

Functional microbial ecology in arctic soils: the need for a year-round perspective

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Abstract

The microbial ecology of arctic and sub-arctic soils is an important aspect of the global carbon cycle, due to the sensitivity of the large soil carbon stocks to ongoing climate warming. These regions are characterized by strong climatic seasonality, but the emphasis of most studies on the short vegetation growing season could potentially limit our ability to predict year-round ecosystem functions. We compiled a database of studies from arctic, subarctic, and boreal environments that include sampling of microbial community and functions outside the growing season. We found that for studies comparing across seasons, in most environments, microbial biomass and community composition vary intra-annually, with the spring thaw period often identified by researchers as the most dynamic time of year. This seasonality of microbial communities will have consequences for predictions of ecosystem function under climate change if it results in: seasonality in process kinetics of microbe-mediated functions; intra-annual variation in the importance of different (a)biotic drivers; and/or potential temporal asynchrony between climate change-related perturbations and their corresponding effects. Future research should focus on (i) sampling throughout the entire year; (ii) linking these multi-season measures of microbial community composition with corresponding functional or physiological measurements to elucidate the temporal dynamics of the links between them; and (iii) identifying dominant biotic and abiotic drivers of intra-annual variation in different ecological contexts.

Keywords: bacteria, fluxes, fungi, seasonality, soil

Introduction

Arctic and subarctic soils play an important role in global biogeochemistry due to the large quantities of organic carbon (C) that they contain—more than twice the amount of atmospheric C (Tarnocai et al. 2009, Blaud et al. 2015). This large C store is a consequence of an imbalance between primary productivity and decomposition over millennial timescales (Mckane et al. 1997, Hobbie et al. 2000, Blaud et al. 2015), due to limited soil microbial activity under the prevailing conditions of low temperature and high soil moisture (Kirschbaum 1995, Ping et al. 1997), and scarcity of available soil nutrients (Weintraub and Schimel 2003, Sistla et al. 2012, Sullivan et al. 2020). Ongoing changes to the climate in high latitudes (ACAI 2004, Graham et al. 2017, Overland et al. 2019) have drawn attention to the possibility of a shift in the current status of northern soils as a C sink to a C source (Shaver et al. 1992, Oechel et al. 1993, Waelbroeck et al. 1997, Brouillette 2021, Clemmensen et al. 2021). This would result from changes in temperature and hydrology of soils—either directly through changes in air temperature and precipitation, or indirectly through processes such as altered frost-heave dynamics, or permafrost thaw. If these changes alleviate the current limitations on soil microbial activity, non-linear shifts in the balance of production and decomposition would act as a positive feedback to global climate warming (Oechel et al. 2000, Schuur et al. 2008). An understanding of the dynamics of arctic soil microorganisms and their associated functions under changing environmental conditions is therefore

important for realistic predictions of future biogeochemical cycles and climate (Wieder et al. 2019).

One of the defining features of arctic and sub-arctic regions is the long, dark and cold winter season (Serreze and Barry 2014). Such long winters mean that even low rates of soil respiration during the ‘cold season’ (Olsson et al. 2003) may add up to be a quantitatively important component of the annual C budget (Fahnestock et al. 1998, Hobbie et al. 2000, Olsson et al. 2003, Webb et al. 2016, Zona et al. 2016, Raz-Yaseef et al. 2017, Taylor et al. 2018). The large fluctuations in environmental conditions across seasons and corresponding variation in the quality and quantity of resource inputs (Loya et al. 2004) also mean that soil microbial communities and their physiological traits will likely vary across the year. If the composition of microbial communities and their associated activities are different during winter than in summer, observations based on measurements in the relatively short and highly productive (plant) growing season will likely not produce accurate estimates of associated year-round fluxes, yet these are critical to predict responses to a changing climate (Hobbie et al. 2000). This presents a challenge for biogeochemistry since there has been little research on year-round seasonal dynamics of soil biogeochemical processes and microbial communities in arctic and sub-arctic environments. This gap in research is partly due to practical difficulties associated with frozen soil, deep snow layers and hazardous weather conditions, and partly because of a historical assumption that biological activity is ‘on hold’ during winter

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(see Aanderud et al. 2013, Hampton et al. 2017). As such, sampling and observations are typically at best limited to a small number of occasions (Björk et al. 2008) or in the majority of studies, microbial communities and their activities are only measured during the summer (Blaud et al. 2015). Indeed, a bibliographic analysis with studies from 1977 to January 2022 (for methods see Appendix S1), shows that measurements taken outside of the summer growing season were reported in only around 10% of studies in arctic soils that characterize soil microbial communities, and 20% of the studies that measure microbially mediated processes. Within the relatively low number of studies that investigate microbial abundance, community composition and/or microbially mediated processes outside of the growing season in arctic soils, there is a large heterogeneity in types of measurements that were performed (Fig. 1, Appendix S2, online supplementary material). This strongly biased focus on summer measurements likely limits our whole-year understanding of soil ecosystem dynamics in arctic systems.

Building a predictive understanding of arctic soil biogeochemistry and its seasonal patterns will require studies that integrate observations of the dynamics of microbial community composition and the chemical transformations they mediate. Microorganisms decompose and mineralize soil organic matter. Together with their role in nitrogen fixation and in transformations between the different forms of nitrogen, this places them at the centre of ecosystem nutrient cycling (Schimel and Bennett 2004). Moreover, aerobic and anaerobic decomposition processes concomitantly result in the release of greenhouse gases carbon dioxide (CO₂), methane (CH₄) and nitrous oxide (N₂O). The importance of microbial community dynamics in driving the response of these processes to environmental changes has been established for some specific aspects of soil function such as methane emissions (Wagner et al. 2017), nitrification and denitrification (Harter et al. 2014, Isobe et al. 2018), and the potential activities of extracellular enzymes involved in C and N cycles (Waldrop et al. 2000). However, the degree to which compositional information is relevant for predicting more generalized soil functions such as soil organic matter storage and soil respiration remains a point of active debate and research (Allison and Martiny 2008, Schimel and Schaeffer 2012, Bier et al. 2015, Graham et al. 2016, Weedon et al. 2017, Doherty et al. 2020; Monteux et al. 2020). Given the central role of soil microbial activity in the biogeochemistry of arctic ecosystems, and the increasing move towards explicitly incorporating microbial dynamics into ecosystem models (Todd-Brown et al. 2012, Wieder et al. 2013, Wieder et al. 2015), and the increasing number of studies reporting data on community composition, it is important to establish the extent to which microbial communities, and their associated functions co-vary intra-annually in natural environments.

