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RESEARCH

Recent Changes in the Use of Phototrophy by a Mixotrophic Testate Amoeba Inferred from δ^{13}C Measurements from an Arctic Peat Core

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Abstract
High-latitude ecosystems are undergoing rapid ecological changes in response to climate warming. While some changes are well studied, the responses of microbial communities remain less understood. Testate amoebae, shell-producing protists well preserved in peat, provide a means to reconstruct past microbial dynamics. Mixotrophic taxa such as *Archerella flavum* host algal endosymbionts (zoochlorellae), allowing both heterotrophic and phototrophic energy acquisition. Previous work has demonstrated that these pathways result in different δ^{13}C values. We applied a novel stable isotope approach to a peat core from the North Slope of Alaska to reconstruct changes in phototrophy by *Archerella flavum*. δ^{13}C values were measured on *Archerella flavum* tests (i.e. shells) and *Sphagnum*, and a two-endmember mixing model was used to estimate relative usage of phototrophy through time. δ^{13}C values were compared with testate amoeba community composition, test size, vegetation, and historical climate. *Archerella flavum* δ^{13}C values were consistently more positive than *Sphagnum* δ^{13}C values in the peat core, and patterns indicated greater phototrophy use after the late 1980s CE. This shift was followed by expansion of *Archerella flavum* populations and a trend of decreasing test length in several testate amoeba taxa. Increased phototrophy was associated with higher peat C:N ratios, indicating more oligotrophic conditions. From 2007 to 2019 CE, the length of the snow-free growing season was correlated with estimates of phototrophy usage, with more phototrophy during longer growing seasons. δ^{13}C analyses of mixotrophic testate amoebae are a powerful tool for reconstructing microbial nutritional strategies and responses to past environmental change.

Keywords Mixotrophy · Paleoecology · Stable isotope analysis · Testate amoebae

Introduction

High-latitude ecosystems are undergoing significant ecological transformation in response to rapid climate warming. Across the Arctic and boreal regions, shifts in vegetation composition, including shrub expansion and peatland development [1–3], along with changes in hydrology, permafrost stability, and wildfire activity, are altering landscape structure and ecosystem processes. While much research has focused on understanding how these changes may influence carbon balance [1, 3], the response of microbial communities remains less understood. Testate amoebae, a polyphyletic group of shell-producing protists, are particularly well suited to examining peatland microbial community responses to past and ongoing environmental changes because their decay-resistant shells are generally well preserved in peat stratigraphy [4–6]. For example, the expansion of *Sphagnum*-dominated vegetation on the North Slope of Alaska over the last several decades has been linked to significant changes in testate amoeba communities recorded in peat cores, and these changes likely reflect broader changes in microbial food webs [3].

Testate amoebae are key components of microbial food webs in peatland ecosystems, comprising a significant portion of the microbial biomass and playing important roles in nutrient cycling, decomposition, and energy flow [4, 7, 8]. Their function in microbial food webs is complex, because some taxa are heterotrophic while others are mixotrophic, harboring endosymbiotic algae (zoochlorellae) that enable

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them to utilize both heterotrophy and phototrophy [9]. Mixotrophs such as *Archerella flavum*, *Hyalosphenia papilio*, and *Heleopera sphagni* are common in nutrient-poor, *Sphagnum*-dominated habitats [10, 11], often outnumbering heterotrophic species under stable, open bog conditions [12, 13]. Experimental results have shown that mixotroph abundance decreases without light [8, 14]; however, partial shading experiments suggest that relationships between mixotroph abundance and light availability are complicated [15]. Furthermore, little is known regarding the relative utilization of heterotrophy and phototrophy by mixotrophic testate amoebae, and whether they vary their use of energetic strategies in response to environmental conditions.

Stable isotope analyses are powerful tools for understanding microbial food web dynamics. For example, $\delta^{13}C$ and $\delta^{15}N$ measurements on microorganisms inhabiting the water film of *Sphagnum* have been used to uncover feeding relationships [13, 16, 17]. Herbert et al. [15] compiled available $\delta^{13}C$ data from various components of the *Sphagnum*-associated microbial food web, and showed that heterotrophic organisms living in *Sphagnum*, including heterotrophic testate amoebae, nematodes, copepods, rotifers, and bacteria, all generally have $\delta^{13}C$ values similar to *Sphagnum*, ranging from about −24 to −30‰. Mixotrophic testate amoebae, on the other hand, have more positive $\delta^{13}C$ values than their heterotrophic food sources and *Sphagnum*. This suggests that they use an energy source with higher $\delta^{13}C$ values, consistent with use of their algal symbiont (*Chlorella*) [13, 15]. High algal $\delta^{13}C$ values occur because of carbon-concentrating mechanisms utilized by *Chlorella*, which reduce the fractionation of carbon isotopes by the RUBISCO enzyme [15]. Given that the $\delta^{13}C$ values of mixotrophic testate amoebae likely reflect the relative usage of their phototrophic and heterotrophic nutritional strategies, such values provide a potential tool to examine past changes in the relative use of phototrophy in paleoecological studies.

In this study, we introduce a novel approach to estimate past changes in the relative use of phototrophy and heterotrophy by a mixotrophic testate amoeba by analyzing the $\delta^{13}C$ values of *Archerella flavum* and *Sphagnum* along the length of a peat core from the North Slope of Alaska. We then assessed how changes in phototrophy utilization were associated with (1) broader patterns of testate amoeba community change, (2) changes in the size of mixotrophic and heterotrophic testate amoebae, and (3) historical records of temperature and the length of the snow-free season. We conclude by discussing the potential of using $\delta^{13}C$ measurements to investigate changes in the relative utilization of heterotrophy and phototrophy, and potential applications in paleoecological studies to assess past changes in phototrophy by mixotrophic testate amoebae through time.

Methods

Study Area and Core Collection

A peat core was collected from the Brooks Range Foothills region of Alaska's North Slope, a landscape characterized by prolonged winters and brief, cool summers. Between 1993 and 2022, the mean annual air temperature at Toolik Field Station, located 137 km east of our site, was approximately −8.0 °C, with average July temperatures near 11 °C and February averages around −21 °C [18]. Snow typically covers the region for a majority of the year, while the snow-free growing season is 2–4 months long. The region has experienced pronounced warming in recent decades, with mean annual temperatures rising by approximately 0.04 °C per year since 1988 [18].

A 27-cm long and ~8-cm wide peat core, extending from the surface to the underlying mineral soil, was collected using a serrated knife from a *Sphagnum*-rich peat patch embedded within a matrix of moist tussock tundra (68.4315°N, −152.9099°W), on August 10, 2019. The core was wrapped in plastic and aluminum foil, transported in a PVC tube, and stored at ~4 °C prior to laboratory analyses.

