cores x 10 samples/core = 40 samples). Three cores in the peatland plateau and three in the thermokarst site will be prepared and analyzed for 210Pb to obtain age-depth relations for the last 100-150 years. Three cores for the thermokarst site are required for the objectives of WP3 (see below). We estimate 15 samples for 210Pb or 226Ra analysis per core (6 cores x 15 samples/core = 90 samples). 226Ra measurements are required to estimate the background 210Pb activity when the profile does not exhibit an exponential decay in activity to a constant 210Pb value.

Comparison of SOC stocks and recent- and long-term C accumulation rates: We will calculate SOC stocks using data for bulk density, LOI, C content and sampling depth of each core. Comparing total SOC stock between the plateau and the thermokarst does not directly inform about the potential losses of previously-frozen SOC. In order to do this, the total SOC stock of each site needs to be assigned to a defined “peat class” corresponding to explicit time intervals. We plan to quantify SOC stock in each site associated to either post-thaw peat, active layer (or previous active layer) peat and permafrost (or previous permafrost) peat (Figure 3a). Plant macrofossils will be used to improve the classification of peat stratification. For this purpose, the depth and the age of these transitions are obtained using the 210Pb and 14C dating results from each site. The 210Pb dating results will be used to determine the time of thaw (i.e. age of the pre-thaw to post-thaw peat transition in the thermokarst core). The 14C dating results will be used to model the age-depth of the plateau and the thermokarst using the *Bacon* software 59. The plant macrofossil identification and age-depth analysis will benefit from the expertise of Prof. Dr. Mariusz Gałka at the University of Lodz. This chronology will be used to estimate the depth associated to the age of thaw in the plateau core to be able to compare the pre-thaw SOC stocks, which include active layer and permafrost peat classes. The age of the top of the permafrost can be estimated from the measured depth of the active layer in the plateau (measurements of thaw depth in late August) and from changes in peat chemical composition 16 and in plant macrofossil taxa indicators associated to drier conditions such as those from ericaceous species 13 in both sites. The associated depth of this age can then be used in both the plateau and the thermokarst to compare permafrost peat SOC stocks.

Characterization of soil organic matter across chronosequence: Freeze-dried, milled samples will be measured for C and N content, XRF elemental analysis and FTIR spectra to characterize organic matter and evaluate potential differences in peat humification between the plateau and the thermokarst site. Increasing humification with depth (age) is common in peatlands. To determine a potential increase in humification in thermokarst vs plateau, we will compare the relation of humification with age in both sites and test whether humification is different between sites after controlling for age using analysis of covariance. The humification index will be calculated after baseline correction as a ratio of intensity in 1600–1650 cm-1 (aromatics) to intensity in 1030–1080 cm-1 (polysaccharides) regions from FTIR spectra 60.

WP2: Soil respiration fluxes and 14C analysis of respired CO2 in intact and thawing peatlands (C Estop Aragonés, U Münster & W Borken, U Bayreuth)

Approach to estimate “old” SOC respiration: We will quantify at the Iškoras and Áidejávri sites rates of “old” SOC respiration (FOLD, g “old” C m-2 d-1). For this we need to measure the total soil respiration flux (FTOTAL) and quantify the fractional contribution of “old” SOC to the flux (XOLD). This fractional contribution of “old” SOC to the flux (XOLD) will be determined from 14CO2 flux measurements using a collar approach shown in Figure 4 14,20. We will insert normal collars (25 cm inner-diameter) down to 30 cm in the soil (full profile collars). The insertion depth of 30 cm is suitable given the active layer of at least 50 cm at these sites and based on the experience in other sites 14,20. Next to the full profile collars, we will extract 30 cm deep cores, place them into collars with sealed bottoms and reinsert the collar in the pit, thus excluding CO2 produced below 30 cm (sealed bottom collars). The soils will be trenched during collar installation and the vegetation will be carefully clipped to reduce the autotrophic component and maximize the heterotrophic component in the CO2 flux. These installations will take place at least a month before the 14CO2 measurements to minimize disturbance effects and better compare the 14CO2 signature of both collars. Therefore, the 14CO2 will be measured in full profile collars that allow deep soil respiration in the flux (14CO2 FULL) and in collars sealed at the bottom that exclude such deep respiration (14CO2 SURFACE). This approach uses the difference in 14CO2 between both collars to determine the influence of “old” SOC respiration and has been used in previous studies 14,20,35. A lower 14CO2 in full profile collars than in sealed bottom collars (14CO2 FULL < 14CO2 SURFACE) is indicative of SOC sources deeper than 30 cm depleted in 14C (“old” SOC) being respired and contributing to the CO2 flux. The age of the “old” SOC source (14COLD) will be defined from soil core 14C dating results at the base of the active layer based on the maximum thaw depth (thaw probe measurements) in the undisturbed location. The 14COLD will reflect the minimum age of previously-frozen SOC at the sites. The 14COLD can also be standardized across all study sites to compare old C release rates across sites and contrast thaw by active layer deepening vs thermokarst. With these data we will calculate XOLD and FOLD following the isotopic mass balance equations shown in Figure 4.