Seasonally-cold environments tend to exhibit strong seasonality in communities and processes at all levels of the food web, and the resulting intra-annual dynamics are expected to be sensitive to changes in climatic conditions (Ernakovich et al. 2014). In this review we synthesize the results of studies in arctic and sub-arctic soils to elucidate the seasonal variation of soil microbial communities and their associated biogeochemical processes. More specifically, we address the following questions: (i) what is the evidence for differences in biomass, community composition and functional potential of soil microbial communities inside and outside of the summer growing season? (ii) What are the consequences of the summer-bias in measurements for our understanding and ability to predict whole-year variability in biogeochemical processes (e.g. microbial growth rate, CO₂ and CH₄ fluxes, nitrogen

cycling and extracellular enzyme activities)? Specifically, we evaluate evidence for three different hypothetical scenarios where seasonality of microbial communities will have consequences for predicting biogeochemical processes; (iii) What are the most urgent remaining research challenges for answering these questions?

Seasonality of (sub)arctic soil microbial communities

Microbial communities and their characteristics are expected to differ between seasons because of large fluctuations in environmental conditions, e.g. average soil temperatures ranging from -15°C to 7°C (Ernakovich et al. 2014) and winter water potentials as low as -10 MPa (Stähli and Stadler 1997). Nutrient availability (e.g. Grogan and Jonasson 2003, Koller and Phoenix 2017) and the quality and quantity of resource inputs from plants also show distinct seasonal patterns (Iversen et al. 2015). This temporal variation will lead to alternating periods of relatively favourable or unfavourable conditions for different community members, depending on their metabolism and physiological tolerances. Because of relatively cold winter soil temperatures in high Northern latitudes (Fig. 2), microbes grow more slowly compared to those in temperate climates, but their ability to grow under cold circumstances does not seem to be specifically adapted to living in cold environments (Ernakovich et al. 2014). Most Arctic species are psychrotolerants (tolerant of cold temperatures, but also able to grow above 20°C) rather than psychrophiles (niche dependent on cold temperatures, see Morita 1975) (Jefferies et al. 2010, Ernakovich and Wallenstein 2015). Alongside the need to tolerate cold temperatures, arctic microbes also face periods of osmotic stress and resource starvation (Stähli and Stadler 1997). Because solutes are excluded from the ice as water freezes, the remaining unfrozen water will have an increasingly low osmotic potential, meaning that winter-active microbes often also need to be halotolerant (Jefferies et al. 2010). Further, substrate availability tends to decline throughout the winter, due to a combination of lack of plant exudates and reduced decomposition rates (Lipson et al. 2000, Ernakovich and Wallenstein 2015, McMahon and Schimel 2017). Conversely, during the spring thaw flush, microbes may have to cope with elevated levels of soil moisture or even anoxia (Björk et al. 2008, Edwards and Jefferies 2013), but at the same time an increase in available nutrients (Zak et al. 1990), also released from microbial cells (Gao et al. 2021). Taken together, all of these factors can be expected to give rise to seasonal patterns in the turnover and relative abundance of different taxa in the soil microbial community.

The biogeochemical activity in a soil can be influenced by the overall abundance or biomass of the microbial community (Moorhead et al. 2013) as well as its taxonomic and functional group composition (Fierer et al. 2007). In this section, we review the published evidence for winter-summer seasonal dynamics in Arctic soils separately for microbial abundance or biomass, and for microbial community composition and functional potential. Since for both aspects there is limited evidence, to draw overall conclusions we also review some similar studies on seasonality in alpine and temperate (i.e. seasonally cold) environments.

Seasonality of microbial biomass and abundance

Based on available evidence, we found that microbial abundance often differs between seasons, and that the winter-spring transition, with associated freeze-thaw cycles, seems to be a critical period. Various measures are typically used to estimate micro-

