Utilization of ancient permafrost carbon in headwaters of Arctic fluvial networks

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Northern high-latitude rivers are major conduits of carbon from land to coastal seas and the Arctic Ocean. Arctic warming is promoting terrestrial permafrost thaw and shifting hydrologic flowpaths, leading to fluvial mobilization of ancient carbon stores. Here we describe 14C and 13C characteristics of dissolved organic carbon from fluvial networks across the Kolyma River Basin (Siberia), and isotopic changes during bioincubation experiments. Microbial communities utilized ancient carbon (11,300 to >50,000 14C years) in permafrost thaw waters and millennial-aged carbon (up to 10,000 14C years) across headwater streams. Microbial demand was supported by progressively younger (14C-enriched) carbon downstream through the network, with predominantly modern carbon pools subsidizing microorganisms in large rivers and main-stem waters. Permafrost acts as a significant and preferentially degradable source of bioavailable carbon in Arctic freshwaters, which is likely to increase as permafrost thaw intensifies causing positive climate feedbacks in response to on-going climate change.
Climate-induced Arctic warming has led to increased soil temperatures, causing a succession of changes associated with permafrost degradation and widespread ground collapse or thermokarst, including deepening of the seasonally thawed surface active layer\(^1,2\) and alterations to watershed hydrology\(^3,4\). These changes threaten to destabilize ancient (Pleistocene-aged) northern circumpolar terrestrial permafrost, which contains vast quantities of organic carbon (OC) comprising as much as 50% of global below-ground soil carbon stocks\(^5\). Consequently, soil OC losses of up to 220 PgC by 2100 are predicted across Arctic regions\(^6,7\), leading to large-scale mobilization of permafrost-derived OC into Arctic inland and coastal waters\(^8\).

The great majority of OC (>80%)\(^9\) supplied to coastal seas from Arctic rivers is in the form of dissolved OC (DOC) and thus readily available to support microbial carbon demand during transit through fluvial networks\(^10\). Growing evidence exists from temperate and sub-tropical regions\(^11–13\) that older aged terrestrial carbon can be an important subsidy to microbial communities, yet the processing and fate of ancient Arctic permafrost OC throughout fluvial networks is currently unknown. Understanding the response of aquatic systems to future increases in permafrost-derived DOC is necessary for determining its effects upon global carbon cycling and predicting its export via high-latitude rivers.

Studies from major Arctic rivers show that higher-order fluvial systems contain predominantly young (\(^14\)C-enriched) DOC\(^14–17\), suggesting that limited mobilization of permafrost has occurred to date or that it has been removed upstream. A number of studies focusing on lower-order and headwater streams provide evidence for ancient DOC mobilization\(^18,19\). Elucidating the fate of permafrost-derived DOC is crucial for establishing if on-going thaw is currently impacting Arctic fluvial networks and in determining whether its turnover may result in a positive carbon feedback to climate change\(^7\).

Here, we examine the quantity, age and source of the OC present, and constrain the nature of OC supporting microbial carbon demand with increasing water residence time through fluvial networks (higher order in the stream network). We base this work in the Kolyma River Basin, Siberia, which represents the largest global watershed (ca. 650,000 km\(^2\)) completely underlain by continuous permafrost. The majority of this permafrost is comprised of frozen Yedoma, organic-rich (1–5% carbon by mass) Pleistocene-aged deposits that can contain 50–90% ice content\(^2,20\). Yedoma–ice complexes measured in the Kolyma Basin suggest that permafrost carbon by mass concentrations are on the order of 1.5 ± 1.4%, with an average carbon inventory amounting to 14 ± 8 kg m\(^{-3}\) (refs 21–23). Thawing Yedoma-derived OC is thought to be highly susceptible to biological degradation in fluvial networks\(^19,24\), and has been proposed as a key feedback upon global climate\(^20\).