Chronology Development

The chronology for the peat core is more thoroughly described in a previous paleoecological study of peatland development on the North Slope [3]. To develop an age-depth model, *Sphagnum* fragments were picked from eight 1-cm depth intervals, rinsed in distilled water, and submitted for radiocarbon (^{14}C) analysis at the Lawrence Livermore National Laboratory. Calendar ages were assigned to the eight depth horizons by calibrating post-bomb ^{14}C measurements using the Northern Hemisphere Zone 1 (NHZ1) dataset [19], and pre-bomb radiocarbon dates were calibrated using the IntCal20 calibration curve [20]. Bayesian age-depth modeling was performed using the software package rbacon [21], with 4000 Markov Chain Monte Carlo iterations. The median age-depth model was used to estimate ages for subsequent paleoecological analyses.

Analytical Methods

The peat core was subsampled into contiguous 1-cm sections. To characterize botanical composition, 1-cm^3 samples were dispersed in distilled water on Petri dishes and the percent abundance of plant macrofossil types was estimated under a dissecting microscope [3]. The plant macrofossil groupings used were *Sphagnum*, herbaceous, woody, other mosses, and unidentified organic material. C:N ratios were

measured from 1-cm^3 subsamples from each depth interval. Dried soil samples were ground in a SPEX SamplePrep 8000D ball mill to analytical fineness, and percent C and N were determined using elemental combustion with a Costech elemental analyzer [22].

Testate amoebae were isolated and their assemblages and morphology analyzed from 1-cm^3 subsamples following standard methods [23]. Subsamples were boiled, sieved to retain material between 15 and 300 µm in diameter, and mounted in glycerol on microscope slides. Individual testate amoebae were identified to morphospecies using taxonomic resources [24, 25], and at least 100 individuals were tallied per sample. The relative abundance of each taxon was then calculated as a percentage of the total number of testate amoebae counted in each sample. *Archerella flavum* was selected for our isotopic analyses due to its consistently high abundance throughout the core, its well-established mixotrophy, and its robust and well-preserved tests. Water table depth (WTD) was reconstructed using a weighted average partial least squares (2-component) model developed from nearly 4000 northern hemisphere surface-samples in the Neotoma Paleoecology Database [6]. Sample-specific error estimates were estimated using a bootstrapping approach with 1000 iterations. To assess changes in test (i.e. shell) length over time, individuals of the most abundant taxa were photographed from each sample along the core and measured using ImageJ software [26]. Taxa included *Archerella flavum* (n = 1054), *Hyalosphenia papilio* (n = 256), *Alabasta militaris* (n = 208), and *Nebela tincta-collaris* type (n = 409). For visualization of changes in test size, data from adjacent centimeters were merged if fewer than 10 individuals of a given species were measured in a sample.

δ^{13}C was measured separately on *Archerella flavum* tests and *Sphagnum* moss from the same peat samples along the core. A 1-cm^3 subsample from each depth interval was washed through sieves using distilled water to isolate material between 20 and 80 µm in diameter, which included the expected size range of *Archerella flavum*. Based on initial measurements, we estimated that analyses of 10–20 empty tests (i.e., devoid of cytoplasm, zoochlorellae, or other materials) of *Archerella flavum* composited together would produce enough carbon (~ 1 nmol) for δ^{13}C analyses with an accuracy and precision of ~ 1‰ [27, 28]. Thus, from each subsample we identified 10–20 empty *Archerella flavum* tests at 200x magnification on a pre-combusted microscope slide using an Olympus CX41 and isolated them with a micropipette. The tests were then rinsed in nanopure water and placed in a ~ 0.4 µl drop of nanopure water. The drop was then applied to a spooling-wire microcombustion device interfaced with a ThermoFinnigan Delta V + isotope ratio mass spectrometer (IRMS; Bremen, Germany) at the Central Appalachians Stable Isotope Facility at the Appalachian Laboratory, following methods outlined by Nelson et al. [29]. Blanks (nanopure water to which tests were added and removed) were measured to ensure that the measured δ^{13}C values were from carbon in the tests. Sample data were normalized to Vienna Peedee Belemnite (VPDB) using 5 nmol aliquots of dissolved in-house standards, leucine and sorbitol, which have δ^{13}C values of −31.7 and − 16.2‰, respectively [30, 31]. A size series (ranging from 5.0 to 0.15625 nmol C) of these standards was used to calculate the expected accuracy and precision of the test δ^{13}C data [32]. In addition, we measured δ^{13}C values of modern *Archerella flavum*, *Hyalosphenia papilio*, and *Hyalosphenia elegans* using the spooling-wire microcombustion approach, and added these measurements to the compilation of Herbert et al. [15] to assist with our downcore interpretations.

In parallel, δ^{13}C values were measured on *Sphagnum* tissue from the same peat core depth intervals to estimate the δ^{13}C values of potential heterotrophic food sources at the time of deposition. *Sphagnum* remains were picked, washed with distilled water, dried at 50 °C, wrapped in tin capsules, and measured for δ^{13}C using a Carlo Erba NC2500 elemental analyzer (CE Instruments, Milano, Italy) interfaced with a ThermoFinnigan Delta V+IRMS. The δ^{13}C data were normalized to the VPDB scale using a normalization curve with three internal standards, corn (−13.61‰), cocoa (−28.64‰), and caffeine (−35.36‰). The analytical precision of an internal spinach standard analyzed alongside the *Sphagnum* samples was 0.1‰ for δ^{13}C.

Differences between testate amoeba δ^{13}C and *Sphagnum* δ^{13}C (the baseline for heterotrophic C uptake) were interpreted as a reflection of the relative use of phototrophy. The estimated proportion of carbon that *Archerella flavum* obtained from phototrophy was estimated using a two-end-member linear mixing model, formulated from two simple mass balance equations:

$$\delta_A = f_S \delta_S + f_C \delta_C$$

$$1 = f_S + f_C$$

where δ_A is the δ^{13}C of *Archerella flavum* at a given depth, δ_S is the δ^{13}C of *Sphagnum* at the same depth, and δ_C is the average value for *Chlorella* δ^{13}C from low-nitrogen and low-phosphorus experiments at ambient CO_2 [33, 34]. f_S is the estimated proportion of carbon which *Archerella flavum* obtained from heterotrophy, and f_C is the estimated proportion of carbon which *Archerella flavum* obtained from phototrophy.

Results

Shifts in Vegetation and Testate Amoeba Community Composition

Peat began accumulating at the site in approximately 1920 CE (Fig. 1). Sedges and shrubs were the dominant vegetation early in the record, with a variable amount of *Sphagnum* and other bryophytes. *Sphagnum* was variable in abundance up until the 1960s CE, but became dominant (>90%) after that time. The relative abundance of mixotrophic testate amoebae, particularly *Archerella flavum*, increased from the bottom to the top of the core, with a particularly large increase in *Archerella flavum* since about 2000 CE (Fig. 2). The relative abundance of heterotrophic taxa, such as *Difflugia pristis* type and *Difflugia pulex*, decreased as *Archerella flavum* increased. Other taxa, such as *Alabasta militaris* and *Nebela collaris-tincta* type, maintained a more stable relative abundance throughout the core (Fig. 2).