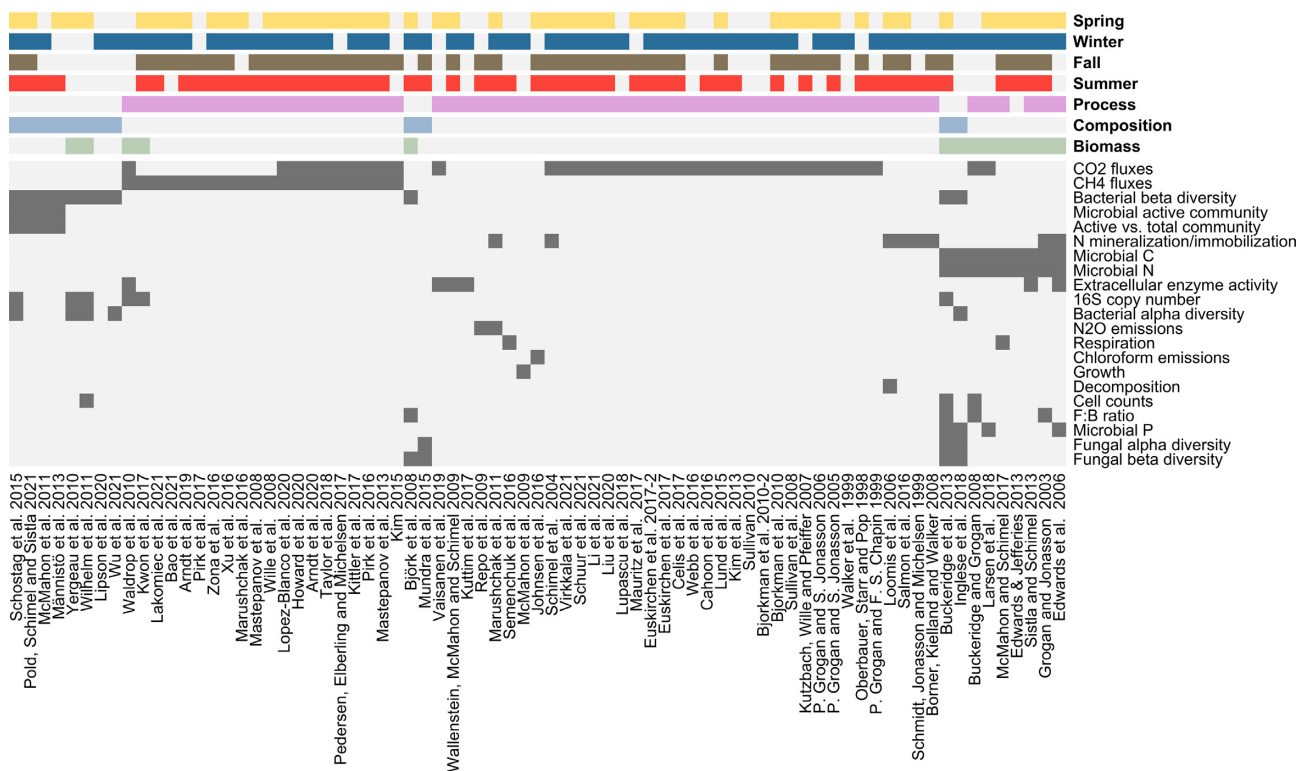


Figure 1. Summary of 75 studies that characterize soil microbial abundance, community composition and/or microbially mediated processes in the (sub-)arctic outside the summer vegetation growing season. Order of studies (in columns) determined by similarity of response variables (grey). Top rows indicate seasonal coverage of measurements. Studies were obtained from a custom search of Web of Science (publication dates range from 1977 to January 2022). See Appendix S1 for description of the literature search methodology and Appendix S2 for a database of all studies included in this heatmap.

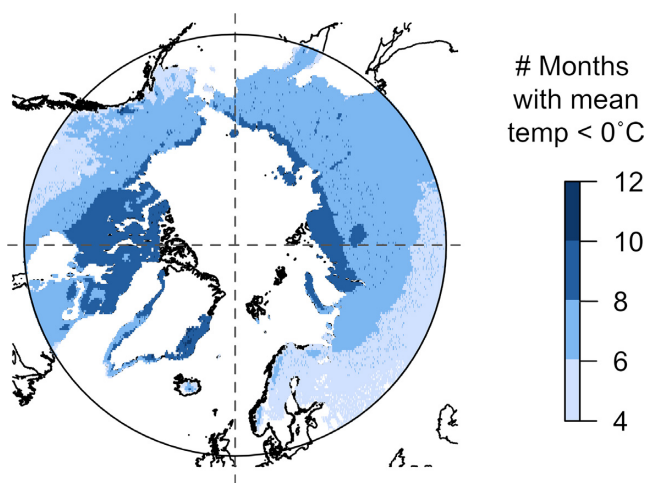


Figure 2. Duration of winter conditions in the pan-Arctic. Pixels are coloured according to the number of months per year with mean monthly soil temperatures $< 0^{\circ}\text{C}$ (0–5 cm depth) as modelled in the SoilTemp model (Lembrechts et al. 2020, 2022).

bial abundance, including quantification of gene copy numbers by quantitative PCR, estimates of microbial biomass (MB) using fumigation-extraction, or substrate-induced respiration methods, direct counts of cell numbers, and extraction and quantification of lipid biomarkers (Nikrad et al. 2016). We identified fourteen studies (annotated with 'Biomass' in Fig. 1) that investigated microbial abundance outside of the growing season. Six of these studies sampled year-round and five in just one season. Information

from studies that sample in one season only is limited by a single sampling time point. Eleven out of fourteen studies sample during the winter-spring transition, while six sample in fall (for additional information on sampling (time) points see Appendix S2). We will discuss seasonal patterns in microbial abundance in more detail below. In two studies carried out in Abisko (Northern Sweden), Grogan and Jonasson (2003) and Larsen et al. (2018) both found clear seasonal patterns in microbial biomass carbon (MBC). In soils of a birch forest, MBC showed a steady increase from October to May, and was lowest in July after snowmelt. Microbial biomass nitrogen (MBN) from the same samples was relatively stable throughout the year (Grogan and Jonasson 2003). Larsen et al. (2018) sampled in Abisko from March until snowmelt in May and found that temporal patterns in biomass differed between vegetation types. In heath tundra there was an approximately 40% decline in MBC during the winter-spring transition but in birch tundra it was constant throughout this period. In both ecosystem types MBN did not show any significant temporal dynamics (Larsen et al. 2018). Edwards et al. (2006 and 2013) studied calcareous fen mires in Manitoba, Canada. They found similar patterns between nutrients, with peaks for microbial C, N and P in late winter (April) and a declines of between approximately 25 and 75% before summer (Edwards et al. 2006, Edwards and Jefferies 2013). Additionally, they showed that these patterns can be highly variable between years, which they attributed to inter-annual variation in mire hydrology (Edwards and Jefferies 2013). In a moist acidic tundra in Alaska, Sistla and Schimel (2013) sampled on five occasions from late winter (April) to early winter (November). Both MBC and MBN were highest in late winter and thaw and then declined by roughly 80% and 70% respectively

by mid-summer (Sistla and Schimel 2013). To investigate microbial abundance at Daring Lake (Northwest Territories, Canada) in a mesic birch hummock ecosystem, Buckeridge et al. (2013) used a variety of different methods. Bacterial cell counts increased in late winter, peaked at thaw and then declined to rise again during summer. Bacterial 16S rRNA gene copy numbers showed somewhat different patterns, with no peak during the thaw peak but but a steady increase during summer that corresponded to the cell count data. Fungal 18S rRNA gene copy numbers showed very large variation among replicates and were not significantly different between sampling dates. The observed abundance changes coincided with changes in community composition (Buckeridge et al. 2013, see below). In contrast, Schostag et al. found no clear seasonal patterns in 16S rRNA gene copy numbers based on a full year of monthly sampling in Svalbard permafrost (Schostag et al. 2015).

Extending our scope to alpine environments reveals a range of studies that confirm the general patterns observed in (sub-)arctic soils: microbial biomass has often been observed to reach maximum annual levels during (late) winter (Schadt et al. 2003, Lipson and Schmidt 2004, Gavazov et al. 2017, Xu et al. 2018) or in winter and spring (Lipson et al. 1999, Puissant et al. 2015). In a sub-alpine grassland, microbial biomass during winter can be as much as double the summer level (Gavazov et al. 2017). However, such patterns are not universal. Löffler et al. (2008) observed bacterial cell counts in dry heath tundra that were lowest in early spring, and increased over the summer to September (Löffler et al. 2008). Similarly, 16S rRNA gene copy numbers were low in February and high in August in meadows and glacier forefields in the Swiss Alps (Lazzaro et al. 2015).