We first assess the initial age and bioavailability of DOC in streams and rivers throughout the basin using measurements of biologically degradable DOC (using DOC loss) and \(^14\)C-DOC measurements. We then determine the source and age of DOC subsidizing microbial demand throughout the network using simultaneous measurements of stable and radiocarbon signatures of DOC (\(^13\)C and \(^14\)C) during short-term (28 days) bioincubation experiments. We show that a preferential loss of permafrost-derived DOC occurs with increasing water residence and inland water transit time, despite similar initially modern DOC ages. Our results demonstrate that microbial communities selectively remove ancient OC during water transit throughout the fluvial network, with a potential to rapidly act as a positive feedback upon climate change with increasing permafrost thaw.

### Results

**Initial \(^14\)C-DOC age and proportion bioavailable DOC.** We conducted 81 individual incubation experiments on samples from 19 streams and rivers throughout the Kolyma fluvial network, grouping by stream size and type (Fig. 1; Supplementary Table 1). Thaw streams, directly draining Yedoma outcrops, contained extremely high concentrations of DOC (10,939 ± 1,278 μM) that are highly depleted in \(^14\)C (Δ\(^14\)C = 883 ± 41‰; Fig. 2a). A large proportion of thaw stream DOC was utilized by microorganisms.
indicating that Pleistocene-age OC (11,300 to 20,000 years) was similar to that found in bioincubations. DOC loss during short-term (28 days, 20 °C) incubations (47 ± 8%), confirming the high bioavailability of permafrost-derived carbon in fluvial systems19,24 (Table 1; Supplementary Table 2). Mean DOC concentrations declined moving downstream through the fluvial network, yet proportions of DOC loss were similar (15–21%) among all other stream and river waters (analysis of variance P > 0.05, Table 1; Supplementary Table 2). Erosion-impacted streams that receive greater amounts of permafrost soil inputs via mechanical erosion of the stream banks contain 14C-depleted DOC (−214 ± 145‰; Fig. 2a), highlighting the strong potential for thermokarst processes to sporadically deliver aged OC to fluvial networks. By contrast, all other stream and river waters contained modern initial bulk DOC pools (Δ14C > −50‰; Fig. 2a), consistent with the majority of available radiocarbon data for DOC from major Arctic Rivers14,15,18.

Radiocarbon age of DOC supporting microbial demand. Microbial communities in Yedoma thaw stream waters consistently utilized highly 14C-depleted DOC (−750 to −1,000‰), indicating that Pleistocene-age OC (11,300 to > 50,000 14C years) is intrinsically biologically labile and immediately available for microbial metabolism after mobilization to freshwaters (Fig. 2b).

Microbial demand in erosion-impacted streams was also supported by highly aged DOC (Δ14C = −405 ± 185‰, Table 1), demonstrating that microbial communities in waters containing mixed modern and ancient OC pools can preferentially utilize an older OC fraction (up to 10,000 14C years). Furthermore, despite similar modern initial bulk DOC ages among other stream and river waters, Δ14C values of the DOC utilized by microorganisms across these sites (that is, the fraction consumed during bioincubations) varied considerably (Δ14C = +300 to −630‰; Fig. 2b), indicating a disconnect between bulk DOC age and the age of DOC fuelling microbial demand11,13. Modern (14C-enriched) DOC periodically utilized by microorganisms through the river network may have been delivered from soil pore waters that can contain high values of Δ14C-DOC25 or may be caused by the upstream preferential loss of aged OC.

Source and radiocarbon age of DOC supporting microorganisms. We used a dual-isotope approach26 to examine the source and age of OC subsidizing DOCloss throughout the stream network. We partitioned source contributions that most likely explained utilized Δ14C and δ13C values of DOCloss assuming three dominant sources of DOC (contemporary terrestrial OC, permafrost-derived terrestrial OC and internally produced in situ OC) (Fig. 3a).