δ^{13}C Measurements and Reconstruction of Phototrophy

δ^{13}C values measured on *Sphagnum* and *Archerella flavum* in the peat core were similar to those observed in modern samples (Fig. 3). δ^{13}C values for *Sphagnum* in the peat core ranged from −31.4 to −25.4‰, and trended more negative over time, with some of this trend likely due to fossil fuel emissions and the well-known Suess Effect [35]. Values for *Archerella flavum* ranged from −26.5 to −19.1‰ and showed the same negative trend seen in the *Sphagnum* values until the mid 1980s CE, but in the last 3 decades the two trends diverged significantly. Our three new modern δ^{13}C measurements on *Archerella flavum* confirmed that the species has a similar range of values as other mixotrophic species (Fig. 3). Prior to this study only one measurement on *Archerella flavum* was reported in the literature [13]. Based on the amount of carbon in the *Archerella flavum* samples, the average expected accuracy and precision of the δ^{13}C data were 1.06‰ (range: 0.61–2.27‰) and 1.14‰ (range: 0.66–2.37‰), respectively. All δ^{13}C measurements can be found in Online Resource 1: Table 1, and 2.

The δ^{13}C of *Archerella flavum* was always more positive than the δ^{13}C of *Sphagnum* from the same depth, differing by 1.3 to 11.4‰. Given that the δ^{13}C of *Sphagnum* reflects the heterotrophic food environment for the microbial community, this difference between the δ^{13}C of *Sphagnum* and the δ^{13}C of *Archerella flavum* should primarily reflect the relative use of the algal symbiont (*Chlorella*) as an energy source. Therefore, increased algal symbiont use should result in a greater difference between *Sphagnum* δ^{13}C and *Archerella flavum* δ^{13}C. From the 1940s to the 1980s CE, this difference averaged 2.2‰, while the difference averaged 6.4‰ after the 1980s CE. Our δ^{13}C measurements (Online Resource 1: Table 1) provide a sub-decadally resolved record of changes in the relative use of C sources by *Archerella flavum*, and suggest that populations shifted toward greater reliance on algal symbionts after the late 1980s CE, just prior to the increased abundance of *Archerella flavum* in the 2000s CE.

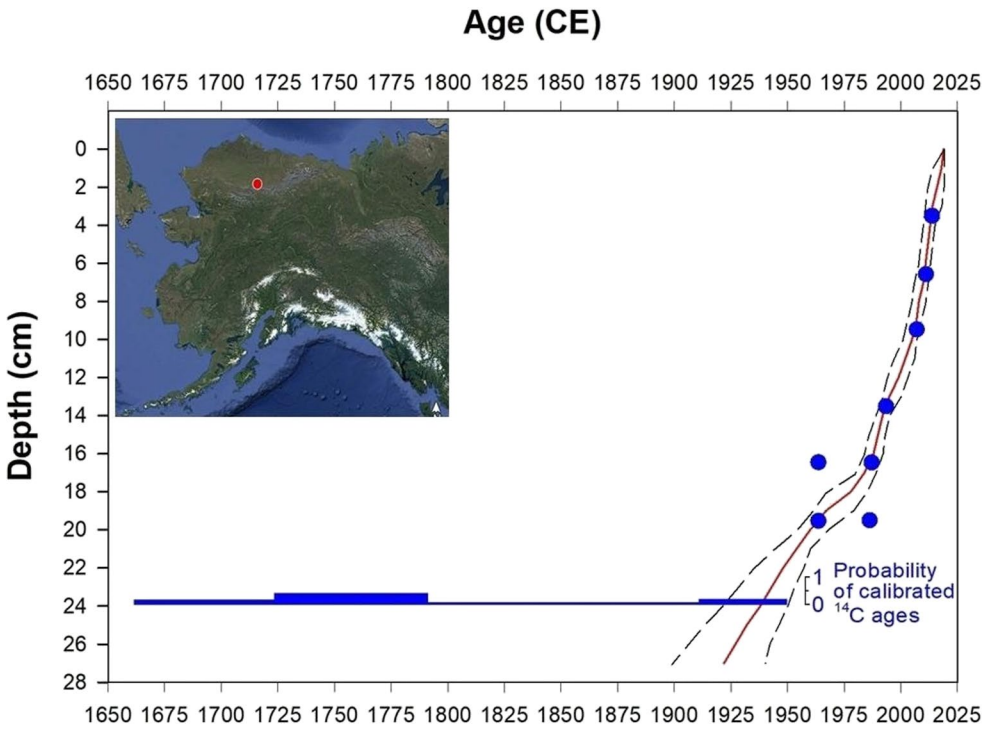

Fig. 1 Median age-depth model (red line) developed using the R package rbacon [21] for our peat core. The inset map shows the site location (red) on the North Slope of Alaska (68.4315°N, −152.9099°W). Dashed lines indicate the 95% confidence interval for the age-depth model. Blue circles indicate post-bomb dates (after 1950 CE) calibrated using the NHZ1 dataset [19]. Blue bars indicate 95% confidence intervals for the pre-bomb date (before 1950 CE) calibrated using the IntCal20 calibration curve [20]