When higher winter microbial biomass is observed, it is often attributed to a simultaneous increase in fungal abundance and decrease in bacterial abundance, leading to an increase in the fungal-to-bacteria ratio (F:B ratio) outside of the growing season. The evidence for this is mostly from alpine systems, where higher fungal abundance and F: B ratios are often observed in winter (Schadt et al. 2003, Lipson and Schmidt 2004, Puissant et al. 2015). This has also been documented in arctic mesic tundra (Buckeridge et al. 2013). In contrast, year-round stability in fungal gene copy numbers have been found in both mesic Arctic tundra and alpine grasslands (Lazzaro et al. 2015).

The preceding examples show that seasonal dynamics of soil microbial biomass are often observed, and that the most common patterns involve a peak in observed biomass in late-winter to early spring, and lowest levels in a range of periods—from just after thaw to late summer, depending on the system. The pattern seems most robust when based on fumigation-extraction techniques. DNA-based and cell-count methods tend to show less distinct seasonality, possible due to the relative stability of the relevant biomarkers (see section below). Greater sampling efforts during the winter-spring transition might be one of the reasons the spring thaw is often identified as a critical period, and the low number of observations per season might hide additional seasonal dynamics.

Seasonality of microbial community composition

We were able to identify seven studies that characterized microbial community composition in Arctic soils at multiple times of the year including the snow-covered period (Table 1). Most sampling occasions occurred around the spring thaw period (May/June, depending on the location) and early in the growing season (Fig. 3). Mid-winter sampling (December-February) was

done in only one study (Schostag et al. 2015). Nevertheless, all studies showed evidence for seasonality in some aspects of microbial community composition, often coinciding with shifts in microbial abundance. Since there is very little evidence available and studies use different (sampling) methods to investigate microbial communities (Table 1), drawing general conclusions on seasonality of microbial community composition is difficult. However, there were some observations shared by multiple studies, which we summarize here.

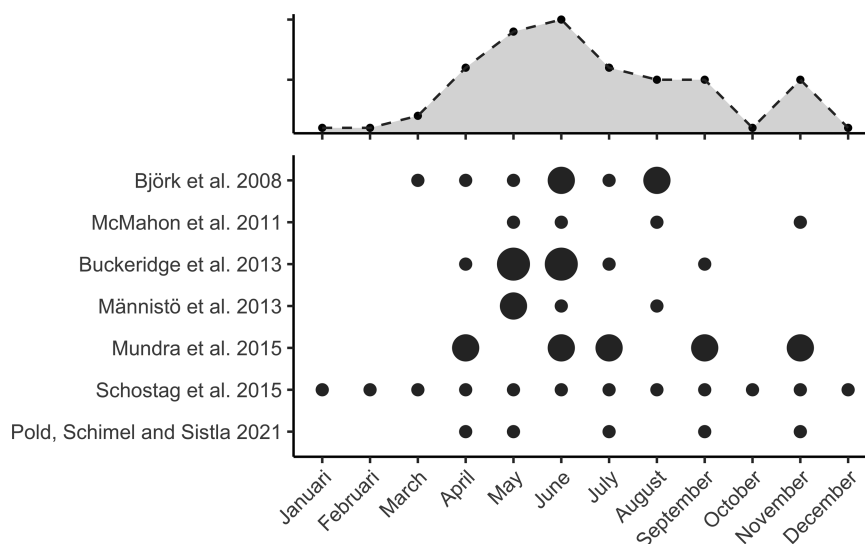
Sampling occasion was often the most important factor explaining differences in microbial community composition; more than vegetation type (Björk et al. 2008, McMahon et al. 2011), soil depth (Pold et al. 2021), or habitat with varying degree of snow cover (Männistö et al. 2013) and/or temperature (Pold et al. 2021). Alpha diversity did not show seasonal differences across seasons in the active layer of permafrost soils at Svalbard (Schostag et al. 2015). The largest turnover of species often occurred when soils thawed in spring (Björk et al. 2008, Buckeridge et al. 2013, Männistö et al. 2013, Schostag et al. 2015) or froze again in fall (Schostag et al. 2015), however, this conclusion could be biased by the fact that most samples were collected around the spring thaw (Fig. 3)—as also seen for evidence on biomass/abundance (see section above). In some studies, the change in community composition at snowmelt was only apparent when investigating the active fraction of the microbial community (McMahon et al. 2011), while the total community was observed to change earlier in the year between April and May (Pold et al. 2021). All in all, the consistently larger effect of seasonal sampling date relative to other potential drivers suggest that microbial communities may be more sensitive to short-term seasonal fluctuations than to long-term changes in vegetation cover and/or temperature, emphasizing the need for more systematic temporal analysis of these systems.

Although it seems seasonal fluctuations in abiotic conditions often influence community composition, spatial effects with accompanied changes in vegetation type and/or temperature regimes are still an important factor. A study focusing on root-derived ectomycorrhizal fungi in a heath ecosystem on Svalbard failed to find clear seasonality in community composition. In this case, spatial effects were more pronounced than seasonality, although after accounting for the former, some differences in richness and community structure between winter and summer became apparent (Mundra et al. 2015). Similarly, in shrub and tussock tundra at Toolik Lake there were differences in seasonal dynamics between vegetation types. Shrub bacterial communities showed greater seasonal dynamics which was linked to a change in active community composition, whereas in tussock soil active microbes were more likely to change their metabolic state (McMahon et al. 2011). There was also a vertical spatial effect in the same location where sampling time point differences were particularly evident in the deepest mineral soil layer (Pold et al. 2021).

Overall changes in community composition could in some cases be attributed to fluctuations in abundance of specific taxa. For example, winter communities in the organic layer of soil in birch hummock at Daring Lake could be distinguished by a *Flavobacterium* with specific membrane adaptations to low temperatures (Buckeridge et al. 2013). At the critical period of snowmelt some studies found a significantly higher proportion of Actinobacteria (Björk et al. 2008; Buckeridge et al. 2013), and a terminal restriction fragment that best matched Actinobacteria became more abundant during the growing season in tussock tundra (McMahon et al. 2011). In contrast, in a study by Pold et al. (2021), Actinobacteria abundance decreased after snowmelt in the organic rhizosphere-associated top soil. Acidobacteria, who favour

Table 1. Overview of methods, locations and vegetation types for studies that investigated microbial community composition at multiple times of the year including the snow-covered period.