Figure 2 | Downstream Δ14C DOC values. Δ14C values of (a) initial DOC, and (b) utilized DOC supporting microbial demand (DOCloss) during bioincubations. Δ14C values of utilized DOC were calculated as outlined in equation (1) (see Methods). Table 1 displays mean values for initial and utilized DOC across sites and sample numbers for each site type.

| Table 1 | DOC and isotope characteristics throughout the fluvial network. |
|---------------------------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|
| n | Initial DOC (µM) | SUVA254 (lmg−1m−1) | DOCloss (%) | Init. Δ14C (‰) | OCloss (%) | Mean OCloss age (14C years) | Permafrost (%) | Contemporary (%) | In situ (%) |
|---------------------------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|
| Yedoma thaw | 5 | 10,939 ± 2,728 | 1.63 ± 0.19 | 47.2 ± 7.6 | −884 ± 41 | 16,576 | 97.2 ± 0.8 | 1.7 ± 0.5 | 1.1 ± 0.5 |
| Erosion streams | 3 | 2,503 ± 518 | 3.25 ± 0.44 | 17.5 ± 4.9 | −214 ± 145 | 4,171 | 42.8 ± 20.7 | 56.4 ± 20.7 | 0.8 ± 0.4 |
| Streams | 25 | 1,691 ± 87.6 | 3.44 ± 0.11 | 13.3 ± 2.0 | 51 ± 6 | 170 | 13.0 ± 4.0 | 85.9 ± 4.0 | 1.1 ± 0.2 |
| Minor tributaries | 7 | 766 ± 110 | 3.56 ± 0.19 | 21.0 ± 5.5 | 25 ± 13 | 26 ± 88 | Modern | 5.7 ± 3.5 | 93.7 ± 3.6 | 0.6 ± 0.2 |
| Major tributaries | 8 | 511 ± 68 | 4.03 ± 0.08 | 12.2 ± 1.8 | 40 ± 3 | 68 ± 32 | Modern | 0.6 ± 0.1 | 73.1 ± 8.4 | 26.3 ± 9.9 |
| Main stem | 6 | 425 ± 46 | 3.67 ± 0.10 | 14.6 ± 1.8 | 22 ± 9 | 74 ± 42 | Modern | 0.7 ± 0.1 | 88.6 ± 2.8 | 10.7 ± 4.4 |

DOC, dissolved organic carbon; OC, organic carbon.

Mean initial DOC concentrations; specific ultraviolet absorbance at 254 nm (SUVA254). Percent DOC loss over 28 days (DOCloss) calculated Δ14C value and mean radiocarbon age of DOCloss (using equation (1) in Methods). The sample size at each site type is provided (n) and individual data are detailed in Supplementary Table 2. Mean percentage (± s.e.m.) contribution of permafrost, contemporary and in situ-derived DOC to DOCloss determined using the dual-carbon isotope mixing model.
Microbial demand in Yedoma thaw waters was almost entirely (97.2 ± 0.8%) supported by permafrost-derived OC, demonstrating that ancient DOC was highly bioavailable when present. Downstream, permafrost subsidized greater proportions of DOCloss in higher-order erosion-impacted (42.8 ± 20.7%) and other small streams (13.0 ± 4.0%) as compared with minor tributary (5.7 ± 3.5%), major tributary (0.6 ± 0.1%) and mainstem waters (0.7 ± 0.1%) (Table 1). This landscape pattern of declining permafrost contribution to DOCloss through the fluvial network proved consistent across both years of investigation (2012 and 2013), showing that microbial demand in thaw streams and headwaters is consistently subsidized by significant proportions of permafrost OC. The fact that DOC character and age altered with increasing water residence time does not simply reflect catchment variability, as evidenced by identical patterns of DOC modernization in sub-basins within the larger Kolyma watershed. Furthermore, most sites drained similar soil and vegetation types, and spanned little elevation change within the Kolyma lowlands. Utilized Δ14C of DOCloss increased exponentially (r² = 0.72, P < 0.001, Supplementary Fig. 1) relative to initial Δ14C values, suggesting that older OC fractions were rapidly and preferentially mineralized when present in bulk DOC (initial Δ14C = –20%). Terrestrially derived contemporary OC contributed towards a minor proportion of DOCloss in thaw waters (1.7 ± 0.5%), but comprised the main OC source (>55%; Table 1) across stream and river waters, emphasizing the dominance of surface soil–water dynamics in determining bulk DOC character and thus biochemical processing and fate (Fig. 3b).