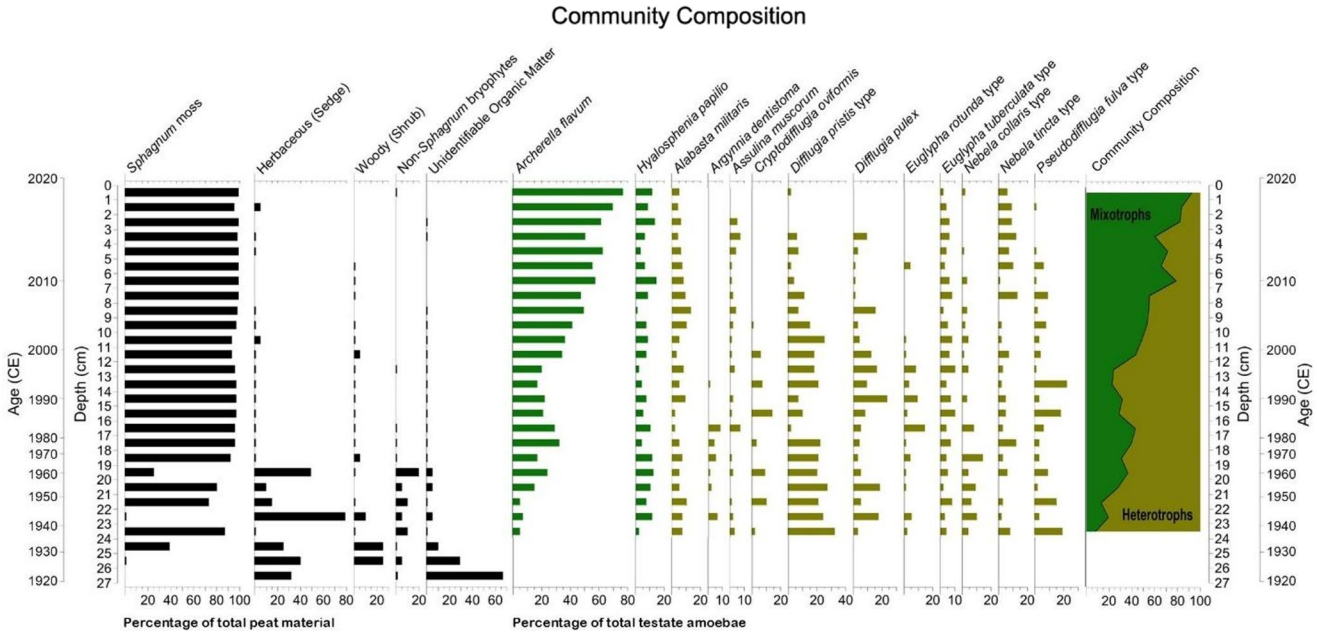

Fig. 2 Stratigraphic records of plant and testate amoeba community composition. Testate amoeba taxa in green are mixotrophs, while taxa in light brown are heterotrophs. Only dominant and/or frequent testate amoeba taxa are shown, and the complete dataset is available through the Neotoma Paleoecology Database. The proportion of mixotrophs and heterotrophs is summarized in the panel on the far right

Comparing Inferred Phototrophy To Measured and Reconstructed Environmental Properties

Our reconstructed record of phototrophy broadly corresponds with variability in the length of the snow-free growing season as measured near Toolik Field Station from 2007 to 2019 CE, at least within the uncertainty of the age-depth model (Fig. 4). The increase in inferred phototrophy seen after the late 1980s CE may also have been in response to increased warming, and the expansion of nutrient-poor conditions as indicated by the dominance of *Sphagnum* (Fig. 4). The highest C:N ratios, consistent with nutrient-poor conditions, occurred toward the top of the core and coincided with high estimated phototrophy usage (Fig. 4). Reconstructed water-table depths, based on testate amoeba communities, remained relatively stable throughout the record, suggesting that moisture changes had little influence on the observed changes in inferred phototrophy (Fig. 4).

Changes in Test Size in Mixotrophic and Heterotrophic Taxa

Measurements of testate amoebae in the core indicated that the recent community changes and increasing use of phototrophy by *Archerella flavum* were associated with changes in the test size of both heterotrophic and mixotrophic testate amoebae. The length of *Archerella flavum* tests was variable prior to 2005, but tests became consistently smaller after this time (Fig. 5). *H. papilio* also decreased in size from the bottom to the top of the core, with tests becoming more variable in size and generally smaller after 1980 CE, coinciding with the onset of increased phototrophy in *Archerella flavum*. *A. militaris* displayed a distinct peak in size centered around 2000 CE, when *Archerella flavum* became more abundant. *N. collaris-tincta* type showed a relatively consistent decrease in size from bottom to top of the core. (Fig. 5). All test measurements can be found in Online Resource 1: Table 3.

Discussion

Community Changes and Increased Phototrophy During the Past Several Decades

Our results indicate that *Archerella flavum*, a mixotrophic species, has increased in abundance over the past several decades at this location on the North Slope, and similar increases in mixotrophic testate amoebae have occurred more broadly within *Sphagnum*-dominated settings on the North Slope of Alaska since the mid-1900s [3, 37]. At our study location, mixotrophs comprised less than 40% of the testate amoeba community prior to the late 1990s CE, but since that time they have made up 50–84%, indicating a dramatic reorganization of the microbial community (Fig. 2). This recent expansion of mixotrophs may be a more widespread phenomenon in high-latitude *Sphagnum*-dominated