Author/year	Method	Location	Vegetation type
Björk et al. 2008	PLFA	Latnjajaure, Northern Sweden	Four tundra types
McMahon et al. 2011	BrdU labelling and T-RFLP (class-level)	Toolik Lake, Alaska	Shrub and tussock tundra
Buckeridge et al. 2013	PLFA	Daring Lake, Canada	Birch hummock
Männistö et al. 2013	BrdU labelling and T-RFLP	North-West Finland	Tundra heath
Mundra et al. 2015	ITS2 sequencing	Svalbard	Tundra heath
Schostag et al. 2015	16S sequencing (DNA and RNA)	Svalbard	Tundra heath
Pold et al. 2021	16S sequencing (BrdU and DNA)	Toolik Lake, Alaska	Shrub and tussock tundra

**Figure 3.** Overview of sampling points for studies that investigated microbial community composition at multiple times of the year including the snow-covered period. Size indicates the number of observations within the same month. Multiple observations within the same month for Mundra et al. (2015) are done in different years.

less labile C sources, grow slowly, and are sometimes found to dominate soil bacterial communities in the Arctic (McMahon et al. 2011, Männistö et al. 2013, Pold et al. 2021), were least abundant in September in the mineral soil at Toolik Lake (Pold et al. 2021), and they dominated the winter-active community in shrub tundra (McMahon et al. 2011). In tundra heath, different phylogenotypes within the Acidobacteria dominated at different sampling dates in the total and active microbial community profiles and the turnover of specific phyla was not always apparent for total communities (Männistö et al. 2013). Seasonal changes were not always strongly related to changes in abundance of any dominant phylum (Pold et al. 2021). It is possible that seasonal differences become only visible at a lower taxonomic level. We want to emphasize that these studies make use of different molecular methods to characterize the communities which measure different aspects of microbial communities and are therefore not directly comparable.

As with seasonal changes in microbial abundance, additional evidence for seasonality in microbial community composition in cold ecosystems comes from alpine systems (Ernakovich et al. 2014, Donhauser and Frey 2018). In alpine environments, intra-annual differences are often observed, and are generally greater than variation between sampling sites. In an alpine dry meadow in the Colorado Rocky Mountains, 16S rRNA gene clone libraries revealed different bacterial communities between winter, spring and summer, where the spring community was most distinct from

the other sampling points (Lipson and Schmidt 2004). In four different sites in the Swiss Alps, seasonal variation in both bacterial and fungal communities based on T-RFLP analysis was greater than differences between sites (Lazzaro et al. 2015). A comparison between early and late snowmelt sites based on SSU libraries revealed a similar pattern (Shahnavaiz et al. 2012) and in Swiss Jura subalpine grassland soils, there was no clear grouping of communities by elevation but only by season (Puissant et al. 2015). In the Colorado Rocky Mountains, fungal communities changed most from spring to summer, which is warmer and dry (Schadt et al. 2003). In contrast, in a boreal mire in Southern Finland, T-RFLP combined with cloning of Archaeal 16S ribosomal DNA and reverse-transcribed RNA revealed relatively small shifts in the total microbial community with season but a more dynamic active microbial community (Juottonen et al. 2008). 16S rRNA gene pyrosequencing of a temperate mountain forest soil community by Kuffner et al. 2012 similarly showed that relative abundances of individual groups displayed moderate shifts but saw no seasonal switches between dominant and rare taxa, and no seasonal differences in diversity indices. In the Austrian Alps, based on PLFA data, a stable bacterial community composition over winter was found (Gavazov et al. 2017), however, no comparison with summer communities was made.

Although based on a relatively small number of studies and a with a clear sampling bias towards the winter-spring transition (Fig. 3), we can conclude that communities are relatively sta-

ble throughout the whole winter period, but the transition from frozen to thawed soils after snowmelt regularly results in the largest change in community composition. This period is also often associated with large changes in microbial biomass (see section 2.1) and is presumably driven by changes in environmental conditions such as substrate availability, soil liquid water content and soil aeration. If shown to be general for more arctic environments these results imply that seasonal variation in microbial communities might be relevant for understanding soil biogeochemistry, and moreover, that changes in environmental conditions that are expected to happen under future climate change might have large effects on microbial communities and their associated functions.

Seasonality of microbial processes

From the perspective of arctic biogeochemistry, the seasonal dynamics of soil microbial community abundance or composition are only important in contexts where composition is a reliable indicator of the seasonality of microbe-mediated biogeochemical functions. Intra-annual variation has been observed in arctic and subarctic soils for a range of soil processes. For example, soil CO₂ efflux at two high latitude sites was typically 10–100 times higher in summer compared to winter (Björkman et al. 2010). Time series of CH₄ emissions from three different arctic sites could be separated into two or three (overlapping) peak periods, related to the timing of snow-melt, the peak vegetation growing season, and ground-freezing (Pirk et al. 2017). Shifts from N immobilisation during the growing season to N mineralisation during winter are commonly observed in arctic and alpine environments (Grogan and Jonasson 2003, Schimel et al. 2004, Weintraub and Schimel 2005, Gavazov et al. 2017, McMahon and Schimel 2017). Intra-annual variation in potential enzyme activity has also been observed (Wallenstein et al. 2009, Weedon et al. 2014), suggesting seasonal variation in the nutritional requirements of soil microbes. On one level, such seasonal dynamics of biogeochemical process rates are to be expected: intra-annual variation in soil moisture (Mintz and Serafini 1992) and temperature (Lembrechts et al. 2022) are universal, and these factors, along with soil chemical properties, all exert strong controls over soil biogeochemical process rates (Booth, Stark and Rastetter 2005, Davidson and Janssens 2006). If these physical controls (temperature and moisture) are dominant relative to biotic factors, then the observational bias towards growing season measurements may not be problematic. In this case it should be possible to define an (empirical) model relating these soil physical and chemical properties to process rates (Fang and Moncrieff 2001, Mikan et al. 2002) and use it to extrapolate from growing season observations to the rest of the year using climatic and soil hydrological data. However, the examples given above (section 2) raise the possibility that seasonal dynamics in the composition of soil microbial communities could lead to seasonally varying mediation and interactions of biological controls of soil process rates with the effects of physical drivers. For example, seasonal variation in the predominance of certain metabolic pathways; competition for nutrients and substrates, uptake and immobilization, antagonistic interactions, and other organism-specific dynamics may cause seasonal variation in the observable relations between soil physical and chemical properties and process rates. If these controls are important, then extrapolation from growing-season sampling and measurements to the rest of the year may result in significant biases in prediction and upscaling. This may be particularly important for phylogenetically narrow functions such as methanogenesis and

denitrification, for which there is relatively less functional redundancy within a local community (Schimel 1995). We propose that seasonality of microbial communities will have consequences for predicting biogeochemical processes in situations where one or more of the following are true: 1) microbial communities active at different times of the year show different process kinetics; 2) the relative importance of different microbial processes and/or their corresponding physico-chemical controls varies throughout the year; and 3) (as a consequence of the previous) the response of communities and their associated processes to perturbations (e.g. climate change) depend on the timing of the perturbation, and/or the timing of observations. In the remainder of this section, we give examples of evidence for each of these scenarios.