Environmental and compositional effects on 14C utilization. Seasonal and spatial trends in OC bioavailability across fluvial networks can be controlled by variations in water temperature, inorganic nutrient availability and OC character. To examine whether downstream variations in water temperature and inorganic nutrient concentrations influenced the fraction and age of DOC utilized, we conducted parallel experiments incubating waters at 4 °C and under nutrient-replete conditions. Total DOCloss was lower at colder incubation temperatures (mean Q10 = 1.21 ± 0.07), but was not significantly different with respect to the mean Δ14C value of DOCloss (paired t-test, P > 0.1; Fig. 4a). Bioenergetic constraints from lower water temperatures therefore did not consistently alter the DOC age or fraction utilized, implying that selective DOC loss continues during colder fall and winter periods, when greater contributions of older DOC could be expected. Similarly, inorganic nutrient concentration did not significantly influence Δ14C values of DOCloss (paired t-test, P > 0.1; Fig. 4b), indicating that stoichiometric differences cannot account for the observed downstream trends. Individual water samples displaying differences in the age of DOC utilized at colder temperatures, or under nutrient-replete conditions, all resulted in the loss of an older (14C-depleted) DOC fraction. This implies that our results represent conservative estimates of permafrost OC loss along the aquatic continuum under our experimental conditions (Fig. 3a,b).

Discussion
Together, our findings indicate that terrestrial permafrost OC export is already underway and actively contributing to DOC turnover in Arctic fluvial networks. The presence of permafrost OC has previously gone undetected in fluvial networks due to the preferential loss of permafrost OC removing headwater signatures dominated by permafrost inputs, combined with the historical dominance of field sampling on larger main-stem rivers. In addition, the persistence of modern DOC sourced from vegetation and surface soils causes permafrost OC dynamics to be effectively masked when examining bulk initial OC alone.

The composition of DOC in Arctic stream and river networks is expected to shift under future climate change scenarios. Permafrost thaw is promoting greater groundwater and subsurface flow from Arctic and sub-arctic watersheds, likely resulting in the export of DOC characterized by lower-molecular weight compounds and a lower degree of aromaticity. Across our sites, we observed a linear increase in the proportion of bioavailable carbon (DOCbio) with decreasing DOC aromaticity as evidenced by lower SUVA254 values (r² = 0.50, P < 0.001; Supplementary Fig. 2), suggesting that increasing future permafrost contributions may promote enhanced microbial metabolism within Arctic fluvial networks. Furthermore, the microbial utilization of older DOC fractions was observed with decreasing OC aromaticity (Fig. 4c) and increasing DOCloss (r² = 0.37, P < 0.001), suggesting that DOC compositional changes will result in greater ancient carbon turnover. Heterotrophic metabolism and respiration is dependent on low-molecular weight DOC from thawing permafrost soils. The lower aromatic content and molecular weight of...
permafrost turnover in northern high-latitude systems\textsuperscript{10,12,41}. Photodegradation has been shown to influence surface water DOC processing in the Arctic\textsuperscript{37}, yet it is currently unclear how sunlight affects the bulk age of DOC. Recognizing that modern bulk DOC signatures in Arctic rivers may disable a rapidly cycling old C fraction, future efforts to detect the impacts of permafrost thaw should focus on headwater systems where the rapid removal and preferential biological utilization of ancient permafrost-derived DOC appears prevalent. As permafrost thaw accelerates\textsuperscript{13-18}, it seems apparent that this will be accompanied by an increase in the amount of bioavailable DOC in aquatic networks, with aged DOC accounting for increased subsidies to aquatic microorganisms ultimately fuelling a positive feedback on climate.