Figure 4 – Field-deployed collars to quantify the fractional contribution of “old” SOC respiration (XOLD) using 14CO2 from collars that either include (Full profile) or exclude (Sealed bottom) respiration of deep/old sources 14. An isotopic mass balance is applied to determine XOLD, and the rate of old SOC loss (FOLD) is calculated using XOLD and the total respiration CO2 flux (FTOTAL) measured in Full profile collars.

Site selection to contrast the effect of mode of thaw on “old” SOC respiration: We will determine rates of “old” SOC respiration (FOLD) at four sites to contrast different modes of thaw: undisturbed vs disturbed peat plateau (thaw by active layer deepening) and peat plateau vs thermokarst (thaw by thermokarst). We will select i) a site with an active layer depth gradient to compare undisturbed areas with areas thicker active layer (Áidejávri site), and ii) a site with an undisturbed area and an adjacent thermokarst area as in WP1 (Iškoras site). The active layer depth gradient will be established from a spatial survey of manual thaw depth measurements at the site (MSc. student assistance). At each of these areas we will select five locations for fluxes, install one “Full profile” collar and one “Sealed bottom” collar in each location (Figure 4), and sample 14CO2 once in 2024 and once in 2025 at the end of the growing season (4 sites/areas x 5 locations x 2 samples per location x 2 sampling times plus 4 samples for atmospheric air – total 84 14CO2 measurements). The locations will be representative of the dominant site vegetation and sampling will take place at the end of the growing season (late August/early September) when thaw depth and deep soil temperature are near their maximum, conditions that are expected to favor “old” SOC respiration and thus yield maximum estimates of previously-frozen SOC loss during the growing season. The level of replication (n=5 per site) is robust compared to other studies 14,36 and ensures a greater coverage of spatial variability in our estimates of old SOC release.

Installations, flux measurements and 14CO2 sampling: CO2 and CH4 fluxes will be measured in five locations at each site using dark chambers (10-15 L) and portable greenhouse gas analyzers (UGGA Los Gatos Research, Uni Münster or LI-COR LI7810, NTNU) at least 5 times during summer to estimate cumulative growing-season soil respiration. We will measure soil respiration from “Full profile” collars and determine fluxes (FTOTAL) from linear regression of increase in CO2 and CH4 concentrations over time. The locations will be selected and trenched prior flux measurement and 14CO2 sampling to ensure dominance of soil heterotrophic respiration. Air and soil temperature (10, 20 and 40 cm) is currently monitored at the sites by the Norwegian colleagues. We will also deploy soil moisture sensors (ECH2O EC-5) connected to data loggers at 10 and 25 cm depth in the peat plateaus and additional temperature sensors (TMC20-HD) at the thermokarst sites to obtain information about the depth of freezing during winter and seasonal dynamics. Additional temperature measurements during gas flux measurements will be performed in each collar with a handheld temperature probe. For 14CO2 sampling in August/early September in 2024 and 2025 (late), we will enclose chambers and remove atmospheric CO2 from the headspace with a CO2 trap (soda lime) by circulating the headspace volume for about 20-30 min using pumps at a rate of ⁓1 L min-1 with 9V block batteries. Thereafter, respired CO2 will accumulate until headspace concentrations are ⁓1500 ppm, enough for 14C analysis. The accumulated CO2 in the chamber will be withdrawn and stored in specific stainless steel flasks (1.5 L, two cocks) arranged at the University of Bayreuth. Atmospheric CO2 samples will be collected for 14C analysis to correct the 14CO2 measurements from the collars following common procedures 14,27. Once returned to Germany, gas samples will be extracted from flasks with digital mass flow controller, purified and then converted to graphite targets using the zinc reduction method at the University of Bayreuth 55. Graphite targets will be analyzed for 14C by the Keck Carbon Cycle AMS facility at UC Irvine.