If microbial communities vary in process kinetics (i.e. different functional relations between rates and physico-chemical controls) at different times of the year, then extrapolations based on relationships observed in one season to other times of year will be biased (Fig. 4). Evidence for such intra-annual variation of kinetic parameters has been found in a range of arctic, alpine and temperate systems. Wallenstein et al. (2009) found that the Q_{10} (temperature sensitivity) of six hydrolytic soil enzymes varied seasonally in arctic tundra soils, meaning that the same enzymatic processes respond differently to variation in soil temperature at different times of the year. They suggested that this is due to differences in the isoenzymes contributing to enzyme activity, most likely caused by different microbes producing them (Wallenstein et al. 2009). Additional relevant evidence comes from alpine and temperate environments. In two different vegetation types in the Rocky Mountains, potential respiration rates at two incubation temperatures (0 and 22 °C) were found to differ between soils sampled in winter and summer. At low temperatures at 0 °C, winter communities showed higher activity, higher growth rates and higher temperature sensitivity than summer communities incubated under the same conditions (Lipson et al. 2002, Monson et al. 2006). In both studies this was for a large part explained by changes in microbial community composition, and the ability of communities collected from under the snow pack to still grow exponentially at 0 °C, while the summer community was not able to grow below 4 °C (Monson et al. 2006). A similar seasonal contrast has been observed in several studies of CH₄ production—a classical narrow function. In a boreal mire, the highest potential rates of archaeal CH₄ production at a standard temperature were measured from winter communities (Juottonen et al. 2008). In a temperate system, incubation of three different peat soil types at the same temperature year-round showed a moderately high CH₄ production potential in soils sampled in January from one site, but the highest potential in June. Addition of different substrates influenced the CH₄ production capacity and methanogen abundance in a season-dependent manner and different taxa were found to be active throughout the year, suggesting that a number of distinct communities of methanogens, each with particular responses to temperature and substrate supply, were responsible for the CH₄ production at different times of the year (Sun et al. 2012). Both of these studies involve laboratory incubations at set temperatures of field collected samples, and deal with systems outside the (sub-)arctic region. Although they provide evidence of seasonally varying process kinetics, more measurements, preferably *in situ*, are needed to confirm whether similar variation exists in boreal and (sub-)arctic environments.

It is important to note that different potential process rates between temporally distinct sub-communities may, at least partially, be due to differences in the types of substrates that predominate in the soil during different seasons (Lipson et al. 2002): mi-

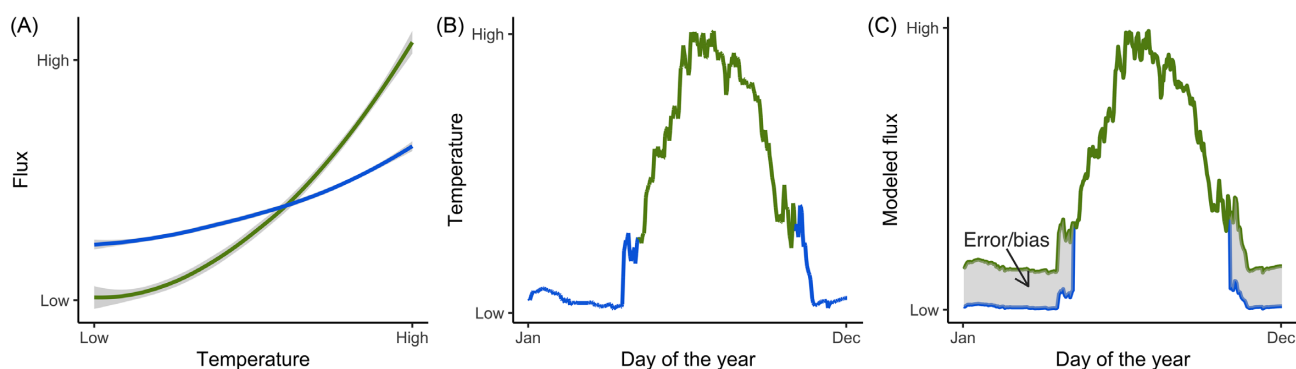


Figure 4. Hypothetical relationship between process kinetics and microbial communities. For simplicity we visualize a situation where there are two distinct microbial communities that are clearly separated in time and have strongly contrasting process kinetics (A) Idealized process kinetics for the growing season (green line) and winter (blue line) microbial communities, represented as a rate of flux as a function of soil temperature (B) Soil temperature throughout the year, colored by season. (C) Modelled flux throughout the year. Green line assumes summer process kinetics persist year-round, blue line shows the 'actual' flux rates outside the growing season. The grey area indicates the potential error or bias when process kinetics measured on the summer community are used to make predictions year-round.

crobes often go from processing more complex carbon polymers and phenolics in winter, to proteins during snowmelt, and use mostly rhizodeposits in the vegetation growing season (Schmidt et al. 2007). These different substrates may have inherently different decay kinetics (Davidson and Janssens 2006), as well as promote the growth and activity of different members of the soil microbial community, with different life history strategies (Fierer et al. 2007). This seasonality in substrate-supply most likely combines with the seasonality of conditions (dis)favoring certain metabolic pathways such as waterlogging and changes in oxygen availability.