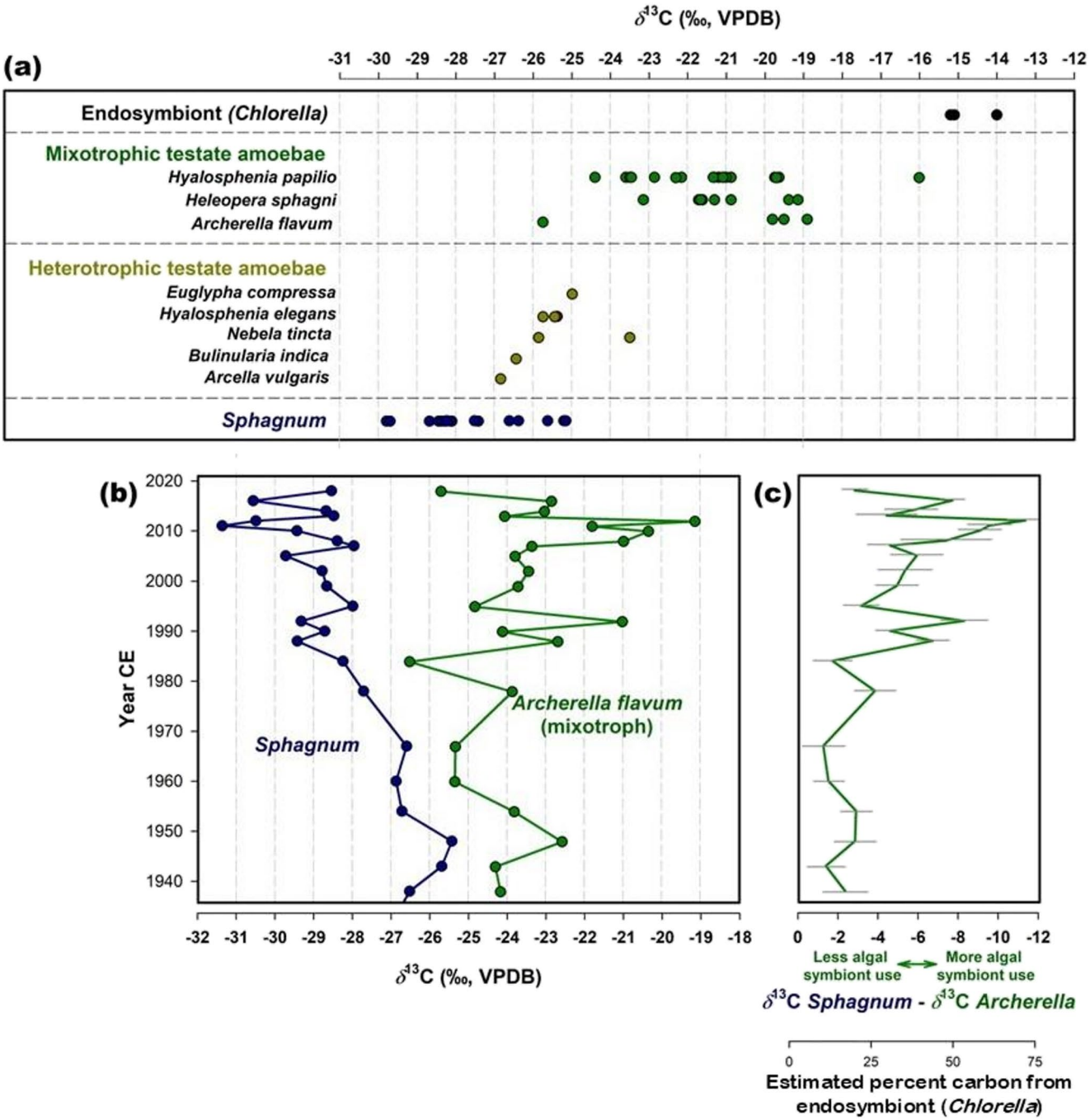

Fig. 3 (**a**) Compilation of literature-derived and new measurements of $\delta^{13}C$ for taxa in the food web of *Sphagnum*-dominated peatlands [13, 15–17]. For *Chlorella*, $\delta^{13}C$ data from low-nitrogen and low-phosphorus experiments, at ambient CO_2 are shown [33, 34]. (**b**) $\delta^{13}C$ of *Sphagnum* and *Archerella flavum* in a peat core from the North Slope of Alaska **(c)** The difference between the *Sphagnum* and mixotroph measurements. Error bars indicate the expected precision of the spooling-wire microcombustion approach. During times of increased algal symbiont use, the difference between *Sphagnum* $\delta^{13}C$ and *Archerella flavum* $\delta^{13}C$ should increase due to an increase in the % C derived from the symbiont

habitats. For example, all 12 peat records studied by Zhang et al. [11] in Quebec showed mixotrophic taxa becoming dominant in recent decades, reaching 50% relative abundance. Of the 79 peat records that contained mixotrophs in the recent northern hemisphere compilation of Zhang et al. [38], mixotrophic taxa became more abundant since 1875 CE in 61 of them, or 77%.

Our downcore $\delta^{13}C$ measurements of *Archerella flavum* and *Sphagnum* suggest that the species increased its reliance on phototrophy after the late 1980s CE, just prior to its

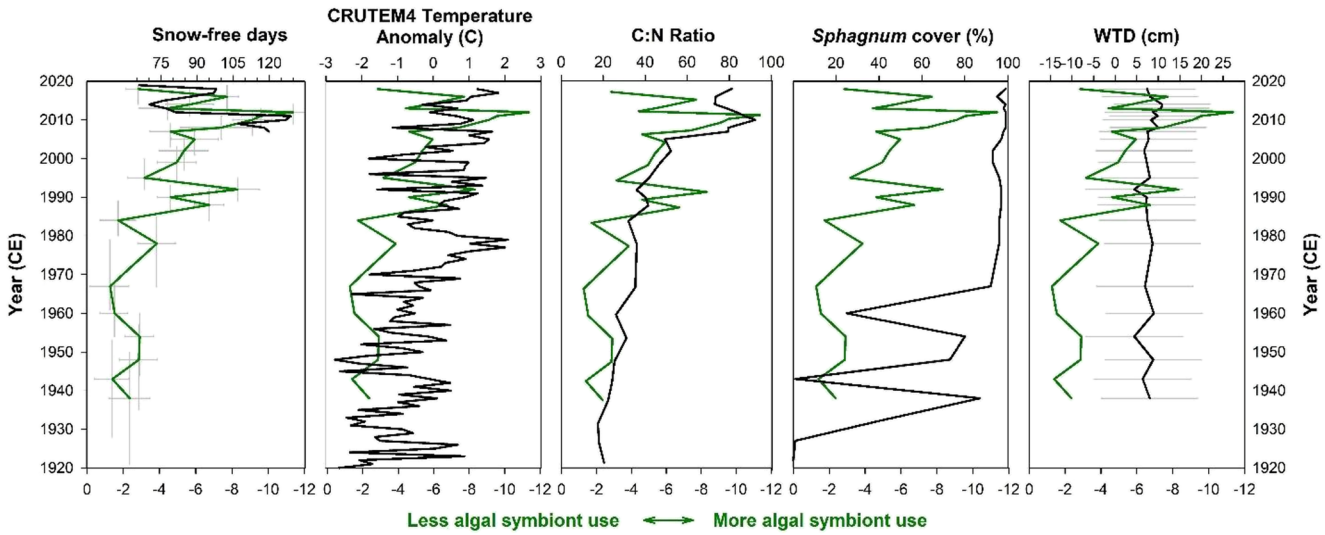

Fig. 4 The reconstructed record of phototrophy in *Archerella flavum* is shown in green with vertical error bars indicating the uncertainty of the age-depth model and horizontal error bars indicating the expected precision of the spooling-wire microcombustion approach. This inferred record was compared with the measured length of the snow-free growing season from 2007 to 2019 from the Toolik Lake region [18], temperature records from the North Slope of Alaska [36], the record of C:N ratios in the core, the record of *Sphagnum* remains in the core, and water table depth (WTD) reconstructed using a weighted average partial least squares (2-component) model developed from nearly 4000 northern hemisphere surface-samples in the Neotoma Paleoecology Database [6]. Horizontal error bars were estimated for each sample using a bootstrapping approach with 1000 iterations

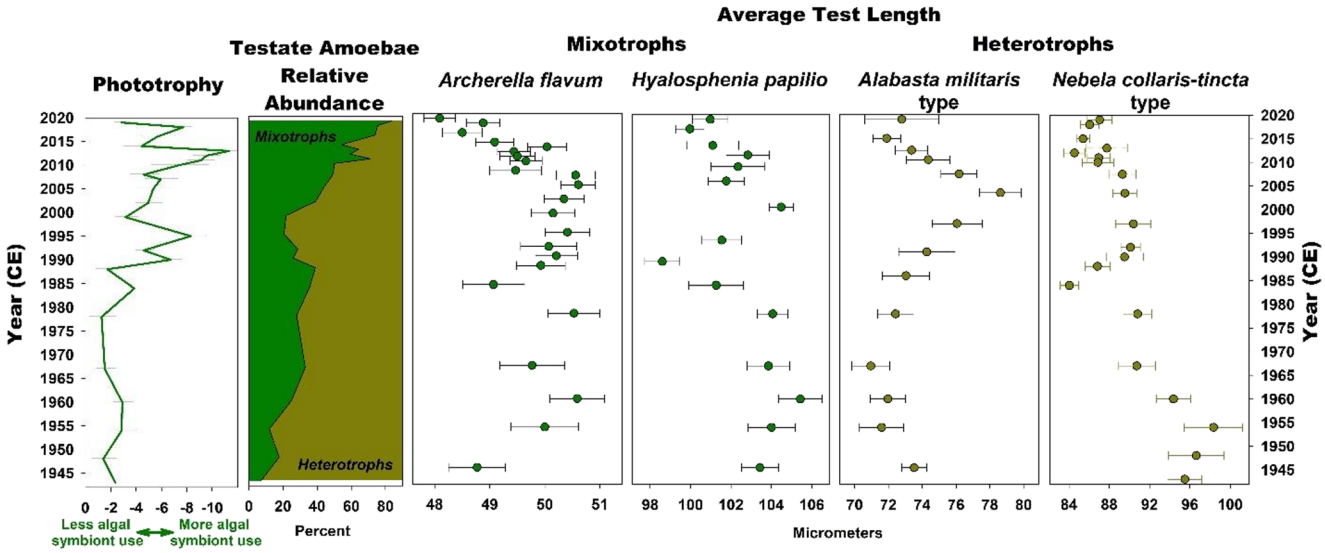

Fig. 5 The reconstructed record of phototrophy in *Archerella flavum* (green line) includes gray horizontal error bars indicating the expected precision of the spooling-wire microcombustion approach. Records of test size are shown for four taxa: *Archerella flavum* ($n=1054$), *Hyalosphenia papilio* ($n=256$), *Alabasta militaris* ($n=208$), and *Nebela tincta-collaris* type ($n=409$). Error bars for test length were calculated using standard error of the mean