WP3: Survey of thermokarst peatlands – Relation of intensity of collapse and post-thaw C accumulation rates (C Estop Aragonés, U Münster)

During 2024 and 2025, we will visit and select five waterlogged thermokarst peatland sites with different collapse intensity to quantify post-thaw C accumulation rates and explore their variability (Figure 3b). We will aim to maximize the variability of depths of collapse across sites to cover the greatest possible range of post-thaw C accumulation rates. Access to sites will require deploying boardwalks to minimize disturbance during soil core sampling. At each site, three soil cores will be sampled down to 1 meter to recover peat stratigraphy including at least the peat accumulated post-thaw and its transition to pre-thaw peat. The depth of collapse in the thermokarst peatlands will be determined by measuring the distance from the top to the transition between plateau and post-thaw peat in each core. We will select sites where soil surface is waterlogged and monitor the water table position during summer in 2024 and 2025 by installing wells with water table loggers (5 needed in total). The water table position will be necessary to correct the depth of collapse in case some cores aggraded peat above the water table. Cores will be sampled and stored as described in WP1. Cores will be shipped to Münster where will be sectioned to 1 cm, processed for bulk density, freeze-dried and milled for further analysis of C and N, and of 210Pb. Samples for 210Pb or 226Ra analysis will be shipped to Dr. Olid. In addition to the site from WP1, we will select 4 more thermokarst sites (4 sites x 3 cores/site x 15 210Pb samples/core = 180 210Pb samples) making a total of five sites for WP3 data analysis. The age-depth relations obtained from 210Pb analysis will be used to determine the age of the collapse (time of thaw). Together with the C stock quantified, we will determine post-thaw C accumulation rates across sites. The relation between post-thaw C accumulation rate and depth of collapse will be explored through regression analysis.

WP4: Potential SOC and DOC mineralization rates (C Estop Aragonés, U Münster)

We will perform incubation experiments to assess site differences in mineralization and solid/dissolved organic matter properties (humification indices, stoichiometry, and aromaticity):

SOC mineralization (CO2 and CH4 production rates and decay constant): CO2 and CH4 production rates will be determined in oxic and anoxic (N2 flushed) incubations at 15 °C in at least five selected depths from permafrost and previously-frozen SOC in the peatland plateau and the thermokarst peatland cores, respectively. Production rates will be monitored during one year and measured at least monthly to determine if gas production rates are lower in the thermokarst site, a potential consequence of the prolonged decomposition in peat of that site following thaw. We will prepare three replicates for each depth (4 depths x 3 replicates x 2 sites x 2 treatments x 14 measurement times – total 672 measurements). Headspace CO2 and CH4 concentrations will be measured in a gas chromatograph SRI 8610. Production rates will account for gaseous and dissolved phase with temperature corrected Henry’s constant. Rates will be standardized per gram of soil carbon to compare organic matter lability between sites. With this dataset, SOC decay constant can be determined using a first-order double exponential decay model 61 to obtain parameters (decay constants and pool sizes) for the labile (Lab) and recalcitrant (Rec) SOC fractions: SOC (t) = SOCLab e -k1 t + SOCRec e -k2 t.