**Methods**

**Study area and sample collection.** Surface water samples were collected directly from streams and rivers on several expeditions in 2012 and 2013 (Supplementary Table 1). Sampling concentrated during the months of August to October, when surface active layer of permafrost and thus maximum export of permafrost-derived OC to inland waters is expected. Additional sampling soon after the spring ice-out period (June) provided insights during a period of minimal surface active layer thaw. We grouped sites by stream width, separating streams (< 1-5 m), minor (> 5 to ≤ 200 m) and major tributaries (> 200 m), and the Kolyma main stem (Fig. 1). A small proportion of highly turbid (> 100 nephelometric turbidity units (NTU’s)) upland streams visibly affected by active mechanical soil erosion were examined independently (erosion streams) from unaffected streams (< 100 NTU), thus providing more robust source determination\textsuperscript{28} (see the Isotope mixing model section). These streams were always small (< 3 m width) and situated within active thermokarst zones with mixed vegetation covered and exposed yedoma banks.

Stream temperature was measured in the field (YSI multi-meter) and sample turbidity immediately upon return to the laboratory (Hach 2100Q). Waters were filtered through pre-combusted glass fibre filters (0.7 μm) on the day of collection under a combusted glass filtration system. After filtration, waters were immediately transferred into pre-combusted amber glass vials (40 ml vials, ~35 ml water) leaving a headspace. Triplicate aliquots for initial DOC concentration were immediately acidified (trace element grade HCl, ≤ pH 2) and stored until analysis. Aliquots for δ\textsuperscript{13}C and δ\textsuperscript{14}C were immediately stored frozen (−20 °C) in pre-leached, acid-cleaned HDPE bottles.

**DOC and bioincubations.** DOC was calculated as the mean of between three and five injections on a Shimadzu TOC-V where the coefficient of variance across measurements was < 2%. Triplicate bioincubations: DOC measurements were typically within <5% of each other. The bioavailability of DOC (DOC\textsubscript{bio}) was determined as the difference in triplicate measures of DOC before and after a 28-day laboratory incubation at ± 0.5 °C or 4 ± 1 °C containing the indigenous microbial community from each site. The absorbance of water at 254 nm was measured (Shimadzu UV-1800) and the specific ultraviolet absorbance (SUVA\textsubscript{254}) determined as a proxy for aromaticity\textsuperscript{34}. After bioincubation, waters were refiltered as above, and samples collected as before for DOC, δ\textsuperscript{13}C and δ\textsuperscript{14}C analyses. Parallel bioavailability incubations conducted with nutrient-amended waters were supplemented with inorganic nitrate and phosphate (KNO\textsubscript{3}/KH\textsubscript{2}PO\textsubscript{4}) according to Redfield ratio, resulting in nutrient-replete incubations in relation to ambient site nutrient concentrations. We used analysis of variance to test between site means and paired t-tests to compare parallel incubations results. All tests using Univariate regression and exponential fits were conducted in SPSS21 (IBM).

**Stable and radiocarbon isotope analyses.** δ\textsuperscript{13}C analyses were conducted at the University of California, Davis Stable Isotope Facility and δ\textsuperscript{14}C analyses at the Eidgenössische Technische Hochschule (ETH) Zürich. δ\textsuperscript{13}C-DOC samples were analysed using an O.I. Analytical Model 1010 TC/TOC analyzer (precision of ± 0.2%) interfaced to a PDZ Europa 20–20 IRMS (Sercon Ltd). 13C-DOC measurements were calibrated against the 13C values of KHP and IHSS Suwannee River humic acid in Milli-Q. Waters for 13C-DOC analyses were freeze-dried (Christ Alpha 2-4, LSC with a low-carbon vacuum hybrid pump, Vacubrand RC-6; Martin Christ, Labex Instrument AB, Sweden) directly in pre-combusted (850 °C) quartz tubes. Samples were acidified under HCl vapours to remove carbonates and flame sealed with pre-combusted CuO under vacuum. CO\textsubscript{2} was cryogenically captured and quantified (~ 30 μg carbon) before measurement using a microscale radio-carbon dating system (MICADAS; http://www.ams.ethz.ch/instruments/micadas) and gas-feeding system\textsuperscript{42-43}. Combusted NIST SRM 4990C oxalic acid was used as a standard for normalization, and blanks were determined using radiocarbon-free CO\textsubscript{2} both at a concentration of 5% CO\textsubscript{2} in He. The modern oxalic acid standard was measured to better than 1% relative error and until samples were fully consumed.
Radiocarbon contents are reported as Δ13C (%) and 14C age. All radiocarbon values are corrected for procedural blanks with the errors propagated. The isotopic signature (Δ13C and Δ14C) of the utilized DOC fraction (DOCfinal) was calculated using triplicate measurements of initial DOC (DOCinit) and final DOC concentrations (DOCfinal) along the associated change in isotopic composition using simple mass balance (equation (1)). Individual errors associated with multiple isotopic measurements were propagated to assess error on Δ3C and Δ14C values:

\[
\text{DOCfinal} = C_{\text{initial}} \times \Delta \text{isotope}_{\text{initial}} / \text{DOCinit} - \text{DOCfinal} \times \Delta \text{isotope}_{\text{final}} / \text{DOCinit}
\]

(1)

Isotopic mixing model. Feasible contributions of three end-member sources (permafrost DOC, contemporary DOC and in situ autochthonous DOC) to DOCfinal at sites were calculated using a dual-isotope mixing model solved using a Monte Carlo simulation approach. Monte Carlo simulations were conducted using the MIXSIAR package (https://github.com/briannock/MIXSIAR/releases) within the R programming environment (http://www.R-project.org). MixSIAR is a Bayesian mixing model44 that allows the contributions of source end-members to isotope data to be estimated, taking into account the uncertainty in source values45 and model error46 (residual and process errors). A dual-isotope approach (14C/13C) was used to increase the accuracy of contribution estimates and to solve for three potential end-member sources.

We defined two separate terrestrial end-members to differentiate between contemporary OC pools (inorganic soil and surface active layer) and permafrost-derived OC that is delivered to freshwaters via ground collapse, or thermokarst processes, bank erosion or deep groundwater flow. An in situ-derived DOC source from aquatic primary producers (for example, phytoplankton algae) was also included. Estimated error values of each DOC source were determined as outlined below with final mean values and ranges defined as follows: permafrost-derived 13C value of the end-member value in the mixing model.

Concentrations (DOCfinal) alongside the associated change in isotopic composition (Δ13C and Δ14C) of algae from DIC and of epilithic algae from across 70 different streams and rivers spanning arctic and temperate environments52. This linear relationship was derived from direct measurements of herbivore δ13C and D13C values52 and demonstrated that a consistent fractionation (for example, 19 or 20%) assumed fixed photosynthetic fractionation did not accurately constrain δ13C of algae from DIC measurements in lotic systems. This resulted in a relative fractionation for this study ranging from 13.1 to 15.5%. Our calculated δ13C for algae closely align with values measured in a single plankton bloom on the Punteldikha River within the Kolyma Basin of ~23.3% (ref. 53) and of the end-members calculated for marine OC in Vonk et al.49 of ~24%. Both values lie within our end-member estimates of the model discrimination factor of up to 1% has been applied for δ14C fractionation. Phyttoplankton should reflect the current δ13C of DIC in waters. A 13C of DIC from the Kolyma River ranged from –77.0 to –79.0% (Raymond et al., unpublished), and was therefore adopted as the mean end-member value in the mixing model.

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Author contributions

R.G.M.S., R.M.H. and T.I.E. conceived the study. Sample collection and incubation set-up was conducted by P.J.M., R.G.M.S., N.Z., A.D. and J.E.V. Radiocarbon analyses of samples was conducted by P.J.M. with assistance from T.I.E. and C.P.M. The manuscript was written by P.J.M. and R.G.M.S. with assistance and comments from T.I.E., C.P.M., N.Z., A.D., R.M.H. and J.E.V.

Additional information

**Supplementary Information** accompanies this paper at http://www.nature.com/naturecommunications

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