rapid increase in relative abundance within the community. Interestingly, prior to 1980 CE the difference between δ^{13}C values of *Archerella flavum* and *Sphagnum* were not only smaller than after this time, but they were also positively correlated, consistent with a strong heterotrophic signature reflected in the *Archerella flavum* δ^{13}C values. After the 1980s CE, the difference between *Sphagnum* and *Archerella flavum* δ^{13}C values increased, and the two values are no longer positively correlated, consistent with increased phototrophy usage. Based on a simple mixing model, we estimate that the changes in δ^{13}C in *Archerella flavum* and *Sphagnum* represent a shift from approximately 10 to 25% of carbon derived from phototrophy, to over 25%, possibly up to 75%, of carbon obtained via phototrophy (Fig. 3). Expansion of

Archerella flavum and its increased reliance on phototrophy may have altered competitive dynamics, leading to changes in microbial community composition and decomposition rates.

Environmental changes of the last several decades may have contributed to the inferred increase in phototrophy and the subsequent population expansion of *Archerella flavum*. These changes potentially included the development of more oligotrophic conditions, variability in light availability due to changes in the length of the snow-free growing season, and changes in water balance or other potential responses to climatic changes. Decreased nutrient availability, potentially enhanced by the expansion and increasing dominance of *Sphagnum* over the past several decades on the North Slope [3], may have favored *Archerella flavum* and promoted its increased use of phototrophy. Across terrestrial and aquatic systems, mixotrophy appears to be favored under nutrient-limited conditions in a range of organism groups [39, 40]. Ecological theory also predicts that mixotrophs will outcompete strict autotrophs and heterotrophs in nutrient-limited settings [41]. Increased abundance of mixotrophic testate amoebae, including *Archerella flavum*, has also been observed in more oligotrophic peatland environments [6, 10, 11]. In our peat record, the highest C:N ratios, consistent with nutrient-poor conditions, occurred toward the top of the core and coincided with high phototrophy usage and the high abundance of *Archerella flavum*.

A longer snow-free growing season and the resulting increased light availability may have also contributed to the expansion of *Archerella flavum* and its increased use of its algal symbiont as a carbon source (Fig. 4). In a seasonal observation of peatland sites in northern Poland, *Archerella flavum* relative abundance and density increased from spring to summer, and this difference was mainly attributed to increased light availability [42]. *Archerella flavum* occurrence was also positively correlated with light intensity in a tropical peatland precipitation-exclusion experiment [43]. In full-shading experiments, abundance and biomass of mixotrophs decreased [8] and death of all endosymbiont *Chlorella* occurred within 3 months [14]. Partial shade with 50–60% light reduction, on the other hand, resulted in viable endosymbionts even after 2 years, and a slight unexpected increase in the relative abundance of mixotrophs, though the reason for this is unclear and absolute densities of testate amoebae were not measured [15]. Although it is not a factor at our Arctic site, studies have also observed a decline in mixotrophic testate amoebae along gradients of increasing tree cover in bogs, suggesting a possible link between shading and mixotroph decline related to decreased light availability [10, 12, 44]. The lack of mixotrophs in tree-shaded plots could also be influenced by hydrology, since drying is one factor that promotes tree expansion in peatlands [12, 44], and mixotrophs are not typically abundant in drier habitats [6].

Hydrological changes may have also contributed to the observed recent changes in mixotrophic testate amoebae on the North Slope, and possibly elsewhere in the arctic. The mixotrophic testate amoebae *Archerella flavum*, *Hyalosphenia papilio*, and *Heleopera sphagni* are typically found in moderately wet peatland habitats, while the species *Amphitrema wrightianum* is typical of very wet peatlands. Mixotrophic testate amoebae are particularly sensitive to drying as they live in the uppermost portion of the *Sphagnum* [42], and prior research conducted in peatlands found that experimental drying led to a loss of mixotrophs [45]. Of course, limited drying of a very wet site can result in moderately wet conditions ideal for mixotrophs. However, the reconstructed moisture at the location examined in this study has not changed considerably over the last century (Fig. 4), so hydrological change was not likely a cause of the increased abundance of *Archerella flavum*. Together, these observations underscore the sensitivity of microbial communities and mixotrophy to multiple co-occurring environmental changes, such as warming, nutrient limitation, growing-season length, and vegetation shifts.

Changes in Test Length

Testate amoeba community changes and increased phototrophy over the past several decades were associated with changes in test length for the dominant testate amoeba taxa in the peat core. All four measured taxa decreased in size from ~ 2000 CE to the present (Fig. 5), a pattern that may be related to the same suite of environmental changes that promoted the expansion of *Archerella flavum* and its increased use of phototrophy. The abundance of taxa with smaller test sizes have been linked to increasingly ombrotrophic or drier conditions in *Sphagnum* peatlands [46], and our observed size changes within individual species may represent a similar response. Analogous patterns have been observed in other protist groups; for example, smaller phytoplankton often dominate under nutrient-poor conditions [47]. Warmer temperatures have also been linked to reductions in protist size [48, 49]. Reduced snow cover in winter may also favor smaller-bodied testate amoebae in peatlands, due to increased exposure to frost events that limit larger testate amoebae [50]. However, in addition to the recent trend toward smaller test sizes, our measurements reveal quite a bit of test-length variability downcore, and three taxa attained their smallest size at an earlier point in the record, when conditions were presumably cooler and less nutrient-poor. The timing of earlier size shifts also varied among taxa.

$\delta^{13}C$ as a Tool To Study Mixotrophy Through Time

Our study demonstrates that the $\delta^{13}C$ values of mixotrophic testate amoebae, when measured alongside $\delta^{13}C$ of *Sphagnum* in a peat core, can be used to estimate changes in the relative use of phototrophy and heterotrophy through time. Previous ecological studies have clearly shown that $\delta^{13}C$ of mixotrophic testate amoebae differs from the $\delta^{13}C$ of the heterotrophic food environment [13, 15], and the consistently higher $\delta^{13}C$ measurements of *Archerella flavum* as compared to *Sphagnum* in our core indicate that this signature is recorded in the paleoecological record. Furthermore, by using a spooling wire-IRMS, we were able to measure $\delta^{13}C$ using just 10–20 tests per sample instead of the 300–800 tests typically required using conventional elemental analyzer-IRMS methods [13, 15–17], which makes the approach much more applicable to high-resolution peat core analyses.