Biological factors can also contribute to seasonal variation in process rates when either fundamentally different processes predominate at different times of year, or the importance of different controlling factors changes seasonally. In this situation, extrapolations based on growing-season observations of microbial physiology could underestimate, or even completely miss important processes and their predominant controls. An example is CH_4 production in arctic environments, where the contribution of methanogenesis to soil fluxes is often largest outside the growing season during the so-called 'zero curtain', when soil temperatures balance around 0°C (Zona et al. 2016, Raz-Yaseef et al. 2017). Year-round measurements suggest that limiting measurements of soil carbon fluxes to the growing season could miss as much as 50% of the annual methane emissions (Zona et al. 2016, Raz-Yaseef et al. 2017, Taylor et al. 2018). Moreover, observations of an hysteresis dynamic in the relationship between soil temperature and CH_4 efflux, when compared between spring and early fall, suggests that different controls besides temperature drive CH_4 efflux in the different seasons (Taylor et al. 2018). Evidence from arctic and alpine systems also suggest that the dominant fluxes in soil nitrogen dynamics contrast strongly between growing and cold seasons. A shift in microbial activity that leads from a predominance of N immobilisation during the growing season (especially at thaw) to net N mineralisation during winter (Grogan and Jonasson 2003, Schimel and Bennett 2004, Weintraub and Schimel 2005, Gavazov et al. 2017, McMahon and Schimel 2017) can lead to as much as twice the amount of soil extractable ammonium and triple the amount of exchangeable organic N at snowmelt compared to the growing season (Gavazov et al. 2017). More generally, the stoichiometry of soil microbes seems to vary seasonally in many cold-climate systems. Microbial C demand may be highest in late winter (Lipson et al. 2000, Brooks et al. 2004, Buck-

eridge and Grogan 2008), which can have consequences for the subsequent growing season's plant growth (Schmidt and Lipson 2004). Seasonal dynamics of macronutrient supply and demand are also detectable in patterns of soil enzyme activity (Weedon et al. 2011). In an Alaskan tundra ecosystem, the highest potential activity of hydrolytic enzymes was found during winter, while oxidative enzymes were found to have a greater potential during summer (Sistla and Schimel 2013), which is likely related to the oxygen status of the soil (Freeman et al. 2001). Similarly, in calcareous fen mires in Manitoba, Canada, the acid and alkaline phosphatase activities (hydrolytic enzymes) were higher during spring melt than during the growing season (Edwards et al. 2006). In subalpine grasslands in the Swiss Jura mountains, microbial potential enzyme activity was three times higher in winter and spring than in summer and autumn (Puissant et al. 2015). All of these examples illustrate the risk of bias if conceptual understanding and quantitative estimates of soil biogeochemical cycles are based on growing season observations only. It should be noted that none of the studies cited here provide direct evidence that the observed seasonality in biogeochemical processes is a result of seasonality in microbial communities. However, this is a consequence of the extreme rarity of studies that combine process and community measurements over the entire year (Fig. 1, Appendix S2); and we propose that the investigation of these potential links forms an important near-term research goal (see section below).

A potential consequence of intra-annual variation in microbial communities, process kinetics, and dominant processes relates to the effect of changes in conditions (e.g. climate change) on the soil system. The effect of a perturbation is likely to depend on both the timing of the perturbation, and the observational period in which the system is monitored for changes. This is very important in experiments investigating the effects of climate change on arctic soil biogeochemistry. This is because it is likely that winter and shoulder season microbial communities and their associated processes respond differently to climate change compared to summer communities; and moreover, changes in temperature or precipitation will have different effects on the soil biota, depending on the timing of the changes (e.g. winter versus summer warming (Williams et al. 2015)).

There are several examples from the recent literature that illustrate this potential for complex interactions between the timing of climate change factors, and the timing of the consequences. At the level of microbial communities, Semenova et al. (2016) found that

winter snow-depth manipulations (simulating increased winter precipitation) altered the functional composition of fungal communities when sampled in summer (Semenova et al. 2016). Similar experimental designs applied failed to find an effect of winter snow cover manipulation on spring and summer microbial communities in a Finnish boreal forest (Männistö et al. 2018) and in Swedish subarctic Tundra (Krab et al. 2019). Evidence from a series of snow-cover manipulation sites, along a precipitation gradient in Inner Mongolia, suggests that the variable effect of snow cover on soil microbes might be associated with differences in ecosystem water status (Liu et al. 2018). Studies of soil enzymes and nutrient cycling have also revealed seasonal variation in the effects of climate change manipulations. In a 22-year long tundra warming experiment at Toolik Lake, Alaska, summer warming resulted in a significant increase in peroxidase activity. However, the main effects of summer warming on extracellular enzyme activities occurred during early and late winter, and thaw (Sistla and Schimel 2013). In subalpine grasslands in the Swiss Jura mountains, transplanting soils to lower altitudes to mimic year-round warming only affected potential overall microbial activity (measured as fluorescein diacetate hydrolysis) during winter, where lower levels of potential activity were measured in warmed soils (Puissant et al. 2015). In another study conducted at Toolik Lake, Alaska, it is suggested that the effect of increased N mineralisation rates under a deeper snow pack could be continued into the growing season by altering the amount and timing of plant-available N in this ecosystem (Schimel, Bilbrough and Welker 2004). Although the examples given here are too heterogeneous to allow general conclusions, they show that temporal asynchrony between perturbations and their corresponding effects may be widespread in (sub-)arctic soils. Ecosystem level experiments in climate change should therefore be designed to detect a range of seasonal effects.

Knowledge gaps and future perspectives

The above discussion has shown that: the majority of soil microbial ecology research in (sub-)arctic environments has been limited to measurements taken during the vegetation growing season (Section 1); there is nevertheless evidence that microbial community composition and abundance show considerable intra-annual variation (Section 2); and this variation can potentially complicate our ability to estimate and predict year-round biogeochemical functioning, and forecast the effects of a changing climate (Section 3). The empirical basis for the latter two points is currently limited by the lack of available data, so further research is necessary to establish generality and relative importance of causal relationships between climatic seasonality, soil microbial communities, and their associated biogeochemical processes. This information will be valuable for parameterizing and validating the new generation of microbe-focussed soil ecosystem models (Wieder et al. 2015, Guo et al. 2020), and for improving projections of soil biogeochemical processes under novel climates with changing timing and magnitude of seasonal variation (Ernakovich et al. 2014). Below we highlight the most important knowledge gaps and opportunities for future research in this field.