Microbial biomass, microbial turnover time and carbon use efficiency: Microbial growth rates are measured based on 18O incorporation from water into genomic DNA 49. For this, H218O (97.0 atom%, Campro Scientific, Germany) and Milli-Q water is added to samples to gravimetric water contents. Before adding labelled water, vials with either flushed with synthetic air or N2 to adjust oxic or anoxic conditions. Then, all samples are incubated for 24 h at 10°C. At the end of the incubation, CO2 and CH4 concentrations in the headspace are measured using a GC. The incubation experiment is stopped by shock freezing in liquid N2 and samples are then ground using a vibration mill. Total DNA will be extracted with FastDNA™ SPIN Kit for Soil (MP Biomedicals, Germany). DNA concentrations are quantified by the Picogreen fluorescence assay using a microplate spectrophotometer (Infinite M200, Tecan, Austria)*.* Aliquots of DNA extracts are pipetted into silver capsules and dried at 60°C. Subsequently, the 18O:16O isotope ratio of DNA is analyzed using a Thermochemical Elemental Analyzer coupled to an IRMS. Based on the 18O abundance in DNA we calculate the amount of DNA that is produced during the 24 h incubation. In the next step, we calculate the microbial growth rate (microbial C g-1 h-1) using a conversion factor which describes the relationship between microbial biomass C and microbial DNA content 50. Microbial biomass is determined by the fumigation extraction method 51. For this purpose, organic C is extracted in 0.5 M K2SO4 from fumigated and non-fumigated soil samples, and is analyzed using a TOC analyzer. Microbial biomass is calculated with a conversion factor of 2.22 52. Microbial C uptake is calculated as the sum of CO2 and CH4 production and C invested into microbial growth. Microbial CUE is then calculated as the ratio of C allocated to growth over microbial C uptake 40 and microbial turnover time is calculated as the ratio of microbial biomass C over microbial growth (further details in 49). In total, 48 samples, i.e., aliquots of peat cores: 4 depths x 3 replicates x 2 sites x 2 treatments will be analyzed.

DOC mineralization through microbial activity: We will perform peat leaching experiments to assess dissolved organic matter (DOM) mineralization differences between permafrost (peatland plateau) and previously-frozen SOC (thermokarst). The same depths as for the SOC incubations will be leached in a 1:5 mass ratio (wet peat:distilled water) for 20 min 62, filtered through pre-combusted glass filters (GF/F Whatmann) and stored at dark at 15 °C to determine biomineralization after 7 and 28 days under oxic and anoxic conditions (5 depths x 3 replicates x 2 sites x 2 treatments x 2 measurement times (day 7 and 28) – total 120 samples). Bacterial inoculum will be added (5% v) and samples will be filtered again in pre-combusted glass filters previous analysis. We will determine DOC and total dissolved nitrogen concentrations and DOM absorption spectra across 200-800 nm using photometry (UV-VIS Agilent Cary 100 E) to calculate aromaticity indices SUVA254 and spectral slope ratios 63. Due to short incubation time, DOC decay constant (k) will be calculated by fitting a two-pool model 64 as DOC (t) = DOCLab e -kt + DOCRec, which only provides a decay constant but provides informative parameters of the labile and recalcitrant DOC fractions (DOCLab and DOCRec, respectively).

Table 1 - Time table of the research program



## Handling of research data

Data gathered in this project will consist of a comprehensive suite of properties from soil cores including humification indices, stoichiometry and radiocarbon and 210Pb dates as well as field greenhouse gas flux measurements and associated radiocarbon dates from sites in Norway, which represents novel data. This data is likely of high interest considering the very limited available information in this region. All results will be published in peer-reviewed journals, preferentially in Open-Access Journals supported by the DFG Open-Access Publishing Program. Relevant original data will be made available alongside the articles as supporting electronic material. We will add our radiocarbon measurements to the International Soil Radiocarbon Database (ISRaD - https://soilradiocarbon.org/), which is an ongoing effort that allows compiling radiocarbon dates for both soils and gases. The age-depth relations from the sampled thermokarst peatlands generated in our project from 210Pb dating provide useful information to assess and upscale the effects of permafrost thaw at larger scales. To this end, we will generate freely accessible datasets through platforms such as zenodo (https://zenodo.org/), PANGAEA (https://www.pangaea.de/) so that this information can be reused in other research efforts.

## Relevance of sex, gender and/or diversity

The members for this project will be solely selected based on the criteria of scientific excellence. We are committed to increase the number of female researchers, the career qualifications of early-career female researchers and make research more family friendly (see section 5.8).

1. Project- and subject-related list of publications

*Works cited from sections 1 and 2, both by the applicant(s) and by third parties. Please include DOI/URL if available. A maximum of ten of your own works cited may be highlighted; font at least Arial 9 pt.*

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