This novel approach could be applied to a range of ecological and paleoecological questions. Ecological studies could be designed to examine the spatial variability in phototrophy across environmental gradients, as well as the impact of disturbance and other potential factors. Work could also be focused on improving our understanding of the ecosystem-level effects of phototrophy usage by mixotrophs. For example, recently it has been suggested that increased carbon fixation by mixotrophs could affect peatland carbon balance. In one experiment, a 50% loss of mixotrophic testate amoebae was associated with a 13% drop in net CO_2 uptake [8], and coupling studies like this with $\delta^{13}C$-based estimates of phototrophy usage would potentially allow for estimates of the range of CO_2 uptake values attributable to the use of phototrophy by mixotrophic testate amoebae. Furthermore, $\delta^{13}C$-based estimates of phototrophy could be applied in paleoecological contexts to assess relationships between mixotroph phototrophy, carbon accumulation, and climate change over the last several thousand years. By exploring how the use of phototrophy by mixotrophs is affected by changing environmental conditions at a range of timescales, both in paleorecords and the present, the method could be applied to better understand the functioning of peatland ecosystems.

Although our results indicate great promise for $\delta^{13}C$-based estimates of phototrophy by mixotrophic testate amoebae, there are assumptions and uncertainties that require further testing and quantification. For example, the $\delta^{13}C$ value of *Chlorella* living within mixotrophic testate amoebae has never been directly measured, so our inferences are based on the value of free-living *Chlorella* in low-nitrogen and low-phosphorus conditions, which are likely similar to oligotrophic peatland environments [33, 34]. However, isolation of endosymbiotic *Chlorella* and measurement of their $\delta^{13}C$ values would allow better quantification of the isotopic contribution of phototrophy, and an assessment of how variable *Chlorella* $\delta^{13}C$ values are along community and environmental gradients. Such measurements would provide estimates of the expected range of variability in these values through time and better constrain uncertainty estimates. Repeated measurements of test $\delta^{13}C$ from the same samples, across multiple mixotrophic species, and across multiple peat cores would also help to quantify uncertainty and assess consistency in response among taxa and across habitats.

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Data Availability Chronological and testate amoeba compositional data are publicly available via the Neotoma Paleoecology Database (neotomadb.org, Site name NSNSF19-6, [link](https:/apps.neotomadb.org/explorer/?siteids=36459)). $\delta^{13}C$ measurements and test size data are available in Online Resource 1. No novel code was used in this study.

Declarations

Competing interests The authors declare no competing interests.

References

1. Euskirchen ES, Bret-Harte MS, Shaver GR, Edgar CW, Romanovsky VE (2017) Long-term release of carbon dioxide from Arctic tundra ecosystems in Alaska. Ecosystems 20(5):960–974