Sampling design for intra-annual variation. The exact timing and dynamics (i.e. gradual vs abrupt transitions) of intra-annual variation are challenging to determine. Ideally, regular sampling throughout the whole year would be conducted, with sufficient sampling moments to determine the timing of important changes, and the duration of periods of relative stability. However, sampling in the (sub-)arctic is usually constrained by logistics, so a more pragmatic approach might be to implement temporally nested

sampling designs (Morrisey et al. 1992). This involves sampling at several sets of time intervals, for example two consecutive days, within two consecutive weeks within four selected moments spread throughout a year. Such a design allows quantification of the relative magnitudes of fluctuations at different timescales. If multiple replicates from multiple locations are taken for each time point, information about the magnitude of spatial variation can also be obtained. This is particularly important for soil organisms, since destructive sampling and a high degree of small-scale spatial variation mean that temporal and spatial variation are always confounded to some degree (De Gruyter et al. 2020). Such a design could target the moments of higher frequency sampling towards times of year that are likely to be particularly dynamic for the communities and processes of interest. Our review above suggests that the late winter/spring/summer transition, and not the growing season, is most important from this perspective, but whenever available researchers can use a combination of historical soil temperature records and prior knowledge about seasonal dynamics (e.g. for methanogenesis the observations of Zona et al. 2016 and Raz-Yaseef et al. 2017) to design the sampling strategy for their particular system.

Alongside the need for higher resolution temporal sampling, observations from multiple years are crucial for confirming the generality of seasonal patterns. This is especially relevant in the light of climate change, where each consecutive year might be drastically different from the last. Without observations from multiple years from the same system, any observed differences cannot be distinguished from confounding spatial variation, nor can properly seasonal (i.e. periodic) dynamics be distinguished from possible longer-term directional changes, or random-walk like dynamics. Indeed, of the primary studies we reviewed in Section 2, only 15% included observations from a period > 12 months.

Once suitable time series data are obtained, they will need to be analysed with appropriate statistical methods. Although tools for detecting seasonality, change-points and long-term trends in time series data are available (Zhao et al. 2019) they require larger numbers of observations than are usually available from soil sampling campaigns, and work best with regularly spaced data. Smoothing techniques such as Generalized Additive Models (GAMs) are more flexible and allow formal tests for non-linearity, trends and periodic fluctuations (Simpson 2018) although the sensitivity of these will depend on the frequency of sampling and length of the time series. For temporally nested designs, mixed-effects models can be used to partition variance between different time scales (Bolker et al. 2009).

Higher frequency, multi-year sampling designs will require considerable more cost and effort than single time point sampling. We recommend that researchers balance these costs against the potential risk of mischaracterizing the system due to sampling moments that are mismatched relative to the process of interest. Improved temporal sampling strategies will allow more robust characterization of the temporal patterns identified above. They will provide answers to questions such as: how common is intra-annual variation in process kinetics? (Lipson et al. 2002, Monson et al. 2006, Wallenstein et al. 2009). Can we better constrain our estimate of the timing of community changes between winter and summer? How stable are community compositions and process rates at different times of year? Answers to these questions will in turn inform the further refinement of sampling strategies for better characterizing the interplay of physical and biotic factors in controlling soil biogeochemistry in seasonally-cold environments.

Linking microbial community composition with biogeochemical processes: both our arctic bibliographic analysis, and a broader meta-analysis by Bier et al. (2015) showed that currently there are very few studies that try to statistically link the microbial community composition to (potential) functionality (Fig. 1). Defining the nature of such a link is important, since many studies describe dynamics in community composition, precisely to make inferences about possible effects on ecosystem functions. Although the challenge of linking composition and function is common to all microbial ecology, it is particularly acute in a rapidly changing arctic winter, where identifying which organisms are able to metabolise the complex substrates available at that time of year might be key to understanding the link between microbial communities and biogeochemical processes. Additionally, because of low turnover of DNA markers in cold soils, rRNA genes isolated during winter might not reflect microbial activity at the time of sampling (Schostag et al. 2020). This emphasises the need to investigate the active part of the microbial community, using e.g. metatranscriptomic analysis, labelling of nucleic acids (e.g. BrdU), labelled PLFA or metaproteomics and metabolomics (Singer et al. 2017).

Dominant drivers: almost all the multi-season studies cited above found that there is at least some degree of seasonality in microbial community composition in (sub-)arctic soils, and that the winter-spring transition is typically the moment of most abrupt change. The precise causal mechanisms driving these dynamics remain largely unknown. Likely candidates include seasonal variation in soil physical-chemical conditions (e.g. temperature, soil moisture and osmotic potential), in the quantity and quality of resources inputs from plants into the soil (e.g. root exudates, fine root turnover, litter fall), and in the degree of top-down control by soil fungi- and bacteriophages (Crowther et al. 2013), or viruses (Kuzyakov and Mason-Jones 2018). Identifying the dominant seasonality drivers, and the degree to which these are site-specific or generalizable across all arctic soils would be an important step in building a predictive understanding of whole-year soil biogeochemistry. If physical-chemical conditions such as temperature override other factors, then modelling efforts can subsequently focus on understanding how changing climate will alter the dynamics of soil conditions, and thereby affect the timing of transitions in microbial communities and functions. If plant-mediated factors are more important, as suggested by Schostag et al. (2015), then research efforts can be directed towards understanding the interplay between microbial communities and the quality and timing of plant inputs, as well as coupling the study of arctic vegetation phenology (Bjorkman et al. 2015) with processes happening in the soil. Experiments to identify the relative importance of different drivers could take advantage of existing climate manipulation experiments (Henry and Molau 1997). For example, by measuring how warming-induced changes in the timing of soil thaw alters microbial temporal dynamics and comparing the magnitude of these effects to changes induced by experimental manipulations of plant cover, litter input, or substrate availability. Alternatively, observational time series of communities and processes, coupled to data on soil physical and chemical parameters, could be analyzed using techniques for causal inference, such as structural equation models and recent extensions to path analysis (Shipley 2000, Douma and Shipley 2021).

Conclusions

The important role of soil microorganisms in regulating the status of the world's largest soil carbon stock lends an urgency to the study of arctic soil microbiology (Wieder et al. 2019). Given

the ubiquitous intra-annual dynamics of microbial communities and the processes they mediate, combined with the strongest expected climate changes at high-latitude taking place during the winter, we argue that future studies should incorporate designs that cover the full annual cycle of arctic soils. Understanding which times of year are particularly critical for soil microbes, and how their sensitivity to changes in the soil environment varies throughout the year, will be essential for predicting the effects of ongoing climate change in all seasons on the microbiology and biogeochemistry of high-latitude systems. Although extending studies beyond the vegetation growing seasons brings daunting logistical and methodological challenges, we are optimistic that the obtained insights will richly compensate for the increased effort and difficulty.

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Supplementary data

Supplementary data are available at [FEMSEC](#) online.

Conflict of interest statement. None declared.

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