2. Crichton KA, Anderson K, Fewster RE, Charman DJ, Garneau M, Väliranta M, Gallego-Sala AV (2025) Satellite data indicates recent Arctic peatland expansion with warming. Commun Earth Environ 6(1):1–9
3. Stansfield AR, Booth RK, Loisel J, Camill P, Yu Z, Xia Z, Gengaro A, Scally A (2025) Recent Sphagnum expansion into the tundra on the North Slope of Alaska. *Ecological Monographs* in press
4. Mitchell EA, Charman DJ, Warner BG (2008) Testate amoebae analysis in ecological and paleoecological studies of wetlands: past, present and future. Biodivers Conserv 17:2115–2137
5. Ireland AW, Booth RK (2012) Upland deforestation triggered an ecosystem state-shift in a kettle peatland. J Ecol 100(3):586–596
6. Booth RK, Stansfield A, Cowper E, Kodero JM (2025) Testate amoebae as paleoenvironmental indicators in peatlands: calibration-dataset synthesis and assessment of modern analogues using the Neotoma Paleoecology Database. Quat Sci Rev 366:109491
7. Wilkinson DM, Mitchell EA (2010) Testate amoebae and nutrient cycling with particular reference to soils. Geomicrobiol J 27(6–7):520–533
8. Jassey VE, Signarbieux C, Hättenschwiler S, Bragazza L, Buttler A, Delarue F, Robroek BJ (2015) An unexpected role for mixotrophs in the response of peatland carbon cycling to climate warming. Scientific reports, 5 1:16931
9. Gomaa F, Kosakyan A, Heger TJ, Corsaro D, Mitchell EA, Lara E (2014) One alga to rule them all: unrelated mixotrophic testate amoebae (Amoebozoa, rhizaria and Stramenopiles) share the same symbiont (Trebouxiophyceae). Protist 165(2):161–176
10. Lamentowicz M, Kajukało-Drygalska K, Kołaczek P, Jassey VE, Gąbka M, Karpińska-Kołaczek M (2020) Testate amoebae taxonomy and trait diversity are coupled along an openness and wetness gradient in pine-dominated Baltic bogs. Eur J Protistol 73:125674
11. Zhang H, Amesbury MJ, Piilo SR, Garneau M, Gallego-Sala A, Väliranta MM (2020) Recent changes in peatland testate amoeba functional traits and hydrology within a replicated site network in northwestern Québec, Canada. Front Ecol Evol 8:228
12. Creevy AL, Andersen R, Rowson JG, Payne RJ (2018) Testate amoebae as functionally significant bioindicators in forest-to-bog restoration. Ecol Indic 84:274–282
13. Jassey VE, Meyer C, Dupuy C, Bernard N, Mitchell EA, Toussaint ML, Gilbert D (2013) To what extent do food preferences explain the trophic position of heterotrophic and mixotrophic microbial consumers in a *Sphagnum* peatland? Microb Ecol 66:571–580
14. Schönborn W (1965) Die sedimentbewohnenden Testaceen Einiger masurischer seen. Acta Protozool 3:19–27
15. Herbert RP, Peters SC, Nelson DM, Booth RK (2019) Light variability and mixotrophy: responses of testate amoeba communities and shell δ13C values to a peatland shading experiment. Eur J Protistol 67:15–26
16. Jassey VE, Shimano S, Dupuy C, Toussaint ML, Gilbert D (2012) Characterizing the feeding habits of the testate amoebae hyalosphenia papilio and Nebela tincta along a narrow fen-bog gradient using digestive vacuole content and 13 C and 15 N isotopic analyses. Protist 163(3):451–464
17. Mieczan T, Michał N, Adamczuk M, Bielańska-Grajner I (2015) Stable isotope analyses revealed high seasonal dynamics in the food web structure of a peatbog. Int Rev Hydrobiol 100(5–6):141–150
18. Environmental Data Center Team (2024) Monthly climate summaries from Toolik, Alaska (1993–2022). Toolik Field Station, Institute of Arctic Biology, University of Alaska Fairbanks, Fairbanks, AK 99775. https://www.uaf.edu/toolik/edc/monitoring/abiotic/climate_summaries.php
19. Hua Q, Barbetti M, Rakowski AZ (2013) Atmospheric radiocarbon for the period 1950–2010. Radiocarbon 55(4):2059–2072
20. Reimer PJ, Austin WE, Bard E, Bayliss A, Blackwell PG, Ramsey CB, Talamo S (2020) The IntCal20 Northern Hemisphere radiocarbon age calibration curve (0–55 cal kBP). Radiocarbon, 62 4:725–757
21. Blaauw M (2010) Methods and code for 'classical'age-modelling of radiocarbon sequences. Quat Geochronol 5(5):512–518
22. Camill P, Umbanhowar CE Jr, Geiss C, Edlund MB, Hobbs WO, Dupont A, Ramos M (2017) The initiation and development of small peat-forming ecosystems adjacent to lakes in the north central Canadian low arctic during the Holocene. Journal of Geophysical Research: Biogeosciences, 122 7:1672–1688
23. Booth RK, Lamentowicz M, Charman DJ (2010) Preparation and analysis of testate amoebae in peatland palaeoenvironmental studies. Mires Peat, *7*
24. Siemensma FJ (2019) Microworld. World-wide electronic publication, Kortenhoef, the Netherlands, World of Amoeboid Organisms https://www.arcella.nl
25. Kosakyan A, Ralf M, Enrique L, Clément D, Edward M, A. D (2025) A taxonomic monograph of hyalospheniid testate amoebae amoebozoa. Arcellinida: Hyalospheniformes
26. Schneider CA, Rasband WS, Eliceiri KW (2012) NIH image to imageJ: 25 years of image analysis. Nat Methods 9(7):671–675
27. Griener KW, Nelson DM, Warny S (2013) Declining moisture availability on the Antarctic Peninsula during the late eocene. Palaeogeogr Palaeoclimatol Palaeoecol 383:72–78
28. Nelson DM (2012) Carbon isotopic composition of ambrosia and *artemisia* pollen: assessment of a C3-plant paleophysiological indicator. New Phytol 195(4):787–793
29. Nelson DM, Hu FS, Scholes DR, Joshi N, Pearson A (2008) Using SPIRAL (single pollen isotope ratio analysis) to estimate C3-and C4-grass abundance in the paleorecord. Earth Planet Sci Lett 269(1–2):11–16
30. Nelson DM, Hu FS, Michener RH (2006) Stable-carbon isotope composition of *poaceae* pollen: an assessment for reconstructing C3 and C4 grass abundance. Holocene 16(6):819–825
31. Nelson DM, Hu FS, Mikucki JA, Tian J, Pearson A (2007) Carbon-isotopic analysis of individual pollen grains from C3 and C4 grasses using a spooling-wire microcombustion interface. Geochim Cosmochim Acta 71(16):4005–4014
32. Korasidis VA, Wing SL, Nelson DM, Baczynski AA (2022) Reworked pollen reduces apparent floral change during the Paleocene-Eocene thermal maximum. Geology 50(12):1398–1402
33. Beardall J, Griffiths H, Raven JA (1982) Carbon isotope discrimination and the CO2 accumulating mechanism in *chlorella emersonii*. J Exp Bot 33(4):729–737
34. Beardall J, Roberts S, Raven JA (2005) Regulation of inorganic carbon acquisition by phosphorus limitation in the green alga chlorella emersonii. Can J Bot 83(7):859–864
35. Tans PP, De Jong AFM, Mook WG (1979) Natural atmospheric 14 C variation and the suess effect. Nature 280(5725):826–828
36. Osborn TJ, Jones PD (2014) The CRUTEM4 land-surface air temperature data set: construction, previous versions and dissemination via Google Earth. Earth Syst Sci Data 6(1):61–68
37. Taylor LS, Swindles GT, Morris PJ, Gałka M, Green SM (2019) Evidence for ecosystem state shifts in Alaskan continuous permafrost peatlands in response to recent warming. Q Sci Rev 207:134–144
38. Zhang H, Väliranta M, Swindles GT, Aquino-López MA, Mullan D, Tan N, Zhao Y (2022) Recent climate change has driven divergent hydrological shifts in high-latitude peatlands. Nat Commun 13(1):4959
39. Selosse MA, Charpin M, Not F (2017) Mixotrophy everywhere on land and in water: the grand écart hypothesis. Ecol Lett 20(2):246–263

40. Stoecker DK, Hansen PJ, Caron DA, Mitra A (2017) Mixotrophy in the marine plankton. Annu Rev Mar Sci 9(1):311–335
41. Schenone L, Aarons ZS, García-Martínez M, Happe A, Redoglio A (2024) Mixotrophic protists and ecological stoichiometry: connecting homeostasis and nutrient limitation from organisms to communities. Front Ecol Evol 12:1505037
42. Marcisz K, Lamentowicz Ł, Słowińska S, Słowiński M, Muszak W, Lamentowicz M (2014) Seasonal changes in sphagnum peatland testate amoeba communities along a hydrological gradient. Eur J Protistol 50(5):445–455
43. Krashevska V, Sandmann D, Maraun M, Scheu S (2012) Consequences of exclusion of precipitation on microorganisms and microbial consumers in montane tropical rainforests. Oecologia 170(4):1067–1076
44. Payne RJ, Creevy A, Malysheva E, Ratcliffe J, Andersen R, Tsyganov AN, Mazei Y (2016) Tree encroachment may lead to functionally-significant changes in peatland testate amoeba communities. Soil Biol Biochem 98:18–21
45. Koenig I, Schwendener F, Mulot M, Mitchell EA (2017) Response of sphagnum testate amoebae to drainage, subsequent re-wetting and associated changes in the moss carpet-results from a three year mesocosm experiment. Acta Protozool 56(3):191–210
46. Marcisz K, Jassey VE, Kosakyan A, Krashevska V, Lahr DJ, Lara E, Fournier B (2020) Testate amoeba functional traits and their use in paleoecology. Front Ecol Evol 8:575966
47. Mousing EA, Richardson K, Ellegaard M (2018) Global patterns in phytoplankton biomass and community size structure in relation to macronutrients in the open ocean. Limnol Oceanogr 63(3):1298–1312
48. Atkinson D, Ciotti BJ, Montagnes DJ (2003) Protists decrease in size linearly with temperature: ca. 2.5% C−1. Proceedings of the Royal Society of London. Series B: Biological Sciences 270(1533):2605–2611
49. Forster J, Hirst AG, Esteban GF (2013) Achieving temperature-size changes in a unicellular organism. ISME J 7(1):28–36
50. Jassey VE, Lamentowicz M, Bragazza L, Hofsommer ML, Mills RT, Buttler A, Robroek BJ (2016) Loss of testate amoeba functional diversity with increasing frost intensity across a continental gradient reduces microbial activity in peatlands. Eur J Protistol 55:190–202